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DIVERSITY OF PLASMODIAL MYXOMYCETES FROM ANDA ISLAND, PANGASINAN, PHILIPPINES

RUDOLF V. KUHN[1], ANTON OLIVER M. JAVIER[1], COLEEN P. RODILLAS[1], CHRISTIAN M. PARRA[1], LIWAYWAY HIYAS M. CORPUZ[1], ANTHONY T. BUAYA[2], and THOMAS EDISON E. DELA CRUZ[1,2]*

[1] *Department of Biological Sciences, College of Science and*
[2] *Fungal Biodiversity and Systematics Group, Research Center for the Natural and Applied Sciences*
University of Santo Tomas España 1015 Manila, Philippines

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ABSTRACT

The unique life cycle and fascinating fruiting bodies of myxomycetes make them ideal model organisms for the study of cellular differentiation and ecological patterns. Our research study then focuses on the diversity and abundance of myxomycetes found in Anda Island, Pangasinan in Northern Philippines. A total of 180 moist chambers were prepared from ground leaf litter and twigs collected from a 15 m^2 quadrat within the study site. Twenty four species of myxomycetes belonging to 11 genera were collected and identified from the moist chambers: *Arcyria* (2), *Collaria* (1), *Comatricha* (1), *Craterium* (2), *Diachea* (1), *Diderma* (2), *Didymium* (1), *Elaeomyxa* (1), *Perichaena* (1), *Physarum* (11), and *Stemonitis* (1). Of all moist chambers, 55% yielded myxomycetes. Ground leaf litter (29%) yielded more myxomycetes than twigs (26%). Assessment of species diversity (H_S=1.15), richness (H_G=5.33) and evenness (E=0.56) showed rich assemblage of myxomycetes. Among the collected species, one for each of the genera *Arcyria*, *Craterium*, *Diderma,* and *Physarum,* were recorded to be abundant. Interestingly, three species of myxomycetes are new records for the Philippines: *Craterium microcarpum, Physarum decipiens* and *Elaeomyxa miyazakiensis*. This is the first report of myxomycetes in Anda Island, Pangasinan, Philippines.

Key words: myxomycetes, species abundance, diversity, biogeography, Philippines

INTRODUCTION

The Philippines is a tropical archipelago of 7107 islands. The geographic isolation of many of these islands resulted in various unique flora and fauna. It has been estimated that more than 6000 species of animals and plants are endemic to the country (Myers *et al.* 2000). Therefore, it is not surprising that new species of plants and animals are still being discovered recently in the country, e.g. forest mice (*Apomys*

* Corresponding author : tedelacruz@mnl.ust.edu.ph

aurorae, *A. banahao*, *A. brownorum*, *A. magnus*, *A minganensis*, *A. sierrae*, and *A. zambalensis)* (Heaney *et al.* 2011), monitor lizard *(Varanus bitatawa)* (Welton *et al.* 2010), giant pitcher plant *(Nepenthes attenboroughii)* (Robinson *et al.* 2009), sundew plant *(Drosera ultramafica)* (Fleischmann *et al.* 2011) including those belonging to the myxomycetes, e.g. *Craterium retisporum* (Moreno *et al.* 2009). However, studies on island myxomycetes are relatively few in spite of their role in wood decay and nutrient cycling in temperate forests and in bio-accumulation of heavy metals (Zhulidov *et al.* 2002; Takahashi 2004). Rojas and Stephenson (2008) surveyed different habitat elevations and substrates types in Cocos Island, Costa Rica and reported 41 species belonging to 19 genera. Beltran *et al.* (2010) also evaluated the species diversity of myxomycetes between arid and semi-arid zones in the Canary Islands in Spain and reported 63 species belonging to 21 genera. The study also highlighted the similarities between slime molds collected from the arid and semi-arid zones in the Canary Islands to the desserts of Central America. In the Philippines, *Hemitrichia serpula, Perichaena depressa, Physarella oblonga, Stemonitis fusca* and species of *Arcyria*, *Cribraria, Lycogala* and *Stemonitis* were reported from the islands of Palawan and Palaui, Cagayan (Reynolds 1981; Quimio 2002). Dela Cruz *et al.* (2011) also reported 30 species of myxomycetes from Hundred Islands National Park, Pangasinan. Macabago *et al.* (2012) identified 45 species and 13 genera from Lubang Island, Occidental Mindoro. Interestingly, this geographically isolated island yielded six new records for the Philippines: *Arcyria globosa*, *Comatricha robusta, Craterium atrolucens, Lamproderma cacographicum, Oligonema schweinitzii* and *Perichaena microspora*. In this research study, we reported 24 species including three new records of myxomycetes from specimens collected in Anda Island, Pangasinan, Philippines. This is the first report of myxomycetes in Anda Island, Pangasinan in Northern Philippines.

MATERIALS AND METHODS

Study site

Anda Island (16°17'00.16''N, 119°58'00.08''E; land area= 83.80 sq.km) is located near the town of Bolinao in the province of Pangasinan, Northern Philippines. Climate data indicate an average monthly rainfall range of 6.1 mm in February to 608.6 mm in August. The island has an average minimum and maximum temperature of 23.3 °C and 32.4 °C, respectively, and average monthly humidity between 71 - 85%. It has a type I climate season, i.e. wet from June to November and dry from December to May. The study site is characterized by a tropical secondary forest, with patches of rice fields and established residential houses. A small patch of this secondary forest along the local highway and near a rice field was used as the collection site. The island is also inhabited by man and may be useful in looking at the effects of human civilization on myxomycete assemblages.

Collection of substrates and preparation of moist chamber set-ups and herbaria

Ground leaf litter (30) and twigs (30) were collected in brown paper bags within a 15 m^2 quadrat. Prior to the preparation of moist chambers, the collected substrates were allowed to air-dry for few days. A total of 180 moist chambers were then set-up following the protocol of Stephenson and Stempen (1994). The moist chambers were placed inside wooden cabinets, not directly exposed to sunlight, and incubated at room temperature for up to 8 weeks. All moist chambers were observed regularly (at least twice a week) for the presence of plasmodia and/or fruiting bodies. The number of moist chambers with plasmodia and/or fruiting bodies was recorded and used in ecological analysis. Fruiting bodies with their respective substrate were removed from the moist chamber set-up, allowed to air dry and glued on previously prepared herbarium boxes. Herbarium boxes were then labeled with the collection number, substrate, collection sites and dates, the species name and the names of the collector. All herbaria were deposited at the Pure and Applied Microbiology Laboratory, Research Center for the Natural and Applied Sciences, University of Santo Tomas.

Characterization and identification of plasmodial myxomycetes

To identify the collected myxomycetes, the specimens for each species were described based on their fruiting body descriptions and spore morphology under a dissecting and a compound light microscope. Identification was done following comparison of these morphological characters with published literature (Stephenson & Stempen 1994; Keller & Braun 1999), web-based identification keys (http://slimemold.uark.edu/) and an electronic, computer-based identification key, *SynKey* (Mitchell 2008). To check for the current valid names of the identified myxomycetes, an online nomenclatural information system (http://nomen.eumycetozoa.com) was used.

Moist chamber productivity and ecological analysis

The productivity of the moist chambers (MC) was computed by determining the percent yield for the collection site and each of the substrate types. Plasmodial/or fruiting body growth on a moist chamber was counted here as positive for myxomycetes and was recorded as one positive collection. Then, the total positive collection was divided by the total number of moist chamber prepared. Thus, the percentage yield (PY) was computed as follows:

$$\text{Percent Yield (PY)} = \frac{\text{number of MC positive for myxomycetes}}{\text{total number of MC prepared}} \qquad (1)$$

Then, the relative abundance (RA) was also determined for each of the collected species of myxomycetes. Initially, the number of moist chambers positive for a species of myxomycetes was counted and divided by the total number of collections.

The relative abundance was computed as follows:
Relative Abundance (RA) =

$$\frac{\text{number of collections for a particular species of myxomycetes}}{\text{total number of myxomycete collection}} \quad (2)$$

Abundance Indices (AI) were then assigned to all of the species represented among the collections. Initially, the percentage of a particular species among the total number of collections, i.e. the Relative Abundance, was determined. Then, a "breaking point" was assigned based on these percentage values (S. L. Stephenson, pers. comm., 04 January 2009). Each species was then categorized as: (1) abundant (A) if their relative abundance (RA) is ≥10% of the total collections, (2) common (C) if RA is ≥5% but <10% of the total collections, (3) occasionally occurring (O) if RA is ≥3 but <5 of the total collections, and (4) rare (R) if the myxomycetes had an RA of <3% of the total collections. Finally, the myxomycete diversity of the study site was also calculated using different diversity indices as described by Dagamac *et al.* (2012). These indices were computed based on RA and as follows:

Shannon Index Diversity $(\mathbf{H_s}) = -\sum_i (\mathbf{p_i} \ln \mathbf{p_i})$ (3)
where p_i = is the proportional abundance of the ^{i}th species

Gleason Index of Species Richness $(\mathbf{H_G}) = \mathbf{N_p}\text{-}\mathbf{1/\ln N_i}$ (4)
where N_p = the total number of species
N_i = the total number of individuals in ^{i}th species

Pielou's Index of Species Evenness $\mathbf{E = H_s/H_{max}}$ (5)
where H_s = Shannon Index of Diversity
H_{max} = the maximum value of Hs

RESULTS AND DISCUSSION

Percent yield

Of 180 moist chambers set-up from ground leaf litter and decaying woody twigs collected from Anda Island, 55% yielded myxomycetes (Fig. 1A). Fruiting bodies were recorded more in moist chambers than plasmodia. Between the two collected substrates, ground leaf litter (29%) yielded more myxomycetes than twigs (26%) (Fig. 1B). Schnittler *et al.* (2002) also found a higher occurrence of myxomycetes on the ground leaf litter, though Macabago *et al.* (2010) recorded a higher yield in aerial than ground leaf litter. It was previously reported that myxomycetes associated with woody twigs are not necessarily same as those associated with ground litter in terms of their occurrence and abundance (Stephenson *et al.* 2008). Stephenson (1989) implicated that the myxomycetes assemblage in ground leaf litter was mediated by factors that is favorable for plasmodial or amoeba development. These factors included acidic pH of the decaying leaf litter, soil nutrients, moist and the available food microorganisms, *e.g.* bacteria, yeast and algae. As shown in this study, the high myxomycetes yield recorded in ground leaf litter as compared to twigs indicated that ground leaf litter is a favorable substrate for myxomycetes.

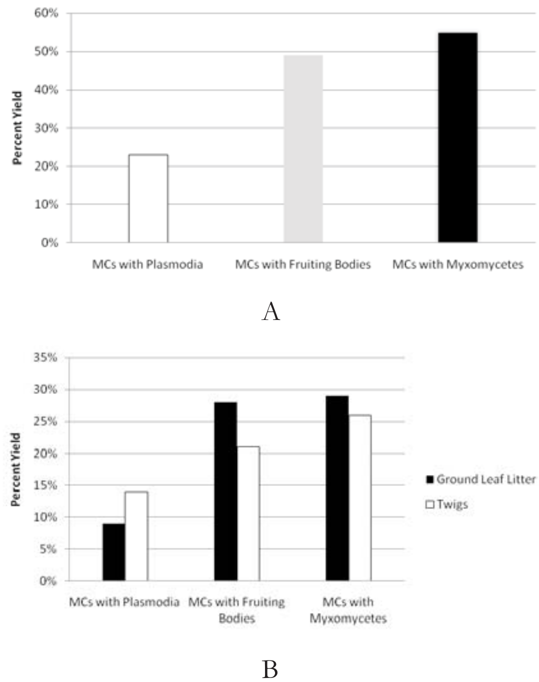

Figure 1. Percent yield of myxomycetes in moist chambers (A) and between the two collected substrates (B)

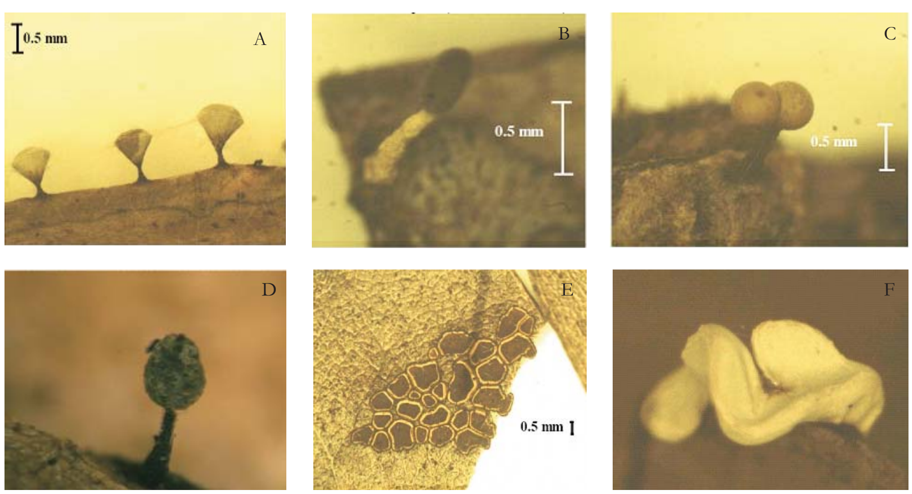

Figure 2. Representative species of myxomycetes recorded in Anda Island, Pangasinan: (A) *Craterium retisporum,* (B) *Diachea leucopodia*, (C) *Perichaena pedata, (D) Elaeomyxa miyazakiensis, (E) Perichaena depressa* and (F) *Physarum bivalve*

Species Occurrence, Abundance and Diversity

A total of twenty four species of myxomycetes were collected from Anda Island, Pangasinan (Table 1). The identified species belong to the following genera: *Arcyria* (2), *Collaria* (1), *Comatricha* (1), *Craterium* (2), *Diachea* (1), *Diderma* (2), *Didymium* (1), *Elaeomyxa* (1), *Perichaena* (1), *Physarum* (11), and *Stemonitis* (1). Of the collected *Physarum* species, six were identified as *Phy. bivalve, Phy. bogoriense, Phy. cinereum, Phy. compressum, Phy. decipiens* and *Phy. echinosporum*. *Arcyria cinerea, Collaria arcyrionema*, *Diachea leucopodia, Diderma hemisphaericum, Didymium nigripes, Elaeomyxa miyazakiensis, Per. depressa, Comatricha* sp. and *Stemonitis fusca* were also reported in Anda Island. Two species of *Craterium*, i.e. *Cra. retisporum* and *Cra. microcarpum,* were also noted among collected substrates. Interestingly, three species of myxomycetes are new records for the Philippines: *Craterium microcarpum, Physarum decipiens* and *Elaeomyxa miyazakiensis*. These species have been previously reported in other countries but not in the Philippines until now.

Of all collected myxomycetes, *A. cinerea, Cr. microcarpum, Diderma hemisphaericum,* and *Phy. echinosporum* were the most abundant (Table 1). Species of *Arcyria*, *Physarum* and *Stemonitis* were also reported to be widely abundant in temperate and tropical forests, and in island habitats occurring in ground leaf litter or parts of living trees such as twigs and old bark (Schnittler *et al.* 2002; Stephenson *et al.* 2004; Everhart & Keller 2008; Stephenson *et al.* 2008; Tran *et al.* 2008; Adamonyte & Kastanje 2011). *Collaria arcyrionema*, *Diachea leucopodia, Physarum decipiens* and *Stemonitis fusca* were recorded as common in this study, while most of the species were recorded as rarely occurring (Table 1). *Comatricha* sp.1, *Craterium retisporum, Diderma* sp.*, Didymium nigripes, Elaeomyxa miyazakiensis, Physarum bivalve, Phy. bogoriense, Phy. cinereum, Phys. compressum, Physarum* sp.1, *Physarum* sp.2, *Physarum* sp.8, *Physarum* sp.9, and two unidentified myxomycetes were all recorded as rare. Similarly, species of *Diderma* and *Comatricha* has been found to be abundant in temperate than in tropical forests (Stephenson 1989; Novozhilov *et al.* 2000). The genera *Arcyria, Physarum, Diderma* and *Stemonitis* are also abundantly diverse and distributed in island habitats (Beltran *et al.* 2004; Rojas & Stephenson 2008). Rojas and Stephenson in 2008 described the genus *Craterium* as rare in Cocos Island, Costa Rica; however, Adamonyte and Kastanje in 2011 abundantly recorded *Craterium leucocephalum* in the island of Saaremaa, Estonia. Differences in climatic conditions in these two islands play a crucial part in the abundance and distribution of *Craterium* species. This may also be a factor for the differences in myxomycete distribution in Anda Island. Though Anda Island is inhabited by man, the influence of anthropogenic activities to myxomycete distribution was not correlated, and therefore, is recommended for future studies.

In this study, the computed diversity indices of myxomycetes in Anda Island, Pangasinan showed high value for species diversity (H_S=1.15), species richness (H_G=5.33) and species evenness (E=0.56). Similarly, high species diversity was also recorded in Lubang Island, Occidental Mindoro (Macabago *et al.* 2012). However, it was observed that geographic isolation does not greatly influence species composition, and endemism seems not to occur in for myxomycetes since species recorded in an island habitat also occur in mainland (Eliasson 1991; Rojas &

Table 1. Species abundance of myxomycetes collected from Anda Island, Pangasinan

Taxa	% Abundance
Arcyria cinerea (A. cinerea var. *digitata)*	A
Arcyria sp. 1	O
Collaria arcyrionema	C
Comatricha sp. 1	R
Craterium microcarpum	A
Craterium retisporum	R
Diachea leucopodia	C
Diderma hemisphaericum	A
Diderma sp.	R
Didymium nigripes	R
Elaeomyxa miyazakiensis	R
Perichaena depressa	O
Physarum bivalve	R
Physarum bogoriense	R
Physarum cinereum	R
Physarum compressum	R
Physarum decipiens	C
Physarum echinosporum	A
Physarum sp. 1	R
Physarum sp. 2	R
Physarum sp. 3	O
Physarum sp. 8	R
Physarum sp. 9	R
Stemonitis fusca	C
Unidentified Myxo 6	R
Unidentified Myxo 7	R

Note :
[a] Abundant Indices, where Abundant (A) = ≥10% of the total collections, Common (C) = ≥5% but <10% of the total collections, Occasional (O) = ≥3 but <5 of the total collections, and Rare (R) = <3% of the total collections

Stephenson 2008). But the wide range of diverse habitats in island ecosystems provides diverse microhabitats for myxomycetes (Rojas & Stephenson 2008). Thus, the diverse assemblage of myxomycetes recorded in this study further supports other studies conducted in island ecosystems.

CONCLUSIONS

A total of 24 species of myxomycetes belonging to 11 genera were collected and identified in Anda Island, Pangasinan Philippines: *Arcyria cinerea* (*A. cinerea* var. *digitata*), *Collaria arcyrionema, Craterium microcarpum, Cra. retisporum*, *Diachea leucopodia, Diderma hemisphaericum*, *Didymium nigripes, Elaeomyxa miyazakiensis, Perichaena depressa, Physarum bivalve, Phy. bogoriense, Phy. cinereum, Phy. compressum, Phy. decipiens, Phy. echinosporum,* and

Stemonitis fusca. One species each of *Arcyria*, *Comatricha*, *Diderma* and five species of *Physarum* were identified up to the genus level only. Three species, i.e. *Craterium microcarpum, Physarum decipiens,* and *Elaeomyxa miyazakiensis,* are new records for the Philippines. Higher productivity was also recorded in the study as well as in ground leaf litter than twigs. Eleven species of *Physarum* and one species for each genus *Arcyria*, *Craterium*, and *Diderma* were recorded abundantly.

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REFERENCES

Adamonyte G, Kastanje V. 2011. Myxomycetes of the island of Saaremaa, Estonia. Folia Cryptog Estonica Fasc 48:1-4.

Beltran E, Lado C, Barrera J, González E. 2004. Myxomycetes diversity in the laurel forest of Garajonay National Park (Canary Islands, Spain). Syst Geogr Pl 74: 159-73.

Beltran E, Mosquera J, Lado C. 2010. Myxomycete diversity from arid and semiarid zones of the Canary Islands (Spain). Mycotaxon 113:439-42.

Dagamac NHA, Stephenson SL, dela Cruz TEE. 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.

dela Cruz TEE, Kuhn RV, Javier AOM, Rodillas CP, Parra CM, Corpuz LHM, McHugh RD. 2011. Occurrence and distribution of plasmodial myxomycetes in Hundred Islands National Park, Pangasinan, Philippines. Acta Manilana 59:65-74.

Eliasson UH. 1991. The myxomycete biota of the Hawaiian Islands. Mycol Res 95: 257-67.

Everhart SE, Keller HW. 2008. Life history strategies of corticolous myxomycetes: the life cycle, plasmodial types, fruiting bodies, and taxonomic orders. Fungal Div 29: 1-16.

Fleischmann A, Robinson AS, McPherson S, Heinrich V, Gironella E, Madulid DA. 2011. *Drosera ultramafica* (Droseraceae), a new sundew species of the ultramafic flora of the Malesian highlands. Blumea 56: 10-15.

Heaney LR, Balete DS, Rickart EA, Alviola PA, Duya MRM, Duya MV, Veluz JM, Vande Vrede L, Steppan SJ. 2011. Seven new species and a new subgenus of forest mice (Rodentia: Muridae: Apomys) from Luzon Island. Fieldiana Life and Earth Science, 2: 1-60.

Keller HW, Braun KL. 1999. Myxomycetes of Ohio: their systematics, Biology, and use in teaching. Ohio Biological Survey 13(2), Columbus, Ohio.

Macabago SAB, dela Cruz TEE, Stephenson SL. 2012. First records of myxomycetes from Lubang Island, Occidental Mindoro, Philippines. Sydowia 64: 109-18.

Macabago SAB, Dagamac NHA, dela Cruz TEE. 2010. Diversity and distribution of myxomycetes (slime molds) from La Mesa, Ecopark in Quezon City, Philippines. Biotropia, 17:51-67.

Mitchell D. 2008. Synoptic Key to the Myxomycetes (SynKey), UK.

Moreno G, Mitchell DW, Stephenson SL, dela Cruz TEE. 2009. A new species of *Craterium* (Myxomycetes) with reticulate spores. Boletín de la Sociedad Micológica de Madrid 33:175-80.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853-858.

Novozhilov YK, Schnittler M, Zemlianskaia IV, Fefelov KA. 2000. Biodiversity of plasmodial slime molds (Myxogastria): Measurement and interpretation. Protistology 1: 161-78.

Quimio TH. 2002. Checklist and database of Philippine Fungi (1806-2001). *ASEAN Regional Center for Biodiversity Conservation, Laguna*.

Reynolds DR. 1981. Southeast Asian myxomycetes II. Philippines. Philipp J Biol 10: 127-50.

Robinson AS, Fleischmann AS, McPherson SR, Heinrich VB, Gironella EP, Peña C. 2009. A spectacular new species of *Nepenthes* L. (Nepenthaceae) pitcher plant from central Palawan, Philippines. Bot J Linn Soc 159: 195-202.

Rojas C, Stephenson SL. 2008. Myxomycete ecology along an elevation gradient on Cocos Island, Costa Rica. Fungal Div 29: 117-27.

Schnittler M, Lado C, Stephenson SL. 2002. Rapid biodiversity assessment of a tropical myxomycete assemblage - Maquipucuna Cloud Forest Reserve, Ecuador. Fungal Div 9: 135-67.

Stephenson SL. 1989. Distribution of myxomycetes in temperate forests II. Patterns of occurrence of bark surface of living tress, leaf litter, and dung. *Mycologia* 81: 608-21.

Stephenson SL, Schnittler M, Lado C. 2004. Ecological characterization of a tropical myxomycete assemblage - Maquipucuna Cloud Forest Reserve, Ecuador. *Mycologia* 96: 488-97.

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

Stephenson SL, Urban LA, Rojas C, McDonald MS. 2008. Myxomycetes associated with woody twigs. Sociedad Mexicana de Micología 27: 21-28.

Takahashi K. 2004. Distribution of myxomycetes on different decay states of deciduous broadleaf and coniferous wood in a natural temperate forest in the Southwest of Japan. Syst Geogr Pl 74:133-42.

Tran HTM, Stephenson SL, Hyde KD, Mongkolporn O. 2008. Distribution and occurrence of myxomycetes on agricultural ground litter and forest floor litter in Thailand. Mycologia 100:181-90.

Welton LJ, Siler CD, Bennett D, Diesmos A, Duya RM, Dugay R, Rico ELB, van Weerd M, Brown RM. 2010. A spectacular new Philippine monitor lizard reveals a hidden biogeographic boundary and a novel flagship species for conservation. Biology Letter 6: 654-8.

Zhulidov DA, Robarts RD, Zhulidov AV, Zhulidova OV, Markelov DA, Rusanov VA, Headley JV. 2002. Zinc accumulation by the slime mold *Fuligo septica* (L.) Wiggers in the former Soviet Union and North Korea. J Environ Q 31:1038-42.