List of species collected and interactive database of myxomycetes (plasmodial slime molds) for Mt. Arayat National Park, Pampanga, Philippines

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Myxomycetes (plasmodial slime molds) are fungus-like protists that are often understudied in a biodiverse country such as the Philippines. As such, the purpose of the project described herein was first to document the species of myxomycetes collected in Mt. Arayat National Park, Pampanga, Philippines and then to develop an interactive database for these organisms using DELTA software. These are the first reports of myxomycetes from Mt. Arayat. A total of 33 species were recorded for the two slopes of the mountain during the wet and dry seasons.Five species of myxomycetes (i.e., *Arcyria afroalpina, Collaria arcyrionema, Craterium concinnum, Enerthenema papillatum* and *Licea biforis*) were new records for the Philippines. As a second component of the project, interactive identification guides were generated from 67 taxonomic characters using the DELTA software. The interactive database recognized spore appearance, colour of the spores by transmitted light, fruiting body colour and the outline of the sporotheca as the most useful characters in recognizing the species of myxomycetes known from Mt. Arayat. The interactive database generated in this study is the first effort along these lines for the Philippines and for Southeast Asia.

Key words – taxonomy – diversity – species listing – protist – identification key

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Introduction

Relatively little is known of the status of myxomycetes in Southeast Asia, and this is particularly true for the Philippines. Most of the earlier published reports from the region were lists of species from countries such as Myanmar and Thailand (Reynolds & Alexopoulos 1971). In the Philippines, Uyenco (1973) reported 18 species representing 10 genera from 314 specimens collected from Luzon (Quezon City and Laguna) and Mindanao (Basilan and Zamboanga) during the period of 1961 to 1973. Dogma (1975) listed 46 species in 20 genera, including those reported previously by Martin & Alexopoulos (1969) for the entire country. Later, Reynolds (1981) provided a more extensive listing of Philippine myxomycetes, based upon published records and his personal collections. A total of 107 species were reported for the Philippines, including the myxomycete collections from Mindanao (Davao, Cotabato and Zamboanga) by E.B. Copeland, and from Luzon by A.D.E. Elmer (Benguet) and E.D. Merrill (Bataan, Manila, Cavite and Laguna). To the best of our knowledge, there have been no publications on Philippine myxomycetes since the paper by Reynolds (1981), until dela Cruz et al (2009)

evaluated the myxomycete collection in the Mycological Herbarium of Museum of Natural History at the University of the Philippines Los Baños, Laguna. These specimens were found to be in good condition. Moreno et al (2009) reported a new species of *Craterium*, *C. retisporum*, from a specimen collected on Anda Island, Pangasinan. Macabago et al (2010) reported 28 species of myxomycetes belonging to 11 genera from La Mesa Watershed EcoPark in Quezon City, Metro Manila. In addition, Daga mac et al (2010) reported five new records of myxomycetes from the bark of *Samanea samans* (Jacq) Merr. collected from 21 different localities in Luzon. Herein, we report 33 species of myxomycetes representing 14 genera collected from different substrata obtained in Mt. Arayat National Park, Pampanga in Central Luzon, Philippines. Moreover, we report the first interactive database on myxomycetes developed for any region of Southeast Asia.

Methods

The study was conducted during 2008 and 2009. All species were identified at least to the genus level on the basis of published descriptions and identification keys (Stephenson & Stempen 1994), and also with web-based electronic databases [e.g., SYNKey (Mitchell 2008) and the Eumycetozoan Project (http://slimemold.uark.edu/)]. Taxonomic names used for the myxomycetes are those available from the online nomenclatural database for the eumycetozoans (http://nomen.eumycetozoa.com).

Study Site (Fig. 1)

Mt. Arayat National Park (15°12'00"N, 120°44'31"E to 15.20°N, 120.742°E) is located in the municipality of Arayat in the province of Pampanga, on Luzon Island in the Northern Philippines. The park has a mid-mountain forest type with an elevation of 1,030 m above sea level (masl). The area has an annual temperature range of 22–31°C and annual rainfall ranges from 284–1,844 mm.

Characterization and identification of collected myxomycetes

Samples of four different types of substrata were randomly collected along the South Trail (Baño) and the North Trail (Magalang), both of which extend to the peak of the mountain. Moist chamber cultures were prepared from these samples. These cultures were maintained under diffuse light at room temperature and observed regularly for the presence of myxomycetes for a period of up to eight weeks. All fruiting bodies of myxomycetes that developed in the moist chamber cultures were removed and then allowed to air-dry gradually. Each myxomycete, together with the portion of the substratum upon which it fruited, was placed in a small box for storage. Photographs of each specimen were obtained with the use of a Moticam 1000 (Motic, USA) camera system. To identify these specimens, the fructification type (plasmodiocarp, aethaloid, sporangiate and pseudoaethaloid) and gross morphological features (e.g., colouration of the peridium or stalk, presence or absence of lime, morphometrics of all vegetative structures, etc.) were then examined under a dissecting microscope (Olympus, USA) at different magnifications. Microscopic slides also were prepared from fruiting bodies to observe the presence of other special characters [e.g., appearance of the capillitium, presence of a calyculus, presence of lime nodes, and spore morphology [size, shape, texture, etc.)]. All slides were prepared using 95% ethanol and lactophenol, following the procedure described by Stephenson & Stempen (1994). Slides were then observed under a brightfield compound microscope (Scanner Objective to OIO, Olympus CX 31).

Development of an interactive database for myxomycetes of Mt. Arayat National Park using the DELTA software.

All possible morphological characters of the 33 species of myxomycetes from Mt. Arayat were initially determined under the stereomicroscope and compound light microscope. Each character was then assigned to any one of the five different categories using the Character Editor of the DELTA software. These were (1) unordered multistate: two or more character states that have no orderly pattern; (2) ordered multistate: 2 or more character states that have orderly pattern, (3) integer numeric: any measurement in whole number (4) real numeric: any measurement in fractional values, and (5) text: additional information (synonyms or geographic distribution/origin of the specimen). Images of myxomycetes were

Fig. 1 – Map of the municipality of Arayat in Pampanga (map source: globalpinoy.com).

also included in the interactive database as a CIMAGES file to further improve the keys that were generated. The data files were then checked for format errors and inconsistencies using the program CONFOR in the DELTA software. Finally, the DELTA software was run to generate (1) an Interactive Key (INTKEY), a user-friendly interactive database for species identification and species information retrieval; (2) Conventional Key, a dichotomous key used for identification of species; and (3) Natural Language, a comprehensive description of all encoded myxomycetes. The interactive database can now be used for the identification of newly collected myxomycetes.

Results and Discussion

Species listing of Mt. Arayat myxomycetes

A total of 33 species representing 14 genera are included in this annotated list of species from Mt. Arayat National Park. These species of myxomycetes were recorded from aerial litter (AL), ground litter (GL), twigs (TW) and bark (BK) collected in the park. Species are arranged alphabetically under each taxonomic order. Examples that are new records for the Philippines are indicated in bold.

Order Stemonitales

Collaria arcyrionema (Rostaf.) Nann.-Bremek. ex Lado (site: Magalang; substratum: AL)

Enerthenema papillatum (Pers.) Rostaf. (site: Baño; substratum: TW)

Lamproderma scintillans (Berk. & Broome) Morgan (sites: Baño and Magalang; substrata: GL, BK)

Stemonitis fusca Roth (site: Magalang; substrata: AL, GL, TW)

Stemonitis pallida Wingate (sites: Baño and Magalang; substrata: TW, BK)

Order Liceales

Licea biforis Morgan (site: Magalang; substratum: AL)

Order Trichiales

Arcyria afroalpina Rammeloo (sites: Baño and Magalang; substratum: GL)

Arcyria cinerea (Bull.) Pers. (sites: Baño and Magalang; substrata: AL, GL, TW, BK)

Arcyria insignis Kalchbr. & Cooke (sites: Baño & Magalang; substratum: TW)

Arcyria pomiformis (Leers) Rostaf. (site: Magalang; substratum: TW)

Perichaena chrysosperma (Curr.) Lister (site: Baño; substratum: GL)

Perichaena depressa Lib. (site: Baño; substratum: GL)

Order Physarales

Craterium concinnum Rex (site: Baño; substratum: AL)

Cribraria microcarpa (Schrad.) Pers. (site: Baño; substratum: BK)

Diachea bulbillosa (Berk. & Broome) Lister (site: Baño; substratum: BK)

Diachea leucopodia (Bull.) Rostaf. (site: Magalang; substratum: AL)

Diderma effusum (Schwein.) Morgan (sites: Baño and Magalang; substrata: AL, GL)

Diderma hemisphaericum (Bull.) Hornem. (sites: Baño and Magalang; substrata: AL, GL)

Diderma subasteroides M.L. Farr (site: Baño and Magalang; substrata: BK, GL, TW)

Didymium iridis (Ditmar) Fr. (sites: Baño and Magalang; substrata: AL, GL, TW)

Didymium nigripes (Link) Fr. (site: Magalang; substratum: GL)

Didymium ochroideum G. Lister (site: Baño; substratum: GL)

Didymium squamulosum (Alb. & Schwein.) Fr. (sites: Baño and Magalang; substratum: AL, GL)

Physarella oblonga (Berk. & M.A. Curtis) Morgan (site: Magalang; substratum: BK)

Physarum album (Bull.) Chevall. (sites: Baño and Magalang; substrata: AL, GL, TW)

Physarum bivalve Pers. (sites: Baño & Magalang; substrata: AL,TW)

Physarum cinereum (Batsch) Pers. (site: Baño; substrata: AL, GL)

Phyarum compressum Alb. & Schwein. (site: Baño and Magalang; substrata: AL, GL, TW)

Physarum echinosporum Lister (site: Baño; substrata: AL, GL)

Physarum leucophaeum Fr. (sites: Baño and Magalang; substrata: AL, GL)

Physarum pusillum (Berk. & M.A. Curtis) G. Lister (site: Magalang; substrata: GL, TW)

Physarum roseum Berk. & Broome (site: Magalang; substratum: TW)

Physarum viride (Bull.) Pers. (site: Baño; substrata: GL, TW)

These are the first reports of myxomycetes from Mt. Arayat National Park. Interestingly, five of the species listed herein are new records for the Philippines. These are *Arcyria afroalpina, Collaria arcyrionema, Craterium concinnum, Enerthenema pappilatum* and *Licea biforis*. The last comprehensive listing of Philippine myxomycetes was published by Reynolds (1981). He presented an annotated list of 107 species based on published and unpublished records. Of these, 53 species were listed as new records for the country, and these were members of the genera *Arcyria, Badhamia, Ceratiomyxa, Clastoderma, Comatricha, Craterium, Cribaria, Diachea, Dictydium, Diderma, Didymium, Echinostelium, Fuligo, Hemitrichia, Lamproderma, Licea, Lycogala, Metatrichia, Perichaena, Physarella, Physarum, Stemonitis, Trichia* and *Tubifera*. Most of these myxomycetes were collected from substrata obtained from several sites in the country, including Luzon, Visayas and Mindanao. The list compiled by Reynolds corresponded to

25% of all known species at the time it was published and represented approximately 60% of the estimated total Philippine myxomycete flora. Recently, Corpuz et al (2009) also studied the myxomycetes flora in selected highland and island localities in Luzon. About 25 species were observed in highland localities in Luzon, while approximately 30 species were recorded from different islands in Pangasinan. Interestingly, four new records for the Philippines, *Elaeomyxa miyazakiensis, Lepidoderma tigrinum, Perichaena pedata* and *Physarum decipiens* were noted. Moreover, a specimen found on Anda Island, Pangasinan was identified as a species of *Craterium* new to science, which was described as *C. retisporum* by Moreno et al (2009). Dagamac et al (2010) also collected five new records for the Philippines. These were *Clastoderma microcarpum, Dianema harveyi, Diderma subasteroides, Physarum leucophaeum* and *Stemonitis pallida.* All five were obtained from bark samples of *Samane samans* (Jacq) Merr. collected from 21 different localities in Luzon. This now brings the total number of myxomycetes recorded from the Philippines to 122. However, the reported number is still considered small for a tropical country. This suggests that there are still many more species remaining to be found in less studied and relatively unexplored parts of the Philippines.

Interactive database for Mt. Arayat myxomycetes using the DELTA software.

A total of 67 characters were encoded using the DELTA software program. Forty-nine were unordered multistate characters, ten were text characters, while six were real numeric characters. Thirty-eight of the 67 characters were independent characters, thus allowing the program to infer that certain characters are applicable only to certain taxa. From the 67 encoded characters, only 55 were found useful in differentiating the different myxomycete taxa from Mt. Arayat, and thus were useful in generating INTKEY (Figure 2b). Characters such as spore appearance, color of the spores by transmitted light, fruiting body colour and the outline of the sporotheca were generally the most useful characters in recognizing species, and thus were scored with high reliabilities. Consequently, these characters were those recommended initial characters that needed to be checked prior to the application of the interactive database. The INTKEY program acts as a functional diagnostic tool for the rapid identification of specimens and for information retrieval (Dallwitz et al. 2000). The INTKEY displays information on the taxonomy of the species, including their illustrations and other relevant data. The program also describes diagnoses, summarizes, and finds similarities and/or differences between members of related taxa (Dallwitz et al. 1995, 2000).

Another identification guide generated with the DELTA software was a conventional or dichotomous key. The dichotomous key categorizes the species based on important characters. These characters can be used to distinguish unknown species from other species in the database. In the present study, 49 of the 67 encoded characters were found to be useful in generating the dichotomous key. On the practical side, the generated dichotomous key can be printed and used as field guide. The Natural Language was another identification guide generated by the DELTA software. The Natural Language listed all information for a particular species encoded in the database. Taxonomic descriptions written in the Natural Language format include synonyms, fruiting body descriptions, vegetative parts and spore morphologies. Since descriptions of all taxa in the database cannot be generated using the INTKEY or the Dichotomous Key, the Natural Language provides descriptions of all previously encoded characters.

The DELTA software has already been applied successfully to myxomycetes in the Order Echinosteliales (Pando 1999) and to several groups of plants (Alejandro & Liede 2003, Watson & Dallwitz 2004, Bean 2005, Watson & Dallwitz 2005, Alejandro & Meve 2008). The interactive database generated for the myxomycetes of Mt. Arayat is user-friendly and contains information on the taxonomy of these organisms and other relevant information (e.g., their biogeography and/or economic importance). Furthermore, this database can be updated regularly and requires minimal maintenance. The DELTA interactive database generated in this project was the first for myxomycetes from the Philippines, or anywhere

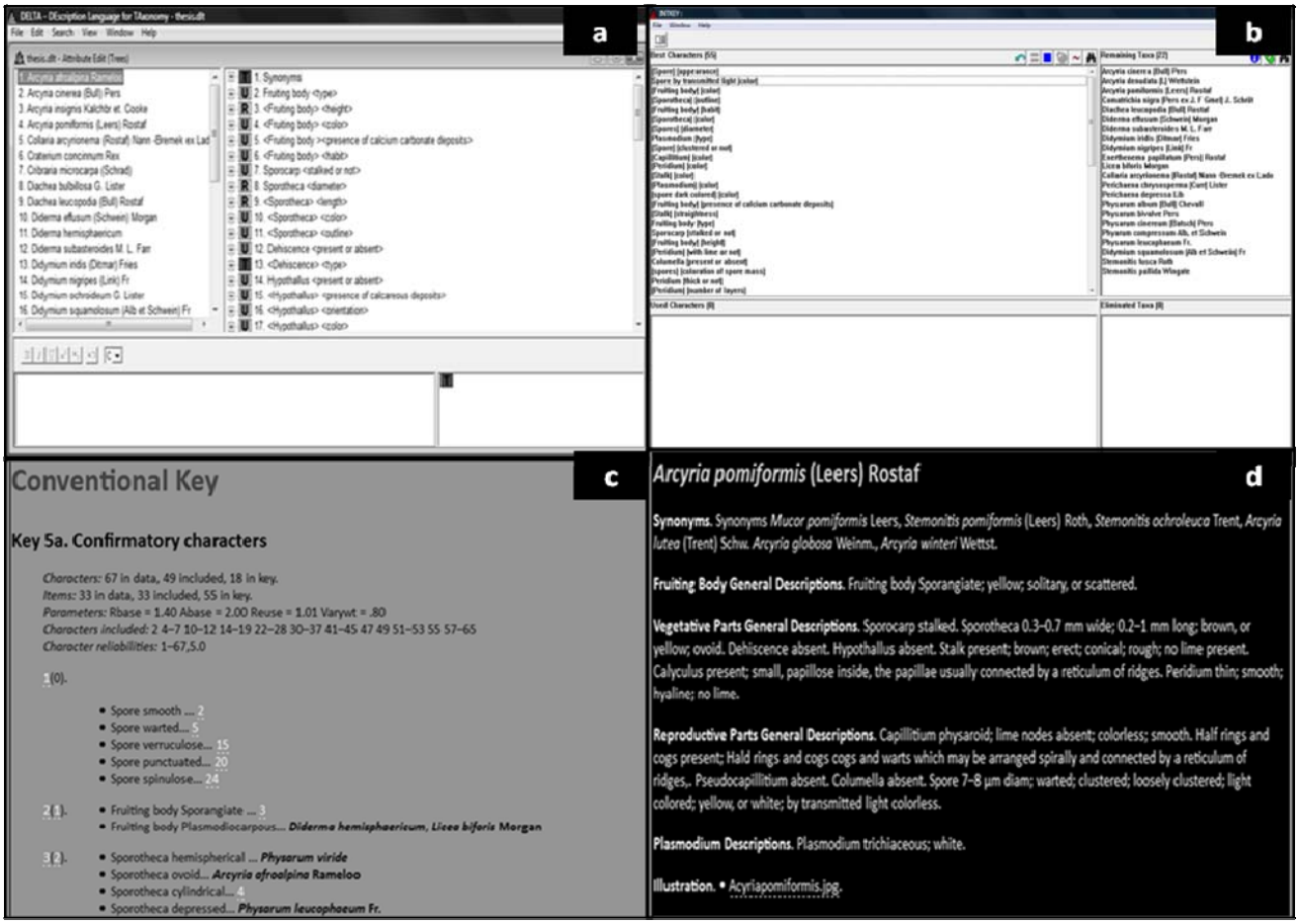

Fig. 2 (a–d) – Features of the interactive database for Mt. Arayat myxomycetes as generated from the DELTA software **a** DELTA editor **b** Interactive Key **c** Conventional key **d** Natural language.

else in Southeast Asia. This online database can help other myxomycologists to easily share new discoveries relating to myxomycetes, most specifically the global distribution patterns of these organisms. It also helps other researchers to overcome some of the difficulties in identifying myxomycetes to species. Since this can be updated at any time, data specifications (e.g., characters and its character states for each taxa) that were introduced in the development of the database can be shared and further developed among researchers interested in the study of myxomycetes. Through the use of the DELTA software, conventional identification keys and interactive keys can be easily generated and can be valuable for the taxonomist working with myxomycetes.

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