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Molecular confirmation and taxonomy of the Rubiaceous *Mycetia apoensis* (Elmer) Govaerts

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The little known endemic Philippine Rubiaceae *Adenosacme apoensis* Elmer was transferred to the genus *Mycetia* Reinw. based on herbarium specimens. Also, *Mycetia apoensis* was once thought as conspecific with *M. cauliflora*. To date, *M. apoensis* lacks comprehensive vegetative and reproductive descriptions to fully understand the species and be able to delineate from other members of *Mycetia*. To verify the generic affiliation of the species with more certitude, two chloroplast markers (*rps*16 intron and *trn*L-F region) were sequenced from the recent collections at Mt. Apo, Davao. Bayesian analysis of the combined plastid (*rps*16 and *trn*L-F) dataset strongly supported (PP = 1.0) the inclusion of *M. apoensis* in the genus *Mycetia* and resolved *M. cauliflora* as its sister-taxa. A comprehensive description and botanical illustrations of *M. apoensis* as well as its conservation status based on IUCN criteria are here provided.

Keywords: *Adenosacme*, *Mycetia*, cpDNA, *rps*16, *trnL-F*, Philippine endemic, conservation

Introduction

Mycetia Reinw. of the family Rubiaceae is composed of about 45 species of small shrubs characterized by the corky white bark of older branches, the yellow or rarely white flowers and the fungus-like, snowy white, juicy berries [1]. The genus is distributed in tropical and subtropical Asia [2] and underwent several tribal affiliations from Mussaendeae [3], Hedyotideae [4], Isertieae [5], to the recently Argostemmateae [6]. In its current tribe, the genus *Argostemma* Wall. is resolved as its sister-taxa [7].

Currently, there are four *Mycetia* species [*Mycetia apoensis* (Elmer) Govaerts, *Mycetia cauliflora* Reinw., *Mycetia javanica* (Blume) Reinw. Ex Korth., and *Mycetia mindanaensis* (Elmer) Govaerts] known to be present in the Philippines [8]. Two of the Philippine endemic species (*M. apoensis* and *M. mindanaensis*) were previously under the genus *Adenosacme* Wall. but transferred to *Mycetia* based on

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morphology of herbarium specimens [9]. Moreover, Elmer [10] noted the close relationship between *M. apoensis* and *M. cauliflora*. There is a need to re-examine the morphology of the incompletely known *M. apoensis*.

In a recent botanical assessment of the Thomasian Angiosperm Phylogeny & Barcoding Group (TAPBG) at Mt. Apo, Brgy. Ilomavis, Kidapawan City, the flowering and fruiting branches of *M. apoensis* were spotted from low to mid elevation of the mountain. The TAPBG took the opportunity to study the plant to: 1) confirm its generic affiliation inferred from two chloroplast makers (*rps*16 intron and *trn*L-F region); 2) resolve whether *M. apoensis* is conspecific with *M. cauliflora*; and, 3) provide comprehensive descriptions, botanical illustration as well as the conservation status of *M. apoensis*.

Materials and methods

Two samples of *M. apoensis* (coded as 14-505 and 14-510) were collected at Mt. Apo National Park, through the Kidapawan-Magpet trail running through the Ilomavis Campsite (1,800 masl) and the Ko'ong Campsite (2,000 masl). Field photographs of the collected plants were taken. Leaf samples were placed in bags containing silica gel for DNA analysis [11]. Vegetative and reproductive branches were likewise collected for herbarium specimens. Preservation of reproductive parts was done by placing the parts in a plastic tube filled with commercial 70% ethyl alcohol.

Silica-dried leaf samples were subjected to DNA extraction following the protocol of Qiagen DNeasy Plant Mini Kit (Qiagen, Germany). The *rps*16 intron was amplified and sequenced using rps16-1F/rps16-2R [12] while the *trn*L-F region was done using the primer pair c/f [13, 14]. PCR reactions were performed on a Biometra T-Gradient in volumes of 25 mL following the PCR parameters and mixture of Alejandro *et al.* [15–17]. In all PCR runs, one sample was run with water instead of DNA as a negative control to test for contamination. Amplified DNA was purified with the QiaQuick PCR purification kit (Qiagen). All sequences were retrieved by the commercial services of Macrogen, Korea.

CodonCode Aligner v.3.0.1 was used to assemble and manually edit the forward and reverse sequences. Subsequently, the sequences were assembled using Seaview v4.5.2 for alignment and the excision of unnecessary bases. Additional DNA sequences were retrieved from Genbank (http://www.ncbi.nlm.nih.gov/) representing members of the tribe Argostemmateae and species from closely related tribes such as Anthospermeae, Danaideae, Dunnieae, Knoxieae, Paederiae, Putorieae, Rubieae, Spermacoceae, and Theligoneae [18]. *Colletoecema* and *Luculia* were used as outgroups for character polarity. A Bayesian analysis of the aligned sequences was conducted using the software Mr. Bayes v3.2.2 [19]. The best performing evolutionary model was determined using MrModelTest v.2.3 [20] under three model selection criteria: a) Akaike Information Criterion (AIC) [21]; b) AICc (second order criterion of AIC); and, c) the Bayesian Information Criterion (BIC) [22]. Bayesian analysis was performed with a sample frequency of 1000, 4 parallel chains and 10 million generations.

Results and discussion

Sequence characteristics and variation. The combined (*rps*16 intron and *trn*L-F region) analysis included 38 sequences. Four new sequences of *M. apoensis* from the two molecular markers are newly published here. Matrix lengths of the two markers are 1,390 base pairs (bp) for the *trn*L-F marker and 1205 bp for the *rps*16 intron. Although the *rps*16 intron data set has the shorter matrix length, it yielded the highest number of informative characters (216 bp) compared to the *trn*L-F region (206 bp). The aligned combined data set consisted of 2,596 bp

and a total of 422 informative characters. Alignment was without difficulty due to low genetic variation across the two cpDNA regions.

***Phylogenetic position of* Mycetia apoensis.** The two sampled *M. apoensis* nested in a subgroup together with *M. cauliflora*. These two *Mycetia* species are sister to another subgroup containing *M. gracilis*, *M. javanica*, and *M. malayana* (Fig. 1). All five included *Mycetia* species formed a monophyletic clade with strong support (PP = 1.0) (Fig. 1). This conforms with the transfer of *Adenosacme apoensis* made by Davis *et al.* [9]. Similar to the findings of Rydin *et al.* [18], our combined tree suggests that *Neohymenopogon* Bennet and *Mouretia* Pit. are closely related with the tribe Argostemmateae along with *Mycetia* and *Argostemma* with high support (PP = 1.0). Both *Neohymenopagon* and *Mouretia* possessed persistent calyx lobes on the fruit that is also common in *Argostemma* and *Mycetia* [18].

Our combined tree (Fig. 1) suggests that *M. apoensis* is closely related to *M. cauliflora* with high support (PP = 1.0). Sequence variation between *M. apoensis* and *M. cauliflora* is 7.70% for the *trn*L-F region but 0.00% for the *rps*16 region. The relatively high divergence in the *trn*L-F region indicates that the two species are not conspecific in contrast to Elmer [10]. Based on morphology, *M. apoensis* is distinct from *M. cauliflora* in having more slender and somewhat longer calyx teeth [23], longer petiole and calyx, smooth yellowish gray bark, scurfy leaf, petiole and peduncle surfaces, 9–12 lateral nerve pairs,

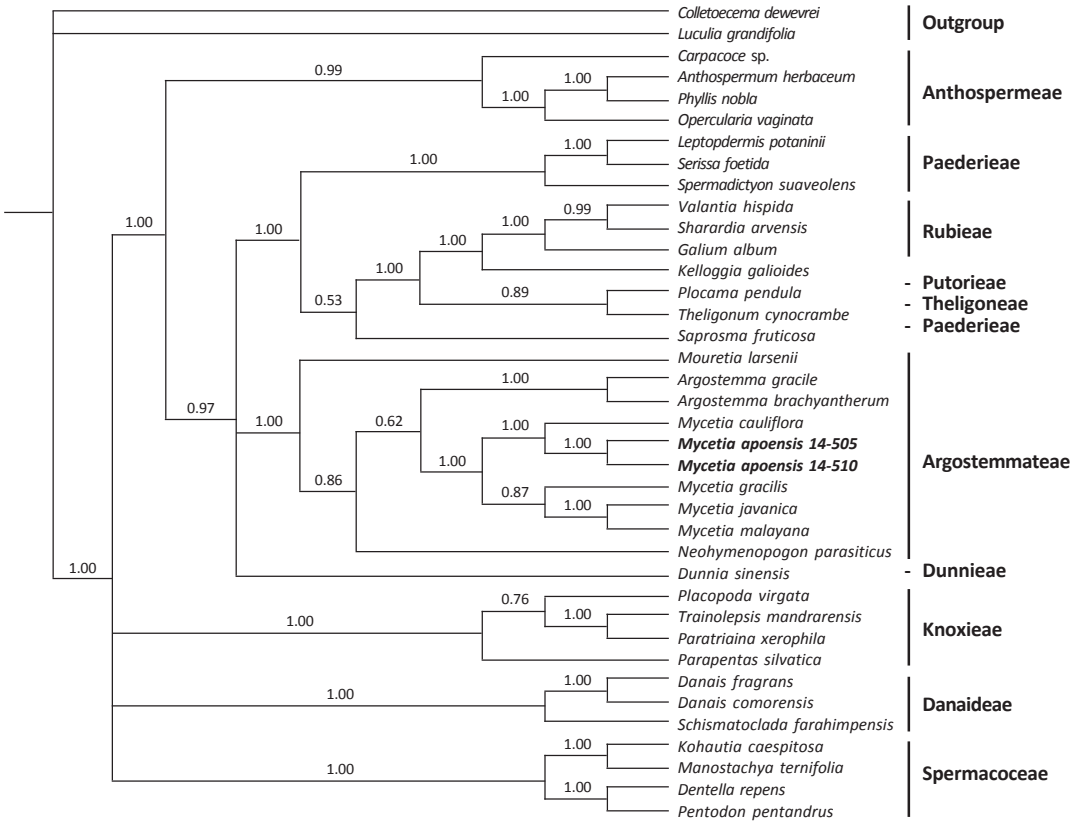

Figure 1. Majority rule consensus tree inferred from the combined *rps*16 and *trn*L-F sequence data. Numbers above nodes are Bayesian posterior probabilities. The highlighted text indicates *Mycetia apoensis.*

a style which is short tomentose and glabrous towards the base and a subglobose fruit shape. Comparative morphology of the two species is presented in Table 1.

***Taxonomy of* Mycetia apoensis.** This section provides the comprehensive vegetative and reproductive morphology as well as the

Table 1. Morphological comparison of *M. cauliflora* and *M. apoensis*
(*The significant differences are in bold fonts.*)

	Mycetia cauliflora	***Mycetia apoensis***
Habit	Shrub	Shrub
Height	1–2 m	1–2 m
Stem		
Thickness	Finger thick	3.9–7.5 mm
Color	**White**	**Covered with a yellowish gray bark**
Stipule		
Texture	Submembranous	Submembranous
Shape	Lanceolate-ovate	Triangularly acuminate to lanceolate
Length	2.5–6 mm long	5.0–8.0 mm long,
Surface	Subglabrous	Subglabrous
Petiole		
Length	**2–10 mm long**	**5.0–20 mm long**
Leaf blade		
Shape	Lanceolate or oblanceolate	Broadly and more or less oblanceolate
Venation	Reticulate	Reticulate
Length	125–160 mm	90–200 mm
Width	35–70 mm	30–50 mm
Apex	Sharply pointed	Acuminate
Base	Gradually tapered	Attenuate
Surface	**Glabrous, puberulous on the nerves beneath**	**Glabrous, dirty brown scurfy**
Lateral nerve pairs	**10–18 pairs**	**9–12 pairs**
Inflorescence		
Type	Thyrses	Loose cymose panicle
Pedicel		
Length	15 mm	17–21 mm
Calyx		
Shape	Turbinate	Elliptic, the base much constricted
Length	**5 mm long**	**7.0–8.0 mm long**
Lobes number	5	5
Lobes length	**1–2 mm long**	**3.0–3.5 mm long**
Corolla		
Color	Yellow	Bright Yellow
Shape	Funnel-tubular	Tubular
Length	10 mm long	12–13 mm long
Surface adaxial (above)	Glabrous outside, rough inside	Glabrous except the strigose hairs in the middle portion of the tube
Anther		
Length/Width	1.5 mm long	2 mm long
Style		
Length	In long-styled form: 4–8 (–11) mm long In short-styled form: 2 mm long	9–10 mm long
Surface	**Puberulous**	**Glabrous towards the base, short tomentose**
Fruits		
Presence of calyx lobes	Crowned by the calyx lobes	Crowned by the calyx lobes
Shape	**Oblong**	**Subglobose**
Length	10–15 mm long	5–12.71 mm
Width	8–10 mm wide	5–12.45 mm

Figure 2. *Mycetia apoensis.* (A) Habit, (B) fruits, and (C) flower. Scale bars in A, B, and C indicate 1 cm. Photos taken by Villanueva JCC and Alejandro GJD.

distribution, habitat and conservation status of *M. apoensis.*

Mycetia apoensis *(Elmer) Govaerts, Fig. 2 and Fig. 3*

Type: Philippines, Mindanao, District of Davao, Todaya (Mt. Apo), v.1909, *Elmer* 10504 (K!).

Lax shrub, 1–2 m tall; stem, 3.9–7.5 mm thick, terete, subglabrous and covered with a smooth yellowish gray bark; branches are sparingly rebranched and reclinate. Leaves thin, paler green beneath, mainly horizontal, flat or only the tips recurved, opposite, glabrous or dirty brown scurfy, membranous or thinly chartaceous, drying green, broadly or more or less oblanceolate, entire margins, 9.0–20 cm long and 3.0–5.0 cm wide just above the middle apex acuminate, base attenuate; nerves more prominent beneath, 9–12 on each side of the prominent midvein, reticulate crossbars quite evident; petiole slender, sub-glabrous or minutely scurfy brown, 0.5–2.0 cm in length, stipules submembranous and subglabrous, 5.0–8.0 mm long, 1.8–2.5 mm wide, entire, brown when dry, triangularly acuminate to lanceolate. Infloresence a loose cymose panicle, descending usually in the leaf axis, once or twice rebranced, 3.5–5.0 cm long and wide; peduncle subcompressed, dirty brown scurfy, arising from

Figure 3. *Mycetia apoensis.* (Elmer) Govaerts. (A) flowering branch, (B) Inflorescence (C) infructescence, (D) open corolla showing anther and style, (E) longitudinal section of fruit, and (F) cross section of ovary. From Villanueva *et al.* 14-505 & 14-510 (USTH). Drawn by Diego N.

a whorl of persistent, dry, straw-colored, 2.0–3.0 mm long triangular bracts, bearing 1 or 2 similar whorls, and about 11–14 mm long, pedicels similar in vestiture, very slender, 17–21 mm long, subtended by similar bracts that are chiefly in whorls of three's, the larger ones occasionally branched; calyx 7–8 mm long, 2.7–3.2 mm wide, elliptic, the base is much constricted, rough puberulent or finely scabrous, abruptly divided into five very thin, 3.0–3.5 mm long calyx lobes which are triangular at the base and very narrowly lanceolate at the apex; corolla bright yellow, 12–13 mm long, tubular, tube strigose adaxially, subglabrous abaxially, 9–10 mm long; lobes 5 triangularly oblong, glabrous adaxially and subglabrouse abaxially, valvate; stamens 5, just below the throat; filament glabrous, 0.5–0.6 mm long, adnate to the corolla; anthers oblong, 2.0 mm

long, dorsifixed, acute at apex, style slightly exceeding the corolla, 9–10 mm and two-forked at apex although occasionally easily separating clear to the narrowed base, glabrous toward the base, short tomentose otherwise; fruit subglobose 5–12.71 mm long 5–12.45 mm wide, slightly scabrous, two-celled, juicy, snow-berry white, sunken at apex and surmounted by the five persistent calyx teeth; seeds 0.5 mm across, dark brown, angularly flattened, very numerous in two dense masses.

Distribution and habitat: Restricted to Mt. Apo National Park, Davao and Mt. Hibok Hibok, Camiguin; from 1200–1300 masl in a very moist densely forested flats.

***Conservation status of* Mycetia apoensis.** This species is restricted to Mt. Apo National Park at 1200–1300 masl and Mt. Hibok Hibok, Camiguin. It was also reported that the same species was collected in Mindoro [10]. Based on the IUCN Red List Categories and Criteria [24], *M. apoensis* is categorized as Vulnerable [VU B2ab(i)]; B2, area of occupancy estimated to be less than 2,000 km^2 (area of Mt. Apo: 550 km^2 and area of Mt. Hibok Hibok: 238 km^2); a, severely fragmented or known to exist at no more than 10 locations (*M. apoensis*: known to exist at two locations); b(i), continuing decline, observed, inferred or projected, in extent of occurrence (*M. apoensis*: a decrease in the extent of occurrence of this species is inferred due to the lack of recent records indicating its presence in Mindoro).

Conclusion

Molecular and morphological analyses confirm the identity and distinctness of *M. apoensis* over *M. cauliflora*.

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