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Srivari Illam, No. 61, Karthik Nagar, 10th Street, Saravanampatti, Coimbatore, Tamil Nadu 641035, India
Registered Office: 3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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continued on the back inside cover

Cover: A bag worm with its beautiful heap of junk. Acrylics on 300 GSM paper by Dupati Poojitha based on a picture by Sanjay Molur.



***Dasymaschalon leilamericanum* (Annonaceae), a new species with evidence of non-monophyly from Mount Lantoy Key Biodiversity Area, Philippines**

**Raamah Rosales¹ , Edgardo Lillo² , Archiebald Baltazar Malaki³ , Steve Michael Alcazar⁴ , Bernardo Redoblado⁵ , John Lou Diaz⁶ , Inocencio Buot Jr.⁷ , Richard Parilla⁸ & Jessica Rey⁹ **

¹College of Arts and Sciences, Cebu Technological University-Main Campus, Cebu City, Philippines.

^{2,3,4,5,6}Cebu Technological University-Argao Campus, Cebu, Philippines.

⁷Forest Biological Sciences, College of Forestry and Natural Resources, University of the Philippines, Los Baños, Laguna, Philippines.

⁸Division of Natural Science and Mathematics, University of the Philippines - Tacloban College, Leyte, Philippines.

⁹Institute of Biological Science, College of Arts and Sciences, University of the Philippines, Los Baños, Laguna, Philippines.

¹raamah.rosales@ctu.edu.ph (corresponding author), ²edgardo.lillo@ctu.edu.ph, ³archlam68@gmail.com, ⁴alcazarsteve@mic@gmail.com,

⁵bnaid7@gmail.com, ⁶diazjohnlou@gmail.com, ⁷iebuot@up.edu.ph, ⁸rbparilla@up.edu.ph, ⁹jdrey@up.edu.ph

Abstract: A new Annonaceae species, *Dasymaschalon leilamericanum* is described from Mount Lantoy Key Biodiversity Area (KBA) in the Municipality of Argao, Cebu, Philippines. *D. leilamericanum* is distinguished from closely related species by morphological characters including laminar size, leaf areolation, and seed/fruit shape and size. Significant differences observed in a multivariate analysis of morphological data for *D. leilamericanum*, *D. clusiflorum*, *D. filipes*, *D. ellipticum*, and *D. blumei* indicate *D. leilamericanum* is a distinct species. A phylogenetic tree analysis performed using maturase K (*matK*) and ribulose 1,5-biphosphate carboxylase (*rbcL*) as molecular markers failed to match *D. leilamericanum* with other *Dasymaschalon* species. Taken together, the results of morphological and molecular analysis indicate a unique evolutionary pathway for *D. leilamericanum* with its genus.

Keywords: Conservation, Cebu Island, evolutionary, forest, limestone, multivariate, non-monophyly, plant, paraphyletic, phylogenetic, sedimentary, shrub.

Cebuano: Espesye sa bag-ong Annonaceae ang *Dasymaschalon leilamericanum* nga gihulagway sa Mount Lantoy Key Biodiversity Area (KBA) sa Munisipyo sa Argao, Cebu, Pilipinas. Ang *D. leilamericanum* gipalahi gikan sa suod nga relasyon nga mga espesye pinaagi sa morphological nga mga karakter lakin ang laminar size, dahon areolation, ug liso/bunga porma ug gidak-on. Mahinungdanon nga mga kalainan nga nakita sa usa ka multivariate nga pagtuki sa morphological data alang sa *D. leilamericanum*, *D. clusiflorum*, *D. filipes*, *D. ellipticum*, ug *D. blumei* nagpaka nga ang *D. leilamericanum* usa ka lahi nga espesye. Usa ka phylogenetic tree analysis nga gihimo gamit ang maturase K (*matK*) ug ribulose 1,5-biphosphate carboxylase (*rbcL*) kay ang mga molecular marker napakyas sa pagpares sa *D. leilamericanum* sa ubang klase sa *Dasymaschalon*. Sa tingub, ang mga resulta sa morphological ug molecular analysis nagpaka sa usa ka talagaon nga evolutionary pathway alang sa *D. leilamericanum* uban sa iyang genus.

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INTRODUCTION

The forest of Cebu island in the Philippines, is home to several endemic species of flora and fauna (Cadiz & Buot 2010; Rosales et al. 2020). Several of the endemic species growing in this area are threatened due to deforestation and continued human pressure (Lillo et al. 2019, 2020, 2021). The forest fragments of Mount Lantoy Key Biodiversity Area (KBA) in Argao, Cebu, listed two Critically Endangered, two Endangered, four Vulnerable, and 16 restricted-range species (CI/DENR-PAWB/Haribon 2006). The Tabunan forest of the Central Cebu Protected Landscape (CCPL) is the largest forest fragment with an area of 40 ha. The Tabunan forest is home to the endemic *Cinnamomum cebuense* Kosterm and among the threatened species in spite its distribution range is within the protected landscape (Quimio 2006).

The genus *Dasymaschalon* is classified under the order Magnoliales of the family Annonaceae. It was initially considered to be a part of section *Unona* auct. non L. (Hooker & Thompson 1855). *Dasymaschalon* was classified as a section of *Desmos* Loureiro's (1790:352) *Desmos* sect by Hooker & Thomson (1855:134) and Safford (1912:507) Saff, *Dasymaschalon* (1912:507) (e.g. Sinclair 1955; Maxwell 1989; Li 1993). Many taxonomists such as Finet & Gagnepain (1906), Merrill (1915), Hutchinson (1923), Fries (1959), Bân (1975), Klucking (1986), Van Heusden (1992), Kebler (1993), Koek-Noorman et al. (1997), Sun et al. (2002) disagreed with the taxonomic treatment of Safford and supported Dalla Torre & Harms' decision to raise *Dasymaschalon* to the generic rank in 1901.

The genus *Dasymaschalon* is primarily found in southeastern Asia (Craib 1912; Merrill 1915; Sinclair 1955; Ast 1938; Tsiang & Li 1979; Bân 2000; Nurmawati 2003; Wang et al. 2009, 2012) particularly in southern China, Philippines, Indonesia, Malaysia, Thailand, and Indochina. Dalla-Torre & Harms (1901) estimated that *Dasymaschalon* (Hook & Thomson 1885) may have up to 30 species (Wang et al. 2009, 2012), with three (<http://www.philippineplants.org/Families/Annonaceae.html>) found in the Philippines and one in India. According to Guo et al. (2018), the genus *Dasymaschalon* is closely related to *Friesodielsia* Steenis s.str. (1948:458) and is morphologically distinct with small trees and shrubs (rarely climbers), shallow conical torus, 2–3-cohering petals arranged in one whorl, echinate ornamentation and pollen with thin exine (Walker 1971; Le Thomas 1980, 1981; Van Heusden 1992; Kebler 1993; Doyle & Le Thomas 2012).

While undertaking a field survey of flora on Mount Lantoy KBA of the Municipality of Argao (Image 1), we came across an interesting species of *Dasymaschalon* which did not match other known species. In this paper, we describe a new species of *Dasymaschalon* from Mount Lantoy Key Biodiversity Area, Cebu Island, Philippines.

MATERIALS AND METHODS

Sampling site

The novel *Dasymaschalon* was collected during a field survey of flora on Mount Lantoy KBA of the Municipality of Argao (Image 1). Mount Lantoy is one of the new sites classified as a key biodiversity area (KBA) among the 117 terrestrial areas in the Philippines based on irreplaceability and vulnerability criteria (Mallari et al. 2001). These sites are inhabited by 16 restricted-range, four Vulnerable, two Endangered, and two Critically Endangered species (CI/DENR-PAWB/Haribon 2006).

Plant morphological characterization

Photographic records were taken, and voucher specimens were collected and subsequently deposited in the DOST-NICER Biodiversity Museum and University of San Carlos (USC)-Terrestrial Species Museum. The description of morphological characters of the species is based on fruit and vegetative traits (LAWG 1999; Pi et al. 2009; Masungsong et al. 2019; Hernandez et al. 2020) (Ngoc-Daido et al. 2018). The vegetative traits were examined and measured from seedlings and press-dried specimens while field characteristics were noted on the spot. Fruit traits were noted in both fresh specimens and those preserved in 70% ethanol.

The third mature leaves, which are the ones that are fully exposed to sunlight, were chosen as the leaf samples for characterization (Pi et al. 2009). The specimen's ten mature leaf samples were employed in the investigation. With a hand lens with greater magnification, the leaf samples were inspected. Based on the Leaf Architectural Working Group Manual, the leaf architecture was described (LAWG 1999). Following Masungsong et al. (2019) and Hernandez et al. (2020), petiole length width and leaf blade were measured using a digital caliper, and venation angles, base and apex were determined using a protractor. Additionally, herbarium specimens were analyzed using online photos of the type specimens (www.philippineplants.org). Except where noted, every photo was taken outdoors where the species were located. The morphological analysis of variance was used

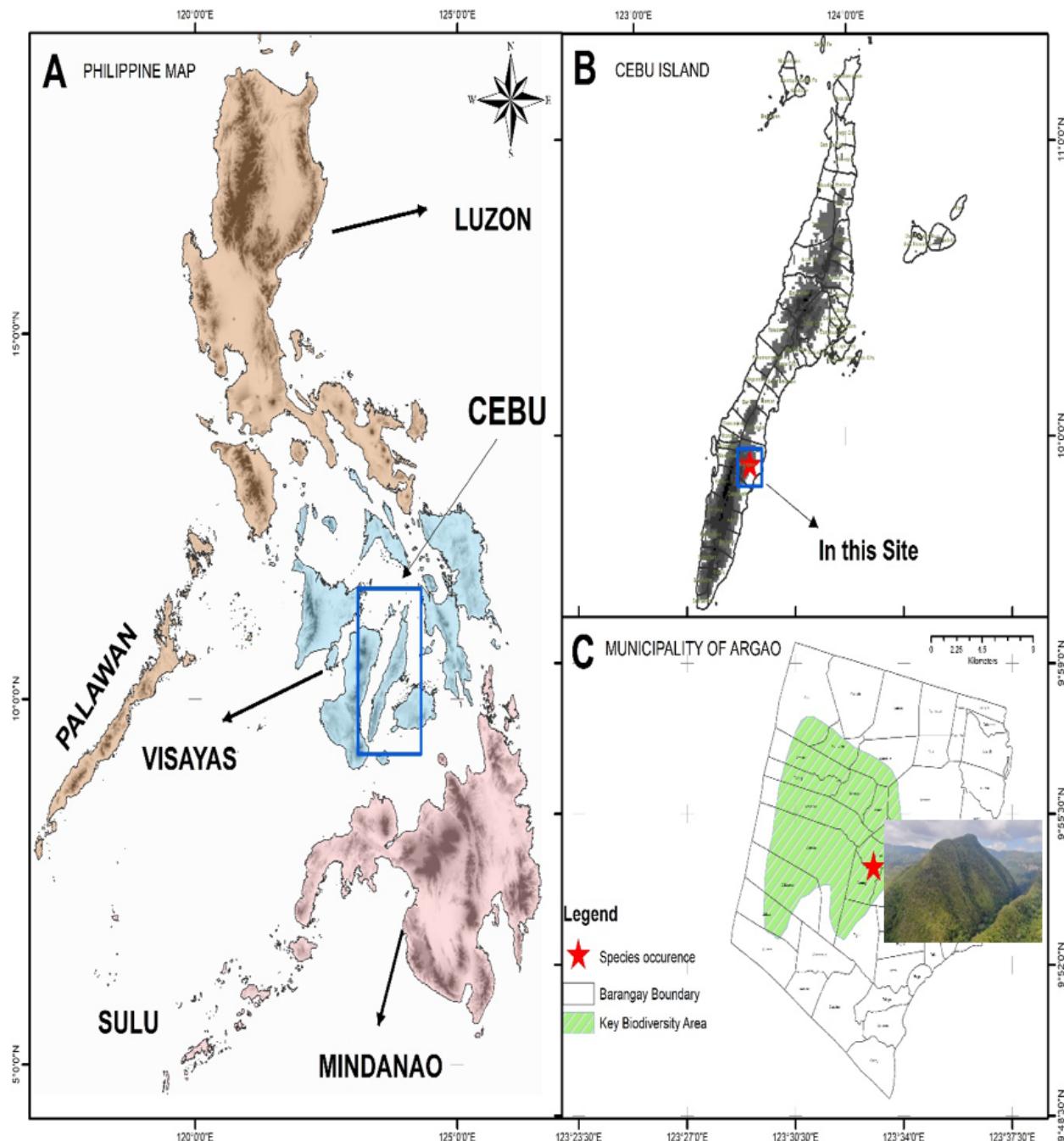


Image 1. Location of the new species in Mount Lantoy of the Municipality of Argao, Cebu at Philippine map (GIS generated map; Landsat 8; www.earthexplorer.usgs.ph; NAMRIA; Philippine GIS data). A—Philippine map and location of Cebu Island within the box | B—Cebu Island and location of the study site within the box | C—specimen collection site indicated by the star mark.

for the morphological traits of the *D. leilamericanum* and the related species (Meeran et al. 2023)

DNA Extraction, amplification, and sequencing

Chloroplast genomic DNA was extracted from dried leaves of the specimens collected from the study site using the QIAGEN DNeasy® Plant Mini Kit while

sample cleanup was done using the QIAGEN DNeasy® PowerClean® Pro Cleanup Kit, following manufacturer's protocols, respectively. For amplification each 50 µl PCR contained 25 µl of MyTaq® HS Red Mix, 2x, 1 µl of each forward and reverse primer, 1 µl of DNA template, and 1 µl nuclease-free water (NFW). The respective thermal cycle conditions were 95°C denaturation, 50°C annealing,

70°C, and 72°C extension. A total of 12 samples were sent for sequencing at the facility of Macrogen, Inc., Seoul, South Korea.

For the molecular authentication of the new species, the most commonly preferred gene loci suggested by the Consortium for the Barcoding of Life (CBOL), maturase K (*matK*) and ribulose 1,5-biphosphate carboxylase (*rbcL*), were chosen as primers (Yu et al. 2011; Tran et al. 2021). These cpDNA were also used by Guo et al. (2018) in demonstrating the incongruence among different gene trees involving *Dasymaschalon* and other closely-related taxa. The forward and reverse primers used to amplify *matK* and *rbcL* genes were adopted from respective authors cited in de Vere et al. (2015). The primer sequence for the *matK*-forward (MatK-3FKIM-r) CGTACAGTACTTTGTGTTACGAG and *matK*-*matK*_1R_kim) ACCCAGTCCATCTGGAAATCTTGGTCC. The primer sequence for *rbcL*-forward (*rbcL*a-F) ATGTCACCACAAACAGAGACTAAAGC and *rbcL*-reverse (*rbcL*aJf634R) GAAACGGTCTCTCCAACGCAT.

Alignment and Phylogenetic Analyses

The complementary chromatogram reads from sequenced samples were checked and trimmed for quality control using Finch TV, then aligned to generate a consensus sequence using BioEdit software (CAP contig assembly). The consensus sequence was then reverse complemented followed by alignment with closelyrelated species in MEGA7 via MUSCLE (Edgar 2004). This is then followed by trimming of gaps between the sequences and deletion of sequences with common undefined base calls and sequences with relatively short alignment. There was also an addition of related plant species and/or replacement of different reference sequences in order to lengthen the generated alignment via MUSCLE. Using the same software, the Best Fit Model was determined via the Maximum Likelihood of the default setting (Model Selection ML – Automatic Neighbor-Joining Tree). The phylogenetic tree was then generated using the best model determined for the aligned sequences, and the test of phylogeny was set to bootstrap (1,000 replicates) and completion for gaps regardless of the method.

The sequences were submitted to nucleotide Basic Local Alignment Search Tools (BLAST) of the National Center for Biotechnology Information (NCBI) as well as to the Barcode of Life Database (BOLD) system for similarity checks and identification. Conversely, for further phylogenetic analyses and evolutionary tree construction, the *matK* and *rbcL* respective gene sequences of species belonging to all related genera

in the family Annonaceae with *D. leilamericanum* were retrieved and accessed from GenBank® of the National Center for Biotechnology Information (NCBI) (Table 1). The *matK* and *rbcL* DNA sequences were submitted to NCBI for the application of accession numbers.

RESULTS AND DISCUSSION

The *D. leilamericanum* is described through morphological and molecular characterization. The morphological diagnostic characters used are plant habit, leaf pattern, apex of leaf, base, veins order and category, flower, petals, sepals, stamen, pedicels, fruits and monocarp, stalks, and seeds.

Morphological diagnostic characters

The analysis clearly showed that *D. leilamericanum* differs from other *Dasymaschalon* in having distinct morphological characteristics such as larger laminar size which belong to notophyll category (LAWG 1999) with leaf area ranging from 2,025–4500 mm², laminar length: width ratio of 4:1, vein orders; Leaf 10 vein category pinnately veined, 20 vein category festooned semicraspedodromous veined, agrophic veins simple, 20 vein spacing irregular, 20 vein angle smoothly decreasing toward the base, inter 20 veins weak intersecondaries, 30 vein category alternate percurrent, 30 vein coarse sinuous, 30 vein angle to 10 obtuse, 30 vein angle variability inconsistent, 40 and 50 vein category regular polygonal reticulate, and the leaf areolation 5 or more sides. Fruit stalk (infructescence) 29 mm, axillary/terminal, Pedicels (per Monocarp) ranges 13–38 mm, Monocarps 50–60 pcs, ellipsoid 9–20 x 8–13 mm, pinkish to black color, 5–7 seeded/monocarp, embedded in pulpy tissue. Seed dimension 0.7–0.9 cm x 0.5–0.6 cm (Table 2; Images 2, 3, 4, & 5).

Taxonomy

Dasymaschalon leilamericanum Rosales & Lillo, sp. nov. (Images 2–6)

Type: Holotype: Lillo 00090 (CTU-DOST NICER Biodiversity Museum). Philippines, Cebu Island, Municipality of Argao, Mount Lantoy KBA (Lat. 9.904229, Long. 123.5513) at 99 m elevation (Image 1). The type specimen was located at the base of Mount Lantoy, along the river of Barangay Usmad, bearing multiple fruits, 2 March 2020.

Isotype: USCBM 2675 (University of San Carlos

Table 1. List of selected species used to reconstruct the phylogenetic tree for *Dasymaschalon leilamericanum* sp. nov. and their GenBank Accession information.

	Species	GenBank Accession	
		<i>rbcL</i>	<i>matK</i>
1	<i>Dasymaschalon leilamericanum</i> sp. nov.	[PQ878320]	[PQ869009]
2	<i>Dasymaschalon macrocalyx</i>	[AY841610.1]	[AB924891.1]
3	<i>Dasymaschalon clusiflorum</i>	[JQ768668.1]	[JQ768548.1]
4	<i>Dasymaschalon filipes</i>	[JQ768672.1]	[MH308078.1]
5	<i>Dasymaschalon ellipticum</i>	[JQ768670.1]	[JQ768550.1]
6	<i>Dasymaschalon oblongatum</i>	[JQ768679.1]	[JQ768559.1]
7	<i>Dasymaschalon megalanthum</i>	[JQ768678.1]	[JQ768558.1]
8	<i>Dasymaschalon longiflorum</i>	[JQ768675.1]	[MH308073.1]
9	<i>Dasymaschalon dasymaschalam</i>	[MT264015.1]	[MT264031.1]
10	<i>Dasymaschalon glaucum</i>	[JQ768673.1]	[JQ768553.1]
11	<i>Dasymaschalon acuminatum</i>	[MT264012.1]	[MT264028.1]
12	<i>Desmos dumosus</i>	[JQ768689.1]	[HG005013.1]
13	<i>Desmos chinensis</i>	[JQ762414.1]	[KP093298.1]
14	<i>Desmos polycarpus</i>	[KF496673.1]	[KX786589.1]
15	<i>Desmos elegans</i>	[HQ214067.1]	[JQ768571.1]
16	<i>Desmos cochinchinensis</i>	[JQ768688.1]	[OL604143.1]
17	<i>Friesodielsia desmoides</i>	[AY841618.1]	[JQ768577.1]
18	<i>Friesodielsia biglandulosa</i>	[MG896081.1]	[MG910438.1]
19	<i>Friesodielsia glauca</i>	[MG896051.1]	[MG910428.1]
20	<i>Schefferomitra subaequalis</i>	[KX786628.1]	[KX786606.1]
21	<i>Uvaria macrophylla</i>	[KP094324.1]	[KP093408.1]
22	<i>Uvaria concava</i>	[JN175197.1]	[JN175167.1]
23	<i>Uvaria cordata</i>	[JN175198.1]	[AB924906.1]
24	<i>Uvaria wrayi</i>	[FJ743821.1]	[AB924778.1]
25	<i>Uvaria siamensis</i>	[FJ743824.1]	[AB925067.1]
26	<i>Uvaria lucida</i>	[MN166687.1]	[MN166628.1]
27	<i>Melodorum fruticosum</i>	[AY319071.1]	[AB924724.1]
28	<i>Monanthotaxis micrantha</i>	[KX761331.1]	[KX761300.1]
29	<i>Monanthotaxis buchananii</i>	[JQ768700.1]	[JX517585.1]
30	<i>Monanthotaxis fornicata</i>	[JQ768702.1]	[JQ768583.1]
31	<i>Monanthotaxis montana</i>	[MF353789.1]	[MF353672.1]
32	<i>Monanthotaxis whytei</i>	[AY841635.1]	[EF179278.1]
33	<i>Cyathostema viridiflorum</i>	[AY841607.1]	[FJ743746.1]
34	<i>Cleistochlamys kirkii</i>	[JX572412.1]	[JX517486.1]
35	<i>Dielsiothamnus divaricatus</i>	[EU169759.1]	[KX146236.1]
36	<i>Meiocarpidium lepidotum</i>	[EU169754.1]	[EU169687.1]
37	<i>Mitrella kentii</i>	[AY841633.1]	[FJ743751.1]
38	<i>Monocyclanthus vignei</i>	[EU169765.1]	[EU169698.1]
39	<i>Ophrypetalum odoratum</i>	[EU169767.1]	[EU169702.1]
40	<i>Pyramidanthe prismatica</i>	[JN175193.1]	[JN175163.1]
41	<i>Toussaintia orientalis</i>	[EU169756.1]	[EU169689.1]

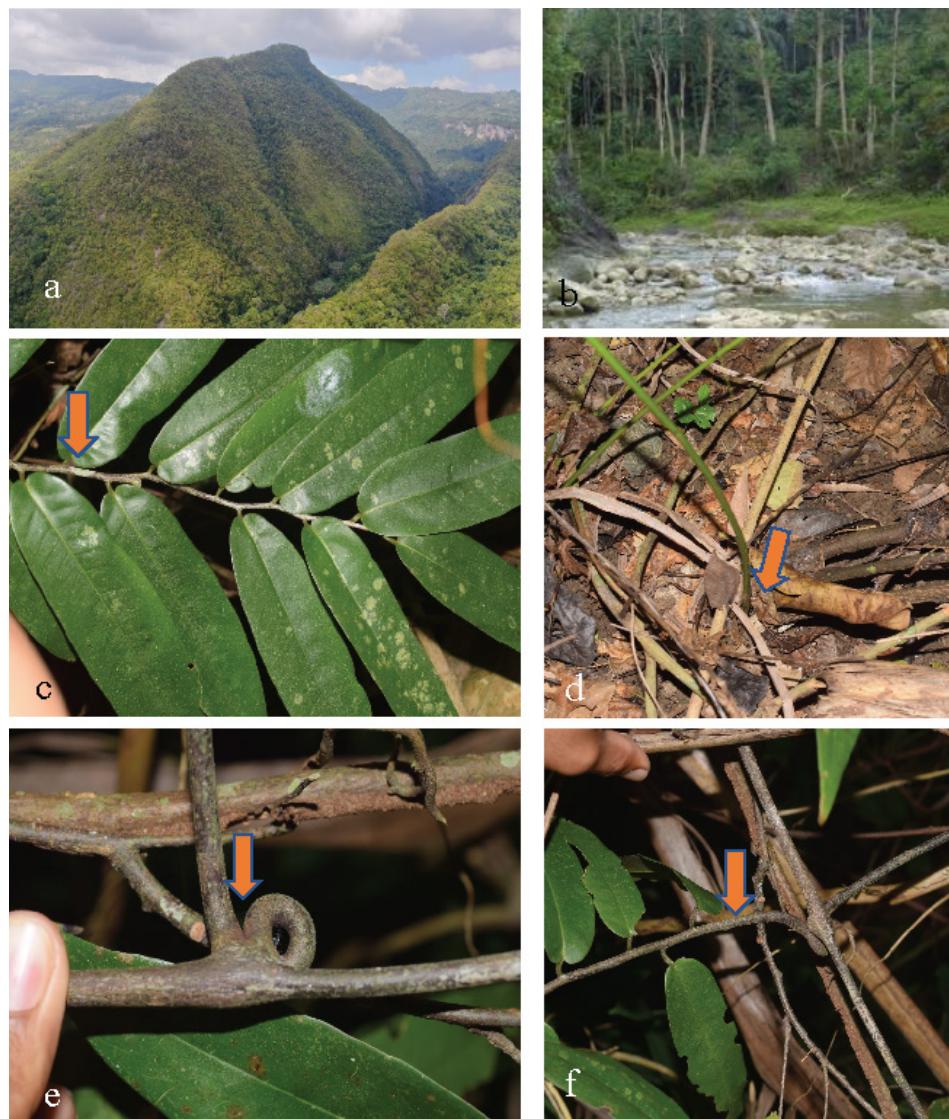


Image 2. Habitat and habit of *Dasymaschalon leilamericanum* sp. nov.: a—panoramic view of Mount Lantoy KBA | b—Argao river at the base of Mount Lantoy | c—Leaf arrangement | d–f—Habit of *Dasymaschalon leilamericanum* sp. nov. © John Lou Diaz.

Biological Museum).

Ecology: Near a height of 99 m, the species is found in creeks with thick vegetation at the foot of Mount Lantoy (Image 1). The Carcar formation dominates the Mount Lantoy KBA, which is defined as a forest over limestone habitat type. Its geological makeup is dominated by elevated sedimentary and metamorphic rocks, with limestone making up a sizeable portion of that composition (Audley-Charles et al. 1979).

The species was found 5 m from the river bed. The species associated with *Dasymaschalon leilamericanum* Rosales and Lillo at the type locality include, among many others, *Rapanea philippinensis* (A. DC.) Mez (Primulaceae), *Ficus benjamina* L. (Moraceae), *Bischofia javanica* Blume (Phyllanthaceae), *Guioa koelreuteria*

(Blanco) Merr. (Sapindaceae), *Melicope triphylla* (Lam.) Merr. (Rutaceae), *Neonauclea calycina* (Bartl.) Merr. (Rubiaceae), *Senna alata* (Fabaceae), *Schefflera obtusifolia* Merr. (Araliaceae), *Guioa acuminata* Radlk. (Sapindaceae), *Goniothalamus elmeri* Merr. (Annonaceae), *Semecarpus cuneiformis* Blanco (Anacardiaceae), *Vitex parviflora* Juss. (Lamiaceae), *Bridelia stipularis* Blume (Phyllanthaceae), and *Canarium asperum* Benth. (Burseraceae).

Distribution: Thus far, Mount Lantoy KBA in the Municipality of Argao, Cebu, is where this new species is known to exist (Image 1). *Dasymaschalon* species are widespread in forests and thickets at low and medium elevations, sometimes on limestone soil, according to Merrill (1923).

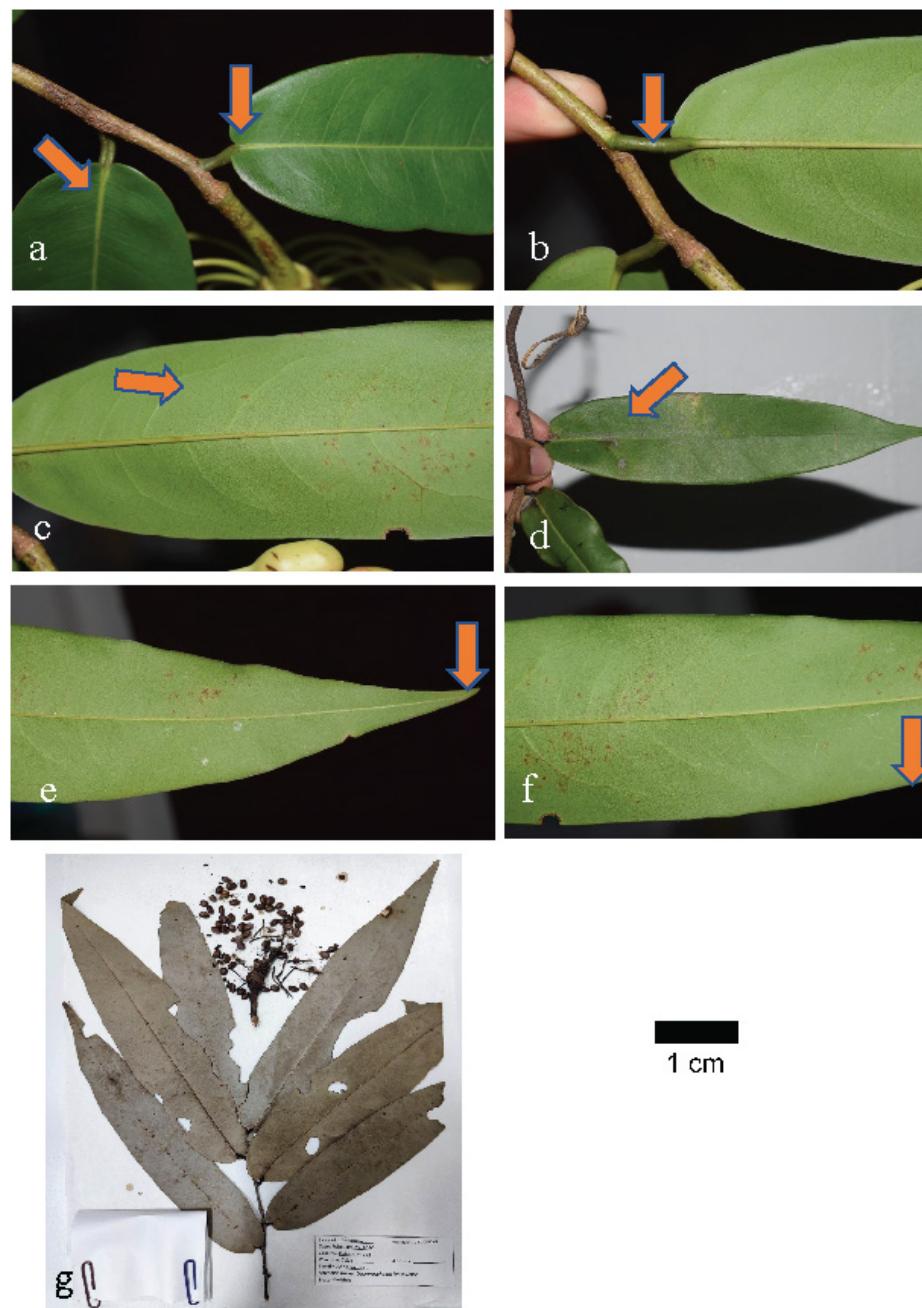


Image 3. Leaf morphology of *Dasymaschalon leilamericanum* sp. nov.: a—alternate leaf arrangement | b—swollen petiole | c—back surface (adaxial) | d—leaf surface (abaxial) | e&f—leaf apex (adaxial) | g—herbarium specimen. © John Lou Diaz.

Etymology: This new species is named in honor of Dr. Leila America who actively promoted biodiversity conservation in the Philippines. Dr. America was the former director of the Forestry and Environment Research Division (FERD), prior to her passing, of the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) under the Department of Science and Technology (DOST) of the Philippines.

This new species would add to the list of important species that serve as the basis for the protection and conservation of the mountain as a habitat for the endemic species of Cebu Island. Pelser & Barcelona (2017) recorded the 'Critically Endangered' *Cynometra cebuensis* species in Mount Lantoy KBA. Other endemic species include *Copsychus cebuensis* Steere, 1890 (Malaki et al. 2018) and *Anixa zebuensis* Broderip, 1841 (Rosales et al. 2020).

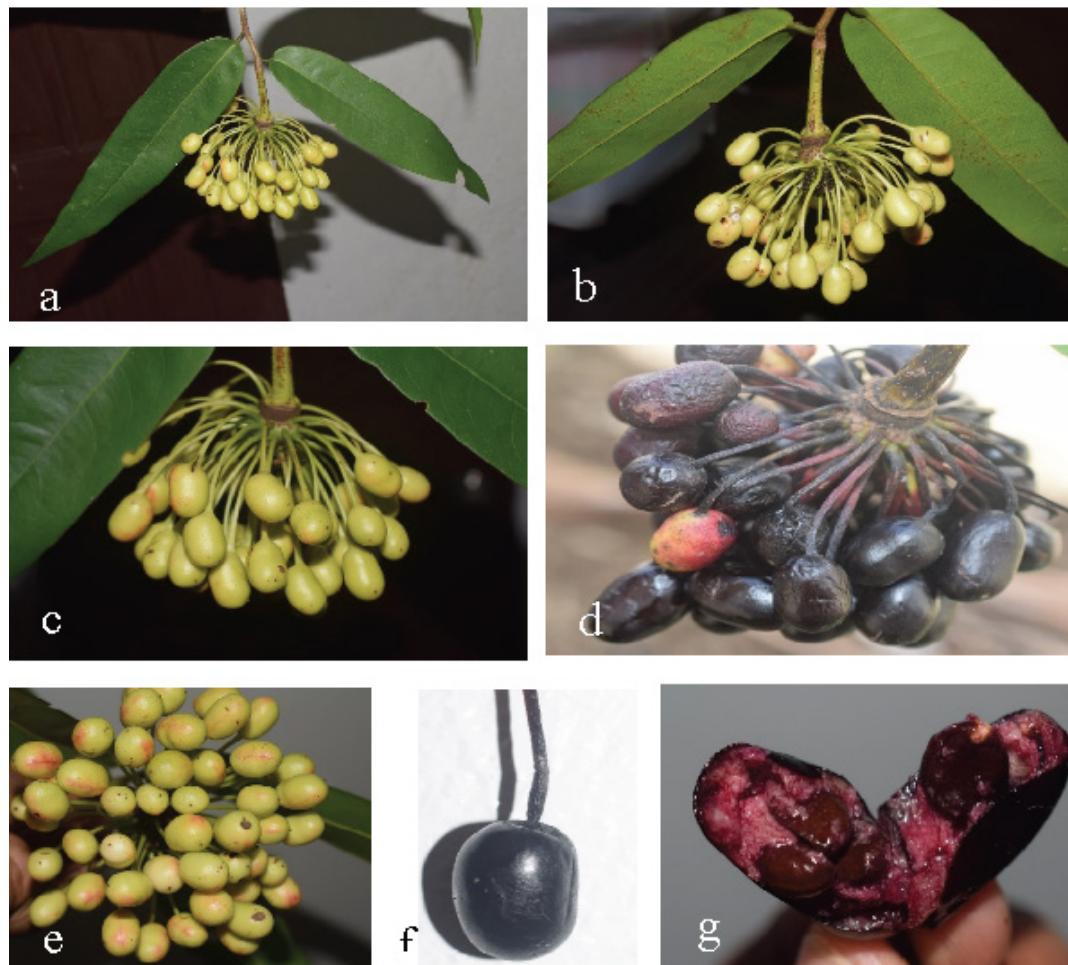


Image 4. Fruiting habit of *Dasymaschalon leilamericanum* sp. nov. © John Lou Diaz.



Image 5. Fruits and Seeds of *Dasymaschalon leilamericanum* sp. nov. © John Lou Diaz.

Table 2. Morphological comparison of *Dasymaschalon leilamericanum* sp. nov., *Dasymaschalon clusiflorum* (Merr.) Merr, *Dasymaschalon filipes* (Ridl.) Ban. *Dasymaschalon ellipticum* Nurmawati, and *Dasymaschalon blumei* (Finet & Gagnep 1906; Nurmawati 2003).

Plant Morphology (LAWG 1999)	<i>Dasymaschalon leilamericanum</i> sp. nov.	<i>Dasymaschalon clusiflorum</i> (Merr.) Merr.	<i>Dasymaschalon filipes</i> (Ridl.) Ban	<i>Dasymaschalon ellipticum</i> Nurmawati	<i>Dasymaschalon blumei</i> Finet & Gagnep
Habit	Small tree 5 m in height.	Small tree up to 10 m high.	Small trees, 5 m high. Branches glabrous	Small trees up to 5 m. Branches glabrous	Branches are either tomentose or glabrous and either a shrub or a small tree with a height of 6 m.
Leaf pattern	Simple and alternate in arrangement, petiolar attachment marginal and swollen appearance, glabrous, lamina chartaceous to sub coriaceous, laminar size notophyll with leaf area ranges from 2,025–4500 mm ² , laminar shape lanceolate, symmetrical, leaf margin entire and wavy	Petiole range is 1–2 x 7–15 mm, it is glabrous; ellipsoidal, lamina can be chartaceous and some are sub coriaceous, ellipsoidal in shape and narrow, some can be lanceolate or can be ellipsoidal while others can be broad, laminar size notophyll with leaf area ranges from 5.5–23.7 x 1.3–9 cm, pale on the lower surface; and glabrous in all sides including the midrib.	Petiole ranges from 9–17 x 2–3 mm; lamina is characterized with sub coriaceous or can be chartaceous, it is oblanceolate in shape or can be ellipsoidal, leaf area ranges from 16.5–35 x 5–9 cm. glaucous in the lower surface,	Petiole ranges from 10–17 x 2–3 mm, described as glabrous; sub-coriaceous for the lamina, oblong in shape, leaf area ranges from 17.5–33.6 x 4–9.6 cm, glaucous in the lower surface and glabrous in all sides in the midrib.	Petiole ranges from 2–3 x 3–5 mm, thickly tomentose or can be glabrous, covered mostly by a leaf base; lamina can be sub coriaceous or some can be chartaceous, leaf area ranges from 9–31 x 4–10 cm, oblanceolate
Apex of Leaf	Apex angle is acute; shape can be acuminate	Apex acute and can be shortly acuminate	Apex acute to acuminate	Apex acute or acuminate	Apex acute, shortly to abruptly acuminate
Base	Base angle obtuse, and base shape rounded	Base cuneate to subrounded	Base rounded	Base cuneate to sub rounded	Base cordate
Veins order					
1 ^o vein category	Pinnately veined	Pinnately veined	Pinnately veined		Pinnately veined
2 ^o vein category	2 ^o vein category festooned semicraspedodromous, 12–14 pairs, agrophic veins simple, 2 ^o vein spacing irregular, 2 ^o vein angle smoothly decreasing toward the base, inter 2 ^o veins weak inter-secondaries	Secondary veins slender raised above, curved, 6–12 pairs, including with the midrib an angle of about 35–55 degree, glabrous; lateral veins anastomosing inconspicuous	Secondary veins slender raised above, curved, including with the midrib an angle of about 55–60 degree, 14–17 pairs; lateral veins anastomosing inconspicuous.	Secondary veins slender raised above, glabrous, curved, 13–19 pairs, including with the midrib an angle of about 50–60 degree; lateral veins anastomosing.	Secondary veins faint, curved, 10–14 pairs, midrib an angle of about 35–55°, glabrous or densely tomentose; lateral veins anastomosing inconspicuous
3 ^o vein category	3 ^o vein category alternate percurrent, 3 ^o vein coarse sinuous, 3 ^o vein angle to 1 ^o obtuse, 3 ^o vein angle variability inconsistent,	Tertiary veins reticulate.	Tertiary veins are scalariform.	Tertiary veins scalariform	Tertiary veins are scalariform.
4 ^o and 5 ^o vein category	4 ^o and 5 ^o vein category regular polygonal reticulate, and the leaf areolation 5 or more sides.				
Flower	Unknown	Axillary or terminal, single, seldom raceme two –six flowers.	Flowers unknown	Solitary, some can be raceme with three–five flowers	Axillary or terminal, solitary, seldom raceme with two–four flowers.
Petals	Unknown	Three coriaceous, ovate, triangular, lanceolate or rhomboid petals, with or without a distinct claw, 1.2–11 x 1–2.4 cm, thick 0.5–3 mm, a sharp or directly acuminate apex, valvate reduplicate, and puberulent.	Petals unknown	Petals 3, coriaceous, linear lanceolate, 3.3–7 cm x 0.8–1.2 mm, thick c. 1mm, apex described as sharp, with valvate reduplicate, not clearly defined claw, and velutinous.	Three–four petals, some are coriaceous others can be sub-coriaceous, it is lanceolate, size range is 3.5–7 x 1.5–2.4 cm, thickness is 0.5–1 mm, apex described as valvate reduplicate, and acuminate
Sepals	Unknown	Sepal is predominantly ovate, size ranges from 2–4 x 2–5 mm, apex can either be acuminate or acute, rarely pubescent.	Sepal is predominantly ovate c. size range is 3–4 x c. 2 mm, apex can be rarely pubescent, and mucronate.	Sepal is predominantly ovate c. size range is 3–4 x c. 2 mm, apex can be rarely pubescent, and mucronate.	Triangular sepal with size ranges from c. 4 x 4–5 mm, apex can be densely pubescent, sparsely tomentose or acuminate

Plant Morphology (LAWG 1999)	<i>Dasymaschalon leilamericanum</i> sp. nov.	<i>Dasymaschalon clusiflorum</i> (Merr.) Merr.	<i>Dasymaschalon filipes</i> (Ridl.) Ban	<i>Dasymaschalon ellipticum</i> Nurmawati	<i>Dasymaschalon blumei</i> Finet & Gagnep
Stamen	Unknown	Stamens 2–4 mm x c. 0.5–1 mm, apex discoid, glandular dots absent.	Stamens unknown	Stamens size ranges from 23 x c. 1 mm, described as convex apex with the presence of dotted glandular	Stamens 4 x 1 mm, apex convex, glandular dots absent
Pedicels	3–4 cm long	Pedicel 1.2–4.2 cm x 1–2 mm, glabrous.	Pedicel 19–33 cm x c. 1 mm, glabrous (bearing carpida).	Pedicel ranges from c. 1 mm x 1.5–2 cm and described as glabrous.	Pedicel rangers from 3.4–15 cm x 1.5–2 mm, predominantly tomentose or densely pubescent, and glabrous
Fruits	Axillary or terminal	Axillary or terminal	Axillary or terminal	Axillary or terminal	Axillary or terminal
Monocarps	30–50, ellipsoid 9–20 x 8–13 mm, pinkish to black color	Ranges from 20–50 and described as globose, with size ranges from 7–17 x 5–8 mm	Ranges from 20–35, ellipsoid, with size ranges from 10–14 x 7–9 mm.	Ranges from 20–30, described as ellipsoidal to sub globose, size ranges from 7.5–10 x 6–7.5 mm	Monocarps 7–30
Stalks	Stalks 13 x 38 mm glabrous	Size ranges 8–21 x 1–2 mm, described as rarely pubescent to glabrous	Size ranges from 8–22 x c. 0.5 mm, described as rarely pubescent.		Rarely pubescent, seldom glabrous, size ranges from 4–15 x 1–2 mm
Seeds	5–7 seeds, ellipsoid, embedded in pulpy tissue. Seed dimension 0.7–0.9 cm x 0.5–0.6 cm.	Granulate with 1 (–2) seeds, rarely pubescent to glabrous	Granulate with 1(–2) seeds, villous apiculum, and rarely pubescent.	1(–2) seeds, slightly smooth, glabrous.	Granulate with 2–7 seeds, described as ellipsoidal but seldom globose on each of its segment, size ranges from 7–9 x 5–8 mm.

Table 3. Maximum likelihood fits of 24 different nucleotide substitution models for the matK genes sequences aligned for *Dasymaschalon leilamericanum* sp. nov.

Model	#Parameter	BIC	AICc	InL	Invariant
T92	77	4397.269592	3755.961766	-1800.758018	n/a
T92+G	78	4388.799433	3749.29861	-1796.420642	n/a
T92+I	78	4389.896853	3750.39603	-1796.969352	0.456754048
T92+G+I	79	4399.004029	3751.310358	-1796.420645	9.76544E-06
HKY	79	4406.668791	3758.97512	-1800.253025	n/a
HKY+G	80	4407.962061	3752.075691	-1795.797364	n/a
HKY+I	80	4409.135406	3753.249036	-1796.384037	0.454509194

Table 4. Maximum Likelihood fits of 24 different nucleotide substitution models for the rbcL genes sequences aligned for *Dasymaschalon leilamericanum* sp. nov.

Model	#Parameter	BIC	AICc	InL	Invariant
K2	64	3048.949444	2546.264332	-1208.914456	n/a
T92	65	3050.818567	2540.285826	-1204.918388	n/a
K2+G	65	3052.503191	2541.97045	-1205.7607	n/a
K2+I	65	3053.728609	2543.195868	-1206.373409	0.45697074
T92+G	66	3054.534347	2536.154189	-1201.845649	n/a
T92+I	66	3055.717657	2537.3375	-1202.437304	0.45697074
K2+G+I	66	3062.2175	2543.837343	-1205.687226	9.24534E-06

Table 5. Multivariate analysis of morphological data *Dasymaschalon leilamericanum* sp. nov..

Plant morphology (LAWG 1999)	SS	df	MS	F	P-value	FCrit
Habit	94.49	4	23.62	45.2	0.00	2.87
Leaf pattern	17935657	4	4483914	4.52	0.00	2.87
2 ^o vein category	189.2	4	47.3	13.75	0.00	2.87
Pedicels	2457.68	4	614.42	19.07	0.00	2.87
Monocarps	2290.24	4	572.56	6.23	0.00	2.87
Stalks	4109.44	4	1027.36	5.58	0.00	2.87
Seeds	96.56	4	24.14	23.21	0.00	2.87

Conservation Status: The new species *Dasymaschalon leilamericanum* is known only in limestone forest of Mount Lantoy along the river, at an elevation of 99 m. Merrill (1923) designated the three *Dasymaschalon* species found in the Philippines (www.philippineplants.org) as endemic to the country, just like this new species. The *Dasymaschalon clusiflorum* (Merr.), described as endemic in the Philippines, was designated as Least Concern (LC) based on the IUCN Redlist (2020–2) (<https://www.iucnredlist.org/search?query=dasymaschalon&searchType=species>).

The morphological comparison between *Dasymaschalon leilamericanum* (a newly described species) and closely related species—*Dasymaschalon clusiflorum* (Merr.), *Dasymaschalon filipes* (Ridl.) Ban., *Dasymaschalon ellipticum* Nurmawati, and *Dasymaschalon blumei* Finet & Gagnep—reveals significant differences across various plant characteristics (Meeran et al. 2023) (Table 5). The p-values for the traits Habit, Leaf pattern, 20 vein category, pedicels, monocarps, stalks, and seeds are all p-value is 0.00, indicating highly significant morphological divergence between *D. leilamericanum* and the other species. These differences suggest *D. leilamericanum* is a distinct species within the genus *Dasymaschalon*, with its own set of ecological adaptations and evolutionary characteristics. The morphological traits that set *D. leilamericanum* apart provide valuable insights into its functional ecology, reproductive success, and evolutionary history, helping to further refine its taxonomic status and improve our understanding of its role within its habitat.

Molecular Analyses

Model Selection for *matK* and *rbcL* genes

Models with the lowest BIC scores (Bayesian Information Criterion) were considered to describe the substitution pattern the best. For each model, the AICc value (Akaike Information Criterion, corrected), the maximum likelihood value (lnL), and the number

of parameters (including branch lengths) are also presented (Nei & Kumar 2000). Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (+G) with five rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+I). Whenever applicable, estimates of gamma shape parameters and/or the estimated fraction of invariant sites were shown. Assumed or estimated values of transition/transversion bias (R) were shown for each model, as well. They were followed by nucleotide frequencies (f) and rates of base substitutions (r) for each nucleotide pair. Relative values of instantaneous r should be considered when evaluating them. For simplicity, the sum of r values is made equal to 1 for each model. For estimating ML values, a tree topology was automatically computed. The analysis involved 39 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 693 positions for *matK* genes and a total of 581 positions for *rbcL* genes in the final dataset (Tables 3 & 4). Evolutionary analyses were conducted in MEGA7 (Kumar et al 2016).

Phylogenetic relationships

The study represents the first molecular analysis of *Dasymaschalon* in Cebu Island, Philippines. As shown in reconstructed phylogenetic tree of the *matK* and *rbcL* gene sequences (Figures 1 & 2), *D. leilamericanum* sp. nov. is a new species molecularly related to *Uvaria* species as with other *Dasymaschalon* species. The position of *D. leilamericanum* sp. nov. in the phylogenetic tree is not unusual. Wang et al. (2012) and Guo et al. (2018) described this non-monophyletic characteristic of *Dasymaschalon*, particularly with regards to cpDNA genes. As also suggested by Guo et al. (2018), the incongruence of *Dasymaschalon* might be a result of hybridization with closely-related genera.

The evolutionary history using the *matK* and *rbcL*

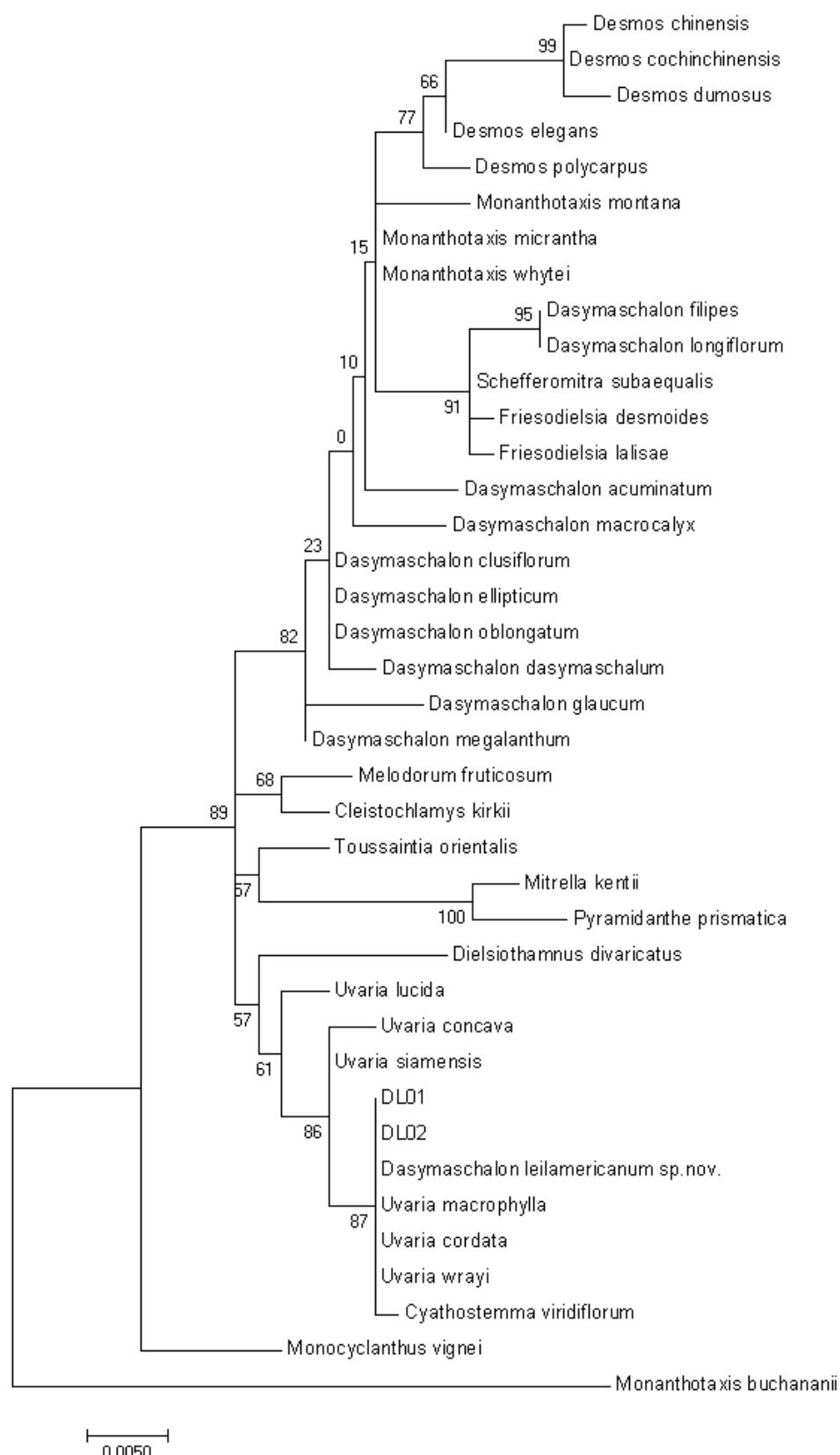


Figure 1. Molecular Phylogenetic analysis of the matK gene sequences aligned for *Dasymaschalon leilamericanum* sp. nov. by maximum likelihood method via Tamura 3-parameter model.

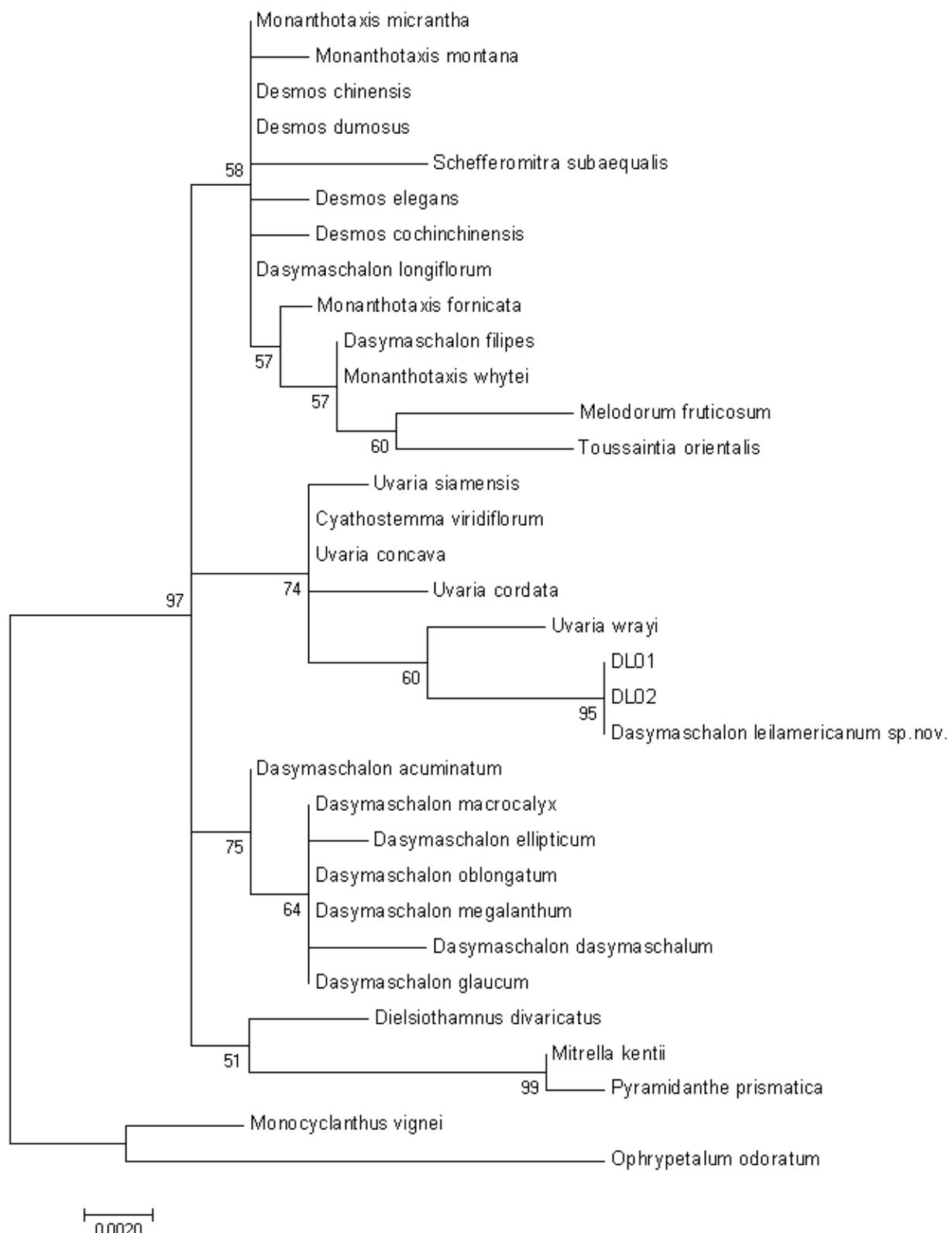


Figure 2. Molecular Phylogenetic analysis of the *rbcL* gene sequences aligned for *Dasymaschalon leilamericanum* sp. nov. by maximum likelihood method via Kimura 2-parameter model.

genes were inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura 1992) (Figures 1 & 2). The tree with the highest log likelihood (-1796.97) for *matK* gene and (-1208.91) for *rbcL* gene were shown (Table 3 & 4). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA7 (Kumar et al 2016).

The phylogenetic analyses of Couvreur et al. (2011) are in agreement with the inferred association of *Dasymaschalon* with another clade, containing species from the genera *Uvaria* and *Cyathostemma*, and the clade made up of species from the genera *Monanthotaxis*, *Melodorum*, and *Desmos*. The majority of *Dasymaschalon* species (*D. macrocalyx*, *D. clusiflorum*, and *D. ellipticum*) belong to a single, monophyletic group that is strongly supported. The *D. leilamericanum* demonstrated non-monophyly as it is more closely related to genus *Uvaria* than to the major clade of *Dasymaschalon* or with the clade of *Desmos* and *Monanthotaxis*.

According to Wang et al. (2012), certain Annonaceae species showed some degree of evolutionary flexibility in their morphological differences. A unique example of evolutionary adaptability may be seen in the pollination chamber of the flower in *Dasymaschalon*. Wang et al. (2012) also noted that some *Dasymaschalon* species (*D. tibetense*, *D. filipes*, and *D. longiforum*) belong to a distinct clade that is closely connected to Asian *Friesodielsia* species. This clade is distinct from the clade that contains the majority of *Dasymaschalon* species. The non-monophyly of the three species indicates a parallel evolution with another lineage where morphological convergence occurs (Zander 2008). The fundamental idea that all species are monophyletic restricts the ability of other species to evolve and their history to be complicated, which could lead to an underestimation of variability within and among closely related taxa (Alström et al. 2011).

CONCLUSION AND RECOMMENDATION

A new species of *Dasymaschalon* (Annonaceae), *Dasymaschalon leilamericanum*, is described and illustrated with diagnostic characters. Several notable morphological characters are the larger laminar size which belong to notophyll category with an area ranges from 2,025–4,500 mm², laminar length : width ratio of 4:1, 2° vein festooned semicraspedodromous, agrophic vein simple, 2° vein spacing irregular, 2° vein angle smoothly decreasing toward the base, inter 2° veins weak intersecondaries, 3° vein category alternate percurrent, 3° vein coarse sinuous, 3° vein angle to 1° obtuse, 3° vein angle variability inconsistent, 4° and 5° vein category regular polygonal reticulate, and leaf areolation five or more sides, Monocarps 50–60, ellipsoid 9–20 x 8–13 mm, pinkish to black color, longer fruit pedicel, and 5–7 ellipsoid seeds/monocarp. The significant differences observed in the plant morphology of *D. leilamericanum* compared to *D. clusiflorum*, *D. filipes*, *D. ellipticum*, and *D. blumei*—as indicated by the p-values of 0.000 across key morphological traits (Habit, leaf pattern, 2° vein category, pedicels, monocarps, stalks, and seeds) suggest that *D. leilamericanum* is a distinct species within the *Dasymaschalon* genus. These differences highlight the species' unique adaptations to specific ecological conditions, reproductive strategies, and evolutionary pathways. Phylogenetic trees of *matK* and *rbcL* genes showed that *D. leilamericanum* did not match to any species under *Dasymaschalon* but it is closer to genus *Uvaria*. Although the *D. leilamericanum* shares some morphological characteristics with other *Dasymaschalon* species, genetically it is closer to another genus thus the non-monophyletic characteristic exhibited in the phylogenetic tree is unexpected. The DNA barcode established in this study can help enrich the literature on molecular data of Philippine flora. The Mt. Lantoy KBA in Argao, Cebu, Philippines, is becoming a hotspot for biodiversity conservation with the addition of new species. It is recommended that future studies on phylogenetic analyses be conducted on other *Dasymaschalon* species found in the Philippines to understand its intergeneric relationships, endemism, and evolutionary history.

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Author details: RAAMAH ROSALES, holds a PhD degree in biology and is the project leader of the study on DNA Barcoding of endemic flora in Cebu Island, Philippines. His focus of research is on biology conservation and ecosystem resiliency. DR. EDGARDO LILLO is a licensed forester who has extensive study on Philippine flora. DR. ARCHIEBALD BALTAZAR MALAKI is the program leader of the flora and fauna assessment in Cebu Island. DR. STEVE MICHAEL ALCAZAR is involved on Cebu Island conservation and passionate about plant-animal interaction. MR. BERNARDO REDOBLADO is a licensed forester and involved in many biodiversity assessment projects. MR. JOHN LOU DIAZ is also a forester and assisted several biodiversity assessment in the island. DR. INOCENCIO BUOT JR. is a highly respected researcher whose expertise include taxonomy, plant biology, and ecosystem management. DR. RICHARD PARILLA is an expert in DNA barcoding and involved in conservation projects on flora and fauna in neighboring islands. DR. JESSICA REY is a widely published researcher with studies on molecular analyses on plant taxonomy, diseases, and gene expression.

Author contributions: RR—project leader, molecular analysis and paper writing; EL—data collection, morphological analysis and paper writing; ABM—guidance in research methodology; SMA—guidance in data collection; BR—specimen collection and morphological analysis; JLD—specimen collection and morphological analysis; IBJr.—guidance in research methodology; RP—molecular analysis and paper writing; JR—DNA extraction, molecular analysis



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Dr. Susan Cheyne, Borneo Nature Foundation International, Palangkaraya, Indonesia
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