

Protosteloid amoebal assemblages as microbial models for elevational diversity gradient in tropical montane landscape

Akira T. Komoda^{1,2}, Lanz Aidan Daryll A. Tan^{1,2}, Allan Miguel G. Tomimbang^{1,2}, Kristian Andrei R. Tan^{1,2}, John Carlo Redeña-Santos^{2,4}, Nikki Heherson A. Dagamac^{1,2,3,4,*} & Christian Elmarc Ocenar-Bautista²

¹ Department of Biological Sciences, College of Science, University of Santo Tomas, España 1008 Manila Philippines

² Initiatives for Conservation, Landscape Ecology, Bioprospecting, and Biomodeling (ICOLABB), Research Center for the Natural and Applied Sciences, University of Santo Tomas, España 1008 Manila Philippines

³ The Graduate School, University of Santo Tomas, España 1008 Manila Philippines

⁴ D'ABOVILLE Foundation and Demo Farm Inc., 1200 Makati City, Philippines

* e-mail: nhadagamac@gmail.com

Komoda A.T., Tan L.A.D.A., Tomimbang A.M.G., Tan K.A.R., Redeña-Santos J.C., Dagamac N.H.A. & Ocenar-Bautista C.E. (2023) Protosteloid amoebal assemblages as microbial models for elevational diversity gradient in tropical montane landscape – *Sydowia* 77: 129–139.

Protosteloid amoebae are small, fungus-like protists belonging to Class Amoebozoa whose diversity and distribution in the Southeast Asian tropics remained little known. A systematic collection of aerial and ground litter collected at different elevation belts (0–300, 301–600, 601–900, 901–1200, 1201–1500 m a.s.l.) on the north- and south-facing slopes of Mt. Calavite Wildlife Sanctuary (MCWS), Philippines was cultivated on weak malt yeast agar (wMYA). A positive wMYA plate where morphologically determinable protosteloid amoebae were distinguished served as a taxonomic unit for the ecological analysis employed in PAST (Paleontological Statistics) and R studio. The following trend was observed amongst the protosteloid amoebal communities: (i) highest species diversity was found on middle elevation, aerial substrates, and south-facing slope; (ii) the cosmopolitan species *Protostelium mycophagum* appeared to be the most abundant; and (iii) the beta diversity clusters lower elevation and higher elevation sites separately indicating variations in species composition. Interestingly, the ecological pattern of elevational diversity gradient (EDG) applied to macroorganisms seems to be a potential pattern even in cultivable fruiting bodies of protosteloid amoebae. This work is the first in-depth species diversity study of protosteloid amoebae in the Philippines and the whole of Southeast Asia.

Keywords: abundance, eumycetozoa, microbial ecology, morphological species concept, slime molds.

Protosteloid amoebae, like dictyostelids and myxomycetes, are a microscopic group of slime molds (Eumycetozoa). They begin as single-celled amoebae, which are typically found on decaying litter of both ground and aerial substrate. These provide them with a sufficient supply of food sources, such as fungi and bacteria, for their proliferation (Spiegel et al. 2007). As such, protosteloid amoebae are usually considered microbial predators. Following this predatory amoeboid stage, they eventually mature to form clear to transparent fruiting bodies that typically consist of one to eight spores held by a non-septate needle-like stalk (Spiegel et al. 2007, Schnittler et al. 2012). Under certain conditions (i.e., environmental condition disturbance), the protosteloid amoebae fruiting bodies undergo spore dispersal until such conditions improve, whereas the spores will be able to

germinate fresh protosteloid amoebae again (Spiegel et al. 2017). Protosteloid amoebae are concentrated in terrestrial ecosystems, with higher prevalence in tropical and temperate forests (Spiegel et al. 2007, Moore & Spiegel 2000, Shadwick 2010). A variety of factors have been shown to affect protosteloid amoebae diversity across these ecosystems, particularly elevation, climatic variables, precipitation seasonality, and temperature range (Aguilar et al. 2011). Interestingly, a majority of species have been found to grow more dominantly in the presence of two or more of these factors. For example, *Cavostelium apophysatum*, *Nematostelium gracile*, and *Schizoplasmodiopsis amoeboides* that predominantly grow on aerial litter microhabitats prefer higher precipitation and lower temperature ranges during both wet and dry months. It has also been suggested that different

microhabitats play an important role in supporting protosteloid amoeba assemblages, with some species whose occurrence tends to correlate on a particular substrate (Zahn et al. 2014). This may suggest that even though protosteloid amoeba can proliferate along varying climatic and seasonal conditions, different species are more readily defined based on their preferred microhabitat (e.g., barks of decaying logs or living trees, decaying plant material, and aerial or ground litter) as outlined by Spiegel et al. (2017). Among all the groups of Eumycetozoa, protosteloid amoebae are the least studied in terms of their biodiversity due to their perplexing morphology and occurrences, making them difficult to identify and cultivate (Shadwick et al. 2017). In fact, up to this date, no single record of its ecology in the Philippines and in tropical Southeast Asia has been reported. Thus, this leaves a wide gap regarding protosteloid amoeba diversity and distribution within the Eumycetozoa group (Spiegel et al. 2007, Schnittler et al. 2012). Therefore, the primary objective of this study is to assess for the first time the diversity and distribution of protosteloid amoebae across varying elevational belts, two different leaf litter types, and aspect or slope direction of a mountain using Mt. Calavite Wildlife Sanctuary (MCWS) as the model site.

Materials and methods

Sampling method and collection

Sample collection was performed on the tropical mountain Mt. Calavite Wildlife Sanctuary, Mindoro, Philippines (13.4365° N, 120.3666° E) via random sampling of 10 aerial leaf litters and 10 ground leaf litters per elevation belt set at least 300 meters apart, yielding a total of five sampled elevation belts above sea level: 0–300, 301–600, 601–900, 901–1200, and 1201–1,500 m a.s.l. (see Fig. 1). Sampling was done on each mountain slope (north and south), cumulatively totaling 200 litter samples used only for this study. All collected leaf litter samples were immediately air-dried to prevent filamentous fungi from growing and the leaves from rotting. They were then placed in a paper bag for transport and storage until further being processed in the laboratory (Spiegel et al. 2007).

Media preparation

The weak malt yeast agar (wMYA) media were prepared in 1 liter batches. Each batch of media was able to supplement 50 plates. To cultivate all samples, four batches of media and 200 culture plates were prepared. For 1 l of the wMYA 0.02 g

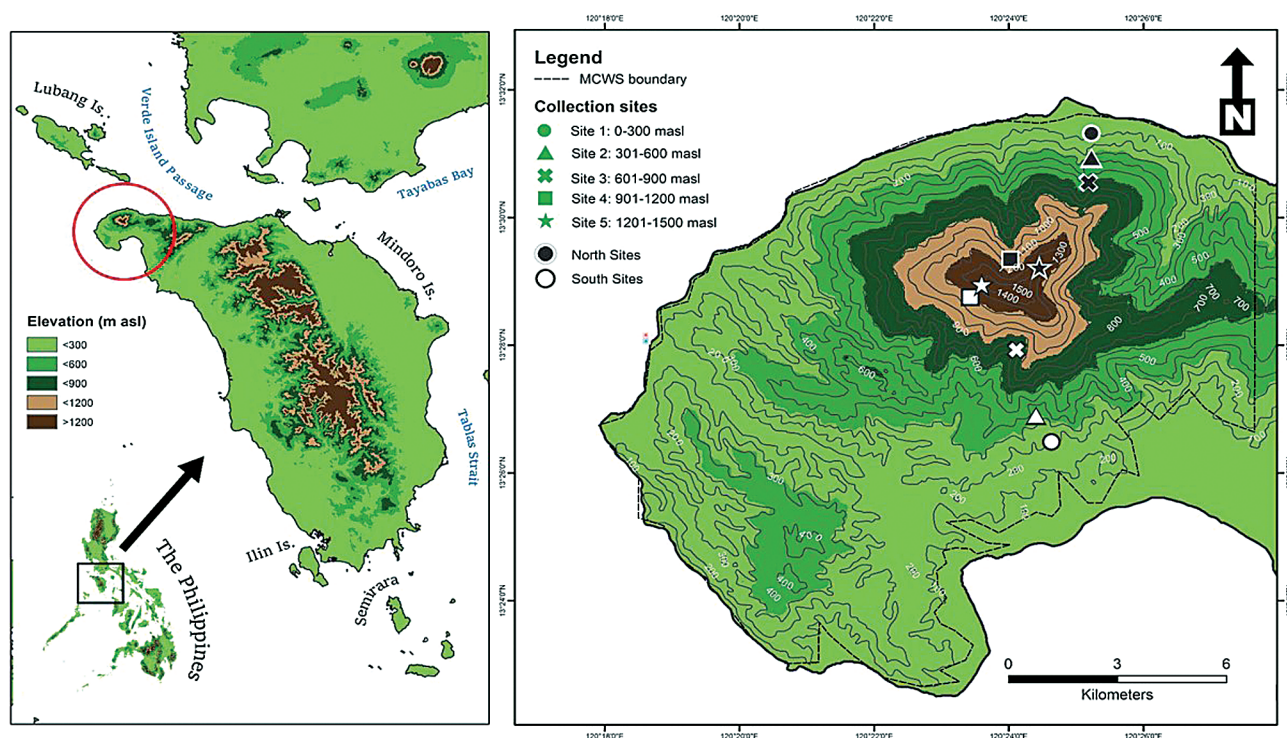


Fig. 1. Map of the sampling site (Mt. Calavite Wildlife Sanctuary, Mindoro, Philippines).

yeast extract powder, 0.02 g malt, 0.75 g K₂HPO₄ (Dipotassium hydrogen phosphate), and 15 g agar were used. The media was then autoclaved at 121 °C, 15 psi for 15 min (Ocenar-Bautista et al. 2024).

Cultivation (Primary isolation plate)

The collected aerial and ground leaf litter were cut into tiny strips (to fit in the disposable Petri dishes) using sterilized scissors (Spiegel et al. 2007) and categorized according to the five elevation belts and the two mountain slopes. Then they were soaked in distilled water for 20 min and transferred to the prepared media plates using sterile forceps. To maintain a consistent number of leaves for observation, eight leaves were picked and radially placed on a Petri plate. The media plates with leaf litter were incubated in a moist environment at room temperature for 5 to 14 days (Ocenar-Nautista et al. 2024).

Observation

The phenotypic observation was focused on the sporoma(ta) of the protosteloid amoebae via microscopic examination using a compound light microscope (10× objective) on the edges of the leaf litter (substrate). Sporomata are the most recognizable and easier to pinpoint than any of its life stages (Spiegel et al. 2007). The observation of sporomata was done between 5 and 14 days (ideal observation days since it is the protosteloid growth at its maximum). Abundance was counted as one abundance count per one positive plate for each species, meaning observation of a particular species in one plate, no matter the number of occurrences on the same plate, is counted as one count.

Species identification

The vast majority of the protosteloid species can be identified with microscopic observation of their sporoma morphology (Spiegel et al. 2007). The typical morphology of protosteloid amoebae includes the sporomata being transparent, similar to a water droplet, stalks delicate, and ending with a hydrophobic basal disk (Spiegel et al. 2007). However, it is also important to take into consideration the spore (shape, number, and size), stalk (length, width, curviness or sinuousness, and flexibility), and their spore sensitivity to air currents. The hand guide “*A Beginner’s Guide to Identifying The Protostelids*” by Spiegel et al. (2007) was used to identify the different morphospecies. The hand guide contains information regarding more than 90 % of the pro-

tosteloid amoebae species, as well as images, the rarity of the specimen, the habitat in which they can be found, and differences between protosteloids, fungi, myxobacteria, and other slime molds. Valid accepted names were checked using the online nomenclature information system of Eumycetozoa (nomen.eumycetozoa.com).

Data analysis

Morphospecies counting was initially emphasized prior to data analysis of the diversity and abundance of protosteloid amoeba species. In contrast, a wMYA that showed growth (evidence of sporomata and stalks), regardless of the evidence in multiple replicates in one plate, is only considered as one positive taxonomic unit. This concept of a countable taxonomic unit for a species was adapted from Dagamac & Dela Cruz (2015) from their review of moist chamber collections of myxomycetes in the Philippines. Herein, the total list of species found in MCWS was accounted for and was then assigned a count based on the aforementioned counting unit. Afterward, the relative abundance was calculated by dividing the number of records per species by the total number of records for the whole study for each of the three variables: (i) ground and aerial litter; (ii) north and south aspects of the sampling site (MCWS); and (iii) the 5 elevational belts. The relative abundance for every species of protosteloid amoebae was then computed and assigned a specific abundance index value wherein those species having >10 % are considered abundant, >5 % common, >1 % occasional, and <1 % rare (Kuhn et al. 2013). For this study, the protocol of Dagamac et al. (2012) was followed, wherein the abundance index was determined by assigning a “breaking point” based on the calculated relative abundances.

In terms of assessing the alpha diversity, the richness was calculated following Bernardo et al. (2018) by constructing a species accumulation curve (SAC) employed using iNEXT package (Hsieh et al. 2016) in R. We were using the vegan package (Oksanen et al. 2012) following with slight modifications the scripts used in Dagamac et al. (2017), and Redeña-Santos et al. (2018). A series of boxplots showing the Shannon diversity index, Simpson diversity index, and their corresponding derivatives were constructed to compare between aspect/slope, substrate type, and elevational belts. Following the calculations from Pecundo et al. (2017), the statistical significance for each diversity index being compared was done to see if the diversity values be-

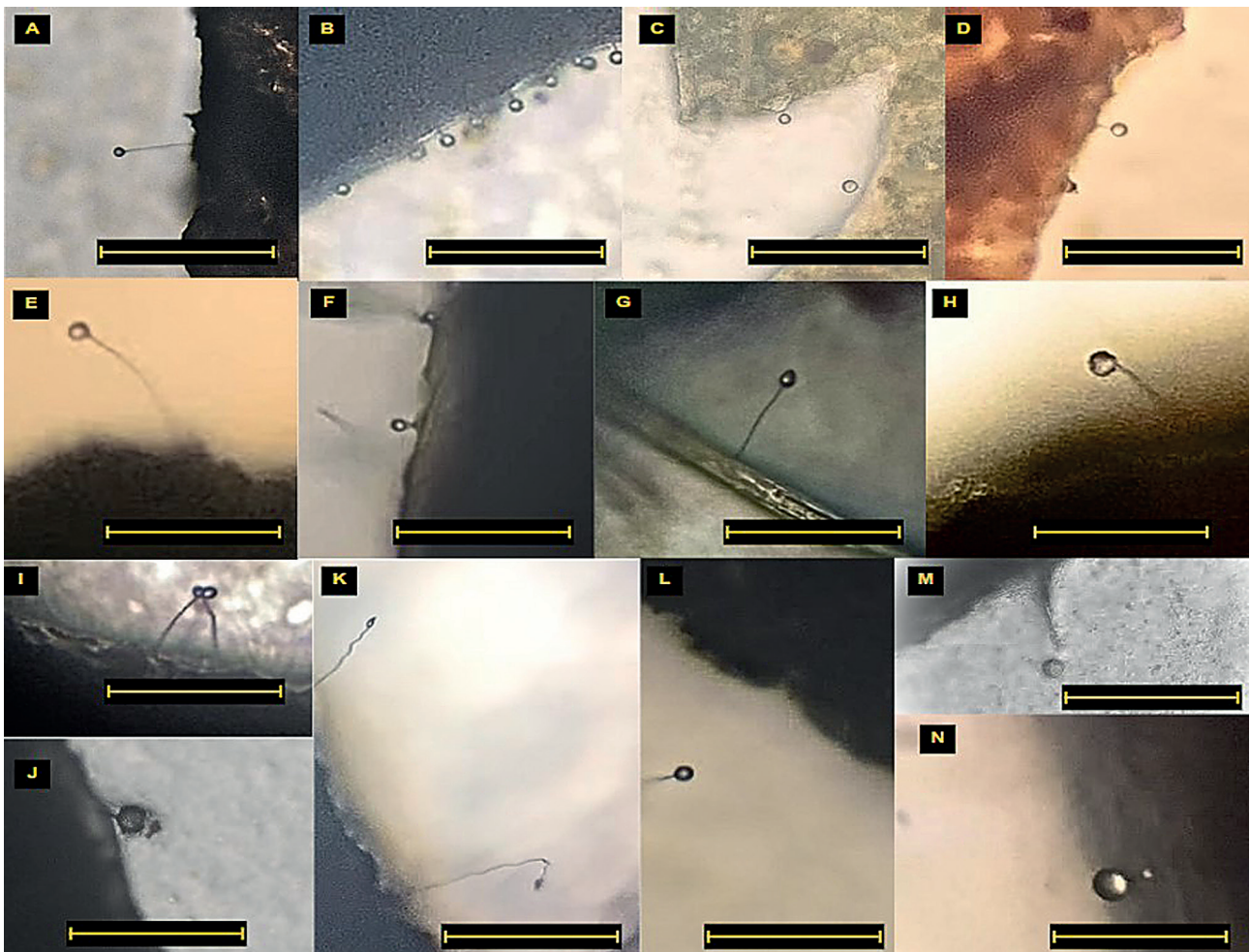


Fig. 2. Protosteloid amoebae species recovered from Mt. Calavite Wildlife Sanctuary, Mindoro, Philippines, viewed at 100× magnification (Scale bar = 100 μm). **A.** *Protostelium mycophagum*. **B.** *Schizoplasmodiopsis pseudoendospora*. **C.** *Microglomus paxillus*. **D.** *Schizoplasmodiopsis amoeboidea*. **E.** *Nematostelium gracile*. **F.** *Cavostelium apophysatum*. **G.** *Nematostelium ovatum*. **H.** *Schizoplasmodiopsis reticulata*. **I.** *Protostelium arachisporum*. **J.** *Endostelium amerosporum*. **K.** *Soliformovum irregulare*. **L.** *Protostelium nocturnum*. **M.** *Tychosporium acutostipes*. **N.** *Schizoplasmodiopsis vulgaris*.

tween the variables are caused by something other than mere chance. The diversity t-test was employed from PAST software (Hammer et al. 2001). Additionally, to depict the number of species being reflected as ranks and evenness being reflected as a slope of a line, a rank abundance curve testing five models was also calculated in the “vegan” package of R using the *radfit* functions. For beta diversity, composition between aspects, between substrate type, and among elevational belts was measured using (1) neighbor-joining (NJ) clustering tree and (2) non-metric multidimensional scaling (NMDS) ordination based on Euclidean distances. The significance of the values was then tested using the *adonis* function to calculate the permutation multivariate ANOVA (PERMANOVA).

Results

Species richness and abundance

From the 200 wMYA plates, 43 % yields a positive fructification of protosteloid amoebae. From these wMYA plates, a total of 16 protosteloid amoebae species from nine genera (105 total records) were morphologically identified from the wMYA plates cultivated from MCWS (Fig. 2), with *Protostelium mycophagum* L.S. Olive & Stoian being the most abundant (23 records). Comparing the relative abundance (Fig. 3), the abundance pyramid revealed three abundant species (*Protostelium mycophagum*, *Schizoplasmodiopsis pseudoendospora* L.S. Olive, M. Martin & Stoian, *Microglomus paxillus* L.S. Olive & Stoian), three common (*Schizoplasmo-*

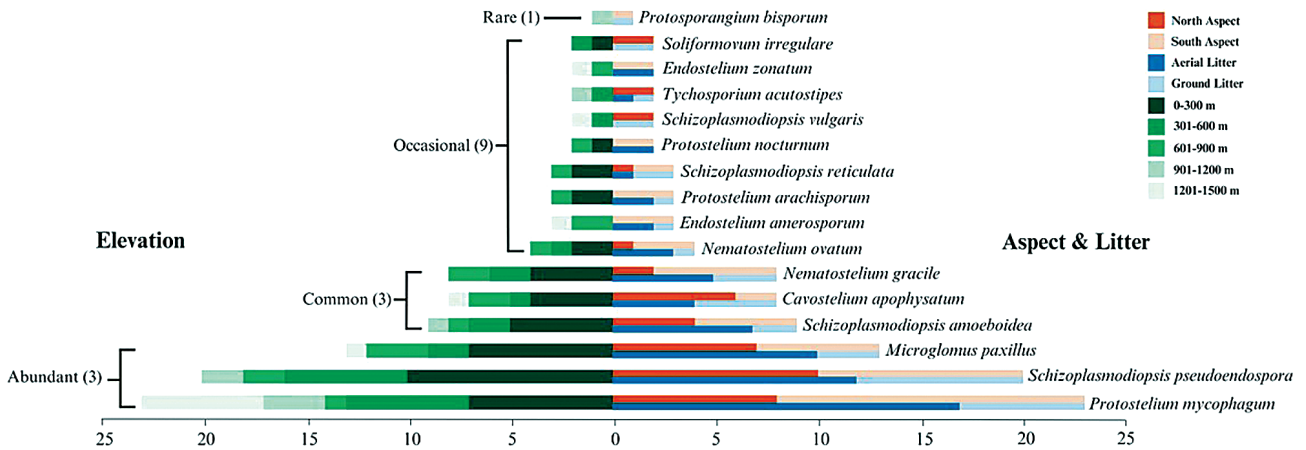


Fig. 3. Pyramid graph showing the relative abundances of all the protosteloid amoebae species found in MCWS across the three variables (red = aspect/slope, blue = substrate type, green = elevation).

diopsis amoeboides L.S. Olive & K.D. Whitney, *Nematostelium gracile* (L.S. Olive & Stoian) L.S. Olive & Stoian, *Cavostelium apophysatum* L.S. Olive), nine occasional (*Nematostelium ovatum* (L.S. Olive & Stoian) L.S. Olive & Stoian, *Schizoplasmodiopsis reticulata* L.S. Olive & Stoian, *Protostelium arachisporum* L.S. Olive, *Endostelium amerosporum* L.S. Olive, *Soliformovum irregulare* (L.S. Olive & Stoian) Spiegel, *Endostelium zonatum* (L.S. Olive & Stoian.) W.E. Benn. & L.S. Olive, *Tychosporium acutostipes* Spiegel, D.L. Moore & J. Feldman, *Schizoplasmodiopsis vulgaris* L.S. Olive & Stoian, *Protostelium nocturnum* Spiegel), and one rare (*Protospo-*

rangium bisporum L.S. Olive & Stoian.) species with only one record. The data also confirms that there are higher abundances in aerial than ground litter, at the south than north aspect, and at the first elevation as well as the highest species richness at the third elevation.

Higher species richness was observed in the ground litter substrate and in the south aspect of the tropical mountain as compared to the aerial litter and north aspect. In terms of the five elevations, elevation three (601–900 m) yields the highest richness, followed by elevation five (1201–1500), elevation one (0–300), elevation two (301–600), and eleva-

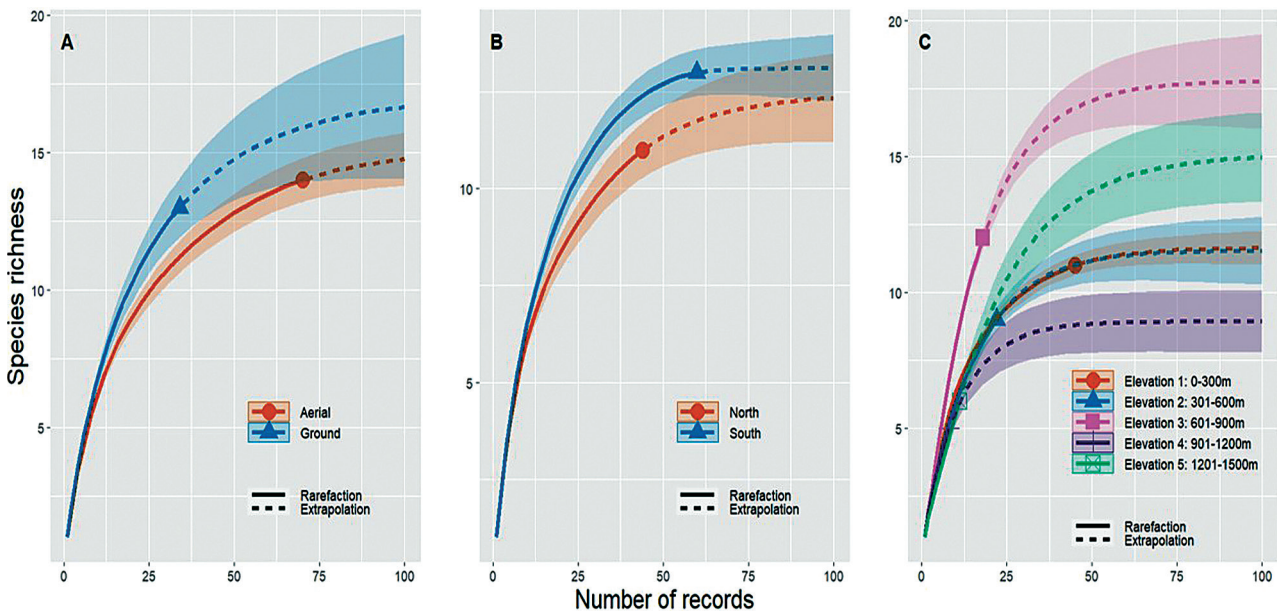


Fig. 4. Species accumulation curve (SAC) of protosteloid species on: **A.** Two types of litter substrates (ground and aerial); **B.** Two types of aspects (north and south); and **C.** Elevation belts based on q=0 plot (ggiNEXT).

tion four (901–1200) (see Fig. 4). On the other hand, higher abundances (number of records) were found in the aerial litter substrate than in the ground litter and also in the south aspect of the mountain (see Fig. 4). Elevation one (0–300 m) generated the highest abundance, subsequently followed by elevation two, elevation three, elevation five, and elevation four.

The rank abundance curve (see Fig. 4) exhibited by the steep slopes of (i) ground litter; (ii) north aspect; and (iii) elevation three (601–900 m) showed higher abundances for dominating species, thus low species evenness. Shallow slopes of (i) aerial litter; (ii) south aspect; and (iii) elevations one, two, four, and five were observed to show higher species evenness and distribution.

Alpha diversity

When comparing the species diversity based on various alpha diversity indices (Fig. 6), results show no significant differences ($p > 0.05$) between aerial and ground litter as established by the overlap of the interquartile ranges of these assemblages (Redeña-Santos et al. 2017, Güler et al. 2016). The aerial litter substrate showed a slightly higher interquartile range due to a higher number of recorded protosteloid amoebae species than in the ground litter. The same trend can also be seen between the north and south aspects, where these assemblages show non-significant differences in terms of their

diversity. However, the same trend cannot be inferred for the species diversity across the elevations, where assemblages in the third elevation (601–900 m) exhibit the highest diversity and evenness, showing significant differences ($p < 0.05$) with elevations four and five established by their non-overlapping interquartile ranges – but have little to non-significant differences with elevations one and two.

Beta diversity

The species composition on the non-metric multidimensional scaling ordination (NMDS) of the litter substrates (aerial and ground) and the five elevations displayed no significant differences (Fig. 7A, C), however, the two mountain aspects (north and south) also present a significant difference (Fig. 7B) in terms of species composition ($R^2 = 0.108$, $P = 0.03$); with the south aspect covering a larger area due to the higher species abundance. Two distinct cluster differences are also observed within the elevational belts (Fig. 7C), which can be further supported by the constructed Neighbor-Joining Clustering Tree (Fig. 7D), whereby bipartite clusters are formed on elevations one & two and elevations four & five, representing similar species composition; while elevation three shares similar protostelid species with all other elevations as well as having a higher degree of similarity to elevations one and two. This implies that clusters one and two have sig-

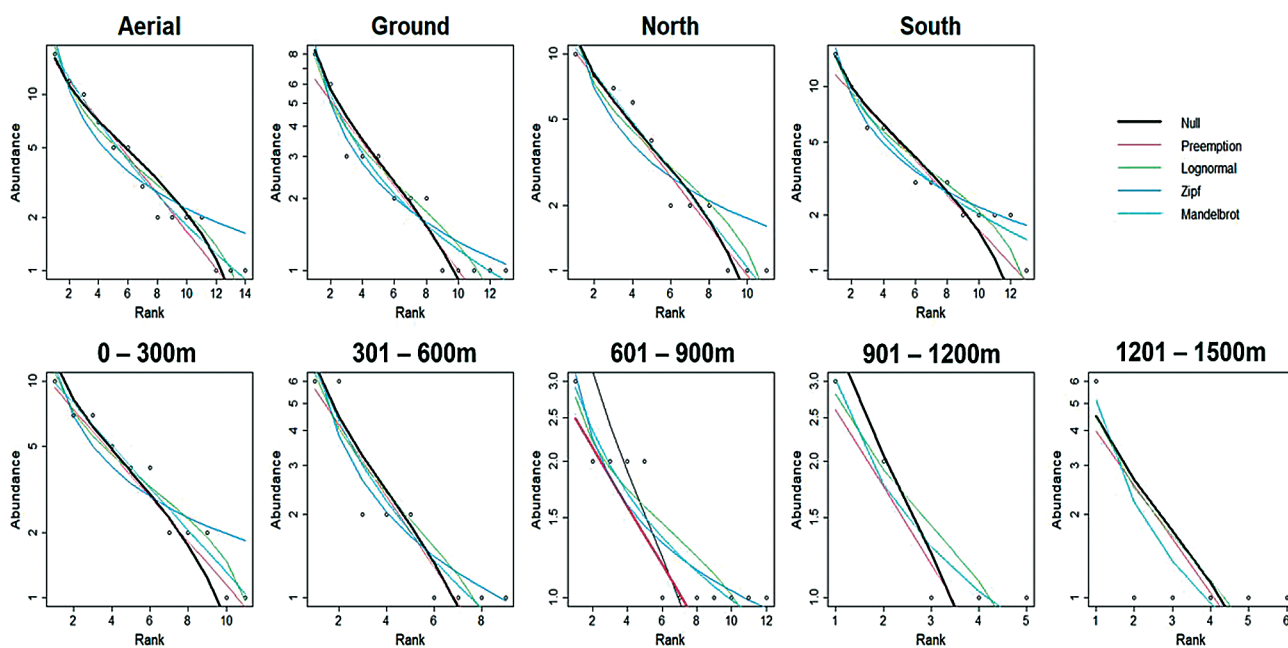


Fig. 5. Rank abundance curves of Aerial versus Ground litter, North versus South mountain aspects, and of the five elevations.

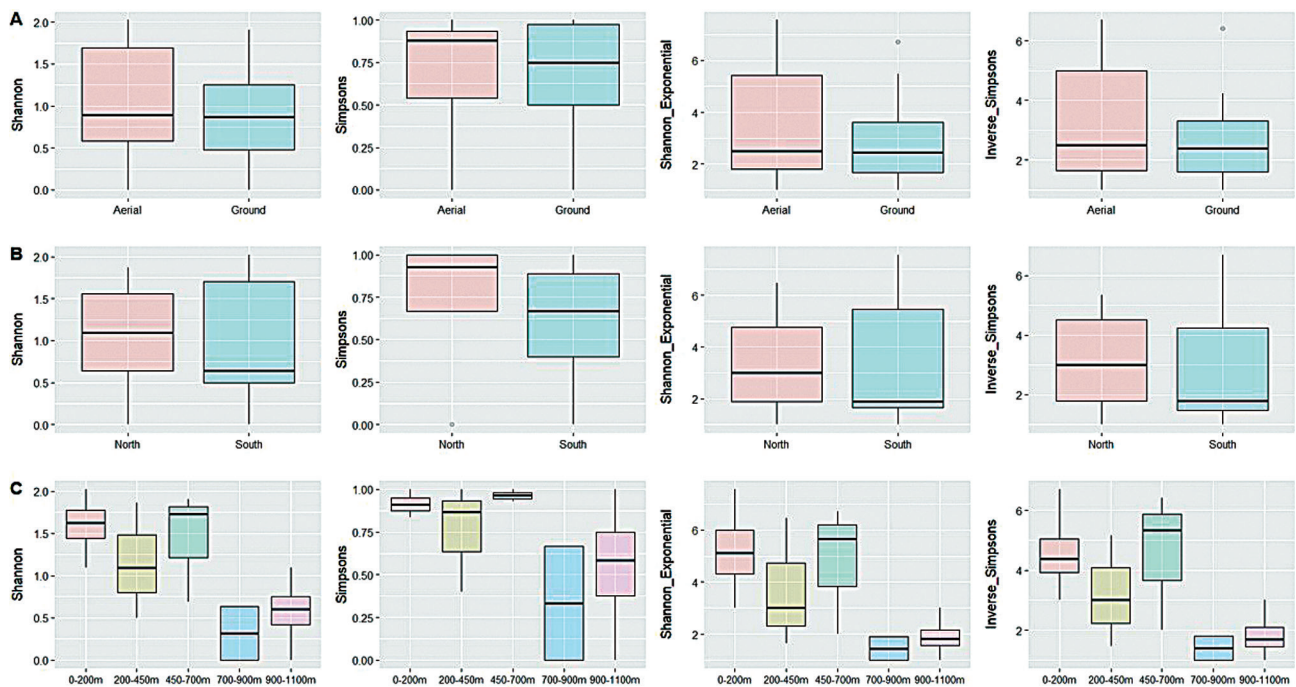


Fig. 6. Alpha diversity boxplots of: **A.** Leaf litter substrates (ground and aerial); **B.** Mountain aspects (north and south); and **C.** Elevational belts.

nificant differences in protosteloid amoebal community occupancy with elevations four and five.

Discussion

Diversity studies on slime molds, specifically on protosteloid amoebae globally are still lacking. Worldwide, diversity and distribution of protosteloid amoebae have been conducted at Great Smoky Mountains National Park (Shadwick et al. 2009), in terrestrial biomes of Australia (Powers & Stephenson 2006), in tropical wet forest of Costa Rica (Moore & Stephenson 2003), as well as in various aquatic environments (Shadwick 2011). Over the last decades, concentrations on understanding the systematic and evolutionary aspects of this enigmatic group of eumycetozoon have drawn more attention in comparison to classical ecological assessment that utilizes morphological species concepts (Mishler 2021). Perhaps, this is due to the difficulty associated with cultivating the species and morphologically assigning species for protists that are also candidates for high cryptic speciation wherein sophisticated molecular tools are a necessity (Walker & Stephenson 2016, Buisan & Dagamac 2021). Nevertheless, like the other eumycetozoon counterparts, the morphologies of protosteloid amoebae fructification are distinguishable albeit, being microscopically conspicuous (Dagamac & dela Cruz,

2015). As such, the classical approach used in species diversity studies of myxomycetes (Balaoro-Banzuela et al. 2023, Limbo-Dizon et al. 2022, Macabago & Stephenson 2021) or dictyostelids (Dagamac & dela Cruz 2019, Liu & Li 2014) of which many ecological hypotheses have been tested (see Schnittler et al. 2022, Bernardo et al. 2018, Dagamac et al. 2017) also merits protosteloid amoebae to be microbial models. Using classical morphological assessment and following the established protocols in conducting diversity studies on the other eumycetozoon counterparts, this pioneering study for the Philippines attempts to understand the distribution of protosteloid amoebae along tropical elevational belts with opposing slopes and different types of substrate collected from MCWS, a locally protected terrestrial landscape in the Philippines (Mindoro Biodiversity Conservation Foundation Inc. 2014).

Litter diversity of protosteloid amoebae

In terms of species diversity of leaf litter substrates (Figs. 3, 4), higher richness was observed from the ground litter than the aerial litter, and on the other hand, the aerial litter was observed to have a higher abundance that contains *Protostelium mycophagum* as the most abundant species. Additionally, the aerial litter exhibited a shallow slope,

while the ground litter exhibited a steep slope (Fig. 5). Despite this, the diversity t-test analysis has indicated no significant difference in the species diversity of ground and aerial litter. This finding was also observed in a litter where other slime mold species were used as a model (Nguyen et al. 2020, Dagamac et al. 2015, Rea-Maminta 2015). If this is the case, protosteloid amoebae diversity is not heavily reliant on the type of leaf litter substrates. This can be attributed to the high microbial load present in the soil (Macabago et al. 2017), which suits the ground litter fructification where protosteloid amoebae predate, while the aerial foliage provides better spore dispersal via wind for the aerial litter fructification (Balaoro-Banzuela 2023). Hence, regardless of the contact on the soil surface or wind dispersal, for as long as the leaf has morphologies that can indirectly trap spores of protosteloid amoebae, expect that the diversity between similar leaf litters (either ground or aerial) to have at least a similar species diversity.

Species diversity between different slope aspects

Higher richness and abundance were observed in the south aspect of the mountain than in the north (Figs. 3, 4). In the rank abundance curve (Fig. 5), the north aspect showed a steep slope, whereas the south aspect resulted in a shallow slope, which is indicative of a higher species evenness established in the latter. Nonetheless, there is no significant difference between the two mountain aspects in terms of their alpha diversity (Fig. 6). Although no studies were done on the species diversity of protosteloid amoebae in the mountain direction, Stephenson (1989) indicated that the north aspect of a temperate forest contains a slightly higher species richness and diversity of myxomycetes, which is in contrast to this study's findings. PERMANOVA showed that there is a significant variation between the species composition in south-facing assemblages versus their northern counterparts. This inter-slope variation, however, may be the result of a differential response of slope-specific species driven by several environmental factors linked with the mountain's slope aspect. Previous studies carried out in montane environments in mid-latitudinal regions revealed pronounced differences in humidity, moisture, precipitation, transpiration, and vegetation between north- and south-facing aspects, which affects community attributes resulting in niche differentiation (Méndez-Toribio et al. 2016, Pepin et al. 2017, Marler & del Moral 2018, Singh 2018). However, regardless of

this finding, the protosteloid amoebae α -diversity did not show significant differences between the two mountainsides (north or south aspects), which may indicate that the conditions (i.e. solar radiation and moisture) are favorable. Nevertheless, this tells us that the protosteloid growth in the two aspects also occurs as a pattern and is non-random. To ascertain these suppositions, comparative studies among accessible vertical trails are highly recommended for further investigations.

Elevational distribution of protosteloid amoebae

Among the five elevations presented (Figs. 3, 4), the third elevation (601–900 m) exhibited the highest species richness, followed by the fifth elevation (1201–1500 m), first elevation (0–300 m), second elevation (301–600 m), and fourth elevation (901–1200 m). Despite this, the alpha diversity (Fig. 6) showed that the third elevation has the highest species diversity. On the other hand, the elevation that produced a highly populated protosteloid area is elevation one, which is to be expected since warmer temperatures at lower elevations are favorable to fungi, which means more decompositions are occurring, and more protosteloid amoebae can inhabit decaying litter substrates (Looby & Martin 2020). Similarly, in the studies of Landolt et al. (2006), Rojas & Stephenson (2008), and Cheng et al. (2013), lower elevations also yield higher abundances of myxomycetes and dictyostelids, while Rojas & Stephenson (2008), Liu et al. (2013), and Dagamac et al. (2017) confirm that species diversity of myxomycetes are the highest at lower elevations since their diversity decreases as elevation increases. In the case of dictyostelids, species richness was observed in the high elevation and low elevation, with the mid-elevation having the least richness (Landolt et al. 2006), which sparks interest since it gives an impression that each of the slime mold groups seems to have a distinct elevational diversity pattern.

The results obtained align with an ecological pattern or phenomena of changing diversity with elevation, or specifically, decreasing species diversity (or species richness) as elevation increases, known as elevation diversity gradient (EDG) (McCain & Grytnes 2010). In the case of slime molds, according to Rojas & Stephenson (2008), Liu et al. (2013), Dagamac et al. (2017) and Novozhilov et al. (2018), myxomycetes also adhere to the EDG trend of decreasing species diversity as mountain elevation increases (highlands) in a tropical mountain setting, which is insightful because protosteloid amoebae shares similarities with myxomycetes. Ad-

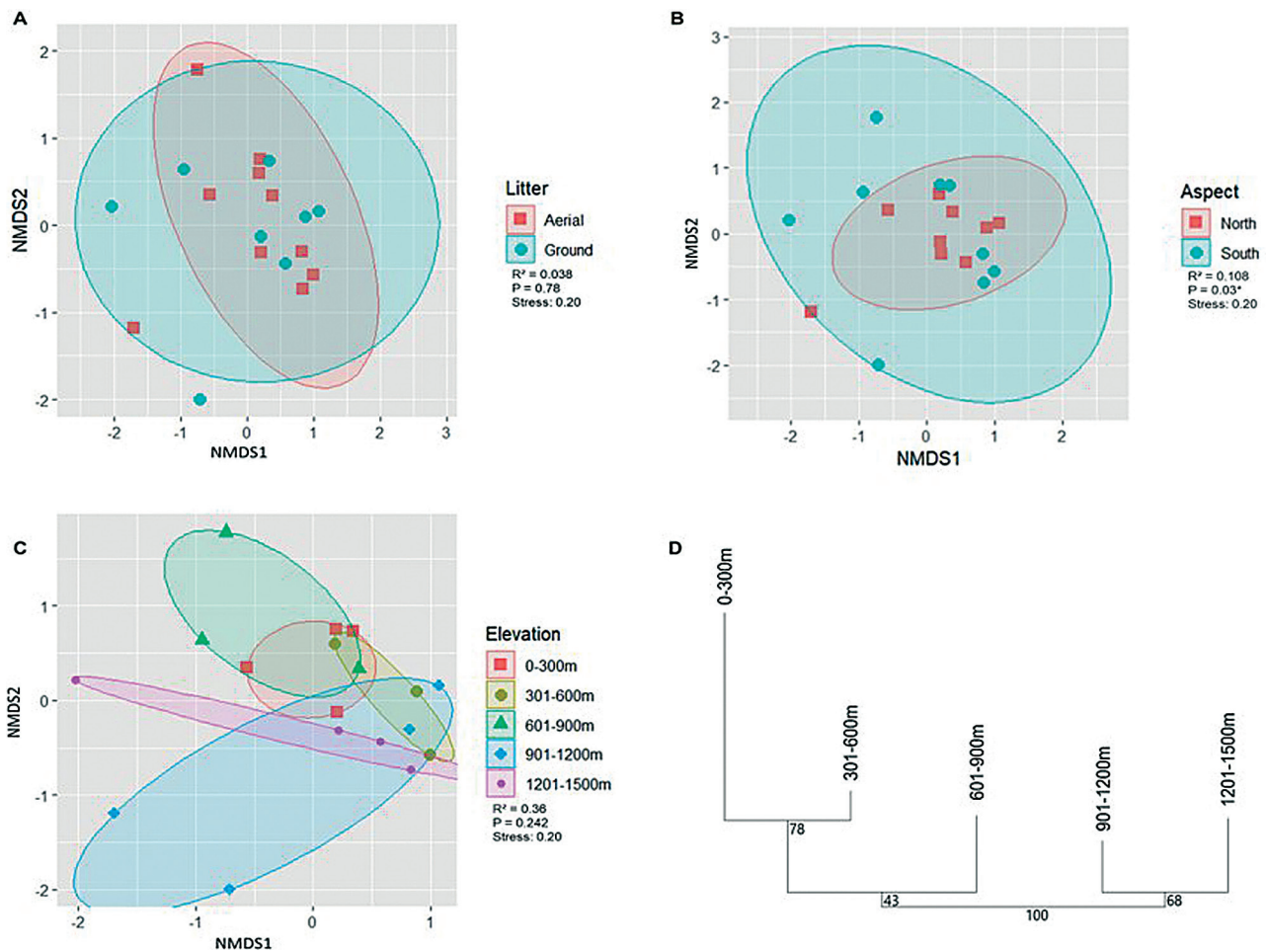


Fig. 7. NMDS ordination plot of: **A.** Two litter substrates (ground and aerial); **B.** Two mountain aspects (north and south), where an asterisk (*) indicates a significant difference in their species composition as established by PERMANOVA; **C.** Elevation belts; and **D.** Neighbor-Joining Clustering Tree (Euclidean) of the different elevational belts.

ditionally, there are four types of trends observed in EDG, which, according to McCain & Grytnes (2010), are: (i) declining richness with increasing elevation; (ii) plateaued richness at low elevations; (iii) plateaued richness at low elevations with a mid-elevation peak; and (iv) unimodal pattern. As mentioned earlier, the results for the species diversity across five elevations (Fig. 4) resemble that of a normal curve, thus conforming to the unimodal pattern trend, which is more common in nature than the decreasing trend.

Five general contributing factors may affect the species diversity, richness, and abundance of protosteloid amoebae in a tropical mountain that exhibits a unimodal trend in an EDG. These are: (i) for every 100 m elevation increase, the temperature decreases by $\sim 0.6^\circ\text{C}$, which could negatively affect the growth of protosteloid amoebae inhabiting higher

elevations (elevation four and five) in the tropics since they are attuned to tropical temperatures (McCain & Grytnes 2010); (ii) decreasing air pressure and increasing solar radiation for every increasing elevation may delay the cell cycle of slime molds (as observed by Devi et al. (1968) when a myxomycete was irradiated) and influence the spore dispersal; (iii) as elevation increases, precipitation increases, however, in tropical mountains, the middle elevation exhibits the highest precipitation, which could be favorable for protosteloid amoebae colonization (Moore & Stephenson 2003, McCain & Grytnes 2010); (iv) lower and higher elevations of a mountain causes the spatial area of the said elevations to overlap with each other, which is the cause for the unimodal trend at mid-elevations—and to supplement, may be due to the fact that lower elevations are more susceptible to anthropological dis-

turbances as compared to higher elevations and that higher elevations are vulnerable to environmental changes (McCain & Grytnes 2010, Looby & Martin 2020, Balaoro-Banzuela et al. 2023); and finally, (v) flora also exhibits a unimodal trend, while bacteria and fungi shows species richness at lower elevations, which may give implication that the protosteloid amoebae also follow the unimodal pattern in order to inhabit the leaf litter substrates found in the flora-rich mid-elevations while also having ample amounts of bacterial or fungal prey (Looby & Martin 2020).

Given that few studies have only been conducted on protosteloid amoebae, future research could be conducted to ascertain many complex specific environmental conditions or extrinsic/abiotic factors such as definite temperature ranges the protosteloid amoebae would most grow in a tropical mountain climate. The more interesting part is to assess if EDG among microbial groups, specifically the fruiting eumycetozoans like the protosteloid amoebae, is more of a rule of thumb rather than just an isolated case.

Acknowledgements

The authors would like to thank the DOST-PCAARRD for the funding granted to conduct this elevational gradient study as part of the Project MATAPAT. NHAD received small financial support from UST-RCNAS and as a Balik Scientist Grantee from PCAARRD. JCR-S and CEO-B acknowledges DOST-ASTHRDP and DOST-SEI for the scholarship grant, respectively. The authors would also like to acknowledge the professional and technical assistance extended by Dr. Sittie Aisha B. Macabago and Prof. Frederick W. Spiegel.

References

- Aguilar M., Spiegel F.W., Lado C. (2011) Microhabitat and climatic preferences of protosteloid amoebae in a region with a mediterranean climate. *Microbial Ecology* **62**: 361–373.
- Balaoro-Banzuela R.C., Ocenar-Bautista C.E., Buebos-Esteve D.E., Claudio-Paragas C.Y., Limbo-Dizon J.E., Heherson N. (2023) Rapid diversity assessment of litter myxomycete assemblages in the upland and coastal terrains of San Fernando City, La Union, Philippines. *Biodiversitas* **24**(5): 2877–2886.
- Bernardo J.L.M., Arioder L.J.Q., Almadrones-Reyes K.J., 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.N., Dagamac N.H.A. (2021) Elucidating hidden slime mold diversity in Southeast Asia: a review of potential methods. *Slime Molds* **1**: V1A3.
- Cheng C.B.T., Yu K.N.T., Campos M.L., Adora J.M.V., Pascua G.C.P., Pangilinan M.V.B., Buaya A.T., dela Cruz T.E.E. (2013) Occurrence and diversity of myxomycetes (plasmodial slime molds) along the northern slope of Mt. Makulot, Cuenca, Batangas, Philippines. *Asian Journal of Biodiversity* **4**(1): Art. #297, 65–83.
- Dagamac N.H.A., dela Cruz T.E.E. (2015) Myxomycete 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 moulds) along two transects in Mt. Arayat National Park, Pampanga, Philippines. *Mycology* **3**(2): 119–126.
- Dagamac N.H.A., Rea-Maminta M.A.D., dela Cruz T.E.E. (2015). Plasmodial slime molds of a tropical karst forest, Quezon national Park, the Philippines. *Pacific Science* **69**(3): 411–422.
- Dagamac N.H.A., Novozhilov Y.K., Stephenson S.L., Lado C., Rojas C., dela Cruz T.E., Unterseher M., Schnittler M. (2017) Biogeographical assessment of myxomycete assemblages from Neotropical and Asian Palaeotropical forests. *Journal of Biogeography* **44**: 1524–1536.
- Devi V.R., Guttus E., Guttus S. (1968) Effects of ultraviolet light on mitosis in *Physarum polycephalum*. *Experimental Cell Research* **50**(3): 589–598.
- Güler B., Jentsch A., Apostolova I., Bartha S., Bloor J., Campetella G., Canullo R., Házi J., Kreyling J., Pottier J., Szabó G., Terziyska T.S., Uğurlu E., Wellstein C., Zimmermann Z., Dengler J. (2016) How plot shape and spatial arrangement affect plant species richness counts: implications for sampling design and rarefaction analyses. *Journal of Vegetation Science* **27**(4): 692–703.
- 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.
- Hsieh T.C., Ma K.H., Chao A. (2016) iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution* **7**(12): 1451–1456.
- 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) Three new records of plasmodial myxomycetes from Anda Island, Pangasinan, Philippines. *Biotropia* **20**(1): 1–9.
- Landolt J.C., Stephenson S.L., Cavender J.C. (2006) Distribution and ecology of dictyostelid cellular slime molds in Great Smoky Mountains National Park. *Mycologia* **98**(4): 541–549.
- Limbo-Dizon J.E., Almadrones-Reyes K.J., Macabago S.A.B., Dagamac N.H.A. (2022) Bioclimatic modeling for the prediction of the suitable regional geographical distribution of selected bright-spored myxomycetes in the Philippine archipelago. *Biodiversitas* **23**(5): 2285–2294.
- Liu P., Li Y. (2014) Dictyostelids from Jilin Province China. *Phytotaxa* **183**: 279–283.
- Liu Q.-S., Yan S., Dai J., Chen S. (2013) Species diversity of corticolous myxomycetes in Tianmu Mountain National Nature Reserve, China. *Canadian Journal of Microbiology* **59**(12): 803–813.
- Looby C.I., Martin P.H. (2020) Diversity and function of soil microbes on montane gradients: the state of knowledge in a changing world. *FEMS Microbiology Ecology* **96**(9): faa122.

- Macabago S.A.B., Stephenson S.L. (2021) Does Huxley's Line Apply to Myxomycetes? *Pacific Science* **75**(4): 531–541.
- 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**: 221–236.
- Marler T.E., del Moral R. (2018) Increasing topographic influence on vegetation structure during primary succession. *Plant Ecology* **219**: 1009–1020.
- McCain C.M., Grytnes J.-A. (2010) Elevational gradients in species richness. *Encyclopedia of Life Sciences* (ELS). John Wiley & Sons, Chichester, pp. 1–10.
- Méndez-Toribio M., Meave J.A., Zermeño-Hernández I., Ibarra-Manríquez G. (2016) Effects of slope aspect and topographic position on environmental variables, disturbance regime and tree community attributes in a seasonal tropical dry forest. *Journal of Vegetation Science* **27**(6): 1094–1103.
- Mindoro Biodiversity Conservation Foundation Inc. (2014) Mt. Calavite Wildlife Sanctuary Resource and Socio-Economic Assessment (RSEA) Final Report. Muntlupa City. Mindoro Biodiversity Conservation Foundation Inc.
- Mishler B.D. (2021) What, if anything, are species? Boca Raton, CRC Press. doi.org/10.1201/9781315119687
- Moore D.L., Spiegel F.W. (2000) The effect of season on protostelid communities. *Mycologia* **92**(4): 599–608. https://doi.org/10.2307/3761417
- Moore D.L., Stephenson S.L. (2003) Microhabitat distribution of protostelids in a tropical wet forest in Costa Rica. *Mycologia* **95**(1): 11–18.
- Nguyen L.T.T., Sanchez-Mahecha O., Almadrones-Reyes K.J., Redeña-Santos J.C., Dagamac N.H.A. (2020) Occurrence of leaf litter inhabiting myxomycetes from lowland forest patches of Northern and Central Vietnam. *Tropical Ecology* **60**: 495–506.
- Novozhilov Y.K., Shchepin O.N., Alexandrova A.V., Popov E.S., Dagamac N.H. (2018) Altitudinal patterns of diversity of myxomycetes (Myxogastria) across tropical forests of Southern Vietnam. *Protistology* **12**(2): 73–80.
- Ocenar-Bautista, C. E., Balaoro-Banzuela, R. C., Claudio-Paragas, C. Y., Buebos-Esteve, D. E., Dagamac, N. H. A. (2024) First records of protosteloid amoebae isolated from coastal litter in the Philippines. *Check List* **20**(2): 249–257.
- Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., Simpson G.L., Sólymos P., Stevens M.H.H., Wagner H. (2012) Vegan: community ecology package. Software. http://CRAN.R-project.org/package=vegan
- Pecundo M.H., Dagamac N.H.A., Stephenson S.L., dela Cruz T.E.E. (2017) First myxomycetes survey in the limestone forest of Puerto Princesa Subterranean River National Park, Palawan, Philippines. *Nova Hedwigia* **104**: 129–141.
- Pepin N.C., Pike G., Schaefer M., Boston C.M., Lovell H. (2017) A comparison of simultaneous temperature and humidity observations from the SW and NE slopes of Kilimanjaro: The role of slope aspect and differential land-cover in controlling mountain climate. *Global and Planetary Change* **157**: 244–258.
- Powers D.M., Stephenson S.L. (2006) Protostelids from tropical forests, woodlands and deserts in Australia. *Mycologia* **98**(2): 218–222.
- 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 from forest patches on ultramafic and volcanic soils. *Chemistry and Ecology* **31**(8): 741–753.
- Redeña-Santos J.C., Van Thao D., Schnittler M., Dagamac N.H.A. (2018) The first report of composition and occurrence of myxomycete assemblages in protected and unprotected plantation forests: a comparative study in Thai Nguyen City, Northern Vietnam. *Plant Ecology and Evolution* **151**(2): 231–240.
- Redeña-Santos J.C., Dunca J.A.U., Thao D.V., Dagamac N.H.A. (2017) Myxomycetes occurring on selected agricultural leaf litters. *Studies in Fungi* **2**(1): 171–177.
- Rojas C., Stephenson S.L. (2008) Myxomycete ecology along an elevation gradient on Cocos Island, Costa Rica. *Fungal Diversity* **29**: 117–127.
- Schnittler M., Dagamac N.H.A., Woyzichovski J., Novozhilov Y.K. (2022) Chapter 10 - Biogeographical patterns in myxomycetes. In Rojas C., Stephenson S.L. (Eds.) *Myxomycetes* (2nd edn.), Academic Press, pp. 377–416.
- Schnittler M., Novozhilov Y., Romeralo M., Brown M.W., Spiegel F.W. (2012) Fruit body-forming protists: Myxomycetes and Myxomycete-like organisms. In: Frey W. (ed.) *Englers Syllabus of Plant Families*, 1/1, 13th ed. Borntraeger, Stuttgart, pp. 40–88.
- Shadwick J.D.L. (2010) Ecological and evolutionary studies of protosteloid amoebae. Master's. Thesis. University of Arkansas.
- Shadwick L.L. (2011) *Systematics of Protosteloid Amoebae*. Theses and Dissertations 221. http://scholarworks.uark.edu/etd/221
- Shadwick J.D., Stephenson S.L., Spiegel F.W. (2009) Distribution and ecology of protostelids in Great Smoky Mountains National Park. *Mycologia* **101**(3): 320–328.
- Shadwick J.D., Silberman J.D., Spiegel F.W. (2017) Variation in the SSUrDNA of the genus *Protostelium* leads to a new phylogenetic understanding of the genus and of the species concept for *Protostelium mycophaga* (Protosteliida, Amoebozoa). *Journal of Eukaryotic Microbiology* **65**(3): 331–344.
- Singh S. (2018) Understanding the role of slope aspect in shaping the vegetation attributes and soil properties in Montane ecosystems. *Tropical Ecology* **59**(3): 417–430.
- Spiegel F.W., Shadwick J.D.L., Shadwick L., Brown M.W. (2007) A beginner's guide to identifying the protostelids. https://slimemold.uark.edu/pdfs/Handbook1_3rd.pdf
- 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 Vanneliida, Centramoebida, and Pellitida). In Archibald J. et al. (eds.) *Handbook of the protists*. Springer, Cham. https://doi.org/10.1007/978-3-319-32669-6_12-1
- 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**(4): 608–621.
- Walker L.M., Stephenson S.L. (2016) The species problem in myxomycetes revisited. *Protist* **167**(4): 319–338.
- Zahn G., Stephenson S.L., Spiegel F.W. (2014) Ecological distribution of protosteloid amoebae in New Zealand. *Peer-Journal* **2**: e296–e296.

(Manuscript accepted 26 July 2024; Corresponding Editor: I. Krisai-Greilhuber)