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Of Biodiversity, Boundaries, and Distribution: The Myxomycetes of the Philippines and Beyond

Sittie Aisha Bustamante Macabago
University of Arkansas, Fayetteville

Of Biodiversity, Boundaries, and Distribution: The Myxomycetes of the Philippines and Beyond

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by

Sittie Aisha Bustamante Macabago
University of Santo Tomas
Bachelor of Science in Microbiology, 2007
University of Santo Tomas- Graduate School
Master of Science in Microbiology, 2010

July 2021
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

_____
Steven L. Stephenson, Ph.D.
Dissertation Director

_____
Frederick W. Spiegel, Ph.D.
Committee Member

_____
Allen L. Szalanski, Ph.D.
Committee Member

_____
J. D. Willson, Ph.D.
Committee Member

Abstract

This dissertation contains a compilation of independently performed studies primarily focusing on the myxomycetes (plasmodial slime molds) from the Philippines and integrating local and worldwide data to demonstrate regional and global trends. The major themes include the following: (I) a review of the diverse group of spore-producing amoeboid protists, including the myxomycetes; (II-IV) diversity assessments in three different groups of islands in the Philippine archipelago; (V) mapping the myxomycetes found in the Philippines for databasing and analyzing the geocoded data; (VI) a study on regional boundaries, including the Philippines, using myxomycete species composition; and, (VII) creating a global species distribution model of the ubiquitous myxomycete morphospecies, *Arcyria cinerea*, and predicting future distributions under different climate scenarios.

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Sainyong lahat, Maraming Salamat po!

Dedication

To learning, unlearning, and relearning.

Table of Contents

Introduction……1

I. A Review on the Dispersal of Spores and Variations in Sporogenic Fruiting Amoeboid Protists……..3

Abstract……3

Sporogenic fruiting amoeboid protists……..……...3

Spore production and dispersal as an adaptive strategy……...……..13

Life stages and reproductive strategies of fruiting amoeboid protists…….……..27

Genetic variation in fruiting amoeboid protists: roots and consequences…….....……...32

Perspectives……41

Literature Cited……..44

II. Implications of the Role of Dispersal on the Occurrence of Litter-inhabiting Myxomycetes in Different Vegetation Types after a Disturbance: A Case Study in Bohol Islands, Philippines……...……...55

Abstract……..55

Introduction……55

Materials and Methods……...57

Results……61

Discussion……..63

Literature Cited……..70

Tables……76

Figures……77

III. Myxomycetes of the Caramoan Islands and an Update on the Species Found in the Bicol Peninsula, Philippines ……...81

Abstract……..81

Introduction……....82

Materials and Methods……...83

Results and Discussion……..86
Conclusions and Recommendations……..92
Literature Cited……..93
Tables……96
Figures……98

IV. Myxomycetes of Coron Island and Additions to the Myxomycetes of Palawan group of Islands in the Philippines.……...……..104
Abstract……104
Introduction……..104
Materials and Methods……105
Results……..108
Discussion……111
Literature Cited……114
Tables……...118
Figures……..120

V. Mapping the Myxomycetes of the Philippines……121
Abstract……121
Introduction……..121
Methods……122
Results and Discussion……125
Literature Cited……128
Tables……...132
Figures……..134

VI. Does the Huxley's line Apply to Myxomycetes?…….…...……...148
Abstract……148
Introduction……..148
Methods……151
Results……..153

Discussion……155
Literature Cited……157
Tables……...162
Figures……..163

VII. Species Distribution Modeling of the Morphospecies *Arcyria cinerea* (Bull.) Pers......166
Abstract……166
Introduction……..166
Methods……168
Results and Discussion……....172
Literature Cited……176
Tables……...180
Figures……..181

Conclusions…….192
Literature Cited…….194

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Introduction

Plasmodial slime molds, also referred to as myxomycetes or myxogastrea, are amoeboid protists that can generate spore-producing fruiting bodies often visible to the naked eye. Current classifications consider the myxomycetes as a monophyletic taxon within Amoebozoa (Adl et al. 2018, Lahr et al. 2011, Fiore-Donno et al. 2010). Contemporary studies are pushing towards a phylogenetic classification of the group as opposed to the traditional classification into five orders (Echinosteliales, Liceales, Trichiales, Stemonitidales, and Physarales) (Leontyev et al. 2019) using mostly noticeable morphological characters, or at least a combination of modern molecular methods and traditional taxonomic techniques (Walker & Stephenson 2016). Whether morphological characterizations are supported by molecular data, it is unsurprising to say that the persistence of species and populations in the myxomycetes are shaped by spore dispersal.

The first part of this dissertation reviews a heterogenous group of fruiting amoeboid protists, including the myxomycetes. These are the amoebozoan dictyostelids, myxogastrids, and protosteloid amoebae, the heterolobosean acrasids, and other spore-generating amoeboid protists. This section also describes differences in their lifestyle and reproductive systems, and examines the role of multiple vector contributions to their dispersal, as well as long-distance dispersal events and the probable consequences of dispersion for the communities and populations.

Chapters II to IV of this work feature independent studies on the diversity of the myxomycetes found in selected groups of islands in the Philippines. Different factors likely affecting their occurrence and diversity have been examined in the separate investigations. These include the following: the implied role of dispersal and associated vegetation in the Bohol group of islands (chapter II), the correlation of geographic and ecological distances and the relevance of the theory of island neutrality to the patterns of myxomycete assemblages in the Caramoan islands

(chapter III), and a survey of myxomycetes in Coron island and updates to the myxomycetes of the Palawan group of islands (chapter IV).

It has already been a few decades since the exploration of myxomycetes in the Philippines began, however collections of myxomycetes are still scattered and lacking a convenient database for sharing and updating existing information. In chapter V of this manuscript, an effort was started to create a repository of myxomycete collections that can be accessed, queried, and analyzed upon publication.

Regional boundaries on myxomycetes have also been examined in chapter VI, such that the recognized biogeographic boundary demarcated by Huxley's modification of the Wallace line was used to see if myxomycete species composition varies within this controversial boundary or transitional zone.

In the last section (VII) of this dissertation, the first effort to create a global environmental niche model of the cosmopolitan species, *Arcyria cinerea* (Bull.) Pers. was made to reveal tendencies in the persistence of the morphospecies under two future climate change scenarios.

I. A Review on the Dispersal of Spores and Variations in Sporogenic Fruiting Amoeboid Protists

Abstract

The ability to form fructifications that carry airborne spores must have evolved multiple times within the tree of life. With the exclusion of plants, true fungi, myxobacteria, the ciliate *Sorogena*, and the stramenopile *Sorodiplophrys*, some groups of amoeboid organisms can generate spore-bearing structures that allow dispersion of their propagules. Dispersal of spores is an essential function of fruiting amoeboid protist species with ecological and evolutionary values, especially with persistence of populations and species under global change. This review aims to describe the variations among the amoebozoan dictyostelids, myxogastrids, and protosteloid amoebae, heterolobosean acrasids, and other fruiting amoebae. This review does not attempt to present models showing spatio-temporal movements and niche colonization, rather offer perspectives on the possible consequences of dispersal and its capacity to affect individuals, populations, and species of selected amoeboid protists that are adept to forming fruiting bodies with spore and/or spore masses.

Sporogenic Fruiting Amoeboid Protists

The fruiting amoeboid organisms, according to Schnittler et al. (2012), are a taxonomically diverse group that share similar traits, such as bacterivory or as predators of other microorganisms, having a unicellular stage that germinates from spores, aggregation of cells or formation of a coenocytic mass, which later transforms to fruiting structures that generate one to thousands of propagules to be dispersed by air or other vectors. Fruiting bodies vary widely in sizes, and spores

are either products of meiosis or mitosis. Botanical nomenclature is frequently used as mycologists customarily studied these organisms (Martin and Alexopoulos 1969; Stephenson 2014).

The inclusive organisms belong or are closely related to at least four original supergroups. These are the Amoebozoa, Excavata, Opisthokonta, and Rhizaria. In a recent tree of eukaryotes (see Burki et al. 2020), Amoebozoa is largely considered as a member of the current supergroup Amorphea, where Opisthokonta is also presently nested within another large taxon, the Obazoa. Excavata is a paraphyletic group referred in Burki et al. (2020) as "Excavates," while Rhizaria is now part of Sar in the new supergroup TSAR (Telonema, Stramenopila, Alveolata, and Rhizaria). Based on this recent phylogeny, the organisms treated in this review belong to three new "supergroups" (TSAR, Amorphea, and "Excavates"). Because there were no major taxonomic corrections, such that all four original supergroups still belong to four distinct taxa (Rhizaria in TSAR, Opisthokonta nested in Obazoa that is sister to Amoebozoa within Amorphea, and "Excavates" forming an uncertain branching order), the original supergroups will be used in this text.

Groups belonging to Opisthokonta and Rhizaria were tentatively considered as "orphans" due to inadequate number of representatives that limited their sampling in previous phylogenetic analyses. Amoebozoa, which is a deep branch in the eukaryotic tree of life (Fiore-Donno et al. 2010), comprise the most diverse members (Stephenson 2014) conventionally called Eumycetozoa (Olive 1975) or "slime moulds," which are amoeboid cells having acutely pointed subpseudopodia and tubular mitochondrial cristae (Dykstra 1977). According to Shadwick et al. (2009) the whole supergroup can be called Eumycetozoa since besides the name having priority over Amoebozoa, the flagellar apparatus of myxogastrids and the flagellated forms of protosteloid amoebae seem to have a shared an origin that may be ancestral in the supergroup (see Spiegel 1990, 1991; Spiegel

et al. 1995; Shadwick et al. 2009). This would make Eumycetozoa as delimited by Olive (1975) paraphyletic. If, however, the name is used by the whole original supergroup (Amoebozoa), it would make a monophyletic taxon (Schnittler et al. 2012). Based on morphological and molecular data, an assemblage of cellular slime molds typically termed as "acrasids" belong to Heterolobosea within the original Excavata (Page & Blanton 1985; Roger et al. 1996; Baldauf et al. 2000; Brown et al. 2010, 2011b). Recent information does not allow the arrangement of all fruiting body-forming amoeboid eukaryotes in a natural system (Schnittler et al. 2012). Known life stages of each major group will be discussed in the latter section of this chapter.

How variable are the fruiting amoeboid organisms and how related are they to each other?

Amoebozoan Slime Molds

Characteristically, slime molds currently grouped within Amoebozoa showcase two fruiting processes: sporocarpy and sorocarpy. The former includes two of Olive's (1975) members of Eumycetozoa, the Myxogastria and Protostelia, while the latter consist of the last of Olive's original Eumycetozoa taxa Dictyostelia, and *Copromyxa*, which belongs in Tubulinea (Adl et al. 2019, Brown et al. 2011a). Due to shortage of molecular data for *Copromyxella,* it is currently treated as a close relative of *Copromyxa* because of its morphological similarities to the previous (Schnittler et al. 2012). It is to be noted that molecular phylogenies have supported the monophyly of sporocarpic Myxogastria (also more commonly known as Myxomycetes) and sorocarpic Dictyostelia in Amoebozoa (Pawlowski & Burki 2009), in fact they have each been shown to be monophyletic (Fiore-Donno et al. 2010; Schaap et al. 2006). More commonly known as dictyostelids and myxomycetes, these organisms are generally ubiquitous and often usually found in the microhabitats in which they typically occur (Stephenson 2014), such as plant material and

soil, and act as major predators of bacteria and other microorganisms (Stephenson & Stempen 1994). On the contrary sporocarpic protosteloid amoebae (more traditionally referred to as protostelids) are a non-monophyletic collection (Spiegel et al. 2017), which was specifically characterized as paraphyletic (Shadwick et al. 2009) with some members grouping with other amoebozoans that have not yet been documented to fruit.

To date, Myxogastria have the greatest number of species, ca. 1000 accepted morphospecies (Lado 2005-2021), in the Eumycetozoa, followed by the Dictyostelia, and then by Protostelia (Schnittler et al. 2012).

Sporocarpic members of the Myxogastria and Protostelia

Sporocarp-forming amoeboid members of Amoebozoa generate a fruiting body from one cell. In the protosteloid amoebae, a cell differentiates into one fructification, while in myxogastrids it is most characteristic that one amoeboid cell will give rise to a fruiting body or fruting bodies after it has likely increased in size (by feeding) and successive nuclear divisions with no cell division (forming a multinucleate cell) has occurred.

Myxogastrids

The term Myxogastria, or Myxogastrea, is interchangeable with Myxomycetes, owing its classification with several major groups, such as plants, animals, or fungi (Stephenson 2014), plus widely used monographs following botanical nomenclature that refer to them as myxomycetes are based on the morphology of the fructifications. Since de Bary's (1862) works, they have been recognized to be protists but only until more recent studies have they been grouped with Amoebozoa (Cavalier-Smith 1998; Bapteste et al. 2002; Yoon et al. 2008; Baudalf 2008; Schnittler 2012; Stephenson 2014). The group is also often referred to as plasmodial slime molds, signifying

its difference with the cellular slime molds (acrasids and dictyostelids) and protostelids (note: some protostelids can form a plasmodium). A ciliate genus (*Sorogena*) produces fruiting structures that resemble those of typical myxogastria (Bardele et al. 1991, Sugimoto & Endoh 2008); however, it will not be treated in this dissertation.

About 1000 recognized species (Lado 2005-2021) can be found mostly in terrestrial habitats, and even retrieved from aquatic environments (Schnittler et al. 2012), uncommon microhabitats such as the cavity of sea urchins (Karpov & Mylnikov 1997; Zaman et al. 1999), and dry zones (Stephenson et al. 2008), probably at a certain life stage other than the fruiting bodies. What defines them from the cellular slime molds is the ability of their amoeba to become a single, often huge, multinucleate (after a series of nuclear divisions not accompanied by cytokinesis) cell. Fruiting bodies, known as sporocarps, are produced from single, amoeboid, vegetative cells. According to Schnittler et al. (2012), fruitings can be discovered in the field or recovered using substrates, which are typically decaying plant matter, by way of making moist chambers, or agar cultures.

Protosteloid amoebae

Protosteloid amoebae, a more preferred term proposed by Shadwick et al. (2009) due to its lack of taxonomic implications, are organisms historically called protostelids, represent another sporocarp-forming amoeboid group that is described by the same authors as a microscopic drop of water resting upon the tip of a fine hair, relating to its simple microspcopic fructifications with refractile spore/s. Like myxogastrids, one amoeba or fragments of a larger amoebae differentiate into a single, stalked fruiting body that is about 5-500 μm tall (Schnittler et al. 2012), a size range smaller than most myxogastrids, supporting one or a few spores (see Olive 1975; Spiegel 1990;

Spiegel et al. 2004; Shadwick et al. 2009). Approximately 35 species of protosteloid amoebae have been described since the 1960s (Spiegel et al. 2004, 2007). As a group, Protostelia is paraphyletic (Schnittler et al. 2012) with at least eight well-supported lineages in Amoebozoa that includes the protostelids (Shadwick et al. 2009; Lahr et al. 2011a). According to Schnittler et al (2012) four of the lineages comprise only protosteloid (fruiting) forms, while the other four are interspersed with non-fruiting amoeboid organisms. So far, there are no strongly supported relationships among any of the protostelid-containing groups or between any of the protostelid containing groups and either the myxogastrids or the dictyostelids. In some phylogenetic analyses, however, one group designated as the Macromycetozoa (Fiore-Donno et al. 2010) contains the myxogastrids, dictyostelids, and protosporangiid protostelids (*Ceratiomyxa*, which is historically described as protostelid). The distribution of the group is insufficiently known (Schnittler et al. 2012) because most of the organisms have been observed only in agar cultures.

Protostelid-like organisms have been used to erect hypotheses relating to the evolution of fruiting within the now claimed as non-monophyletic Eumycetozoa (see Shadwick et al. 2009). Molecular phylogenies have been produced for both myxogastrids and dictyostelids, but only a few works have been done on the protostelids (Shadwick et al. 2009). It is asserted that protostelids that have been included in phylogenies do not cover the extent of the supposed morphological groups of protostelids (Spiegel 1990; Spiegel et al. 1995). Shadwick et al. (2009) stated that the ribosomal small subunit RNA gene (SSU) sequences that come from only two closely related species have been used as examples in most studies that include protostelids. Furthermore, the same authors express that the term protostelid directed misperception in the literature because it suggests an evolutionarily cohesive taxonomic unit (Spiegel 1990; Adl et al. 2005), while likewise, the term is used to describe morphology (Spiegel 1990). To avoid the confusion, it is recommended

to use protosteloid amoebae to refer to a highly diverse group of amoeboid cells developing protostelid-like fruiting bodies.

Sorocarpic Dictyostelia and *Copromyxa*

Sorocarp-forming amoebae are a diverse assemblage of amoeboid organisms that are able to form fructifications by the aggregation of individual cells. Essentially a polyphyletic group, it has members scattered along the eukaryotic tree of life. Amoebozoan members (dictyostelid cellular slime molds and *Copromyxa*), however, are supported by molecular phylogeny and some, but not all, morphological characters unified within the supergroup, such as the acutely pointed pseudopodia in the amoeba of dictyostelids and the slug-shaped amoeba of *Copromyxa*. Nevertheless, their nesting in Amoebozoa is reinforced by molecular phylogeny.

Dictyostelids

More commonly known as social amoebae or cellular slime molds, the dictyostelids are a relatively homogeneous (Stephenson 2014) monophyletic group of soil microbes (Schaap et al. 2006) inclusive of approximately 160 designated species (Romeralo et al. 2011) that were described first by Brefeld (1869, 1884). Since the first comprehensive monograph of 50 morpho-species by Raper (1984), more species have been added by a number of individuals, including Hagiwara (1989), Landolt et al. (2008), Cavender et al. (2010), and Vadell et al. (2011) to name a few. Separate taxa previously assigned to a single species have also been presented by Romeralo et al. (2010a). Earlier treatments on the taxonomy of the group used characters such as stalk tip and base morphology, aggregation patterns, and spore morphology improved the species concept for dictyostelids (Stephenson 2014). Species have been delimited by putting greater emphasis on

the early developmental stages (Cavender et al. 2013) and by the application of both morphological and molecular characters, giving us an increased understanding of the variation that exists within the group (Stephenson 2014). The species *Dictyostelium discoideum* has been used as a model organism (Bonner 1959) for cellular processes, and its genome completely sequenced (Eichinger et al. 2005), followed by another three taxa (Schnittler et al. 2012).

Found commonly in the soil microhabitat, mostly in the surface humus or litter layers (Cavender & Raper 1965b, c; Cavender 1973, 1990; Raper 1984; Feest 1987; Hagiwara 1989; Stephenson & Landolt 1996; Stephenson 2014), the sorocarpic fruting bodies are formed by the aggregation of trophic amoebae, but unlike myxogastrids, no evidence of flagellated stages has been found (Schnittler et al. 2012) and are seldom observed in the field but are easily found by culture using diluted soil on agar plates (Raper 1984; Romeralo et al. 2010a).

Copromyxa and *Copromyxella*

Copromyxa is a genus of fruiting, slug-shaped (limax) amoebae that has been molecularly shown to belong in Tubulinea within Amoebozoa (Brown et al. 2011a). The sorocarps are described to be columnar to arborescent, with many branches (Schnittler et al. 2012), and like the dictyostelids are formed by individual amoeba coming together and as explained by Schnittler et al. (2012) as crawling up one another to form a mound and subsequently encysting (sorocyst). The organisms identified to be *Copromyxa* are found mostly on animal, more particularly herbivore, dung.

Resemblance in amoebae and sorocarp form (Raper et al. 1978; Raper 1984), presumes *Copromyxella* to be a close relative of *Copromyxa* (Schnittler et al. 2012). Lack of molecular data for the genus hampers proving this relationship. Unlike *Copromyxa*, *Copromyxella* has been found

in soil and on decaying mushrooms, but they were also recovered from bald eagle dung (Schnittler et al. 2012). In the recent classification of eukaryotes, *Copromyxella* was still grouped in Tubulinea nested in Euamoebida (*sensu* Smirnov et al. 2011) along with other naked amoebae described with tubular, subcylindrical pseudopodia, or entirely monopodial and subcylindrical with no adhesive uroidal structures or changes to the form of locomotion (Adl et al. 2019). Sorocarpy, as mentioned, was observed for *Copromyxella*.

Heterolobosean sorocarpic amoebae

Acrasidae

This group of sorocarpic amoebae placed in the Heterolobosea within the original supergroup Excavata used to share a morphologically defined taxon with the amoebozoan dictyostelid cellular slime molds and other sorocarpic amoebae from Amoebozoa and Opisthokonta. These were collectively called cellular slime molds, or acrasids, or more particularly sorocarpic amoebae (emphasizing fruiting) that includes some, if not all, of the following genera: (Stephenson 2014) *Acrasis*, *Pocheina*, *Copromyxa* (now in Amoebozoa), *Copromyxella* (believed to be related to *Copromyxa*), *Fonticula* (closely related to a lineage in Opisthokonta), and *Guttulinopsis*. Molecular and morphological data denoted heterolobosean affinity for *Acrasis* and *Pocheina* (Page & Blanton 1985; Roger et al. 1996; Baldauf et al. 2000; Brown et al. 2010, 2011b), which formed a strongly supported monophyletic group that is sister to a clade containing *Allovahlkampfia* and several other amoebae (Brown et al. 2011b). They have a life cycle analogous to the dictyostelids, but particularly *Acrasis* and *Pocheina* (Schinttler et al. 2012) have limax-type amoebae that have an eruptive pseudopodium (compared to an acutely pointed subpseudopodia in dictyostelids, and a non-eruptive one in *Copromyxa*) and plate-like

mitochondrial cristae (tubular for the amoebozoans).

From this point forward, the taxon Acrasidae that includes *Acrasis* and *Pocheina* will be referred to as "acrasid cellular slime molds" or "acrasids." Of these two genera only *Acrasis* has been studied in detail (Stephenson 2014). The acrasid cellular slime molds are typically associated with plant material, particularly dead parts of aerial and ground litter for *Acrasis*, while *Pocheina* (having amoebae similar to *Acrasis*) have been recovered from substrate culture of living tree barks (Schnittler et al. 2012). *Allovahlkampfia* was described from a variety of microhabitats such as in cave soil, on decaying wood, and on bark of living trees. According to Brown et al. (2011b), the amoebae are similar to *Acrasis*, which gather together to form simple and small sorocarps compared to either globose to pointy or arborescent (Stephenson 2014) with spores arranged in chains in *Pocheina* and *Acrasis*, respectively.

Orphan sorocarpic amoebae

To date, members of the artificial assemblage of sorocarpic amoebae have been mostly placed in the eukaryotic tree of life. *Fonticula* and *Guttulinopsis*, although having been placed in Opisthokonta (see Brown et al. 2009) and Nucletmycea (see Adl et al. 2019) in Amorphea and Rhizaria (see Schuler et al. 2018, Brown et al. 2012), respectively, will be referred to as orphan sorocarpic amoebae for the time being. Both genera share the aggregative behavior of the amoeba that ultimately forms a globose sorocarp. Despite both having plate-like mitochondrial cristae as in the acrasid cellular slime molds, the genus *Fonticula* develops a volcano-shaped, acellular stalk that forces out spores using an unknown mechanism from amoebae with filose pseudopodia, while *Guttulinopsis* has a conical, cellular stalk that forms from amoebae with generally lobose, somewhat eruptive pseudopodia (Schnittler et al. 2012) that is reminiscent of the acrasids.

Typically found on canine dung and soil, *Fonticula* was found to be closely related to the nucleariid amoebae, which collectively places them as a close relative of Fungi in the supergroup Opisthokonta (Brown et al. 2009). The dung inhabiting (Stephenson 2014) *Guttulinopsis* can be considered as to having indeterminate phylogenetic position (despite appearing close to Rhizarians) because of the lack of more rigorous molecular data (Schnittler et al. 2012), even its relationship to the other sorocarpic amoeba is relatively unknown.

Spore Production and Dispersal as an Adaptive Strategy

The fruiting body-forming amoeboid organisms included in this treatment are capable of producing minute propagules that have the tendency of being released from their raised fructifications. These propagules, which will generally be called spores, serve as germline cells that can act either as vegetative cells or gametes or both. The colonization of new habitats or recolonization seem to be only possible by the facility of dispersal of at least some of the spores. Beijerinck's (1913) "in microorgansims, everything is everywhere" led to the development of the notion that generally prokaryotes, unicellular eukaryotes, and multicellular microorganisms have a cosmopolitan distribution due their small sizes and their abilities to form dormant stages, such as spores, eggs, and cysts, which aid dispersal by air, dust, or animals (Foissner 2006). Why is it, though, that some, if not most, of these fruiting amoeboid eukaryotes have species that seem to have a limited distribution? In this section details will be presented on the process of spore and sporocarp formation for most, if not all, of the organisms included. Multiple vector contributions and implications on community processes and patterns will also be deliberated.

How variable are the mechanisms of sporocarp and spore production for these fruiting amoebae?

As tackled previously, the fruiting amoeboid protists covered in this treatment produce two major fruiting bodies- sporocarp and sorocarp. The former, as exemplified by myxogastrids and protosteloid amoebae, develops from one cell that develops into a sporophore (commonly stalked) containing one to many spores depending on the group. The latter, as shown by a diverse lineage such as amoebozoan dictyostelids and *Copromyxa*, acrasid cellular slime molds and the orphan sorocarpic amoebae *Fonticula* and *Guttulinopsis*, arises from the aggregation or cooperation of cells holding more than one spore.

Protosteloid amoebae. In the protosteloid amoebae one amoeba or portions of a larger amoeba differentiates into a single, stalked, sporocarp that is about 5–500 μm tall (Schnittler et al. 2012), which supports one or few spores (see Olive 1975; Spiegel 1990; Spiegel et al. 2004; Shadwick et al. 2009). This starts from a prespore cell (Schnittler et al. 2012), which creates a stalk, that becomes a sporogen, which after laying down a cell wall later becomes a spore in single-spored species, or cleaves into cells that will each make their own walls and become individual spores. In a study of 30 taxa by Schnittler and Tesmer (2008), protosteloid amoebae spores were found to be the most heterogenous among the "Eumycetozoans" as spore diameters range from 4-42.6 μm.

Myxogastrids (myxomycetes). The myxogastrids have a variety of fruiting bodies—stalked (sporangiate), unevenly shaped typically following the shape of the coenocytic plasmodium (plasmodiocarpous), or compound fruitings where individual sporocarps may not be discernable (aethaloid) or may be identifiable (pseudoaethaloid). Despite the variation in sporocarp formation, all follow a certain sequence that is all myxogastrid fruiting bodies develop from a single,

multinucleate (coenocytic) plasmodium (explains the name plasmodial slime mold). The cell will later on undergo meiosis followed by cytokinesis to generate multiple spores within the spore casing (peridium), while the stalk (if present) remains acellular (Kalyanasundaram 1973; Schnittler et al. 2012). Apart from the stalk, spores, and spore casing, tubular threads called capillitium (singular) that hold the spores together are differentiated parts of a single sporocarp. Majority of the myxomycetes produce fruiting bodies that are usually not more than 1-2 mm tall (Stephenson 2014), however some can produce fruitings that are more than a centimeter (like in Order Stemonitales) and are over a meter wide that makes them easily visible in the field. Since they have a wide variety of fructification dimension, the number of spores produced is also within an extensive range. In the same study by Schnittler and Tesmer (2008), they have presented that the small species *Echinostelium* develop approximately 100 spores per sporocarp, while average-sized sporocarps produce between 10^5 and 10^6 spores. Spore sizes are usually 5 to 15 μm in diameter (Stephenson 2014).

Dictyostelids. Also known as social amoebae, the dictyostelids form sorocarps from the aggregation of amoeboid cells forming one or more slug-shaped, multicellular assembly that (Stephenson 2014) either migrates or directly transforms to a fruiting body, which comprises a stalk (may be branching or not) and one or more masses of spores, with each spore having a mean diameter of 5± 0.1 mm (Schnittler & Tesmer, 2008). The stalk length varies from 1.5–27.2 mm in length.

Acrasid cellular slime molds (Acrasidae). Like the dictyostelids, the acrasid cellular slime molds aggregate their cells forming a sorogen that arranges its cells to produce encysted stalk cells

(Schnittler et al. 2012), while the remaning cells of the sorogen form into chains or globose spores. In *Acrasis*, the sorocarps are typically pink to orange and are either branched or uniseriate. The sorocarp is consist of two cell types: cuboidal to flattened, thin, living stalk cells, which distinguishes them from dictyostelids with either hollow or dead cells in the stalk (Stephenson 2014); and spore cells, developing one to a few filiform chain branches on top of the stalk (Schnittler et al. 2012). Like *Acrasis*, the sorocarps of *Pocheina* are pink to orange in color, but their stalks are short with an apical, globose structure (sorus) containing the spores (Stephenson 2014). Based on micrographs available (see Brown et al. 2011b), the sorocarps of the acrasids do not grow higher than 1 mm.

Copromyxa and ***Copromyxella***. Aggregation of amoebae and development of fructifications is similar in *Copromyxa* and *Copromyxella*. Sorocarps consist of a single type of cell called sorocysts (Schnitler et al. 2012). These are encysted forms of the amoebae that have gathered or aggregated before the development of the fruiting body, which starts from one amoeba encysting followed by other amoebae of the aggregate as they crawl up and encyst on top. Sorocarps of *Copromyxa* are thread-like (filiform) or tree-like (arborescent) that are white to tan in colour (Schnittler et al 2012). Despite similarities with *Copromyxa*, there are a few features noted by Schnittler et al. (2012) that distinguishes *Copromyxella*, such as smaller amoeba cells without large contractile vacuoles (instead having many smaller vacuoles). This implies that without looking at molecular data and without familiarity to the amoebae of both taxa, *Copromyxa* and *Copromyxella* are indistinguishable by their sorocarps. Based on a few micrographs, a sorocarp of *Copromyxa* is below 1 mm tall.

Fonticula. The aggregating amoebae of *Fonticula* secrete an extracellular matrix (Schnittler et al. 2012) that later on creates the stalk. Majority of the amoebae on top of the mound encyst to make the spores, which will later on form the globose, mucoid spore mass (sorus). Sorocarps (approximately no more than 0.5 mm tall, based on monographs) are described to be white, with volcano-shaped, acellular tube that is the stalk.

Guttulinopsis. The sorocarps of *Guttulinopsis*, which are often macroscopic, are characterized to be stalked or, more seldom, sessile, globose to somewhat elongated (Stephenson 2014). The stalk is cellular containing degenerated, amoeboid, and encysted cells (Schnittler et al. 2012) that were members of the amoebae that have aggregated prior to forming the fruiting body. Like *Fonticula*, they have a mucoid sorus that makes them look shiny when found on dung.

Compared to myxogastrids, dictyostelids, and protosteloid amoebae, there are fewer studies characterizing the rest of the sorocarpic amoebae, especially *Copromyxa* and *Copromyxella*, *Fonticula*, and *Guttulinopsis*. Until then the productivity of their spore-generation will not be quantitatively presented.

What are the influences of different vectors to the dispersal of fruiting amoeboid protists?

Martiny et al. (2006) raised an inquiry regarding an organism's size influencing its dispersal ability. Mainly interested on long-distance dispersion events moving propagules across barriers, they indicated that this relies on whether an organism disperses by active propulsion or by passive transport, with vehicles such as ocean currents or migrating birds. The vast distribution of microorganisms has often been credited to their small sizes that come in high numbers. Besides these traits, fruiting amoeboid protists have the capability to form spores and/or cysts that can be

dispersed by a number of vectors, and are presumed to withstand unfavorable conditions during their non-dormant stages. Whatever group of organisms considered, dispersal could essentially be placed in two categories: active or passive. Fruiting amoeboid protists considered in this study has not been found yet to actively disperse its spores, unlike some taxa in other major groups such as basidiomycetes that are known for their ballistospores that can be actively discharged by propulsion from their fruiting bodies. The nearest event indicative of a semblance to some other fungal taxa (gasteromycetes) is the raindrop ballist (Dixon 1963) spore dispersal of myxogastrid *Lycogala* and *Reticularia* (Schnittler et al. 2012). However, this still reflects passive dispersal using raindrops as a means to propel spores out of the sporocarp. Having said these, dispersal of spores by these fruiting amoeboid protists always need an external agent.

The role of wind and animals as vectors of dormant stages and trophic cells has been broadly assumed for microorganisms (Foissner 2006). Investigations on organisms other than the ones covered here that are supporting this concept have also been performed (see Corliss & Esser 1974; Foissner 1987). This seems to be also true for the fruiting amoeboid protists, especially the more studied Eumycetozoans (dictyostelids, myxogastrids, and protostelids).

Since most Eumycetozoans develop aerial spores, they have always been thought to be carried by the wind together with other particulates (dust and other microorganisms). The spores of myxomycetes, for example, have often been considered as largely wind dispersed (Alexopoulos 1963; Schnitter & Tesmer 2008; Schnittler et al. 2012; Stephenson 2014). Stephenson et al. (2008) stated that if this proves to be case then the global wind patterns would give them great potential for long-distance dispersal over intercontinental gaps. In their study of the distribution of four taxonomically distinct species of myxomycetes (*Barbeyella minutissima*, *Ceratiomyxa morchella*, *Leocarpus fragilis* and *Protophysarum phloiogenum*) they have found distributional patterns

across continents that somehow contradict the cosmopolitan claim for myxomycetes. They have concluded that long-distance dispersal by wind may not be as common for myxomycetes as expected; that although most myxomycetes are considred to have wide distributional ranges, with many species seeming to be cosmopolitan or virtually like it, outcomes from other studies (e.g., Stephenson et al. 1993) have shown that spatial distribution of these slime molds may largely be affected by other ecological conditions. In order to shed more light on understanding the ubiquity of slime molds, Winsett and Stephenson (2011) looked specifically for genetic identity of one morphospecies (*Didymium difforme*) by examining intraspecific variation using a region of the mitochondrial small subunit DNA sequences. Fifty-six collections from three geographically distant regions of the world (Africa, Central America, and North America) revealed that the sequences from these collections did not separate into geographic groups. In fact, all but one of the six major groups contained sequences from collections in at least two different regions. The only group with sequences from one region, Kenya, without the other two regions was made up of sequences from collections made at localities as far as 40 km apart. Although the results of the study may somehow imply provincialism of myxomycete species, thus probably suggests limited dispersal, this also supports long distance dispersal of spores due to the fact that collections from distant localities grouped together. An interesting point to note is that they found that sequences from isolates collected in the same location do not necessarily group together. A possible explanation for this would be long-distance dispersal of spores actually happened, but once they were deposited they gave rise to a new population of the species. A previous study by Winsett and Stephenson (2008) of geographically disconnected collections of *Didymium squamulosum* using intraspecific DNA sequences of the nuclear ribosomal internal transcribed spacers (ITS) led to a similar conclusion, wherein most closely related sequences were not determined by geography.

Dispersal by animals is a more encompassing factor for most, if not all, fruiting amoeboid protists presented here. Dictyostelids are thought to be dispersed by vertebrates (Stephenson & Landolt 1992), such as deer, and yak (Sathe et al. 2009), songbirds (Suthers 1985), and most possibly invertebrates dwelling in soil (Cavender 1990), such as earthworms (Stephenson et al. 2007; Schnittler et al. 2012) that can actively transfer spores to new habitat islands (Schnittler & Tesmer 2008). Crickets and other birds (Stephenson 2007) are also supposed to be common agents of distribution. Grazing animals can pick up cellular slime molds with grass and soil, but Sathe et al. (2009) indicated that it is not known if a carnivore ingests them in the same way (like cats and dogs occasionally eating grass) or catches them indirectly by feeding on herbivores. They have noted that a considerable distance travelled by these animals has been observed (about 20 km^2 for the deer they have spotted to approximately 1,000 km^2 for the elephant). Like the dictyostelids myxomycetes are also inferred to be dispersed by invertebrates, mainly insects (Schnittler & Tesmer 2008). Blackwell et al. (1982) have noted a group of beetles specializing in feeding on myxomycete sporocarps. Dung-inhabiting fruiting amoeboid organisms such as *Acrasis*, *Pocheina*, *Copromyxa*, and *Guttulinopsis* are known to be globally distributed (Olive 1902, 1975; Cavender 1973; Spiegel et al. 2004). The more rarely encountered *Fonticula alba*, which was isolated only once in Kansas, USA (Worley et al. 1979) was also found on canine faeces. No study has yet focused on actual observations of the mechanisms of their dispersal or implications of such. However, it may be congruently supposed that animals, in a direct or indirect fashion, most likely transport them.

Like other macro- and microorganisms, fruiting amoeboid protists may also be artificially transferred by human activities. Similar to the way noted by Foissner (2006) when tropical and indopacific species of foraminifera may have entered the Mediterranean Sea through the Suez

Canal as carried by immigrants, and rotifers, *Brachionus havanaensis* and *Keratella Americana,* being introduced to the southeast (Segers 2001). Shipping, construction of canals (Foissner 2006), and transporting of soil across the world may be major reasons for introduction and reintroduction of such organisms due to the fact that they are typically associated with soil, plant debris, and ground litter.

Owing to the minute sizes of the spores produced by fruiting amoeboid protists and their ideal positioning on the apical region of their fruiting structures they are more than likely anticipated to be easily dispersed. It is to be emphasized that these organisms have motile stages, especially the sporocarpic amoebozoans known to have flagellated stages and the ability to form plasmodia which can relatively migrate a considerable distance. Studies on the dispersal of trophic stages (amoeba or plasmodium) are lacking and it is presumed to be that movement of such stages is mostly dependent on environmental conditions (e.g. moisture, food) or the need to cooperate with other cells, which is true for social amoebae and other aggregating slime molds. It is worth noting that some of these organisms are capable of forming other dormant stages, generally called cysts, which may be deposited on substrates (e.g. soil, plant material), that may be relocated by a number of vectors (e.g. water, animals, human activities).

Increased production and dispersal of spores, regardless of the vector escalates the chance for a species to colonize new habitats (Schnittler & Tesmer 2008). However, their success depends on a number of other factors such as, but not limited to, differences in climate or vegetation (Stephenson et al. 2008), ecological differences in a specific habitat, and their lifestyle preferences (mating and reproductive system, fruiting conditions).

What is the importance of spore dispersal in determining community processes and patterns?

When dealing with the comparative existence of ubiquitous organisms one can argue that some organisms have developed adaptions for dispersal, have ideal vectors, and have found appropriate habitats at the right time. It has been expected that small sizes and high numbers perform a key role in the distribution of organisms (Fenchel 1993; Finlay et al. 1996, Finlay 2002). Foissner (2006) expressed that microorganisms have distribution patterns comparable to those known from higher plants and animals, and that these reveal historical (Gondwanan/Laurasian), ecological (tropical/temperate), and local conditions.

Dispersal will only be successful if it is subjected to favorable conditions. This feat undergoes a certain, assumingly long, evolutionary, and ecological process and will in turn create an effect on the persistence of the population and on the community.

Colonization of new habitats. Dispersion capacity and density of populations play a role in the ability of fruiting amoeboid protists to exploit new and/or distant territories. Despite these, they seem to show limited geographic distribution. Stephenson et al. (2008) indicated that myxomycetes are generally opportunistic or "fugitive" organisms (Hutchinson 1951) because they have a high reproductive potential, show efficient means of dispersal, and are depicted by rapid development. They further claimed that these permit them to colonize habitat islands occurring both temporally and spatially in nature. Schnittler and Tesmer (2008) stated that just like true fungi, airborne spores allow eumycetozoans to inhabit different habitat islands by way of having available suitable microhabitats (commonly decaying plant matter). These microhabitats denote habitat islands for microorganisms where the island biogeography theory employs (Andrews et al. 2005). Therefore, depending on habitat islands, these fruiting amoeboid protists create

metapopulations in the logic set by Hanski (1994), and the number of populations that can be established effectively should be dependent on their capability to invade such habitats (Schnittler & Tesmer 2008).

It is not to be neglected, however, that habitats differ, and this affects the dispersal of such organisms. Martiny et al. (2006) asserted that cells in subsurface soils and sediment would not disperse as far as those in water and surface soils. This explains the convergent evolution of fructifications that produce spores, which can be airborne—a trait possessed by all of the fruiting amoeboid protists. Several factors can be tied up with the evaluation of success of colonization, such as population density, efficiency of dispersal (mostly by vectors), environmental conditions, sexual systems (see next section of the dissertation), and the means of assessing the actual presence of such organisms.

In a review of microbial biogeography, Martiny et al. (2006) elaborated that colonization rate depends on the taxon's population density as much as its dispersal ability. They mentioned that for passively dispersed macroorganisms most propagules are transported only very short expanses, but a small proportion can disperse over immense distances; which they believe to be the same case for smaller organisms. Therefore, great numbers of potential propagules increase the chance that at least one will travel a long distance that may establish a persistent population. Low densities, however, minimize the chances.

Some studies support the idea that the availability of certain microhabitats seemingly enforces effects on the occurrence of these fruiting amoeboid protists. Stephenson et al, (2008) found very fragmented range of *Barbeyella minutissima,* which is a species that appears to be almost exclusively restricted to *Picea* and/or *Abies* in montane forests (Stephenson et al. 2019), as one example. They have related this to the "moderate endemicity model" proposed by Foissner

(2006), who suggested that about 30% of the protist species are morphological, and/or genetic, and/or ecological endemics. This zonal patterns of distribution has also been recognized by Schnittler et al. (2012) with less notable support to the long distance dispersal of spores by myxomycetes as proposed by Kamono et al. (2009); but with agreement that dispersal events facilitates species to inhabit large ranges with the availability of suitable habitat (Schnittler et al. 2000) that suffice certain conditions such as temperature, as demonstrated by the formation of fruiting bodies by tropical species in greenhouses in Europe.

Mating systems or the absence of it also play a role in the establishment of populations by fruiting amoeboid protists. Although most have not been thoroughly studied for the existence of sexuality, some groups have been known to exhibit sexuality, while some have detected to show a shift from sexual to asexual or vice versa. Schnittler and Tesmer (2008) estimated that asexual species of Eumycetozoans need a minimum spore rain of approx. 0.7 spores per habitat island to attain a frequency of 50%, while in sexual species it is at least 2.4-fold higher than the previous depending on the incompatibility system (mating type) assumed. In summary they have presented that species with sexual "reproductive" systems often produce more spores than asexual ones, with many morphospecies having sexual and asexual strains, and that back-conversion from sexual to asexual reproduction occasionally happens. Theoretically, in organisms having asexual systems every spore has the potential to establish a population, whereas in sexual organisms there is a need to establish compatibility with another cell in the same habitat; therefore, an optimum spore density (Schnittler & Tesmer 2008) is required, which declines with higher number of mating types. In a study of reproductive systems of *Didymium iridis* populations, it has been suggested that sexual and asexual strains in Central America are sympatric (Betterley 1983).

Habitat preferences of fruiting amoeboid protists are currently only known by fruiting

bodies (Schnittler 2012), as these can reach macroscopic sizes or may at least be the most recognizable stage. There is, however, a most likely possibility that many habitats harbor populations of these organisms other than the fruiting stage. As suggested by Stephenson et al. (2008), direct environmental sampling with the use of molecular techniques such as DNA probes or ePCR (Schnittler et al. 2012) would be a possible way to detect covert populations in the form of amoeba or plasmodium that would be considered "sink" populations (relative to dispersal potential).

The distribution of an organism or a taxon, which may be linked to its dispersal patterns, almost only reveals the accounts in proximity to where the ones who study them live. Such is also the case for most fruiting amoeboid protists as stated by Stephenson et al. (2008), who estimated that about less than 25% of all herbarium specimens of myxomycetes have records that are systematically digitalized (in databases) where only the fruiting stage was considered (further emphasizing the need for other methods that can capture these group of organisms in other phases of their life, such as their trophic stages). In this regard they have asserted that a species could have a wider "true" distribution compared to the ones provided by maps.

Populations and structure. A study of wild isolates of cellular slime molds by Sathe et al. (2009) conveyed that spatial structure is determined by patterns of dispersal, with populations inclining to be either more or less viscous; while another idea is that dispersal is widespread, and what thrives is dictated by adaptations to local conditions. They added that with social organisms, such as the dictyostelids, an important aspect of spatial structure is whether or not groups consist of close relatives or clones. Bonner (2009) articulated that both spatial structure, as related to dispersal, and kinship bear on the evolution of the life cycle in the social amoebae. In a molecular

population genetics study of *Dictyostelium discoideum* by Flowers et al. (2010), they found no geographically distinct subgroups in eastern United States lacking a clear pattern of geographic structuring of nucleotide variation and absence of support for isolation-by-distance, but despite that their results suggest that vectors cause a high rate of dispersal, which describes the relative genotype diversity within small population patches like the Virginia population (also see Fortunato et al. 2003; Gilbert et al. 2007). A similar study from an independent sampling in several localities showed that there still is differentiation among populations of *D. discoideum* (Smith 2004). In an attempt to report molecular genetics of myxogastrian populations in deep ravines in Saxony, Germany and France, Fiore-Donno et al. (2011) found no correlation between genetic patterns and geographic distribution, as most genotypes were present in numerous localities. This, however, indicates high dispersal of spores as specifically exhibited by one genotype that is present in Germany and in France. The authors concluded that just like the case for *D. discoideum*, where coexistence of closely related strains promote the evolution of mechanisms for kin recognition making it challenging for non-related (probably opportunistic) amoebae to join the aggregate, sympatric speciation appears to be a scheme for genetic isolation of some myxogastrians.

Adaptation. When a propagule has been actually released or dispersed from its parent entity it's establishment in a new habitat is not always guaranteed, especially if it ends up on an uncommon locality. Besides surviving the conditions during the dispersal process, it has to be able to adapt to its new environment in ways that would promote its own persistence and would ultimately foster greater odds for the founding of populations to come. One thing to consider when starting in a new habitat is the capacity to outcompete local populations that may have better adaptation to certain living conditions (Martiny et al. 2006). This is a familiar situation to some aggregating protists,

specifically dictyostelids, where in the formation of colonies closely related cells seem to be preferred over non-related cells by means of kin recognition. This local adaptation somehow hinders the chances of migrant cells. Reproductive systems of fruiting amoeboid protists (covered more in detail in the next section) appear to also play a chief role in the establishment of populations of isolated cells. A switch to an asexual mode offers at the least a short-term advantage for isolated spores as indicated by Schnittler and Tesmer (2008) since outcrossing will not be a requirement at that point. The results of their colonization model study implied that if a sexual system develops, the number of mating types per locus ought to also increase. Nevertheless, the evolution of a more complex reproductive and sexual system also forces an evolutionary potential to produce more spores. Not only will a population be able to adapt to survival conditions and reproductive requirements with higher evolutionary potential, it also enhances the likelihood for them to adjust to forthcoming climate changes. It is widely assumed that a population's evolutionary tendency increases if there is more genetic variation in the population.

Life Stages and Reproductive Strategies of Fruiting Amoeboid Protists

The fruiting amoeboid protists covered in this treatment undergo similar life stages, which fundamentally starts from a spore that germinates into an amoeba that either aggregate with other amoebae or grow to become a multinucleated cell, and eventually differentiate to or raise a structure that bears a single spore or some multiple spores depending on the organism. Collectively these spore-bearing fruiting amoeboid organisms reproduce using a variety of sexual, asexual, or interplay of both strategies. For the sake of technical arguments, the term reproduction signifies the assembly of a new generation of cells that carry prospect to continue the population. It was reasoned by Spiegel (2011) as inspired by the work of Lahr et al. (2011b) that reproduction and

sex (or the absence of it) could be mutually exclusive, as reproduction should be narrowed down to the generation of more cells from a lesser number of such; while sex, as is the case for fertilization (gametes), results to a reduced number of founding cells that involves syngamy and karyogamy. An evidence of sex has been recorded in all major groups of eukaryotes (Moriyama 2009), with most of them having two mating types (or sexes) but with some (like myxogastrids and some fungi) exhibiting multiple mating types and mating loci (Kawano et al. 1995; Casselton 2008; Moriyama & Kawano 2009). Sexual organisms can either be heterothallic or homothallic. The former are those that develop mating types and have a ploidal change (haploid-diploid), while the latter have no mating types but with ploidal change. Together with homothallic organisms, apomictic (asexual, no ploidal change) groups are considered to be non-heterothallic (Clark & Haskins 2010, 2013). Despite the costs of sex, relative to asexual reproduction (de Meeûs et al. 2007; Otto 2006; Fiore-Donno et al. 2011), many organisms still employ sex (Fiore-Donno et al. 2011) or a little bit of it (Green 1995) probably due to the evolutionary potential (Dacks 1999) that comes with it.

How different are the developmental and reproductive schemes of spore-producing fruiting amoeboid organisms?

Sporocarpic slime molds

Myxogastria. As in most, if not all, spore-producing fruiting amoeboid protists myxogastrids have dormant, vegetative, and fruiting stages. Apart from the spores, they can form microcysts (encysted myxamoebae) and/or sclerotium (hardened plasmodium) as other dormant stages due to unfavorable conditions. Spores germinate to become unicellular amoeboflagellates (Stephenson

2014), which are also called myxaflagellates (Schnittler et al. 2012) that can switch to being myxamoebae (non-flagellated amoebae) or back to flagellated unicells that can undergo succeeding cell divisions generating sizeable asexual populations. Sexual strains that basically have a one-locus, multiple allelic heterothallic systems (Clark & Haskins 2010; Stephenson 2014), where some forms like in *Physarum polycephaulm* have a three-loci multiple allelic system (Kawano et al. 1987), undertake syngamy of two compatible haploid myxamoebae. This zygote progresses to a coenocytic plasmodium, which when activated by starvation and/or phototrophy (Schnittler et al. 2011) differentiates into a single fructification or a number of fructifications. A fruiting body undergoes meiosis prior to sporulation. Asexual forms of the fruiting body cannot be distinguished from sexual forms (Fiore-Donno et al. 2011), and follow the same stages of development. However, being asexual they skip meiosis and produce diploid spores that germinate to diploid amoeboflagellates, therefore omitting syngamy of myxamoebae and leading to the development of a diploid plasmodium.

Protosteloid amoebae. Both sexual and asexual forms have been observed for protosteloid amoebae. Most groups that have complex life cycles seem to be sexual (Lahr et al. 2011b; Spiegel 2011; Schnittler et al. 2012), and meiosis is often linked with spore formation (Spiegel 1990, 1991). A simple life cycle involves the germination of a uninucleated amoeba, which may asexually divide into nucleated daughter cells, that has the ability to be converted to being flagellated or non-flagellated to form a prespore cell (Schnittler et al. 2011). A stalk is secreted, and when completed a cell wall is created by single-spored species; while multiple-spored species cleave, followed by wall formation. Species having complex life cycles (assumed to be customarily sexual) may involve multinucleate amoebae, fusion of flagellated amoebae, or formation of cysts (Spiegel et al.

1995). The general arrangement of stages is comparable to the simple life cycle, which only differs in the nuclear number of germinating amoeba from a spore (multinucleate amoeba) and/or the encystation of plasmodial amoeba, which will eventually release amoeboflagellates that may fuse (sexual) to form a plasmodium and raise a fruiting body or fruiting bodies (from a fragmented plasmodium).

Sorocarpic slime molds

Dictyostelids. Formation of fruiting bodies and aggregation of amoebae characterize an asexual course for dictyostelid cellular slime molds, however sex is known for a number of species (Raper 1984; Cavender 1990; Kessin 2001; Stephenson 2014). Non-flagellated amoebae germinate from spores and undergo successive cell division. Starvation promotes the amoebae to aggregate, which stops the cleavage of amoebae (Kessin 2001), and form a slug that explores until fruiting is triggered by ideal conditions (Schnittler et al. 2011). A multicellular fructification is formed by the differentiation of cells as to being stalk cells or spores. The sexual part of the life cycle, which appears to regularly arise in natural conditions (Flowers et al. 2010), initiates with the union of two compatible amoebae that is followed by the cannibalistic consumption of chemically attracted independent cells by the zygote (Szabo et al. 1982; Ishida et al. 2005; O'Day & Keszei 2012). A macrocyst, which is a dormant stage, releases recombinant haploid amoebae (Kessin 2001), which may follow the asexual cycle that leads to aggregation of individual amoebae, fruiting, and sporulation.

Copromyxa. Life cycle of *Copromyxa* is based on the illustration of *Copromyxa protea* by Brown et al. (2010) that was founded on the works of Nesom (1973) and Spiegel and Olive (1978).

Amoebae germinate from sorocysts (spores) and form an aggregate. A cell encysts and the other amoebae of the aggregate crawl up, encyst, and form sorocysts. Before aggregation some amoebae may encyst and/or excyst back and/or initiate the presumed sexual stage, which is the formation of sphaerocyst (thick-walled zygote) that is a product of fusion and karyogamy by two amoebae. As expressed by the authors, the next stages after the formation or germination of the sphaerocyst is unknown.

Acrasis. The representative life cycle for the acrasid cellular slime molds is patterned from the illustration of *Acrasis rosea* by Schnittler et al. (2011). Trophic amoebae germinate from spores, and may encyst (microcyst) and excyst back to an amoeboid form. Aggregation of amoebae leads to the formation of a sorogen, followed by encystation of one cell as the first stalk cell. The sorogen rises as other cells encyst to become further cells of the stalk. The remaining cells form chains of amoeboid cells, which later on encyst developing as spores. No sex has been observed so far in the studies of acrasid cellular slime molds.

Fonticula. The life cycle of *Fonticula* is based on the illustration of *Fonticula alba* by Brown et al. (2009). Spores enveloped by a mucoid matrix germinate as trophic amoebae that can either encyst, which are morphologically similar to spores (Schnittler et al. 2011), or aggregate with other amoebae. The mound formed by the aggregating cells develops a slime covering, while the sorogenic amoebae discharges an extracellular matrix that will comprise the stalk. The amoebae on the upper layer encyst and form the spores, which are raised into a swollen sorus.

Guttulinopsis. To date there is no illustrated life cycle of *Guttulinopsis*. The life stages presented here are based on descriptions by Schnittler et al. (2012) and illustrations and descriptions by Brown et al. (2012). Amoebae germinate from mucoid spores, aggregate with other amoebae, and form multicellular fruiting bodies. Mature sorocarps, which may have a single or multiple sori on top of a compartmentalized stalk cells made up of degenerated, amoeboid or encysted cells.

For most of the sorocarpic amoebae, fruiting involves an asexual process. Spiegel & Olive (1978) documented possible sexuality in *Copromyxa protea* with an indeterminate process (Brown et al. 2011a; Schnittler et al. 2011), or commencement.

Genetic variation in fruiting amoeboid protists: Roots and Consequences

Simply put, variation may be defined as the differences among individuals in a population or between one generation and the next (parents and offsprings). What brings about variation can be categorically placed into three general causes: mutation, recombination, and gene flow. Mutation, a process by which a gene (or a form of it: an allele) or chromosome different from the wild type is created, is said to cause changes but does not alone drive rapid evolution of populations and species because mutation rates are so low at a given time in a given generation (Griffiths et al. 2000). Recombination can be mainly defined as the rearrangement or formation of new combination of genes or genetic material. In natural conditions this happens during crossing-over of homologous chromosomes in meiosis or independent assortment. Having said that, recombination will only produce variation if genes are segregating at different locations (loci) in the genetic material (e.g., chromosomes). Therefore, theoretically recombination will not account for variation in asexual organisms. Genes can be transferred from one population to another (gene flow) and may lead to a proportion of an allele that is intermediate between the donor populations.

However, gene flow will only cause variation if there is an actual difference in the form of the gene (heterozygous) between the populations within a species. Gene flow may occur through sex or horizontal gene transfer. Considering these, sexual organisms are more likely to acquire variation than asexual organisms.

What are the implications of genotypic and phenotypic variations on the populations and communities of fruiting amoeboid protists?

Variation, which is comparable between individuals, populations, species, and even higher order lineages, is the center of adaptation and evolution in general. Differences can be presented as a set of observable characteristics (phenotype) that typically reflect the genetic make-up of an individual (genotype). It is to be noted, however, that interplay between the genotype and the individual's environment may affect its phenotype. Given the right tools (such as the availability of credible descriptions and characterizations of organisms, monographs and/or micrographs and/or any physical inspection of actual individuals) and good judgment, it is relatively more manageable to discern phenotypic variations than genotypic variations. What makes it challenging to study, more so measure, genetic variation is the requirement that the genotypes of organisms being studied must be identified. In here comes the need for molecular markers that show variability among the subjects. Molecular phylogenetics has improved greatly over the past decades as researchers were allowed to analyze differences among groups of organisms down to the individual level, at least for some lineages. This is also gradually being undertaken in spore-bearing fruiting amoeboid organisms.

Sporocarpic amoeboid protists

Phylogenetic studies have revealed the Myxogastria to be a monophyletic taxon (e.g. Lahr et al. 2011a) within Amoebozoa. Relative to the original members of Eumycetozoa (Olive 1970), Myxogastria is sister to the dictyostelids as depicted using SSU rRNA gene, while combined with EF-1α gene it is sister to *Ceratiomyxa* that is together sister to dictyostelids (Fiore-Donno et al. 2010). The group Protostelia, however appearing related to myxogastrids and dictyostelids, emerges paraphyletic as members of which are interspersed among other non-fruiting members of Amoebozoa; although it is presented that some of its members collectively is sister to the clade Dictyostelia + *Ceratiomyxa* + Myxogastria. Previous works on the myxogastrid phylogeny (Spiegel et al. 1995; Fiore-Donno et al. 2005) showed that Echinosteliales are the ancestral group, appearing basal to the dark-spored clade (Physarales and Stemonitales) and an assortment of bright-spored clade (Trichiales and the genus *Cribraria* in order Liceales). Relating this to the study of Schnittler and Tesmer (2008), which aimed to compare spore numbers among the slime molds, they found that spore numbers per fruiting body increases seemingly consistent along these lines, with the *Echinostelium* having the lesser mean number of spores followed by dark-spored representatives, then by the bright spored taxa. Compared to the myxogastrids, protosteloid amoebae have received less attention from molecular phylogeneticists. Most, if not all, of the molecular work on protosteloid amoebae seem to have been trapped on the recovery of the group as a monophyletic taxon. A previous study by Shawick et al. (2009) using SSU rRNA gene that shows polyphyly of the group as a whole was supported by the work of Lahr et al. (2011a) using SSU rDNA and actin genes. Separate but well supported clades containing classical protostelids are distributed among Amoebozoan lineages with no evident relationships among each other.

It was estimated that only about 10% of the most widely used gene for phylogenetics and

barcoding (Fiore-Donno et al. 2013), SSU rRNA, of myxogastrids or myxomycetes is available in the database, and that the resolution of phylogenetic position of the lineages comprising it is restricted by the lack of sequences especially for the bright-spored group (Lucisporidia). Using two genes (SSU rRNA and EF-1α), they have found the para/polyphyly of two classical orders (Liceales and Trichiales) and some families and genera. They have concluded that no apomorphy is provided by the currently used characters for higher-order classification of the bright-spored group, with several monophyletic taxa only described by a collection of few characters (such as the features of the capillitium). This incidentally calls for the use of a combination of characters in classifying myxogastrids. The results of their study also implied that stalked, individual fructifications from a large plasmodium are ancestral characters, while small plasmodium producing a single fruiting body is derived. Probably due to their unarguably smaller size, the protosteloid amoebae do not have numerous morphological characters (as in fruiting) that will allow one to easily distinguish them from each other. However, if their life stages (as described in the previous section) are carefully deliberated the five clades of fruiting protostelids (protosporangiid clade, protosteliid clade, soliformoviid clade, cavosteliid clade, and schizoplasmodiid clade) presented in Shadwick et al. (2009) seem to have distinct morphological identities.

The idea that the transmission of biological information from one generation to another (inheritance) occurs only through the DNA is debatable. Evidences for non-genetically inherited traits have been explained (see Danchin et al. 2011 and references cited therein), and have been described to be ecological, cultural, and epigenetic. At least for the myxomycetes, changes in the phenotype have been documented and have been contemplated if these are effects of phenotypic plasticity, which is the ability of a phenotype to vary relative to environmental influences on the

genotype. It is not to be confused, though, that in this context phenotypic plasticity is being used to equate to or be directly linked to non-genetic inheritance. One way to justify their association is to say that environmental conditions may cause epigenetic or developmental differences that may lead to phenotypic variations, which may be in the form of phenotypic plasticity. Forsman (2015) reviewed phenotypic plasticity and its effects on individuals, populations, and species. It was emphasized that an increased understanding of the roles of plasticity warrants a 'whole organism' approach, rather than using a single trait. He claimed the need to discriminate between the effects linked with individual phenotypic variation due to plasticity and the effects of individual variations to direct plasticity, further asserting that elaborate methods are needed to study developmental and phenotypic plasticity independent of genetic variation. In light of this, classifications have to be critically thought of and that these should consider mechanisms that may influence the morphology of organisms. In the myxomycetes, studies determining the stability of morphological variations in *Didymium difforme* (Lister 1901) have been conducted, where environmental factors such as moisture and the assemblages of other microorganisms that co-exist in the culture have been associated to morphological variations in *D. difforme* (see Cayley 1929). There may be different environmental conditions that could be affecting the typical and atypical development of the species (Winsett & Stephenson 2011). Other studies concerning isozymes (Franke et al. 1968; Franke & Berry 1972; Franke 1973; Berry & Franke 1973; Betterley & Collins 1983) stipulated general conclusions of intraspecific variation within other species, however they did not show correlation between morphological variation and protein variation. With these gaps, it may be reasonable to say that observable differences in fructifications are relatable to phenotypic plasticity rather than genotypic diversity. On that thought, dissimilarities in microhabitats and/or habitats and/or climate may play a role on the populations of fruiting amoeboid protists.

To show if there will be geographical patterns that will come out of using sequences from different geographical units, Winsett and Stephenson (2011) analysed 56 sequences of mitochondrial SSU of *D. difforme* from Central America, Central United States, and Africa that revealed 13 haplotypes indicating no clear geographical pattern; however, there are some groups that include collections from the same location implying these sequences to be more alike. A similar study resulted to the same conclusion when Fiore-Donno et al. (2011) investigated 52 specimens of *Lamproderma* species. However, genetic variations seen among the samples pointed to an asexual mode of reproduction. Reporting findings on populations of fruiting amoeboid protists is quite tricky due to a variety of reproductive strategies as presented on the previous section. Their ability to produce a large number of amoebae that can result from mitosis, with each amoeba having the capability to develop a plasmodium (at least for myxogastrids and some protostelids), and the huge number of spores that can be produced by a single fruiting account for the difficulty in establishing of what an individual or a population is.

Reproductive systems and the presence of absence or mating types play a role in variations observed on these fruiting amoeboid protists. A couple of studies indicated that some morphospecies of myxogastrids, such as of *Didymium iridis, D. megalosporum*, *D. ovoideum,* and *D. squamulosum,* may be complexes of heterothallic lines (Collins 1963; Clark 1984; Clark et al. 2013) that are associated with non- heterothallic (presumably apomictic, clonal) lines (Collins 1980; Collins et al. 1983; El Hage et al. 2000). A more recent study by Feng and Schnittler (2015) on the myxogastrid *Trichia varia*, the use of three independent gene markers (SSU rRNA, EF-1α, and COI) revealed three sexual but reproductively isolated species. A similar study by Fiore-Donno et al. (2011) on several collections of *Lamproderma species*, they found asexual modes of reproduction as isolates having the same genotype for SSU rRNA and ITS1 also showed to be

identical in the EF-1α gene marker. Sexual forms of the protosteloid amoebae (Lahr et al. 2011b; Spiegel 2011; Schnittler et al. 2012) are typically associated with having a complex life cycle (see descriptions in the previous section). However, the genetic divergence of the populations that they may form have not been quantified or described in detail. Considering that sexual lines have more sources of variation (recombination and migration of genes, apart form mutations), it seems likely to be assumed that asexual forms will show less differences in their genetic makeup.

Sorocarpic amoeboid protists

Among the fruiting amoeboid protists discussed in this treatment, the sorocarpic cellular slime molds show the most diversity, as they are found on different major groups of eukaryotes-dictyostelids and *Copromyxa* in Amoebozoa, *Acrasis* in Excavata, *Fonticula* in Opisthokonta (Adl et al. 2005; Baldauf et al. 2000; Brown et al. 2009; Brown et al. 2010; Roger et al. 1996; Schaap et al. 2006), and *Guttulinopsis* in Rhizaria (Brown et al. 2012). Dictyostelids branch sister to myxogastrids in Amoebozoa and forms a monophyletic group (Fiore-Donno et al. 2009). *Copromyxa* has been recovered to within Tubulinea in Amoebozoa (Lahr et al. 2011; Lahr et al. 2013) that is sister to the hartmanellids (with amoeboid member not yet been observed to fruit). Heterolobosean acrasid cellular slime molds have been in placed in Excavata that comprises the two fruiting genera *Acrasis*, and *Pocheina* (Baldauf et al. 2000; Brown et al. 2010b). *Fonticula* branches sister to *Nuclearia*, which is together sister to the Fungi in Nucletmycea within the Opisthokonta (see tree by Brown et al. 2009). *Guttulinopsis* is placed within the Rhizarian group (see tree by Brown et al. 2012) within SAR (Stramenopiles-Alveolates-Rhizaria). If treated altogether this morphologically defined group obviously depict a polyphyletic taxon.

Among all the cellular slime molds, the dictyostelids had most of the molecular work; from

the establishment of the monophyly of the taxon, to the delimitation of a number of species, until population studies. Phylogenetic studies on dictyostelids produced four major groups based on SSU rRNA data (Schaap et al. 2006; Romeralo et al. 2013). Genomes of the representative species from each major group have now been sequenced (Heidel et al. 2011; Eichinger et al. 2005; Sucgang et al. 2011; Romeralo et al. 2013). An earlier experiment of Schnittler and Tesmer (2008) estimating the spore number and dimension and sorocarp size indicated an evolutionary trend towards larger spore numbers per fruiting body among the groups, with group four having the highest values. As altruism ("sacrificial" dying) is exhibited by stalk cells of dictyostelids, evolutionary implications of this social behavior have gained some interest on this group (Fortunato et al. 2003; Li & Purugganan 2011). The recent work of Romeralo et al. (2013) investigated the evolution of multicellularity using mapping of phenotypic traits and linking to genome-based phylogeny. They have shown a fairly scattering character states with several states reappearing multiple times in different groups. They have accounted this both to morphological differences between species and within species upon exposure to different conditions that further imply environmentally adaptive systems for morphogenetic control and the consequent plasticity, owing to the need to assemble variable number of cells with different functions. However, they have seen trends in phenotypic evolution by reconstructing the ancestral states to all Dictyostelia and groups 1–3, which they described to be have small, unbranched fruiting structures, containing elliptical spores with polar granules. The last common ancestor (LCA) to clade 2B developed regular whorls of side branches in its fructifications, while the LCA of group 4 evolved large, unbranched fruiting bodies. They concluded that factors leading to phenotypic variations might be due to habitat, geographical origin, local conditions of the species, and their interactions with the biota of their environment.

Population studies of dictyostelids in North America revealed various coexisting haplotypes with no geographic structure (Flowers et al. 2010; Fortunato et al. 2003). This affirms the idea that the presence of related strains favors the evolution of processes leading to kin selection that limits non-related amoebae to co-aggregate (Flowers et al. 2010), which should promote sympatric speciation among aggregating amoebae (Fiore-Donno et al. 2011). Studies involving populations of dictyostelid species led to the discovery and/or confirmation of sexual forms. Prior findings reported a mating system for some species, such as *Polysphondylium pallidum* (Eisenberg & Francis 1977; Kawakami & Hagiwara 2002), and *Dictysotelium giganteum* (Erdos et al. 1973), although their reproduction as detailed on the previous section is mainly asexual (Schnittler & Tesmer 2008). The same study by Flowers et al. (2010) found low levels of nucleotide variation across the strains, but sex is implicit by the occurrence of recombinant genotypes.

Amoeboid organisms capable of forming sorocarps by aggregation of individual cells generally describe cellular slime molds or "acrasids." Brown et al. (2011) argued the use of acrasids to members of Acrasidae in Heterolobosea. Covered in this treatment are two genera: *Acrasis* and *Pocheina*. The first described species of the genus *Acrasis* is *A. granulata*. Brown et al. (2010) proposed a new species, *Acrasis helenhemmesae*, which notwithstanding small differences between some other strains and the originally described to be highly plastic *A. rosea* once in culture (Olive & Stoianovitch 1960) is supported by SSU rRNA gene sequences in phylogenetic trees. The plasticity of acrasid cellular slime molds was further emphasized by Brown et al. (2010) when they have observed that growth on primary plates varies from succeeding culture plates. A year after, Brown et al. (2011b) used a combination of morphological characteristics and SSU rRNA gene sequences to show the diversity of *Acrasis* and *Pocheina*, which together produced a well-supported monophyletic group that is sister to a clade comprising *Allovahlkampfia*

and other non-fruiting amoebae. Each having a distinct fruiting morphology, four lineages of *Acrasis* were resolved. Furthermore, an isolate classified as *Pocheina rosea* nests within the clade containing isolates of *A. rosea*. As they hypothesize that morphological variants of *A. rosea* may be genetically different and may as well be placed in separate groups, the authors have called for a more rigorous study with better sampling to signify a good diversity of the acrasid cellular slime molds, which includes more of the pochenoid members. Additionally, despite the seeming discrimination among major clades offered by fructifications, the use of molecular data for taxonomic resolution was suggested.

Perspectives

The wide distribution of amoeboid organisms capable of forming spore-bearing fruiting bodies suggests that this trait might have evolved numerous times among the eukaryotes. In theory dispersal of spores may act as both an agent of divergence, as in the matter for vicariance, or a force that balances it, making populations more similar or more different depending on the circumstances. Whatever the case is, dispersal events impact genetic differentiation, which plays a major role in the evolution of lineages, species, and populations. Despite the concept that evolution, as a general idea, involves populations it is undeniable that its basis, which is genetic variation, commences on the individual level. Individuals in a population may have different genotypes that have variable fitness under different environments; such is the basis for natural selection. The evolutionary success of a population, therefore, lies on genetic variation among its individual members.

Despite seeming restrictions on the distribution of fruiting amoeboid protists, their ability to produce high population densities with relatively flexible life strategies have allowed them to

develop adaptations to establish themselves in new habitats. The idea that these organisms can cross geographical barriers by means of long–distance dispersal of spores, as evidenced by cosmopolitan morphospecies, may be detached from the observation of delimited occurrence of some species. As some of the lineages are believed to have a deep phylogeny (see Fiore-Donno et al. 2009; Wegener-Parfrey et al. 2011; Estrada et al. 2013), their current distribution may have been affected by continental separation events that may be supported by morphospecies comprised of biological species complexes, which may show reproductive isolation and limited distribution (Clark & Haskins 2010). Perceived genetic diversity and apparent cryptic speciation may very well align with this or may just convey local adaptations of populations.

Phenotypic variations within a morphospecies have been recorded. Their possible causes, which may be due to geographic origin, habitat characteristics, and certain local environmental conditions that include their associations with the biotic components (see Romeralo et al. 2013), speculated. At present, however, it is difficult to circumscribe direct causation of such likely plasticity. This setting correspondingly poses a challenge among taxonomists regarding demarcating species. It is not disputable that a universal principle in species delimitation is non-existent, so far, as a variety of concepts are flexibly used among discovered taxonomic units. Despite the ability of forming testable hypotheses, the tendency to center such on more conspicuous traits creates bias and a few times result to phylogenies that appear to not be encompassing the diversity of lineages under study.

Adding on to the variations exhibited by fruiting amoeboid protists ideas, such as independent evolution may be due to different lifestyles and reproductive strategies, arise. Asexual (and/or clonal) strains may accumulate variations unlike those of the sexual lines. Hypothetically asexual lines may complete their life cycle without the need for other individuals. Asexuality has

been observed in all the lineages treated here; and studies have reinforced that it brings an advantage to them, even temporarily. That reason, taken alone, why do they still pursue or at least switch to having sex? However crudely, sex increases their chances of obtaining more variations; and therefore, will allow them to have more adaptive traits during selection. This is, without a doubt, essential when they colonize new habitats: an event that is not surprising among the spore-producing, fruit-forming amoeboid organisms. How about the influence of lateral gene transfers, such as when myxomycetes take up bacteria or yeasts?

For some of the lineages, social behaviors have been a vital part of their life cycle. Several studies have shown the relationship of genetic relatedness to their aggregating behavior, which influences their fruiting formation. The seemingly scarce population structure within a certain lineage of fruiting aggregating amoebae may be rooted from their co-evolved kin recognition capability. This, however, does not disregard the actuality of co-existence of genetically variable individuals or groups in one spatial range due to their shared habitats and/or microhabitats (such as dung, soil, plant materials) that may be transported by some vectors (e.g., animals, such as grazers).

Points stated above, among others, make this heterogeneous group of organisms as good specimens to initiate and/or elaborate on various aspects of developmental and evolutionary biology. They have been used to identify mechanisms of differentiation, cell signaling, multicellularity, and social behavior. With a lot of potential in providing answers in biogeography, diversity, and speciation, the necessity to thoroughly explore them arises; much of which involve the extensive development of good molecular markers (or genome-based approaches) coupled with adequate sampling that will show their holistic ecology.

Literature Cited

Adl, S. M., Bass, D., Lane, C. E., Lukeš, J., Schoch, C. L., Smirnov, A., Agatha, S., Berney, C., Brown, M. W., Burki, F., Cárdenas, P., Čepička, I., Chistyakova, L., del Campo, J., Dunthorn, M., Edvardsen, B., Eglit, Y., Guillou, L., Hampl, V., Heiss, A. A., Hoppenrath, M., James, T. Y., Karnkowska, A., Karpov, S., Kim, E., Kolisko, M., Kudryavtsev, A., Lahr, D. J., Lara, E., Le Gall, L., Lynn, D. H., Mann, D. G., Massana, R., Mitchell, E. A., Morrow, C., Park, J. S., Pawlowski, J. W., Powell, M. J., Richter, D. J., Rueckert, S., Shadwick, L., Shimano, S., Spiegel, F. W., Torruella, G., Youssef, N., Zlatogursky, V. & Zhang, Q. 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. Journal of Eukaryotic Microbiology 66: 4-119.

Adl, S. M., Simpson, A. G. B., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bonser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, L. H., Mann, D. G., McCourt, R. M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W. & Taylor, M. F. J. R. 2005. The new higher-level classification of eukaryotes with emphasis on the taxonomy of protists. Journal of Eukaryotic Microbiology 52:399–451.

Alexopoulos, C. J. 1963. The myxomycetes II. The Botanical Review 29:1–78.

Baudalf, S. L. 2008. An overview of the phylogeny and diversity of eukaryotes. Journal of Systematics and Evolution 46:263–273.

Baldauf, S. L., Roger, A. J., Wenk-Siefert, I. & Doolittle, W. F. 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290: 972–977.

Bapteste, E., Brinkmann, H., Lee, J. A., Moore, D. V., Sensen, C. W., Gordon, P., Durufle´, L., Gaasterland, T., Lopez, P., Mu¨ller, M. & Philippe, H. 2002. The analysis of 100 genes support the grouping of three highly divergent amoebae: Dictyostelium, Entamoeba, and Mastigamoeba. Proceedings of the National Academy of Sciences USA 99:1414–1419.

Bardele, C. F., Foissner, W. & Blanton, R. L. 1991. Morphology, morphogenesis and systematic position of the sorocarp forming ciliate *Sorogena stoianovitchae* Bradbury & Olive, 1980. The Journal of Protozoology 38: 7–17.

Beijerinck, M. W. 1913. De infusies en de ontdekking der backteriën. Jaarboek van de Koninklijke Akademie v. Wetenschappen. Müller, Amsterdam. The Netherlands.

Berry, J. A. & Franke, R. G. 1973. Taxonomic significance of intraspecific isozyme patterns of the slime mold *Fuligo septica* produced by disc electrophoresis. American Journal of Botany 60: 976–986.

Betterley, D. A. & Collins, O. R. 1983. Reproductive systems, morphology, and genetical diversity in *Didymium iridis* (Myxomycetes). Mycologia 75: 1044–1063.

Blackwell, M., Laman, T. G. & Gilbertson, R. L. 1982. Spore dispersal in *Fuligo septica* (Myxomycetes) by lathridiid beetles. Mycotaxon 14: 58–60.

Bonner, J.T. 1959. The cellular slime molds. Princeton University Press, Princeton.

Bonner, J. T. 2009. The social amoebae. Princeton University Press, Princeton.

Brefeld, J. O. 1869. *Dictyostelium mucoroides*. Ein neuer Organismus aus der Verwandschaft der Myxomyceten. Abh Senckenb Naturf Ges 7: 85–107.

Brefeld, J. O. 1884. *Polysphondylium violaceum* und *Dictyostelium mucoroides* nebst Bemerkungen zur Systematik der Schleimpilze. Unters Gesamtgeb Mykol 6: 1–34.

Brown, M. W., Kolisko, M., Silberman, J. & Roger, A. J. 2012. Aggregative multicellularity evolved independently in the eukaryotic supergroup Rhizaria. Current Biology 12: 1123-1127.

Brown, M. W., Silberman, J. D. & Spiegel, F. W. 2011a. "Slime Molds" among the Tubulinea (Amoebozoa): Molecular systematics and taxonomy of *Copromyxa*. Protist 162: 277–287.

Brown, M. W., Silberman, J. D., Spiegel, F. W. 2011b. A contemporary evaluation of the acrasids (Acrasidae, Heterolobosea, Excavata). European Journal of Protistology 48: 103–123.

Brown, M. W., Spiegel, F. W. & Silberman, J. D. 2010. A morphologically simple species of *Acrasis* (Heterolobosea, Excavata), *Acrasis helenhemmesae* n. sp. Journal of Eukaryotic Microbiology. 57: 346–353.

Brown, M. W., Spiegel, F. W. & Silberman, J. D. 2009. Phylogeny of the "forgotten" cellular slime mold, *Fonticula alba*, reveals a key evolutionary branch within Opisthokonta. Molecular Biology and Evolution 26:2699–2709.

Burki F., Roger A. J., Brown, M. W. & Simpson, A. G. B. 2020. The New Tree of Eukaryotes. Trends in Ecology & Evolution 35(1): 43-55.

Cayley, D. 1929. Some observations on myce- tozoa of the genus *Didymium*. Transactions of the British Mycological Society 14: 227–248.

Casselton, L. A. 2008. Fungal sex genes-searching for the ancestors. Bioessays 30:711–714.

Cavalier-Smith, T. 1998. A revised six-kingdom system of life. Biol. Rev. 73: 203–266. Cavender, J. C. 1990. Phylum Dictyostelida. In: Cavender JC, Margulis L, Corliss JO, Melkonian M, Chapman DJ (eds), Handbook of Protoctista. Jones and Bartlett, Boston, pp. 88–101.

Cavender, J. C. 1973. Geographical distribution of Acrasiae. Mycologia 65: 1044–1054.

Cavender, J. C., Vadell, E. M., Landolt, J. C., Winsett, K. E., Stephenson, S. L., Rollins, A. W. & Romeralo M. 2013. Ten new small dictyostelids from seasonal rain forests of Central America. Mycologia 105(3):610–635.

Cavender, J. C., Landolt, J. C., Ndiritu, G. G. & Stephenson, S. L. 2010. Dictyostelid cellular slime molds of Africa. Mycosphere 1:147–152.

Cavender, J. C. & Raper, K. B. 1965a. The Acrasieae in nature. II. Forest soil as a primary habitat. American Journal of Botany 52:297–302.

Cavender, J. C. & Raper, K. B. 1965b. The Acrasieae in nature. III. Occurrence and distribution in forests of eastern North America. American Journal of Botany 52:302–308.

Clark, J. & Haskins, E. F. 2013. The nuclear reproductive cycle in the myxomycetes: a review. Mycosphere 4(2), 233–248.

Clark, J. & Haskins, E. F. 2010. Reproductive systems in the myxomycetes: a review. Mycosphere 1: 337–353.

Collins, O. R. 1980. Apomictic-heterothallic conversion in a myxomycete, *Didymium iridis*. Mycologia 72: 1109– 1116.

Collins, O. R. 1963. Multiple alleles at the incompatibility locus in the myxomycete *Didymium iridis*. American Journal of Botany 50: 477–480.

Collins, O. R., Gong, T. & Grantham, M. 1983. Genetical analysis of an apomictic- heterothallic convertant of *Didymium iridis* (Myxomycetes). Mycologia 75: 683–672.

Corliss, J. O. & Esser, S. C. 1974. Comments on the role of the cyst in the life cycle and survival of free-living protozoa. Transactions of the American Microscopical Society 93: 578-593.

Dacks, J. B. & Roger, A. J. 1999. The first sexual lineage and the relevance of facultative sex. Journal of Molecular Biology 48: 779–783.

Danchin, E., Charmantier, A., Champagne, F. A., Mesoudi, A., Pujol, B. & Blanchet, S. 2011. Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. Nature Reviews Genetics 12: 475-486.

De Meeuˆs, T., Prugnolle, F. & Agnew, P. 2007. Asexual reproduction: genetics and evolutionary aspects. Cellular and Molecular Life Sciences 64: 1355–1372.

Dixon, P. A. 1963. Spore liberation by water drops in some myxomycetes. Transactions of the British Mycological Society 46: 615–619.

Dykstra, M. J. 1977. The possible significance of mitochondrial configurations in the acrasid cellular slime molds with reference to members of the Eumycetozoa and the fungi. Mycologia 69: 579–591.

Eichinger, L. et al. (97 authors). 2005. The genome of the social amoeba *Dictyostelium discoideum*. Nature 435: 43–57.

Eisenberg, R. M. & Francis, D. 1977. The breeding system of *Polysphondylium pallidum*, a cellular slime mold. Journal of Protozoology 24: 182–183.

El Hage, N., Little, C., Clark, J. D. & Stephenson, S. L. 2000. Biosystematics of the *Didymium squamulosum* complex. Mycologia 92: 54–64.

Erdos, G. W., Raper, K. B. & Vogen, L. K. 1973. Mating types and macrocyst formation in Dictyostelium discoideum. Proceedings of the National Academy of Sciences USA 70: 1828–1830.

Estrada-Torres, A., Wrigley de Basanta, D. & Lado, C. 2013. Biogeographic patterns of the myxomycete biota of the Americas using a parsimony analysis of endemicity. Fungal Diversity 59: 159-177.

Feest, A. 1987. The quantitative ecology of soil Mycetozoa. Progress in Protistology 2:331–361.

Fenchel, T. 1993. There are more small than large species? Oikos 68: 375-378.

Finlay, B. J., Corliss, J. O., Esteban, G. & Fenchel, T. 1996. Biodiversity at the microbial level: the number of free-living ciliates in the biosphere. Quarterly Review of Biology 71: 221-237.

Finlay, B. J. 2002. Global dispersal of free-living microbial eukaryote species. Science 296: 1061-1063.

Fiore-Donno, A. M., Clissmann, F., Meyer, M., Schnittler, M. & Cavalier-Smith, T. 2013. Two-Gene Phylogeny of Bright-Spored Myxomycetes (Slime Moulds, Superorder Lucisporidia). PLoS ONE 8(5): e62586.

Fiore-Donno, A. M., Novozhilov, Y. K., Meyer, M. & Schnittler, M. 2011. Genetic structure of two protist species (Myxogastria, Amoebozoa) suggests asexual reproduction in sexual amoebae. PLoS ONE 6(8): e22872.

Fiore-Donno, A. M., Nikolaev, S. I., Nelson, M., Pawlowski, J., Cavalier-Smith, T. & Baldauf, S. L. 2010. Deep phylogeny and evolution of slime moulds (Mycetozoa). Protist 161: 55–70.

Fiore-Donno, A. M., Berney, C., Pawlowski, J. & Baldauf, S. L. 2005. Higher-order phylogeny of plasmodial slime moulds (Myxo- gastria) based on elongation factor 1-A and small subunit rRNA gene sequences. Journal of Eukaryotic Microbiology 52: 1–10.

Flowers, J. M., Li, S., Stathos, A., Saxer, G., Ostrowski, E., Queller, D., Strassmann, J. & Purugganan, M. 2010. Variation, sex, and social cooperation: Molecular population genetics of the social amoeba *Dictyostelium discoideum*. PLoS Genetics 6: e1001013.

Foissner, W. 2006. Biogeography and dispersal of micro-organisms: a review emphasizing protests. Acta Protozoologica 45:111–136.

Foissner, W. 1987. Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. Progress in Protistology 2: 69-212.

Forsman, A. 2015. Rethinking phenotypic plasticity and its consequences for individuals, populations and species. Heredity 115: 276–284.

Fortunato, A., Strassmann, J. E., Santorelli, L. L. & Queller, D. C. 2003. Co-occurrence in nature of different clones of the social amoeba, *Dictyostelium discoideum*. Molecular Ecology 12: 1031–1038.

Franke, R. G. 1973. Symposium on the use of electrophoresis in the taxonomy of algae and fungi V. Electrophoresis and the taxonomy of saprophytic fungi. Bulletin of the Torrey Botanical Club 100: 287– 296.

Franke, R. G. & Berry, J. A. 1972. Taxonomic application of isozyme patterns produced with disc electrophoresis of some myxomycetes, order Physarales. Mycologia 64: 830–840.

Franke, R. G., Balek, R. W. & Visentin, L. P. 1968. Taxonomic significance of isozyme patterns of some myxomycetes, order Physarales, produced with starch gel electrophoresis. Mycologia 60: 331–339.

Gilbert, O. M., Foster, K. R., Mehdiabadi, N. J., Strassmann, J. E. & Queller, D. C. 2007. High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. Proceedings of the National Academy of Sciences USA 104: 8913–8917.

Green, R. F. & Noakes, D. L. G. 1995. Is a little bit of sex as good as a lot? Journal of Theoretical Biology 174: 87–96.

Griffiths, A. J. F., Miller, J. H., Suzuki, D. T., Lewontin, R. C. & Gelbart, W. M. 2000. An Introduction to Genetic Analysis. 7th edition. New York: W. H. Freeman.

Hagiwara, H. 1989. The taxonomic study of Japanese dictyostelid cellular slime molds. National Science Museum, Tokyo.

Hanski, I. 1994. A practical model of metapopulation dynamics. Journal of Animal Ecology 63: 151–162.

Heidel, A., Lawal, H. M., Felder, M., Schilde, C., Helps, N. R., Tunggal, B., Rivero, F., John, U., Schleicher, M., Eichinger, L., Platzer, M., Noegel, A. A., Schaap, P. & Glöckner, G. 2011. Phylogeny-wide analysis of social amoeba genomes highlights ancient origins for complex intercellular communication. Genome Research 21: 1882 – 1891.

Hutchinson, G. E. 1951. Copepodology for the ornithologist. Ecology 32:571–577.

Andrews, J. H., Kinkel, L. L., Berbee, F. M. & Nordheim, E. V. 2005. Fungi, leaves, and the theory of island biogeography. Microbial Ecology 14: 227–290.

Ishida, K., Hata, T. & Urushihara, H. 2005. Gamete fusion and cytokinesis preceding zygote establishment in the sexual process of *Dictyostelium discoideum*. Development, Growth & Differentiation 47: 25–36.

Kalyanasundaram, I. 1973. Capillitial development in *Stemonitis*. Taxonomy of Fungi I: 9–13. Madras Univ., India.

Karpov, S. A. & Mylnikov, A. P. 1997. Ultrastructure of the colorless flagellated *Hyperamoeba flagel- lata* with special reference to the flagellate apparatus. European Journal of Protistology 33: 34 –355.

Kawakami, S. & Hagiwara, H. 2002. Two mating groups of *Polysphondylium pallidum*, a dictyostelid cellular slime mould. Mycoscience 43: 453–457.

Kawano, S., Takano, H. & Kuroiwa, T. 1995. Sexuality of mitochondria: fusion, recombination, and plasmids. International Review of Cytology 161:49–110.

Kawano, S, Kuroiwa, T. & Anderson, R. W. 1987. A third multialletic mating-type locus in *Physarum polycephalum*. Journal of General Microbiology 133:2539–2546.

Kessin, R. H. 2001. *Dictyostelium*: evolution, cell biology, and the development of multicellularity. Cambridge Univ. Press, Cambridge.

Lado, C. 2005-2021. An online nomenclatural information system of Eumycetozoa. Real Jardín Botánico, CSIC. Madrid, Spain. https://eumycetozoa.com (Accessed March 22, 2021).

Lado, C. 2005–2019. An online nomenclatural information system of Eumycetozoa. Real Jardín Botánico, CSIC. Madrid, Spain. http://www.nomen.eumycetozoa.com (Accessed October 5, 2019).

Lahr, D. J. G., Grant, J. R. & Katz, L. A. 2013. Multigene phylogenetic reconstruction of the Tubulinea (Amoebozoa) corroborates four of the six major lineages, while additionally revealing that shell composition does not predict phylogeny in the Arcellinida. Protistology 164: 323–339.

Lahr, D. J. G, Grant, J., Nguyen, T., Lin, J. H. & Katz, L. A. 2011a. Comprehensive phylogenetic reconstruction of Amoebozoa based on concatenated analyses of SSU-rDNA and actin genes. PLoS One 6(7): e22780.

Lahr, D. J. G., Parphrey, L. W., Mitchell, E. A. D, Katz, L. A. & Lara, E. 2011b. The chastity of amoe- bae: re-evaluating evidence for sex in amoeboid organisms. Proceedings of the Royal Society B 278: 2081–2090.

Landolt, J. C., Cavender, J. C., Stephenson, S. L. & Vadell EM. 2008. New species of dictyostelid cellular slime moulds from Australia. Australian Systematic Botany 21:50–66.

Li, S. I. & Purugganan, M. D. 2011. The cooperative amoeba: *Dictyostelium* as a model for social evolution. Trends in Genetics 27: 48–54.

Lister, A. 1901. On the cultivation of mycetozoa from spores. The Journal of Botany 39, 5–8.

Martin, G. W. & Alexopoulos, C. J. 1969. The myxomycetes. University of Iowa Press, Iowa City, IA.

Moriyama, Y. & Kawano, S. 2009. Maternal inheritance of mitochondria: multipolarity, multiallelism and hierarchical transmission of mitochondrial DNA in the true slime mold *Physarum polycephalum*. Journal of Plant Research 123: 139–148 .

Nesom, M. G. 1973. Life Cycle and Ultrastructure of the Cellular Slime Mold, *Copromyxa arborescens* [dissertation]. University of North Carolina, Chapel Hill, North Carolina.

Olive, L. S. 1975. The Mycetozoans. Acad. Press, New York, San Francisco, London.

Olive, E. W. 1902. Monograph of the Acrasieae. Proceedings of the Boston Society of Natural History 30: 451–510.

Olive, L. S. & Stoianovitch, C. 1960. Two new members of the Acrasiales. Bulletin of the Torrey Botanical Club 87(1): 1.

Otto, S. & Gerstein, A. 2006. Why have sex? The population genetics of sex and recombination. Biochem Soc Trans 34: 519–522.

O'Day, D. H. & Keszei, A. 2012. Signalling and sex in the social amoebozoans. Biological Reviews of the Cambridge Philosophical Society 87:313–329.

Page, F. C. & Blanton, R. L. 1985. The Heterolobosea (Sarcodina: Rhizopoda), a new class uniting the Schizopyrenida and the Acrasidae (Acrasida). Protistologica 21: 121–132.

Pawlowski, J. & Burki, F. 2009. Untangling the phylogeny of amoeboid protists. Journal of Eukaryotic Microbiology 56: 16–25.

Raper, K. B. 1984. The Dictyostelids. Princeton University Press. Princeton.

Raper, K. B. Worley, A. C. & Kurzynski, T. A. 1978. *Copromyxella*: a new genus of Acrasidae. American Journal of Botany 65: 1011–1026.

Roger, A. J., Smith, M. W., Doolittle, R. F. & Doolittle, W. F. 1996. Evidence for the Heterolobosea from phylogenetic analysis of genes encoding glyceraldehyde-3-phosphate dehydrogenase. Journal of Eukaryotic Microbiology 43: 475–485.

Romeralo, M., Skiba, A., Gonzalez-Voyer, A., Schilde, C., Lawal, H., Kedziora, S., Cavender, J. C., Glo¨ckner, G., Urushihara, H. & Schaap, P. 2013. Analysis of phenotypic evolution in Dictyostelia highlights developmental plasticity as a likely consequence of colonial multicellularity. Proceedings of the Royal Society B: Biological Sciences 280: 20130976. http://dx.doi.org/10.1098/rspb.2013.0976.

Romeralo, M., Cavender, J., Landolt, J., Stephenson, S. L. & Baldauf, S. 2011. The expanding phylogeny of social amoebae defines new major lineages and emerging patterns in morphological evolution. BMC Evolutionary Biology 11: 84.

Romeralo, M., Spiegel, F. W., Baldauf, S. L. 2010a. A fully resolved phylogeny of the social amoebas (Dictyostelia) based on combined SSU and ITS rDNA sequences. Protist 161:539–548.

Romeralo, M., Landolt, J., Cavender, J., Larsen, G. & Baldauf, S. 2010b. New species of Dictyostelids from Alaska. Mycologia 102: 588–595.

Sathe, S., Kaushik, S., Lalremruata, A., Aggarwal, R. A., Cavender, J. C. & Nanjundiah, V. 2009. Genetic Heterogeneity in Wild Isolates of Cellular Slime Mold Social Groups. Microbial Ecology 60 (1): 137-148.

Schaap, P. et al. (11 authors) S.L. 2006. Molecular phylogeny and evolution of morphology in social amoebas. Science 314: 661–663.

Schnittler, M., Novozhilov, Y., Romeralo, M., Brown, M. W., Spiegel, F. W. 2012. Fruit body-forming protists: Myxomycetes and Myxomycete-like organisms Acrasia, Eumycetozoa. In book: Syllabus of Plant Families - A. Engler's Syllabus der Pflanzenfamilien Part 1/1: Blue-green Algae, Myxomycetes and Myxomycete-like organisms, Phytoparasitic protists, Heterotrophic Heterokontobionta and Fungi, Edition: 13, Chapter: 4, Publisher: Borntraeger Science Publishers, Berlin, Editors: Frey, pp.40-88.

Schnittler, M. et al. (7 authors) 2011: Rote Liste und Gesamtartenliste der Schleimpilze (Myxomycetes) Deutschlands. Natursch Biol Vielfalt 70: 125–234.

Schnittler, M. & Tesmer, J. 2008. A habitat colonisation model for spore-dispersed organisms – does it work with eumycetozoans? Mycological Research 112: 697–707.

Schnittler, M. & Mitchell, D. W. 2000. Species diversity in Myxomycetes based on the morphological species concept: a critical examination. Stapfia 73: 55–61.

Segers, H. 2001. Zoogeography of the Southeast Asian Rotifera. Hydrobiologia 446-447 (1): 233-246.

Shadwick, L. L., Spiegel, F. W., Shadwick, J. D. L., Brown, M. W. & Silberman, J. D. 2009. Eumycetozoa = Amoebozoa?: SSUrDNA phylogeny of protosteloid slime molds and its significance for the amoebozoan supergroup. PLoS ONE 4: e6754.

Smith, M. H. 2004. Genotypic diversity and population structure in *Dictyostelium discoideum*. M.A. Thesis, Rice University, Houston, Texas.

Spiegel, F. W. 2011. Commentary on the chastity of amoebae: re-evaluating evidence for sex in amoeboid organisms. Proceedings of the Royal Society B 278: 2096–2097.

Spiegel, F. W. 1991. A proposed phylogeny of the protostelids. BioSystems 25: 113–120.

Spiegel, F. W. 1990. Phylum plasmodial slime molds: Class Protostelids. In: Margulis L, Corliss JO, Melkonian MJ, Chapman D, eds. Handbook of Protoctista. Boston: Jones and Bartlett Publishers, Inc. pp 484–497.

Spiegel, F. W. 1990. Phylum Plasmodial slime molds, Class Protostelia. – In: Margulis, L., Corliss, J.O., Melkonian, M. & Chapman, D.J. (eds.): Handbook of Protoctista. Pp. 484–497. Jones & Bartlett, Boston.

Spiegel, F. W., Shadwick, J. D., Lindley, L. L., Brown, M. W. & Ndiritu, G. 2007. A beginner's guide to identifying the protostelids. Publ. at http://slimemold.uark.edu/.

Spiegel, F. W., Stephenson, S. L., Keller, H. W., Moore, D. L. & Cavender, J. C. 2004. Mycetozoans. In: Mueller, G.M., Bills, G.F. & Foster, M.S. (eds.). Biodiversity of Fungi, Inventory and monitoring methods. Pp. 547–576. Elsevier Acad. Press, Burlington, MA.

Spiegel, F. W., Lee, S. B. & Rusk, S. A. 1995. Eumycetozoans and molecular systematics. Canadian Journal of Botany 73: 738–746.

Spiegel, F. W. & Olive, L. S. 1978. New evidence for the validity of *Copromyxa protea*. Mycologia 70:843–847.

Stephenson, S. L., Novozhilov, Y. K., Almadrones-Reyes, K. J., Dagamac, N. H. A. & Schnittler, M. 2019. New records of *Barbeyella minutissima* (Myxomycetes, Echinosteliales) with an updated distribution map. Nova Hedwigia 109 (1-2): 177-186(10).

Stephenson, S. L. 2014. Excavata: Acrasiomycota; Amoebozoa: Dictyosteliomycota, Myxomycota. In book: Systematics and Evolution, 2nd Edition The Mycota VII Part A. D. J. McLaughlin and J.W. Spatafora (Eds.).

Stephenson, S. L., Schnittler, M. & Novozhilov, Y. K. 2008. Myxomycete diversity and distribution from the fossil record to the present. Biodiversity and Conservation 17:285–301.

Stephenson, S. L., Skay, M. E., Slay, C. A. & Tuggle, A. E. 2007. Cave crickets (Orthoptera: Rhaphidophoridae) as vectors of dictyostelids (Protista: Dictyosteliida). Entomological News 118: 292–295.

Stephenson, S. L. & Landolt, J. C. 1996. The vertical distribution of dictyostelids and myxomycetes in the soil/ litter microhabitat. Nova Hedwigia 62:105–117.

Stephenson, S. L. & Stempen, H. 1994. Myxomycetes: a handbook of slime molds. Timber, Portland, OR.

Stephenson, S. L. & Landolt, J. C. 1992. Vertebrates as vectors of cellular slime moulds in temperate forests. Mycological Research 96: 670–672.

Sucgang, R. et al. 2011. Comparative genomics of the social amoebae *Dictyostelium discoideum* and *Dictyostelium purpureum.* Genome Biology 12: R20.

Sugimoto, H. & Endoh, H. 2008. Differentially expressed genes during fruit body development in the aggregative ciliate *Sorogena stoianovitchae* (Ciliophora: Colpodea). Journal of Eukaryotic Microbiology 55: 110–116.

Suthers, H. B. 1985. Ground-feeding migratory songbirds as cellular slime mold distribution vectors. Oecologia 65: 526–530.

Szabo, S. P., O'Day, D. H. & Chagla, A. H. 1982. Cell fusion, nuclear fusion, and zygote differentiation during sexual development of *Dictyostelium discoideum*. Developmental Biology 90: 375–382.

Vadell, E. M., Cavender, J. C., Romeralo, M., Edwards, S. M., Stephenson, S. L. 2011. New species of dictyostelids from Patagonia and Tierra del Fuego, Argentina. Mycologia 103:101–117.

Wegener-Parfrey, L., Lahr, D. J. G., Knoll, A. H. & Katz, L. A. 2011. Estimating the timing of early eukaryotic diversification with multigene molecular clocks. Proceedings of the National Academy of Sciences 108:13624–13629.

Winsett, K. E. & Stephenson, S. L. 2011. Global distribution and molecular diversity of *Didymium difforme*. Mycosphere 2:135–146.

Winsett, K. E. & Stephenson, S. L. 2008. Using ITS sequences to assess intraspecific genetic relationships among geographically sepa- rated collections of the myxomycete *Didymium squamulosum*. Revista Mexicana de Micología 27: 59–65.

Worley, A. C., Raper, K. B. & Hohl, M. 1979. *Fonticula alba*: a new cellular slime mold (Acrasiomycetes). Mycologia 71: 746–760.

Yoon, H. S., Grant, J., Tekle, Y. I., Wu, M., Chaon, B. C., Cole, J. C., Logsdon, J. M. Jr, Patterson, D. J., Bhattacharya, D. & Katz, L. A. 2008. Broadly sampled multigene trees of eukaryotes. BMC Evolutionary Biology 8:14.

Zaman, V., Zaki, M., Howe, J., Ng, M., Leipe, D. D., Sogin, M. L., Silberman, J. D. 1999. *Hyperameoba* isolated from human feces: description and phylogenetic affinity. European Journal of Protistology 35: 197–207.

II. Implications of the role of dispersal on the occurrence of litter-inhabiting myxomycetes in different vegetation types after a disturbance: a case study in Bohol islands, Philippines

Abstract

The assemblages of myxomycetes associated with ground litter in seven different study sites situated along a disturbance gradient on the Bohol Islands in the Philippines were studied using exclusively the moist chamber culture technique. The disturbance gradient, which was also characterized by differences in plant community types, resulted from a major typhoon (Haiyan) that affected the islands following a 7.2 magnitude earthquake. These assemblages were analyzed using several software and abundance-based data for the evaluation of species richness, composition, and α and β diversity of myxomycetes in the various study sites. The results suggested that environmental alterations could be a factor affecting the abundance of myxomycetes in different habitats, and that dispersal plays a role in shaping the patterns of distribution of assemblages of myxomycetes associated with particular plant communities. In addition, the present study pointed out the need for an extensive survey to assess the impacts of conservation and restoration for areas that are susceptible to ecological shifts, such as events caused by the changing global climate.

Introduction

As a general observation, the diversity of myxomycetes (plasmodial slime molds or myxogastrids) is considered to be directly related to the diversity and numbers of available vascular plants, which provide the substrates supporting the microorganisms that serve as food resource for the two trophic stages (amoeboflagellate and plasmodia) in the myxomycete life cycle

(Madelin 1984, Stephenson 1989), although their occurrence in a particular locality is considered to be controlled largely by environmental factors such as temperature and moisture (Alexopoulos 1963). Since their discovery, the majority of the studies have been focused on their biology and taxonomy, while ecological and biogeographical studies of these organisms have been carried out only recently.

The archipelagic landscape of the Philippines serves as an ideal system for island biogeographical studies of myxomycetes. However, most previous studies have been directed towards updating the myxobiota of the country, including diversity assessments in lowland (dela Cruz et al. 2014) and montane forests (Mt. Kanlaon [Alfaro et al. 2015], Mt. Arayat [Dagamac et al. 2014]), national parks (Quezon National Park [Dagamac et al. 2015a], La Mesa Ecopark [Macabago et al. 2010]), and certain coastal islands (Lubang Islands [Macabago et al. 2016, 2012], Hundred Islands [Kuhn et al. 2013, dela Cruz et al. 2011], and the Polilio Islands [Viray et al. 2014]). An appreciable increase in overall myxomycete research in the Philippines during the past decade is noteworthy, but few detailed ecological studies have been carried out. However, the body of data that has been obtained has greatly increased our understanding of myxomycetes in the Paleotropics. Myxomycete diversity in some localities such as Puerto Galera (Dagamac et al. 2015b) and the Bicol Peninsula (Dagamac et al. 2015c) appear to be following the intermediate disturbance hypothesis (Connell 1978), since anthropogenic or natural disturbances are presumed to have affected the assemblages of myxomycetes present. Having a total of 7, 107 islands of mostly volcanic origin (Hall 2002) located just above the equator and with these supporting distinct plant community types, the islands of the Philippines are still understudied with respect to their potential for investigations of patterns of myxomycete biodiversity.

Bohol Island is a region in the mid-southern part of the archipelago, a portion of the Philippines that underwent a natural disaster. A typhoon hit the islands and nearby provinces after a 7.2 magnitude earthquake in the last quarter of 2013 (Lagmay et al. 2013). The primary objective of the study reported herein was to carry out a survey of the assemblages of myxomycetes occurring in different study sites that are defined on the basis being situated at different points along a disturbance gradient and are characterized by different types of plant communities. The major question being addressed was first to determine whether or not it was possible to relate these different plant community types to differences in patterns of myxomycete distribution and diversity, and then to evaluate the impact of disturbances on these patterns.

Materials and Methods

Collecting protocols: The study described herein was carried out during 2014 in the Bohol Islands of the Philippines seven months after these and other nearby islands were subjected to a magnitude 7.2 earthquake followed by the super typhoon Haiyan in 2013. The study sites (Fig. 1) are all, except one (reef woodland), underlain by limestone and were selected to represent each of the major types of plant communities available in the different major habitats. The grassland site (Chocolate Hill) was noted to have mostly tall grasses and a few woody shrubs, but no trees. The indigenous woodland (Carmen forest patch) was situated near the foot of the grassland site and was characterized as a mixed forest patch having mostly native plants with a mixture of tropical trees, shrubs, woody and herbaceous plants, and saplings, seedlings, and grasses on the forest floor. The canopy was relatively open, and the floor was observed to have trails for humans and smaller vehicles. The monotypic, man-made plantation (Mahogany forest) was a fairly homogenous stretch of non-native tropical, timber hardwood Mahogany trees (*Swietenia* sp.), with sparse

saplings and seedlings on the forest floor. Heterogeneous natural forest (Rajah Sikatuna National Park) was the most structured of all the sites. It is a protected area with mostly secondary growth forest, having a few emergent layers, and a generally closed canopy for the most part of the forest with trees comprising canopy and understory layers. There were fewer shrubs than the indigenous woodland, rather other vascular plants, such as ferns, were present along with indiscriminately scattered seedlings and vines on the forest floor. The satellite islands sites were mostly composed of an assortment of trees and other woody shrubs. Coastal mixed forest (Panglao Island) is connected to mainland Bohol by a land bridge and was noted to have patches of mixed trees and shrubs within a developed touristic area having buildings, roads, and active human activities. The same was true for the smallest island site, reef woodland (Virgin Island), as such that this non-limestone stretch of sandy mound was a regularly visited area with loosely distributed trees maintained by the inhabitants; however, there was no apparent vegetation structure. The Islet mixed forest (Balicasag Island) was another touristic site, with less recognizable human activities on land except for a community of inhabitants and the visitors for recreational water activities, such as diving and snorkeling. Mixed, broadleaf trees and shrubs lined the coast of this second smallest island. Additional descriptions of the study sites are given in Table 1.

Collection of substrates, laboratory isolation techniques, and determination of myxomycetes: Samples of ground leaf litter (GL) and twigs (TW) were haphazardly collected from the different study sites. No specimens that had developed under natural conditions in the field were collected. All samples were air-dried for two weeks in the Research Center for the Natural and Applied Sciences at the University of Santo Tomas in the Philippines, after which they were sent to the University of Arkansas (UARK) in the United States of America for processing. A total of 168 samples (three plates per sample, pooled) were used to prepare moist chamber cultures,

following the protocol described by Stephenson and Stempen (1994). Samples were placed in Petri dishes lined with filter paper, distilled water was added to soak the substrates for about 24 hours, the pH was measured, and then the excess water was poured off. Cultures were observed for two months and/or until fruiting bodies were evident. Despite the limitations of solely using the moist chamber culture technique, since the fruiting bodies for some taxonomic groups of myxomycetes do not appear in such cultures, the technique has been shown to be effective for carrying out some recent rapid biodiversity surveys for myxomycetes, as has been demonstrated in a number of instances (e.g., Ethiopia [Dagamac et al. 2016], Oman [Schnittler et al. 2015], El Salvador [Rojas et al. 2013]). On some instances it is necessary to carry out such surveys because of bureaucratic, financial and time constraints. In the present study, when fruiting bodies of myxomycetes appeared in moist chamber cultures, they were carefully transferred to small pasteboard boxes and then deposited in the herbarium of the UARK for future study. The morphological concept of species was used to classify and identify the myxomycetes listed herein, and the nomenclatural treatment follows Lado (2005-2016).

Exhaustiveness of the survey: The software program EstimateS (Version 9.1, Colwell 2013, 100 randomizations) was used to construct species accumulation curves (SAC) that make it possible to assess the completeness of our survey for (1) each of the seven study sites that are different with respect to their plant community and/or habitat types, and (2) the pooled dataset from all seven study sites. Initially, a species-record input file was generated and subsequently loaded in the program. The results of the Chao 1 estimator, an estimator for target richness for individual-based data as such that one record of a species in a certain culture is considered as one individual (*sensu* Stephenson 1988), were then used to calculate the percentage exhaustiveness by dividing the actual number of species recorded by the mean number of species expected as

estimated by the Chao 1 estimator. To compare the richness of species, rarefaction curves for each of the seven study sites were constructed from the Coleman rarefaction values generated from EstimateS.

Analysis of myxomycete assemblages: To assess the dataset obtained from the records of myxomycetes appearing in the moist chambers, various software and abundance-based species data were used for the evaluation of species richness, diversity, and composition.

The relative abundance of each species was obtained by dividing the total number of collections for each species of myxomycetes by the total number of myxomycetes collected (Stephenson et al. 1993). The computed values were then translated to an abundance index described by Stephenson et al. (1993), for which species < 0.5% of the total number of collections are defined as rare; species > 0.5% but < 1.5% of the total number of collections defined as occasional; species > 1.5% but < 3% of the total number of collections defined as common; and species > 3% of the total number of collections defined as abundant.

Using the vegan package in R (R Core Team 2016), the most plausible species abundance distribution (SAD) model for each of the vegetation types was defined from the rank-abundance plots (Whittaker 1965), following the concept of Wilson (1991) that suggested five models. These are Null (fits the broken stick model), Preemption (fits the geometric series or Motomura model), log-Normal, Zipf, and Mandelbrot. A species abundance distribution (SAD) is regarded as a vector of the abundances of all species present in a community, where it describes the abundance (number of individuals observed) for each different species found within a community (McGill et al. 2007). Taxonomic diversity was calculated by obtaining the ratio of the number of species to the number of genera. The value of this ratio was inversely proportional to the taxonomic diversity (Stephenson et al. 1993), where the lower ratio indicates a more diverse biota.

The software SPADE (Chao & Shen 2010) was used to generate the Fisher's alpha and maximum likelihood estimator for the more intuitive Inverse Simpson index. The former index is a logarithmic series model of species richness (Fisher et al. 1943) while the latter is a heterogeneous measure of species diversity that accounts for both the species richness and evenness. In addition, to visualize the patterns of species composition in the different plant community types, a clustering analysis was employed in PAST (Hammer et al. 2001) using the paired group algorithm in the Morisita similarity measurement. Subsequently, a principal coordinate analysis (PCoA) based on the Bray Curtis dissimilarities of myxomycete abundance records was performed in the R environment.

Results

The present study increased the number of species of myxomycetes known to the Philippines to 158, with eight new records for the islands. These were *Comatricha elegans* (Racib.) G. Lister, *Comatricha laxa* Rostaf., *Didymium bahiense* Gottsb., *Physarina echinospora* K.S. Thind & Manocha, *Physarum bitectum* G. Lister, *Physarum sulphureum* Alb. & Schwein., *Physarum serpula* Morgan, and *Physarum straminipes* Lister.

Approximately 69% of the laboratory cultures produced myxomycetes, with twigs (75%) more productive than leaf litter (62%). Relative to the plant community types, the indigenous woodland in a forest patch yielded the highest productivity (100%), followed by the grassland (88%), coastal mixed forest (83%), islet mixed forest (75%), heterogeneous natural forest (70%), and reef woodland (50%), with the monotypic man-made forest (41%) being the least productive. The myxomycetes identified from fruiting bodies accounted for a total of 54 different morphospecies (Supplementary file 1).

Using EstimateS (Colwell 2013), the entire study area showed 56% survey exhaustiveness, a value lower than most comparable studies that have been carried out. The specific survey completeness for each study site is given in Table 2, along with actual and expected numbers of taxa found. Values for the reef woodland (32% survey completeness) were not included in the table, since the exceedingly limited data (only six species represented by one record each) skewed further diversity assessments.

Among the 54 morphotaxa identified, seven were found to be abundant, while 13 were common, and 14 were occasional. Twenty rare species not included in Fig. 2 were all singletons from five collection sites. These were six species from heterogeneous natural forest: (*Arcyria denudata* (L.) Wettst., *Collaria* sp., *Hemitrichia serpula* (Scop.) Rostaf. ex Lister, *Perichaena pedata* (Lister & G. Lister) G. Lister, *Stemonitis smithii* T. Macbr., *Willkommlangea reticulata* (Alb. & Schwein.) Kuntze; one species from indigenous woodland: *Physarum lakhanpalii* Nann.-Bremek. & Y. Yamam.; two species from grassland: *Physarina echinospora* K.S. Thind & Manocha, *Physarum decipiens* M.A. Curtis; one species from reef woodland: *Physarum compressum* Alb. & Schwein.; four from the islet mixed forest: *Arcyria insignis* Kalchbr. & Cooke, *Badhamia sp.*, *Diderma sp.*, *Didymium clavus* (Alb. & Schwein.) Rabenh.; and six from coastal mixed forest *Comatricha elegans* (Racib.) G. Lister, *Comatricha* sp., *Dictydiaethalium plumbeum* (Schumach.) Rostaf., *Physarum bitectum* G. Lister, *Physarum sulphureum* Alb. & Schwein., and *Physarum globuliferum* [Bull.] Pers.).

Using the *radfit* command in R, a rank abundance plot for each study site was constructed. Five out of the 7 plant communities showed that their species abundance distribution follows the Zipf model, and this implies that they are comparable with each other following a generalized

linear model. Only the monotypic plantation and reef woodland both followed the geometric series of the Preemption model.

In terms of taxonomic diversity, the 54 morphotaxa found in the whole study area belonged to 20 genera (S/G = 2.7). Among all study sites, the most taxonomically diverse was found to be the grassland site, while the least was the islet mixed forest (Table 2). Reef woodland yielded six morphospecies from three genera.

The α diversity in each collecting site was calculated using Fisher's alpha and Simpson's indices (Fig. 3). Based on mathematical computations, the most species rich is the grassland among the six study sites. Results of Fisher's alpha are congruent to the rarefied values of species richness as shown on Table 2. The inverse of Simpson's index of diversity showed the coastal mixed forest to be the most diverse. For both indices, heterogeneous natural forest was analyzed to be the least species rich and least species diverse. Reef woodland, which is not included in the table and in further community analysis, recorded zero Fisher's alpha result and the lowest (0.83) species diversity.

Ecological distances among the six sites were examined using clustering and ordination (Fig. 4). Both the Morisita similarity index clustering from PAST and ordination from R showed that the monotypic man-made plantation was the most ecologically distant site. Heterogeneous natural forest was most similar to the grassland community, as the indigenous woodland had more similar assemblages of myxomycetes to the satellite island communities, islet mixed forest and coastal mixed forest.

Discussion

This study delivered results that were comparable to those reported for temperate forests. For example, Wrigley de Basanta et al. (2008) estimated 23-43% productivity for twigs in tropical

and 67-85% productivity in temperate forests. Some species of myxomycetes have been observed to have a limited occurrence on woody fragments, such as the case for *Willkommlangea reticulata* (Stephenson et al. 2008) and *Cribraria microcarpa.* In the present study, the former species was recorded again three decades (Dagamac & dela Cruz 2015) after it was listed by Reynolds (1981) as *Cienkowskia reticulata* (Alb. & Schwein.) Rostaf. In addition, this is the second study reporting a species of *Macbrideola,* a taxon which was first documented by Macabago et al. (2010). Unfortunately, both collections are of poor quality; therefore, not allowing a confident identification to the species level. Interestingly, a species of myxomycete (*Physarina echinospora*), which appears to have a distribution centered in the tropics and was known originally from Africa and has since been reported from Mexico (Lado & Wrigley de Basanta 2008), India (Tembhurne et al. 2013), and Ethiopia (Dagamac et al. 2016), was recorded for the first time for both the Philippines and all of Southeast Asia. Since the most current list of myxomycetes in the Philippines includes 150 taxa (Dagamac & dela Cruz 2015), this study adds to the relatively meager number relative to other tropical countries, such as Costa Rica in the Neotropics with at least 225 species (Rojas et al. 2015a). However, it should be noted that Neotropical areas have been subjected to more rigorous spatial and temporal sampling efforts than the Paleotropics, such as the Philippines.

Several studies have indicated that biodiversity seems to be higher in temperate regions (see Schnittler et al. 2001, Schnittler & Stephenson 2000) than in tropical, specifically the Neotropics, or boreal areas (Schnittler & Novozhilov 1995). However, some studies on myxomycetes have suggested that richness in the Paleotropics may be even greater (Tran et al. 2006). Particularly when looking at different plant debris microhabitat, it appears that aerial litter substrates harbor more species of myxomycetes species than ground litter substrates (Rojas & Stephenson 2008, Rojas & Stephenson 2013). The Bohol Islands in the Philippines has a typical

seasonably distributed annual rainfall and with the occurrence of natural calamities such as earthquake and typhoon before the conduct of this study, this small scale attempt to assess the diversity of myxomycete assemblages operated on the notion that previously elevated substrates have been mixed with the ground litter microhabitats–a likely case for natural disturbance, along with the inevitable integration of substrates from one area to another due to wind, water flow, and direct and/or indirect animal (e.g., human) transport.

Despite the low values for survey completeness, the noteworthy observation of previously unrecorded morphospecies of myxomycetes provides evidence of the substantial value of the study reported herein especially with respect to vulnerability assessments of insular ecosystems. Distinct occurrences of some morphospecies in isolated islands like Bohol warrant an investigation to determine if distribution patterns can be accounted for by environmental gradients such as different plant community types or if these are mostly influenced by natural and/or anthropogenic disturbances. There are a number of definitions associated with disturbance. The most appropriate would seem to be the one by Grime (1977), who described it to be a partial or total destruction of biomass. More elaborate definitions have been given by Pain & Levin (1981), Sousa (1984), Pickett & White (1985), Reynolds et al. (1993), Mackey & Curie (2000), and Shea (2004), most of which dealt with operational values of disturbance. In relation to diversity assessments, it seems the definition given by Sousa (1984), which states that a disturbance is a "…discrete, punctuated killing, displacement, or damaging of one or more individuals (or colonies) that directly or indirectly creates an opportunity for new individuals (or colonies) to become established" appears to be considered by many as the more practical, since it implies that without new species occupying the space unrestricted by disturbance diversity will not increase (Osman 1977, Collins et al. 1995, Huxham et al. 2000). This seems to conform to the outcomes of the present study, such that despite

having more available variety of microhabitats on the forest floor litter of the heterogeneous natural forest, the restricted access to the protected area resulted to less richness and overall diversity. It is possible that after having been disturbed by the calamities, there was a less likely chance of bringing in myxomycetes from other areas as a result of human activities and natural forces, such as wind, because the forest has less open canopies and is characterized by vegetation layers in most parts, therefore possibly impeding the thriving of myxomycetes on the forest floor. A study on the effects of disturbance on tropical forests found that moderate levels of disturbance did not affect the species diversity nor functional diversity of tropical trees within an eight-year period after a disturbance (logging), such that species richness is conserved; however, at high disturbance intensity the tree species shifted from a 'slow', shade-tolerant, and conservative species to a 'fast', light-demanding, and acquisitive species that will functionally enhance productivity and nutrient cycling (decomposition rate) (Carreno-Rocabado et al. 2012). It was also noted that temporal changes in the community were mostly affected by recruitment, such that at high disturbance the 'recruits' varied from surviving or dead individuals compared to low disturbance treatments that had similar traits. However, it was noted that recruitment was low in the latter and in undisturbed control forests. If these were parallel to the disturbance in Bohol islands, it may be implicit that the changes in vegetation traits, such as wood density, leaf toughness and area, dry matter content, and concentration of organic nutrients in the plants may be affecting the community of myxomycetes that depend on plant and plant debris, such that at high disturbance despite probably recruiting new plant species the functional changes in vegetation may be prompting the myxomycetes to find a more suitable microhabitat. If the disturbance intensity was relatively low the lower recruitment rate of new plants should not have caused a bigger impact on plant functional traits, and therefore should ideally have less impact on myxomycete assemblages and diversity.

On another note, the disturbance (whether low or high intensity) should have caused an increase in available microhabitats on the forest floor in the form of plant debris; however, the likely displacement of myxomycetes and the arguably limited modes of dispersal to and from other sites via natural forces or anthropogenic events may have negatively affected the species diversity of myxomycetes. Another perspective would be that at this point after the disturbance the myxomycetes were dwelling in microhabitats other than ground litter.

The other areas, such as the more species diverse coastal mixed forest, indigenous woodland, islet mixed forest, and monotypic man-made plantation, are more accessible to agents of dispersal, specifically anthropogenic factors since all are available for touristic explorations. Biodiversity being affected by disturbances has been reported in recent studies in which assemblages of myxomycetes, primarily those in the Neotropical southwestern Peruvian Amazon, have been affected by habitat loss and forest disturbance (Rojas & Stephenson 2013). Comparable remarks were also noticed in disturbed areas of the Bicol Peninsula in the Philippines (Dagamac et al. 2015c) and in the forests of Puerto Galera, Philippines (Dagamac et al. 2015b), where highest diversity, specifically species richness, was seen in intermediately disturbed areas following the intermediate disturbance hypothesis (Connell 1978). In relation to this study, it would be very speculative to say which sites are the most highly or least likely disturbed due to the lack of parameters to identify and gauge the factors of disturbance (natural, as in earthquakes and typhoons, or anthropogenic elements).

Despite being more constrained from entry by humans and other larger animals the grassland area, which is situated on limestone hills, may have obtained its high number of species and a moderately diverse ecosystem due to other agents of dispersal such as wind. If spatial dispersal were to be considered, it is to be expected that the grassland community would be more

likely similar to the indigenous woodland due to their proximity than to the heterogeneous natural forest, which was not the case. Both heterogeneous natural forest and indigenous woodland were observed to have a similar vegetation structure as such that tall trees and shrubs were present. However, the indigenous woodland was a mixed forest patch having an observably more open canopy that has less canopy and sub-canopy layers. There was also more noticeable vegetation in the shrub layer of the indigenous woodland, as opposed to a mostly fern-inhabited layer of the heterogeneous natural forest. Ordination using a species matrix and clustering using species abundances indicated that there is less ecological distance between the grassland community and heterogeneous natural forest despite the remarkable differences in plant communities. This may well be linked to the transfer of myxomycete propagules or of actual substrates between these two distinct communities through agents of dispersal other than animals in general. It was noted that most of the leaf litter collected in the grassland was not from vegetation that was present in the grassland area, a similar finding in the study conducted by Fischer & Stephenson (2014) in two grassland types in Northwest Arkansas, where they shared that some samples contained a mixture of broadleaf plants. This dispersal event may also be displayed by the relatedness in species composition as shown by the β diversity of the two satellite islands, coastal mixed forest and islet mixed forest, although this may also be relevant to the fact that both satellite islands contained similar vegetation. In addition, the dispersion of myxomycetes that was most plausibly of their spores from mainland to the satellite islands is also denoted by the affinity of indigenous woodland and satellite islands. Although possible as these areas are close to touristic sites, it could not be clearly pointed out if this is more correlated with anthropogenic factors by means of humans going from one island to another or by wind dispersal as these sites have more open canopies. On that note, the disturbance caused by the earthquake and typhoons may have generated an opportunity

for new individuals of myxomycetes to be established in these communities, as such that the natural disturbances paved the way for an increase in plant debris that serve as microhabitats and the further opening of the canopies in these areas so that dispersal (either by means of natural factors such as wind, water flow, transport of animals from mainland to coastal mixed forest via land bridge, etc., or by anthropogenic activities via people going to these sites) of myxomycetes from mainland to satellite islands and vice versa was facilitated. .

In the case of the monotypic plantation, being man-made already signifies an intervention to the natural ecosystem. The Preemption model (SAD) followed by this community and another satellite community, which is the reef woodland, is a resource partitioning model among the rank abundance plots exhibiting that the most competitive species takes more resources, leaving other species to have less (Gardener 2014). There is a seeming disparity, though, since the species richness given by Fisher's alpha index and the rarefied values generated from EstimateS did not show the predicted low values for the monotypic plantation. As in the case of a comparative study between a monotypic agricultural land and a heterogeneous plant community in Negros Oriental in the Philippines, the protected forest was seen to have higher myxomycete diversity than the monotypic plantations (Alfaro et al. 2015). In this situation, it is likely that the role of available microhabitats in the plant communities could be a factor in the characteristics of the myxomycete assemblages as similarly noted in other litter myxomycete studies in Asia (mixed forest type in Japan, Takahashi 2013; lowland tropical forests in Vietnam, Tran et al. 2014). The non-native mahogany trees, introduced in the 1970s, were broad and thick-leaved. The sparse presence of seemingly decaying litter posed a difficulty during the time of the collection. Most, if not all, collected substrates were from fallen leaves and twigs of mahogany trees and a few seedlings on the forest floor. The scarcity of more suitable habitats for myxomycetes may have played a part.

Notwithstanding the low to moderate survey exhaustiveness, which may have led to the inability to depict a more convincing role of the differences in plant community types and prompted us to treat the data with caution, the distributional patterns of myxomycete assemblages in a disjunct area with environmental gradients such as the Bohol Islands calls for a more thorough examination. Along with previous studies, this survey implies that dispersal of myxomycetes is occurring in Bohol islands. What would be interesting in the future is to do a more comprehensive sampling, which involves a more exhaustive method using other available microhabitats and field collections of myxomycetes and incorporating other islands that have similar plant community types and evaluate if the myxomycete communities will be analogous or not. In this way, a conclusion on the roles of geographical barriers on dispersal will be well supported. The case in Bohol could not be solely correlated to a stronger influence by natural and/or anthropogenic disturbances or by dispersal alone. What was implicit in the study was that dispersal likely influences the assemblages of myxomycetes in disturbed habitats. A species distribution modeling (similar to selected myxomycetes in Costa Rica, Rojas et al. 2015b) emerges to be a potential project to extend this perspective to see if environmental factors based on world climate datasets also impact distributional patterns, especially in disjointed areas (like islands) that are vulnerable to ecological alterations and are subjected to threats posed by climate change.

Literature Cited

Alexopoulos, C. J. 1963. The Myxomycetes II. Botanical Review 29: 1–77.

Alfaro, J. R. D., Alcayde, D. L. I. M, Agbulos, J. B., Dagamac, N. H. A. & Dela Cruz, T. E. E. 2015. The occurrence of myxomycetes from a lowland montane forest and agricultural plantations of Negros Occidental, Western Visayas, Philippines. Fine Focus 1: 7–20.

Carreno-Rocabado, G., Pena-Claros, M., Bongers, F., Alarcon, A., Licona, J.-C. & Poorter, L. 2012. Effects of disturbance intensity on species and functional diversity in a tropical forest. J. Ecol. doi: 10.1111/j.1365-2745.2012.02015.x.

Chao, A. & Shen, T. J. 2010. Program SPADE (Species Prediction And Diversity Estimation). Program and User's Guide; http://chao.stat.nthu.edu.tw (accessed 13 February 2016).

Collins, S. L., Glenn, S. M. & D. J. Gibson. 1995. Experimental Analysis of intermediate disturbance and initial floristic composition - decoupling cause and effect. Ecology 76: 486-492.

Colwell, R. K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 7. User's Guide and application; http://purl.oclc.org/estimates (accessed 13 February 2016).

Connell, J.H. 1978. Diversity in tropical rain forests and coral reefs. Science 199: 1302–1310.

Dagamac, N. H. A. & dela Cruz, T. E. E. 2015. Myxomycetes research in the Philippines: Updates and opportunities. Mycosphere 6(6): 784-795.

Dagamac, N. H. A., Stephenson, S. L. & dela Cruz, T. E. E. 2014. The occurrence of litter myxomycetes at different elevations in Mt. Arayat, National Park, Pampanga, Philippines. Nova Hedwigia 98: 187-196.

Dagamac, N. H. A., Rea-Maminta, M. A. D. & dela Cruz, T. E. E. 2015a. Plasmodial slime molds of a tropical karst forest, Quezon National Park, the Philippines. Pacific Science 69: 407-418.

Dagamac, N. H. A., Rea-Maminta, M. A. D., Batungbacal, N. S., Jung, S. H. & Bulang, C. R. T et al. 2015b. Diversity of plasmodial slime molds (myxomycetes) on coastal, mountain, and community forests of Puerto Galera, Oriental Mindoro, Philippines. J. Asia Pac. Biodivers.8: 322-329.

Dagamac, N. H. A., dela Cruz, T. E. E., Rea-Maminta, M. A. D., Aril- dela Cruz, J. V. & Schnittler, M. 2015c. Rapid assessment of myxomycete diversity in Bicol Peninsula. Nova Hedwigia doi: http://dx.doi.org/10.1127/nova_hedwigia/2015/0252.

Dagamac, N. H. A., Hoffman, M., Novozhilov, Y. K. & Schnittler, M. 2017. Myxomycetes from the highlands of Ethiopia. Nova Hedwigia 104(1-3):111-127.

Dela Cruz, T. E. E., Kuhn, R. V., Javier, A. O. M., Rodillas, C. P., & Parra, C. M. *et al.* 2011. Occurrence and distribution of plasmodial myxomycetes in Hundred Islands National Park, Pangasinan, Philippines. Acta Manilana 59: 65-74.

Dela Cruz, T. E. E., Rea, M. A. D., Tran, H. T. M., Ko Ko, T. W. & Stephenson, S. L. 2014. A comparative species listing of myxomycetes from tropical (Philippines) and temperate United States) forests. Mycosphere 5(2): 299-311.

Fischer, H. E. & Stephenson, S. L. 2014. Myxomycetes associated with two types of grasslands in northwest Arkansas Austrian J. Mycol. 23: 125-129.

Fisher, R. A., Corbet, A. S. & Williams, C. B. 1943. The relation between the number of species and the number of individuals in a random sample of an animal population. J. Anim. Ecol. 12: 42-58.

Gardener, M. 2014. Community ecology: Analytical methods using R and Excel. - Pelagic Publishing.

Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111: 1169-1194.

Hall, R. 2002. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. Journal of Asian Earth Science 20: 353-431.

Hammer, Ø., Harper, D. A. T. & Ryan, P. D. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1-9.

Huxham, M., Roberts, I., & Bremner, J. 2000. A field test of the intermediate disturbance hypothesis in the soft-bottom intertidal. International Review of Hydrobiology 85: 379-394.

Kuhn, R. V., Javier, A. O. M., Rodillas, C. P., Parra, C. M. & Corpuz, L. H. M. *et al.* 2013. Diversity of plasmodial myxomycetes from Anda Island, Pangasinan, Philippines. Biotropia 20: 1-9.

Lado, C. 2005-2016. An on line nomenclatural information system of Eumycetozoa. http://www.nomen.eumycetozoa.com.

Lado, C. & Wrigley de Basanta, D. 2008. A review of Neotropical Myxomycetes (1828-2008). Anales Jard. Bot. Madrid 65(2): 211-254.

Lagmay, A. M. F., Eco, R. N. & Yban˜ez, R. 2013. Magnitude 7.2 temblor rocks Bohol, Philippines (Initial assessment). Project NOAH Open-File Reports 1(3): 5-8.

Macabago, S. A. B., Dagamac, N. H. A., dela Cruz, T. E. E. 2010. Diversity and distribution of plasmodial myxomycetes (slime molds) from La Mesa Ecopark, Quezon City, Philippines. Biotropia 17: 51-61.

Macabago, S. A. B., dela Cruz, T. E. E., Stephenson, S. L. 2012. First records of myxomycetes from Lubang Island, Occidental Mindoro, Philippines. Sydowia 64: 109-118.

Macabago, S. A. B., Stephenson, S. L., dela Cruz, T. E. E. 2016. Diversity and distribution of myxomycetes in coastal and mountain forests of Lubang Island, Occidental Mindoro, Philippines. Mycosphere 7(1): 18-29.

Mackey, R. L. & Currie, D. J. 2000. A re-examination of the expected effects of disturbance on diversity. Oikos 88: 483-493.

Madelin, M. F. 1984. Myxomycetes, microorganisms and animals: a model of diversity in animal-microbial interactions. In: ANDERSON, J.N., RAYER, A.D.A. and WALTON, W.H. (eds.): Invertebrate-microbial Interactions, pp. 2-33. - Cambridge University Press, New York, USA.

McGill, B., Etienne, R., Gray, J., Alonso, D., Anderson, M., Kassa, H., Dornelas, M., Enquist, B., Green, J., He, F., Hurlbert, A., Magurran, A., Marquet, P., Maurer, B., Ostling, A., Soykan, C., Ugland, K. & White, E. 2007. Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. Ecology Letters 10: 995-1015.

Osman, R. W. 1977. The establishment and development of a marine epifaunal community. Ecological Monographs 47: 37-63.

Paine, R. T. & Levin, S. A. 1981. Intertidal landscapes: disturbance and the dynamics of pattern. Ecological Monographs 51: 145-178.

Pickett, S. T. A. & White, P. S. 1985. The ecology of natural disturbance and patch dynamics. - Academic press, London.

R Core Team. 2016. R: a language and environment for statistical computing. https://www.R-project.org (accessed 15 February 2016).

Reynolds, D. R. 1981. Southeast Asian myxomycetes II. Philippines. Philippine Journal of Biology 10 (2-3): 127-150.

Reynolds, C. S., Padisak, J. & Sommer, U. 1993. Intermediate Disturbance in the Ecology of Phytoplankton and the Maintenance of Species Diversity – a Synthesis. Hydrobiologia 249:183-188.

Rojas, C. & Stephenson, S. L. 2008. Myxomycete ecology along an elevation gradient on Cocos Island, Costa Rica. Fungal Diversity 29:117-127.

Rojas, C. & Stephenson, S. L. 2013. Effect of forest disturbance on myxomycete assemblages in the southwestern Peruvian Amazon. Fungal Diversity 59: 45-53.

Rojas, C., Morales, R. E., Calderon, I. & Clerc, P. 2013. First records of myxomycetes from El Salvador. Mycosphere 4(6): 1042-1051.

Rojas, C., Valverde, R. & Stephenson, S. L. 2015a. New additions to the myxobiota of Costa Rica. Mycosphere 6(6): 709-715.

Rojas, C., Zuniga, J. M. & Stephenson, S. L. 2015b. Ecological niche modeling of some Costa Rican myxomycetes. Current Res. Env. App. Mycol. 5(2): 153-159.

Schnittler, M. 2001. Ecology of myxomycetes from a winter-cold desert in western Kazakhstan. Mycologia 93: 653–669.

Schnittler, M. & Novozhilov, Y. K. 1995. The myxomycetes of boreal woodlands in Russian northern Karelia: a preliminary report. Karstenia 36: 19-40.

Schnittler, M. & Stephenson, S. L. 2000. Myxomycete biodiversity in four different forest types in Costa Rica. Mycologia 92: 626-637.

Schnittler, M., Novozhilov, Y. K., Shadwick, J. D. L., Spiegel, F. W., García-Carvajal, E., König, P. 2015. What substrate cultures can reveal: Myxomycetes and myxomycete-like organisms from the Sultanate of Oman. Mycosphere 6(3): 356-384.

Shea, K., Roxburgh, S. H. & Rauschert, E. S. J. 2004. Moving from pattern to process: coexistence mechanisms under intermediate disturbance regimes. Ecology Letters 7: 491-508.

Sousa, W. P. 1984. The role of disturbance in natural communities. Annual Review of Ecology and Systematics 15: 353-391.

Stephenson, S. L. 1988. Distribution and ecology of Myxomycetes in temperate forests. I. Patterns of occurrence in the upland forests of southwestern Virginia. Can. J. Bot. 66: 2187-2207.

Stephenson, S. L. 1989. Distribution and ecology of myxomycetes in temperate forests. II. Patterns of occurrence on bark surface of living trees, leaf litter, and dung. Mycologia 81: 608-621.

Stephenson, S. L., Kalyanasundaram, I. & Lakhanpal, T. 1993. A comparative biogeographical study of myxomycetes in the mid-appalachians of eastern North America and two regions of India. Journal of Biogeography 20: 645-657.

Stephenson, S. L. & Stempen, H. 1994. Myxomycetes A Handbook of Slime Molds. - Timber Press Inc., USA.

Stephenson, S. L., Urban, L. A., Rojas, C. & McDonald, M. S. 2008. Myxomycetes associated with woody twigs. Revista Mexicana de Micologia 27: 21-28.

Takahashi, K. 2013. Myxomycete distribution varies among leaf litter of different vegetation in a local secondary forest of warm-temperate western Japan. Mycoscience 54: 368–377.

Tembhurne, R. R., Nanir, S. P. & Nasarin, S. 2013. The four new species of Myxomycetes of South-East Maharashtra, India. Int J Med Biomed Res 2(1): 36-39.

Tran, H. T. M., Stephenson, S. L., Hyde, K. D. & Mongkolporn, O. 2006. Distribution and occurrence of myxomycetes in tropical forests of northern Thailand. Fungal Diversity 22: 227-242.

Tran, D. Q., Nguyen, H. T. N., Tran, H. T. M. & Stephenson, S. L. 2014. Myxomycetes from three lowland tropical forests in Vietnam. Mycosphere 5(5): 662- 672.

Viray, A. T., Rotap, D. D. S., Migraso, L. L., Sibbaluca, N. C. I., Escobar, E. C. V., Buaya, A. T. & dela Cruz, T. E. E. 2014. Occurrence and diversity of myxomycetes (slime molds) in Polillo Island, Quezon Province, Philippines. Acta Manilana 62: 9-17.

Whittaker, R. H. 1965. Dominance and diversity in plant communities. Science 147: 250–260.

Wilson, J. B. 1991. Methods for fitting dominance/diversity curves. J. Veg. Sci. 2: 35-46.

Wrigley de Basanta, D., Stephenson, S.L., Lado, C., Estrada-Torres, A. & Nieves-Rivera, A.M. 2008. Lianas as a microhabitat for myxomycetes in tropical forests. Fungal Diversity 28: 109-125.

Tables

Table 1. Description and geographic coordinates of the study sites in the Bohol Islands.

Site name	Vegetation type	Latitude	Longitude	Landmass location
Chocolate Hill	Grassland	N9°48'48.1"	E124°10'22.1"	mainland Bohol
Carmen forest patch	Indigenous woodland, mixed forest patch	N9°47'38.9"	E124°10'17.3"	mainland Bohol
Mahogany forest	Monotypic man-made plantation	N9°39'51.3"	E124°04'30.3"	mainland Bohol
Rajah Sikatuna National Park	Heterogeneous natural forest	N9°41'50.1"	E123°57'54.3"	mainland Bohol
Panglao Island	Coastal mixed forest	N9°32'48.8"	E123°45'10.0"	satellite peninsula
Virgin Island	Reef woodland	N9°33'34.5"	E123°43'14.5"	satellite island/reef
Balicasag Island	Islet mixed forest	N9°31'1.9"	E123°41'4.9"	satellite island

Table 2. Statistics of individual-based species accumulation curves for the entire study area (Bohol Islands) and the different study sites showing numbers of taxa, records, and rarefied species, the values for expected species according to Chao1 estimation (mean ± SD) computed in EstimateS and the values for the measure of taxonomic diversity (S/G) of each site.

Site	Species found			Species expected			Taxonomic Diversity	
	Taxa	Records	Species Rarefaction	Chao 1 Mean	SD	Survey Completeness	Genera	S/G Ratio
Bohol Islands	54	308	12.1	95.9	25.9	56%	20	2.70
Heterogeneous natural	24	76	9.6	79.5	49.2	30%	14	1.71
Monotypic man-made	13	18	13.0	60.2	56.3	22%	9	1.44
Grassland	18	25	13.6	72.0	47.9	25%	13	1.38
Indigenous woodland	20	34	12.9	31.7	8.91	63%	11	1.82
Islet mixed	28	65	11.8	51.7	15.8	54%	10	2.80
Coastal mixed	34	84	12.5	69.7	23.6	49%	14	2.43

Figures

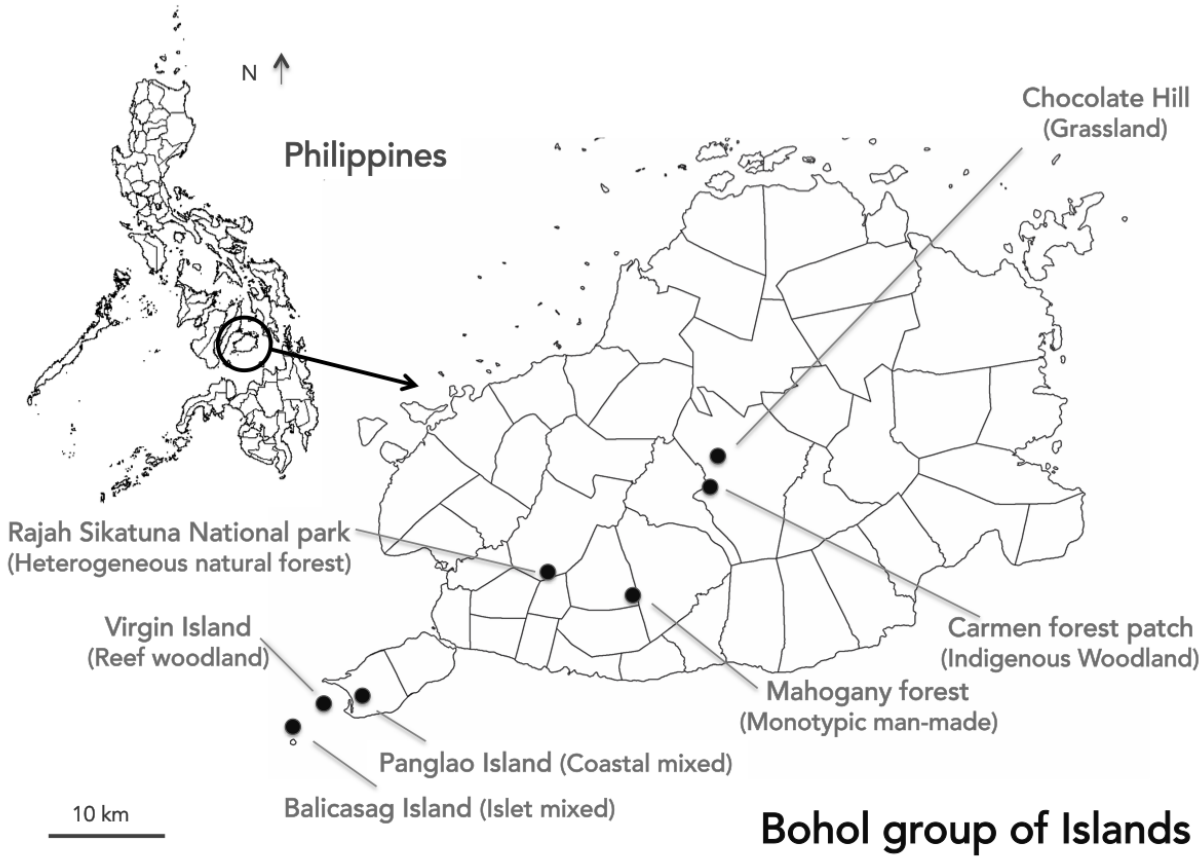

Figure 1. Study area in Bohol Islands, Philippines, with specific study sites marked by black dots (illustration modified from maps generated by DIVA-GIS).

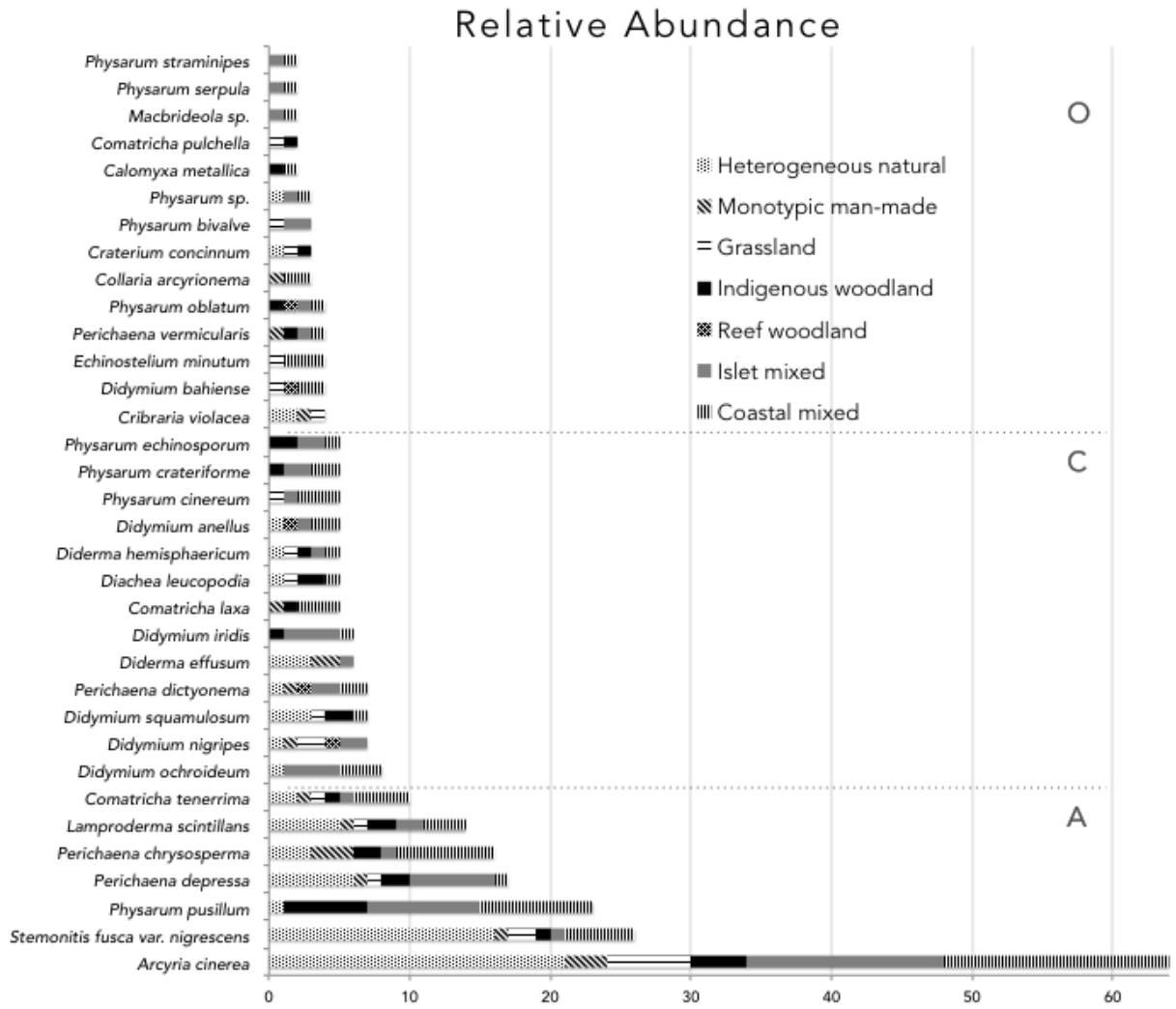

Figure 2. Occurrence of myxomycetes and their respective abundance indices (A = abundant, C = common, O = occasional) based on the number of records in the different study sites. Rare species (20) are excluded from this graph.

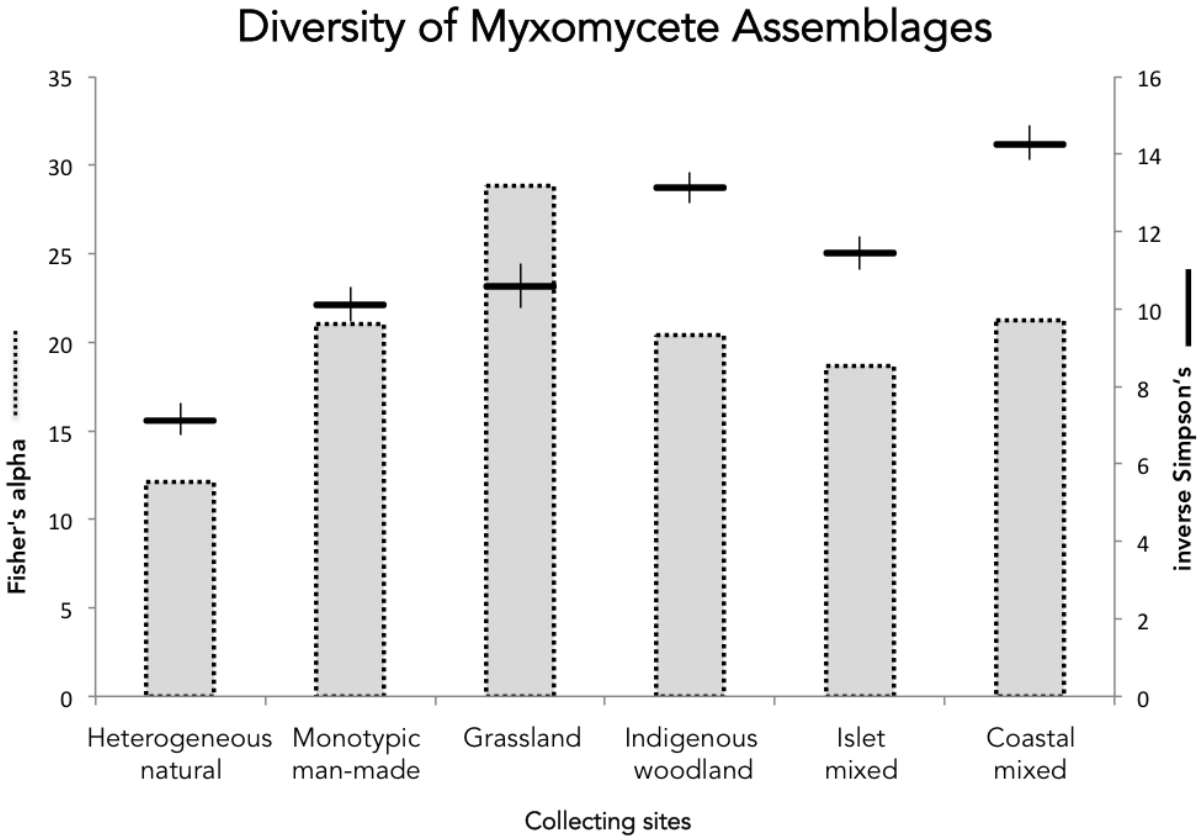

Figure 3. The α diversity in the six study sites. Dotted gray bars represent Fisher's alpha index of diversity; horizontal black lines show the inverse of Simpson's index of diversity, with its upper and lower 95% confidence interval indicated by thin vertical lines across as computed by SPADE.

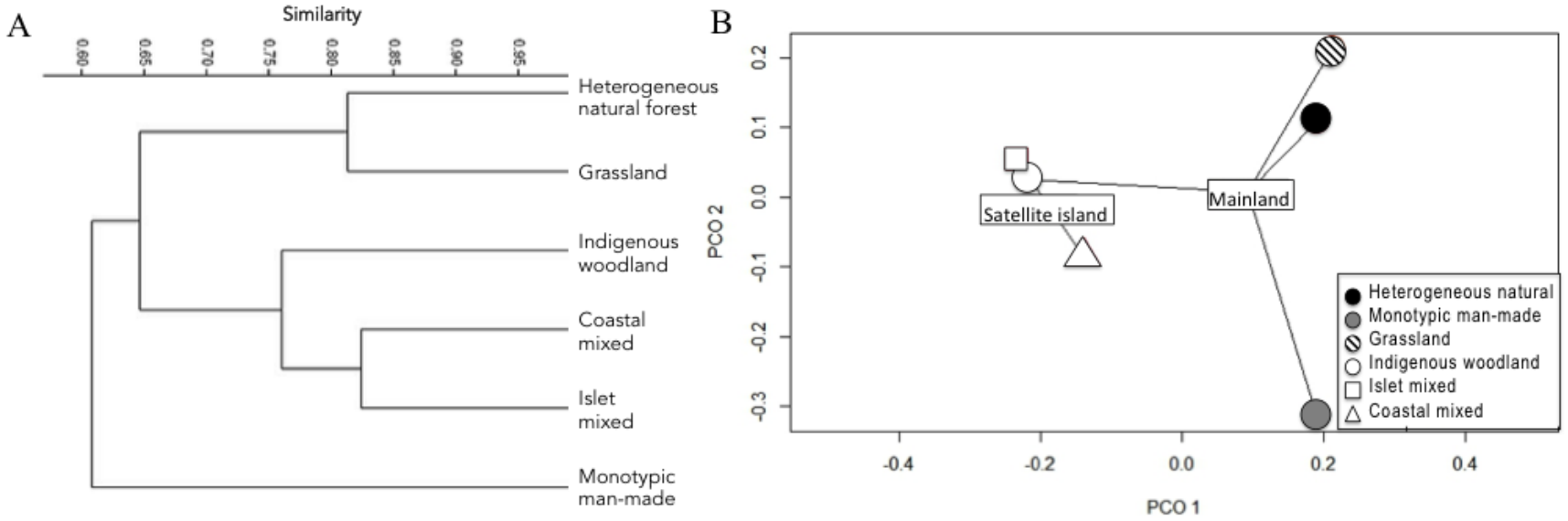

Figure 4. Clustering (A) and ordination (B) analyses showing β diversity investigation of the study area using the Morisita index for similarity of myxomycete communities between collecting sites and Principal coordinate analysis (PCoA) using the Bray Curtis index of dissimilarities of myxomycete abundance records, respectively.

III. Myxomycetes of the Caramoan Islands and an update on the species found in the Bicol Peninsula in the Philippines

Abstract

The main objective of this study was to characterize the assemblages of myxomycetes on isolated Philippine islands by way of a correlational study relating to the geographical and ecological distance in the Caramoan Islands, with an updated checklist of the myxomycetes of the Bicol Peninsula in the second section. Four islands of varying sizes and distances from each other, but all within relatively close proximity to the main island of the Bicol Peninsula, were chosen from among the Caramoan group of islands. A combination of traditional and more contemporary ecological tools was used to analyze diversity indices among and between the islands. Among the four islands, Matukad Island recorded the highest species richness (46.8) and taxonomic diversity index (2.6), while ranking next to Lahos island in terms of species diversity (7.9). Pairwise comparisons using community similarity indices and clustering analysis consistently showed that Lahos and Matukad are the most similar to each other, while also being closer to each other but situated farthest from the mainland. On the other hand, the two smallest islands, which were also closest to each other and to the mainland, grouped together using clustering analysis but recorded the lowest pairwise percentage similarity value. The ecological patterns in this study appear to follow the unified neutral theory of biodiversity and biogeography more than the insular biogeography theory. In addition, this study adds 16 new morphospecies to the list of myxomycetes known from the Bicol Peninsula, which brings the total to 73, including one new record for the country (*Lamproderma arcyrioides*).

Introduction

In the Philippine archipelago, there have been a number of studies conducted on myxomycetes- a group of eukaryotic fruiting amoebozoans. Some of these are listings published to summarize the records of myxomycetes in certain time periods, while the more recent ones incorporated more information and considered associations with certain ecological phenomena or factors. In the recent decade, myxomycete research was conducted in Bohol Islands (Macabago et al. 2017), Palawan Island (Pecundo et al. 2017), parts of Mindoro (Dagamac et al. 2015a) and Negros Islands (Alfaro et al. 2015), Lubang Islands (Macabago et al. 2016, 2012), the Anda and Hundred Islands in Pangasinan Province (Kuhn et al. 2013, dela Cruz et al. 2011), and the Polilio Islands in Quezon (Viray et al. 2014). In the present study, an effort was made to collect myxomycetes from the Caramoan group of islands, which is comprised of more than 20 islets and islands that are both inhabited and uninhabited and are located in the eastern part of the Philippines facing the Pacific Ocean (Fig. 1). These islands have a type II climate and is characterized as to having no distinct dry season with maximum rainy period from November to April associated with the northeast monsoon (Basconcillo et al. 2017). This group of islands belongs to the municipality of Caramoan in the province of Camarines Sur that lies on the Bicol Peninsula (Fig. 6). Much of which is mountainous and dominated by volcanoes and tablelands, the Bicol Peninsula is situated in the southeastern part of Luzon Island (Linis 2013).

The major objective of the study herein was to conduct a rapid survey of the myxomycetes associated with forest floor debris in limestone forests located in nearby islands. The main query was to determine if it was possible to relate geographical distances to differences in the assemblages of myxomycetes, their distribution, and their diversity.

The second part of this research aimed to provide an update to the myxomycetes of the Bicol Peninsula following the study by Dagamac et al. (2017) and to the current records of myxomycetes from the Philippines (Dagamac & dela Cruz 2015, 2019).

Materials and Methods

***Study area, collection protocols, setup of moist chamber cultures, and determination of myxomycetes**:* The samples collected for this study were obtained in 2013 on selected islands/islets of the Caramoan group of islands in the Philippines (Fig. 1), which is located on the eastern part of the archipelago and opening up to the Pacific Ocean. The documentation of vegetation in the Caramoan Islands is still in its infancy. However, during the collection some plant species and other vegetation were spotted in the collection areas, e. g. trees, such as Narra (*Pterocarpus indicus*), Molave (*Vitex*), Ipil (*Intsia bijuga*), and other dipterocarp trees, bamboos, and some shrubs, grasses, and mangroves in some of the beach forests. Some of these plants were also noted in a study by Balete et al. (2013) in the central and eastern portion of the Bicol Peninsula within mainland Caramoan. A combination of mostly dry and a few damp ground leaf litter (GL, 57) and twigs (TW, 83) were randomly collected along the coastal forests and woodlands from selected accessible islands of varying sizes that were all characterized by the presence of limestone (Fig. 1, Table 1). There were no evident layers of litter on the microsites. Field specimens that have developed under normal conditions were not collected given the fact that field collections are only available if conditions have been suitable for myxomycetes to fruit, but the moist chamber culture approach is effective regardless of recent conditions in the study sites. All samples were air-dried at the Research Center for the Natural and Applied Sciences of the University of Santo Tomas in Manila, Philippines for several weeks until there was no evidence of moisture on either the samples

or the bags. These samples were then sent to the Department of Biological Sciences, University of Arkansas for processing and observation. Necessary permits from the United States Department of Agriculture (USDA) were obtained by one of the coauthors (SLS) prior to transport of substrata. A total of 140 samples were used to prepare moist chamber cultures following the protocol described by Stephenson and Stempen (1994). Overall taxonomic treatment followed Martin & Alexopoulos (1969), with consideration of taxonomic changes that have been made since then, using the morphological species concept by noting characteristics of the fruiting body and spore morphologies, Nomenclature followed the nomenclatural information system of Eumycetozoa (Lado 2005-2020).

Exhaustiveness of the survey*:* The software program EstimateS (Version 9.1, Colwell 2013, 100 randomizations) was used to construct species accumulation curves (SAC) to evaluate the completeness of our survey for (1) each of the study sites that were differentiated in terms of relative island size and distances among each other, and (2) the pooled dataset from all four study sites using a species-record input file. The results of the Chao 1 estimator, an estimator for target richness for individual-based data such that one record of a species in a certain culture is considered as one individual (*sensu* Stephenson 1988), were then used to calculate the percentage exhaustiveness by dividing the actual number of species recorded by the mean number of species expected as estimated by the Chao 1 estimator.

Analysis of myxomycete assemblages*:* To examine the dataset obtained from the records of myxomycetes appearing in the moist chamber cultures, various software and abundance-based species data were used for the evaluation of species richness, diversity, and degree of composition. Here, a moist chamber positive for a particular species of myxomycetes is considered as one collection or record.

The relative abundance of each species was obtained by dividing the total number of collections for each species of myxomycetes by the total number of myxomycetes collected (Stephenson et al. 1993). The values were then translated to an abundance index described by Stephenson et al. (1993), for which species < 0.5% of the total number of collections are defined as rare; species > 0.5% but < 1.5% of the total number of collections defined as occasional; species > 1.5% but < 3% of the total number of collections defined as common; and species > 3% of the total number of collections defined as abundant.

To show the distribution of myxomycetes collected among and the density in each island a heat map was constructed using the software ArcGIS Pro, where a sparse to high density is represented by a range of cool to warm color schemes, respectively, such that the warmer the color in an area the more myxomycete occurrences were recorded.

Pairwise comparisons of myxomycete assemblages were carried out using Sorensen's coefficient of community (CC) and percentage similarity (PS) indices as described by Stephenson (1989). The coefficient of community (CC) index is based on the presence or absence of species in the two communities being compared. In contrast, the percentage similarity (PS) index considers not only the presence or absence of a species but also its relative abundance. The CC and PS values range from 0 to 1. The higher the value, the more similar the communities are in terms of their species composition and abundance. In order to check if there is a correlation between geographic and ecological distances (using PS and CC values), a statistical test using the Mantel test (Hood 2010) was conducted.

The taxonomic diversity Index (TDI), which is also known as the S/G ratio, was calculated by obtaining the ratio of the number of species to the number of genera. The value of this ratio was inversely proportional to the taxonomic diversity (Stephenson et al. 1993), where the lower ratio

indicates a more diverse biota.

The online software Species-Richness Prediction and Diversity Estimation with R (SpadeR) (Chao et al. 2016) was used to generate the maximum likelihood estimator for the more intuitive Inverse Simpson index, which is a heterogeneous measure of species diversity that accounts for both the species richness and evenness, and Chao 1-bc estimator, a bias-corrected form of Chao 1, for species richness. In addition, to visualize the patterns of species composition in the different islands, a clustering analysis was performed based on the Bray-Curtis dissimilarities of myxomycete abundance records in the R environment.

Results and Discussion

The myxomycetes identified from fruiting bodies obtained from moist chamber cultures accounted for a total of 38 different morphospecies. Approximately 76% of the moist chamber cultures produced fruiting bodies after 12 weeks of observation, with ground leaf litter (86%) being more productive than twigs (69%). Among the islands, Matukad Island (II) yielded the highest productivity (92%), followed by Lahos (I) Island (79%), Busdak (IV) Island (72%), and Minalahos (III) Island (42%).

The entire study area showed 46% survey exhaustiveness using EstimateS (Colwell 2013). This value is lower than Palawan Island (92%, Pecundo et al. 2017) and Lubang Island (96%, Macabago et al. 2016), albeit comparable to studies that have been carried out in Mindoro Island (47%, Dagamac et al. 2015), in Bohol Islands (56%, Macabago et al. 2017), and in other parts of Bicol Peninsula (58%, Dagamac et al. 2017). The specific survey completeness for each island in Caramoan ranged from 50-68% (Fig. 2), where Lahos recorded the lowest and Minalahos the highest. The actual and expected numbers of taxa found are shown on Table 1.

Among the 38 morphospecies identified, six were found to be abundant, while nine were common, and six were occasional. Seventeen rare species not included in Fig. 3 were all singletons from three islands (I-III). These were eight species from Lahos Island (I): *Badhamia utricularis*, *Diachea leucopodia*, *Diachea splendens*, *Diachea subsessilis*, *Didymium nigripes*, *Perichaena microspora*, and *Physarum cinereum*; seven species from Matukad Island (II): *Badhamia macrocarpa*, *Badhamia* sp., *Dictydiaethalium plumbeum*, *Didymium minus*, *Lamproderma arcyrioides*, *Physarum didermoides*, and *Physarum echinosporum*; and two species from Minalahos Island (III): *Comatricha nigra* and *Diderma effusum*. The two collections only identified to the genus level (*Badhamia* sp. and *Physarum* sp.) had materials that were too poorly developed to identify beyond the level of species, but they were clearly different from any other species recorded in the same genus. Of the 17 singletons, nine were not recorded from mainland Bicol (see Dagamac et al. 2017 and Table 3).

The majority of the myxomycetes were found in microhabitats collected from Matukad Island as shown by the heat map on Figure 4. Heat maps illustrate the relative density of points or data, in this case a record of a collection, on a map as a dynamic visualization employing a scheme typically composed of gradually varying colors from cool-to-hot to indicate low-to-high density values, respectively. The heat map shows that Matukad had the richest and most abundant collection of myxomycetes (96) as indicated by the bright yellow center inside a red ring within a thin, blue circle, and was followed by Lahos, then Busdak, and Minalahos, with the smallest number of collections as shown by a faint red center inside a diffused blue ring. The trend in the ranking of the number of collections followed the same pattern as the highest number of different taxa found on each island, computed species richness, and taxonomic diversity (Table 1). A point to consider is that the larger islands showed more records of myxomycetes, which could be

correlated to the number of samples collected in each island. It is also noteworthy that the farther islands (Matukad and Lahos) from the mainland revealed the higher occurrence density.

In terms of taxonomic diversity (TDI), Table 1 shows that the highest value was recorded for Minalahos (1.4) and the lowest was for Matukad (2.6), which means that although there was a higher number of morphospecies (26) found on Matukad, the variety of species on Minalahos (10) and the other two islands (Lahos with 22 and Busdak with 11) were distributed among more genera. It is clear, though, from the study that proximity of the islands to each other does not necessarily show similar number of species as also observed in the islands and islets surveyed in Hundred Islands, Pangasinan (dela Cruz et al. 2011). It is to be emphasized that this study only utilized morphological data assessment, and so the interpretation of the results has been conducted with caution considering that our survey is solely based on the fruiting propensity of the myxomycetes.

The α diversity in each collecting site was calculated using Chao 1-bc and Simpson's diversity indices (Table 1). Species richness was measured using the Chao 1-bc estimator as it is the bias-corrected form of the lower bound of species richness estimator referred to as Chao 1 (Colwell & Coddington 1994). Chao and Chiu (2016) expressed that it seems statistically impossible to get a good estimate of species richness when there are many undetectable species in a highly diverse collection. They elaborated that compared to a seemingly uncertain point estimate, an accurate lower bound for species richness is more practical to use. As such, this lower bound of undetected species richness, in terms of the numbers of singletons and doubletons (Chao 1984; 1987), was intuitively used in this study. Among the four islands, Lahos was found to be the most species diverse (11.1) and the second most species rich (43.7) next to Matukad. Consistently Minalahos and Busdak exhibited the two least species diverse and species rich islands. If we will

use this observation in reference to proximity with results showing different patterns of species composition and species diversity for studies in islands conducted so far in the Philippine archipelago, these seem to be defying the insular biogeography model. Perhaps, the capability of long-distance dispersal among myxomycetes possibly holds true as was also implied in another insular habitat study in Bohol island (Macabago et al 2017). The only thing that still seems to not be clearly showcased is how capable these spore-forming organisms (like myxomycetes) are in terms of dispersal. This topic, in itself, is an interesting field for myxomycete ecology exploration.

Ecological distances among the four islands were examined using clustering analysis and pairwise comparisons using community similarity indices. The Bray-Curtis index of dissimilarity using species abundance records in the R environment indicated that the assemblages of myxomycetes are more similar for Lahos and Matukad, and in Minalahos and Busdak (Fig. 5) in a pairwise manner. This was somewhat supported by the community similarity indices for percentage similarity (PS) and Sorensen's coefficient of community (CC), as shown in Table 2. An interesting observation was that Minalahos and Busdak were the closest to each other (420 m) and also the two smallest islands; however, the PS was recorded to be the lowest (4.5%) pairwise within the islands. Lahos and Matukad, the two largest islands and relatively close to each other (see Table 2), consistently recorded the highest community similarity for both PS and CC values. Such pattern of β diversity on collection sites that are in close proximity have been also observed on recent studies about forest patches with different degrees of disturbances in the Philippines (Bernardo et al., 2018) and in Vietnam (Redeña-Santos et al., 2018). However, to further ascertain the relationship of geographical distances with the species composition among the collection sites, Mantel test was performed. The calculation of the Mantel test analysis returned a positive correlation coefficient for both geographic and CC distances (0.001) and geographic and PS

distances (0.996) within the 95% confidence interval obtained for 999 permutations. On a statistical point of view these indicate the absence of correlation between geographic and ecological distances. If geographic proximity does not dictate the ecological similarities of these insular environments, then some other factors may play a more important role. However, without evidence regarding the roles of other factors, such as the different substrates or vegetation types present, on the differences in species diversity and composition, that would be another speculation.

A more likely explanation of these data seems to fit the unified neutral theory of biodiversity and biogeography (Hubbell 2001). This was essentially a hypothesis aiming to explain the diversity and relative abundance of species in communities and implying that biodiversity arises at random due to random birth and death events, species turnover due to local extinction and migration, and large-scale changes due to speciation, dispersal, and global extinction (Jetschke 2002). This theory essentially states that complex ecological interactions and characteristics happen, but every individual of each species in an area are per capita equivalents in a trophic community, therefore assuming neutrality. Accordingly, the island biogeography or insular biogeography theory (MacArthur & Wilson 1967), which is commonly used as an explanation for some studies to see patterns of ecological communities in islands, this current study using myxomycetes as model species seemingly defy this theory. The theory of insular biogeography suggests that species richness in an undisturbed insular habitat is shaped by migration and extinction, such that the movements of individuals into and out of the population are affected by the distance of an island from a source of colonists, where the source is typically the mainland, but it can also be other islands. Furthermore, the rate of extinction once a species colonizes an island is affected by island size, such that bigger islands have larger areas and offer more opportunities for a variety of habitat, and therefore reduces the probability of extinction due to chance.

Differences in habitats also increase the number of species that will be successful after immigration, therefore achieving equilibrium in the end. Following this logic, more isolated islands like Matukad and Lahos are less likely to receive immigrants (in this case spores or other forms of myxomycetes that could potentially survive and thrive). This could be explained with the assumption that from the main island two islands of similar sizes but have different distances will have different species richness. The island that is closer in proximity to the main island is intuitively going to receive higher random dispersion hence, higher richness (and therefore likely higher species diversity) in comparison to an island that is farther from the main island, like Matukad and Lahos. This is obviously not the case for this study such that the two least species diverse and species rich islands (Minalahos and Busdak) are the nearest to the main land. More so, based on the principle that two equidistant islands from the main island but different island size will also affect the random dispersion pattern, the smaller islands can only accommodate limited species in comparison to a bigger landscape that can have wider area to be occupied by any organism, given that it satisfies the condition of neutrality. The unified neutral theory shows the value of dispersal limitation, speciation, and ecological drift in the natural world and therefore presents models for evaluating the role of natural selection and adaptation in general. Interestingly, this observed pattern can be augmented using metadata from myxomycetes studies conducted in many other islands in the Philippines.

The Bicol Peninsula constitutes four provinces (Camarines Norte, Camarines Sur, Albay, and Sorsogon) covering about 12, 070 km^2 of land in the eastern part of the Philippines (Fig. 6). A rapid assessment of myxomycetes in the peninsula conducted by Dagamac et al. (2017) recorded 57 morphotaxa of myxomycetes collected from seven sites within the four provinces.

The present study adds new records of myxomycetes to the province of Camarines Sur, which geographically and politically includes the Caramoan group of islands, and one new record for the country. Among the 38 morphospecies of myxomycetes recorded, 16 were new to the Bicol peninsula excluding two morphotaxa that were only identified to the genus level and *Stemonitis fusca* var. *nigrescens* which is a synonym of *Stemonitis fusca*. These are *Badhamia macrocarpa, Badhamia utricularis, Comatricha laxa, Diachea splendens, Diachea subsessilis, Dictydiaethalium plumbeum, Didymium anellus, Didymium iridis, Didymium minus, Didymium ochroideum, Fuligo cinerea, Lamproderma arcyrioides, Perichaena microspora, Physarum crateriforme, Physarum gyrosum,* and *Physarum straminipes* (Table 3). Twenty morphospecies were shared with the mainland. This now brings the updated records of myxomycetes for the Bicol Peninsula to 73 (Table 3), collected from 11 sites encompassing the study by Dagamac et al. (2017) and the present study. The species *Lamproderma arcyrioides* is a new record for the country, which now also brings the total number of records to 162 for the Philippines.

Conclusions and recommendations

Philippines, being archipelagic in nature, is an ideal model to test different island biogeography hypothesis for myxomycetes. Surprisingly at a small-scale level as shown in this study, it seems that myxomycetes follows the unified neutral theory of biodiversity and biogeography model instead of the insular biogeography theory. Long-term studies, wherein diversity studies of myxomycetes from other widespread remote local islands in the Philippines are included, would be exciting future directives to explore and disentangle biogeographic patterns of slime molds in the context of island biogeography.

Literature Cited

Alfaro, J.R.D., D.L.I.M. Alcayde, J.B. Agbulos, N.H.A. Dagamac & T.E.E. dela Cruz, 2015. The occurrence of myxomycetes from a lowland montane forest and agricultural plantations of Negros Occidental, Western Visayas, Philippines. FineFocus, 01: 07–20.

Balete, D.S., L.R. Heaney, P.A. Alviola, & E.A. Rickart, 2013. Diversity and distribution of small mammals in the Bicol Volcanic Belt of southern Luzon Island, Philippines. National Museum of the Philippines: Journal of Natural History, 1: 61-86.

Basconcillo, J., G. Duran, A. Francisco, R. Abastillas, F. Hilario, E. Juanillo, A. Solis, A. Lucero & S. L. Maratas, 2017. Evaluation of Spatial Interpolation Techniques for Operational Climate Monitoring in the Philippines. Scientific Online Letters on the Atmosphere, 13: 114–119.

Bernardo, J.L.M., J.L.Q. Arioder, K.J. Almadrones-Reyes & N.H.A. Dagamac, 2018. Myxomycetes communities occurring in fragmented forest patches in two municipalities of Laguna, Philippines. Community Ecology, 19: 289–299.

Chao, A., 1984. Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics, 11: 265–270.

Chao, A., 1987. Estimating the population size for capture-recapture data with unequal catchability. Biometrics, 43: 783–791.

Chao, A. & C. H. Chiu, 2016. Species richness: estimation and comparison. In: Balakrishnan, N., T. Colton, B. Everitt B, W. Piegorsch, F. Ruggeri, J. Teugels, (ed.), Wiley StatsRef: Statistics Reference Online. John Wiley & Sons, Hoboken, New Jersey. pp. 1–26.

Chao, A., K. H. Ma, T. C. Hsieh & C. H. Chiu, 2015. Online Program SpadeR (Species-richness Prediction And Diversity Estimation in R). Program and User's Guide published at http://chao.stat.nthu.edu.tw/wordpress/software_download/.

Colwell, R.K. & J. A. Coddington, 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical transactions of the Royal Society of London, Series B, 345: 101–118.

Colwell, R. K., 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 7. User's Guide and application; http://purl.oclc.org/estimates.

Dagamac, N. H. A. & T. E. E. dela Cruz, 2015. Myxomycete research in the Philippines: Updates and opportunities. Mycosphere, 6: 784–795.

Dagamac, N. H. A. & T. E. E. dela Cruz, 2019. The Philippine slime molds after Dogma's 1975 list–How far have we been?. Philippine Journal of Systematic Biology, 13: 58–65.

Dagamac, N. H. A., M. A. D. Rea-Maminta, N. S. Batungbacal, S. H. Jung & C. R. T. Bulang, A.G. R. Cayago & T. E. E. dela Cruz, 2015a. Diversity of plasmodial slime molds

(myxomycetes) on coastal, mountain, and community forests of Puerto Galera, Oriental Mindoro. Philippines. Journal of Asia-Pacific Biodiversity, 8: 322–329.

Dagamac, N.H.A., M. A. D. Rea-Maminta & T. E. E. dela Cruz, 2015b. Plasmodial slime molds of a tropical karst forest, Quezon National Park, Philippines. Pacific Science, 69: 411–422.

Dagamac, N. H. A., T. E. E. dela Cruz, M. A. D. Rea-Maminta, J. V. Aril- dela Cruz & M. Schnittler, 2017. Rapid assessment of myxomycete diversity in Bicol Peninsula. Nova Hedwigia, 104: 31–46.

Dela Cruz, T. E. E., R. V. Kuhn, A. O. M. Javier, C. P. Rodillas, C. M. Parra, L. H. M. Corpuz & R. D. McHugh, 2011. Occurrence and distribution of plasmodial myxomycetes in Hundred Islands National Park, Pangasinan, Philippines. Acta Manilana, 59: 65–74.

Hood, G. M., 2010. PopTools version 3.2.5. Available on the internet [http://www.poptools.org (accessed last July 1, 2020).

Hubbell, S. P., 2001. The unified neutral theory of biodiversity and biogeography. Monographs in Population Biology, Volume 32. Princeton University Press, Princeton, New Jersey.

Jetschke, G., 2002. The Unified Neutral Theory of Biodiversity and Biogeography. Ecology, 83(6): 1771–1772.

Kuhn, R. V., A. O. M. Javier, C. P. Rodillas, C. M. Parra, L. H. M. Corpuz, A. T. Buaya & T. E. E. dela Cruz, 2013. Diversity of plasmodial myxomycetes from Anda Island, Pangasinan, Philippines. Biotropia, 20: 1–9.

Lado, C., 2005-2020. An online nomenclatural information system of Eumycetozoa. Real Jardín Botánico, CSIC. Madrid, Spain. http://www.nomen.eumycetozoa.com.

Macabago, S. A. B., T. E. E. dela Cruz & S. L. Stephenson, 2012. First records of myxomycetes from Lubang Island, Occidental Mindoro, Philippines. Sydowia, 64: 109–118.

Macabago, S. A. B., S. L. Stephenson & T. E. E. dela Cruz, 2016. Diversity and distribution of myxomycetes in coastal and mountain forests of Lubang Island, Occidental Mindoro, Philippines. Mycosphere, 7(1): 18–29.

Macabago, S. A. B., N. H. A. Dagamac, T. E. E. dela Cruz & S. L. Stephenson, 2017. Implications of the role of dispersal on the occurrence of litter-inhabiting myxomycetes in different vegetation types after a disturbance: a case study in Bohol Islands, Philippines. Nova Hedwigia, 104 (1-3): 221–236.

MacArthur, R. H. & E. O. Wilson, 1967. The Theory of Island Biogeography. Princeton University Press, Princeton, New Jersey.

Martin, G.W. & C.J. Alexopoulos, 1969. The Myxomycetes. University of Iowa Press, Iowa City, Iowa.

Pecundo, M.H., N. H. A. Dagamac, S. L. Stephenson & T. E. E. dela Cruz, 2017. First myxomycete survey in the limestone forest of Puerto Princesa Subterranean River National Park, Palawan, Philippines. Nova Hedwigia, 104 (1-3): 129–141.

Redeña-Santos, J.C., D.V. Thao, M. Schnittler & N. H. A. Dagamac, 2018. The first report of composition and occurrence of myxomycetes assemblages in protected and unprotected plantation forest: a comparative study in Thai Nguyen City, northern Vietnam. Plant Ecology and Evolution, 151: 231–240.

Stephenson, S. L., 1988. Distribution and ecology of myxomycetes in temperate forests. I. Patterns of occurrence in the upland forests of southwestern Virginia. Canadian Journal of Botany, 66: 2187-2207.

Stephenson, S. L., I. Kalyanasundaram & T. Lakhanpal, 1993. A comparative biogeographical study of myxomycetes in the mid-Appalachians of eastern North America and two regions of India. Journal of Biogeography, 20: 645-657.

Stephenson, S. L. & H. Stempen, 1994. Myxomycetes A Handbook of Slime Molds. Timber Press Inc., USA.

Viray, A. T., D. D. S. Rotap, L. L. Migraso, N. C. I. Sibbaluca, E. C. V. Escobar, A. T. Buaya & T. E. E. dela Cruz, 2014. Occurrence and diversity of myxomycetes (slime molds) in Polillo Island, Quezon Province, Philippines. Acta Manilana, 62: 9–17.

Tables

Table 1. Summary of data for the different islands (I-IV) within Caramoan group of islands, Camarines Sur showing the approximate sizes of islands, numbers of taxa, records, the values for species richness according to Chao1-bc estimator and species diversity according to Simpson's inverse index computed in SpadeR, and the values for the taxonomic diversity index (TDI) of each site.

Locality	Island size (m)	Records	Taxa found	Genera	TDI	Species Richness	Species Diversity
Caramoan Islands	-	201	38	14	2.7	83.1	10.2
Lahos (I)	415 x 190	64	22	9	2.4	43.7	11.1
Matukad (II)	560 x 320	96	26	10	2.6	46.8	7.9
Minalahos (III)	350 x 140	18	10	7	1.4	14.7	6.8
Busdak (IV)	60 x 37	23	11	6	1.8	19.9	3.9

Table 2. Community similarity using percentage similarity (PS) and Sorensen's coefficient of community (CC) indices and the approximate distances between two islands.

Sites	Approximate distance (m)	Percentage Similarity (*PS*)	Coefficient of Community (*CC*)
Lahos vs. Matukad	1300	17.9%	0.50
Lahos vs. Minalahos	2100	5.5%	0.31
Lahos vs. Busdak	2500	7.5%	0.42
Matukad vs. Minalahos	900	7.0%	0.39
Matukad vs. Busdak	1250	9.5%	0.49
Minalahos vs. Busdak	420	4.5%	0.48

Table 3. Updated list of the myxomycetes of Bicol Peninsula following the initial report of Dagamac et al. (2017) and including the 16 new morphospecies (marked by *) from this study for the Bicol Peninsula with the new record for the peninsula and the country as marked by **. "+" signifies a synonym of *Hemitrichia pardina* (Minakata) Ing; and "++" signifies a synonym of *Stemonitis fusca* var. *nigrescens* (Rex) Torrend.

Myxomycetes of the Bicol Peninsula	
Arcyria cinerea (Bull.) Pers.	***Fuligo cinerea*** (Schwein.) Morgan *
Arcyria cinerea var. ***digitata*** Schwein.	***Hemitrichia calyculata*** (Speg.) M.L. Farr
Arcyria cinerea, dwarf form (Bull.) Pers.	***Hemitrichia serpula*** (Scop.) Rostaf.
Arcyria cinerea, yellow form (Bull.) Pers.	***Lamproderma arcyrioides*** (Sommerf.) Rostaf. **
Arcyria denudata (L.) Wettst.	***Lamproderma scintillans*** (Berk. & Broome) Morgan
Arcyria globosa Schwein.	***Lycogala exiguum*** Morgan
Arcyria incarnata (Pers.) Pers.	***Perichaena chrysosperma*** (Currey) Lister
Arcyria insignis Kalchbr. & Cooke	***Perichaena depressa*** Libert
Badhamia affinis Rostaf.	***Perichaena dictyonema*** Rammeloo
Badhamia macrocarpa (Ces.) Rostaf. *	***Perichaena microspora*** Penz. & Lister *
Badhamia utricularis (Bull.) Berk. *	***Perichaena minor*** var. ***pardina*** (Minakata) Hagelst +
Ceratiomyxa fruticulosa var. ***fruticulosa*** (Müll.) T. Macbr.	***Perichaena pedata*** (Lister & G. Lister) G. Lister
Clastoderma debaryanum A. Blytt	***Perichaena vermicularis*** (Schwein.) Rostaf.
Collaria arcyrionema (Rostaf.) Nann.-Bremek. ex Ing	***Physarum album*** (Nees) Fr.
Comatricha fragilis Meylan	***Physarum bivalve*** Pers.
Comatricha laxa Rostaf. *	***Physarum cinereum*** (Batsch) Pers.
Comatricha nigra (Pers. ex J.F. Gmel.) Schroet.	***Physarum crateriforme*** Petch *
Comatricha pulchella (C. Bab. & Berk.) Rostaf.	***Physarum compressum*** Alb. & Schwein.
Comatricha tenerrima (M.A. Curtis) G. Lister	***Physarum decipiens*** M.A. Curtis
Craterium leucocephalum (Pers.) Ditmar	***Physarum didermoides*** (Pers.) Rostaf.
Cribraria microcarpa (Schrad.) Pers.	***Physarum echinosporum*** Lister
Cribraria violacea Rex	***Physarum globuliferum*** (Bull.) Pers.
Diachea bulbillosa (Berk. & Broome) Lister ex Penzig	***Physarum gyrosum*** Rostaf. *
Diachea leucopodia (Bull.) Rostaf.	***Physarum melleum*** (Berk. & Broome) Massee
Diachea splendens Peck *	***Physarum*** cf. ***oblatum*** T. Macbr.
Diachea subsessilis Peck *	***Physarum pezizoideum*** (Jungh.) Pavill. & Lagerh.
Dictydiaethalium plumbeum (Schumach.) Rostaf. *	***Physarum pulcherrimum*** Berk. & Ravenel in Berk.
Diderma effusum (Schwein.) Morgan	***Physarum pusillum*** (Berk. & M.A. Curtis) G. Lister
Diderma hemisphaericum (Bull.) Hornem	***Physarum stellatum*** (Massee) G.W. Martin
Didymium anellus Morgan *	***Physarum straminipes*** Lister *
Didymium floccosum G.W. Martin, K.S. Thind & Rehill	***Physarum superbum*** Hagelst.
Didymium iridis (Ditmar) Fr. *	***Physarum tenerum*** Rex
Didymium minus (Lister) Morgan *	***Stemonaria fuscoides*** Nann.-Bremek. & Y. Yamam.
Didymium nigripes (Link) Fr.	***Stemonitis flavogenita*** Jahn
Didymium ochroideum G. Lister *	***Stemonitis fusca*** Roth ++
Didymium squamulosum (Alb. & Schwein.) Fr. (Alb. & Schwein.) Fr.	***Stemonitis pallida*** Wingate
Didymium verrucosporum Welden	

Figures

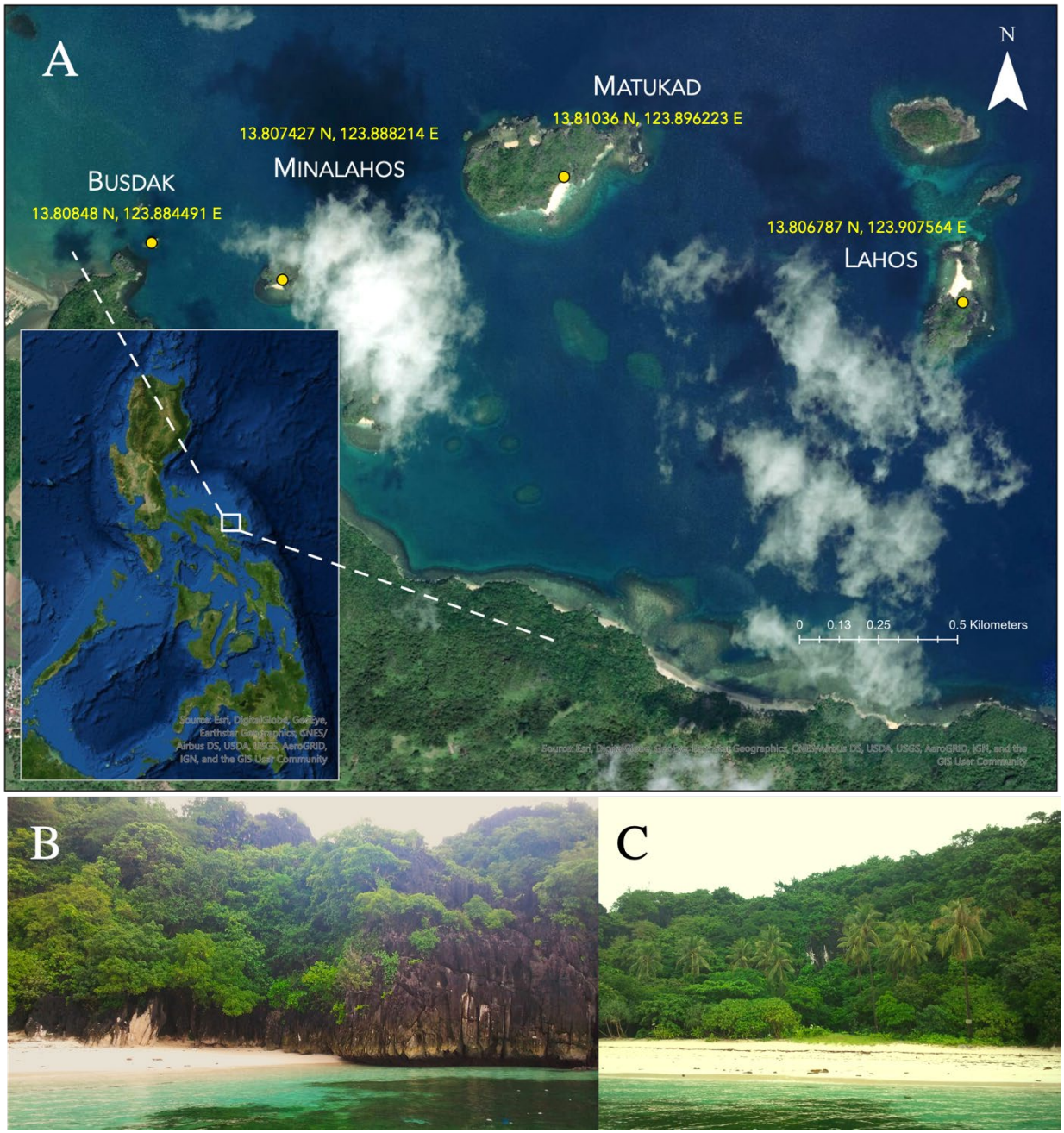

Figure 1. (A) Map of the collecting sites on the Caramoan Islands in the Philippines, showing the four selected islands marked with yellow dots. The inset on the lower left provides a map of the Philippines. (B, C) Some images of the island collection sites. Map generated using ArcGIS Pro utilizing the satellite imagery map settings.

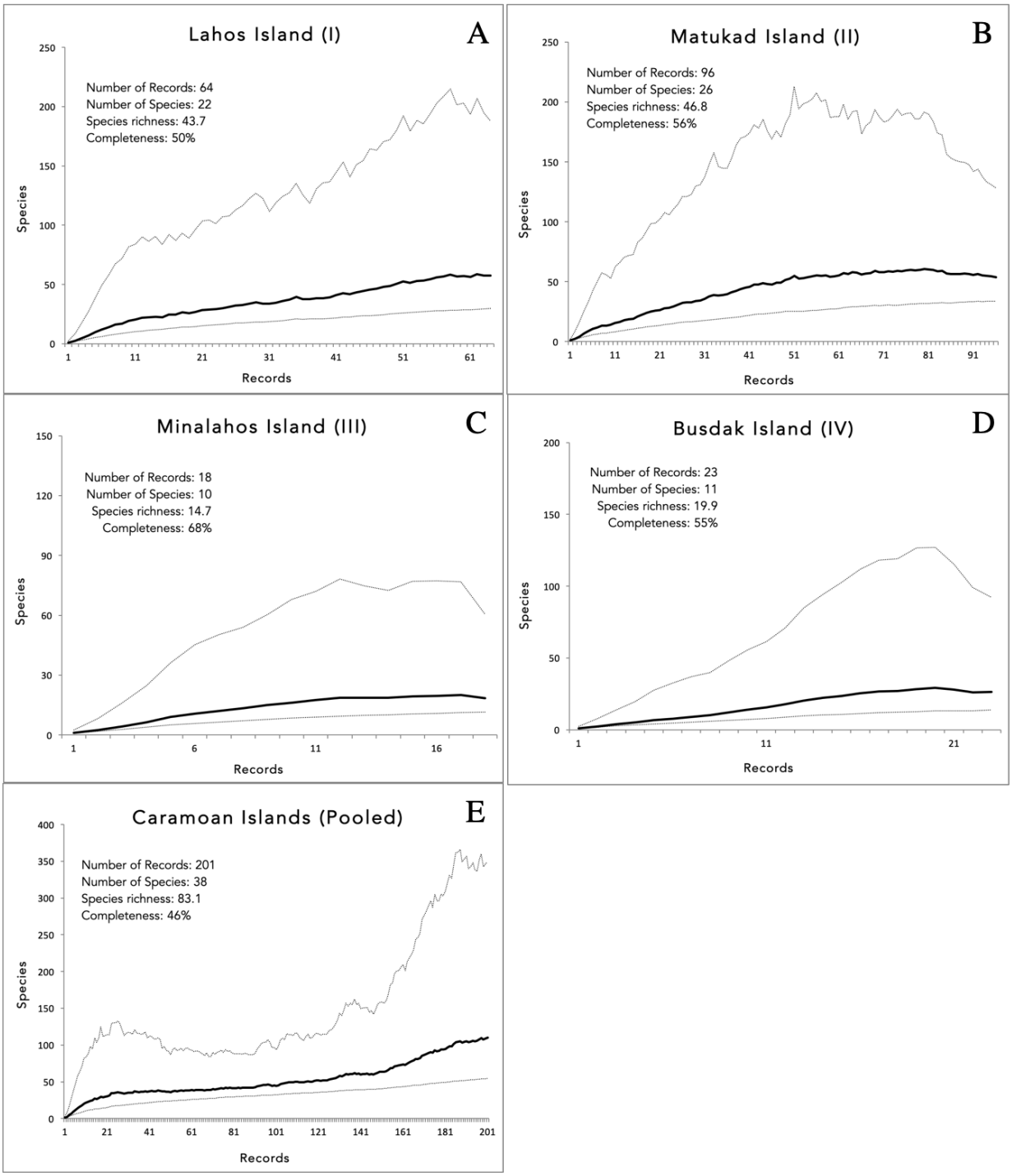

Figure 2. Species Accumulation Curves (SAC) for each of the four islands/islets (A-D) and for the pooled data set in Caramoan Islands (E).

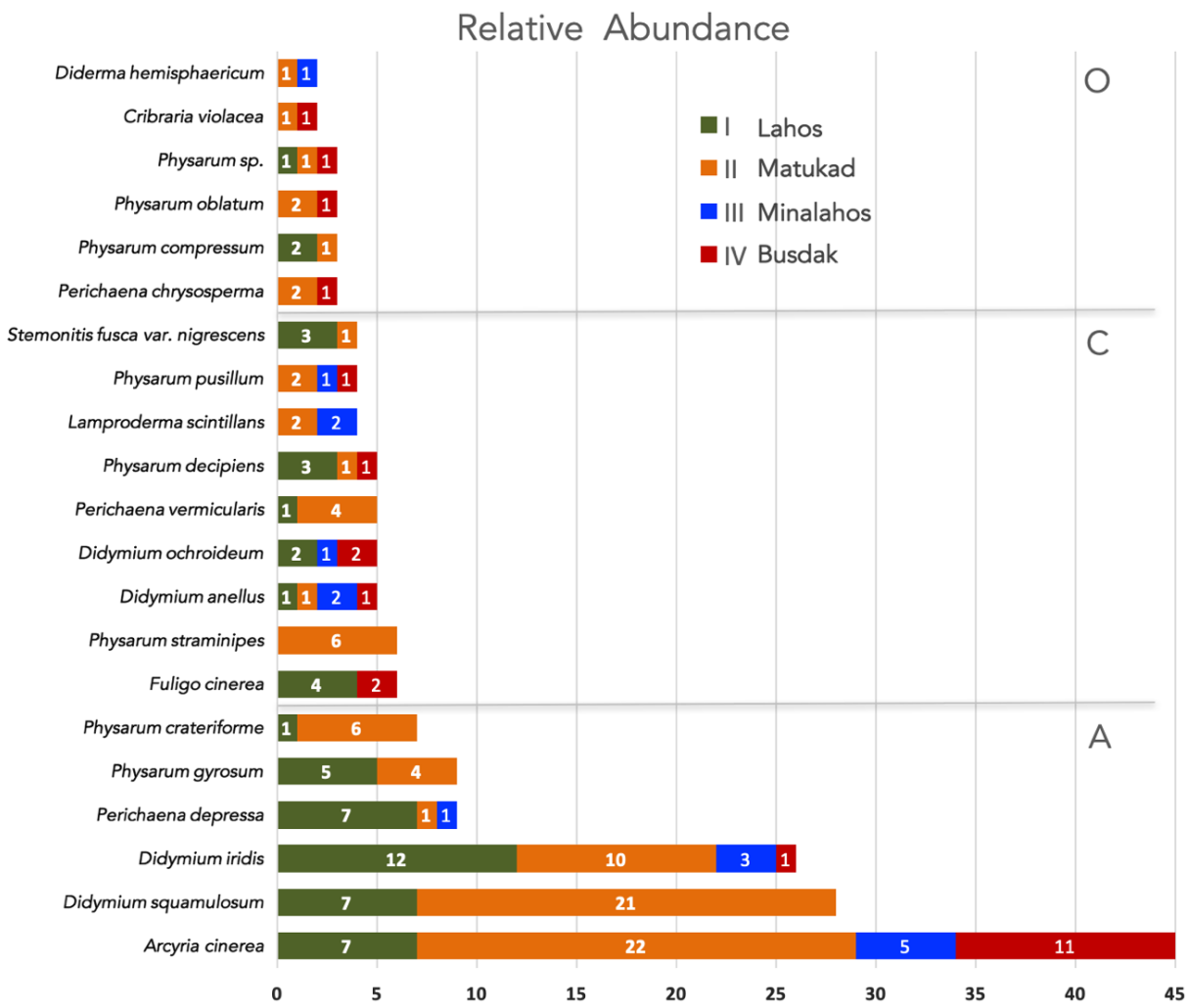

Figure 3. Relative abundance of myxomycete morphospecies collected in Caramoan islands showing the abundant (A), common (C), and occasional (O) species. Seventeen species were found to be rare and were not shown on the figure.

Figure 4. Density of myxomycete collections found in Caramoan group of islands showing the most records in Matukad as illustrated by the huge radius of yellow center within a red ring, followed by Lahos, Busdak, and Minalahos (faintest red core within a washed blue ring), respectively. Map generated using ArcGIS Pro utilizing the satellite imagery map settings.

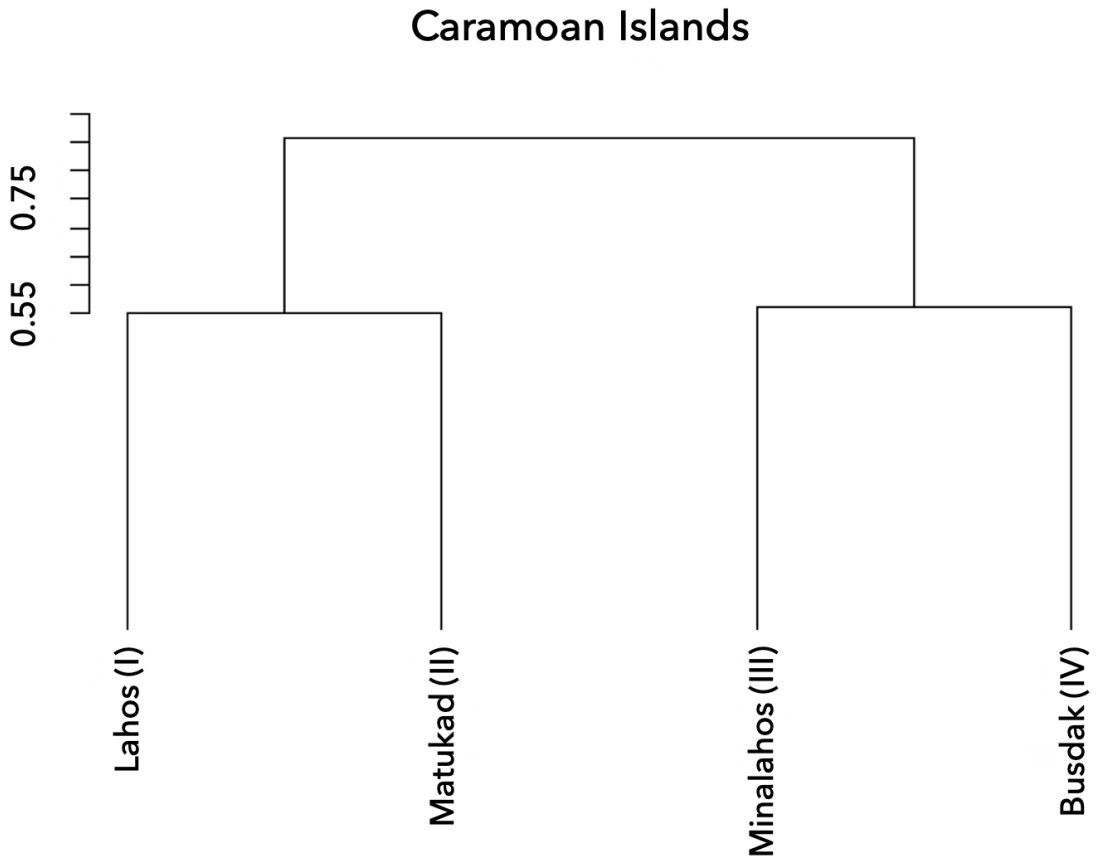

Figure 5. Clustering analysis based on Bray-Curtis dissimilarities of myxomycetes showing that the two largest islands (Lahos and Matukad) and the two smallest islands (Minalahos and Busdak) were more similar to each other.

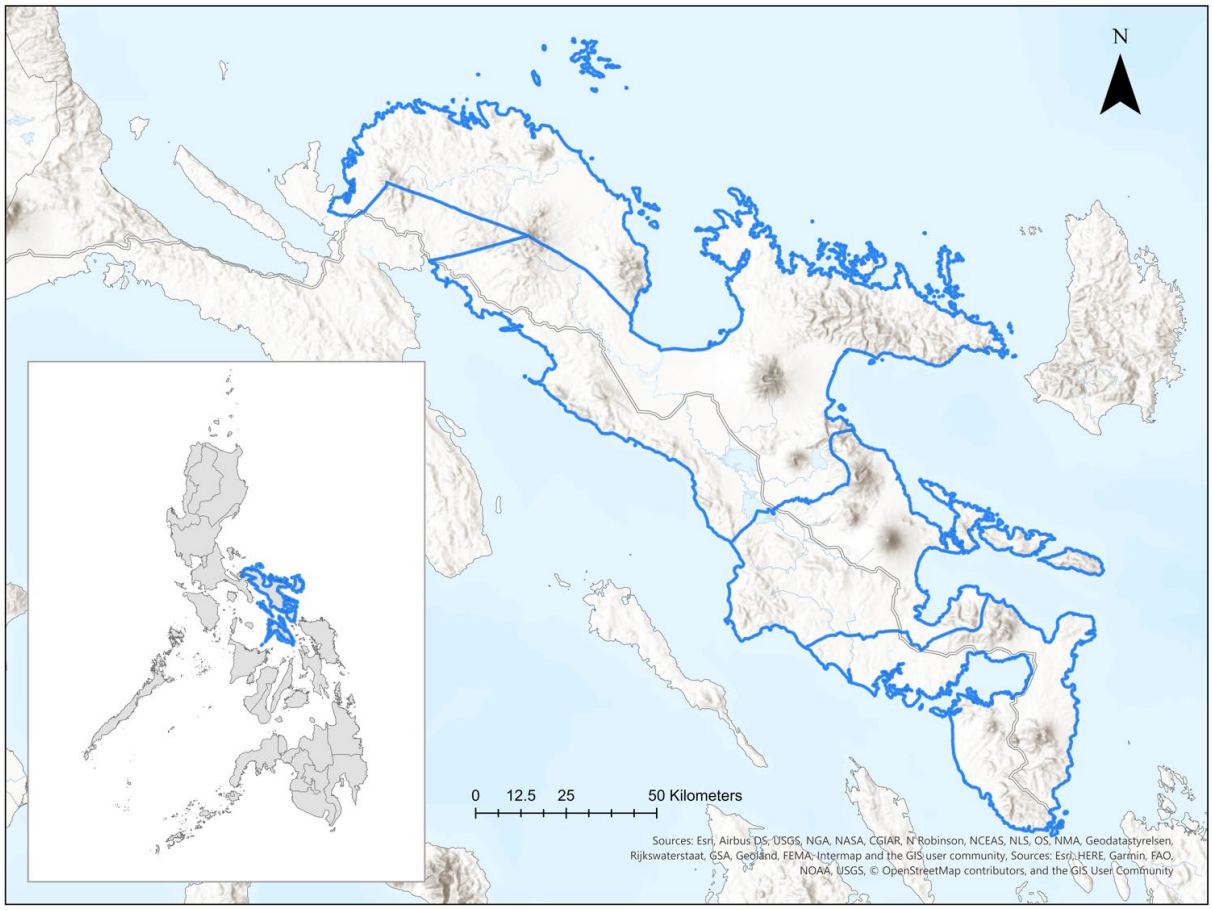

Figure 6. Map of the Bicol Peninsula as marked by the blue lines. The inset shows the Philippine map, where the entire Bicol region including the Bicol Peninsula is also delimited by blue lines.

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

Abstract

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

Introduction

There have been a number of previous studies of the myxomycetes, a diverse group of eukaryotic amoebozoans that are capable of producing fruiting bodies containing microscopic spores, of tropical and subtropical islands outside the Philippines (e.g., Eliasson & Nannenga-Bremekamp 1983, Eliasson 1991, Pando 1997; Nieves-Rivera & Santos-Flores 1998; Novozhilov et al. 2001; Rojas & Stephenson 2008; Stephenson 2009; Kryvomaz et al. 2017; Stephenson & Stephenson 2019). In the Philippines, the study of myxomycetes from insular habitats has increased substantially over the last decade, such as examples as the Hundred islands in Pangasinan (dela Cruz et al. 2011), Lubang island in Occidental Mindoro (Macabago et al. 2012, 2016), Anda

island in Pangasinan (Kuhn et al. 2013), Polilio island in Quezon (Viray et al. 2014), Bohol Island (Macabago et al. 2017), and in Puerto Princesa in mainland Palawan (Pecundo et al. 2017). Before the study reported herein no other myxomycete exploration has been documented for Coron Island, Palawan.

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

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

Materials and Methods

Study area, collecting protocols, laboratory isolation techniques, and determination of myxomycetes: The collecting effort for this study was conducted in 2013 in randomly selected, accessible sites on Coron island (Fig. 1A), in the Palawan group of islands in the Philippines (Fig. 1B), which is located on the western part of the archipelago on the West Philippine Sea (a.k.a.

South China Sea). Coron island is characterized by rolling and steep hills with 5,954 hectares of forest areas dominated by *Pterocymbium tinctorium* (Malvaceae), *Instia bijuga* (Fabaceae), *Koordersiodendron pinnatum* (Anacardiaceae), and *Heritiera sylvatica* (Sterculiaceae) (PCSDS 2006). Samples of mostly dry ground leaf litter (GL) and twigs (TW), and a combination of relatively fresh and mostly dry woody vines (V) were collected in a haphazard manner along the coasts and within areas that were characterized by limestone forests that had a mixture of tall shrubs and mostly dipterocarp and evergreen trees with the common presence of woody vines (lianas). Some palms were spotted in the island but were not collected in the largely carbonatic forest sites that were fairly adjacent to other ecosystems such as inland lakes, brushlands, and mangrove forests to name a few. Field specimens that developed under natural conditions were excluded from the study.

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

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

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

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

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

the island and on which substrates they were found.

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

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

Results

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

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

List of Species

Arcyria cinerea (Bull.) Pers. [A, 32] GL: 5, TW: 13, V: 14

Arcyria denudata (L.) Wettst. [R, 1] V: 1

Badhamia macrocarpa (Ces.) Rostaf. * [O, 2] TW: 2

Clastoderma debaryanum A. Blytt [A, 7] TW: 1, V:6

Comatricha cf. *pulchella* (C. Bab.) Rostaf. [O, 2] V: 2

Comatricha tenerrima (M.A. Curtis) G. Lister [R, 1] TW: 1

Cribraria violacea Rex * [O, 2] GL: 2

Diachea leucopodia (Bull.) Rostaf. [C, 4] GL: 4

Diderma effusum (Schwein.) Morgan [C, 4] GL: 4

Diderma hemisphaericum (Bull.) Hornem. [A, 9] GL: 9

Didymium ochroideum G. Lister * [R, 1] GL: 1

Didymium iridis (Ditmar) Fr. [A, 7] GL: 2, TW: 5

Didymium nigripes (Link) Fr. [C, 5] GL: 3, TW: 2

Didymium squamulosum (Alb. & Schwein.) Fr. & Palmquist [C, 5] GL: 2, TW: 1, V: 2

Lamproderma scintillans (Berk. & Broome) Morgan [A, 9] GL: 9

Perichaena depressa Lib. [A, 58] GL: 23, TW: 14, V: 21

Perichaena dictyonema Rammeloo * [O, 3] GL: 1, TW:2

Perichaena vermicularis (Schwein.) Rostaf. * [R, 1] TW: 1

Perichaena sp. (Trichiida: Trichiidae) [O, 3] GL: 2, V: 1

Physarum echinosporum Lister * [O, 6] GL: 4, V: 2

Physarum oblatum T. Macbr. * [R, 1] TW: 1

Physarum pusillum (Berk. & M.A. Curtis) G. Lister * [R, 1] TW: 1

Physarum sp. (Physarales: Physaraceae) [R, 1] TW: 1

Stemonitis fusca Roth [A, 37] TW: 25, V: 12

Stemonitis sp. (Stemonitidales: Stemonitidaceae) [R, 1] TW: 1

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

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

Discussion

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

Among the microhabitats examined, the woody vines (V) produced the highest myxomycete yield, followed by twigs (TW), and lastly ground leaf litter. Comparing the results to the recent mainland Palawan study (Pecundo et al. 2017), twigs (86% vs 72% in this study) also yielded more myxomycetes than ground litter (57% vs 65% in this study). However, it is not surprising that woody vines recorded the highest taxonomic diversity index (TDI), as other studies also have indicated their high level of productivity as a substrate (e.g., Kryvomaz et al. 2017, Dagamac et al. 2015, Wrigley de Basanta et al. 2008).

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

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

As mentioned in the results section, the objective of this paper was to examine the data for the island as a whole. However, since the substrates were independently collected from different sites, it would seem likely to consider the values obtained for each site. In terms of species richness,

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

There have been two major papers (Reynolds 1981; Pecundo et al. 2017) that considered myxomycetes from the province of Palawan prior to the present study. Reynolds (1981) noted 26 species, while Pecundo et al (2017) listed 33 species, with 22 of these new to the Palawan group of islands. With the exception of the one unassigned species [*Perichaena* (Trichiida: Trichiidae)], this study adds eight new morphospecies for the collective islands of the province of Palawan, updating the total known to 56 species. *Badhamia macrocarpa* also represents as a new record for the country, which brings the new total for the Philippines to 159. Dagamac and dela Cruz (2019)

already tallied 159 for the country after eight additions by Macabago et al. (2017) and one by Bernardo et al. (2018) to the previous summary of 150 by Dagamac and dela Cruz (2015). In the latter, however, the species *Didymium anellus* Morgan was counted twice; therefore, the corrected total then was 149. This now amended the total number of myxomycetes for the Philippines to 159.

Literature Cited

Bernardo, J. L. M, Arioder, L. J. Q., Almadrones-Reyes, K. J. & Dagamac, N. H. A. 2018: Myxomycete communities occurring on fragmented forest patches in two municipalities of Laguna, Philippines. Community Ecology 19: 289–299.

Chao, A., Ma, K. H., Hsieh, T. C. & Chiu, C. H. 2015: Online Program SpadeR (Species-richness Prediction And Diversity Estimation in R). Program and User's Guide published at http://chao.stat.nthu.edu.tw/wordpress/software_download/.

Chao, A. & Chiu, C. H. 2016: Species richness: estimation and comparison. Wiley StatsRef: Statistics Reference Online. 1-26.

Colwell, R. K. 2013: EstimateS: Statistical estimation of species richness and shared species from samples. Version 7. User's Guide and application; http://purl.oclc.org/estimates.

Dagamac, N. H. A. & dela Cruz, T. E. E. 2015: Myxomycetes research in the Philippines: Updates and opportunities. Mycosphere 6 (6): 784-795.

Dagamac, N. H. A. & dela Cruz, T. E. E. 2019: The Philippine slime molds after Dogma's 1975 list – How far have we been?. Philippine Journal of Systematic Biology 13 (2): 58-65

Dagamac, N. H. A., Stephenson, S. L. & dela Cruz, T. E. E. 2012: Occurrence, distribution, and diversity of myxomycetes (plasmodial slime molds) along two transects in Mt. Arayat, National Park, Pampanga, Philippines. Mycology: International Journal of Fungal Biology 3(2): 119–126.

Dagamac, N. H. A., Rea-Maminta, M. A. D., Batungbacal, N. S., Jung, S. H., Bulang, C. R. T., Cayago, A.G. R. & dela Cruz, T. E. E. 2015: Diversity of plasmodial slime molds (myxomycetes) on coastal, mountain, and community forests of Puerto Galera, Oriental Mindoro, Philippines. Journal of Asia-Pacific Biodiversity 8: 322-329.

Dela Cruz, T. E. E., Kuhn, R. V., Javier, A. O. M., Rodillas, C. P., Parra, C. M., Corpuz, L. H. M. & McHugh, R. D. 2011: Occurrence and distribution of plasmodial myxomycetes in Hundred Islands National Park, Pangasinan, Philippines. Acta Manilana 59: 65-74.

Eliasson, U. & Nannenga-Bremekamp, N. 1983: Myxomycetes of the scalesia forest, Galapagos Islands, Ecuador. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C Biological and Medical Sciences 86: 143-154.

Eliasson, U. 1991: The myxomycete biota of the Hawaiian Islands, USA. Mycological Research 95: 257-267.

Kiessling, W. & Flügel, E. 2000: Late Paleozoic and Late Triassic limestones from north Palawan block (Philippines): Microfacies and Paleogeographical implications. Facies 43: 39-77.

Kryvomaz, T., Michaud, A. & Stephenson, S. L. 2017: First survey for myxomycetes on Mahe Island in the Seychelles. Nova Hedwigia 104: 65-84.

Kuhn, R. V., Javier, A. O. M., Rodillas, C. P., Parra, C. M. & Corpuz, L. H. M., Buaya, A. T. & dela Cruz, T. E. E. 2013: Diversity of plasmodial myxomycetes from Anda Island, Pangasinan, Philippines. Biotropia 20: 1-9.

Lado, C. 2005-2020: An on line nomenclatural information system of Eumycetozoa. Real Jardín Botánico, CSIC. Madrid, Spain. http://www.nomen.eumycetozoa.com

Macabago, S. A. B., dela Cruz, T. E. E. & Stephenson, S. L. 2012: First records of myxomycetes from Lubang Island, Occidental Mindoro, Philippines. Sydowia 64: 109-118.

Macabago, S. A. B., Stephenson, S. L. & dela Cruz, T. E. E. 2016: Diversity and distribution of myxomycetes in coastal and mountain forests of Lubang Island, Occidental Mindoro, Philippines. Mycosphere 7(1): 18-29.

Macabago, S. A. B., Dagamac, N. H. A., dela Cruz, T. E. E. & Stephenson, S. L. 2017: Implications of the role of dispersal on the occurrence of litter-inhabiting myxomycetes in different vegetation types after a disturbance: a case study in Bohol Islands, Philippines. Nova Hedwigia 104 (1-3): 221-236.

Ndiritu, G. G., Spiegel, F. W. & Stephenson, S. L. 2009: Distribution and ecology of the assemblages of myxomycetes associated with major vegetation types in Big Bend National Park, USA. Fungal Ecology 2: 168–183.

Nieves-Rivera, Á. M. & Santos-Flores, C. J. 1998: New records of the myxomycetes from Mona Island, Puerto Rico. Journal of Agriculture- University of Puerto Rico 82: 189-199.

Novozhilov, Y. K., Schnittler, M., Rollins, A. & Stephenson, S. L. 2001: Myxomycetes from different forest types in Puerto Rico. Mycologia 77: 285-299.

Pando, F. 1997: Catálogo preliminar de los Mixomicetes del Parque Nacional de Coiba (Panamá). In: Flora y fauna del Parque Nacional de Coiba (Panamá). (ed. S. Castroviejo). Agencia Española de Cooperación Internacional. Madrid. 191-204.

PCSDS. 2006: Flora Survey and Biodiversity Assessment for Core Zoning Municipality of Coron. Palawan Council for Sustainable Development, Puerto Princesa City.

Pecundo, M., Dagamac, N. H. A., Stephenson, S. L. & dela Cruz, T. E. E. 2017: First myxomycete survey in the limestone forest of Puerto Princesa Subteranean River National Park, Palawan, Philippines. Nova Hedwigia 104 (1-3): 129-141.

Reynolds, D. R. 1981: Southeast Asian myxomycetes II. Philippines. Philippine Journal of Biology 10 (2-3): 127-150.

Rojas, C. & Stephenson, S. L. 2008: Myxomycete ecology along an elevation gradient on Cocos Island, Costa Rica. Fungal Diversity 29: 117-127.

Samonte-Tan, G. P. B., Pido, M. D., Abesamis, N. P., Naguit, S., Pontillas, M. C. A. & Trono, R. B. 2008: Monitoring and Evaluation: Lessons from Tubbataha Reef National Park and Coron Island Ancestral Domain, Philippines. Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008 Session number 23.

Stasolla, G. & Innocenti, G. 2014: A new species of cavernicolous crab from Coron Island, Palawan, the Philippines (Decapoda: Brachyura: Varunidae). The Raffles Bulletin of Zoology 62: 591-599.

Stephenson, S. L. 1988: Distribution and ecology of myxomycetes in temperate forests. I. Patterns of occurrence in the upland forests of southwestern Virginia. Canadian Journal of Botany 66: 2187-2207.

Stephenson, S. L. 2009: First records of myxomycetes from Ascension Island. Sydowia 61: 105-115.

Stephenson, S. L., Kalyanasundaram, I. & Lakhanpal, T. 1993: A comparative biogeographical study of myxomycetes in the mid-Appalachians of eastern North America and two regions of India. Journal of Biogeography 20: 645-657.

Stephenson, S. L. & Stempen H. 1994: Myxomycetes A Handbook of Slime Molds. Timber Press Inc., USA.

Stephenson, S. L. & Stephenson, B. C. 2019: Distribution and ecology of myxomycetes on Christmas Island, Indian Ocean. Phytotaxa 416: 138-148.

Unterseher, M., Schnittler, M., Dormann, C. & Sickert, A. 2008: Application of species richness estimators for the assessment of fungal diversity. FEMS Microbiology Letters 282: 205–213.

Viray, A. T., Rotap, D. D. S., Migraso, L. L., Sibbaluca, N. C. I., Escobar, E. C. V., Buaya, A. T. & dela Cruz, T. E. E. 2014: Occurrence and diversity of myxomycetes (slime molds) in Polillo Island, Quezon Province, Philippines. Acta Manilana 62: 9-17.

Wrigley de Basanta, D., Stephenson, S. L., Lado, C., Estrada-Torres, A. & Nieves-Rivera, A. M. 2008: Lianas as a microhabitat for myxomycetes in tropical forests. Fungal Diversity 28:109-125.

Tables

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

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

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

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

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

Myxomycetes of the Palawan group of islands	Synonym (as reported)
Arcyria cinerea (Bull.) Pers. +	
Arcyria denudata (L) Wettst.+	
Arcyria incarnata (Pers.) Pers. +	
Arcyria obvelata (Oeder) Onsberg +-	*Arcyria nutans*
Badhamia macrocarpa (Ces.) Rostaf. *	
*Ceratiomyxa fruticulos*a var. *fruticulosa* (Müll.) T. Macbr. +	
Clastoderma debaryanum A.Blytt ++	
Collaria arcyrionema (Rostaf.) Nann.-Bremek. ex Lado.++	
Comatricha nigra (Pers. ex J.F.Gmel.) J.Schröt. ++	
Comatricha pulchella (C.Bab. & Berk.) Rostaf. ++	
Comatricha tenerrima (M.A.Curtis) G.Lister. ++	
Cribraria atrofusca G.W. Martin & Lovejoy +	
Cribraria cancellata (Batsch) Nann.-Bremek. +-	*Dictydium cancellatum*
Cribraria microcarpa (Schrad.) Pers. +	
Cribraria violacea Rex *	
Diachea leucopodia (Bull.) Rostaf. +	
Diachea subsessilis Peck ++	
Diderma effusum (Schwein.) Morgan ++	
Diderma hemisphaericum (Bull.) Hornem. +	
Didymium iridis (Ditmar) Fr. ++	
Didymium nigripes (Link) Fr. +	
Didymium ochroideum G. Lister *	
Didymium squamulosum (Alb. & Schwein.) Fr. & Palmquist +	
Echinostelium minutum de Bary ++	
Fuligo aurea (Penz.) Y. Yamam. +-	*Erionema aurea*
Hemitrichia calyculata (Speg.) M.L. Farr +-	*Hemitrichia stipitata*
Hemitrichia pardina (Minakata) Ing ++	
Hemitrichia serpula (Scop.) Rostaf. ex Lister ++	
Lamproderma scintillans (Berk. & Broome) Morgan ++	
Lycogala epidendrum (L.) Fr. +	
Perichaena chrysosperma (Curr.) Lister +	
Perichaena depressa Lib. +	
Perichaena dictyonema Rammeloo *	
Perichaena vermicularis (Schwein.) Rostaf. *	
Physarum album (Bull.) Chevall. ++	
Physarum bivalve Pers. ++	
Physarum cinereum (Batsch) Pers. ++	
Physarum compressum Alb. & Schwein. ++	
Physarum decipiens M.A.Curtis ++	
Physarum echinosporum Lister *	
Physarum leucophaeum Fr. ++	
Physarum melleum (Berk. & Broome) Massee ++	
Physarum nicaraguense T. Macbr. +	
Physarum notabile T. Macbr. +	
Physarum oblatum T. Macbr. *	
Physarum pezizoideum (Jungh.) Pavill. & Lagerh. +	
Physarum pusillum (Berk. & M.A. Curtis) G. Lister *	
Physarum stellatum (Massee) G.W. Martin +	
Physarum viride (Bull.) Pers. ++	
Reticularia lycoperdon (Bull.) +	
Stemonitis axifera (Bull.) T.Macbr. + -	*Stemonitis smithii*
Stemonitis fusca Roth +	
Stemonitis herbatica Peck +	
Stemonitis splendens Rostaf. ++	
Stemonitopsis typhina (F.H. Wigg.) Nann.-Bremek. +-	*Comatricha typhoides*
Willkommlangea reticulata (Alb. & Schwein.) Kuntze ++	

Legend:

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

Figures

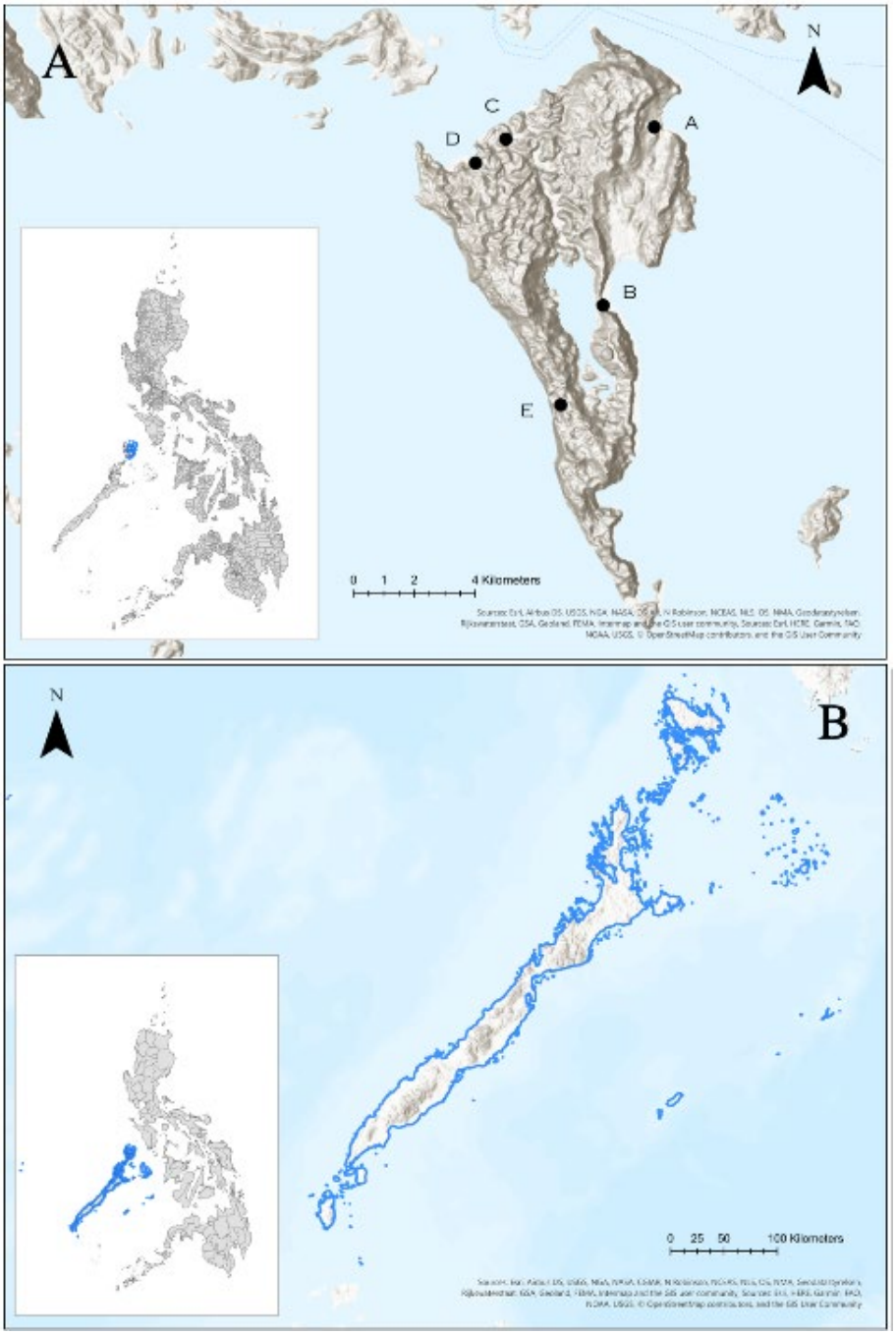

Figure 1. (A) Map of Coron Island with the collecting sites [A-E] marked by black dots (•). The inset on the lower left shows a map of the Philippines, with Coron delimited in blue. (B) Map of the Palawan group of islands including Coron Island. The inset on the lower left shows a map of the Philippines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

V. Mapping the Myxomycetes of the Philippines

Abstract

The main objective of this chapter is to create an accessible repository and map of the myxomycete collections in the Philippines. This allows the georeferencing of collections that were not previously tied to geographic coordinates and/or not available online and, therefore, permits them to be viewed, queried, and analyzed with other geographic data. Tabulated data were gathered from data contributors. After successive verification, the information was then stored in a spreadsheet database containing specific fields for georeferencing. The data was visually projected as a map that can be queried for several geographic and ecological analyses such as a species distribution modeling of *Arcyria cinerea* in the Philippines, where it was shown that the morphospecies has a high presence probability in the central areas of the Philippines and coastal areas on the northwestern Luzon. This venture also updates the current list of myxomoycetes ascribed to the Philippines to 163.

Introduction

The Philippines is an archipelago of more than 7000 islands of various sizes (see Fig. 1). As much as it is a thrilling endeavor, the challenges accompanying conducting scientific research, such as biodiversity assessments, in discontinuous land masses (i.e., islands) are extant especially for microorganisms. It is not just their sizes that makes it tricky, the relative infrequency of background research and other studies that layout the foundation of more intricate scientific efforts impedes the advancement of the studies on these organisms, such as the myxomycetes.

Despite the fairly growing number of reports since the revival of myxomycete investigations in the Philippines from the earlier annotated findings by Dogma (1975) and

Reynolds (1981), as shown by at least 24 publications from 2009 to 2019 (Dagamac & dela Cruz 2019), the need to advance new techniques and scope of studies from largely descriptive ones is imminent. A recent review on the developments of taxonomic work on the Philippine myxomycetes (Dagamac & dela Cruz 2019) stated a tally of 159 myxomycetes accounted for the country. This number was recently updated to 162 (see Macabago et al. 2020a) but after verification of the 2015 listing (Dagamac & dela Cruz 2015), this number stands at 161. The updating of current records for the country and the expansion towards new approaches and topics such as, but not limited to, the ecology of dispersal, island biogeography, and future and current risk assessments, will be more manageable and/or expedient if there is a convenient way to access myxomycete data.

Considering the reasons above and more, this study aims to produce an extensive collection of myxomycetes from the Philippines that can be viewed, updated, queried, and analyzed with other geographical and environmental data by collating and geocoding already available information, and making it accessible for other suitable analyses, such as the species distribution modeling of the most collected morphospecies, *Arcyria cinerea* (Bull.) Pers., in the Philippines.

Methods

This study involves the accumulation of myxomycete collections, inspection and verification of datasets, and analyses and visualization of the myxomycetes found so far in the Philippines.

Data gathering

Tabulated data were independently gathered directly from researchers of Philippine myxomycetes and were tallied/confirmed with published reports, if available. Currently this compilation includes field and moist chamber collections made between 2009 and 2015 and which were published from 2010 to 2020 by the contributors.

Contributors

The following individuals served as record contributors both for published and unpublished collections: Dagamac N. for Pampanga, Quezon Province, Laguna, Bicol, Oriental Mindoro, Negros Occidental, and Cotabato (Buisan & Dagamac 2019, Bernardo et al. 2018, Dagamac et al. 2017, Dagamac et al. 2015a & b, Alfaro et al. 2015, Dagamac et al. 2014, Dagamac et al. 2011), Pecundo M. for Batangas, Bulacan, Palawan, Bohol, and Oriental and Occidental Mindoro (Pecundo et al. 2020, Isagan et al. 2020, Pecundo et al. 2017, Carascal et al. 2017), Rea-Maminta A. for Central Luzon (Rea-Maminta et al. 2015), Buaya A. for Cavite and Nueva Vizcaya, and the author for Quezon City, Occidental Mindoro, Bicol, Palawan, and Bohol (Macabago et al. 2020a, Macabago et al. 2020b, Macabago et al. 2017, Macabago et al. 2016, Macabago et al. 2012, Macabago et al. 2010).

Data cleaning

The necessary information from the tabulated data gathered from the contributors were filtered for the preferred fields [1- Taxon/Species name, 2- Collector No., 3- Collector Name, 4- Substrate/Microhabitat, 5- Date of collection, 6- pH of substrate, 7- Latitude, 8- Longitude, 9- Country, 10- State/Province, 11- Exact locality, and 12- Habitat description] and verified for the

accuracy of the coordinates and precision of entries. Table 1 shows the list of field descriptions and acronyms used in the databasing of the myxomycete collections.

Taxonomy followed the online nomenclatural information system of Eumycetozoa (Lado et al. 2021), where synonyms of a myxomycete taxa were corroborated and rendered to represent only one unique taxon (morphospecies). The geographic coordinate systems (latitude and longitude) were transformed to decimal degrees for uniformity. The rest of the entries by each contributor were retained to maintain integrity of data.

After successive checking of the accuracy of information from the compiled collections, the dataset comprised a total of 5,033 occurrence records belonging to 120 unique myxomycete taxa. The database was saved as a Microsoft Excel spreadsheet file (.xls).

Mapping

The database was imported to ArcGIS Pro ver. 2.7 for processing and visualization. The map base was overlaid with boundaries of Philippine provinces to show confines of the locality. For georeferencing the occurrences, the geographic coordinate system World Geodetic System (WGS) 84 was used.

The entry features were displayed as an XY data on the map base (Fig. 2). To confirm if georeferencing worked, several points on the map were randomly selected to show the field information attached to them as a pop-up window (see Fig. 3).

The points were then presented as unique values, where every taxon is symbolized by an assigned color, plotted in accordance with the coordinate system on the chosen base map. Figure 4 shows that unique symbols were designated for each myxomycete morphospecies, and that the attributes table demonstrated the field entries for each occurrence.

To show the geographic density of myxomycete collections in the area a heat map was constructed, where a high to low density is represented by a range of hot to cool color such that the cooler the color in an area the less myxomycetes were recorded (Macabago et al. 2020a).

Using the attributes tables queries were made in the georeferenced points that allowed analyses of the digitized data. The map and all its raster files will be publicly available for ease of updating the entries and access to the data for further analyses in the future.

Species distribution modeling of *Arcyria cinerea* (Bull.) Pers.

The analysis started with making a query on the presence records of *A. cinerea* in the Philippines using the geocoded database, followed by cleaning of the presence records of *A. cinerea*, then by the modeling of current predicted species distribution with the bioclimatic variables obtained from the Worldclim database at 2.5' resolution for the period of 1970-2000 (Fick & Hijmans 2017) using the program Maxent (Phillips et al. 2006; Phillips & Dud´ık 2008; Elith et al. 2011). Visualization of the Maxent simulation was done by importing the ASCII results to the program ArcGIS Pro ver. 2.7 as a classified data with defined intervals of 0.20 as represented by a color scheme of cool (blue) to warm (red) indicating very low (<0.2), low (<0.4), medium (<0.6), high (<0.8), and very high probabilities (0.80-1.0). All data preparation and MaxEnt procedures followed the protocol of Banag et al. (2015) and as described in Chapter VII of this manuscript, except this section used all 19 bioclimatic variables.

Results and Discussion

A total of 5,033 records belonging to 120 unique myxomycete taxa (see Fig. 6) collected from at least 20 provinces (Albay, Bataan, Batangas, Bohol, Camarines Sur, Cavite, Cotabato,

Laguna, Maguindanao, Manila, Negros Occidental, Nueva Vizcaya, Occidental Mindoro, Oriental Mindoro, Palawan, Pampanga, Pangasinan, Quezon, Sorsogon, and Zambales) in the Philippines (Fig. 2) and were included in at least 17 published reports were incorporated and georeferenced in the database. This database serves as a digital repository of several collections made between specified years but can be updated as needed when more data becomes available. In addition, no images and other information beyond the specified 12 fields in Table 1 are included, unlike an interactive database developed to identify and retrieve information on specific collections of myxomycetes in Mt. Arayat in the Philippines (see Dagamac et al. 2011).

This endeavor emphasized several things about the study of myxomycetes in the Philippines within the time frame of this research: 1) the most heavily sampled area is the south-western part of Luzon as shown by the warm colors on the heat map (see. Fig. 5), while the rest of the country are still necessitating surveys, 2) the top ten most collected species are *Arcyria cinerea* (Bull.) Pers., *Diderma hemisphaericum* (Bull.) Hornem., *Diderma effusum* (Schwein.) Morgan, *Perichaena depressa* Lib., *Didymium squamulosum* (Alb. & Schwein.) Fr. & Palmquist, *Stemonitis fusca* Roth, *Lamproderma scintillans* (Berk. & Broome) Morgan, *Perichaena chrysosperma* (Curr.) Lister, *Collaria arcyrionema* (Rostaf.) Nann.-Bremek. ex Lado, and *Physarum cinereum* (Batsch) Pers. (see Table 2), respectively, 3) sixteen species were collected only once, such as the following: *Badhamia utricularis* (Bull.) Berk., *Comatricha robusta* (T.N. Lakh. & K.G. Mukerji) Nann.-Bremek. & Y. Yamam., *Craterium aureum* (Schum.) Rostaf., *Diachea splendens* Peck, *Dianema harveyi* Rex, *Enerthenema papillatum* (Pers.) Rostaf., *Lamproderma arcyrioides* (Sommerf.) Rostaf., *Lamproderma cacographicum* Bozonnet, Mar. Mey. & Poulain, *Physarum flavicomum* Berk., *Physarum javanicum* Racib., *Physarum pezizoideum* (Jungh.) Pavill. & Lagarde, *Physarum sulphureum* Alb. & Schwein., *Stemonitis*

flavogenita E. Jahn, *Trichia botrytis* (J.F. Gmel.) Pers., *Trichia decipiens* (Pers.) T. Macbr., and *Tubifera ferruginosa* (Batsch) J.F. Gmel. (Table 2), and 4) from published reports, there are currently 163 myxomycetes attributed to the Philippines, including two that were not included in recent listings, *C. aureum* (see Dagamac et al. 2015a) and *P. javanicum* (see Pecundo et al. 2020).

The digitization of collections makes it convenient to conduct queries, and therefore perform deeper analyses of data. Figures 7-11 shows a series of nested queries within the features of the geocoded data, where at least five identifiers (genus, then species, followed by substrate associated, year of collection, and the specific province/locality of the collection) were utilized to narrow down a search. Figure 12 displays that you can discriminate the selected features/collection to the actual point on the map and exhibit information on a specific collection/point on the map as shown on Fig. 13.

Once this data is available online, anyone with access can update the information and even add fields pertinent to a collection. Further analyses with other geographic and environmental data will also be possible such as the species distribution/environmental niche modeling of a common morphospecies in the country, *A. cinerea* (Fig. 14). In this simulation, it shows that the myxomycete has a high chance of presence in central Philippines and the northwestern coasts, while the greater Mindanao (southern islands) and central Luzon (north) have a low probability of harboring *A. cinerea.* Performance of the model evaluated using the receiver operating characteristic (ROC) curve, which showed an area under the curve (AUC) value of 0.995, was found to be robust. The top five variables with the greatest percent contribution to the model were annual precipitation (43.9), minimum temperature of coldest month (34.7), isothermality (8.9), temperature seasonality (5.7), and precipitation of warmest quarter (3.0). In an environmental niche model for *A. cinerea* in Costa Rica (Rojas et al. 2015) isothermality and precipitation were

also found to be top influencing factors, while Almadrones-Reyes and Dagamac (2018) likewise enumerated four among the five above-mentioned environmental variables, except annual precipitation, to be top contributors to the species distribution modeling of another myxomycete, *Diderma hemisphaericum* (Bull.) Hornem., in the Philippines. Jackknife test of variable importance revealed that temperature seasonality contained the most information by itself compared to other variables, a result which was parallel to the findings of Almadrones-Reyes and Dagamac (2018). The simulation also showed that the variable precipitation of the coldest quarter has the most information that is absent in the other variables, where temperature seasonality was a close second.

Besides distribution/niche modelings, other analyses could be done such as, but not limited to, climate projections, habitat risk assessments, summaries of "bigger" data, and other geospatial imaging and ecological assessments.

Literature Cited

Alfaro, J. R. D., Alcayde, D. L. I. M., Agbulos, J. B., Dagamac, N. H. A. & dela Cruz, T.E.E. 2015. The occurrence of myxomycetes from a lowland montane forest and agricultural plantations of Negros Occidental, Western Visayas, Philippines. FineFocus 01:07-20.

Almadrones-Reyes K. J. & Dagamac N. H. A. 2018 Predicting local habitat suitability in changing climate scenarios: Applying species distribution modelling for Diderma hemisphaericum. Current Research in Environmental & Applied Mycology 8(5): 492–500.

Banag, C., Thrippleton, T., Alejandro, G. J., Reineking, B. & Liede-Schumann, S. 2015. Bioclimatic niches of selected endemic Ixora species on the Philippines: predicting habitat suitability due to climate change. Plant Ecology 216(9): 1325–1340.

Bernardo, J., Arioder, L., Almadrones-Reyes, K. & Dagamac, N. H. A. 2018. Myxomycete communities occurring in fragmented forest patches in two municipalities of Laguna, Philippines. Community Ecology. 19. 289-299.

Buisan, P. N. H. & Dagamac, N. H.A. 2019. Records of cosmopolitan myxomycetes occurring on banana litter from Southern Mindanao, Philippines. Philippine Journal of Systematic Biology. 13. 55-57.

Carascal, M. B., Rea, M. A. D., Dagamac, N. H. A. & dela Cruz, T. E. E. 2017. Myxomycetes associated with grassland litter of the Philippines. Current Research in Environmental & Applied Mycology 7(2):56-63.

Dagamac, N. H. A. & dela Cruz, T. E. E. 2019. The Philippine slime molds after Dogma's 1975 list – How far have we been? Philippine Journal of Systematic Biology 13(2): 58-65.

Dagamac, N. H. A. & dela Cruz, T. E. E. (2015). Myxomycetes research in the Philippines: Updates and opportunities. Mycosphere 6(6): 784–795.

Dagamac, N. H. A., dela Cruz, T. E. E., Rea–Maminta, M. A. D., Aril–dela Cruz, J. V. & Schnittler M. 2017. Rapid assessment of myxomycete diversity in the Bicol Peninsula, Philippines. Nova Hedwigia 104 (1-3): 31-46.

Dagamac, N. H. A., Rea–Maminta, M. A. D. & dela Cruz, T. E. E. 2015a. Plasmodial slime molds of a tropical karst forest, Quezon National Park, Philippines. 69: 411-422. DOI:10.2984/69.3.9.

Dagamac, N. H. A., Rea-Maminta, M. A. D., Batungbacalm, N. S., Jung, S. H., Bulang, C. R. T., Cayago, A. G. R. & dela Cruz, T. E. E. 2015b. Diversity of plasmodial slime molds (myxomycetes) on coastal, mountain and community forests of Puerto Galera, Oriental Mindoro, Philippines. Journal of Asia-Pacific Biodiversity 8: 322–329.

Dagamac, N. H. A., Stephenson, S. L. & dela Cruz, T. E. E. 2014. The occurrence of litter myxomycetes at different elevations in Mt. Arayat National Park, Pampanga, Philippines. Nova Hedwigia 98 (1-2): 187-196.

Dagamac, N. H. A., Stephenson, S. L. & dela Cruz, T. E. E. 2012. Occurrence, distribution, and diversity of myxomycetes (plasmodial slime molds) along two transects in Mt. Arayat, National Park, Pampanga, Philippines. Mycology: International Journal of Fungal Biology 3 (2): 119-126.

Dagamac, N. H. A., Pangilinan, M. V. B., Stephenson, S. L. & dela Cruz, T. E. E. 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.

Fick, S. E. & Hijmans, R. J. 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37 (12): 4302-4315.

Isagan, M. D. E., Carbonell, M. K. C., Dalangin, Y. A., Lapira, A. J. L., Pecundo, M. H. & dela Cruz, T. E. E. 2020. Species listing and diversity of myxomycetes from Mt. Makulot and Napayong Island in Taal Lake, Batangas, Philippines. MycoAsia 2020/05.

Lado, C. (2005-2021). An online nomenclatural information system of Eumycetozoa. Real Jardín Botánico, CSIC. Madrid, Spain. https://eumycetozoa.com (March 22, 2021).

Macabago, S. A. B., Dagamac, N. H. A., dela Cruz, T. E. E. & Stephenson, S. L. 2020a. Myxomycetes of the Caramoan Islands and update on the myxomycetes of the Bicol Peninsula in the Philippines. Philippine Journal of Systematic Biology 14, 1.

Macabago, S. A. B., dela Cruz, T. E. E. & Stephenson, S. L. 2020b. Myxomycetes of Coron Island and additions to the Myxomycetes of Palawan group of islands in the Philippines. Karstenia 58(2): 157-167.

Macabago, S. A. B., Dagamac, N. H. A., dela Cruz, T. E. E. & Stephenson, S. L. 2017. Implications of the role of dispersal on the occurrence of litter-inhabiting myxomycetes in different vegetation types after a disturbance: a case study in Bohol Islands, Philippines. Nova Hedwigia 104 (1-3): 221-236.

Macabago, S. A. B., Stephenson, S. L. & dela Cruz, T. E. E. 2016. Diversity and distribution of myxomycetes in coastal and mountain forests of Lubang Island, Occidental Mindoro, Philippines. Mycosphere 7(1): 18-29.

Macabago, S. A. B., Stephenson, S. L. & dela Cruz, T. E. E. 2012. First records of myxomycetes from Lubang Island, Occidental Mindoro, Philippines. Sydowia 64 (1):109-118.

Macabago, S. A. B., Dagamac, N. H. A. & dela Cruz, T. E. E. 2010. Diversity and distribution of plasmodial myxomycetes (slime molds) from La Mesa Ecopark, Quezon City, Philippines. Biotropia 17 (2): 51-61.

Pecundo, M. H., Dagamac, N. H. A., dela Cruz, T. E. E. 2020. A comparative diversity study of myxomycetes in the lowland forests of Mt. Malasimbo and Mt. Siburan, Mindoro Island, Philippines. Karstenia 58 (2): 275-291

Pecundo, M. H., Dagamac, N. H. A., Stephenson, S. L. & dela Cruz T. E. E. 2017. First myxomycete survey in the limestone forest of Puerto Princesa Subterranean River National Park, Palawan, Philippines. Nova Hedwigia 104 (1-3): 129-141.

Phillips, S. J., Dudík, M. & Schapire, R. E. [Internet] Maxent software for modeling species niches and distributions (Version 3.4.1). Available from url: http://biodiversityinformatics.amnh.org/open_source/maxent/.

Rea-Maminta, M. A. D., Dagamac, N. H. A., Huyop, F. Z., Wahab, R. A. & dela Cruz, T. E. E. 2015. Comparative diversity and heavy metal biosorption of myxomycetes (slime molds) from forest patches on ultramafic and volcanic soils. Chemistry and Ecology 31 (8): 741-753.

Rojas, C., Zuñiga, J. M. & Stephenson, S. L. 2015. Ecological niche modelling of some Costa Rican myxomycetes. Current Research in Environmental and Applied Mycology 5: 153–159.

Tables

Table 1. List of field descriptions and names/acronyms used in the databasing of the myxomycete collections.

Field Name	Field Description
Collector No.	Individual collection number assigend by each collector, if available
Collector	Name of the collector/collectors
Taxon	Taxonomic name of the specimen, usually a morphospecies
Substrate	Microhabitat where specimen was found
DateText	Date of collection, if noted
pH	pH of substrate, where specimen was found
LatDec	Latitude in decimal degrees
LonDec	Longitude in decimal degrees
Country	Country of collection (Philippines)
State/Province	Province or region associated with the collection
Exact Locality	Specific area associated with collection, usually a town
Habitat	Description of the environment associated with the collection

Table 2. The updated list of myxomycetes recorded in the Philippines. Names in bold are included in the current digitized collection (120). * = unpublished survey, ** = published, but not included in recent listing, numbers in parentheses () are actual occurrence records.

Myxomycetes of the Philippines		
Alwisia bombarda	*Diderma saundersii*	***Physarum cinereum*** (100)
Arcyria afroalpina (38)	***Diderma subasteroides*** (4)	***Physarum compressum*** (73)
Arcyria cinerea (1268)	***Didymium anellus*** (11)	***Physarum crateriforme*** (19)
Arcyria denudata (63)	***Didymium bahiense*** (4)	***Physarum decipiens*** (53)
Arcyria globosa (28)	***Didymium clavus*** (2)	***Physarum didermoides*** (3)
Arcyria incarnata (11)	***Didymium flocossum*** (2)	***Physarum echinosporum*** (58)
Arcyria insignis (6)	***Didymium iridis*** (68)	***Physarum flavicomum*** (1)
Arcyria magna	*Didymium leoninum*	***Physarum globuliferum*** (9)
Arcyria marginoundulata	*Didymium megalosporum*	***Physarum gyrosum*** (9)
Arcyria obvelata	***Didymium melanospermum*** (8)	***Physarum javanicum*** ** (1)
Arcyria* cf. *oerstedioides ** (2)	***Didymium minus*** (4)	***Physarum lakhanpalii*** (6)
Arcyria virescens	***Didymium nigripes*** (99)	***Physarum leucophaeum*** (15)
Badhamia affinis (2)	***Didymium ochroideum*** (52)	***Physarum melleum*** (98)
Badhamia macrocarpa (2)	*Didymium serpula*	*Physarum nicaraguense*
Badhamia utricularis (1)	***Didymium squamulosum*** (217)	***Physarum notabile*** (16)
Calomyxa metallica (3)	***Didymium verrucosporum*** (6)	***Physarum nucleatum*** (2)
Ceratiomyxa fruticulosa (30)	***Echinostelium minutum*** (16)	***Physarum oblatum*** (30)
Clastoderma debaryanum (47)	*Elaeomyxa miyazakiensis*	***Physarum pezizoideum*** (1)
Clastoderma microcarpum	***Enerthenema papillatum*** (1)	*Physarum polycephalum*
Collaria arcyrionema (114)	*Fuligo aurea*	*Physarum psittacinum*
Collaria rubens (5)	***Fuligo cinerea*** (6)	***Physarum pulcherrimum*** (6)
Comatricha elegans (3)	*Fuligo septica*	***Physarum pusillum*** (64)
Comatricha fragilis (2)	***Hemitrichia calyculata*** (19	*Physarum retisporum*
Comatricha laxa (10)	*Hemitrichia intorta*	*Physarum rigidum*
Comatricha longipila	*Hemitrichia leiocarpa*	***Physarum roseum*** (5)
Comatricha nigra (30)	***Hemitrichia minor*** (2)	*Physarum rubiginosum*
Comatricha pulchella (19)	***Hemitrichia pardina*** (6)	***Physarum serpula*** (2)
Comatricha robusta (1)	***Hemitrichia serpula*** (24)	***Physarum stellatum*** (3)
Comatricha tenerrima (92)	***Lamproderma arcyrioides*** (1)	***Physarum straminipes*** (8)
Craterium atrolucens (2)	***Lamproderma cacographicum*** (1)	***Physarum sulphureum*** (1)
Craterium aureum ** (1)	***Lamproderma scintillans*** (154)	***Physarum superbum*** (8)
Craterium concinnum (15)	*Lepidoderma tigrinum*	***Physarum tenerum*** (5)
Craterium leucocephalum (11)	***Licea biforis*** (3)	***Physarum viride*** (28)
Craterium microcarpum	*Licea erecta*	*Reticularia lycoperdon*
Craterium minutum (5)	*Licea floriformis*	***Stemonaria fuscoides*** (7)
Craterium paraguayense	*Licea operculata*	*Stemonaria longa*
Craterium retisporum	***Lycogala epidendrum*** (2)	***Stemonitis axifera*** (24)
Cribraria atrofusca	***Lycogala exiguum*** (4)	***Stemonitis flavogenita*** (1)
Cribraria cancellata (8)	*Meriderma cribrarioides*	***Stemonitis fusca*** (196)
Cribraria lepida (2)	***Metatrichia vesparia*** (2)	***Stemonitis herbatica*** (10)
Cribraria microcarpa (47)	***Oligonema schweinitzii*** (6)	***Stemonitis pallida*** (27)
Cribraria piriformis	***Perichaena chrysosperma*** (140)	***Stemonitis splendens*** (9)
Cribraria violacea (90)	***Perichaena corticalis*** (23)	*Stemonitis uvifera*
Diachea bulbillosa (8)	***Perichaena depressa*** (267)	***Stemonitopsis subcaespitosa*** (11)
Diachea leucopodia (97)	***Perichaena dictyonema*** (57)	*Stemonitopsis typhina*
Diachea radiata (5)	***Perichaena microspora*** (10)	***Trichia botrytis*** (1)
Diachea splendens (1)	***Perichaena pedata*** (76)	*Trichia contorta*
Diachea subsessilis (12)	*Perichaena reticulospora*	***Trichia decipiens*** (1)
Dianema harveyi (1)	***Perichaena vermicularis*** (33)	*Trichia erecta*
Dictydiaethalium plumbeum (2)	***Physarella oblonga*** (3)	*Trichia favoginea*
Diderma chondrioderma	***Physarina echinospora*** (3)	*Trichia favoginea*
Diderma effusum (270)	***Physarum album*** (30)	***Trichia papillata*** * (10)
Diderma fallax	***Physarum bitectum*** (3)	***Tubifera ferruginosa*** (1)
Diderma hemisphaericum (323)	***Physarum bivalve*** (55)	*Tubifera microsperma*
Diderma rugosum	***Physarum bogoriense*** (2)	***Willkommlangea reticulata*** (8)

Figures

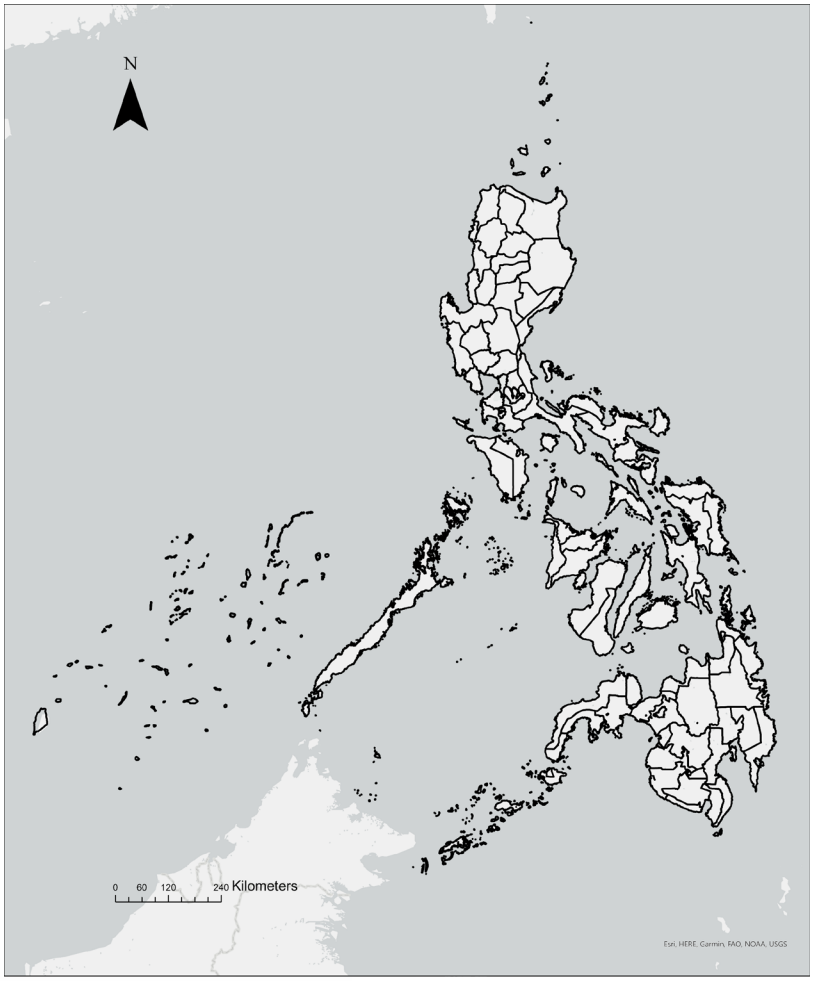

Figure 1. Map of the Philippine archipelago.

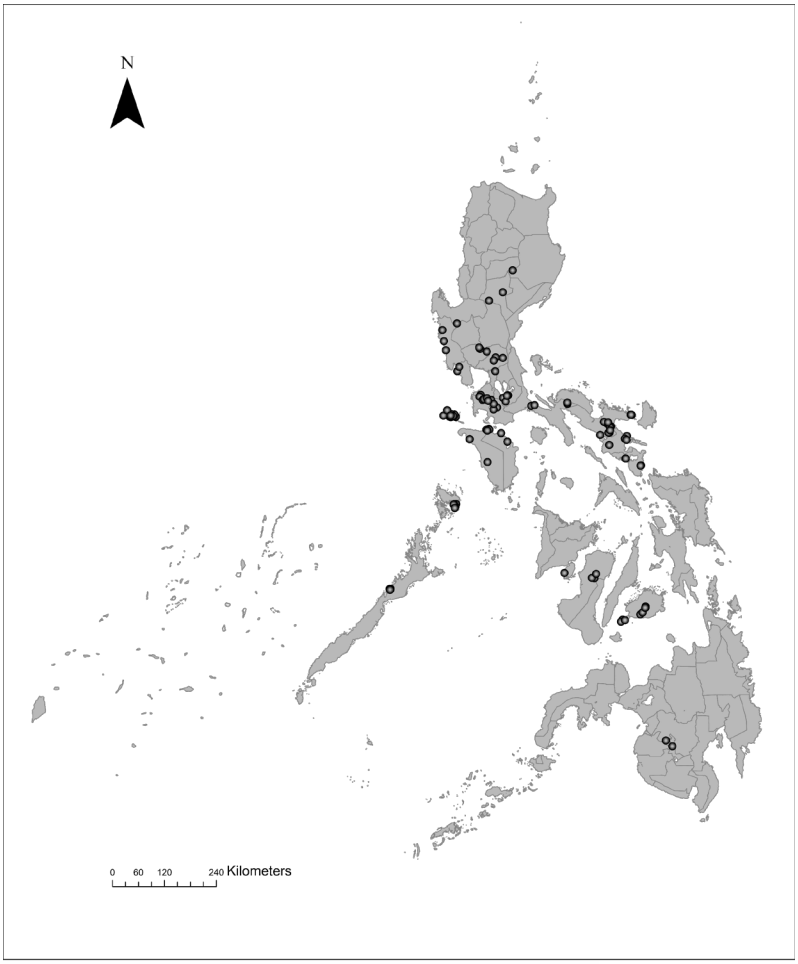

Figure 2. Map of the myxomycete collections in the Philippines.

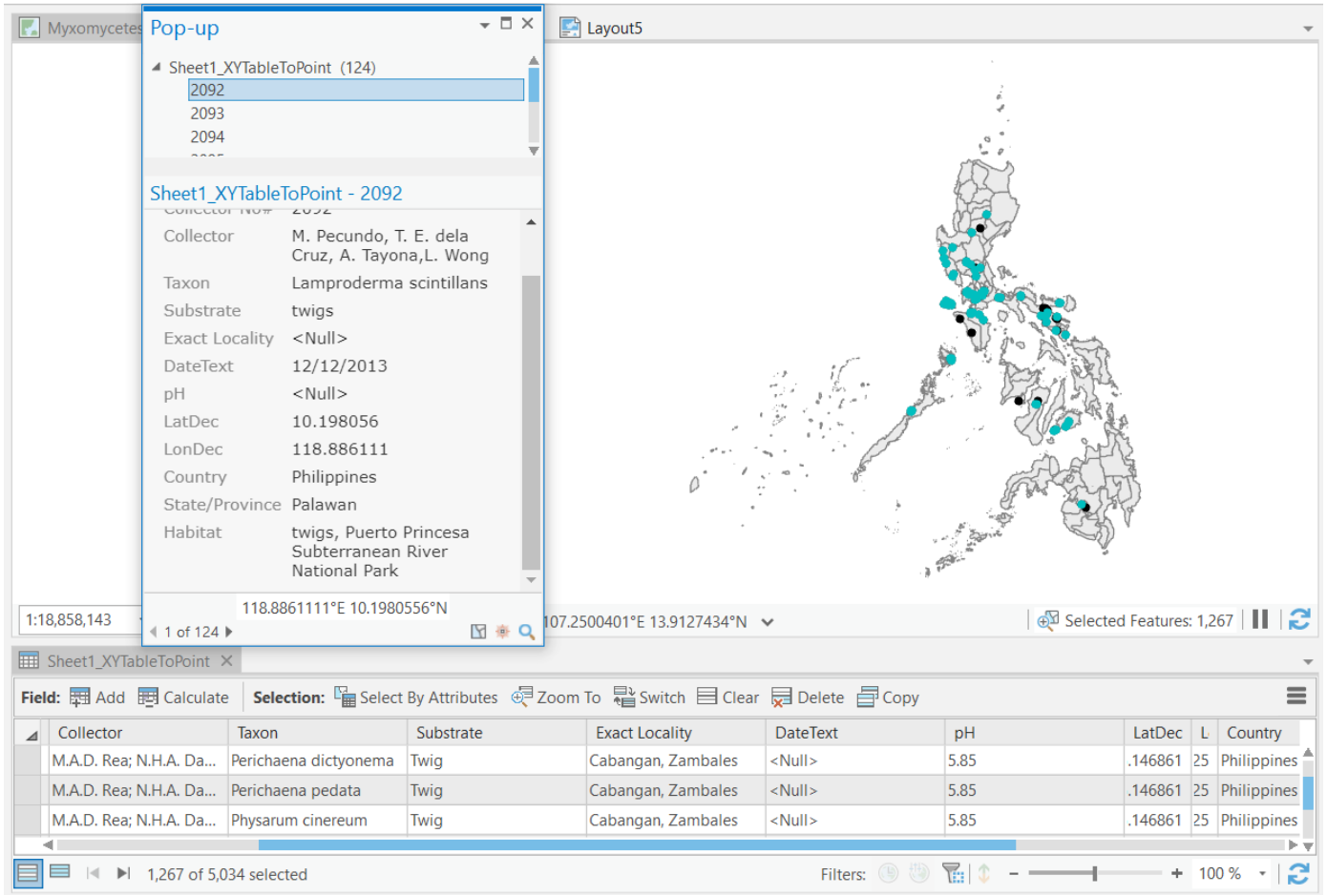

Figure 3. Interface of the geocoded information and map projection of the myxomycetes of the Philippines.

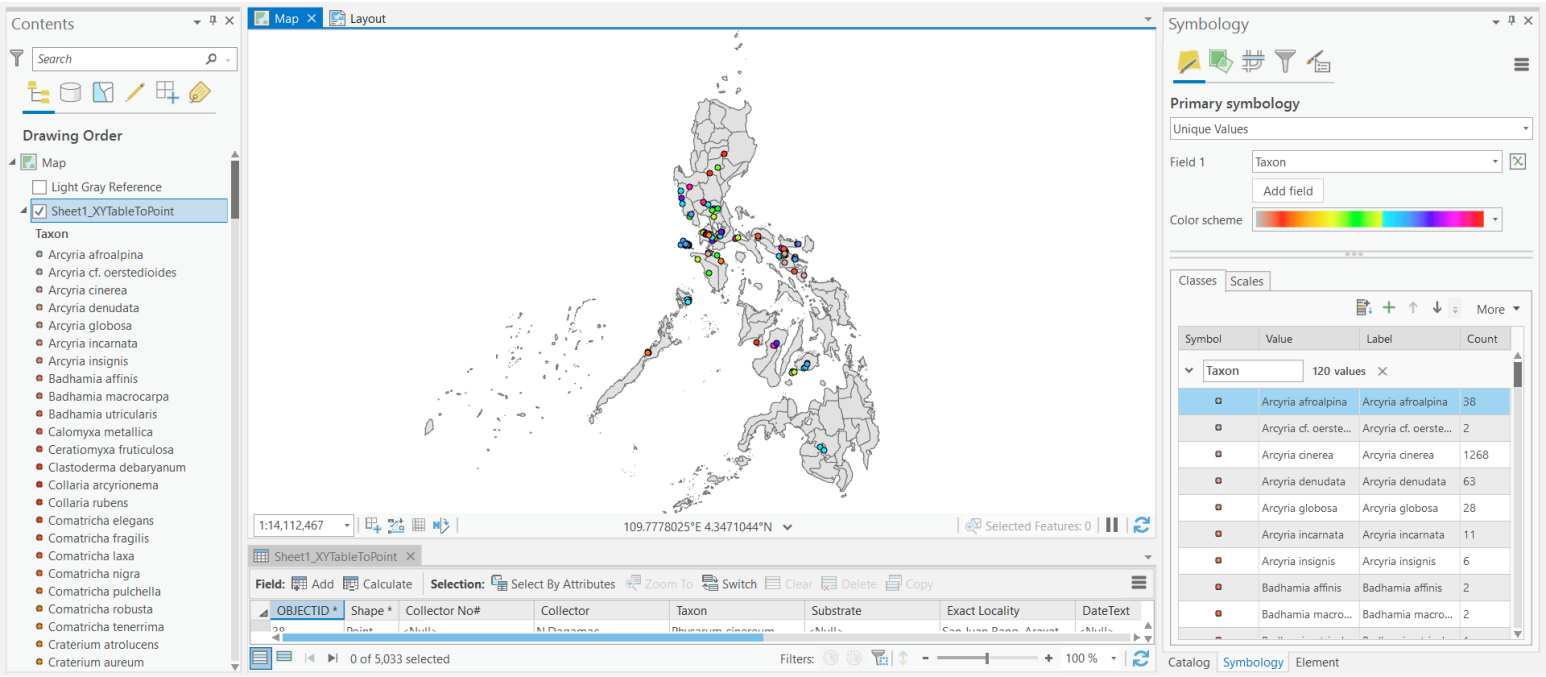

Figure 4. Interface of the mapping of the myxomycetes of the Philippines in ArcGIS Pro.

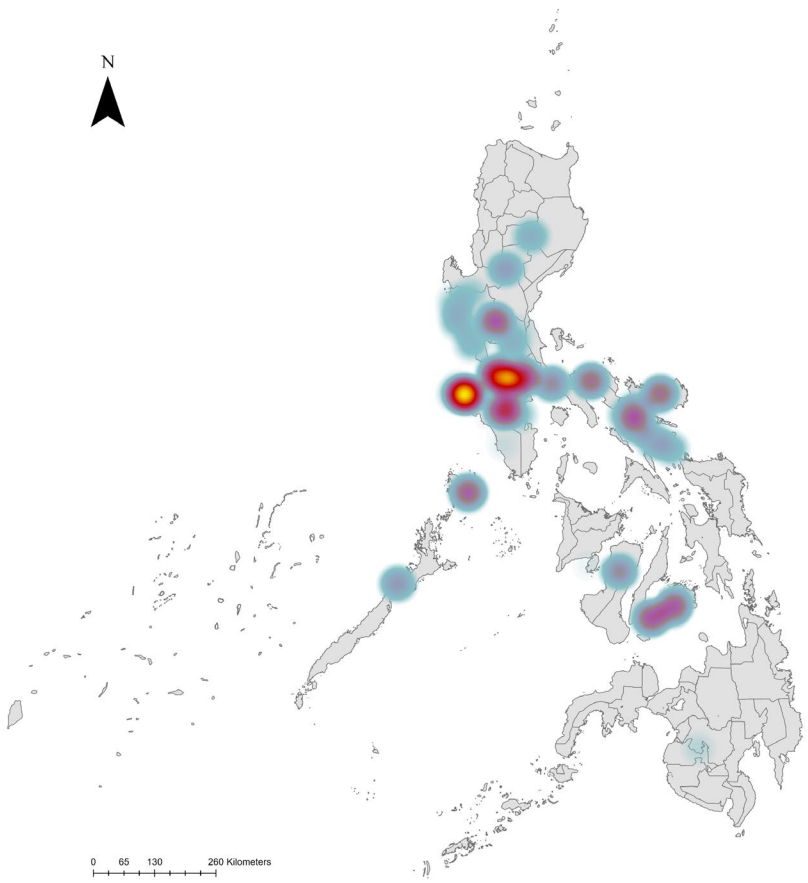

Figure 5. Heat map of the myxomycete collections in the Philippines.

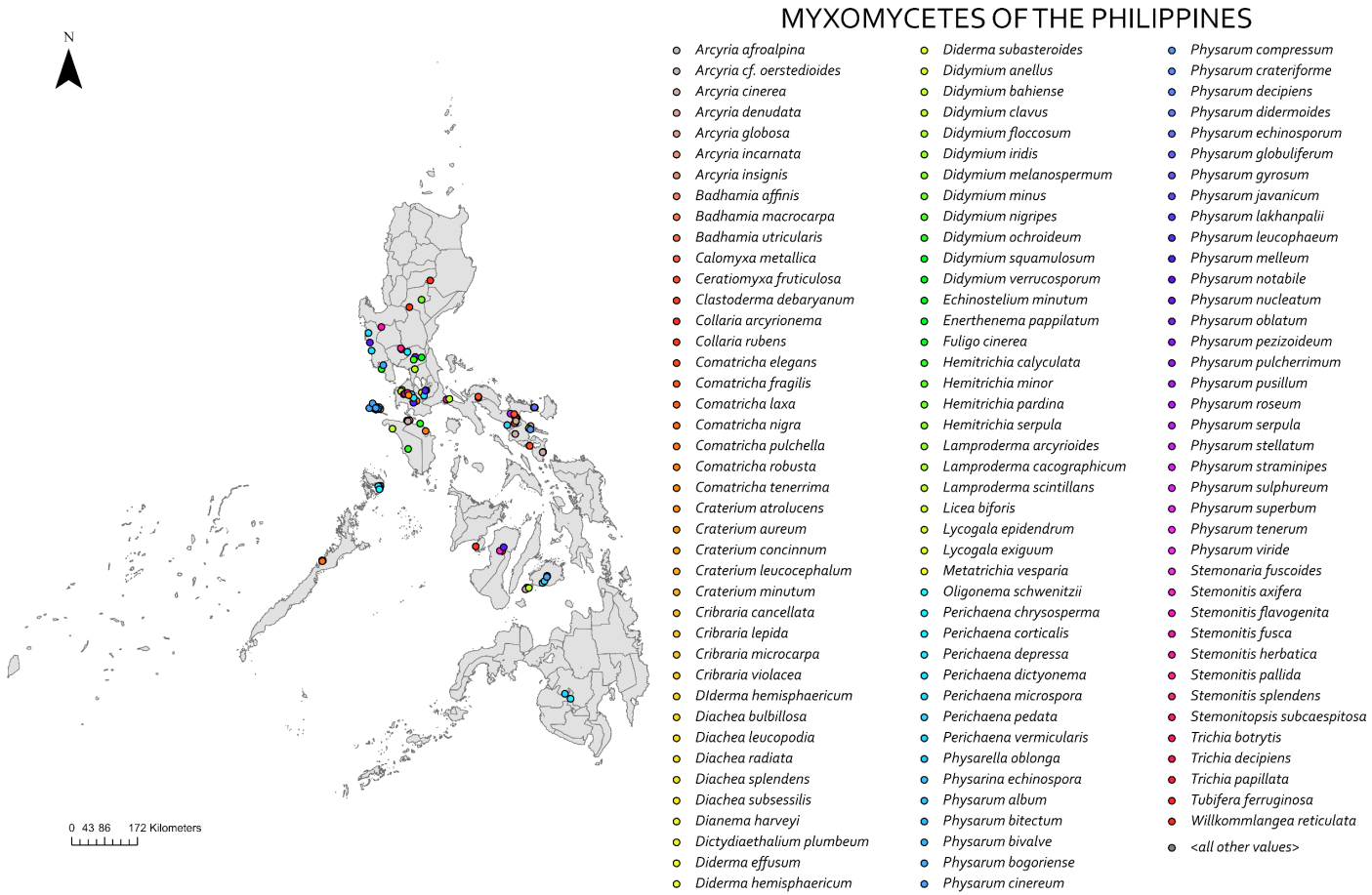

Figure 6. Map of the myxomycetes of the Philippines.

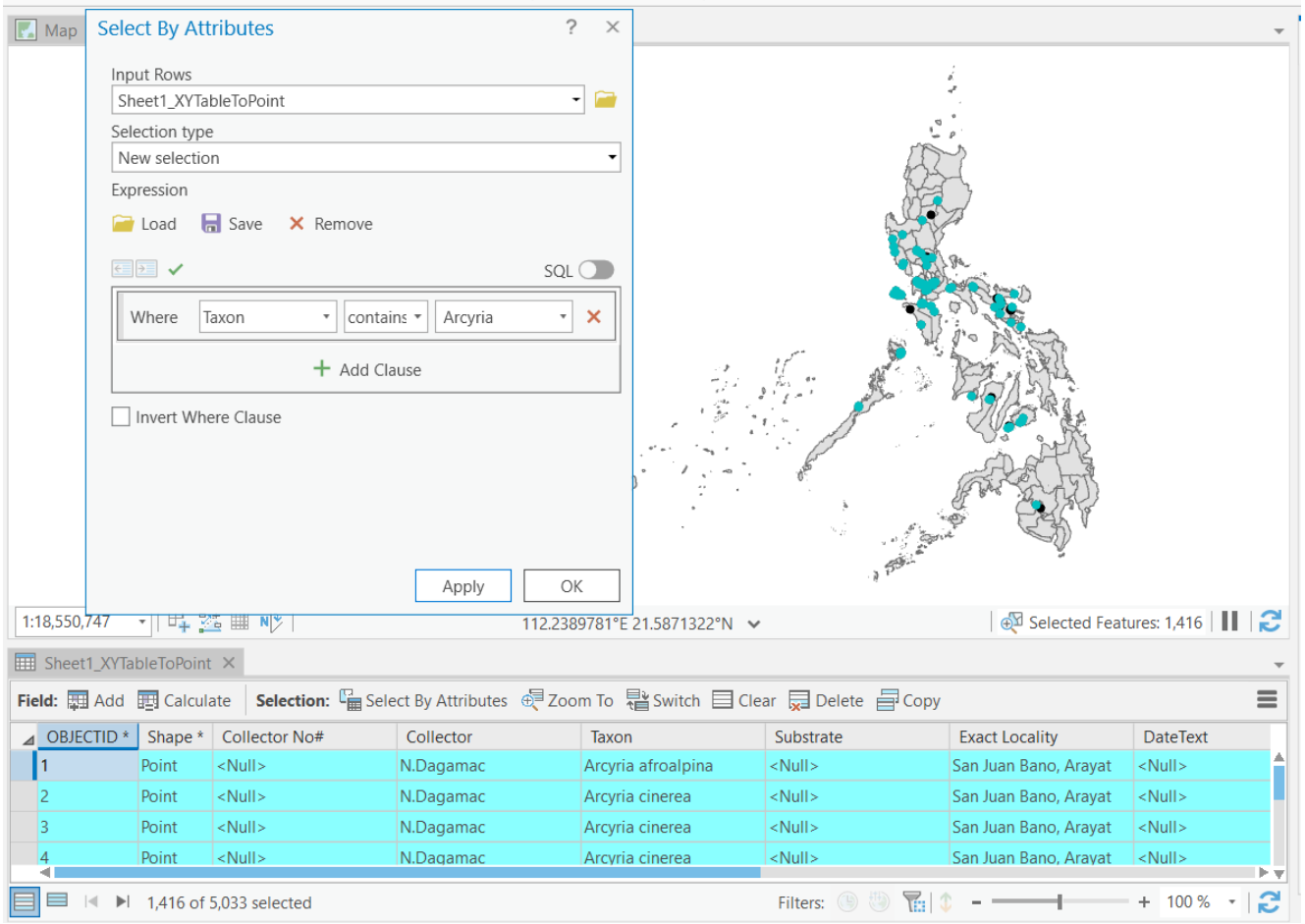

Figure 7. A sample query using features of the geocoded data, where the genus *Arcyria* was used to show how many are there within the database.

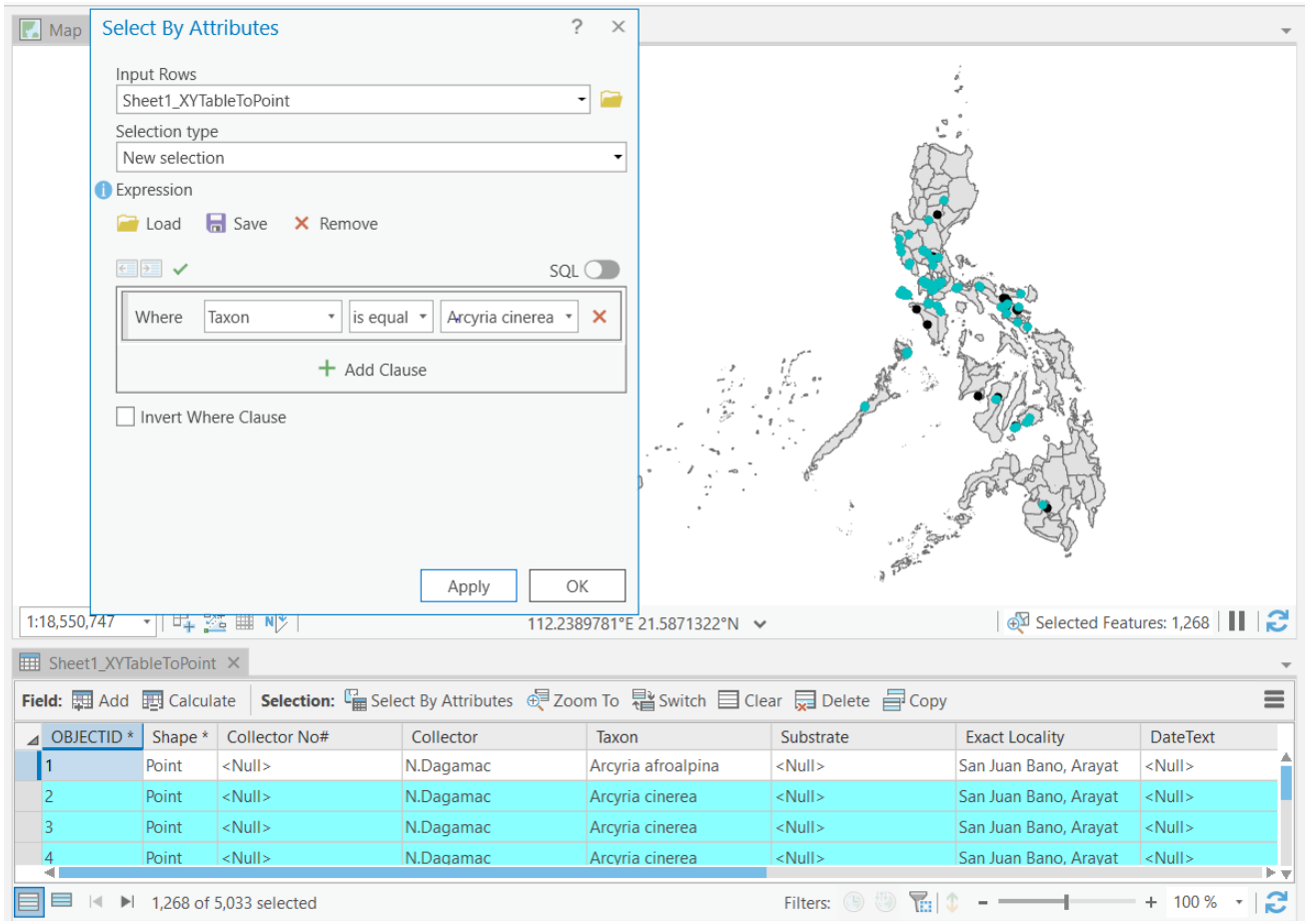

Figure 8. A sample query using features of the geocoded data, where the taxon *Arcyria cinerea* was used to show how many are there within the database.

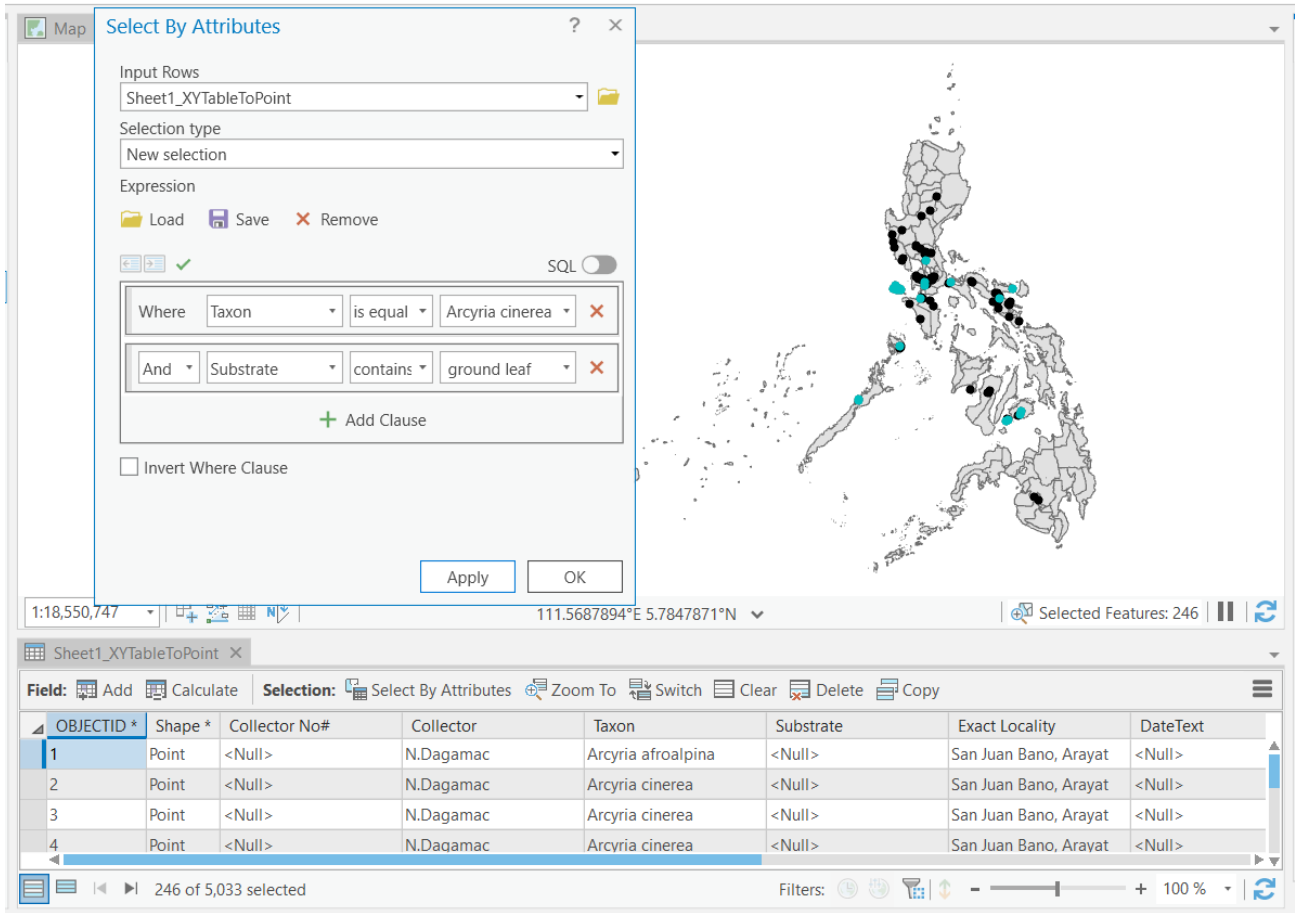

Figure 9. A nested query within the features of the geocoded data, where species followed by substrate associated were the identifiers utilized to narrow down a search.

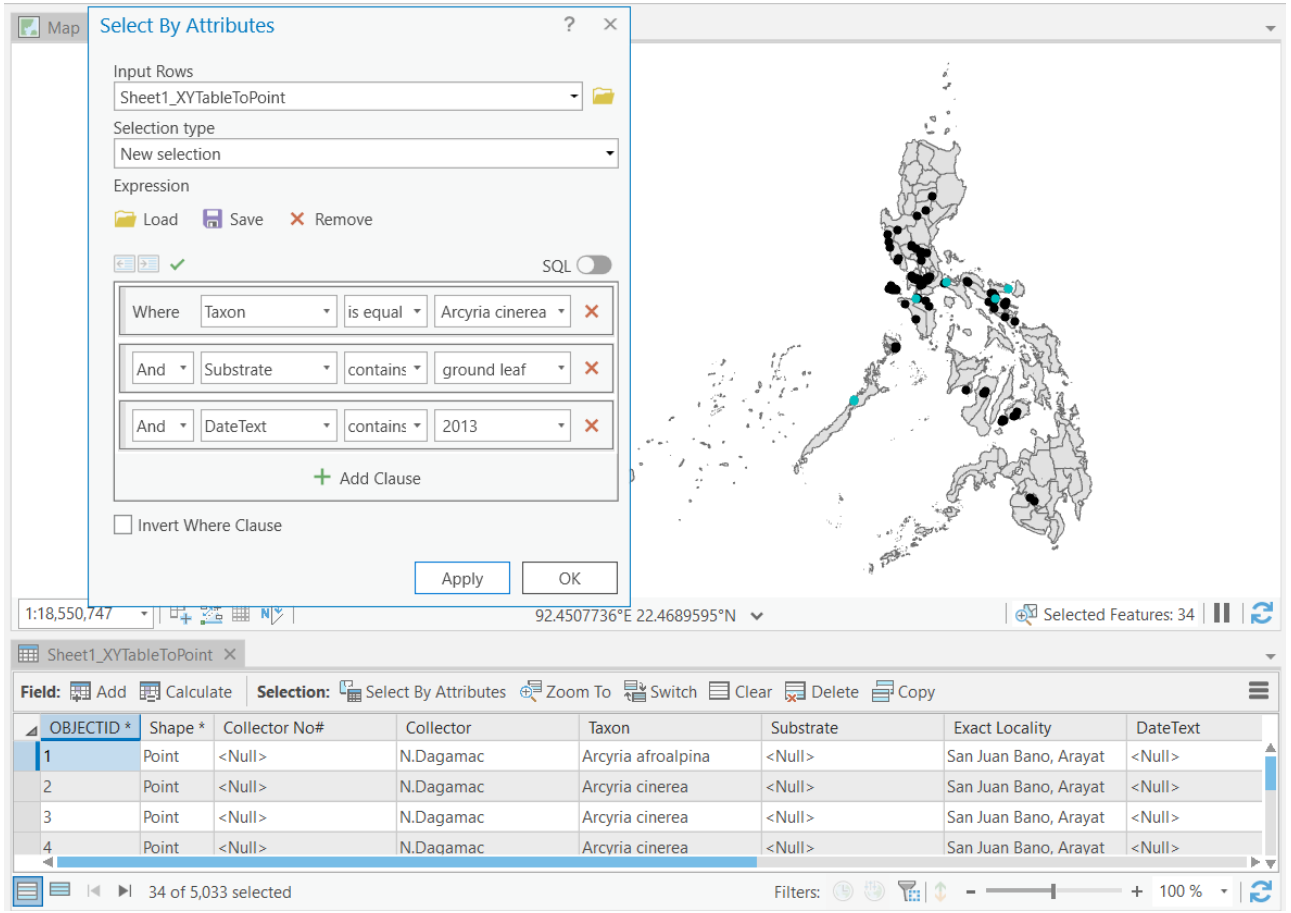

Figure 10. A nested query within the features of the geocoded data, where species followed by substrate associated and year were the identifiers utilized to narrow down a search.

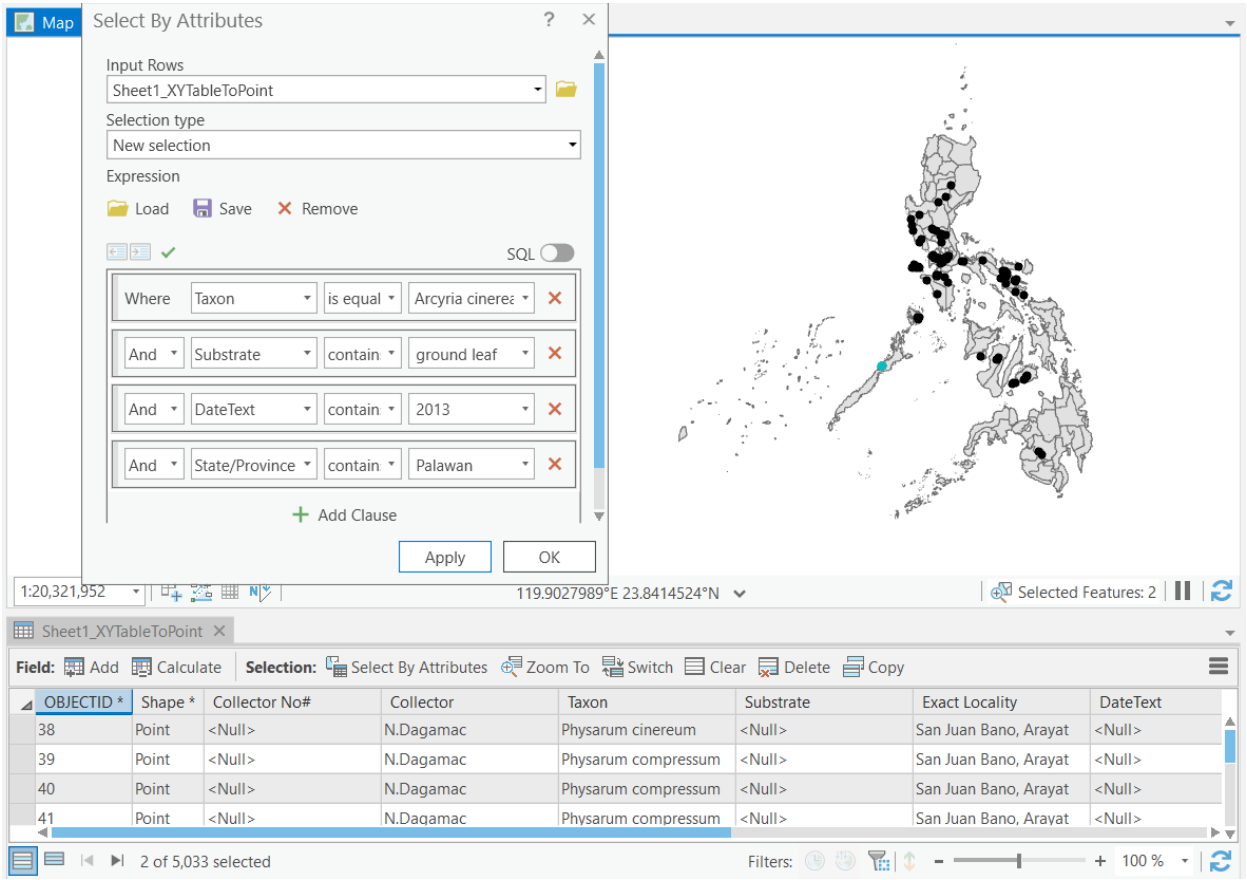

Figure 11. A nested query within the features of the geocoded data, where species followed by substrate associated, year, and State/Province were the identifiers utilized to narrow down a search.

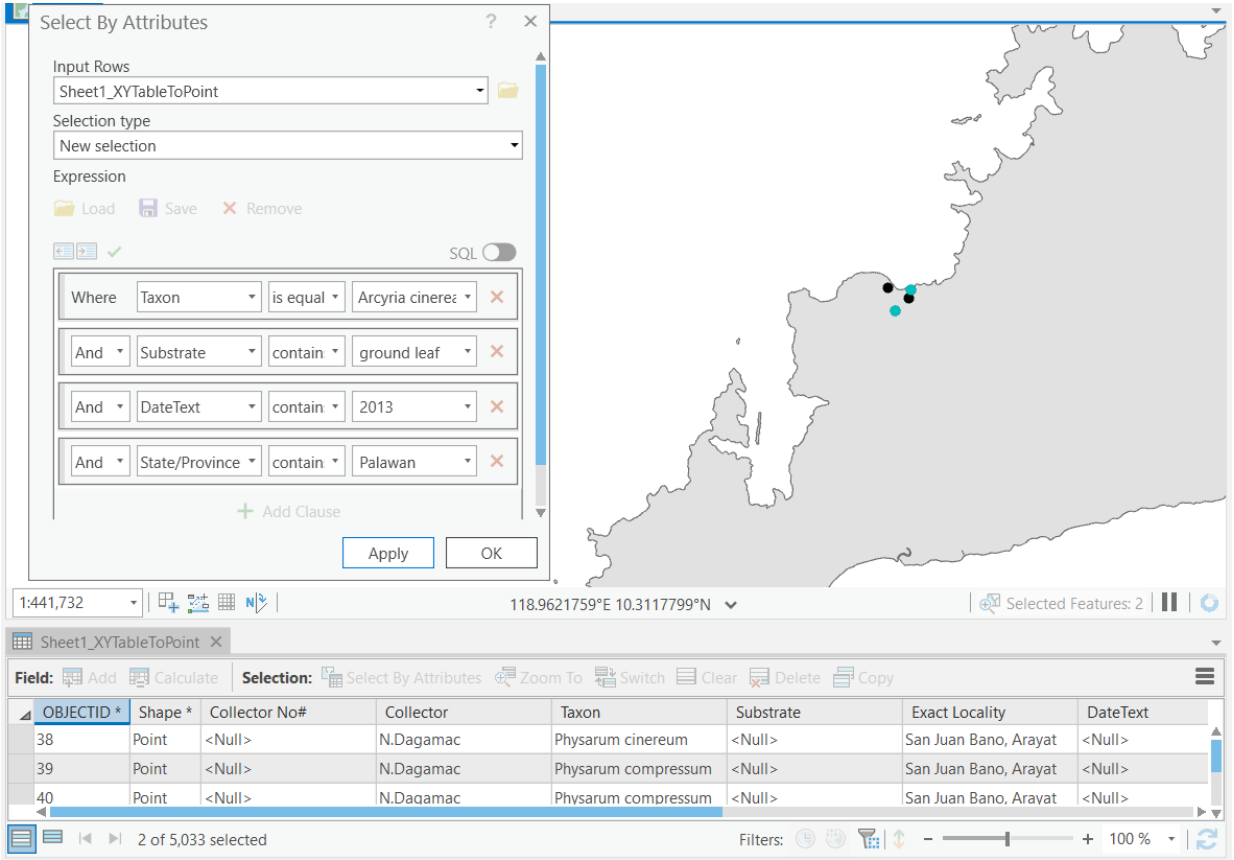

Figure 12. A sample image showing that the attributes are interactive by clicking on the “selected features” after a series of nested queries to narrow down a search.

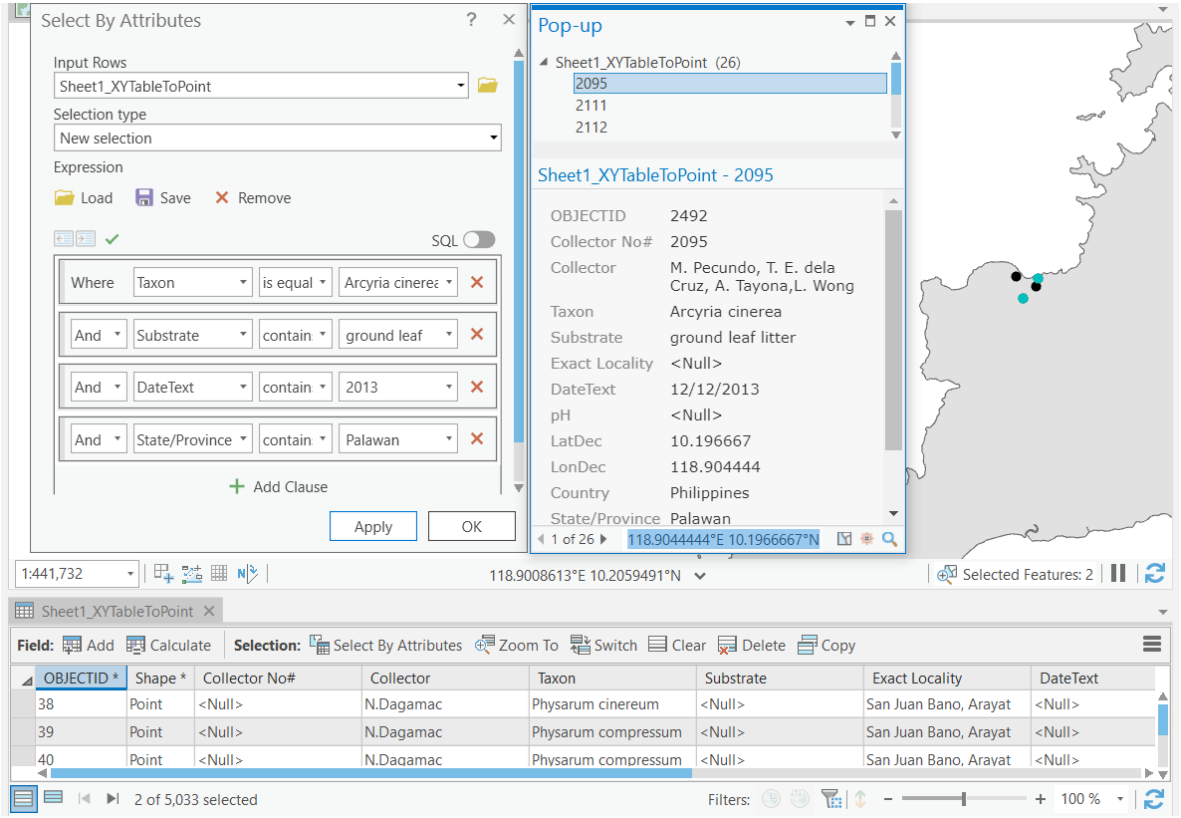

Figure 13. A sample image showing that the attributes are interactive, such that each point on the map displays the geocoded information when clicked/ticked.

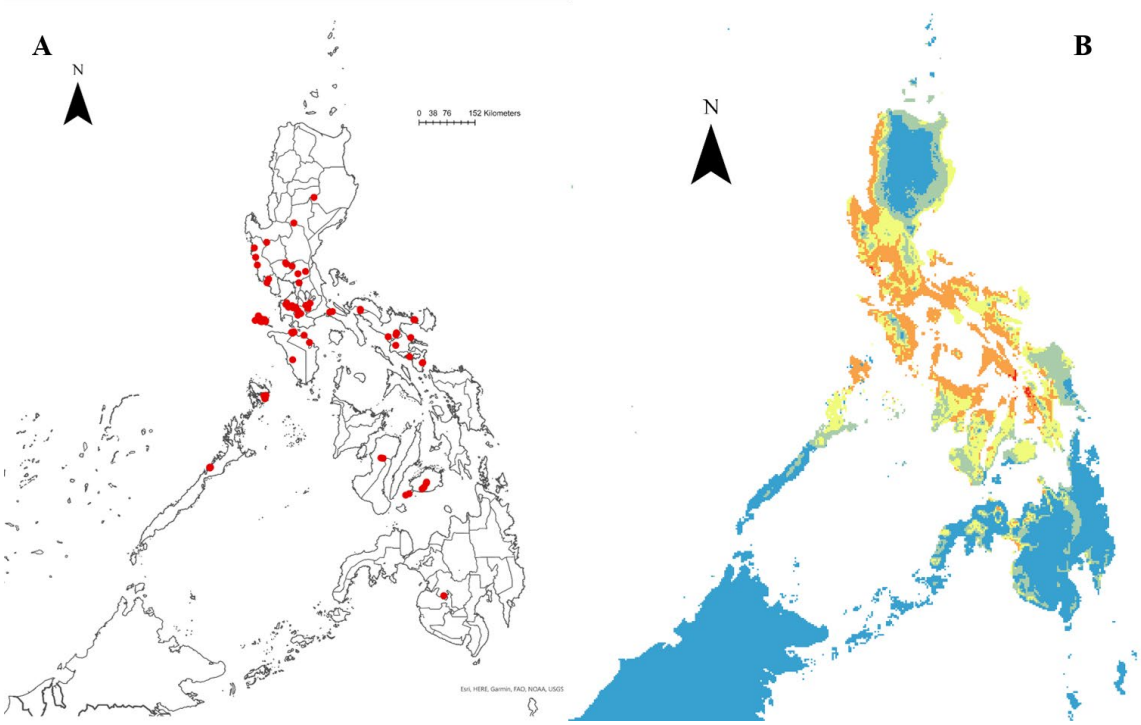

Fig. 14. Species distribution modeling of the morphospecies *Arcyria cinerea* (Bull.) Pers. collected in the Philippines based on the geocoded data in this study. (A) Mapped presence records used in training the model. (B) Predicted current species distribution of *A. cinerea* in the Philippines, where warmer colors indicate higher probability of occurrence.

VI. Does the Huxley's Line apply to myxomycetes?

Abstract

This paper considers whether Huxley's modification of the Wallace line represents a regional boundary affecting distribution in myxomycetes, using patterns of species composition from the territories of Borneo, Palawan, and the remainder of the Philippine archipelago. With a total of 30 species of myxomycetes belonging to 16 genera (TDI = 1.88), Borneo recorded the highest taxonomic diversity compared to Palawan with 56 species (TDI = 2.67) and the oceanic Philippines with 159 species (TDI = 4.18). Based on species composition, Borneo is more similar to Palawan (CC = 0.395) than it is to oceanic Philippines (0.254). However, Palawan is more similar to oceanic Philippines (0.502) than it is to Borneo. This suggests that Borneo and Palawan have a certain affinity in terms of species composition of myxomycetes, but Palawan still seems to have a higher community similarity to the remainder of the Philippine archipelago when compared to Borneo.

Introduction

Biogeographic studies in the Philippines have been limited to a relatively few taxa. The Philippines, an archipelago of more than 7,100 islands in the Western Pacific has three major island groupings—Luzon, Visayas, and Mindanao—which are all part of the Philippine biodiversity hotspot (Heaney 1998). The position of this region in "Wallacea" (*sensu* Dickerson et al. [1928] and defined below) remains a subject for biogeographical debate (Vallejo 2011). The greater islands of Luzon and Mindanao were formed during the Pleistocene. Islands such as Mindoro, Palawan, Sibuyan, Romblon, Tablas, Camiguin, the Batanes Islands, and the Babuyan Islands have

never been connected to Luzon, Mindanao, or other Visayan islands (Vallejo 2011). These islands are oceanic islands, except for Mindoro and Palawan.

The Palawan group of islands represents a fragment of the continental crust which became separated during the formation of the basin of the South China Sea. The tectonic histories of the Philippine islands have been described by Hall (1996, 1998) and Yumul (2008), and the Visayan islands by Dimalanta et al. (2006) and Yumul (2000). The evolution of the archipelago began in the Mesozoic when a fragment of the Asian continent separated, giving rise to Palawan. Continued seafloor spreading and formation of oceanic crust in the Oligocene and the Miocene gave rise to the South China Sea (Vallejo 2011). There is tectonic evidence that Palawan was connected to Borneo (Heaney 1998) for a time. Earlier studies and descriptions of the tectonic history of the Philippine islands suggest that this region could be a key to the basic concepts inherent in the biogeography of Wallacea.

Regarded as a relatively underdeveloped faunal transition zone in the Asian and Australian regions (Wallace 1880, Mayr 1976) and characterized by a notable degree of endemism, Wallacea is delimited as the region between Weber's Line to the east and Wallace's line to the west (Fig. 1). Huxley modified Wallace's line by including all the oceanic islands of the Philippines east of the line (Mayr 1976, Simpson 1977). Huxley (1868) supported Wallace's designation of Selat Lombok as the regional boundary in the lesser Sundas but extended the line as shown in Figure 1—northward between Kalimantan and the Sulu Archipelago, through the Mindoro Strait between Mindoro and the Calamian Group including Palawan, which is a part of the Philippines but generally agreed by researchers to be Asiatic in fauna, then stretching between the Philippine Batan Islands and Taiwan into the Pacific. Wallace never accepted the northern extension of this line; thus it is more properly referred to as "Huxley's Line," as was done by Scrivenor et al. (1943). The

main point relevant to this paper is that it assigns the Philippines (except for Calamian, Palawan, and adjacent small islands) either to the Australian Region or to a separate region between the Oriental and Australian. According to Simpson (1977), Huxley's line when readjusted to coincide with the edge of the Sunda shelf is a clear-cut boundary such that all faunas and islands to the west of that line definitely belong to the Oriental Region.

Earlier biogeographical studies are in agreement that Palawan served as an Asian corridor to the Philippines (Vallejo 2011). The Palawan group of islands is situated on the Sunda Shelf, separated from Borneo by a channel less than 150 m deep and from Mindoro by a channel more than 400 m deep. A number of researchers (e.g., Heaney 1986, Delacour & Mayr 1946) suggested that the Palawan group is faunistically a part of Borneo. There have been several organisms studied to illustrate patterns of species distribution in this region, but never myxomycetes.

Myxomycetes are eukaryotic amoebozoans that give rise to fruiting bodies, which are often visible to the naked eye and capable of producing spores that could be dispersed to potentially long distances. Studies by Kamono et al. (2009) and Ronikier & Lado (2015) have supported the long-distance dispersal of myxomycetes, even suggesting that cross continental dispersal events can take place (Stephenson et al. 2008, Dagamac et al. 2017a). Furthermore, studies on isolated islands show the lack of endemism due to dispersal in the Galapagos (Eliasson & Nannenga-Bremekamp 1983), Hawaii (Eliasson 1991), Macquarie Island (Stephenson et al. 2007), and Bohol (Macabago et al. 2017), to name a few. Throughout a few decades of often widely interrupted myxomycete surveys carried out in the Philippines, only a handful have included the islands of Palawan. Similarly, in Borneo there have only been two published studies recording myxomycetes prior to 1998.

The present study attempted to determine if regional boundaries occur in known assemblages of myxomycetes relative to Huxley's modification of the Wallace line by way of assessing distributional patterns, including similarities and disparities in species composition among the assemblages of myxomycetes in three different localities—Palawan, Borneo, and the oceanic Philippines.

Materials and Methods

Collection of data. A combination of published and digitally sourced data was used in this study. These sources were tallied separately for each of the three localities considered (Fig. 2): Borneo, the Palawan group of islands (or sometimes referred to as Palawan in the succeeding text), and the oceanic Philippines (i.e., the Philippine archipelago excluding the islands of Palawan).

Borneo. There were three major sources of data for Bornean myxomycetes. These were a published paper by Ing and Spooner (1998), the Global Biodiversity Information Facility (www.gbif.org), and the Malaysian Borneo website (http://www.fungiofmalaysianborneo.com/). The paper by Ing and Spooner (1998) cross-referenced two other studies that added to the list of myxomycetes from Borneo—a study by Peregrine and Kassim bin Ahmad (1982) that noted three species presumably associated with plant diseases and Pegler (1997) noting two myxomycetes, one of which was identified only to the genus level and the other reported previously. These three studies listed a total of 28 species of myxomycetes. The virtual repositories of the Planetary Biodiversity Inventory Eumycetozoan Database (1994) through the Global Biodiversity Information Facility (www.gbif.org) noted the occurrence of *Paradiachea cylindrica*, and the personal website: http://www.fungiofmalaysianborneo.com/ added *Lycogala epidendrum* to the list of 30 Bornean myxomycetes.

Palawan. The papers that were the sources of data for Palawan were Reynolds (1981), with the first 26 species of myxomycetes including collections from previous studies, Pecundo et al. (2017), which added 22 species, and Macabago et al. (2020a), with eight more species, for a total of 56 species of myxomycetes.

Oceanic Philippines. There were four major papers used as sources. These were Dagamac and dela Cruz (2015), in which the authors summarized 149 species from previous studies, Macabago et al. (2017, 2020b), with nine new records of myxomycetes, and Bernardo et al. (2018), with one addition, for a total of 159 species. The combination of collections from Palawan and oceanic Philippines totaled 161 species for the entire Philippines.

Analysis of data. A list of all known or recorded species of myxomycetes for each locality was compiled by collating the various sources mentioned above. These lists included both natural field collections and moist chamber culture collections. Whether a myxomycete record from every source was associated with a certain substrate (specific plant material or other organic microhabitats) or with other parameters considered in the specific independent research and/or digital repository was not taken into account in this study because of the evident disparities either in sampling technique or overall data gathered in most, if not all, sources. These lists were then used as a basis for comparisons of species composition and taxonomic diversity, and also to analyze the similarities in the assemblages of myxomycetes in the three localities.

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

Pairwise comparisons of myxomycete assemblages were performed using Sorensen's coefficient of community (CC) index as described by Stephenson (1989). The CC index is based on the presence or absence of species in the two communities being compared, with values ranging from 0 to 1, where lower CC values imply less similar communities.

Results

A list of myxomycetes exclusively present in each locality and shared by two or all three localities are illustrated on Fig. 3. Table 1 shows the total number of species collected thus far from all the territories.

In terms of taxonomic diversity, Borneo recorded a higher taxonomic diversity index (TDI) value than Palawan or the remainder of the Philippines (Table 1), which indicates that although Borneo has the fewest number of species (30), these are distributed in relatively more diverse genera (16) than in either of the other regions.

A total of 167 species were collectively recorded for all three localities (Borneo, Palawan, and the oceanic Philippines). Seventeen of these species (10.2% of total number listed) were shared among the three localities as shown on the intersection of all three circles in the Venn Diagram (Fig. 3). These were *Arcyria cinerea, Arcyria denudata, Ceratiomyxa fruticulosa, Comatricha nigra, Cribraria cancellata, Cribraria microcarpa, Diderma effusum, Hemitrichia serpula, Lycogala epidendrum, Perichaena depressa, Physarum cinereum, Physarum compressum, Physarum pusillum, Physarum stellatum, Physarum viride, Stemonitis splendens,* and *Stemonitopsis typhina*. These are the same species shared between Borneo and Palawan (24.6% of the total 69 species), which means there are no myxomycetes present exclusively in both Borneo and Palawan that are not recorded in oceanic Philippines.

However, between Borneo and oceanic Philippines there are more species shared (24, or 14.5% of 165 total). These were the seventeen that were present in all three localities plus *Didymium megalosporum, Didymium melanospermum, Fuligo septica, Physarella oblonga, Physarum flavicommum, Physarum globuliferum,* and *Physarum tenerum.* These were the same species shared between Borneo and the Philippines as a whole (14.4% of the 167 total).

In contrast, Palawan shared 54 species (33.5% of 161 total) of myxomycetes with the oceanic Philippines. These were the seventeen shared by all three above and the 37 shared exclusively by Palawan and oceanic Philippines (see Fig. 3).

Six species of myxomycetes (20% of species listed for Borneo) were recorded exclusively from Borneo. These were *Cribraria languescens, Erionema aureum, Lycogala terrestre, Paradiachea cylindrica, Physarum digitatum*, and *Stemonitopsis gracilis*. Two species (3.6% of species listed thus far for Palawan) were recorded only for Palawan. These were *Badhamia macrocarpa* and *Diachea subsessilis*. Ninety-eight (61.6%) of the total species listed for the oceanic Philippines were unique to this locality, meaning they were not found in either Borneo or Palawan, while 137 (85.1%) species were found only in the Philippines but not in Borneo.

In order to more intuitively measure similarities in the assemblages of myxomycetes in each locality, the Sorensen's coefficient of community (CC) was used. Since CC considers the species of myxomycetes common to both communities being compared relative to the total number of species occurring in both, this calculation is appropriate for a presence/absence data such as the one used in this study. The communities displaying CC values closest to 1 indicate higher similarity. Taking into consideration the records for each locality and the species they share with the other localities, CC values revealed that the assemblage of myxomycetes in Borneo is more

similar to Palawan (Table 2) than it is to oceanic Philippines or the remainder of the Philippines (including Palawan). However, Palawan is more similar to the oceanic Philippines than it is to Borneo.

Discussion

The Philippines has a largely Sunda biotic component with notable Wallacean, New Guinea, and Australian elements (Vallejo 2011) in its biota. The apparent and at times conflicting interpretations from previous studies that mostly featured macro-organisms (i.e., animals and plants) concur with the seemingly debatable and unsettled place of the Philippines in Wallacea. A study on mosses by Tan (1996) indicated that the Palawan moss flora is mainly an extension of the flora of the Philippines, with some affinity with Java and the lesser Sundas, while showing little influence from Borneo. Likewise, earlier studies by Holloway & Jardine (1968) and Holloway (1987) showed through a phenetic study of Indo-Australian butterflies that Palawan did not exclusively cluster with Borneo (or even the Greater Sundas). Instead, it grouped together with the collective Greater and Lesser Sunda islands like Burma (now Myanmar), Indochina, and Malaya. These coincide with Cracraft's (1989) conclusion that Palawan lacked a "special" relationship with Borneo but was instead a peripheral part of Sundaland. On the contrary, a study by Schuh & Stonedahl (1986) presented an area cladogram, based on the distributions of various members of the order Hemiptera, wherein some parts of southern Philippines are shown linking with North Borneo before linking with northern Philippines (Vane-Wright 1990). In addition, Tan (1998) stated that some areas of Borneo and Palawan possessing an assemblage of uncommon moss taxa sharing similar ecological preferences and same pattern of distribution have been

interpreted as a refugium for species in the Tertiary moist and the Quaternary dry forests (Meijer 1982, Tan 1996).

Based on the results obtained in the present study, the myxomycetes show an extent of similarity between Borneo and Palawan, but not one that exhibits convincing endemism. This could very well be a provisional statement due to the intricate nature of fruiting for myxomycetes (Schnittler et al. 2017), such that when conditions are deemed inapt they can, in theory, exist in other forms (i.e., amoeboid) that will inhibit morphological identification of existing species. Borg Dahl et al. (2018) showed that there is greater diversity of myxomycetes in the soil than the fructifications show. They also provided evidence that fruiting success of nivicolous myxomycetes depended on previous temperature and snow regime. In a biogeographic study of protosteloid amoebae, Aguilar and Lado (2012) also displayed that differences in climatic conditions and microhabitat preferences caused variations in species composition and structure of assemblages from one area to another.

Despite the shared geologic history of Palawan and Borneo there is evidence of disjunction in species composition between these two territories. Whether or not the Philippines' Palawan is faunistically and/or floristically Bornean, or its islands more likely served as a refugia of Sundaland species, it is possible that subsequent climate changes after the Pleistocene have led to habitat, vegetation, abiotic and biotic environment differences, and possible vicariant speciation events contributing to the variances in species composition and diversity in this area. A phylogeographic study of a myxomycete population (Dagamac et al. 2017b) showed limited gene flow that may lead to allopatric speciation because of geographical barriers and reproductive isolation between ribotypes, and ultimately displaying an evidence of an on-going speciation.

Considering the size of Palawan, it has been proven to be rich in plant and animal species while still being explored more in terms of extant myxomycetes and other microscopic taxa. Based on this study alone Borneo seems to have a stronger affinity to Palawan than the rest of the Philippines; however, it does not seem to show Palawan as the northern limit to a regional boundary, such as the Huxley's modification of Wallace's line. To conclude that the known biogeographic region delineated by Huxley's line does or does not apply to myxomycetes is not yet substantiated at this point. Initial studies, such as this, allows for the conception of more relevant approaches in answering biogeographic questions. Although myxomycetes are microorganisms and are likely capable of long-distance dispersal, which would in theory render oceans ineffective as barriers, the results here suggest that more intricate methods such as environmental niche modeling (e.g., Aguilar et al. 2014, Rojas et al. 2015) and other more indicative protocols, such as phylogeographic endeavors (see Dagamac et al. 2017b) discerning if myxomycete species are restricted by ecological/environmental conditions or by geographic barriers, are needed to reach conclusive results. Clearly, this is a subject that warrants additional study. What could prove to be useful in this endeavor is to include metadata of Australian and Papuan biota and other Oriental/Asian territories to demonstrate if indeed islands to the west of Huxley's line, like Palawan and Borneo, cluster with the oriental (Simpson 1977) region and in the process, provide explanation on the factors driving species diversification or lack thereof.

Literature Cited

Aguilar, M., A. M. Fiore-Donno, C. Lado, and T. Cavalier-Smith. 2014. Using environmental niche models to test the 'everything is everywhere' hypothesis for *Badhamia*. The ISME journal 8(4):737–745.

Bernardo, J. L. M., J. L. Q. Arioder, K. J. Almadrones-Reyes, and N. H. A. Dagamac. 2018. Myxomycetes communities occurring in fragmented forest patches in two municipalities of Laguna, Philippines. Community Ecology 19:289–299.

Borg Dahl, M., O. Shchepin, C. Schunk, A. Menzel, Y. Novozhilov, and M. Schnittler. 2018. A four-year survey reveals a coherent pattern between occurrence of fruit bodies and soil amoebae populations for nivicolous myxomycetes. Scientific Reports 8 (1).

Cracraft, J. 1988. From Malaysia to New Guinea: Evolutionary biogeography within a complex continent-island arc contact zone. Pages 2582-2593 Vol. 2 in H. Ouellet, ed. Proceedings of the 19th International Ornithological Congress. Univ. Ottawa Press, Ottawa.

Dagamac, N. H. A., and T. E. E. dela Cruz. 2015. Myxomycetes research in the Philippines: Updates and opportunities. Mycosphere 6(6):784-795.

Dagamac, N. H. A., Y. Novozhilov, S. L. Stephenson, C. Lado, C. Rojas, T. E. E. dela Cruz, M. Unterseher, and M. Schnittler. 2017a. Biogeographical assessment of myxomycete assemblages from Neotropical and Asian Palaeotropical forests. Journal of Biogeography 44:1524-1536.

Dagamac, N. H. A., C. Rojas, Y. Novozhilov, G. Moreno, R. Schlüter, and M. Schnittler. 2017b. Speciation in progress? A phylogeographic study among populations of *Hemitrichia serpula* (Myxomycetes). PLOS ONE 12(4):e0174825.

Delacour, J., and E. Mayr. 1946. Birds of the Philippines. Macmillan, New York. 309 pp.

Dickerson R., E. D. Merrill, R. C., McGregor, W. Schultze, E. H. Taylor, and A. W. Herre. 1928. Distribution of Life in the Philippines. Manila, Bureau of Science. 322 pp.

Dimalanta C. B., L. Suerte, G. Yumul, R. Tamayo, and E. Ramos. 2006. A Cretaceous supra-subduction oceanic basin source for Central Philippine ophiolitic basement complexes: Geological and geophysical constraints. Geosciences Journal 10 (3):305-320.

Eliasson, U., and Nannenga-Bremekamp, N. 1983. Myxomycetes of the scalesia forest, Galapagos Islands, Ecuador. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C Biological and Medical Sciences 86:143-154.

Eliasson, U. 1991. The myxomycete biota of the Hawaiian Islands, USA. Mycological Research 95:257-267.

Fungi of Malaysia web site. http://www.fungiofmalaysianborneo.com/ (accessed on 20 July 2020).

Global Biodiversity Information Facility. https://www.gbif.org/occurrence/78622228 (accessed on 20 July 2020).

Hall, R. 1996. Reconstructing Cenozoic SE Asia. Pages 153-184 in Hall R. and D. Blundell, eds. Tectonic Evolution of Southeast Asia 1. Geological Society of London, London.

Hall, R. 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. Pages 99-131 in R. Hall and J. D. Holloway, eds. Biogeography and Evolution of SE Asia. Backhuys Publisher, Leiden.

Heaney, L. R. 1986. Biogeography of mammals in SE Asia: estimates of rates of colonization, extinction and speciation. Biological Journal of the Linnean Society 28:127-165.

Heaney, L. R. 1998. The origins and dimensions of biodiversity in the Philippines. Pages 12-22 in: L. R. Heaney and J. C. Regalado Jr., eds. Vanishing Treasures of the Philippine Rainforest. Chicago, Field Museum of Natural History.

Holloway, J. D., and N. Jardine. 1986. Two approaches to zoogeography: a study based on the distributions of butterflies, birds and bats in the Indo- Australian area. Proceedings of the Linnean Society of London 179:153-188.

Holloway, J. D. 1987. Lepidoptera patterns involving Sulawesi: what do they indicate of past geography? Pages 103-118 in T.C. Whitmore, ed. Biogeographical evolution of the Malay Archipelago. Clarendon Press, Oxford.

Huxley, T. H. 1868. On the Classification and Distribution of the Alectoromorphae and Heteromorphae. Proceedings of the Zoological Society of London 1868:294- 319.

Ing, B., and B. Spooner. 1998. Myxomycetes from Brunei Darussalam. Kew Bulletin 53(4):829-834.

Kamono, A., H. Kojima, J. Matsumoto, K. Kawamura, and M. Fukui. 2009. Airborne myxomycete spores: detection using molecular techniques. Naturwissenschaften 96:147–151.

Macabago, S. A. B., N. H. A. Dagamac, T. E. E. dela Cruz, and S. L. Stephenson, 2017. Implications of the role of dispersal on the occurrence of litter-inhabiting myxomycetes in different vegetation types after a disturbance: a case study in Bohol Islands, Philippines. Nova Hedwigia 104 (1-3):221–236.

Macabago, S. A. B., T. E. E. dela Cruz, and S. L. Stephenson. 2020a. Myxomycetes of Coron Island and additions to the myxomycetes of Palawan group of islands in the Philippines. Karstenia 28 (58):157-167.

Macabago, S. A. B., N. H. A. Dagamac, T. E. E. dela Cruz, and S. L. Stephenson. 2020b. Myxomycetes of the Caramoan Islands and an update on the species found in the Bicol Peninsula, Philippines. Philippine Journal of Systematic Biology 14(1).

Mayr E. 1976. Wallace's Line in the Light of Recent Zoogeographic Studies. Pages 626-645 in E. Mayr, ed. Evolution and Diversity of Life. Harvard University Press, Cambridge Massachusetts.

Meijer, W. 1982. Plant refuges in the Indo-Malesian region. Pages 576-584 in G.T. Prance, ed. Biological Diversification in the Tropics. Columbia University Press, New York.

Pecundo, M. H., N. H. A. Dagamac, S. L. Stephenson, and T. E. E. dela Cruz, 2017. First myxomycete survey in the limestone forest of Puerto Princesa Subterranean River National Park, Palawan, Philippines. *Nova Hedwigia* 104 (1-3):129–141.

Pegler, D. N. 1997. The Larger Fungi of Borneo. Natural History Publications, Sabah.

Peregrine, W. H., and Kassim bin Ahmad. 1982. Brunei: a first annotated list of plant diseases and associated organisms. Phytopathological Papers 27.

Reynolds, D. R. 1981. Southeast Asian myxomycetes II. Philippines. Philippine Journal of Biology 10 (2-3):127-150.

Rojas, C, J. M. Zuñiga, and S. L. Stephenson. 2015. Ecological niche modeling of some Costa Rican myxomycetes. Curr Res Environ App Mycol 5:153–159.

Ronikier, A., and C. Lado. 2015. Nivicolous Stemonitales (Myxomycetes) from the Austral Andes. Analysis of morphological variability, distribution and phenology as a first step towards testing the large-scale coherence of species and biogeographical properties. Mycologia 107:258–283.

Schnittler, M., N. H. A., Dagamac, and Y. K. Novozhilov. 2017. Biogeographical Patterns in Myxomycetes. Pages 299-331 in S. L. Stephenson and C. Rojas, eds. Myxomycetes: Biology, Systematics, Biogeography, and Ecology. Academic Press, Cambridge, Massachusetts. 474 pp.

Schuh, R.T., and G. M. Stonedahl. 1986. Historical biogeography in the Indo-Pacific: a cladistics approach. Cladistics 2:337-355.

Scrivenor, J. B., I. H. Burkill, M. A. Smith, A. S. Corbet, H. K. Airey Show, P. W. Richards, and F. E. Zeuner. 1943. A Discussion of the Biogeographic Division of the Indo-Australian Archipelago with criticism of the Wallace and Weber Lines and of any other dividing lines and with an attempt to obtain uniformity in the names used for the divisions. Proc. Linnean Soc. London 154:120-165.

Simpson, G.G. 1977. Too many lines. Proceedings of the American Philosophical Society 121 (2):107-120.

Stephenson, S. L. 1989. Distribution and ecology of myxomycetes in temperate forests. II. Patterns of occurrence on bark surface of living trees, leaf litter, and dung. Mycologia 81:608-621.

Stephenson, S. L., I. Kalyanasundaram, and T. Lakhanpal. 1993. A comparative biogeographical study of myxomycetes in the mid-Appalachians of eastern North America and two regions of India. Journal of Biogeography 20:645-657.

Stephenson, S.L., G. A. Laursen, and R. D. Seppelt. 2007. Myxomycetes of subantarctic Macquarie Island. Australian Journal of Botany 55:439–449.

Stephenson, S.L., M. Schnittler, and Y. K. Novozhilov. 2008. Myxomycete diversity and distribution from the fossil record to the present. Biodiversity and Conservation 17:285-301.

Tan, B. C. 1996. Biogeography of Palawan mosses. Australian Systematic Botany 9:193:203.

Tan B. C. 1998. Noteworthy disjunctive patterns of Malesian mosses. Pages 235-241 in: R. Hall and J. D. Holloway, eds. Biogeography and Evolution of SE Asia. Backhuys Publisher, Leiden.

University of Arkansas. Planetary Biodiversity Inventory Eumycetozoan Database. Occurrence dataset https://doi.org/10.15468/zxmhsy accessed via GBIF.org on 13 July 2020.

Vallejo, B. 2011. The Philippines in Wallacea. Pages 27-42 in D. M. Telnov, ed. Biodiversity, Biogeography and Nature Conservation in Wallacea and New Guinea. Entomological Society of Latvia, Riga.

Vane-Wright, R. I. 1990. The Philippines — Key to the Biogeography of Wallacea. Pages 19-34 in W. J. Knight and J. D. Holloway, eds. Insects and the rain forests of South East Asia (Wallacea). Royal Entomological Society, London.

Wallace, A. R. 1880. Island Life: The Phenomena and Causes of Insular Faunas and Floras. Macmillan and Co., London.

Yumul, G. P., C. B. Dimalanta, R. A. Tamayo, and J. A. L. Baretto. 2000. Contrasting morphological trends of islands in Central Philippines. The Island Arc 9(4):627-637.

Yumul G.P., C. B. Dimalanta, V. B. Maglambayan, and E. J. Marquez. 2008. Tectonic setting of a composite terrane: a review of the Philippine island arc system. Geosciences Journal 12:7-17.

Tables

Table 1. Taxonomic diversity index (TDI) values for the localities being studied, where the number of species was divided among the number of genera (S/G). A higher value for TDI indicates a lower taxonomic diversity.

	Species	**Genera**	**TDI**
Borneo	30	16	1.88
Palawan	56	21	2.67
Oceanic Philippines	159	38	4.18
Philippines	161	38	4.24

Table 2. Sorensen's Coefficient of Community (CC) values showing similarities in community composition, where the number closest to 1 shows the highest similarity.

Localities	**CC**
Borneo - Palawan	*0.395*
Palawan - Oceanic Philippines (oPH)	*0.502*
Borneo - Oceanic Philippines (oPH)	*0.254*
Borneo - Philippines (PH)	*0.251*

Figures

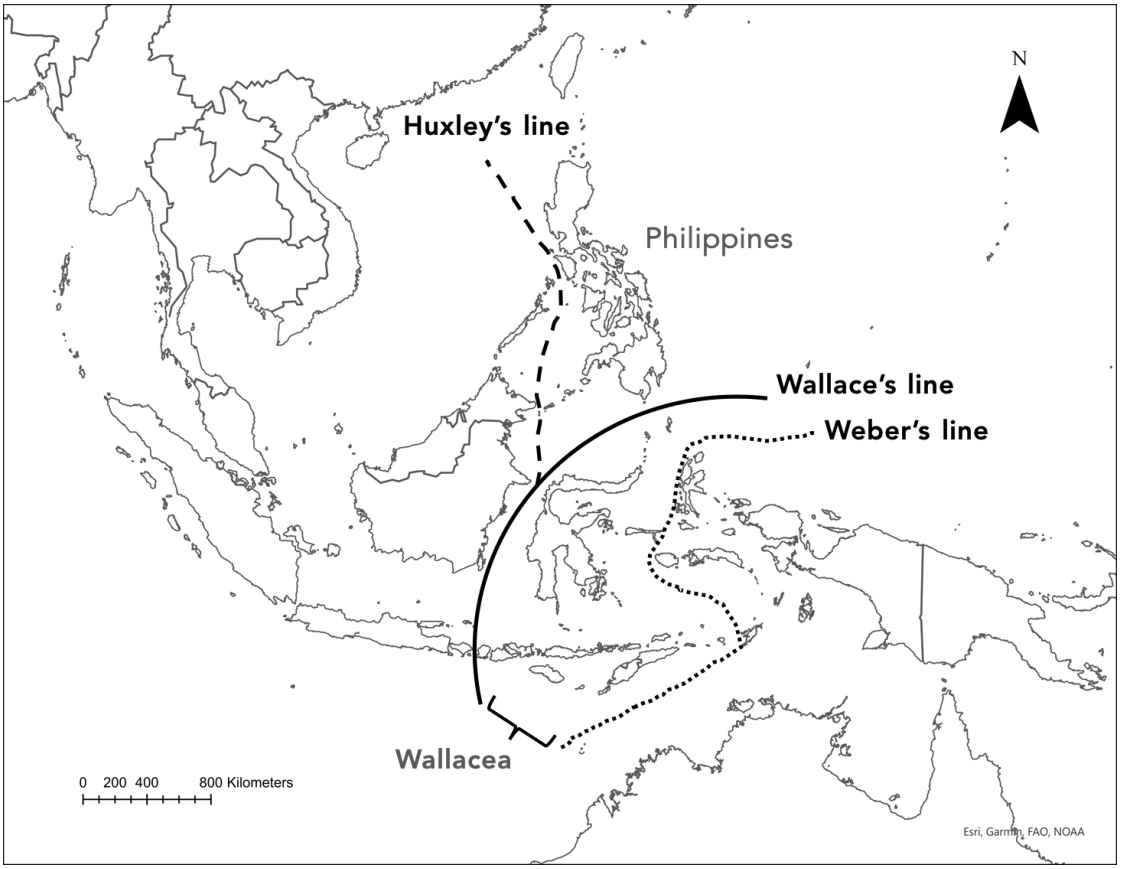

Figure 1. Map showing the Philippines relative to some biogeographical boundaries/lines in the Southeast Asian region. Image generated using the software ArcGIS Pro.

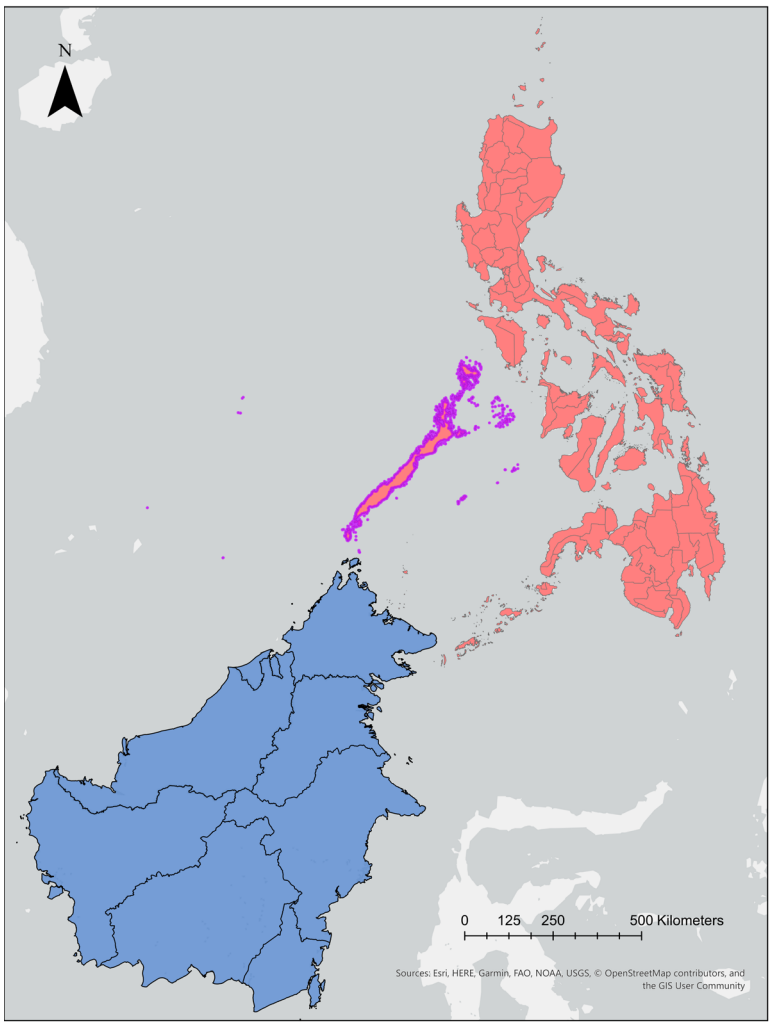

Figure 2. Map showing the respective positions of Borneo (blue, lower left) and the Philippines (red, upper right), including the Palawan group of islands (lined in purple, mid-frame). Image generated using the software ArcGIS Pro.

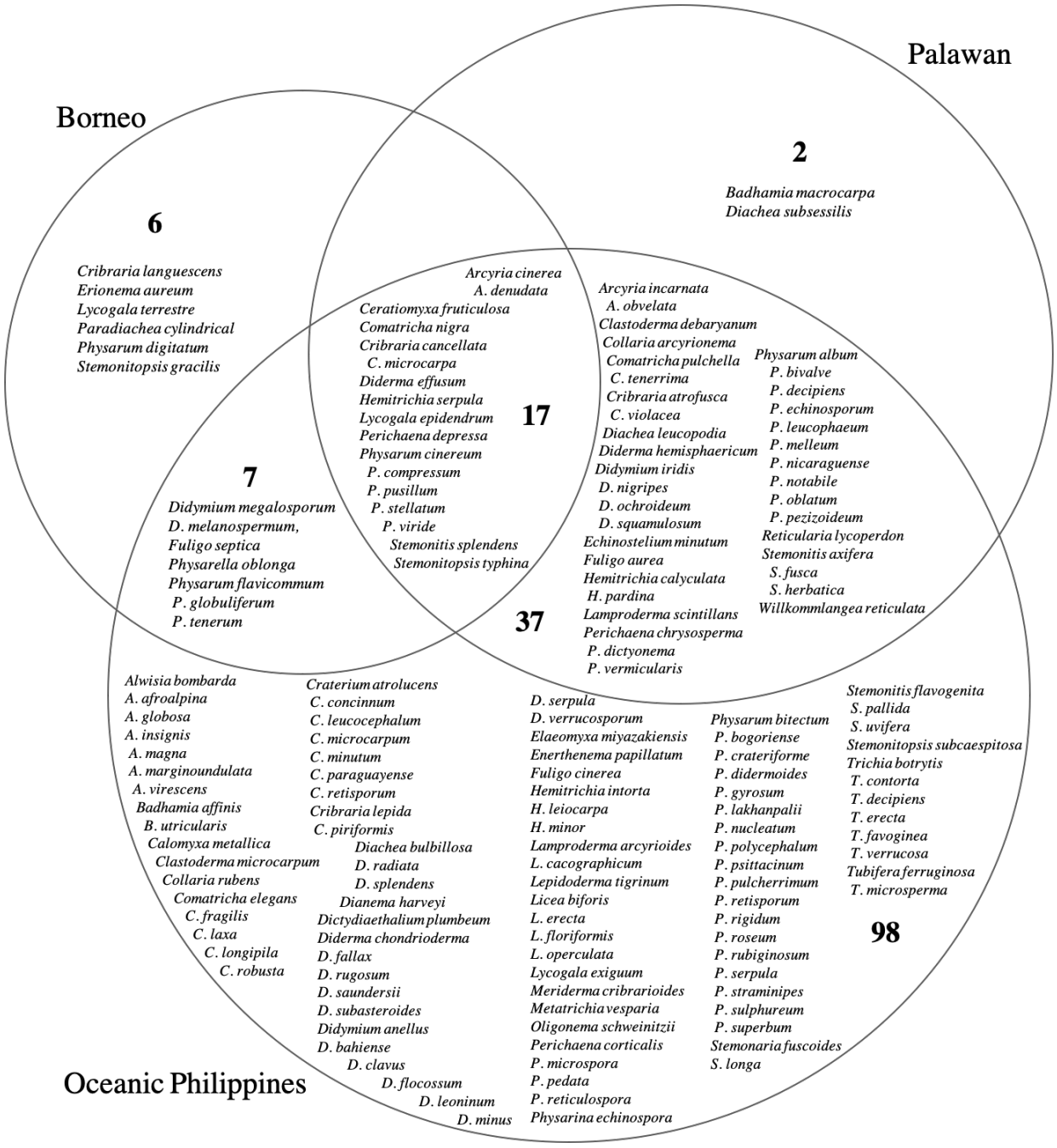

Figure 3. Venn diagram showing the occurrences of myxomycetes in each locality. Shared species are shown where the circles intersect.

VII. Species distribution modeling of the morphospecies *Arcyria cinerea* (Bull.) Pers.

Abstract

The main objective of this study is to create an environmental niche model of the myxomycete morphospecies *Arcyria cinerea* using global database collections of occurrence data to predict species ranges using historical climate data and two different future climate scenarios. Using the program Maxent, the worldwide distribution of *A. cinerea* including the bioclimatic factors that most likely influence its distribution have been predicted. Future climate scenarios (SRES A2 and SRES B1) were independently projected on the baseline model to demonstrate how environmental conditions during these climate change schemes will affect the distribution of the myxomycete. Based on the results of the simulation, the global range of *A. cinerea* will be remarkably narrow and restricted to a few terrestrial areas in the year 2050, except for low-medium probability gains in parts of northern and eastern Europe, central Asia, Russia, and northern Indonesia, and medium-high probability increases in northeastern territories of South America, southern tip of Africa, small sections of central Africa, southwestern and southeastern Australia, and majority of Borneo and New Guinea. The future climate conditions appear to specifically be detrimental to the myxomycete in smaller land masses and isolated islands, and this diminishing tendency will continue in 2080.

Introduction

A persistent challenge to scientists and conservationists is predicting how species will react to the changing global climate. If climatic conditions notably deviate from ideal, continuous climate change is expected to influence the distributions of plant species (Miller & Urban 1999,

Banag et al. 2015) and cause alterations in species composition and interactions in plant communities (Yang & Rudolf 2010). It was shown to affect both the reduction and expansion of the ranges of various animals, and ultimately changed the dynamics and composition of communities (e.g., Parmesan 2006, La Sorte & Thompson 2007, Moritz et al. 2008). It is therefore unassuming to state that it specially threatens species with narrow ranges or with limited dispersal capacities.

To predict the effects of climate change on species ranges a common methodology used is species distribution modeling (SDM) or sometimes referred to as environmental niche modeling or ecological niche modeling, a technique that incorporates the relationships between climatic conditions and the occurrence of a species (e.g., Peterson et al. 2002, Thomas, et al. 2004, Guisan & Thuiller 2005, Pearson 2010) to analyze potential species distributions. The resulting models employ the associations between the two components (environmental layers and species presence records) to generate the most plausible environments where populations of a species being studied can be found (Almadrones-Reyes & Dagamac 2018). SDM has been used for several purposes including monitoring species over a period (Adhikari et al, 2012, Broennimann et al. 2012), identifying suitable environments for species to estimate their geographic distributions (Elith & Leathwick 2009, Anand et al. 2021), evaluating ecological potential (Tingley et al. 2014), making recommendations or decisions for future management (Khanum et al. 2013, Gelviz-Gelvez et al. 2015), and other crucial reasons relating to biodiversity assessments and mitigation.

Despite the usefulness of SDM, there have only been a few studies conducted using microorganisms in niche modeling. To date, SDM research on fungi and lichens (see Wolfe et al. 2010, Dymytrova et al. 2016, Braidwood & Ellis 2012, Guo et al. 2017, Tianxiao et al. 2020), protosteloid amoebae (see Aguilar & Lado 2012), and viruses (see Araujo & Naimi 2020, Larson

et al. 2010) have been conducted (Almadrones-Reyes & Dagamac 2018). However, on myxomycetes, an established group of amoebozoans that have been found on seemingly all places on earth (Stephenson 2003), the use of SDM has been quite occasional. Recent published reports include studies by Aguilar et al. (2014), Rojas et al. (2015), Dagamac et al. (2017), and Almadrones-Reyes & Dagamac (2018).

The myxomycete morphospecies *Arcyria cinerea* (Bull.) Pers. is a good candidate for a global distribution analysis because it has been found in a variety of habitats in almost all continents. With its prevalence and the impending impacts of climate change, the primary goals of this study are the following: 1) to identify the factors that are affecting the distribution of the myxomycete morphospecies *Arcyria cinerea* (Bull.) Pers., and 2) to determine the possible effects of future climate scenarios on the rate of persistence of the myxomycete by illustrating the global distribution of *A. cinerea* using the two climate change settings at two different periods. This will also allow the determination of suitable habitats for the myxomycetes, as well as distinguish conservation strategies of the habitats currently being occupied by the myxomycete.

Methods

This study includes collections of the myxomycete morphospecies *Arcyria cinerea* that either naturally fruited in the field or have matured in a moist chamber culture.

The analysis started with gathering and cleaning of presence records of *A. cinerea*, followed by the modeling of current predicted species distribution and future potential distributions under specified climate scenarios with the program Maxent (Phillips et al. 2006; Phillips & Dud´ık 2008; Elith et al. 2011).

Distribution data

Occurrence records of *A. cinerea* were derived from the database of the University of Arkansas Myxomycete Herbarium under the research laboratory of Dr. Steve Stephenson. A total of 4,012 presence records from 42 countries [Algeria, Argentina, Australia, Belize, Brazil, Cambodia, Canada, Chile, Colombia, Costa Rica, Dominican Republic, Ecuador, France, Guatemala, Honduras, India, Israel, Japan, Kenya, Laos, Madagascar, Malawi, Mexico, Morocco, Myanmar, Namibia, New Zealand, Panama, Peru, Philippines, Puerto Rico, Russia, Seychelles, Singapore, South Africa, Sweden, Tanzania, Thailand, Trinidad, UK Overseas Territory, United States, and Vietnam] were collated. Duplicate observations were removed, and occurence data were spatially filtered at a resolution of 4.5km x 4.5 km. Data cleaning and filtering resulted to a total of 513 presence-only data (see Fig. 1) that was later used in model training and development.

Environmental data

For generating the species' current predicted distribution, bioclimatic variables were obtained from the Worldclim database at 2.5' resolution for the period of 1970-2000 (Fick and Hijmans, 2017). To make sure that predictive power will be maintained and potential difficulties in results interpretation will be avoided (Banag et al. 2015), predictor pairs with Pearson's correlation coefficient $|r| >= 0.8$ were identified for the 19 bioclimatic variables. We chose 10 variables out of the 19 to avoid model overfitting due to collinearity. The analyses followed the protocol of Banag et al. (2015) using R Version 3.1.2 (R Development Core Team, 2014) with the packages *raster* (Hijmans 2014), *dismo* (Hijmans et al. 2014), *rgdal* (Bivand et al. 2014), *sp* (Pebesma & Bivand 2005), *rJava* (Urbanek 2013), *gplots* (Warnes et al. 2013), and *spThin* (Aiello-Lammens et al. 2015).

For future projections, the climate scenarios SRES A2 and SRES B1 for the years 2050 and 2080 from the GCM data portal (http://www.ccafs-climate.org/data_spatial_downscaling/, Delta Method IPCC AR4 file set, "ukmo_hadcm3" models) were selected. These chosen climate scenarios represent varying environmental conditions, such that SRES A2 corresponds to a world with high heterogeneity represented by continuously increasing global population and regionally oriented economic growth that is more fragmented and slower than in other storylines; while SRES B1 describes a convergent world with the same global population as in the A2 storyline but with rapid changes in economic structures toward a service and information economy, with reductions in material intensity, and the introduction of clean and resource-efficient technologies. ASCII Grid formats of the environmental layers for these scenarios were downloaded at a global 2.5' resolution (Navarro-Racines et al. 2020).

Species distribution modeling

Models were created using Maxent version 3.4.4 (https://biodiversityinformatics.amnh.org/open_source/maxent/). Maxent, a machine-learning method based on the maximum entropy algorithm, is among the most frequently used techniques for species distribution modeling (Banag et al. 2015) and has been shown to generally operate better than many other species distribution models (Phillips et al. 2006; Elith et al. 2006; Fischer et al. 2011). It performs by approximating the relative density of species presence records based on the environmental conditions at occurrence locations, representing the used environmental conditions, and at background locations representing the available environmental conditions; thus, giving an estimate of the species' realized niche, and projects it into geographic space (Phillips et al. 2006). Our models include only the 10 continuous bioclimatic variables and not other potentially important factors such as biotic interactions, elevation, dispersal barriers, etc.

For predictions under the two climate scenarios at two different periods, the same conditions between environmental variables and relative species density are applied in generating the maps to appropriately estimate the potential distribution of the morphospecies *A. cinerea* under these future conditions.

Maxent simulations were run for present and future climate scenarios SRES A2 and SRES B1 using the default settings. Model performance was evaluated with a fivefold cross validation using the Area Under the Curve (AUC) of the receiver operating characteristic (ROC) as described in Banag et al. (2015) and Hanley and McNeil (1982). To measure the importance of each environmental variable, model runs were performed with a Jackknife test to identify the variables with the most useful information by itself (Elith et al. 2011) and output format was set to logistic. Response curves were created, and under the Advanced tab "Write plot data" was ticked, as well as "Write background predictions" under the Experimental tab. The ASCII output files from the Maxent runs were each exported to ArcGIS Pro version 2.7 for detailed and standardized visualization of predicted current and future models, which were all classified under defined intervals of 0.20 from values ranging from 0-1. Intervals are illustrated by a color scheme of cool (blue) to warm (red) representing very low, low, medium, high, and very high probabilities.

For possible extrapolation into novel climates, which can be challenging (Elith et al. 2010), Multivariate Environmental Similarity Surfaces (MESS) were calculated, and MESS maps were generated during the Maxent simulations.

Results and Discussion

Species distribution modeling with Maxent shows that the most important bioclimatic variable in the current predicted distribution of *A. cinerea* is temperature seasonality (TS), with 30.4 percent contribution, followed by precipitation of wettest month (PWM), with 28.2 percent contribution (see Table 1). This happens to be supported by the Jackknife test, which suggested that the environmental variable with the highest gain when used in isolation is TS, denoting that it has the most useful information by itself. The variable that decreases the gain the most when it is removed is PWM, which means that it seems to have the most information that is not present in the other variables. The variable with the least contribution to the niche model was temperature annual range. Fig. 2 shows how each environmental variable affects the Maxent prediction, reflecting the dependence of predicted suitability both on the selected variable and on dependencies induced by correlations between the selected variable and other variables. In another report on environmental niche modeling of some Costa Rican myxomycetes, Rojas et al. (2015) found other environmental variables, such as elevation and temperature range, to be greater contributors to the model using *A. cinerea*. This was somehow not comparable to our study because we considered the collinearity of the environmental layers that led to the exclusion of isothermality, one of the five variables they have chosen to include in their simulation, and other bioclimatic layers.

Model performance for *A. cinerea* was found to be quite robust, with an AUC value of 0.91 (1.0 = perfect fit). If the analysis using the ROC was used as a measure of the predictive value of the model in terms of the AUC as suggested by Phillips et al. (2006), then results from this run were found to be equally or even more accurate in discerning presence or absence of the species in a certain raster cell (Rojas et al. 2015) than previous studies on SDM of myxomycetes [see

Almadrones-Reyes & Dagamac 2018 (AUC = 0.80), Dagamac et al. 2017 (AUC range = 0.79-0.92), and Rojas et al. 2015 (AUC range = 0.80 and 0.84). The predicted current distribution shown on Fig. 3 suggests suitable (high-very high probability) territories for *A. cinerea*, such as the following: South American areas like the southeastern part of Brazil, sections of Uruguay, Chile, Peru, and Bolivia, and patches from Guyana, Venezuela, Colombia, and Ecuador; Central American countries such as portions of Panama, Costa Rica, Nicaragua, Guatemala, and the southern tip of Mexico; Southeastern part of the continental United States of America, almost the entire Hawaiian islands and other South Pacific islands, and minute portions in western Canada; Ireland, western United Kingdom, northern Spain, most of France, small districts of southwestern Europe, Georgia, a minor part of Northern Turkey, and north Atlantic islands, such as the Azores and Madeira islands; narrow strips of coastal areas in Côte d'Ivoire, Cameroon, and South Africa, scattered ranges in the central-eastern Africa, eastern covers of Madagascar, and surrounding islands in the Indian ocean such as Seychelles, Comoros, and Mauritius; the Maldives, southwestern parts of Sri Lanka, and patches on the southern tip of India; small parts of Nepal, Bhutan, disjointed southern Myanmar islands, and small fragments in Northern Malaysia and Vietnam; portions of the Sunda islands of Indonesia, Timor Leste, and areas in New Guinea; scattered regions in the northern, central, and portions of southern Philippines, almost the entirety of Taiwan, strips on southwestern Australia including Tasmania, almost the entire New Zealand, and other smaller Pacific islands. The territories (Fig. 3) covered in yellow are predicted to have medium probability, while the ones in green and blue are deemed to have low and very low odds, respectively, in terms of *A. cinerea* occurrence.

For both future climate scenarios (SRES A2 and SRES B1) in both time periods (year 2050 and year 2080), the range of distribution of *A. cinerea* has become narrower. In the year 2050, the

models (Fig. 4 and 6) show that the only way this myxomycete would benefit from both climate scenarios would be from an increase in presence probability in particular areas, such that the distribution likelihood will rise from very low to low-medium in areas of Northern Europe, and small parts of Eastern Europe, Central Asia, Russia, and Northern Indonesia, from very low-low to high probability in parts of Colombia-Ecuador-Peru, a coast in western Saudi Arabia, parts of Central Africa, and Borneo, and from low-medium to high-very high probabilities in the southern tip of South Africa, southern parts of western and eastern Australia, and central parts of New Guinea. In SRES A2 (Fig. 4) very high probabilities are notably predicted for the southern parts of western and eastern Australia, while in SRES B1 (Fig. 6) it is in a small area in the region around Salvador in northeast Brazil. In the year 2080, under both climate scenarios (Fig. 5 and 7), the range of *A. cinerea* is predicted to even be narrower. In the year 2080, under SRES A2 (Fig. 5) the predicted medium-high probability at 2050 on the coastal parts of Louisiana and southern USA would have become low-medium. The same trend is evident in the northeast parts of South America, UK, parts of western, northern, and central Europe, central Africa, and southern regions of Australia. The only notable increases in probabilities are in small coasts in the southern and western regions of Brazil. Under SRES B1 condition, in the year 2080 (Fig. 7), similar trends in probability drops have been observed, even for regions that were projected to gain from climate scenarios at 2050 (Fig. 6) namely parts of northern Europe, Central Asia, and Russia. Despite the increase in probability in a few territories, *A. cinerea* will have a more limited distribution in both climate scenarios and will continue to decline after thirty years. Apart from the prominent distribution restriction in bigger land masses such as North, Central, and South America, Europe, Africa, East Asia, and Australia, it is remarkable to note the rapid decline in smaller territories such as the Caribbean islands, Ireland, Madagascar, Sri Lanka, Philippines, Japan, Taiwan, Sunda

islands, Tasmania, New Zealand, and the rest of the isolated islands and islets in the Atlantic, Indian, and the Pacific regions.

Predictive ecological models based on algorithms such as Maxent should be taken with caution, that is why Maxent includes new features informing users about predicting to novel environments (Elith et al., 2010). Multivariate Environmental Similarity Surfaces (MESS) maps were obtained to compare between the environmental variables used for training and for prediction. Figures 8A, 9A, 10A, and 11A display novel climates for all future predictions, especially the ones shown in red, i.e., for both SRES A2 and B1 at years 2050 and 2080 there are values for the environmental layers that are outside the range of the baseline or current model. Figures 8B, 9B, 10B, and 11B show which variable is most novel at each period in the geographical space. It shows that novel climate conditions in climate scenario SRES A2 in 2050 are mainly due to annual precipitation (yellow), mostly encompassing the globe, and annual mean temperature (orange), mostly on north-central Africa, parts of the Middle East and Central Asia, and small coasts on Chile and Peru and Namibia, with some influence by temperature annual range (green) in parts of Central Asia and similarly by precipitation seasonality (brown) in a small section in Chile and Central Asia. Novel climate conditions in scenario SRES A2 in 2080 (Fig. 9B) and scenario SRES B1 in the years 2050 (Fig. 10B) and 2080 (11B) follow the same trend as in SRES A2 in 2050 (Fig. 8B), except for the more pronounced impact of temperature annual range in future climate scenario SRES B1 in 2050 and 2080. These suggest that because of the novel environmental conditions in the future climate, the global distribution of the myxomycete morphospecies *A. cinerea* will most likely be shaped by annual precipitation and annual mean temperature. It is important to note, however, that these results were generated from presence-only data that makes them prone to bias (Dagamac et al. 2017) and so they should be interpreted with prudence. This is also true for SDM

applications including predictions to new environments (i.e., new locations or times; Elith & Leathwick 2009, Elith et al. 2010). If these are translated as strong assumptions as to being a truly predictive method, they can be highly debatable (Dormann 2007) specially if they entail projections to environments outside of the range by the training data.

Literature Cited

Adhikari, D., Barik, S., & Upadhaya, K. 2012. Habitat distri-bution modelling for reintroduction of Ilex khasiana Purk., a critically endangered tree species of northeastern India. Ecological Engineering 40: 37–43.

Aguilar, M., Fiore-Donno, A.M., Lado, C. & Cavalier-Smith, T. 2013. Using environmental niche models to test the 'everything is everywhere' hypothesis for *Badhamia*. The ISME Journal 8(4): 737–745.

Aguilar, M. & Lado, C. 2012. Ecological niche models reveal the importance of climate variability for the biogeography of protosteloid amoebae. The ISME journal 6(8): 1506–1514.

Aiello-Lammens M. E., Boria R. A., Radosavljevic A., Vilela B. 2015. spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. Ecography 38(5), 541–545.

Almadrones-Reyes, K.J. & Dagamac, N. H. A. 2018. Predicting local habitat suitability in changing climate scenarios: Applying species distribution modelling for Diderma hemisphaericum. Current Research in Environmental & Applied Mycology 8(5): 492–500.

Anand, V., Bakimchandra, O. & Hamilton, S. 2021. Predicting the current and future potential spatial distribution of endangered Rucervus eldii eldii (Sangai) using MaxEnt model. Environmental Monitoring and Assessment 193(3).

Araújo, M. & Naimi, B. 2020. Spread of SARS-CoV-2 Coronavirus likely to be constrained by climate. medRxiv 2020.03.12.20034728; doi: 10.1101/2020.03.12.20034728.

Banag, C., Thrippleton, T., Alejandro, G. J., Reineking, B. & Liede-Schumann, S. 2015. Bioclimatic niches of selected endemic Ixora species on the Philippines: predicting habitat suitability due to climate change. Plant Ecology 216(9): 1325–1340.

Bivand, R., Keitt, T. & Rowlingson, B. 2014. rgdal: bindings for the geospatial data abstraction library. R package version 0.8- 16. http://CRAN.R-project.org/package=rgdal.

Braidwood, D. & Ellis, C.J. 2012. Bioclimatic equilibrium for lichen distributions on disjunct continental landmasses. Botany 90(12): 1316–1325.

Broennimann, O., Fitzpatrick, M.C., Pearman, P.B., Petitpierre, B., Pellissier, L., Yoccoz, N. G., Thuiller, W., Fortin, M. J., Randin, C., Zimmermann, N. E., Graham, C.H. & Guisan, A. 2012. Measuring ecological niche overlap from occurrence and spatial environmental data. Global Ecology and Biogeography 21: 481–497.

Dagamac, N. H. A., Rojas, C., Novozhilov, Y.K. & Moreno, G. H. et al. 2017. Speciation in progress? A phylogeographic study among populations of Hemitrichia serpula (Myxomycetes). PloS One 12(4): e0174825.

Dormann, C.F. 2007. Promising the future? Global change projections of species distributions Basic and Applied Ecology 8: 387–397.

Dymytrova, L., Stofer, S., Ginzler, C., Breiner, F. T. & Scheidegger, C. 2016. Forest-structure data improve distribution models of threatened habitat specialists: Implications for conservation of epiphytic lichens in forest landscapes. Biological Conservation 196: 31–38.

Elith, J., Phillips, S. J., Hastie, T., Dudík, M., Chee, Y. E. & Yates, C. J. 2011. A statistical explanation of MaxEnt for ecologists. Diversity and Distributions, 17: 43-57.

Elith, J. & Leathwick, J. R. 2009. Species distribution models: Ecological explanation and prediction across space and time. Annual Review of Ecology, Evolution, and Systematics 40: 677–697.

Fick, S. E. & Hijmans, R. J. 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37 (12): 4302-4315.

Gelviz-Gelvez, S. M., Pavón, N. P., Illoldi-Rangel, P. & Ballesteros-Barrera, C. 2015. Ecological niche modeling under climate change to select shrubs for ecological restoration in Central Mexico.Ecological Engineering 74: 302–309.

Guisan, A. & Thuiller, W. 2005. Predicting species distribution: offering more than simple habitat models. Ecology letters 8(9): 993–1009.

Guo, Y., Li, X., Zhao, Z., Wei, H. et al. 2017. Prediction of the potential geographic distribution of the ectomycorrhizal mushroom Tricholoma matsutake under multiple climate change scenarios. Scientific report 7: 46221.

Hanley, J.A. & McNeil, B.J. 1982. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 143:29–36.

Hijmans, R. J. 2014. Raster: geographic data analysis and modeling. R package version 2.3-12. http://CRAN.R-project.org/package=raster.

Hijmans, R.J., Phillips, D., Leathwick, J. & Elith, J 2014. dismo: species distribution modeling. R package version 1.0-5. http://CRAN.R-project.org/package=dismo.

Khanum, R., Mumtaz, A. S., & Kumar, S. 2013. Predicting impacts of climate change on medicinal asclepiads of Pakistan using Maxent modeling. Acta Oecologica 49: 23–31.

Larson, S.R., Degroot, J.P., Bartholomay, L.C. & Sugumaran, R. 2010. Ecological niche modelling of potential West Nile virus vector mosquito species in Iowa. Journal of Insect Science 10(1): 110.

La Sorte, F. A., and F. R. Thompson 2007. Poleward shifts in winter ranges of North American birds. Ecology 88:1803-1812.

Moritz, C., J. L. Patton, C. J. Conroy, J. L. Parra, G. C. White, & S. R. Beissinger. 2008. Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. Science 322:261-264.

Miller, C. & Urban, D.L. 1999. Forest pattern, fire, and climatic change in the Sierra Nevada. Ecosystems 2:76–87.

Navarro-Racines, C., Tarapues, J., Thornton, P., Jarvis, A. & Ramirez-Villegas, J. 2020. High-resolution and bias-corrected CMIP5 projections for climate change impact assessments. Sci Data 7(7).

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology Evolution and Systematics 37:637-669.

Pearson, R.G. 2010. Species distribution modelling for conservation educators and practitioners. Lessons in Conservation, American Museum of Natural History 3: 54–89.

Pebesma, E.J. & Bivand, R.S. 2005. Classes and methods for spatial data in R. R News 5(2) http://cran.r-project.org/doc/ Rnews/.

Peterson, A. T., M. A. Ortega-Huerta, J. Bartley, V. Sanchez-Cordero, J. Soberon, R. H. Buddemeier, and D. R. B. Stockwell. 2002. Future projections for Mexican faunas under global climate change scenarios. Nature 416:626-629.

Phillips, S. J., Dudík, M. & Schapire, R. E. [Internet] Maxent software for modeling species niches and distributions (Version 3.4.1). Available from url: http://biodiversityinformatics.amnh.org/open_source/maxent/.

Phillips, S.J. & Dudı´k M. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31:161–175

Phillips, S.J., Anderson, R.P. & Schapire, R.E. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231–259.

Proosdij, A. S. J., Sosef, M. S. M., Wieringa, J.J. & Raes, N. 2016. Minimum required specimen records to develop accurate species distribution models. Ecography 39: 542–552.

Rojas, C., Zuñiga, J. M. & Stephenson, S.L. 2015. Ecological niche modelling of some Costa Rican myxomycetes. Current Research in Environmental and Applied Mycology 5: 153–159.

Stephenson, S.L. 2003. The Fungi of New Zealand Volume 3: Myxomycetes of New Zealand. Fungal Diversity Press, Thailand.

Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. F. De Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. Van Jaarsveld, G. F. Midgley, L. Miles, M. A. Ortega-Huerta, A. T. Peterson, O. L. Phillips & S. E. Williams. 2004. Extinction risk from climate change. Nature 427:145- 148.

Tianxiao, H., Guillera-Arroita, G., May, T. M., Lahoz-Monfort, J. J. & Elith, J. 2020. Using Species Distribution Models for Fungi, Fungal Biology Reviews 34(2): 74-88.

Tingley, R., Vallinoto, M., Sequeira, F. & Kearney, M. R. 2014. Realized niche shift during a global biological invasion. PNAS 111: 10233–10238.

Urbanek, S. 2013. rJava: low-level R to Java interface. R package version 0.9-6. http://CRAN.R-project.org/ package=rJava.

Warnes, G.R. 2013. Includes R source code and/or documentation contributed by: Bolker B, Bonebakker L, Gentleman R, Huber W, Liaw A, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M. & Venables B. *gplots*: Various R programming tools for plotting data. R package version 2.11.0.1. http://CRAN.R-project.org/package= gplots.

Wolfe, B.E., Richard, F., Cross, H. B. & Pringle, A. 2010. Distribution and abundance of the introduced ectomycorrhizal fungus Amanita phalloides in North America. New Phytologist 185(3): 803–816.

Yang, L. H. & Rudolf, V. H. W. 2010. Phenology, ontogeny and the effects of climate change on the timing of species interactions. Ecology Letters 13:1–10.

Tables

Table 1. Percentage contribution, permutation importance, and training gain (with and without the corresponding variable) for the bioclimatic variables used in the niche model of *Arcyria cinerea*.

Environmental variable	% Contribution	Permutation importance	Training gain without	Training gain with only
Temperature Seasonality	30.44	2.73	1.28	0.75
Precipitation of Wettest Month	28.17	26.86	1.19	0.60
Min Temperature of Coldest Month	27.37	15.27	1.26	0.75
Annual Mean Temperature	7.96	20.85	1.27	0.74
Precipitation Seasonality	3.78	14.74	1.27	0.55
Annual Precipitation	0.72	2.18	1.26	0.11
Mean Temperature of Coldest Quarter	0.46	0.82	1.27	0.62
Mean Temperature of Warmest Quarter	0.40	9.16	1.27	0.64
Precipitation of Warmest Quarter	0.35	4.70	1.28	0.42
Temperature Annual Range	0.34	2.69	1.27	0.41

Figures

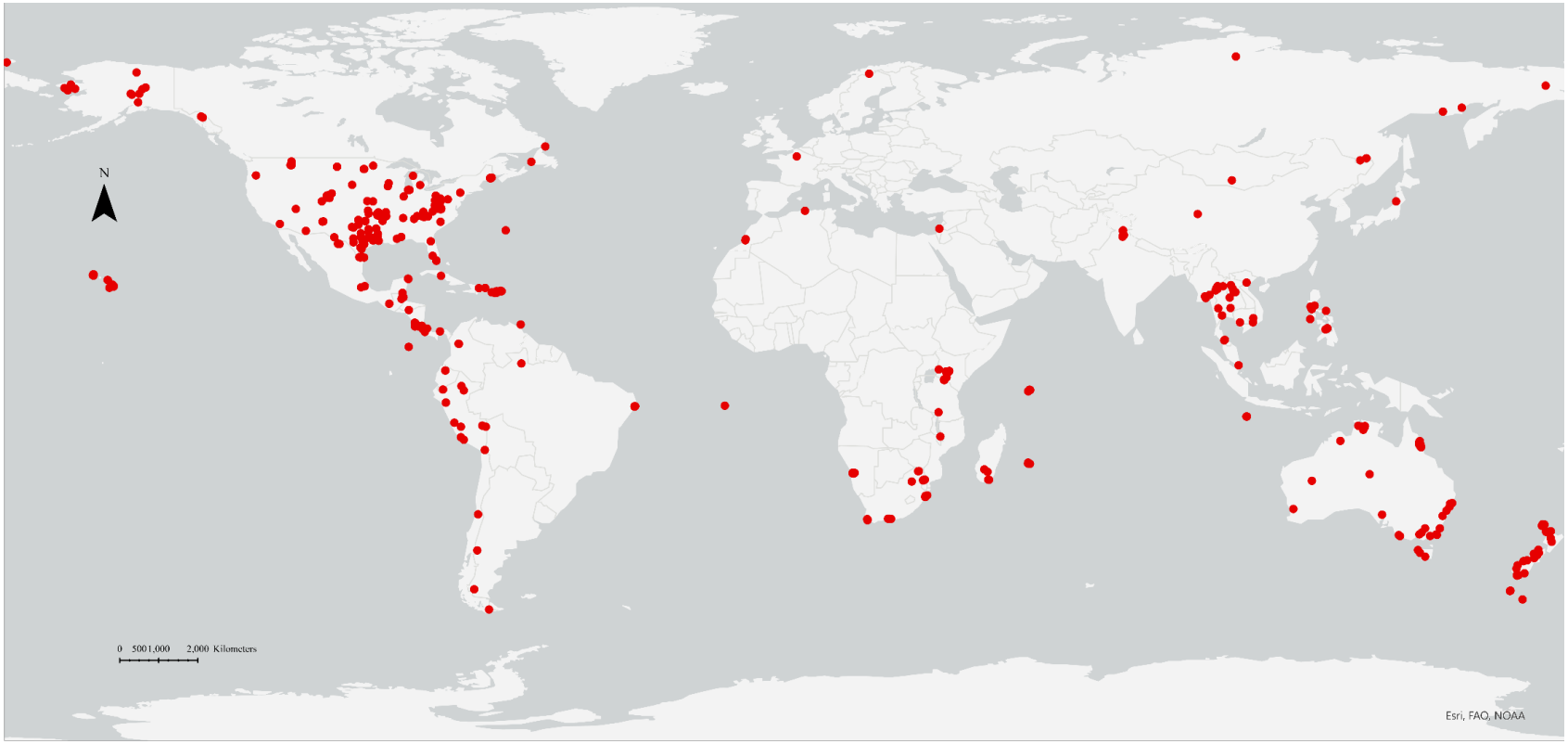

Fig 1. Presence map of *Arcyria cinerea* used in the training of the distribution model.

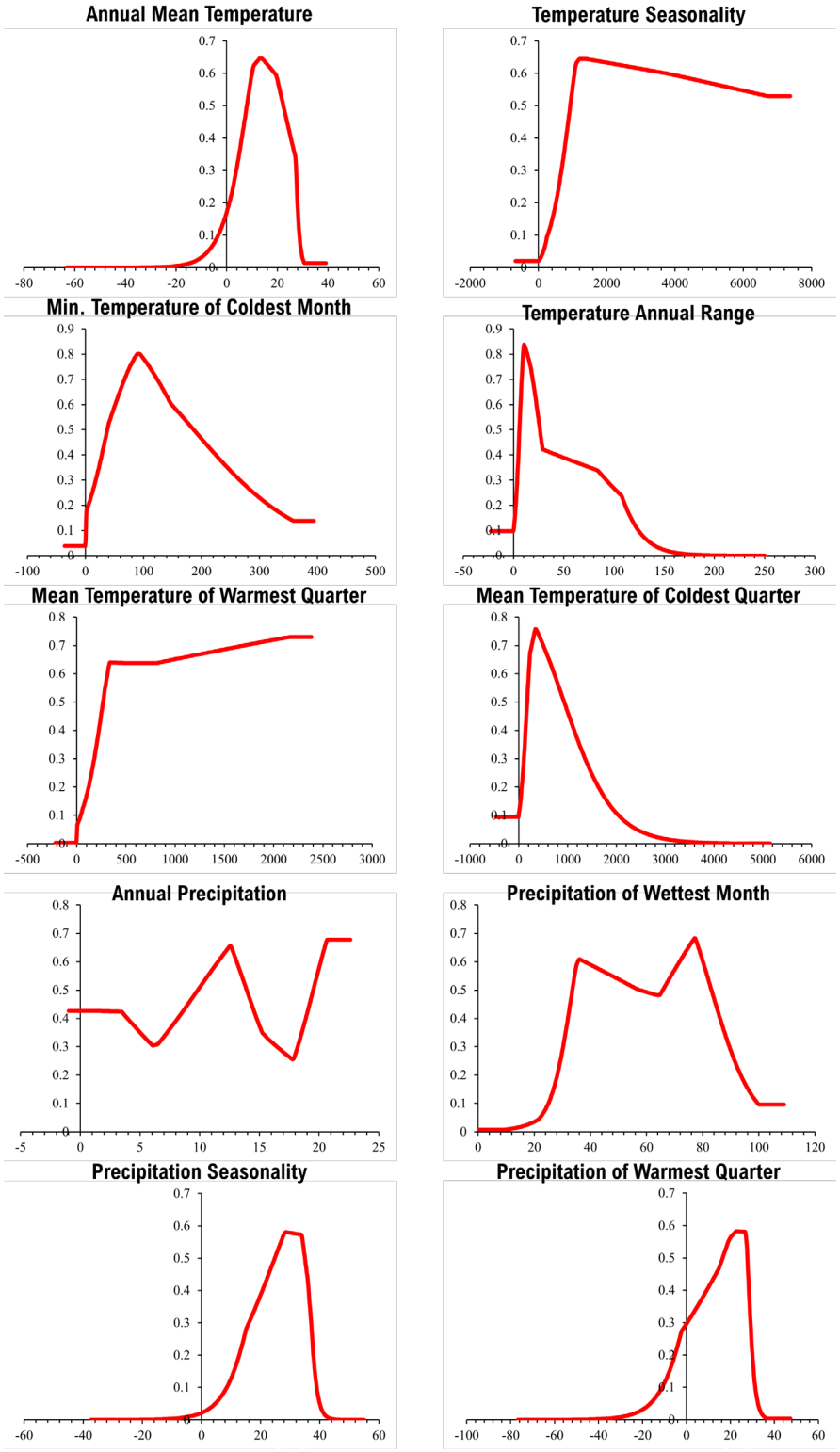

Fig 2. Response curves of each of the environmental variables used in the current predicted distribution model.

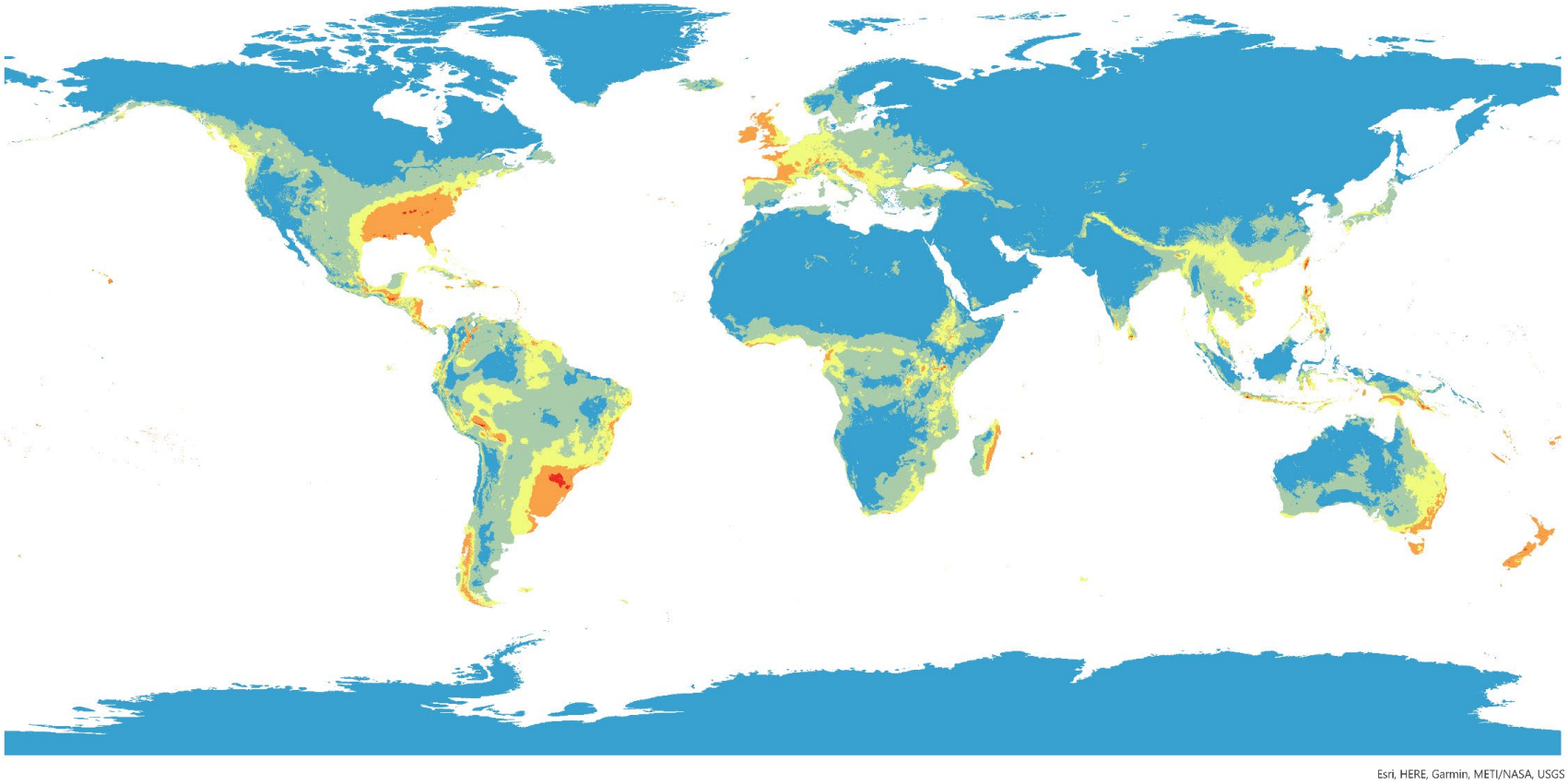

Fig 3. Predicted current global distribution of the myxomycete morphospecies *Arcyria cinerea*.

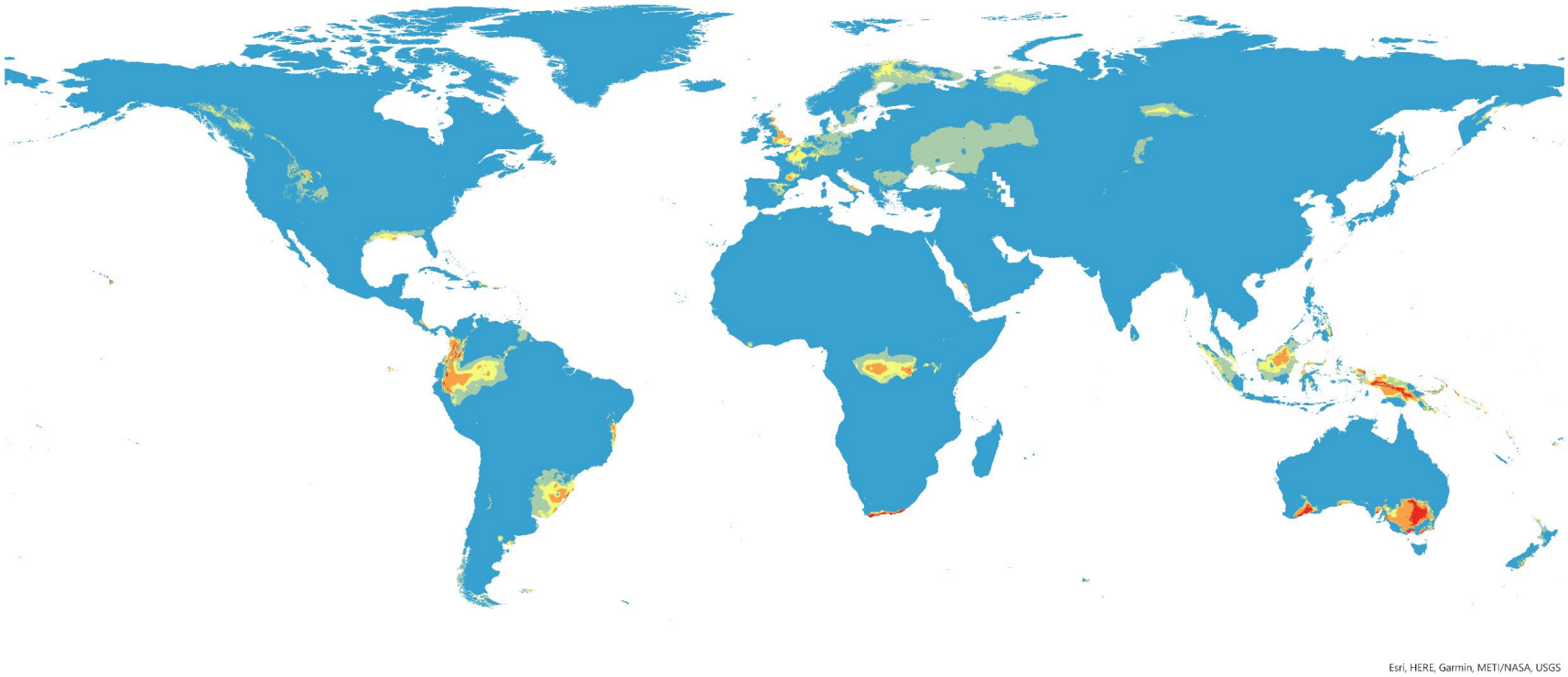

Fig 4. Future predicted distribution of the myxomycete morphospecies *Arcyria cinerea* under climate scenario SRES A2 (2050).

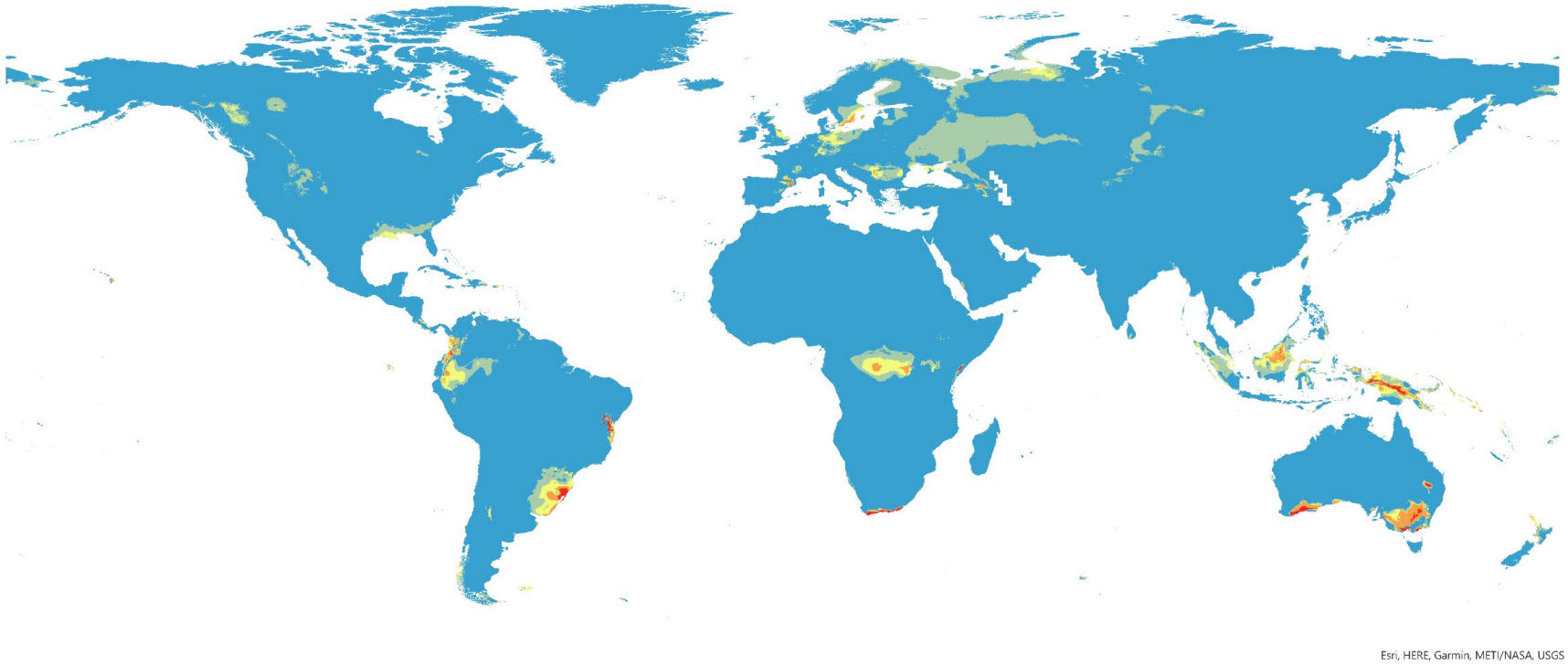

Fig 5. Future predicted distribution of the myxomycete morphospecies *Arcyria cinerea* under climate scenario SRES A2 (2080).

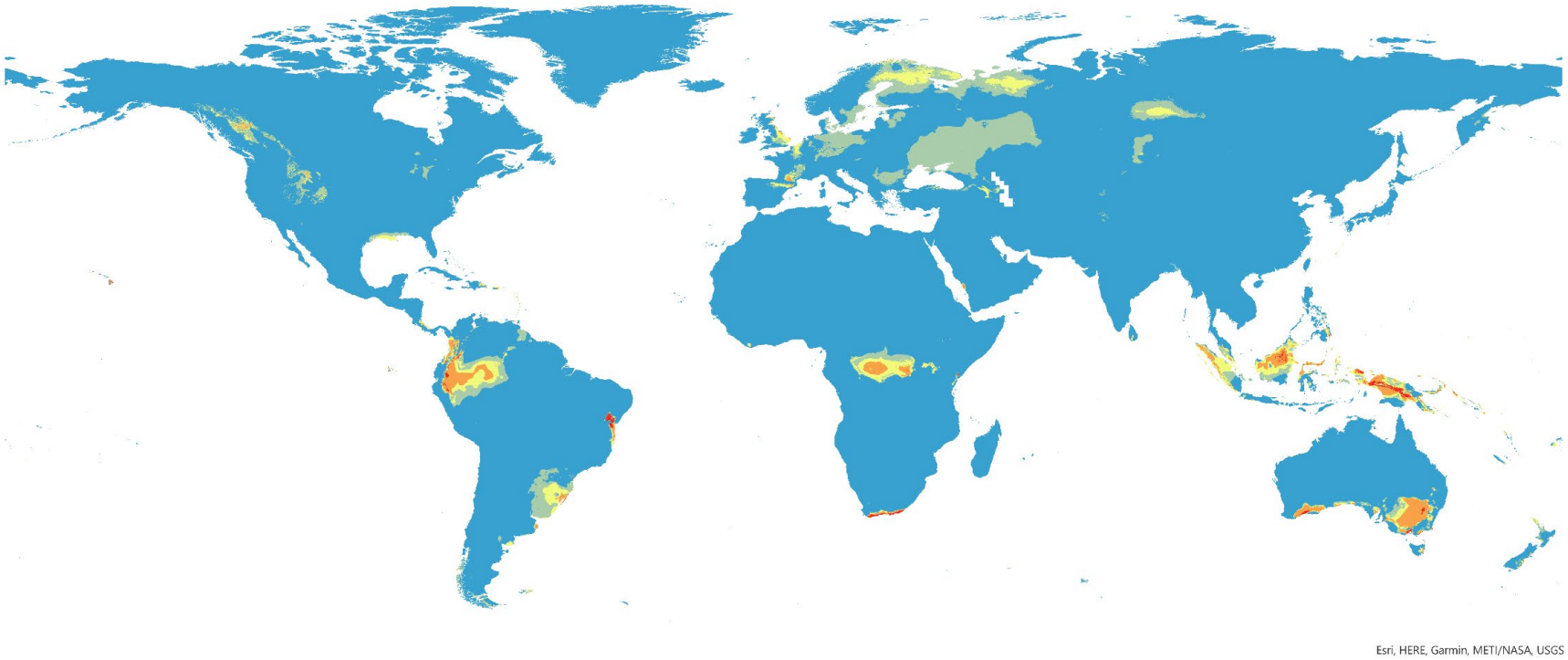

Fig 6. Future predicted distribution of the myxomycete morphospecies *Arcyria cinerea* under climate scenario SRES B1 (2050).

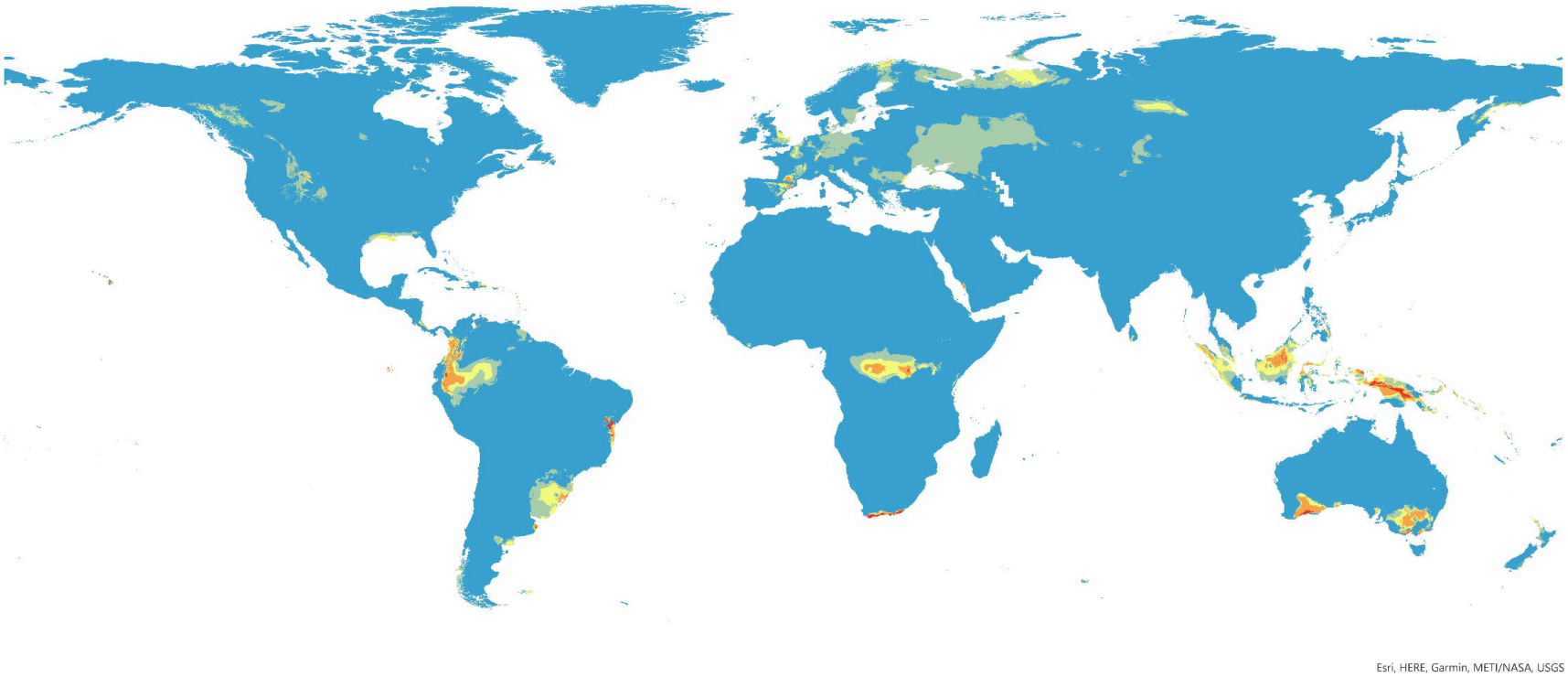

Fig 7. Future predicted distribution of the myxomycete morphospecies *Arcyria cinerea* under climate scenario SRES B1 (2080).

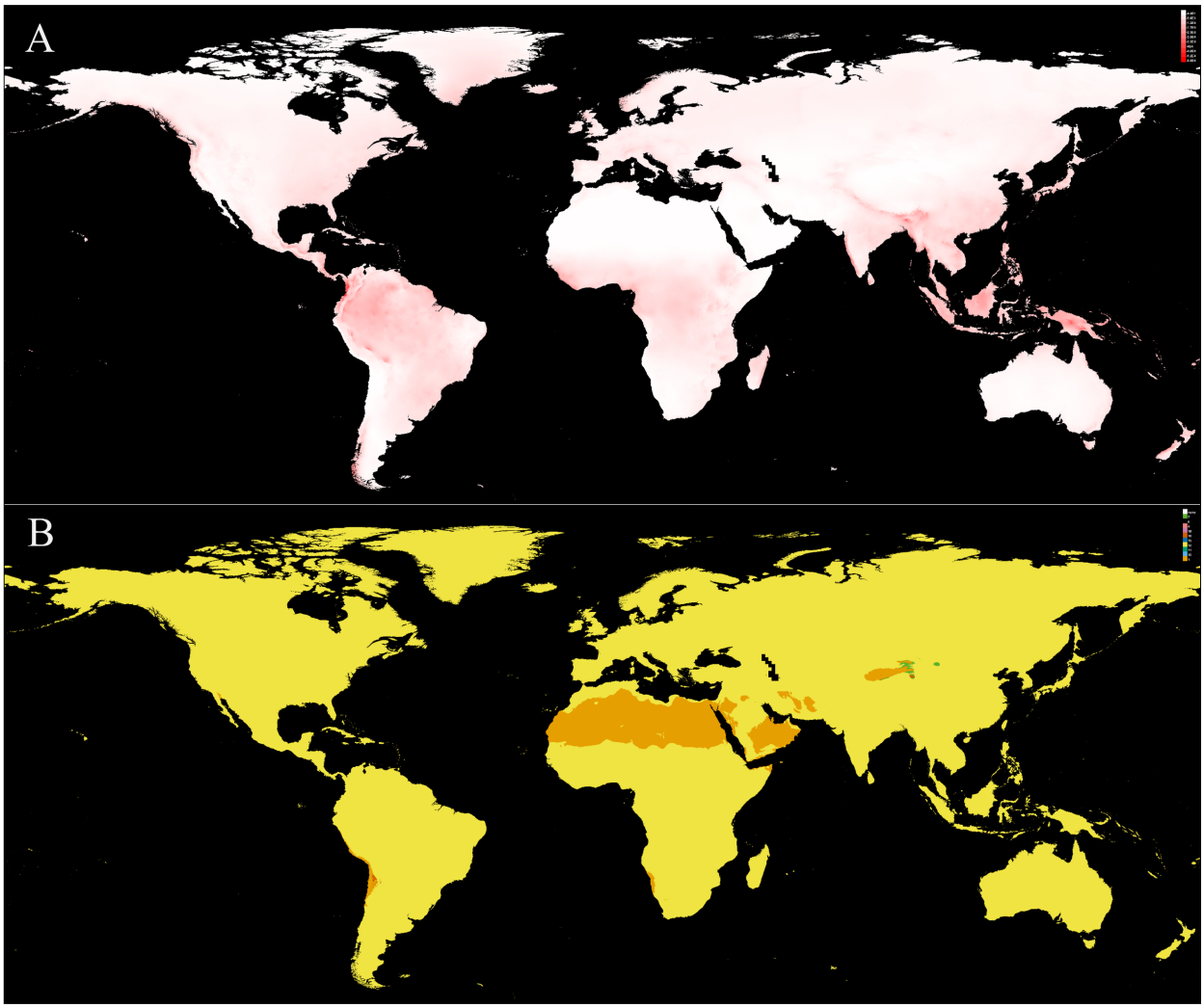

Fig. 8. Multivariate Environmental Similarity Surfaces (MESS) maps comparing the environmental variables in SRES A2 in year 2050 with the environmental layers used for training the model. In A, areas in red (negative values) have one or more environmental variables outside the range, signifying a novel climate, while values similar to the training model layers usually appear in shades of blue. B shows which variables are most novel at each point.

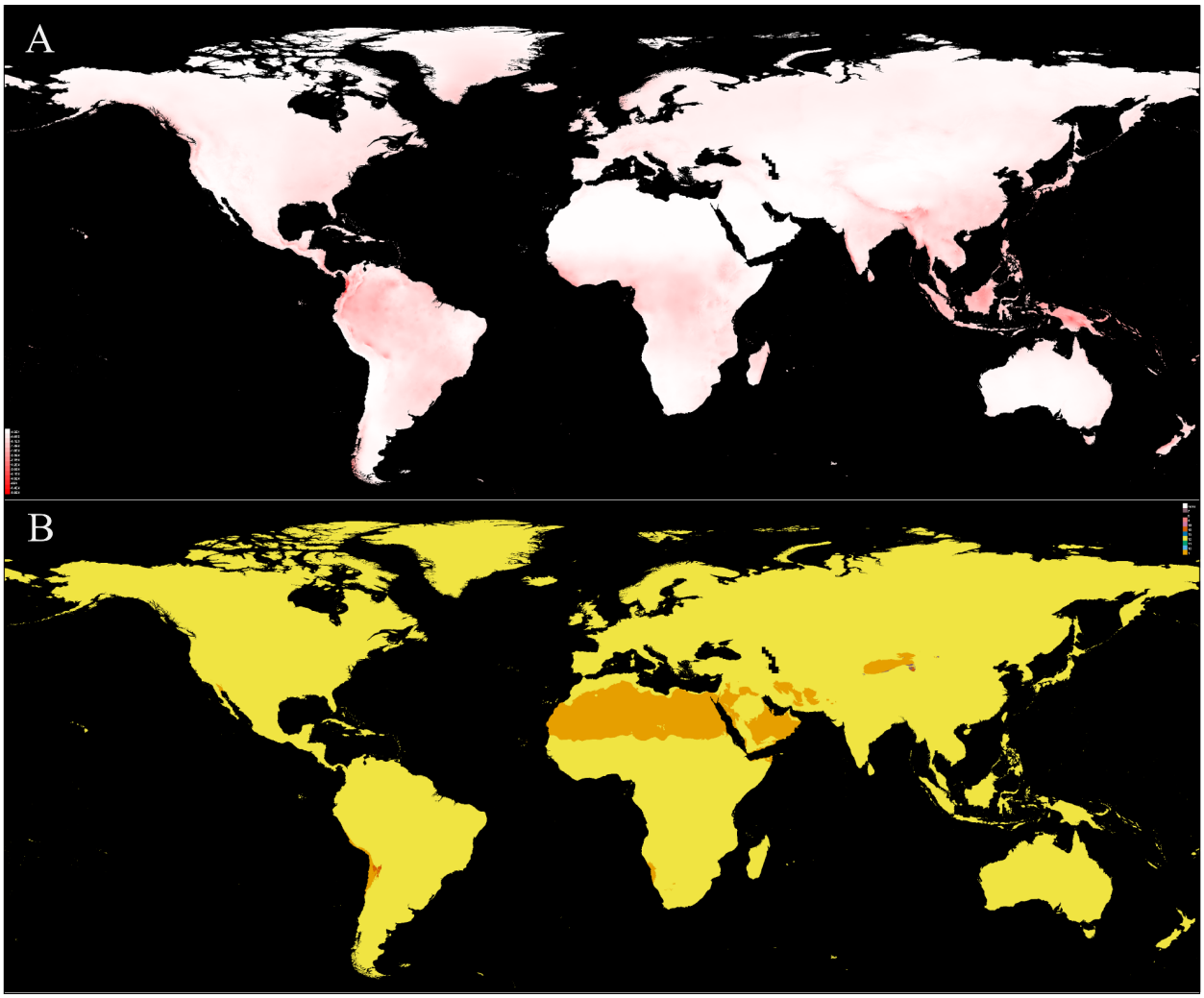

Fig. 9. Multivariate Environmental Similarity Surfaces (MESS) maps comparing the environmental variables in SRES A2 in year 2080 with the environmental layers used for training the model. In A, areas in red (negative values) have one or more environmental variables outside the range, signifying a novel climate, while values similar to the training model layers usually appear in shades of blue. B shows which variables are most novel at each point.

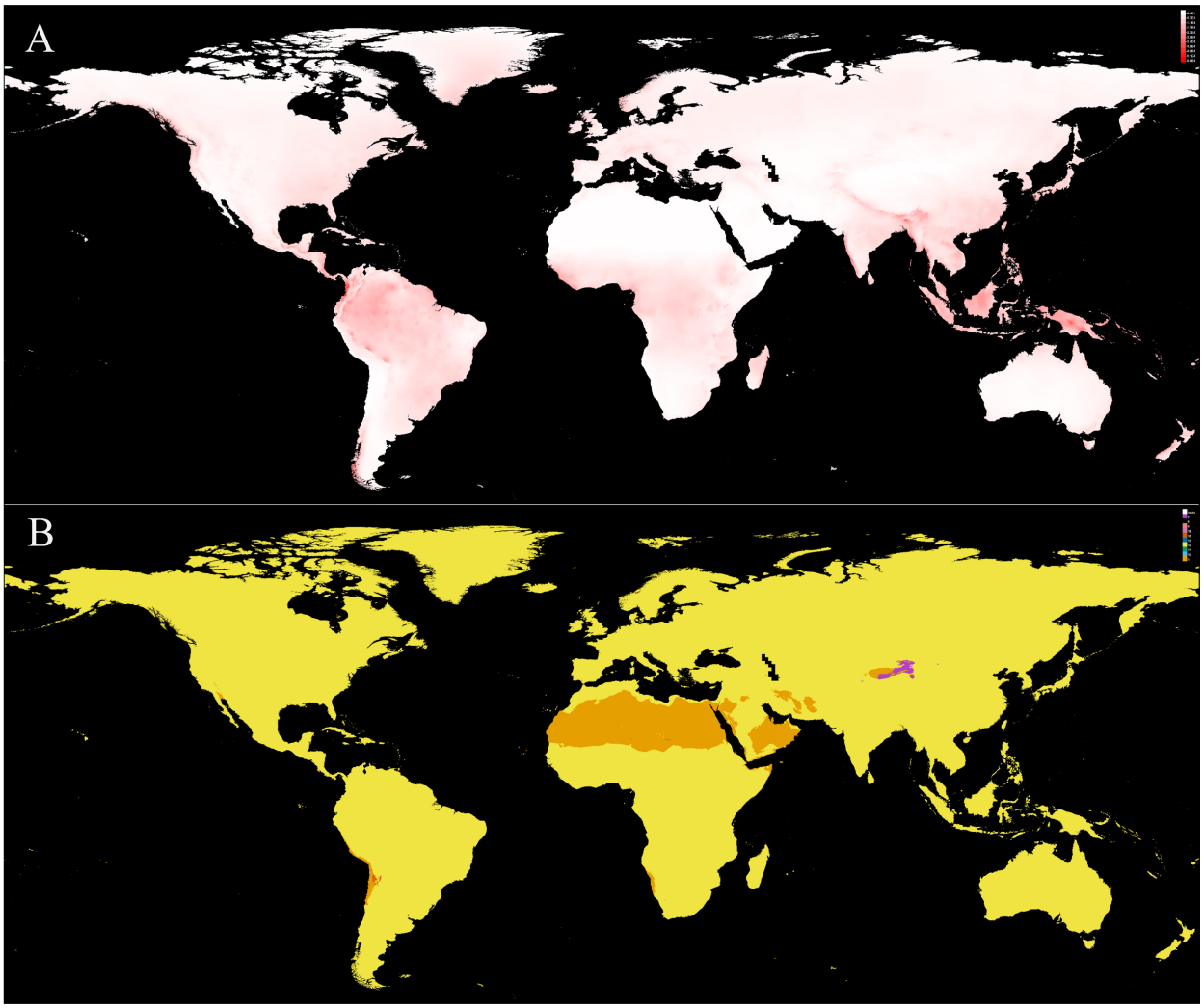

Fig. 10. Multivariate Environmental Similarity Surfaces (MESS) maps comparing the environmental variables in SRES B1 in year 2050 with the environmental layers used for training the model. In A, areas in red (negative values) have one or more environmental variables outside the range, signifying a novel climate, while values similar to the training model layers usually appear in shades of blue. B shows which variables are most novel at each point.

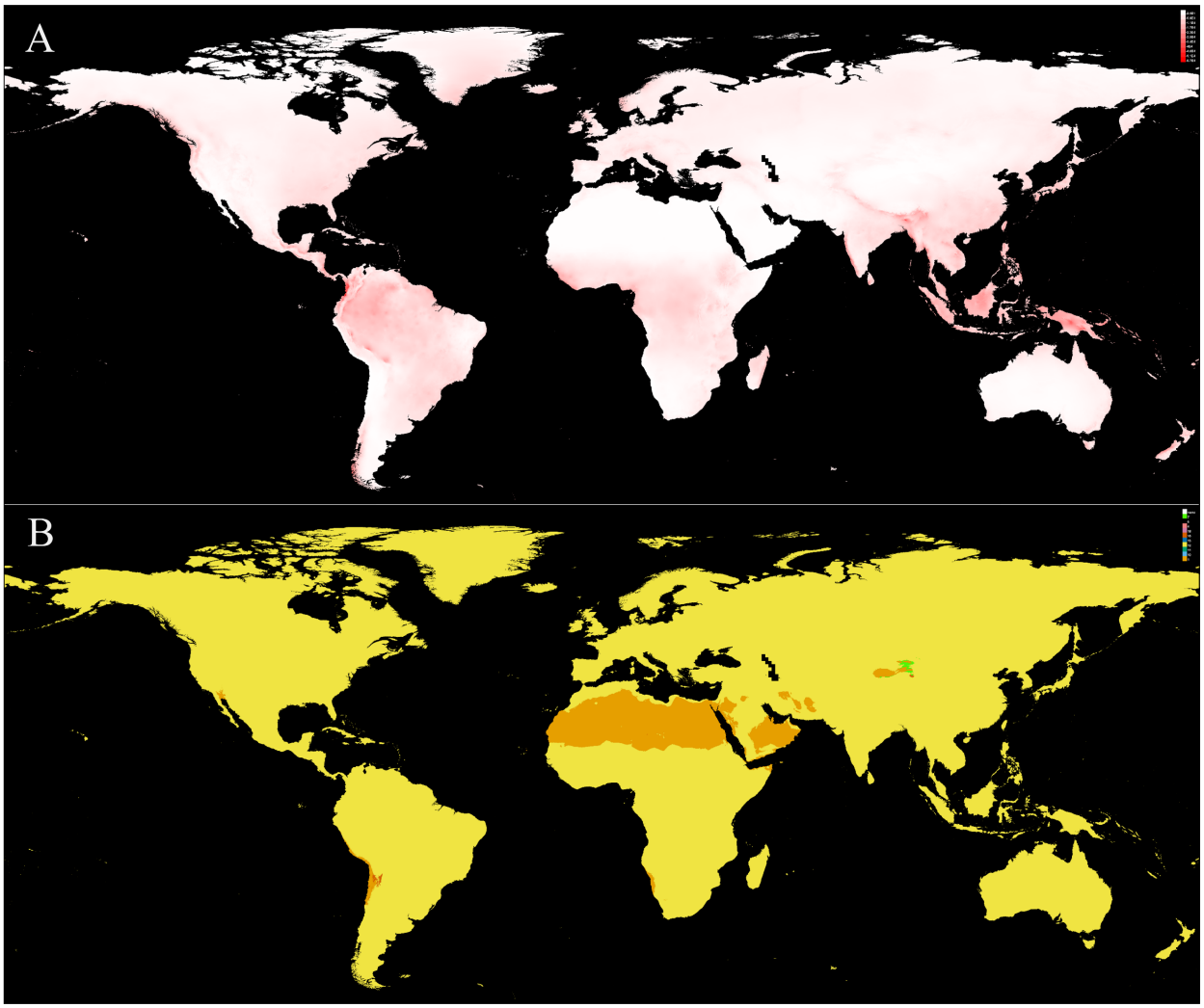

Fig. 11. Multivariate Environmental Similarity Surfaces (MESS) maps comparing the environmental variables in SRES B1 in year 2080 with the environmental layers used for training the model. In A, areas in red (negative values) have one or more environmental variables outside the range, signifying a novel climate, while values similar to the training model layers usually appear in shades of blue. B shows which variables are most novel at each point.

Conclusions

Dispersal events influence genetic differentiation, which plays a key role in the evolution of lineages, species, and population, either by behaving as an instrument of divergence or likeness. The pertinent variations among amoeboid organisms capable of fruiting and producing spores, like the myxomycetes, indicate that this collective set of traits may have evolved multiple times among the eukaryotes. The first chapter of this dissertation depicted the variations among sporogenic amoeboid protists and the likely independent evolution possibly arising from different reproductive and life strategies. Despite asexuality being observed in all the lineages covered in chapter I, it is conjectured that sexuality improves the likelihood of obtaining more variations within the population, therefore rendering them with increased chances of having adaptive traits especially during selection- a vital attribute during spore dispersal and colonization of novel habitats. A noteworthy endeavor would be to examine if dispersal influences vicariance and promotes disjunct distributions of self-dispersing or dispersible organisms, and therefore impact speciation.

The next three chapters in this manuscript examined the assemblages of myxomycetes in three different groups of islands in the Philippines. In the Bohol islands, interpretation of the results suggested that ecological changes could be a component affecting the distribution of myxomycetes in different habitats and that dispersal may be influencing the patterns of distribution of myxomycetes associated with plant communities. The study in Bohol islands added eight new records for the country, such as *Comatricha elegans* (Racib.) G. Lister, *Comatricha laxa* Rostaf., *Didymium bahiense* Gottsb., *Physarina echinospora* K.S. Thind & Manocha, *Physarum bitectum* G. Lister, *Physarum sulphureum* Alb. & Schwein., *Physarum serpula* Morgan, and *Physarum straminipes* Lister. In the Caramoan islands, it was shown that there was no correlation between

and geographical and ecological distances and that at a small scale, it appears that myxomycetes adhere to the unified neutral theory of biodiversity and biogeography instead of the insular biogeography theory. This assessment added 16 new records to the myxomycetes found in the Bicol region peninsula, and one new record for the country: *Lamproderma arcyrioides* (Sommerf.) Rostaf. Chapter IV produced the first survey of myxomycetes in Coron island, where eight new morphospecies were added to the Palawan group of islands including a new record for the country: *Badhamia macrocarpa* (Ces.) Rostaf. Altogether these three surveys increased the myxomycetes collected in the Philippines by 10. The ecological analyses performed on these groups of islands also emphasized the need for extensive investigations and ample sampling in assessing diversity patterns and impacts of conservation efforts in areas that are vulnerable to ecological and climate alterations.

In an advent to map the myxomycetes of the Philippines, more than 5,000 collections of myxomycetes were geocoded with information that could be filtered, queried, and analyzed with other geographic and ecological data in chapter V of this dissertation. This study summarized the areas with the most collections done, the most and least frequently collected morphospecies, and the update of current myxomycetes recorded in the Philippines to 163 with the inclusion of *Craterium aureum* (Schumach.) Rostaf. and *Physarum javanicum* Racib. This undertaking also demonstrated an evaluation that can be done with the data, which is modeling the predicted current distribution of the morphospecies *Arcyria cinerea* (Bull.) Pers. that was found to be occurring with high probability in the central and some northwestern areas of the country.

Results of the study on the biogeographic region known as the Huxley's line suggested that although there is an affinity between Palawan (in the Philippines) and the island of Borneo in terms of composition of myxomycete species, Palawan still exhibits a higher community similarity with

the rest of the Philippine archipelago compared to Borneo. It was recommended to include metadata from the Australo-Papuan region and the oriental regions to see if Palawan and Borneo will convincingly cluster with the rest of the areas west of the line.

In the last section of this dissertation, the current global distribution of the myxomycete morphospecies *A. cinerea* was predicted to be moderately to largely abundant in several areas, except for the Antarctic and Arctic regions. The most important bioclimatic variable contributing to the current model was temperature seasonality. Future predicted models under different climate change scenarios, however, illustrated a continuously diminishing distribution that is dramatically evident in smaller land masses and islands. Novel environmental conditions were also anticipated in the future climate, where the global distribution of *A. cinerea* was projected to probably be influenced by annual precipitation and annual mean temperature more than other bioclimatic variables. Nonetheless, outcomes of predictive ecological models/algorithms should be interpreted with caution, especially when projecting/extrapolating to spatio-temporal environments outside of the training data.

Literature Cited

Adl, S. M., Bass, D., Lane, C. E., Lukeš, J., Schoch, C. L., Smirnov, A., Agatha, S., Berney, C., Brown, M. W., Burki, F., Cárdenas, P., Čepička, I., Chistyakova, L., del Campo, J., Dunthorn, M., Edvardsen, B., Eglit, Y., Guillou, L., Hampl, V., Heiss, A. A., Hoppenrath, M., James, T. Y., Karnkowska, A., Karpov, S., Kim, E., Kolisko, M., Kudryavtsev, A., Lahr, D. J., Lara, E., Le Gall, L., Lynn, D. H., Mann, D. G., Massana, R., Mitchell, E. A., Morrow, C., Park, J. S., Pawlowski, J. W., Powell, M. J., Richter, D. J., Rueckert, S., Shadwick, L., Shimano, S., Spiegel, F. W., Torruella, G., Youssef, N., Zlatogursky, V. & Zhang, Q. 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. Journal of Eukaryotic Microbiology 66: 4-119.

Fiore-Donno, A. M., Nikolaev, S. I., Nelson, M., Pawlowski, J., Cavalier-Smith, T. and Baldauf, S. L. 2010. Deep phylogeny and evolution of slime moulds (Mycetozoa). Protist 161: 55–70.

Lahr, D. J. G, Grant, J., Nguyen, T., Lin, J. H. and Katz, L. A. 2011. Comprehensive phylogenetic reconstruction of Amoebozoa based on concatenated analyses of SSU-rDNA and actin genes. PLoS One 6(7): e22780.

Leontyev, D., Schnittler, M., Stephenson, S., Novozhilov, Y. & Shchepin, O. 2019. Towards a phylogenetic classification of the Myxomycetes. Phytotaxa 399(3): 209–238.

Smirnov, A. V., Chao, E., Nassonova, E.S. & Cavalier-Smith T. 2011. A revised classification of naked lobose amoebae (Amoebozoa: Lobosa). Protist 162(4): 545-70.

Spiegel, F. W., Shadwick, L. L., Ndiritu, G. G., Brown, M. W., Aguilar, M. & Shadwick, J. D. 2017. Protosteloid Amoebae (Protosteliida, Protosporangiida, Cavosteliida, Schizoplasmodiida, Fractoviteliida, and Sporocarpic Members of Vannellida, Centramoebida, and Pellitida). In: Archibald J. et al. (eds) Handbook of the Protists. Springer, Cham.

Walker, L. & Stephenson, S. 2016. The Species Problem in Myxomycetes Revisited. Protist 167: 319-338.