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Journal of Plant Taxonomy and Geography (*Webbia*) is a peer-reviewed journal on Plant Taxonomy, Nomenclature, Phylogeny, Phytogeography and Palaeobotany of the Vascular Plants.

The journal aims to allow research in botanical topics such as taxonomy, systematics, nomenclature, molecular phylogeny, conservation, biogeography, and history of botany, and botanical collections.

It was founded in **1905** in Florence by **Ugolino Martelli** (1860-1934), a botanist well known for his studies of and contributions to the systematics of the tropical genus *Pandanus* and on the Flora of Sardinia.

In the 19th century Florence represented one of the most important European centres in Plant Taxonomy and Phytogeography with several notable Italian botanists worth mentioning such as Filippo Parlatore, Teodoro Caruel, Eugenio Baroni, Stefano Sommier, Odoardo Beccari and Ugolino Martelli himself. In 1842 **Filippo Parlatore** (1817-1877) founded in Florence the *Herbarium Centrale Italicum (FI)*, which soon became one of the most important herbaria in the world. Most of the specimens described and/or cited in *Webbia* are still kept in it.

In 1905, and as a consequence of this multitude of activities in Plant Systematics and Phytogeography, Ugolino Martelli established the journal *Webbia-Raccolta di Scritti Botanici*, firstly published annually in a single issue, and later twice a year. Webbia also began to be a place of publication of contributions from Tropical Botany, especially after the Royal Colonial Herbarium founded in 1904 in Rome was moved to Florence in 1914, currently named Tropical Herbarium Study Center (Centro Studi Erbario Tropicale - Herbarium FT) belonging to the Department of Biology of the University of Florence.

Webbia had been created in honor of **Philip Barker Webb** (1793-1845), a close friend of Filippo Parlatore, who before passing away entrusted his personal herbarium and a library rich of old botanical books and publications to the then Botanical Museum in Florence.

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Species diversity and vegetation structure of woody plants in regenerating *Juniperus* forest replacing *Eucalyptus* in the conservation area of Gullele Botanic Garden, Addis Ababa, Ethiopia

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Abstract. The Gullele Botanic Garden (GBG), on the slope of the Entoto hills, adjoins the north-western limit of the city of Addis Ababa, capital of Ethiopia. The forested area of GBG is home to a mixture of semi-natural forest and *Eucalyptus* plantations with *Eucalyptus* having been removed in the recent past. The floristic composition, species diversity and vegetative structure of the woody plant species in the forested area are here presented from 81 plots (20 × 20 m). A total of 104 woody plant species (90% native, the rest planted or naturalised) of 83 genera and 47 families are recorded; a species richness higher than what researchers have found in nearby natural forests, though the definitions of woody species have varied between studies. The number of individuals of the dominant species, *Juniperus procera*, decreases with increasing DBH and height class, which indicates a healthy regeneration inside the GBG; the appearance of seedlings and young plants of *Juniperus* after the removal of *Eucalyptus* is comparable to the regeneration of *Juniperus* forests described from East Africa after forest fires or clear-felling. Clustering analyses on the floristic data result in four partly overlapping clusters, but the distribution of the plots on clusters changes notably if data on *Eucalyptus* is in- or excluded. Many woody species in nearby natural or semi-natural forests, where *Juniperus procera* is dominant, occur also in GBG but plant communities defined for those forests are not identified in the plot data from GBG. The findings in this paper can serve as a baseline dataset to follow the regeneration of the conservation area towards natural forest, useful for future management and sustainable utilization of Ethiopian forest species at local and national levels.

Keywords: Baseline study, Central Ethiopian Plateau, conservation of biodiversity, conservation of vegetation, Dry Afromontane Forest, frequency, *Juniperus procera*, plant community analyses, population structure.

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INTRODUCTION

Ethiopia has a very diverse flora with a high number of endemic species particularly in the Shewa Uplands (SU) floristic region (Demissew et al. 2021). Just north of Addis Ababa is the Entoto mountain chain and the Gullele Botanic Garden (GBG) with remains of previously continuous dry mountain forest. In the central Ethiopian highlands, only fragmented forests remain (Bekele 1993; Darbyshire et al. 2003). The conservation challenges are significant (Egziabher 1989; Shibru and Balcha 2004; Lemenih and Teketay 2004; Asmelash and Rannestad 2022). Efforts to study and restore the forests have been gaining momentum since the late 1980s (Demissew 1988; Bekele 1993; Hylander and Hylander 1995; Reusing 1998; Institute of Biodiversity Conservation 2005; Shibru and Woldu 2006; Woldemariam et al. 2016; Aerts et al. 2016; Kindu et al. 2022; Masresha and Melkamu 2022), and research on forest structure and composition is being encouraged to guide the restauration of forests (Tesfaye et al. 2002). Asmelash and Rannestad (2022) provide a list of forests and associated vegetation studies in the central Ethiopian highlands. The present study of the forested area in the Gullele Botanic Garden (GBG) intends to contribute to such studies and the conservation efforts.

The natural forests around Addis Ababa

The potential natural vegetation of the central highlands of Ethiopia between 1800 and 3000 (upper limit 3400) m a.s.l. was described and mapped by Friis et al. (2010, 2011, 2022). The natural vegetation is characterised by a mosaic of montane woodland and wooded grassland and dry Afromontane forest (referred to as DAF). One of the first scholarly studies of DAF forests near Addis Abeba was of Menagesha Suba Forest (Demissew 1988). A detailed study of forest remnants on the central Ethiopian plateau around Addis Ababa was produced by Bekele (1993) and covered Jibat Forest south of Gedo in the west (a more humid forest with a somewhat different species composition), Chilimo Forest north of Ginchi, Menagesha Forest west of Addis Ababa, and Wof-Washa Forest to the east of Debre Birhan. In the three drier of these forests, *Juniperus procera* occurs as the dominant woody species in three phytosociological communities out of ten (Bekele 1993).

Regeneration of Juniperus forests in East Africa and Ethiopia

Regeneration of forest is important in connection with the conservation efforts of the central Ethiopian plateau (Asmelash and Rannestad 2022). The regeneration of trees in dry Afromontane forest, particularly of *Juniperus procera*, has been studied in East Africa. Gardner (1926) found that the nearly complete destruction of the old *Juniperus procera* forest was necessary before any natural regeneration with that species took place. This was confirmed from both Kenya and Tanzania by Wimbush (1937) and Hall (1984). Hall summarised the typical life cycle for a *Juniperus procera* forest following clear felling or a major forest fire: "*J. excelsa* [= *Juniperus procera*] has been reported to assume dominance at around 20 years, when it reaches a height of about 8 m. The trees reach full height – 30 m or more in favourable sites – by the time they are 80-90 years old [and] are believed to remain the dominant canopy constituent for a further 200-300 years before showing signs of senescence, … allowing more light into the forest and favouring vigorous growth of late-successional broad-leaved species. These replace the dying *J. excelsa* and constitute the climax forest [which] includes *Ekebergia*, *Olea*, *Olinia* and *Rapanea* [*Myrsine*]." Bussmann (2001) confirmed that these observations applied to *Juniperus* forests in both East Africa and southern Ethiopia, and Bussmann and Beck (1995) had previously provided experimental evidence for poor germination of *Juniperus procera* seeds in the dark or when covered by humus.

Lack of regeneration has been observed in the Wof Washa forest in central Ethiopia, where Teketay and Bekele (1995) saw very few *Juniperus procera* seedlings in *Juniperus* dominated forest. They concluded that this species did not regenerate under the canopy of mature parent trees. And this despite the presence of abundant viable seeds of *Juniperus procera* in the soil seed bank of DAF forests (Teketay and Granström, 1995). Wassie (2007) studied the regeneration of Ethiopian church forests and found that the number of germinated seeds of *Juniperus procera* was very low under closed canopy and in canopy gaps.

The DBH classes for *Juniperus procera* from the hill with the Menagesha Amba Mariam forest, between the main Menagesha Suba forest and the GBG indicate a regenerating forest with trees of comparable age (Tilahun et al. 2015). In Tigray a study on the regeneration from the soil seed bank of *Juniperus procera* and *Olea europaea* subsp. *cuspidata* after disturbance in fenced and unfenced areas found that fencing made no change for the regeneration of *Juniperus procera*, while there was a significantly better regeneration of *Olea europaea* subsp. *cuspidata* in the fenced areas (Aynekulu et al. 2009).

General objectives of this study

The objectives of the present study are:

(1) To present an image of the current woody species composition, diversity, and population structure (DBH, height, density, frequency) in the forested area in GBG and to see this in the light of the history of the area.

(2) To provide a preserved baseline dataset with floristic and structural data on the woody species from the GBG conservation area for future monitoring and analyses of the changes in the vegetation.

(3) To illustrate the replacement of *Eucalyptus* plantations with regenerating native forest and to briefly discuss this process in relation to nearby dry Afromontane forests and their phytosociological plant communities.

MATERIALS AND METHODS

History, geographical location, geology and soil of the GBG on the slope of the Entoto hill; establishment and removal of Eucalyptus

It is not customary to study vegetation in managed botanic gardens, but the removal of the *Eucalyptus* trees from the forested area in GBG presents an opportunity to study the succession of the vegetation and compare this with findings from *Juniperus* forests elsewhere. The process was initiated slightly more than 10 years ago, when the GBG was officially established with the signing of a memorandum of understanding between the town authorities and the Addis Ababa University on October 9th 2009, ensuring the allocation of 705 hectares of land for a botanic garden with a conservation area of ca. 621 ha and approximately 100 ha of cultivated garden (Reeder 2013). A legal proclamation on the GBG, No. 18 in the official Addis Negari Gazeta (2009), appeared soon after the signing of the memorandum.

The GBG was established on the site of a mosaic of regenerating forest patches and *Eucalyptus* plantations. The planting of *Eucalyptus* on the slopes of the Entoto hill began soon after the foundation of Addis Ababa in 1885, when Emperor Menelik II in 1895 ordered the planting of *Eucalyptus* imported from Australia to provide wood and fuel for the growing Addis Ababa population (Pohjonen and Pukkala 1990; Pankhurst 2001). In the first years after this introduction, tax relief for land planted with *Eucalyptus* and free distribution of seed rapidly increased planting. During the Italian occupation in 1935-1940, Italian foresters estimated that over 5000 ha around Addis Ababa were covered with *Eucalyptus*. A study of aerial photographs in 1:20,000 allowed Horvath (1968) to draw a map of the *Eucalyptus* plantations, which then extended over 24,500 ha around the city, particularly on Entoto and the western side of the city, and of this area 13,500 ha were densely planted with *Eucalyptus*. Horvath further pointed out that the intensive cultivation of *Eucalyptus* caused serious erosion on the slopes of Entoto, where there was no undergrowth. The major objectives of the GBG, are now conservation, research, education, and ecotourism (Seta and Belay 2021; Borsch and Löhne 2014). Some non-native species have been introduced in the conservation area during tree planting initiatives (Ayele 2020), including the “green legacy programs”.

The outer limits of the GBG boundary are between latitudes 9°03’36”(= 9.060°) and 9°05’42” (= 9.095°) N and between longitudes 38°41’24” (= 38.69°) and 38°44’24” (= 38.740°) E and the altitude range is between 2575 and 2950 m a.s.l. The rock type and soil of GBG (silicics) is described in Agonafir and Worku (2017). The southern half of the GBG is comparatively flat while the northern half is quite mountainous. Two perennial watercourses originate from the mountainous area and flow southwards towards the city centre (Figure 1).

Beginning before 2019 and continuing after the field work for this study, stumps and coppices of *Eucalyptus* were being debarked until the trees were dead, and native trees were allowed to regenerate naturally. Images from different stages in the removal of *Eucalyptus* are presented here. Figure 2 and 3 show views of the upper part of the GBG conservation area before the systematic removal of *Eucalyptus* began. Figure 2 shows the upper boundary road of GBG. Figure 3 shows a view from a prominent rocky outcrop slightly lower down with the line of sight towards Entoto. Figure 4 shows an early stage in the regeneration of the natural vegetation after removal of the *Eucalyptus*. Figure 5 shows a slightly later stage after removal of the *Eucalyptus* with older specimens of *Juniperus procera*.

Previous studies of the vegetation in the GBG and adjacent vegetation on Mt Entoto

An open vegetation in which young regenerating *Juniperus procera* (Debushe 2008) make up 95.16% of the Basal Area (Debushe et al. 2015) is described from Entoto. Data from the *Eucalyptus* plantations with regeneration of natural vegetation on Mt. Entoto were gathered and analysed by Atinafe et al. (2020). By then, Entoto was still dominated by *Eucalyptus* in terms of number of individuals; with 952 individuals/ha for *Eucalyptus globulus*, 369 individuals/ha for *Juniperus procera* and 304 individuals/ha for *Carissa spinarum*.

In a study of the conservation area of GBG (Reeder 2013), it was attempted to provide a baseline dataset

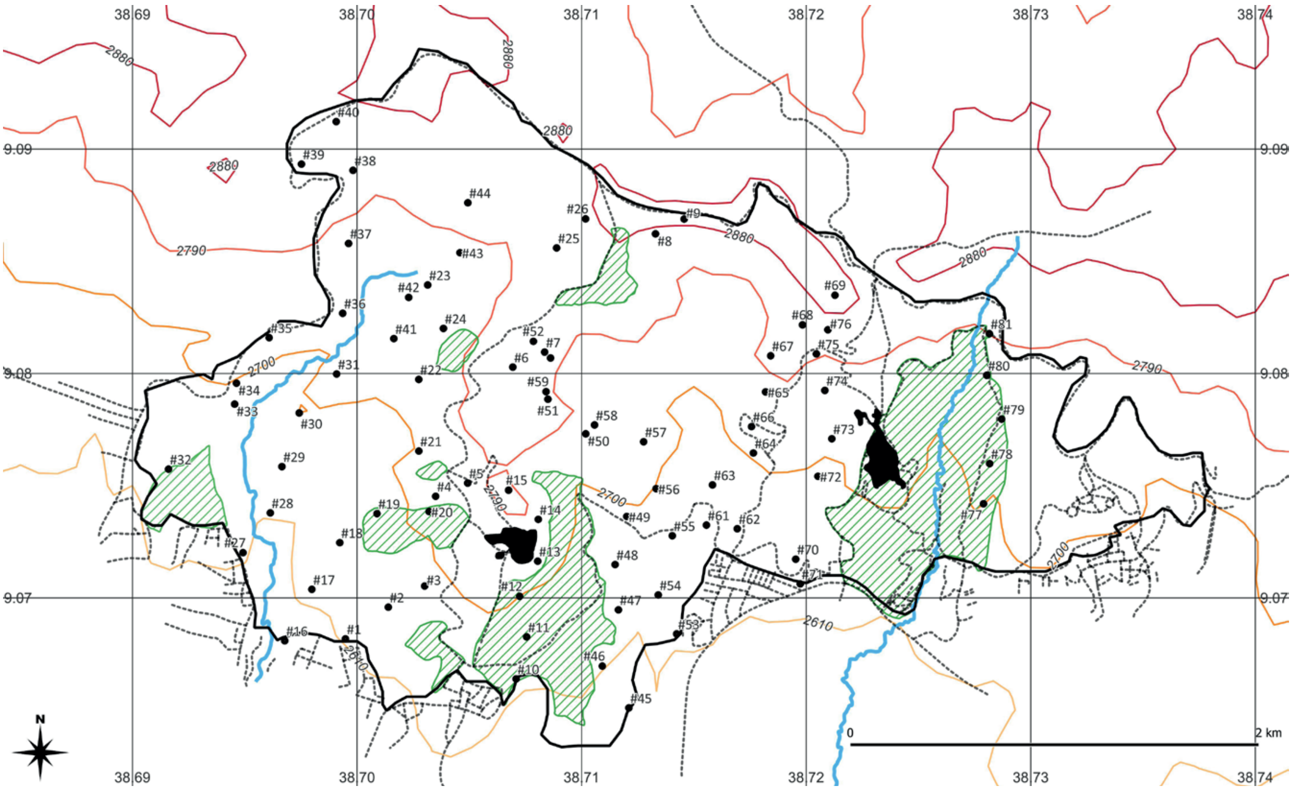

Figure 1. Map of the Gullele Botanic Garden, showing altitude as contour lines (90 m spacing), management areas (green hashed polygons) and the location of the plots (with numbering). The two permanent steams are represented by blue lines; the roads network is represented by dashed lines. The areas in black are the visitor centre in the eastern part of the GBG and the Kalu Beterara Abune Habtemariam Monastery and church in the centre-south-western part. The contour lines are derived from CGIAR-CSI SRTM 90m Digital Elevation Database on https://cgiarcsi.community/data/srtm-90m-digital-elevation-database-v4-1/. The management areas have had work or combination of works carried out in them including terracing, planting and/or enclosure and mulching of seedlings.

Figure 2. Trail along the upper boundary of the GBG with mixture of *Eucalyptus* and indigenous trees before the systematic removal of *Eucalyptus* began. Young plants of *Eucalyptus globulus* with characteristic glaucous leaves are seen in the background to the left of the trail and nearer the foreground to the right. (Ca. 9.0939° N, 38.7028° E; ca. 2900 m a.s.l.). Photo by Ib Friis, 2005.

Figure 3. View towards forests and plantations on the Entoto towards the east from the rocky ledge above 2790 m a.s.l. that divides the upper part of GBG. Photo taken before the removal of *Eucalyptus* began. A few tall trees of *Eucalyptus globulus* can be seen to the left in the foreground and underneath these trees there is prolific regrowth of glaucous resprouting from tree stumps. (Ca. 9.0892° N, 38.7042° E). Photo by Ib Friis, 2005.

Figure 4. An early stage in the regenerating forest in the rocky western part of GBG being reclaimed by *Juniperus procera*. The image was taken some time after cutting and debarking of *Eucalyptus*. Older trees of *Juniperus* are seen in the background surrounding stipes of *Eucalyptus*. [9.0702° N, 38.6999° E; ca. 2650 m a.s.l. Photo by Ergua Atinafe, April 2023].

Figure 5. Later stage of regenerating *Juniperus* forest in an area previously with *Eucalyptus*. In the background the westernmost part of Addis Ababa can be seen. Also visible is the isolated volcanic hill with the Menagesha Amba Mariam Forest, a northern outlier of the much larger Menagesha Suba Forest on Mt. Wuchacha (9.0693° N, 38.7005° E; ca. 2650 m a.s.l.). Photo by Ergua Atinafe, April 2023.

for future research and management practices, but the main focus was on biomass and carbon storage, and floristic analyses or a classification into local community types was not presented. Based on trees with a DBH above 5 cm, *Eucalyptus globulus* (the only *Eucalyptus* species mentioned) accounted for 63% of the number of trees per hectare (with average DBH of 16.6 cm and on average 14.6 m tall) compared to 25.1% of the number of trees per hectare for *Juniperus procera* (with average DBH of 24.9 cm and on average 9.3 m tall) (Reeder 2013). A later study confirmed the finding by Reeder that *Eucalyptus* was the most important taxon for carbon storage in GBG (Woldegerima et al. 2017).

Sampling design

In the present study a total of 81 vegetation plots the size of 20 × 20 m (400 m^2) were positioned across GBG to cover almost the whole extent of the area (Figure 1). Systematic sampling design used to collect vegetation data and topographic variables followed the methods used by previous authors of similar studies (Bogale et al. 2017; Yineger et al. 2008). Altitude and geographical position were recorded for each plot with a Garmin GPSMAP 62. Woody species inside and outside the plots were recorded to produce a complete inventory of the woody plants in GBG. Nomenclature and taxonomy of all species followed the Flora of Ethiopia and Eritrea (with exception of *Osyris lanceolata* Hochst. & Steud.). The identification of the material was confirmed against specimens at the National Herbarium (ETH), Addis Ababa University, and duplicates of herbarium specimens were deposited at ETH, and at the GBG Herbarium. In addition, the presence of different disturbance types (e.g. grazing/browsing, erosion and anthropogenic disturbance) in and around each quadrate were recorded, following Asefa et al. (2015). Data collection took place from October to November 2019 and February to April and June 2020.

Vegetation data collection

The number of individuals per species (of tree, shrub, and woody climber) were counted in each plot. Diameter at Breast Height (DBH) was measured at about 1.3 m from the ground using a tree calliper and recorded if the trees or shrubs had a DBH > 2.5 cm or a height above 3m. Individual trees and shrubs with multiple stems which forked below 1.3 m height were treated as a single individual (Ayanaw and Dalle 2018) by summing up the DBH of the stems; this is particularly relevant in the cases where stumps of *Eucalyptus* have regenerated by producing stubble shoots or suckers. The tree height (from ground level to the top of the crown) was measured using a clinometer.

Structural data analysis

The DBH and height measurements were compiled into an Excel file to calculate the sum DBH per plot, the Basal Area (BA), the number of individuals that had a

DBH and height measurement, plot DBH against height and to calculate the ratio of small sized individuals (< 10 cm) to bigger sized individuals (> 10 cm) for selected species. The BA of the plot and per ha was calculated from the sum of all measured trees (using the DBH as the diameter in assumed circular trunks). The structural data of DBH and height are summarised per plot and per selected species, and these findings were compared to findings by Reeder (2013).

Species and number of individuals per species per plot were compiled into an Excel file in preparation for the clustering and ordination analyses. The frequency (F, the number of quadrats in which the species is encountered in %) of species, including singletons, was calculated from this dataset.

Plant Community Analyses: clustering and ordination

The Excel file with the number of individuals per species was imported into PC-ORD7 (McCune and Mefford 1999; McCune et al. 2002). The resulting plot/species matrix (matrix1) was used to calculate for each plot the number of species; number of individuals; Shannon–Wiener diversity index ($H' = -\Sigma p_i \times (\ln p_i)$, where p_i is the proportion of each species in the sample); Simpson's reciprocal diversity index ($DS = N \times (N-1) / \Sigma ni \times (ni-1)$, where N is the total number of individuals and n is the number of individuals of each species), and Simpson evenness index, calculated by dividing the Shannon diversity index by its maximum value ($=H'/H'_{max}$). A second data matrix (plot/factor matrix; matrix2) was set up with these data in addition to topographical parameters for each plot: altitude; latitude; longitude; and disturbance types recorded in the field. The number of individuals and sum of DBH were added for the dominant species (*Eucalyptus* spp. and *Juniperus procera*).

The clustering for the plant community analysis is based on the number of individuals per species per plot for species occurring in at least three plots. The space conserving linkage method flexible beta (with a beta value of -0.25 and -0.80) was used in combination with Sørensen's compatible distance measure. The high negative value of flexible beta is space expanding, it groups plots more intensely and reduces chaining in relation to the more commonly used group average or nearest neighbour methods (McCune et al. 2002).

Senbeta (2006) stated that in community analysis, a common goal is to detect and describe the value of different species for indicating environmental conditions. An indicator species of a particular group should be faithful to that group. An indicator species analysis (ISA) was carried out in PC-ORD7 (quantitative or binary response, ISA eqn. 1 in Dufrêne & Legendre 1997) on the previously identified clusters. A Monte Carlo test of significance with 4999 permutations was carried out. The average p-values from the Monte Carlo test for different numbers of clusters were used to determine the optimal number of clusters and to identify possible significant indicator species for those clusters.

The non-metric multidimensional scaling (NMS) facility in PC-ORD7 was used to produce ordinations based on a matrix1 with two versions of the same plots, once with and once without data on *Eucalyptus*. The default settings of the autopilot on slow speed and thoroughness with 250 runs on the real data were selected in the setup in PC-ORD7. The stability of the final solution was confirmed by looking for a flat line when plotting stress versus iteration number, and the number of iterations was selected accordingly. The NMS ordination method was preferred since it can handle data that has no known parametrical distribution. We chose the Sørensen distance measure to express floristic dissimilarity, as it gives no weight to species with double absence in plot pairs and because Sørensen distance was used in the clustering. The species included in our ordinations are those present in at least three plots. Species occurring in one or two plots only were considered statistically meaningless.

Vegetation types in nearby natural forests as identified by previous authors were compared to the plots in GBG by running an NMS ordination based on presence/absence data of 32 species. The species data for the different vegetation types were extracted from Table 1 in Bekele (1993) and Table 4 in Beche (2011). The number of axes was set to 2, with 250 runs.

RESULTS AND DISCUSSIONS

Floristic composition and life forms of woody species

In the course of this study, 104 woody plant species belonging to 84 genera and 47 families were recorded in GBG, of which 79 were recorded inside the 81 plots. A species-area curve was constructed in order to see if the plots adequately represented the diversity of the vegetation, and the curve levelled out within the number of plots studied in this paper. The average number of species per plot is 17.4 with a minimum of 4 (plots 19 and 20) and a maximum of 30 species (plot 81). There are 18 species that occur in one plot only. These singletons and species occurring in only two plots were omitted from the ordination and clustering analyses. Appendix 1 provides recorded and derived information about the plots including, altitude, slope, number of species, number of individuals (all, *Juniperus* only, other native species, individuals with a DBH and height measurement), ratio of smaller to bigger individuals, DBH (sum, maximum, for *Juniperus*), BA, height of the tallest tree, diversity indices and disturbances recorded in the field. Appendix 2 lists the species recorded in GBG and provides additional information derived from the data collected for this study or found in the literature. The values obtained for the Simpson Diversity index are shown in Figure 6 – the most diverse plots are located in the north western part of the garden where the old *Eucalyptus* stands had not yet been destroyed when this study was made. The life form distribution of the species is 38 trees (36.54 %), 50 shrubs (48.1 %), 10 tree/shrubs (9.6 %), and 6 climbers (5.77 %). Eleven species are endemic to Ethiopia, and 14 are non-native species that have been introduced to Ethiopia. Fabaceae and Asteraceae are the most dominant families with 11 and 10 species respectively, followed by Myrtaceae and Rosaceae with 6 and 5 species respectively. One *Acacia*, one *Helichrysum* and one *Indigofera* species could not be determined to species level. There may be a doubt about the identity of the *Pittosporum* species in GBG, but it is either *P. abyssinicum* Del. or *P. viridiflorum* Sims (See Appendix 2).

Table 1. Number of native woody species from forests on the highland plateau. The species lists provided in the studies were reassessed to follow the same concept for woody species. Species considered as woody by one author may not have been recorded by a different author. Climbers are only counted if they are shared with GBG.

Forests and studies	Shrubs and/or trees	Climbers shared with GBG	Cumulative total	Shared with the 87 species in GBG
Wof Washa			**126**	**68**
Bekele 1993	28	0	28	21
Teketay & Bekele 1995	54	4	58	37
Fisaha et al. 2013	57	0	57	40
Tilahun 2018	90	7	97	61
Yirga et al. 2019	72	4	76	47
Menagesha			**113**	**71**
Demissew 1988	47	2	49	39
Bekele 1993	30	0	30	27
Fetene et al. 2010	72	8	80	58
Beche 2011	80	5	85	57
Tilahun et al. 2015	32	0	32	30
Chilimo			**81**	**57**
Bekele 1993	28	0	28	24
Soromessa & Kelbessa 2014	71	6	77	55
Siraj & Zhang 2018	40	1	41	31
Entoto			**69**	**61**
Debushe et al. 2015	30	4	34	30
Atinafe et al. 2020	58	9	67	60

GBG shares many of its native woody species with nearby natural and semi-natural forests (Table 1). On one hand, the percentage of species that nearby regenerating or mature forests share with GBG are in decreasing order: 88% for Entoto (61 out of 69 species), 70 % for Chilimo, 63% for Menagesha (both Suba and Amba Mariam) and 54% for Wof Washa. On the other hand GBG shares 82% of its species with Menagesha (71 out of 87), 78% with Wof Washa, 70% with Entoto and 65% with Chilimo.

Density of woody species

The plant species in GBG with the highest density is *Juniperus procera* (with 405.9 individuals/ha) and the least dense species for which DBH data was recorded are the singletons (with less than one individual/ha). The ten woody species with the highest abundance of individuals based on the floristic analysis data and in order of decreasing density are: *Juniperus procera*, *Eucalyptus globulus*, *Rosa abyssinica*, *Eucalyptus camaldulensis*, *Olinia rochetiana*, *Myrsine africana*, *Vernonia leopoldii*, *Sideroxylon oxyacanthum*, *Smilax aspera* and *Carissa spinarum*. See Appendix 2 for the density of the species.

Frequency

The frequency gives an approximate indication of the homogeneity and heterogeneity of a forest stand. A high number of species with high frequency values and few species with low frequency values show homogeneity in forest composition (Ayanaw and Dalle 2018; Mahajan and Fatima 2017). Conversely, a low number of species with high frequency values shows heterogeneity. Our data shows a high number of species with low values for frequency (many not-common species; 33 species occur in less than 5% of plots) and relatively few species have a high frequency (few common species; only two species occur in more than 75% of the plots). These results suggest a high degree of floristic heterogeneity in GBG. *Juniperus procera* and *Rosa abyssinica* are the two most frequent species, followed by *Maytenus arbutifolia*, *Myrsine africana*, *Maytenus addat*, and *Olinia rochetiana*. The species with the lowest frequency (1.2%) were often species that according to Friis et al. (2010, 2011: 81-88 & 103) are characteristic of forest margins of both DAF forests and to some extent also margins of Moist evergreen Afromontane Forest (MAF), such as *Calpurnia* *aurea*, *Erythrina brucei*, *Leonotis ocymifolia*, *Phytolacca dodecandra*, *Salix subserrata*, and *Vernonia adoensis*. See data for frequencies in Appendix 2.

Diameter at Breast Height (DBH), size-structure and Basal Area (BA)

The sum of DBH measurements is presented per plot in Appendix 1, and per species in Appendix 2. The sum of DBH per plot for all species and the sum of DBH of the *Eucalyptus* per plot is shown in the map in Figure 6. The population structure of *Juniperus procera*, *Eucalyptus* and 34 native woody species in all plots in the GBG is presented in Figure 7 in terms of the number of individuals in different DBH classes. A high number of individuals was observed in the lowest DBH class (≤10 cm) with consecutive reduction towards the highest DBH class for both *Juniperus procera*, for *Eucalyptus* and for the group of 34 native species. This trend with many young and few old and large trees depicts healthy populations with good recruitment (Wassie et al. 2005; Siraj and Zhang 2018). Some species have a naturally low DBH and do not grow further once they reach the maximum limit of trunk-size for the species, this is for example the case with *Maytenus arbutifolia*, *Olinia rochetiana* and *Rosa abyssinica*. The results presented by Reeder (2013) based on data collected 10 years ago show a different trend in the distribution of DBH classes for *Juniperus procera*, *Eucalyptus* (only one species of *Eucalyptus* mentioned) and 12 other native species. Reeder reports more individuals per unit area in the 10.1-20 cm DBH class than in the ≤10 cm DBH class for *Juniperus procera* and *Eucalyptus*. It is difficult to assess if this is due to a different sampling protocol or reflects real changes in the population structure of these species. Reeder may have started to measure individuals from 5 cm in diameter and above (not from 2.5 cm) and he did not study the more disturbed parts of the forest where young specimens of *Juniperus procera* are most prominent (the present study covers nearly all parts of GBG). The predominance of small sized individuals in plots can be due to

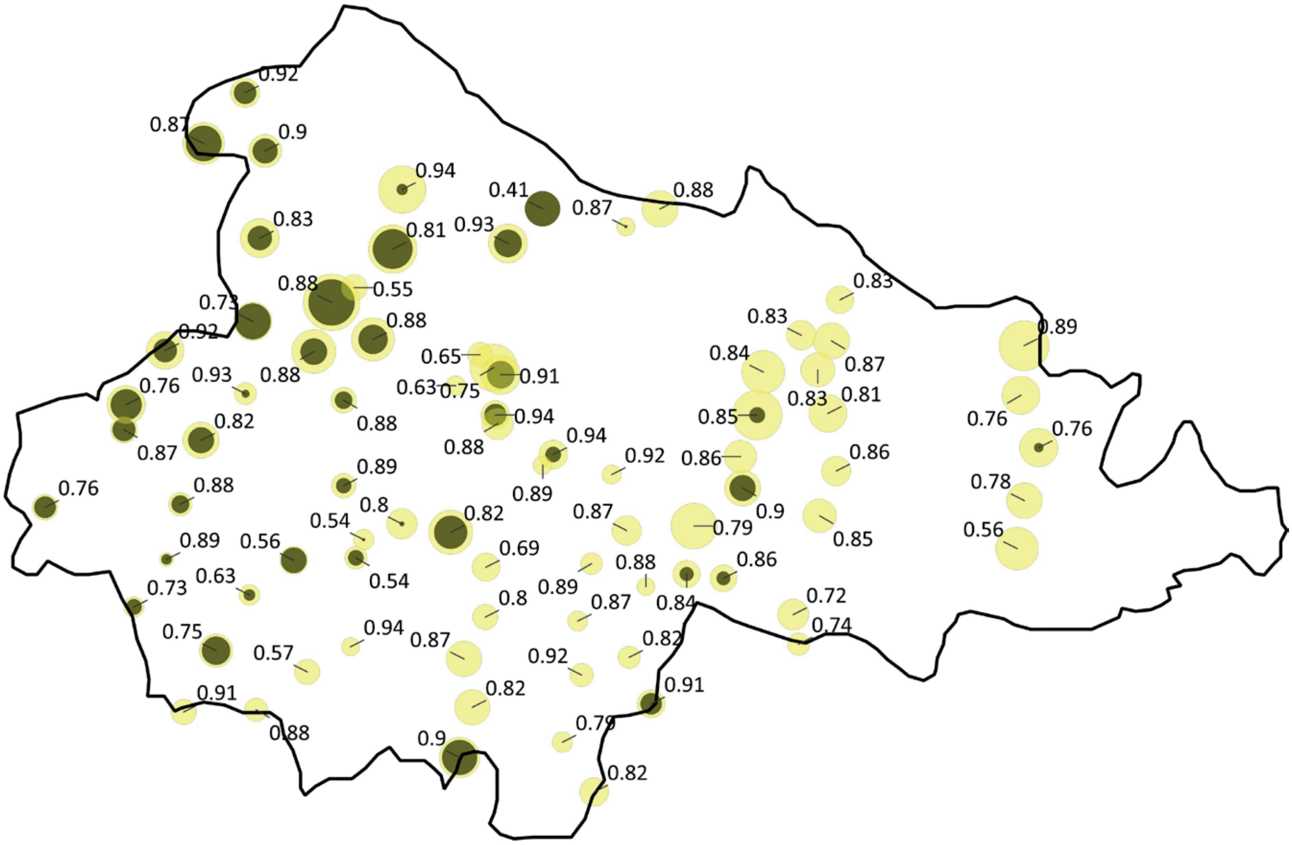

Figure 6. Map showing DBH and the Simpson Diversity index per plot (numbers next to the plots). The size of the plot symbol is relative to the total DBH of all species measured in the plot (open yellow circles) and of the two species of *Eucalyptus* in the plot (filled darker circles). Some plots in the eastern part of the GBG already had all *Eucalyptus* removed at the time of data collection.

recruitment of individuals from the soil seed bank or to the removal of bigger trees in the past (Siraj and Zhang 2018). More native woody species were recorded during this study than were recorded by Reeder (2013) and by Woldegermia et al. (2017) (see Appendix 2), but it is difficult to say if there has been a progressive addition of species to the study area or if species have been missed in previous studies.

Basal Area (BA) per plot for trees that have DBH > 3 cm is listed in Appendix 1, and the total basal area (BA) of woody plants in GBG is 12.91 m^2/ha, which is much lower compared with what has been reported from natural forests in the highlands, suggesting a large number of young individual trees. In the central Ethiopian plateau Chilimo Gaji forest has a BA of 27.3 m^2/ha, and Menagesha Suba forest has a BA of 32.4 m^2/ha (Bekele 1993). Chilimo Gaji forest is reported to have a BA= 454.52 m^2/ha (Siraj & Zhang 2018), but this seems unlikely, because it is about 17 times as high than the figure given elsewhere (Bekele 1993; Table 10) for individuals with a DBH > 10 cm. The BA/ha is dependent on what size of woody plants is included in the calculation of the BA (all individuals or individuals above a certain DBH) and what part of the forest is studied. The BA in the GBG is greater than that of other ecologically similar areas on Mt Entoto where Debushe et al. (2015) report a BA of 4.9 m^2/ha. The BA of species is thought to provide a better measure of the relative importance of the species than simple stem count (Endris et al. 2017). In this study, BA analysis across individual species revealed that there was high dominance in the woody vegetation by either very few and large individuals or by small and numerous individuals of different species. Differences in growth forms could be important; the shrubby and slender species *Maytenus addat* is quite dense but its contribution to the basal area is minimal, the same result is reported by Ayanaw & Dalle (2018). The following four species make the largest contribution to the basal area: *Juniperus procera* has the largest BA with 8.8 m^2/ha, followed by *Eucalyptus globulus* 2.6 m^2/ha, *Eucalyptus camaldulensis* 0.9 m^2/ha and *Olinia rochetiana* 0.11 m^2/ha. The remaining 35 species have a cumulative BA of 0.48 m^2/ha.

Height class distributions

Woody plant height measurements provide valuable insight into the vertical structure of forest stands (Stepper et al. 2015). Height can be used as an indicator of the age of the forest (Hall 1984; Boz and Maryo 2020). In GBG the density of individuals gradually decreases with increasing height. The individuals measured for height, excluding *Eucalyptus*, count 280 individuals/ha that are up to 9 m tall, 246.3 individuals/ha that are up to 21 m tall and only 5.5 individuals/ha that are over 21 m tall. Similar results were reported from natural forests by Bogale et al. (2017) and Bekele (1993). Plots sampled across the entire GBG show a fair amount of variability in average DBH and height as can be seen in Figure 8, where the average height/DBH ratios are shown for all woody species, *Juniperus procera* native woody species excluding *Juniperus procera* and *Eucalyptus*. Most plots have an average height for *Juniperus procera* that is much lower than the maximum height of 30 m or more indicated by Hall (1984). The variation in DBH and height between the different parts of GBG is almost certainly due to difference in history.

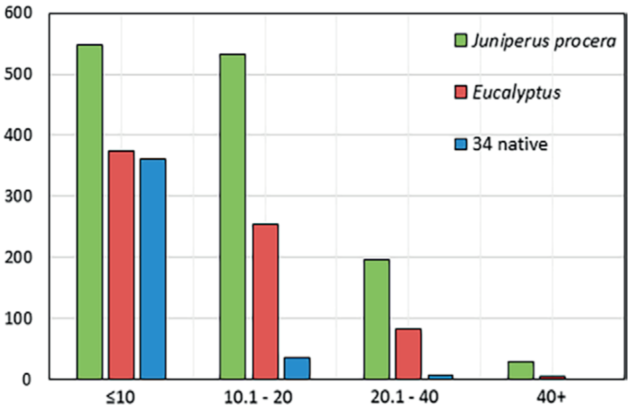

Figure 7. Population structure showing the DBH in cm of *Juniperus procera*, *Eucalyptus* and a group of 34 native species. The y axis represents the number of individuals from all 81 plots (= 3.24 ha).

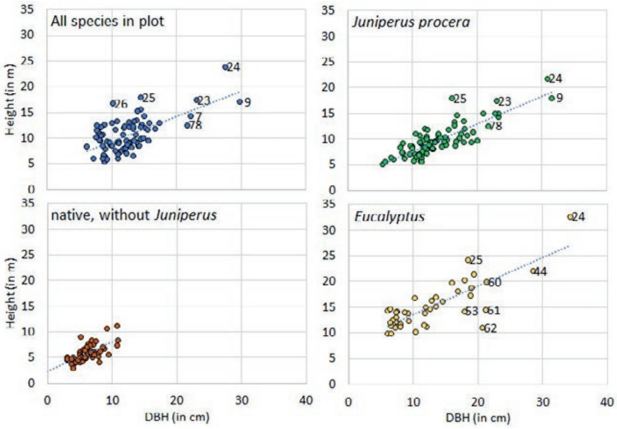

Figure 8. Average height against average DBH by plot. The plot-numbers for plots with high DBH and/or height are given.

Plant Community Analyses: clustering and ordination; influence of removal of Eucalyptus

Plant community analysis is done as a standard procedure in many forest studies. The floristic records from 81 plots in GBG were initially analysed with various clustering and ordination methods and there was notable variation in the resulting clusters. It must be an important part of a community analysis to decide if the clusters reflect natural groups. The clustering method (beta flexible linkage) was selected because this combination gave the lowest amounts of chaining.

None of the species that occur in at least three plots were identified as outliers in the outlier analysis in PC-ORD7. For the study units, plots 19, 26 and 48 were identified as outliers. Clustering including and excluding data from these plots didn't change the overall architecture of the dendrograms, but all three plots featured on very long branches as singleton-sister to a cluster of several other plots.

The best number of high-level clusters was identified by an Indicator Species Analysis (ISA). The lowest average p-value was obtained for four clusters when flexible beta was -0.80 and for 10 cluster when flexible beta was -0.25. The solution with few plots and low average p-values was selected. The four clusters are hierarchically arranged like this: first dichotomy between 1 and 2; second dichotomy between 1.1 and 1.2, and third dichotomy between1.1a and 1.1b.

The ISA yields values for relative frequency, relative abundance, and significance (p-value in the Monte Carlo test) for all the species used in the analysis. Although some species scored as significant in the Monte-Carlo test, none of them were specific to a particular cluster. This suggests that the woody vegetation in the plots across GBG is heterogeneous and without well-defined plant communities, and we have therefore not named the clusters 1.1a, 1.1b, 1.2 and 2 with plant names derived from Indicator Species. Figure 9 shows that in some parts of the study area some plots belonging to the same cluster are found in proximity to each other, for example a row of plots belonging to cluster 1.2 (plots 17-20) in the southwestern part of the GBG and the plots around the visitors' centre (plots 65-81) belonging to cluster 2, but there is no consistent pattern.

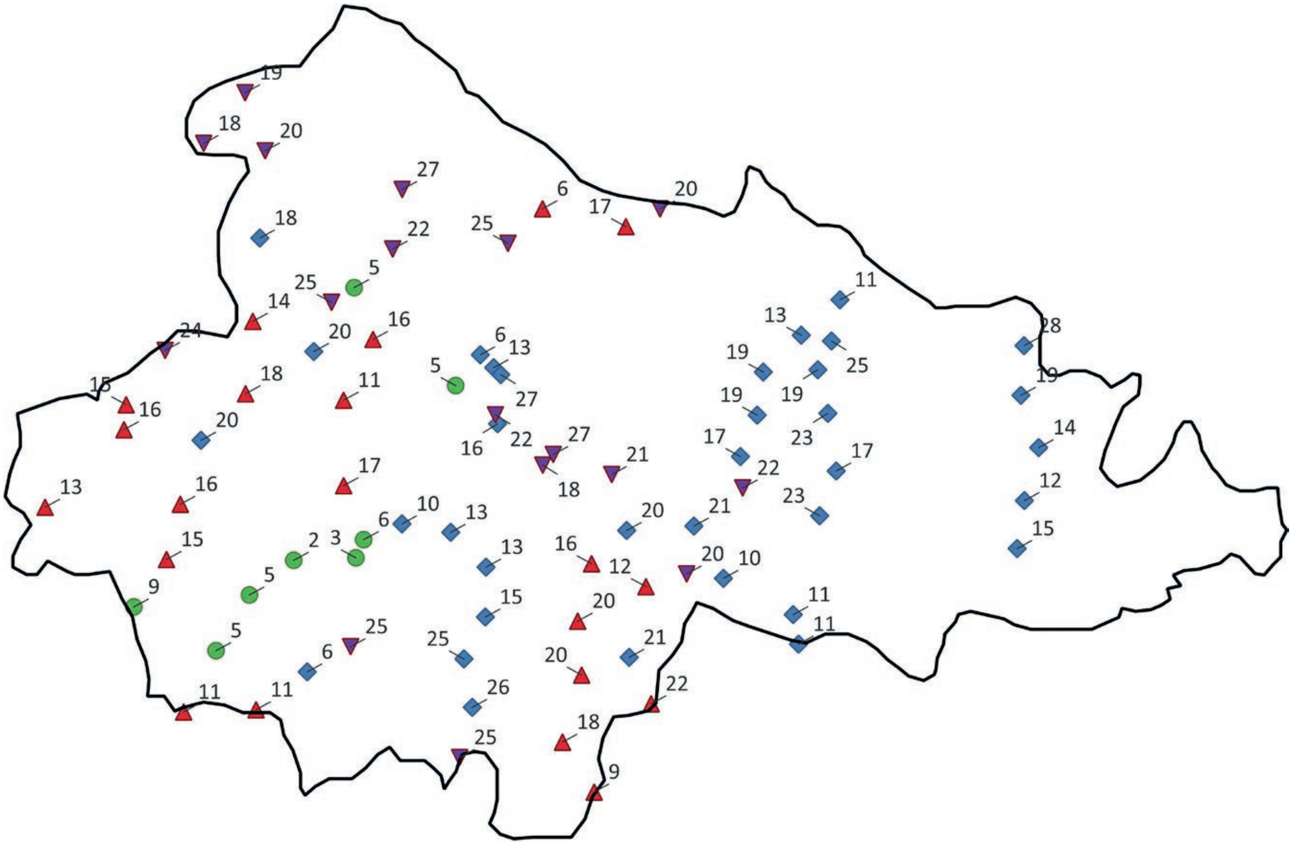

Figure 9. Map with assignment of the plots to the four clusters in our analysis (red triangles to cluster 1.1a, purple inverse triangles to cluster 1.1b, dark green circles to cluster 1.2, dark blue diamonds to cluster 2). The figures at each plot indicate the number of species that were used in the clustering and ordination (only species present in at least three plots and without data for *Eucalyptus*).

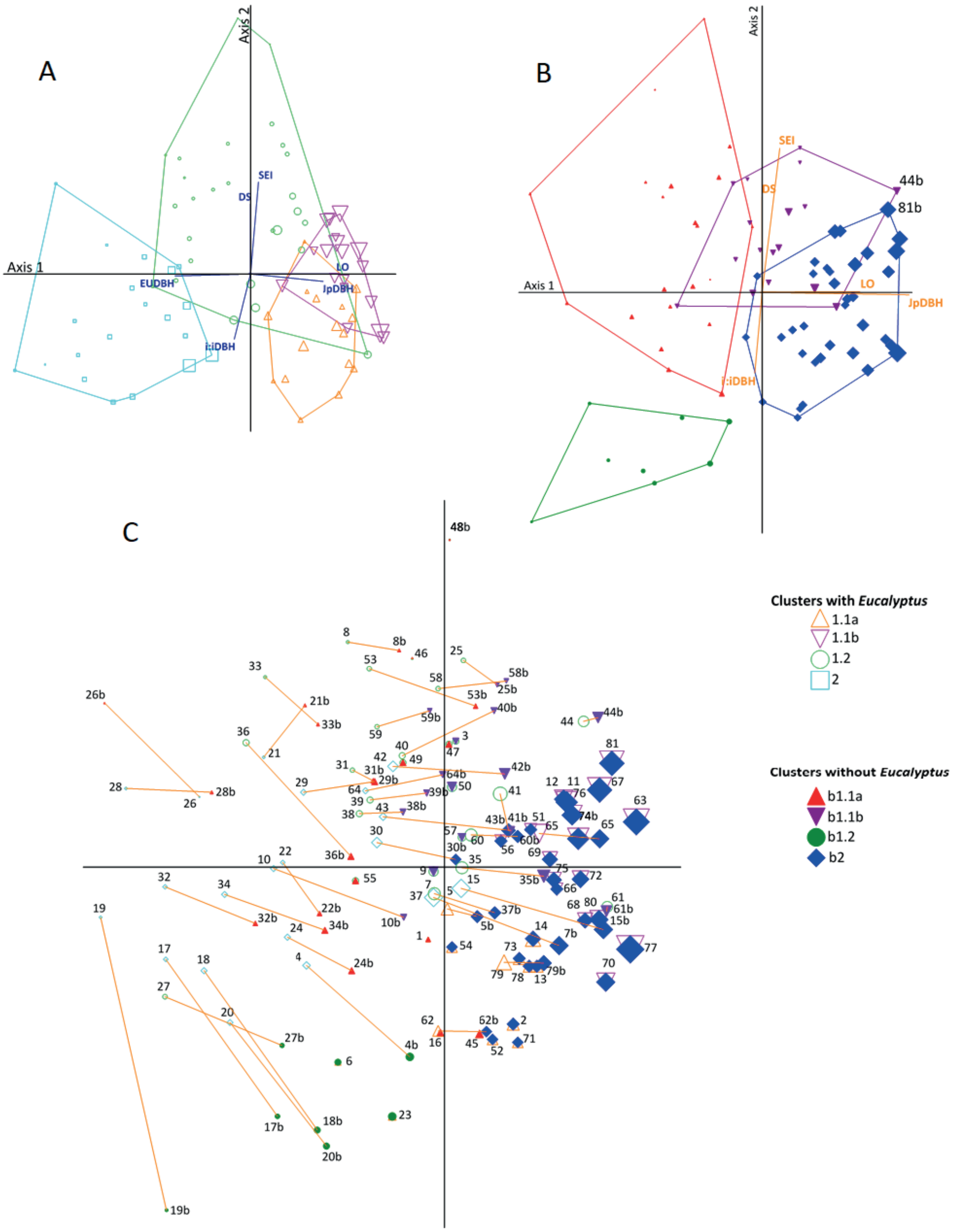

Figure 10. NMS ordination graphs based on numbers of individuals of species present in three plots or more. The points represent plots, the symbols indicate assignment to clusters (with convex hulls around the clusters in A and B). The size of the symbols is proportional to the number of individuals of *Juniperus procera* present in the plots. The axes are rescaled proportionate to the axis with the maximum range, and axis 1 has been rotated to be parallel with the vector longitude (LO). The vector EUDBH represents the sum DBH from *Eucalyptus* spp.; other vector abbreviations are explained in Appendix 1 (not all vectors with a cutoff r^2 value of 0.200 are shown). 10-A: Plot data including *Eucalyptus globulus* and *Eucalyptus camaldulensis*. The final stress for 2-dimensional solution is 23.179, and for 3-dimensional solution: 16.368. The average initial stress is 34.6118. MEASURES OF FIT R^2n (nonmetric fit): 0.9732. 10-B: Plot data excluding all records of *Eucalyptus*. The final stress for 2-dimensional solution is 20.629, and for 3-dimensional solution: 14.976. The average initial stress is 34.9922. MEASURES OF FIT R^2n (nonmetric fit): 0.9776. 10-C: Change in NMS ordination graph caused by the removal of Eucalyptus data. The plots are represented twice in the ordination: plots with numbers only represent all species data, plots with numbers marked with "b" represent the plots without data for *Eucalyptus*. Note that some plots, mainly to the right in the graph, were without *Eucalyptus* when the study was made and are only represented with plots with "b" numbers. For the other plots, the orange lines represent "pseudo-successional vectors" between the plots with *Eucalyptus* and those with the data on *Eucalyptus* removed. The initial stress in the real data is 38.502. The final stress for 2-dimensional solution is 24.100 and for 3-dimensional solution: 14.976. The average initial stress is 34.9581. MEASURES OF FIT R^2n (nonmetric fit): 0.9712.

An NMS calculation yields a graph of the cloud of plot points in 2 or 3 dimensions. The results in PC-ORD7 (autopilot settings) consistently recommend a three-dimensional representation. For the sake of brevity, only axes 1 and 2 are shown here (Figure 10A-C). The NMS point cloud clusters relatively uniformly around its centroid for the graph with *Eucalyptus*, the graph without *Eucalyptus* and the combined graph showing both versions of the plots when the *Eucalyptus* data was removed from the data. Given the results from the ISA a strong division of the cloud is not expected. *Juniperus procera* is particularly prominent in cluster b1.1b and cluster b2 (Figure 10-A and 10-B respectively). The comparison of the cluster analysis with the result of the ordination was carried out because in the study of Ethiopian forests cluster analyses are standard procedure, leading to designation of plant communities named from the names of the indicator species. It is relevant to document that in the GBG we did not find clearly defined vegetation types by clustering. The changes to the position of the plots to be expected by the physical removal of *Eucalyptus* across the GBG is illustrated by Figure 10-C.

The plots with high diversity and evenness (DS and SEI vectors in Figure 10A and 10B) may represent the oldest parts of the forest in GBG. The identical left to right direction of the vectors LO and JpDBH suggests higher dominance of *Juniperus procera* in the eastern part of the GBG (Figure 10-B), and the opposite trend is indicated by the direction of vector EUDBH (Figure 10-A). The exclusion of *Eucalyptus* in the dataset provoques a shift of the plots from the left side of the graph to the right (Figure 10-C). High values of the parameter i:iDBH suggest regenerating vegetation with many young plants in the lower strata and fewer in the canopy.

Comparison of GBG clusters and of nearby forest types of Tamrat Bekele and Dinkissa Beche

As indicated in the paragraph on the natural forests around Addis Ababa and in combination with Table 1, many of the species present in GBG are shared with forests nearby. As shown by the community and Indicator Species Analysis, the clusters identified in GBG are deeply influenced by the removal of *Eucalyptus*. We wanted none the less to comment on the similarity of GBG plots and clusters to the community types identified by Bekele (1993) and Beche (2011). The floristic contents of their vegetation types are here compared via an ordination of presence or absence of 32 species shared with GBG plots, as shown in Figure 11. This crude method does not take into account differences in sampling methodology and analysis. A number of GBG plots from different clusters (25, 44 and others) have a species composition similar, but not identical to that of vegetation type D3 described by Tamrat Bekele (*Erica arborea-Myrica salicifolia*). These are from the upper limit of the Afromontane forests, with a well-defined layer formed by *Erica arborea* up to 8 m and a discontinuous, presumably emergent layer formed by *Juniperus*). Plot 35 is closest to vegetation type C3 – *Dovyalis abyssinica-Myrsine africana* described by Dinkissa Beche. It is notable that the vegetation types of Tamrat Bekele have a species assemblage different from those described by Dinkissa Beche (though this may be due to incomplete data used in this analysis). D8 (*Juniperus procera-Maytenus arbutifolia-Peucedanum winkleri*) with plots from Wof-Washa is most similar to C2 *Sideroxylon oxyacanthum-Dovyalis verrucosa*) from Menagesha. The convex hull surrounding Tamrat Bekele's communities has only marginal overlap with the hulls surrounding the GBG clusters from the present study and there is no overlap of Dinkissa Beche communities with GBG plots. This agrees with our previous observation that the clusters found in the GBG data do not represent natural plant communities.

CONCLUSIONS AND RECOMMENDATIONS

Our findings on the distribution of DBH classes and height of trees suggest that *Juniperus procera* is recruiting well following the removal of *Eucalyptus* and that the recruitment appears to be similar to that happening after clear-felling or forest fires described in studies in East Africa (Gardner 1926; Wimbush 1937; Hall 1984; Bussmann 2001). Although some plots have trees of *Juniperus procera* that are more than 20 m high, very many *Juniperus* trees are currently much smaller and will according to the stated growth rates (Hall 1984; Pohjonen and Pukkala 1992) reach 8-10 m around or just before 2030. The diagram for *Juniperus procera* in Figure 8 shows an almost equal proportion of plots with *Juniperus* trees above and below ca. 10 m high, whereas the average height of other native species is nearly always less than 10 m. The following part of the life cycle of *Juniperus procera* until the trees reach their maximal height will (again according to Hall 1984; Pohjonen and Pukkala 1992) take another ca. 60 years and several hundred years before broad-leaved angiospermous trees gradually take over and more mixed forests with more broad-leaved species appear. If it is possible to find control plots in areas elsewhere on Entoto with a species composition similar to plots in GBG, it might be possible to evaluate the development in GBG better. Future studies would show how quickly more species get added to the

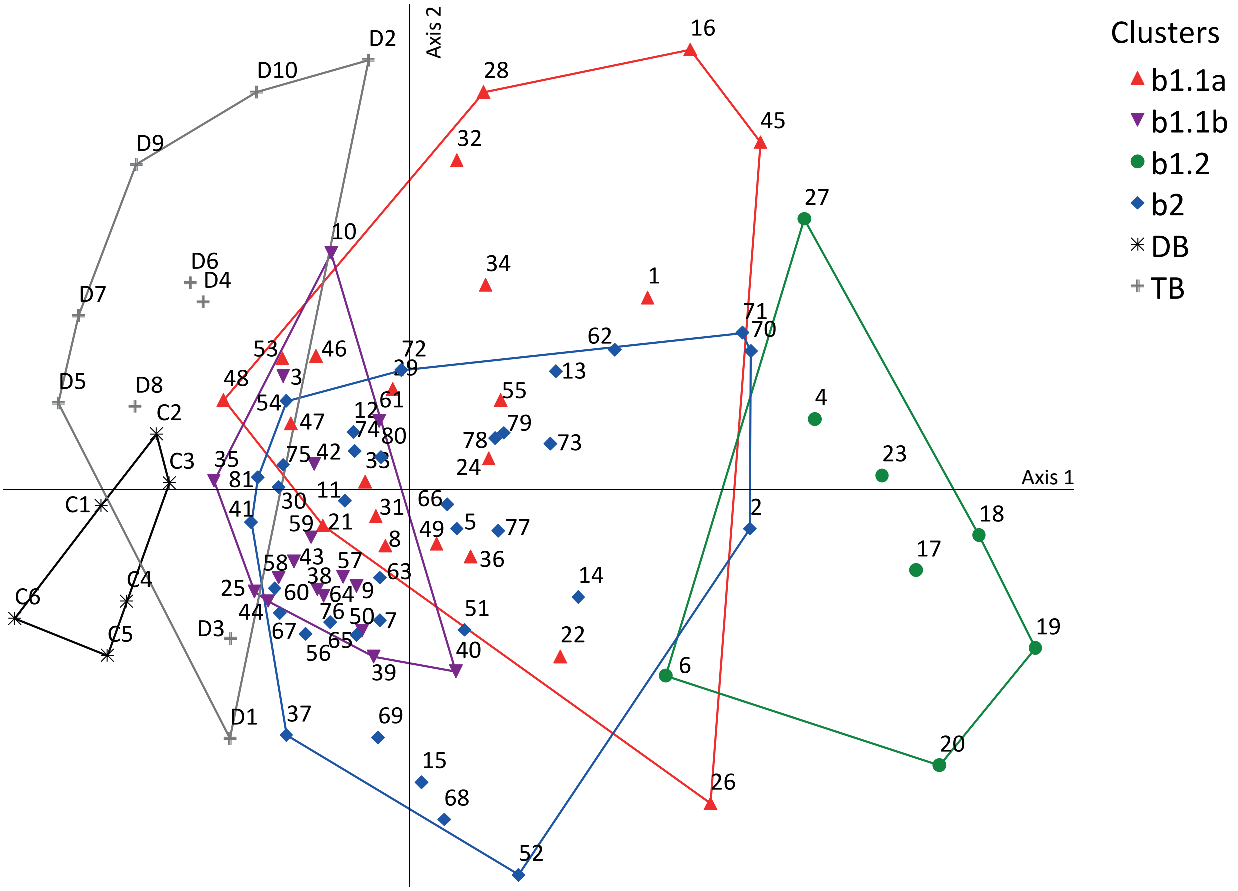

Figure 11. NMS ordination graph of the 81 plots (without *Eucalyptus* data) in the GBG and representative data from nearby natural forests studied by Tamrat Bekele and Dinkissa Beche (see text). The matrix used in the analysis is based on presence/absence data from 32 species. To the left, the grey clusters and the grey hull line represent vegetation types from the studies by Tamrat Bekele (TB = Bekele 1993) and the black clusters and the black hull line represent the vegetation types from Dinkissa Beche (DB = Beche 2011). The final stress for the 2-dimensional solution is 21.8079 from an average initial stress of 42.7674.

floristic mix in the plots that are currently dominated by *Juniperus procera* or if species disappear as more *Juniperus* specimens grow to taller height. It is well known from forests in other tropical countries that the species diversity is usually higher in regenerating forests than in mature forests (Eggeling 1947; Catford et al. 2012).

Currently, the vegetation of GBG is very heterogeneous, probably due to the history with *Eucalyptus* plantations being felled and the reclaiming of cleared areas by *Juniperus* at different times. Attempts in this paper to identify local community types gave the result that groups found by clustering analyses change very notably when the data on *Eucalyptus* are removed, and the clusters found inside the study area are not well defined and different from those found in nearby natural forests. The repositioning of the plots between clusters when *Eucalyptus* is removed, in combination with the life cycle of *Juniperus*, suggests that the conservation area will continue to see notable changes before a more stable equilibrium is reached, but the known life-spans for *Juniperus procera* forests (Hall 1984; Pohjonen and Pukkala 1992) suggest that these changes will take a long time. In order to follow the development in floristic composition, density, frequency, BDH, height class distribution and distribution of the plots on clusters, we suggest that repeat studies like the one documented in this paper be carried out at regular intervals, and with methods and plots closely comparable to those used in this study. Such repeat studies would provide valuable data both on the vegetation and the behaviour of the individual species. For these studied to be useful, it is necessary that the floristic dataset in the Appendices is preserved.

Unfortunately, we have no observations about the original vegetation of the GBG or data about older *Juni-*

perus trees from before the destruction of *Eucalyptus*, but it is known from other sources. when *Eucalyptus* was introduced around Addis Ababa in order to compensate for the destructive cutting of natural fuelwood, and we know that areas with *Eucalyptus* continued to expand until very recently (Horvath 1968). From the available information, we assume it likely that the situation at Gullele and Entoto sometime after 1895 was comparable to clear-felling of the forest, that very little natural forest can have regenerated while the *Eucalyptus* plantations expanded, and therefore most current populations of *Juniperus* on Entoto and at Gullele are much less than 100 years old.

We consider that a few recommendations derived from our practical experience with this project but not directly derived from our plot data are important for the sustainability of the biodiversity in the conservation area of the BGB. This is also in agreement with an established tradition in Ethiopian environmental studies:

(1) Although 70% of the GBG is set aside as a conservation area and basically left to natural regeneration, there are areas where forest management activities are being implemented, as shown in Figure 1. The management measures consist of terracing, planting and/or enclosure and mulching of planted seedlings, and activities necessary for soil conservation on sloping terrain. The management measures should be monitored, and planting of trees should only involve species belonging to the natural forest. In future studies of the development of the vegetation in GBG, special note should be taken of the development in the plots at the managed areas.

(2) Conservation is dependent on ecological, economical and related knowledge, so staff in scientific centres involved in conservation should continue utilizing their field knowledge and experience to conduct research and follow up on previous studies.

(3) As the conservation area inside the GBG is intended for conservation and research on natural forests, the GBG should discourage planting of non-indigenous species inside the conservation area of GBG. Planting during the “green legacy programs” should be done in degraded and unprotected areas around the city.

(4) Awareness on the executive and community levels should be promoted through programs informing about the objectives, goals, ecological and economical value of the garden and the result of research relevant to conservation, including results of studies such as this. In order to raise awareness of the resources in natural forests it is suggested that ethnobotanical studies of the plants in the GBG be carried out to explore the wealth of indigenous knowledge on the diversity of plants, thereby raising awareness of the uses of these plants.

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APPENDIX 1 – INFORMATION ABOUT PLOTS

The altitude was recorded in the field. Slope is derived from a GIS-layer provided by Reeder (2013) **SP** is the number of species and **i** is the number of individuals recorded for the floristic analysis and used to calculate the Shannon-Wiener diversity index (**H'**), the Shannon-Wiener evenness index (**SEI**) and the Simpson's reciprocal diversity index (**DS**). It includes species that occur in 2 or one plot. **iJp**, **inat** and **iDBH** are the numbers of individuals of *Juniperus procera*, of native species other than *Juniperus procera* and individuals with DBH measurements, respectively. The ratio **i:iDBH** is indicative of the number of small individuals in the shrub stratum in relation to number of bigger individuals. **Σ DBH** is the sum DBH of all DBH individuals in the plot. **Max DBH** and **Max H** are measurements of the thickest and tallest tree respectively measured in the plot. Values in brackets of Max H are for *Juniperus procera*, if the tallest species is a different species. **JpDBH** is the sum DBH from *Juniperus procera*. **BA** is the basal area. Erosion **(Er)**, grazing/browsing pressure by wildlife (**Gr/br**) and Anthropogenic factors (**Anthro.**) are types of disturbance recorded during the field work.

Plot	Altitude (m)	Clusters with *Eucalyptus*	Clusters without *Eucalyptus*	Slope	SP	i	iJp	inat	iDBH	i:iDBH	Σ DBH (cm)	Max DBH (cm)	JpDBH	BA	Max H (m)	H'	SEI	DS	Er	Gr/br	Anthro.
1	2611	1.1a	b1.1a	14	11	53	6	17	23	0.43	205.5	18	68	0.17	11 (9)	2.22	0.72	9.37	yes		
2	2644	1.1a	b2	7	6	32	20	0	20	0.63	238	32	238	0.29	20	1.2	0.39	2.43		yes	yes
3	2665	1.2	b1.1b	17	25	73	10	5	15	0.21	134.4	14	95	0.1	10	2.98	0.97	20.69			
4	2713	2	b1.2	4	7	68	13	0	14	0.21	165.4	20	158	0.17	15	1.1	0.36	2.19		yes	yes
5	2756	1.1a	b2	18	11	51	19	1	21	0.41	331	41	312	0.53	19	1.94	0.63	5.52			yes
6	2820	1.1a	b1.2	1	5	18	9	1	10	0.56	151	26	148	0.22	16	1.19	0.39	3		yes	yes
7	2822	2	b2	8	15	102	30	0	32	0.31	711.9	60	697	1.65	19	1.75	0.57	4.2		yes	
8	2858	1.2	b1.1a	23	18	67	4	6	11	0.16	135.3	33	93	0.21	18	2.4	0.78	8.54			
9	2980	1.2	b1.1b	27	21	49	14	1	15	0.31	447	65	442	1.3	26	2.64	0.85	10.32			
10	2623	2	b1.1b	11	29	70	10	1	36	0.51	543	32	112	0.76	29 (8)	1.82	0.59	12.08	yes		yes
11	2650	1.1b	b2	19	26	93	37	11	48	0.52	427.5	24	378.5	0.39	9	2.48	0.8	5.86			
12	2695	1.1b	b2	12	26	100	31	17	48	0.48	432.3	21	347.8	0.38	15	2.61	0.85	8.15	yes		yes
13	2737	1.1a	b2	7	15	50	20	2	22	0.44	238	19	232	0.25	15	2.1	0.68	5.37	yes		
14	2757	1.1a	b2	14	13	49	26	0	26	0.53	287.5	27	287.5	0.36	20	1.79	0.58	3.44	yes		yes
15	2795	2	b2	8	15	99	31	0	68	0.69	633.5	54	249.5	0.77	20	2.05	0.66	5.71	yes		yes
16	2575	1.1a	b1.1a	12	16	69	8	15	29	0.42	238.2	24	43	0.21	20 (6)	2.56	0.83	12.51			yes
17	2575	2	b1.2	9	9	48	7	3	43	0.9	383.1	65	59.75	0.56	26 (12)	1.64	0.53	4.19	yes		
18	2646	2	b1.2	6	7	49	9	1	20	0.41	167.2	19	100.3	0.14	22 (17)	1.27	0.41	2.78			
19	2685	2	b1.2	9	4	37	4	0	35	0.95	280.1	20	34	0.21	17 (10)	1	0.32	2.36	yes		
20	2735	2	b1.2	8	4	27	9	0	25	0.93	193.1	18	90	0.15	20 (15)	0.92	0.3	2.25			
21	2743	2	b1.1a	13	18	57	4	9	28	0.49	225.3	25	84	0.22	20 (16)	2.55	0.83	10.57			
22	2765	2	b1.1a	17	13	50	7	0	24	0.48	242.6	22	118	0.26	22	2.31	0.75	9.8	yes		
23	2780	1.1a	b1.2	7	5	17	11	0	11	0.65	254	36	254	0.51	21	1.12	0.36	2.39		yes	

Plot	Altitude (m)	Clusters with *Eucalyptus*	Clusters without *Eucalyptus*	Slope	SP	i	iJp	inat	iDBH	i:iDBH	Σ DBH (cm)	Max DBH (cm)	JpDBH	BA	Max H (m)	H’	SEI	DS	Er	Gr/br	Anthro.
24	2780	2	b1.1a	7	17	42	9	4	22	0.52	607.4	62	278	1.78	42 (32)	2.44	0.79	10.01			
25	2839	1.2	b1.1b	9	26	96	7	13	35	0.36	509.4	25.5	113	0.7	29 (22)	2.97	0.96	18.1			
26	2887	2	b1.1a	9	7	54	0	0	41	0.76	420.5	20	0	0.4	30 (0)	0.97	0.31	1.73	yes		yes
27	2620	1.2	b1.2	3	11	37	7	2	26	0.7	160.1	13	49	0.09	13 (8)	1.75	0.57	4.06			
28	2618	2	b1.1a	20	16	45	3	0	11	0.24	83.6	16.5	31.5	0.06	14	2.48	0.8	11	yes		
29	2660	2	b1.1a	19	17	72	8	0	27	0.38	207.3	16	77.5	0.14	20 (11)	2.46	0.8	9.4	yes		
30	2702	2	b2	9	21	96	16	1	52	0.54	448.7	21	194.5	0.35	17 (15)	2.31	0.75	5.95			
31	2727	1.2	b1.1a	11	19	53	7	7	19	0.36	186.3	42	120.5	0.27	16	2.74	0.89	17.67	yes		
32	2630	2	b1.1a	15	14	51	6	0	29	0.57	242.7	19	59.5	0.19	24 (12)	1.99	0.64	4.51	yes	yes	yes
33	2671	1.2	b1.1a	10	17	48	3	0	18	0.38	260	23	57.5	0.33	24 (10)	2.44	0.79	8.61	yes	yes	yes
34	2681	2	b1.1a	11	17	63	8	0	41	0.65	493.5	32	110	0.61	22 (18)	2.03	0.66	4.31			
35	2707	1.2	b1.1b	8	26	98	19	1	36	0.37	478	55	244	0.97	28 (20)	2.93	0.95	15.14	yes	yes	yes
36	2739	1.2	b1.1a	8	15	75	8	0	45	0.6	487.5	20	46	0.48	20 (8)	1.92	0.62	3.83	yes	yes	yes
37	2772	1.2	b2	8	19	63	20	6	40	0.63	509.5	27	255	0.65	25 (17)	2.28	0.74	6.47			
38	2809	1.2	b1.1b	12	21	60	9	5	27	0.45	379.2	23	111.2	0.51	25 (20)	2.66	0.86	12.04	yes		
39	2851	1.2	b1.1b	13	19	81	9	8	41	0.51	570.3	37	75	0.82	25 (14)	2.52	0.82	8.71	yes		
40	2874	1.2	b1.1b	11	20	89	9	5	29	0.33	306.5	20	60	0.3	22 (10)	2.77	0.9	15.3			
41	2776	1.2	b2	11	21	90	23	6	47	0.52	627.3	41	322.8	0.91	24 (20)	2.53	0.82	8.69	yes		
42	2775	2	b1.1b	10	27	138	17	5	73	0.53	982.4	40	260.5	1.26	24 (18)	2.68	0.87	8.99	yes	yes	
43	2784	2	b1.1b	7	24	105	13	4	59	0.56	748.5	37	186	0.92	22 (16)	2.37	0.77	5.5			
44	2812	1.2	b1.1b	9	28	133	18	23	43	0.32	717.4	51	409.2	1.28	24 (22)	3.04	0.98	18.56	yes		
45	2579	1.1a	b1.1a	3	10	51	12	7	19	0.37	297.8	35	232	0.49	18	1.97	0.64	6.25	yes		yes
46	2612	1.2	b1.1a	6	19	69	2	17	19	0.28	164	18	36	0.14	12	2.22	0.72	4.95	yes	yes	
47	2621	1.2	b1.1a	11	20	83	9	8	17	0.2	205.5	28.5	161.83	0.27	12	2.68	0.87	13.72	yes		
48	2641	1.2	b1.1a	20	20	76	0	22	22	0.29	159.5	22	0	0.12	9 (0)	2.5	0.81	8.61			
49	2691	1.2	b1.1a	21	16	68	9	12	21	0.31	183	27	105.5	0.19	16	2.42	0.78	9.9			
50	2781	1.2	b1.1b	16	18	58	12	1	13	0.22	142.2	16.5	137.2	0.13	9	2.51	0.81	10.53			
51	2801	1.1b	b2	24	16	65	19	1	20	0.31	348.6	34	341.6	0.6	20	2.48	0.8	9.24			
52	2852	1.1a	b2	1	6	29	16	1	17	0.59	232.2	24	223.7	0.29	18	1.37	0.44	3.05			
53	2619	1.2	b1.1a	12	23	86	7	11	28	0.33	281.9	29	61	0.37	20 (14)	2.74	0.89	12.1			yes
54	2630	1.1a	b2	40	21	41	16	1	17	0.41	192.1	27	184.1	0.21	12	2.37	0.77	6.07	yes		

Plot	Altitude (m)	Clusters with *Eucalyptus*	Clusters without *Eucalyptus*	Slope	SP	i	iJp	inat	iDBH	i:iDBH	Σ DBH (cm)	Max DBH (cm)	JpDBH	BA	Max H (m)	H’	SEI	DS	Er	Gr/br	Anthro.
55	2660	1.2	b1.1a	14	12	36	9	5	14	0.39	129.5	20	109	0.13	10	2.28	0.74	10	yes		
56	2696	1.1b	b2	21	20	67	21	1	22	0.33	311	35	305	0.45	17	2.56	0.83	8.67	yes	yes	yes
57	2734	1.2	b1.1b	16	21	57	11	6	17	0.3	146.5	18	114	0.13	10	2.8	0.91	15.96	yes		
58	2774	1.2	b1.1b	5	28	103	6	14	31	0.3	300.5	33	111	0.33	15 (12)	3.09	1	20.68	yes		yes
59	2789	1.2	b1.1b	27	23	76	6	4	20	0.26	293	26	78	0.43	20 (15)	2.94	0.95	19.93	yes		
60	2806	1.2	b2	14	29	91	19	4	36	0.4	520	34	221	0.77	26 (15)	2.89	0.94	13.08			
61	2630	1.2	b1.1b	11	21	70	17	2	23	0.33	271	26	180	0.35	16 (11)	2.37	0.77	6.94			
62	2655	1.1a	b2	6	14	53	16	0	20	0.38	266	35	183	0.37	18 (10)	2.3	0.74	8.15			
63	2686	1.1b	b2	10	21	101	43	18	61	0.6	674	42	565	0.83	14	2.24	0.72	4.97	yes		
64	2703	2	b1.1b	20	23	82	10	9	40	0.49	443	37	152	0.53	15 (12)	2.72	0.88	10.85			
65	2765	1.1b	b2	19	20	96	30	16	55	0.57	780	46	599	1.47	15 (13)	2.4	0.78	7.3			
66	2732	1.1b	b2	21	17	70	20	10	30	0.43	370	44	333	0.66	13	2.37	0.77	7.84			
67	2802	1.1b	b2	13	19	114	41	12	53	0.46	603	44	542	0.83	13	2.4	0.78	6.54	yes		yes
68	2832	1.1b	b2	13	13	68	24	0	24	0.35	313	45	313	0.46	21	2.2	0.71	6.55			
69	2903	1.1b	b2	3	12	69	25	0	25	0.36	274	26	274	0.31	12	2.13	0.69	6.14	yes		yes
70	2639	1.1b	b2	0	13	63	31	0	31	0.49	340	24	340	0.35	10	1.82	0.59	3.73			
71	2644	1.1a	b2	7	11	34	16	0	16	0.47	194	25	194	0.22	12	1.82	0.59	4.19			yes
72	2686	1.1b	b2	13	23	83	28	2	30	0.36	389.7	44	378.7	0.62	16	2.5	0.81	7.26			yes
73	2703	1.1a	b2	6	17	60	20	0	20	0.33	310.5	43	310.5	0.51	20	2.42	0.78	7.7			
74	2744	1.1b	b2	11	23	84	35	3	38	0.45	481	27	470	0.59	15	2.43	0.79	5.48	yes		yes
75	2767	1.1b	b2	23	19	67	25	4	29	0.43	400	40	373	0.56	20	2.36	0.76	6.39	yes		yes
76	2814	1.1b	b2	12	25	103	33	9	42	0.41	444	31	391	0.51	15	2.68	0.87	8.36	yes		yes
77	2683	1.1b	b2	3	16	72	47	1	48	0.67	596	49	592	0.87	20	1.52	0.49	2.32	yes		yes
78	2707	1.1a	b2	7	12	49	20	0	20	0.41	434	50	434	0.99	22	1.97	0.64	5.03	yes		yes
79	2723	1.1a	b2	10	15	54	25	4	34	0.63	492	54	420	0.89	20	2.02	0.65	4.39	yes		yes
80	2749	1.1b	b2	8	19	64	30	6	36	0.56	485	34	450	0.67	16	2.13	0.69	4.34	yes	yes	yes
81	2772	1.1b	b2	17	30	136	41	12	53	0.39	787	55	717	1.43	22	2.83	0.91	9.29	yes		yes

APPENDIX 2 – INFORMATION ABOUT SPECIES

List of all woody plant species recorded from GBG, with family, growth habit, several metrics from this study, characteristic vegetation type (based on Friis et al. 2010, 2011) and mention if the species has not been reported in previous studies (Reeder 2013; Woldegerima et al. 2017). The nomenclature follows the *Flora of Ethiopia and Eritrea*. Frequency and Density is based on the floristic dataset used in the clustering and ordination analysis. Diameter at breast height (DBH) was measured on individuals with a minimum diameter at breast height of 2.5 cm. Species with individuals too small to be measured for DBH have no value in the DBH column; other values are very small. T = tree, Sh = Shrub, Cl = Climber; * = planted in GBG.

Scientific name and family	Habit	status	Frequency (%)	Density (i/ha)	DBH (m/ha)	Vegetation type	Prior studies
Acacia abyssinica Hochst. ex Benth. (Fabaceae)	T	native	35.8	22.8	1.1	DAF; MAF	
Acacia decurrens (J.C.Wendl.) Willd. (Fabaceae)	T	introduced	1.2	1.5	0.1		absent
Acacia melanoxylon R.Br. (Fabaceae)	T	introduced	2.5	1.9	0.2		absent
Acacia negrii Pic.Serm. (Fabaceae)	T	endemic	1.2	0.6	0.0	DAF	absent
Acacia saligna (Labill.) H.L.Wendl. (Fabaceae)	T	introduced	1.2	0.3	0.0		absent
Acacia sp. (Fabaceae)	T		recorded outside plots				
Albizia schimperiana Oliv. (Fabaceae)	T	native	1.2	1.2		DAF; MAF; TRF	absent
Allophylus abyssinicus (Hochst.) Radlk. (Sapindaceae)	T	native	recorded outside plots			DAF; MAF; RV	absent
Apodytes dimidiata E.Mey. ex Arn. (Icacinaceae)	T	native	9.9	8.0	0.3	DAF; MAF; RV	
Arundinaria alpina K.Schum. (Poaceae)	T	native*	recorded outside plots			DAF; EB; MAF	absent
Arundo donax L. (Poaceae)	Sh	introduced	recorded outside plots				absent
Asparagus africanus Lam. (Asparagaceae)	Sh	native	69.1	42.0		ACB; DAF; EB	
Azadirachta indica A.Juss. (Meliaceae)	T	introduced	recorded outside plots				absent
Bersama abyssinica Fresen. (Melianthaceae)	T	native	19.8	11.1	0.2	DAF; MAF; RV	
Buddleja polystachya Fresen. (Loganiaceae)	Sh	native	4.9	2.5		DAF; EB	
Callistemon citrinus (Curtis) Skeels (Myrtaceae)	T	introduced	recorded outside plots				absent
Calpurnia aurea (Aiton) Benth. (Fabaceae)	Sh	native	1.2	1.9		DAF; MAF	absent
Carissa spinarum L. (Apocynaceae)	Sh	native	48.1	42.3	0.1	ACB; DAF; MAF; RV	
Clematis simensis Fresen. (Ranunculaceae)	Cl	native	4.9	1.9		DAF; EB; MAF	
Clerodendrum myricoides (Hochst.) R.Br. ex Vatke (Lamiaceae)	Sh	native	2.5	1.2		CTW; DAF	absent
Clutia abyssinica Jaub. & Spach (Euphorbiaceae)	Sh	native	4.9	3.1		DAF	
Coffea arabica L. (Rubiaceae)	T	native*	recorded outside plots			MAF; TRF	absent
Conyza pyrrhopappa Sch.Bip. ex A.Rich. (Asteraceae)	Sh	native	16.0	4.0		ACB; DAF	absent
Crotalaria exaltata Polhill (Fabaceae)	Sh	endemic	recorded outside plots			DAF; EB	absent
Cupressus lusitanica Mill. (Cupressaceae)	T	introduced	2.5	1.2	0.0		
Discopodium penninervium Hochst. (Solanaceae)	Sh	native	7.4	8.6		AA; DAF; EB	
Dodonaea angustifolia L.f. (Sapindaceae)	Sh	native	recorded outside plots			DAF	absent
Dombeya torrida (J.F.Gmel.) Bamps (Sterculiaceae)	T	native	3.7	2.5	0.0	DAF; MAF	absent
Dovyalis abyssinica (A.Rich.) Warb. (Flacourtiaceae)	Sh	native	54.3	16.4	0.1	DAF; RV	
Dovyalis caffra (Hook.f. & Harv.) Warb. (Flacourtiaceae)	Sh	introduced	1.2	0.3			absent
Dovyalis verrucosa (Hochst.) Lign. & Bey (Flacourtiaceae)	Sh	native	38.3	15.4	0.0	DAF; EB	absent
*Dracaena steudner*i Engl. (Dracaenaceae)	T	native*	recorded outside plots			DAF; MAF; TRF	absent
Echinops longisetus A.Rich. (Asteraceae)	Sh	endemic*	recorded outside plots			AA; DAF; EB	absent
Ekebergia capensis Sparrm. (Meliaceae)	T	native	9.9	4.0	0.2	DAF; MAF	
Embelia schimperi Vatke (Myrsinaceae)	Cl	native	recorded outside plots			DAF; RV	
Erica arborea L. (Ericaceae)	Sh	native	32.1	24.1	0.0	AA; DAF; EB	
Erythrina brucei Schweinf. (Fabaceae)	T	endemic	1.2	1.2	0.0	DAF; MAF	
Eucalyptus camaldulensis Dehnh. (Myrtaceae)	T	introduced	19.8	70.1	6.9		absent
Eucalyptus citriodora Hook. (Myrtaceae)	T	introduced	recorded outside plots				absent
Eucalyptus globulus Labill. (Myrtaceae)	T	introduced	42.0	196.3	19.9		
Euphorbia abyssinica J.F.Gmel. (Euphorbiaceae)	T	native	recorded outside plots			DAF	absent
Ficus exasperata Vahl (Moraceae)	Sh	native*	recorded outside plots			RV; TRF	absent

Scientific name and family	Habit	status	Frequency (%)	Density (i/ha)	DBH (m/ha)	Vegetation type	Prior studies
Ficus sur Forssk. (Moraceae)	T	native*	1.2	0.3	0.1	DAF; MAF; RV; TRF	absent
Ficus vasta Forssk. (Moraceae)	T	native*	recorded outside plots			DAF; RV	absent
Galiniera saxifraga (Hochst.) Bridson (Rubiaceae)	Sh	native	21.0	11.7	0.0	DAF; MAF	
Grevillea robusta A.Cunn. ex R.Br. (Proteaceae)	T	introduced	recorded outside plots				absent
Grewia ferruginea Hochst. ex A.Rich. (Tiliaceae)	Sh	native*	recorded outside plots			CTW[1]	absent
Hagenia abyssinica (Bruce) J.F.Gmel. (Rosaceae)	T	native	1.2	0.9	0.1	DAF; EB	
Halleria lucida L. (Scrophulariaceae)	Sh	native	2.5	1.2		DAF; EB	
Helichrysum schimperi (Sch.Bip. ex A.Rich.) Moeser (Asteraceae)	Sh	native	4.9	1.2			
Helichrysum sp. (Asteraceae)	Sh		11.1	2.8			
Hypericum revolutum Vahl (Guttiferae)	Sh/T	native	34.6	32.7		AA; DAF; EB	
Indigofera sp. (Fabaceae)	Sh		1.2	0.3			
Inula confertiflora A.Rich. (Asteraceae)	Sh	endemic	29.6	20.1		AA; DAF; EB	
Jasminum abyssinicum Hochst. ex DC. (Oleaceae)	Cl	native	55.6	32.7		DAF	
Jasminum stans Pax (Oleaceae)	Sh	endemic	46.9	40.7		DAF	
Juniperus procera Hochst. ex Endl. (Cupressaceae)	T	native	100	405.9	56.6	DAF; EB	
Laggera tomentosa (A.Rich.) Sch.Bip. ex Oliv. & Hiern (Asteraceae)	Sh	native	12.3	9.9			
Leonotis ocymifolia (Burm.f.) Iwarsson (Lamiaceae)	Sh	native	1.2	0.3		AA; CTW; DAF; EB	
Lippia adoensis Hochst. ex Walp. (Verbenaceae)	Sh	native	29.6	19.8		DAF	
Maerua aethiopica (Fenzl) Oliv. (Capparidaceae)	Sh	native	recorded outside plots			CTW	absent
Maesa lanceolata Forssk. (Myrsinaceae)	Sh/T	native	40.7	24.7	0.4	DAF; MAF; RV	
Marsdenia abyssinica (Hochst.) Schltr. (Asclepiadaceae)	Cl	native	6.2	1.5			absent
Maytenus addat (Loes.) Sebsebe (Celastraceae)	Sh	endemic	74.1	32.1	0.0	DAF; MAF	
Maytenus arbutifolia (Hochst. ex A.Rich.) R.Wilczek (Celastraceae)	Sh	native	74.1	32.7	0.3	DAF	
Millettia ferruginea (Hochst.) Hochst. ex Baker (Fabaceae)	T	endemic*	1.2	0.3		DAF; MAF; RV	absent
Myrica salicifolia Hochst. ex A.Rich. (Myrtaceae)	T	native	6.2	4.6	0.5		absent
Myrsine africana L. (Myrsinaceae)	Sh	native	71.6	60.2	0.0	AA; DAF; EB	
Myrsine melanophloeos (L.) R.Br. ex Sweet (Myrsinaceae)	Sh/T	native	8.6	6.8	0.1	AA; DAF; EB	
Nuxia congesta R.Br. ex Fresen. (Loganiaceae)	Sh/T	native	49.4	29.9	0.4	DAF; MAF; EB	
Olea europaea subsp. *cuspidata* (Wall. & G.Don) Cif. (Oleaceae)	T	native	53.1	16.4	0.1	DAF	
Olinia rochetiana A.Juss. (Oliniaceae)	Sh/T	native	67.9	63.3	2.0	DAF; EB	
Osyris quadripartita Salzm. ex Decne. (Santalaceae)	Sh	native	49.4	30.6	0.2	DAF	
Pentas schimperi (Hochst.) Wieringa (Rubiaceae)	Sh	native	30.9	20.7		DAF; EB; MAF	
Phoenix reclinata Jacq. (Arecaceae)	T	native	recorded outside plots			DAF; MAF; RV; FLV	absent
Phytolacca dodecandra L'Hér. (Phytolaccaceae)	Sh	native	1.2	1.2		DAF	absent
Pinus patula Schiede ex Schltdl. & Cham. (Pinaceae)	T	introduced	recorded outside plots				absent
Pittosporum abyssinicum Delile (Pittosporaceae)	T	native	23.5	10.5		DAF; EB	absent
Pittosporum viridiflorum Sims (Pittosporaceae)	T	native	3.7	0.9	0.3	DAF; MAF	
Podocarpus falcatus (Thunb.) Endl. (Podocarpaceae)	T	native	1.2	0.3		DAF; MAF	absent
Prunus africana (Hook.f.) Kalkman (Rosaceae)	T	native	11.1	3.7	0.1	DAF; MAF	
Rhamnus prinoides L'Hér. (Rhamnaceae)	Sh	native	4.9	1.2		DAF; EB; MAF; RV	
Rhamnus staddo A.Rich. (Rhamnaceae)	Sh	native	37	17.9	0.0	DAF; MAF	
Rhus glutinosa Hochst. ex A.Rich. (Anacardiaceae)	Sh/T	endemic	14.8	8.3	0.0	DAF; EB	
Rhus vulgaris Meikle (Anacardiaceae)	Sh/T	native	6.2	2.5	0.1	ACB; CTW; DAF	
Ricinus communis L. (Euphorbiaceae)	Sh	native	1.2	1.2			absent
Rosa abyssinica R.Br. ex Lindl. (Rosaceae)	Sh	native	88.9	79.3	0.0	DAF; EB	

Scientific name and family	Habit	status	Frequency (%)	Density (i/ha)	DBH (m/ha)	Vegetation type	Prior studies
Rubus apetalus Poir. (Rosaceae)	Sh	native	28.4	19.4		DAF	absent
Rubus steudneri Schweinf. (Rosaceae)	Sh	native	3.7	4.0		DAF	
Rumex nervosus Vahl (Polygonaceae)	Sh	native	recorded outside plots				absent
Salix subserrata Willd. (Salicaceae)	Sh	native*	1.2	1.2		RV	absent
Satureja punctata (Benth.) R.Br. ex Briq. (Lamiaceae)	Sh	native	16	5.2			
Scolopia theifolia Gilg (Flacourtiaceae)	Sh	native	3.7	1.5	0.0	DAF	absent
Sida schimperiana Hochst. ex A.Rich. (Malvaceae)	Sh	native	22.2	5.6			
Sideroxylon oxyacanthum Baill. (Sapotaceae)	Sh/T	native	34.6	53.1	1.2	ACB; DAF	
Smilax aspera L. (Smilacaceae)	Cl	native	51.9	46.0		DAF	
Solanecio gigas (Vatke) C.Jeffrey (Asteraceae)	Sh	endemic	2.5	0.9		DAF; EB; MAF	
Solanum incanum L. (Solanaceae)	Sh	native	recorded outside plots				absent
Syzygium guineense (Willd.) DC. (Myrtaceae)	T	native*	recorded outside plots			DAF; MAF; CTW; RV; FLV	absent
Tacazzea conferta N.E.Br. (Asclepiadaceae)	Cl	native	1.2	0.6		DAF; MAF; RV	absent
Vernonia adoensis Sch.Bip. ex Walp. (Asteraceae)	Sh	native	1.2	0.3			absent
Vernonia amygdalina Delile (Asteraceae)	Sh/T	native	3.7	2.8		DAF; MAF; RV	
Vernonia leopoldii Vatke (Asteraceae)	Sh	endemic	49.4	59.3		DAF	
Washingtonia filifera (Rafarin) H.Wendl. ex de Bary (Arecaceae)	Sh/T	introduced	recorded outside plots				absent

[1] Vegetation type according to Friis et al. (2010, 2011, 2022): **AA** = Afroalpine belt, **DAF** = Dry evergreen Afromontane forest and grassland complex, **EB** = Ericaceous belt, **ACB** = *Acacia-Commiphora* woodland and bushland, **RV** = Riverine vegetation, **CTW** = *Combretum-Terminalia* woodland and wooded grassland, **MAF** = Moist evergreen Afromontane forest, **FLV** = Fresh-water lakes, lake shores, marsh and floodplain vegetation, and **TRF** = Transitional rainforest.

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A new species of *Rigiolepis* (Ericaceae: Vaccinioideae) from the Gayo Plateau, Aceh Province, Indonesia

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Abstract. A new species of Ericaceae from the Gayo Plateau, Aceh Province, Indonesia is described. *Rigiolepis gayoensis* resembles *R. lanceolata* but differs by having shorter leaves, non-protruding basal leaf glands, longer flowering pedicels, a glabrous hypanthium, and a dark red or maroon corolla. A detailed morphological description, ecological notes, conservation status, and photographs are presented. The new species represents the third species of *Rigiolepis* found in the island of Sumatra. A key to the species of *Rigiolepis* in Sumatra is provided.

Keywords: epiphyte, *Rigiolepis*, Sumatra, taxonomy, Vaccinioideae.

INTRODUCTION

Rigiolepis is a genus of Malesian Ericaceae originally described by Hooker (1873). However, Smith (1935) treated some species of *Rigiolepis* as belonging to *Vaccinium*, citing strong resemblance between the two genera. Consequently, as part of Sleumer's (1936) treatment for the Ericaceae of Flora Malesiana, he relegated *Rigiolepis* as a section of *Vaccinium*. Argent (2019) later reinstated *Rigiolepis* as a genus of Malesian Ericaceae through his treatment of *Rigiolepis* and *Vaccinium* of Borneo. As per this treatment, including the more recent discovery of *R. argentii* Mustaqim & Ardi (Mustaqim and Ardi 2021), the number of species recognized for this genus stands at 26. *Rigiolepis* sensu Argent (2019) is characterized as shrubs, usually epiphytic with a sprawling or hanging habit, with a short corolla (up to 4 mm long or rarely slightly longer), anthers opening by long oblique slits, an inferior ovary that is truly 10-locular, and yellowish or orange immature fruits that turn red at maturity. *Rigiolepis* has a center of distribution in the island of Borneo, with some species also found in Peninsular Malaysia, including the Indonesian islands of Java, Sulawesi, and Sumatra (Argent 2019; Mustaqim and Ardi 2021). The island of Sumatra is hitherto known as the westernmost distribution of *Rigiolepis* in Malesia with confirmed occurrence of *R. lanceolata* (Blume) J.J.Sm. and *R. leptantha* (Miq.) J.J.Sm. (Sleumer 1966–1967; Argent 2019; POWO 2023).

During fieldwork in the tropical mountains of Sumatra Island, some unknown specimens of *Rigiolepis* were documented and vouchered from areas in the mid-montane vegetation of the Gayo Plateau, Aceh Province, Indonesia. This region is a component of the northern montane region of Sumatra in Aceh Tengah Regency. The vouchered specimens were examined and compared with those of closely similar species. Relevant literature (e.g., Smith 1935; Sleumer 1936, 1961, 1966–1967; Vander Kloet 2005; Argent 2019; Mustaqim and Ardi 2021) was also consulted. From the initial data and observation of the plant *in situ*, we concluded that this plant is a species new to science.

TAXONOMIC TREATMENT

Rigiolepis gayoensis Mustaqim, M.N.Tamayo & P.W.Fritsch, **sp. nov.** (Figure 1).

Type: Indonesia. Sumatra, Aceh Province, Aceh Tengah Regency, Linge, Kute Robel, Bur Ni Lintang, 4°31’50.7”N 96°50’44.7”E, 1850 m elevation, 17 October 2022, *W.A. Mustaqim 2563* (holotype LGS; isotype MEDA).

Diagnosis

Rigiolepis gayoensis closely resembles *R. lanceolata* (Blume) J.J.Sm. but differs by having shorter leaves [4.6–5.5 cm vs. c. 9.0 cm (or longer)], non-protruding basal leaf glands (vs. protruding), longer flowering pedicels (c. 11 mm vs. 2–3 mm), a glabrous hypanthium (vs. densely hairy), and a dark red or maroon corolla (vs. creamy white).

Description

Epiphytic shrubs, terrestrial, evergreen clambering, subdensely branched, c. 1 m tall, branches weak, flexuose. Roots or stem swollen at base, subglobose, 3–7 cm diam. Young branchlets reddish green, white-hirsutulous, with simple erect trichomes 0.2–0.3 mm long and stipitate trichomes 0.5–0.6 mm long. Mature branchlets grayish brown or gray, glabrescent or with occasional simple erect and stipitate trichomes, 1.0–3.5 mm wide, sparsely lenticellate; perennating buds triangular, 2–3 mm long, with several obscurely overlapping scales, scales with margin entire, minutely ciliolate, apex acuminate. Leaves persistent on older branchlets, laxly crowded, alternate, distichous; petiole white and waxy when young, reddish green at maturity, 2.0–4.0 × 1.0–2.0 mm, with simple erect trichomes same as branchlets and occasional stipitate glands, in cross section abaxially rounded, adaxially flat; leaf blade ovate or lanceolate, convex, larger leaves on each branchlet 4.6–5.5 × 2.2–2.5 cm, coriaceous, adaxial surface glossy green, glabrous, abaxial surface whitish and covered with a waxy coat when young, pale green at maturity, sparsely white-hirsutulous at ½ length from petiole, base rounded to shallowly cordate, margin entire, thinly revolute, apex acuminate, acumen 5–10 mm long, the very tip with an inconspicuous gland, marginal glands 1 per side, c. 1–2 mm from petiole, sunken, 0.3–0.4 mm diam., midvein raised abaxially, sunken adaxially or nearly so, secondary veins 3 or 4 (or 5) on each side of midvein with first two or three pairs arising from base and remainder along midvein, arc-ascending, obscure on both surfaces, tertiary veins inconspicuous. Inflorescences axillary, racemose, developing beyond confines of perennating bud, 1 per axil, 1.5–2 cm long, 4- or 5-flowered, with ± persistent, broadly triangular scales at base; peduncle green or reddish green, 8–10 mm long, pubescent, trichomes and glands same as young branchlets, rachis green or reddish green, 8.0–8.5 mm long, non-ridged, pubescent, trichomes and glands same as peduncle; bracts light brown or reddish, triangular-ovate, subtending pedicels, ± persistent until anthesis, 1.0–1.5 × 0.5–0.6 mm, margin minutely ciliolate, apex acuminate, marcescent, cucullate. Flowers red or maroon, articulated at junction with pedicel, 1.5–1.7 cm long. Pedicel light green with a tinge of red, turning red towards the dilated junction with hypanthium, nodding, 6.0–10.5 × 0.8–1.0 mm at anthesis, with trichomes and glands same as rachis up to ½ to $^{3}/_{5}$ of its length; bracteoles 2, ± persistent until anthesis, borne at 1.0–1.3 mm from pedicel, light brown or reddish, acicular or narrowly elliptic, 0.8–1.0 × 0.3–0.4 mm, margin entire, minutely ciliolate, apex acuminate. Hypanthium green or yellowish green, cupuliform, 2.0–2.5 × 2.0–2.3 mm, glabrous, occasionally covered with a waxy coat; calyx limb 1.0–1.2 mm long, glabrous; calyx lobes broadly triangular, 0.8–1.0 × 1–1.5 mm, glabrous, margin entire, apex acute or obtuse, sessile terminal gland absent. Corolla dark red or maroon, broadly urceolate, shallowly 5-sulcate, 3.0–4.0 × 3.0–4.0 mm, glabrous on both surfaces; corolla lobes 5, recurved

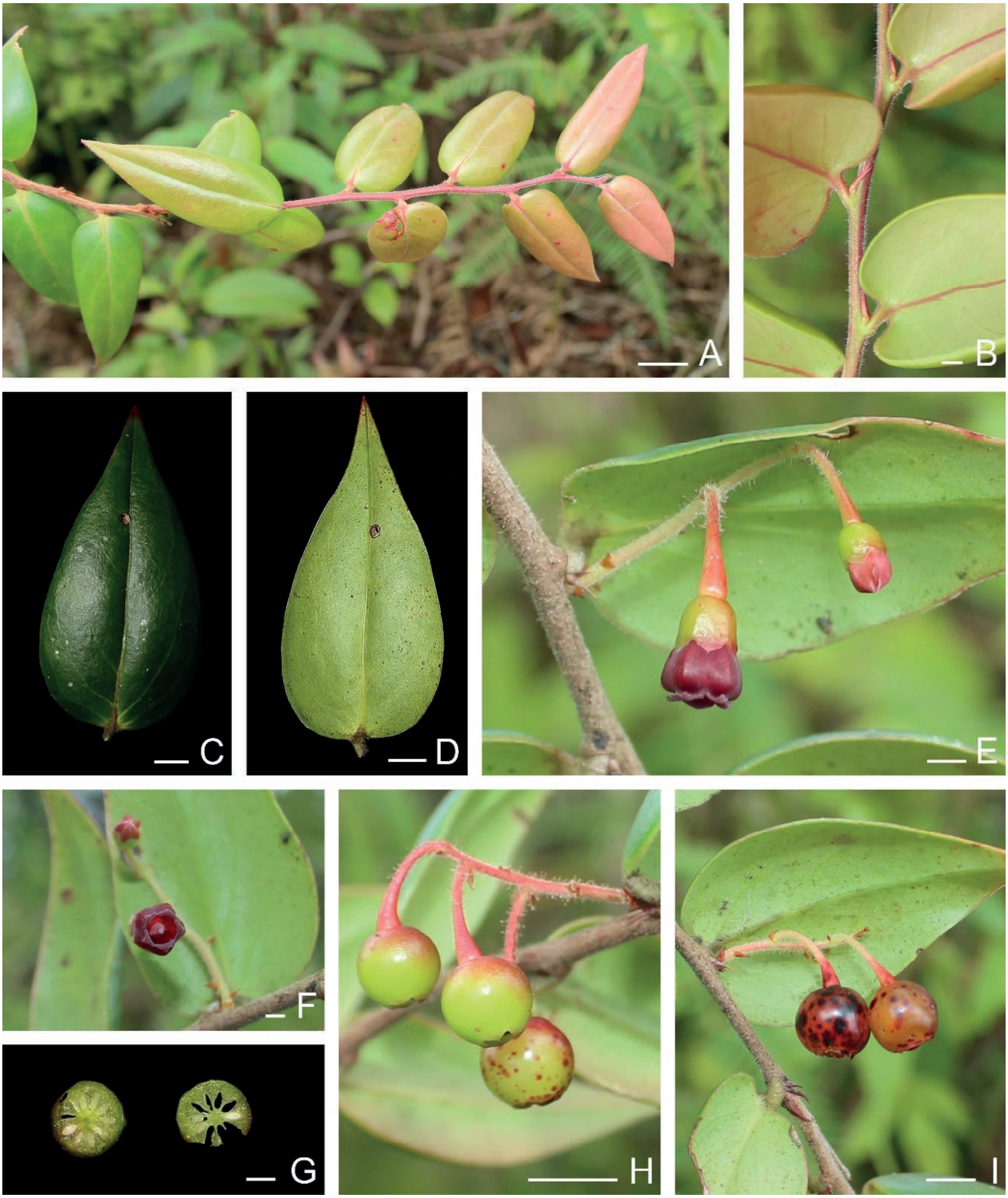

Figure 1. *Rigiolepis gayoensis* Mustaqim, M.N.Tamayo & P.W.Fritsch. A. Leafy branchlet. B. Portion of stem showing indumentum, perennating buds, and cordate leaf blade base. C. Adaxial leaf surface. D. Abaxial leaf surface. E. Inflorescence. F. Anterior view of flower. G. Ovary cross-section. H. Immature fruits. I. Mature (left) and submature (right) fruits. Scale bar: A = 1 cm, B, G = 2 mm, C, D, H, I = 5 mm, E = 2 mm, F = 1 mm. Photographs by Wendy A. Mustaqim. A–F, H, I from the holotype *W.A. Mustaqim 2563*; G from *W.A. Mustaqim 2575*.

at anthesis, 1.0–1.2 × 0.8–1.2 mm, apex obtuse. Stamens 10, monomorphic, distinct, 1.5–1.7 mm long; filaments undulate, dilated at base, 0.8–1.0 mm long, glabrous, anthers 1.3–1.5 mm long, cells 0.8–1.2 mm long, minutely echinulate, *tubules* parallel, erect, cylindric, 0.5–0.6 mm long, opening by oblique laterally oriented apical pores, pore apex oblong, spurs present, erect to slightly upcurved, 0.5–0.6 mm long. Ovary 5-locular but appearing pseudo-10-locular; *ovules* in two columns per locule, each column separated by false partition; *disk* annular, non-bulky, with ridges on margin, 1.0–1.2 mm × 0.5–0.6 mm, glabrous; *style* tubular, slightly dilated at the middle, not exserted from corolla, 2.8–3.0 mm long, glabrous, stigma truncate, with minute papillae. Fruiting pedicels 8–11 mm long, trichomes persistent. Mature fruit red or orange, fleshy and shiny, glabrous, globose, 7.0–7.5 × 7.5–8.0 mm, with dark irregularly-sized blotches, sweet to taste.

Etymology

The epithet *gayoensis* is derived from the Gayo Plateau, the name of the area where the species was found.

Phenology

Flowering in June and October; fruiting in January, June, and October.

Distribution and Habitat

Rigiolepis gayoensis is endemic to the Gayo Plateau, Aceh Province, Indonesia (Figure 2). It was observed growing in tropical mid-montane rainforests at 1770 to 1998 m elevation.

Conservation status

Rigiolepis gayoensis is only known from four unprotected areas in the northern Gayo Plateau. All localities are threatened by logging, forest conversion for coffee plantations, and clearing for development and maintenance of provincial roads. However, since the surrounding vegetations of the areas are poorly explored, we believe that other populations can be discovered once botanical explorations are conducted. Here, we provisionally assess the conservation status of *R. gayoensis* as Data Deficient (DD) (IUCN SPC 2022), as nearby areas with the same elevation and vegetation could harbor additional occurrences of this species. Further detailed population survey highly likely warrant in a threatened status.

Notes

Rigiolepis lanceolata is a variable species, especially its leaf morphology, which caused Smith (1935) to pro-

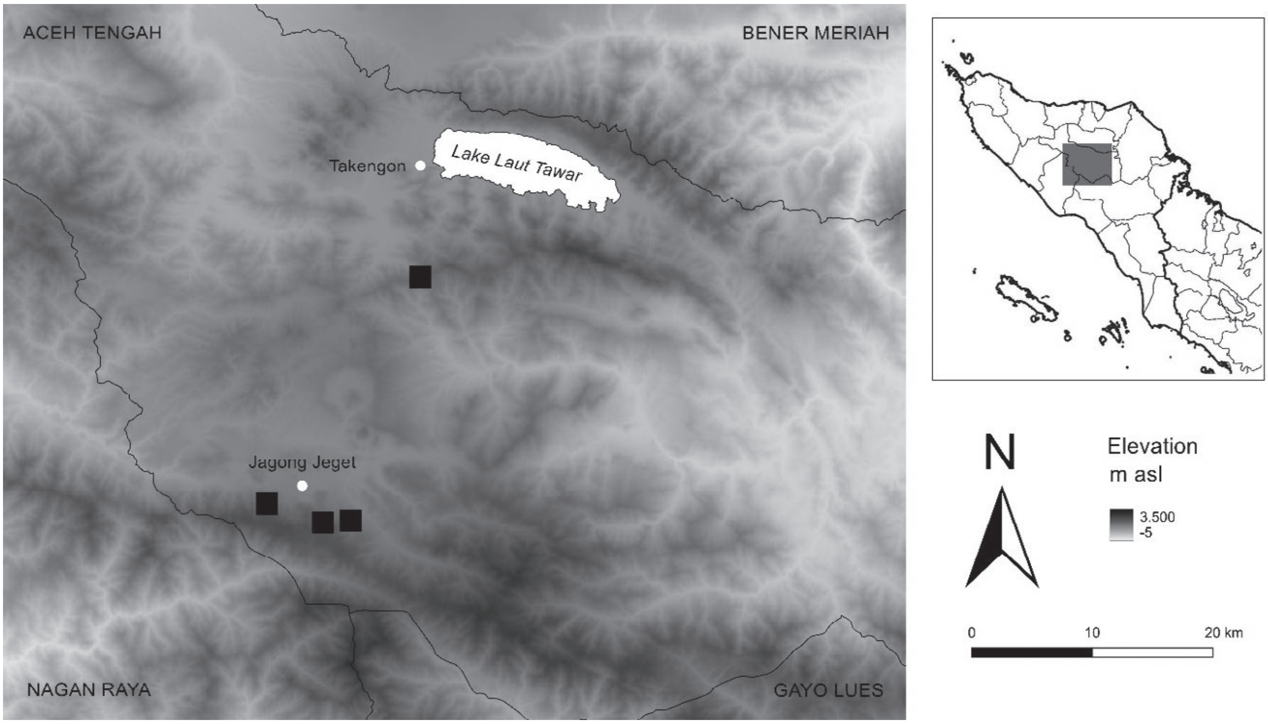

Figure 2. Distribution map of *Rigiolepis gayoensis* in northern Gayo Plateau, Sumatra.

pose three forms; later, two of them were retained by Sleumer (1966–1967), i.e.: (1) f. *marapiense* (J.J.Sm.) Sleumer mainly in having more coriaceous but smaller leaves and (2) f. *ellipticum* (J.J.Sm.) Sleumer mainly in having elliptic leaves, acuminate or subacute apex, and obtuse base. *Rigiolepis gayoensis* differs from the former by having an ovate (vs. lanceolate) and broader leaf blade (ca. 22 mm vs. ca. 20 mm), and from the latter by having an ovate (vs. oblong-elliptic or unequally elliptic) and smaller leaf blade (46–55 × 22–25 mm vs. 70–130 × 25–47 mm) with rounded to subcordate (vs. obtuse) leaf blade base. Furthermore, *R. gayoensis* grows at a higher elevation compared to *R. lanceolata* (1770–1998 m vs 360–1400 m).

Aside from *Rigiolepis lanceolata*, the other species of *Rigiolepis* to occur in Sumatra is *R. leptantha* var. *leptantha* (Argent 2019). *Rigiolepis gayoensis* is distinct from *R. leptantha* var. *leptantha* by having shorter leaves (4.6–5.5 cm vs. 4.5–15 cm), a rounded to shallowly cordate leaf blade base (vs. cuneate), more flowers per inflorescence (4–5-flowered vs. 10–15-flowered) and a dark red or maroon corolla (vs. yellow or cream). In the key to the Bornean species of *Rigiolepis* (Argent 2019), *R. gayoensis* best keys to *R. lobii* var. *lobii*; however, *R. gayoensis* is distinct by having thicker petioles (ca. 2 mm vs. c. 0.5 mm), larger leaves (4.6–5.5 × 2.2–2.5 cm vs. 2.0–5.0 × 0.8–2.0 cm), longer pedicels (10.5 mm vs. 1 mm), and a glabrous corolla (vs. pubescent).

Argent (2019) strongly argued for a truly 10-locular ovary for *Rigiolepis*; a character that greatly supports its generic reinstatement next to *Vaccinium*. However, previous fieldwork by PWF reveals that *Rigiolepis uroglossa* has a pseudo-10-locular ovary (P.W. Fritsch, unpubl. data). This character is also observed for *R. gayoensis* (Figure 1G). Thus, it appears that this character might not be stable to determine generic status of *Rigiolepis*. Although *R. gayoensis* would exhibit such placentation, we recognized it as belonging to *Rigiolepis* through other characters divergent from *Vaccinium* [i.e., mostly epiphytic or climbing shrubs, rarely > 4 mm long flowers (corolla), yellow or orange immature fruits turning red when ripe]. A more detailed morpho-anatomical investigation of the ovary of *Rigiolepis* across varying stages of development (flower buds to ripe fruits) will be essential in evaluating this crucial characteristic.

In recognizing *Rigiolepis* as a separate genus, Argent (2019) also used the frequent presence of a fusiform swollen stem bases or roots (vs. usually absent in *Vaccinium*). *Rigiolepis gayoensis* possesses a subglobose swollen stem base or root (Figure 3). The function of this organ is likely an adaptation to an epiphytic lifestyle, e.g. storing water (see Harsberger 1908; Benzing 1987).

Additional specimens examined (paratypes)

INDONESIA. Paratypes. Sumatra: Aceh Province, Aceh Tengah Regency, Jagong Jeget Subdistrict, Jeget Ayu village, Pengairan (4°20’52.5”N 96°46’30.6”E), 1998 m elevation, 11 January 2023, *W.A. Mustaqim 2575* (LGS); *ibid*. Jagong Jeget Subdistrict, Paya Dedep vil-

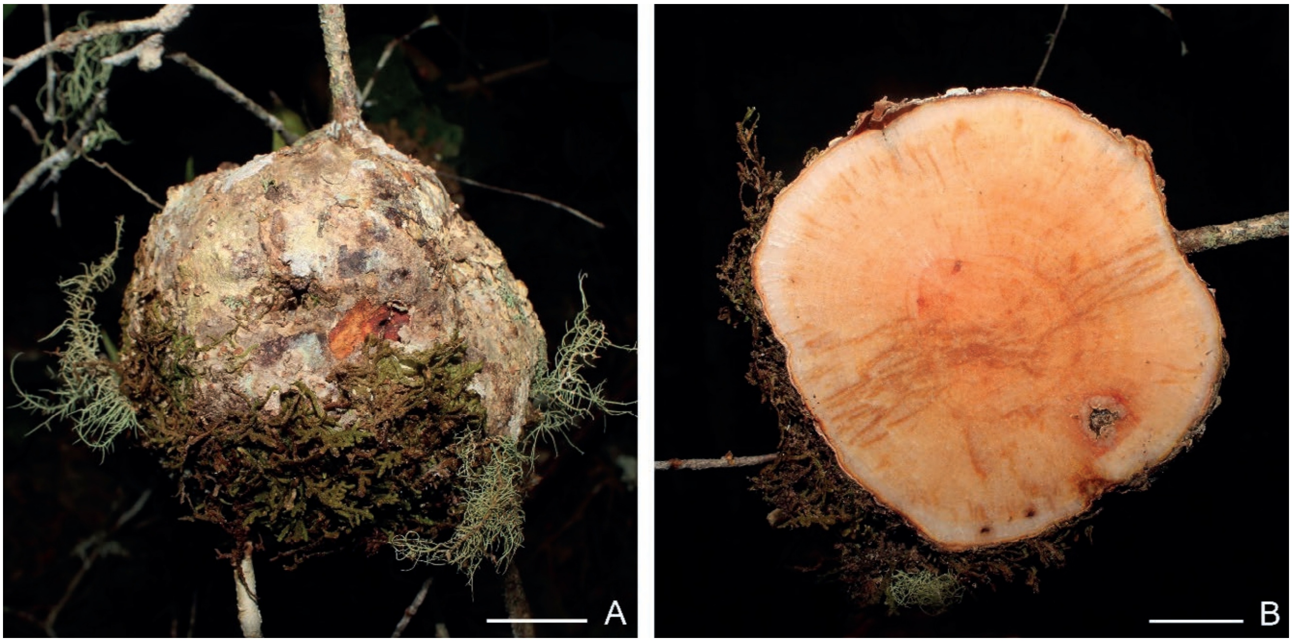

Figure 3. The subglobose swollen stem base or roots of *Rigiolepis gayoensis*. A. Outer surface. B. Cross-section showing growth rings.. Scale bar: 2 cm. All from *W.A. Mustaqim et al. 2475*.

lage, road to Bur Ni Paya (4°21’48.5”N 96°43’58.1”E), 1860 m elevation, 15 June 2023, *W.A. Mustaqim et al. 2724* (LGS); *ibid*. Linge Subdistrict, Gemboyah village (4°21’00.7”N 96°47’48.5”E), 1770 m elevation, 17 June 2023, *W.A. Mustaqim et al. 2745* (LGS).

Key to the species of *Rigiolepis* in Sumatra

1a. Corolla white, yellow or cream; hypanthium pubescent; anther spurs shorter than tubules, c. 0.3 mm long 2

1b. Corolla dark red or maroon; hypanthium glabrous; anther spurs as long as tubules, 0.5–0.6 mm long ..... ***R. gayoensis***

2a. Pedicels stout, 4–8 mm long; hypanthium > 0.5 mm long; basal leaf glands protruding............................ ***R. lanceolata***

2b. Pedicels slender, 2–3 mm long; hypanthium c. 0.5 mm long; basal leaf glands sunken............***R. leptantha*** var. ***leptantha***

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Argostemma kamjongense (Rubiaceae), a new species from Manipur, Northeast India

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Abstract. A new species, *Argostemma kamjongense* (Rubiaceae) confined to Kamjong District of Manipur (Northeast India) is described and illustrated. It is akin to closely allied species, *A. verticillatum* and *A. sarmentosum*, however can be easily distinguishable by the number of lateral veins on the leaves, size of the leaves, fewer number of bracts, slightly reflexed corolla lobes at maturity, style not exserted and enclosed within anther cone. The taxon's potential as a good ecological indicator is discussed along with its ecological adaptations for survival. The new species is also provisionally assessed here as Critically Endangered (CR) according to IUCN Red List Categories and Criteria.

Keywords: *Argostemma kamjongense*, new species, critically endangered, ecological indicator, Manipur (Northeast India).

INTRODUCTION

Argostemma Wall. (Wallich 1824) is a genus of Rubiaceae (Rubioideae–Argostemmateae) with about 178 species (POWO 2023). It is mainly distributed in the tropical and sub–tropical regions of Asian countries and few species in West Africa (Tanaka et al. 2010; Mabberley 2017). Most of the species are mainly lithophytes or occasionally epiphytes, and prefers to grow in shady areas with humid conditions, near streams, waterfalls, wet riverbeds with moss laden rocks, boulders etc. (Sridith and Puff 2000). In India, the genus is among the least studied member of Rubiaceae, mainly because of its small size. Altogether, twelve (12) species so far have been recorded from various parts of India, with maximum species diversity confined to Western Ghats and parts of Andaman and Nicobar Islands (Pandey and Diwakar 2008; Balan et al. 2021). From Northeast India, three (3) species have been described, *viz A. khasianum* C.B.Clarke in Hooker (1880: 43), *A. sarmentosum* Wall. in Roxburgh (1824:324) and *A. verticillatum* Wall. in Roxburgh (1824: 325).

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In one of the botanical surveys conducted in the Kamjong District of Manipur (Northeast India) which is a part of the Indo–Burma Hotspot, the authors came across a waterfall hidden within a forest canopy. On further exploration, we came across a small epilithic species growing along the slimy rocks facing the waterfall. On critical examination, it was revealed that the taxon is a new species under the genus *Argostemma*. We also collected *A. verticillatum* from Manipur which is also a new State record. Another allied species, *A. sarmentosum* was also collected from Meghalaya State, and both were used for comparison with the new species.

MATERIALS AND METHODS

The new species was collected from Yeasom Hills of Kamjong District, Manipur (Northeast India) during repeated field trips conducted from April 2021 to October 2022 (Figure 1). Relevant literatures and various herbaria (ARUN, ASSAM, CAL, K) were consulted to study the existing species and study the differences in their morphological parameters (Ridley 1927; Bremer 1989; Sridith 1999; Chen and Taylor 2011; Lanorsavanh et al. 2020; Vu et al. 2020). The new species is compared with two closely allied species, *A. verticillatum* Wall. and *A. sarmentosum* Wall. found in Northeast India (Table 1). Microphotographs of morphological characters were taken by using phase–contrast microscope (Motic BA 210), LED–USB Cooling Tech Microscope and Sony DSC–W610. All the voucher specimens were deposited at Botanical Survey of India (BSI), Eastern Regional Centre (herbarium code ASSAM), and Manipur University Museum of Plants (MUMP) and Mizoram University Herbarium (MZUH), the latter still under indexing process.

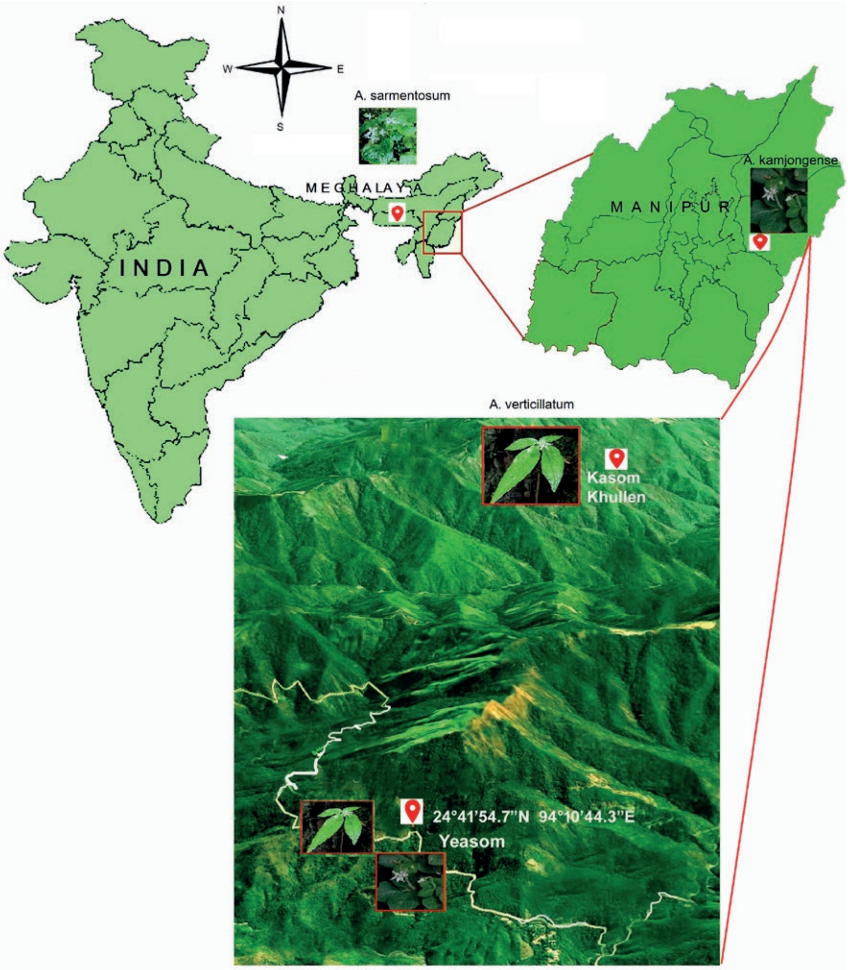

Figure 1. Map showing type locality of *A. kamjongense* B. S. Sadokpam, S. D. Khomdram & S. D. Yumkham, Yeasom Hills at Kamjong District, Manipur (Northeast India) and sites showing distribution of *A. verticillatum* in Manipur & *A. sarmentosum* in Meghalaya.

TAXONOMIC TREATMENT

Argostemma kamjongense B.S.Sadokpam, S.D.Khomdram & S.D.Yumkham, **sp. nov.** (Figure 2).

Type**:** India, Manipur, Kamjong District, Yeasom Hills, 24°41′54.7”N; 94°10′44.3”E, a.s.l. 950 m, 19 September 2022, *Bimolkumar Singh Sadokpam & Sanatombi Devi Yumkham 000998* (holotype ASSAM!; isotypes MUMP!, MZUH!).

Diagnosis

Argostemma kamjongense differs morphologically from *A. verticillatum* and *A. sarmentosum* by having lesser pairs of lateral veins (3–4 pairs versus 4–7 pairs), generally smaller leaves (less than 2.5 cm versus more than 2 cm and up to 10 cm), fewer bracts (1–2 versus in groups of 4 or basally fused in *A. verticillatum* or more than 2 in *A. sarmentosum*), corollas yellowish at base (versus greenish at base), corolla lobes slightly reflexed at maturity (versus strongly reflexed at maturity in *A. verticillatum* and rarely reflexed in *A. sarmentosum*) and style not exserted and enclosed with anther cone (versus exserted).

Description

Plants perennial, herbaceous, 4–6 cm tall, epilithic. Rhizome tuberous, globose, 0.2–0.4 cm, 4–7 roots arising from tuber surface. Stem erect, unbranched, 0.2–1.5 cm long, pubescent, green, terete. Leaves opposite, decussate, anisophyllous in 2 pairs, sometimes in pseudo–whorls, 0.5–2.5 × 0.3–1.5 cm, sessile, ovate to elliptic, leaf base oblique to cuneate, margin entire, apex acute to obtuse, pubescent on both side, 3–4 alternate lateral veins on each side, green, rudimentary leaves in pair present at nodes, acute, 0.1–0.2 cm long. Stipule linear, minute, deciduous, interpetiolar, entire. Inflorescence umbelliform, single, terminal, 1–8 flowered. Peduncle 1.5–3 cm long, pubescent. Bract 1–2, 0.2 × 0.1 cm, acute, pubescent, green, persistent. Flowers pedicellate, pubescent, 4–merous, rarely 5–merous, actinomorphic, 0.4–0.6 cm long, 0.6–0.8 cm in diameter. Calyx 4–lobed, ovate, gamosepalous, campanulate with short tube, 1/3 free, persistent, green, pubescent, 0.4 × 0.1 cm, sepals alternate to petals. Corolla star shaped, white, yellowish at base, broadly rotate, 4–lobed, rarely 5–lobed, lanceolate, apex acute, outer upper lobe pubescent, inner lobe glabrous, slightly reflexed at maturity, uncoiled tip, 0.5–0.6 × 0.25–0.3 cm, corolla tube short, ca. 0.1 cm long. Stamens 4, glabrous, white, basifixed, 0.3–0.4 cm long inserted to the base of corolla tube, filaments free, loosely agglutinated, anthers coherent into a cone, opens up widely at maturity, dehiscence poricidal. Ovary inferior, pubescent, 2–locular, 0.4–0.5 cm long, ovules numerous, axile placentation, style filiform, up to 0.3 cm long, not exserted, enclosed within anther cone, stigma capitate (globular). Capsule small, 0.2 cm in diameter, locules 2, ca. 15–25 seeds per locule.

Etymology

The specific epithet ‘*kamjongense*’ refers to the type locality Kamjong District of Manipur State (Northeast India). It was previously a subdivision under Ukhrul district, and upgraded to the status of district on 8 December 2016.

Phenology

Flowering from June to August; fruiting from late August to early October.

Distribution and Habitat

The new species was collected from the type locality on Yeasom Hills located in Kamjong District, Manipur. It was found thriving as an epilithic herb on an isolated rough hilly terrain mainly composed of rocks and big boulders, near a small waterfall located at an elevation of 950 m. The main part of the study site was covered by a thick forest canopy and therefore blocks direct sunlight. This limits the vegetation of the area and only a few shade loving species which can endure the low light intensity like *Selaginella* P. Beauv., *Riccia* L., *Zingiber* Mill., *Adiantum* L. etc. were found growing. Moving a little further away from the waterfall site (ca. 5 meters), the forest canopy is opened (ca. 2 meters diameter) from where sunrays penetrated and directly illuminated the forest floor. At this spot, there grows a population of around 25 matured plants of the new species, and they tend to become dry and dehydrated when exposed to direct sunlight. This means the new species can thrive only in shady moist area or/and in partial sunlit area. Temperature (moderate), humidity, soil moisture content, rainfall and sunlight play a key role in the growth and survival of the new species (Figure 3).

Preliminary conservation status

The new species is extremely delicate, small in size and currently collected only from its type locality (Yeasom, Kamjong District, Manipur). It grows in a very restricted small population within a radius of around 7 meters. The area is mainly composed of rocks, boulders, small gravels etc. with little or no humus deposition. At the present context, the species is assessed as Critically Endangered (CR) according to the criteria B2ab (i, ii, iii), D of IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022).

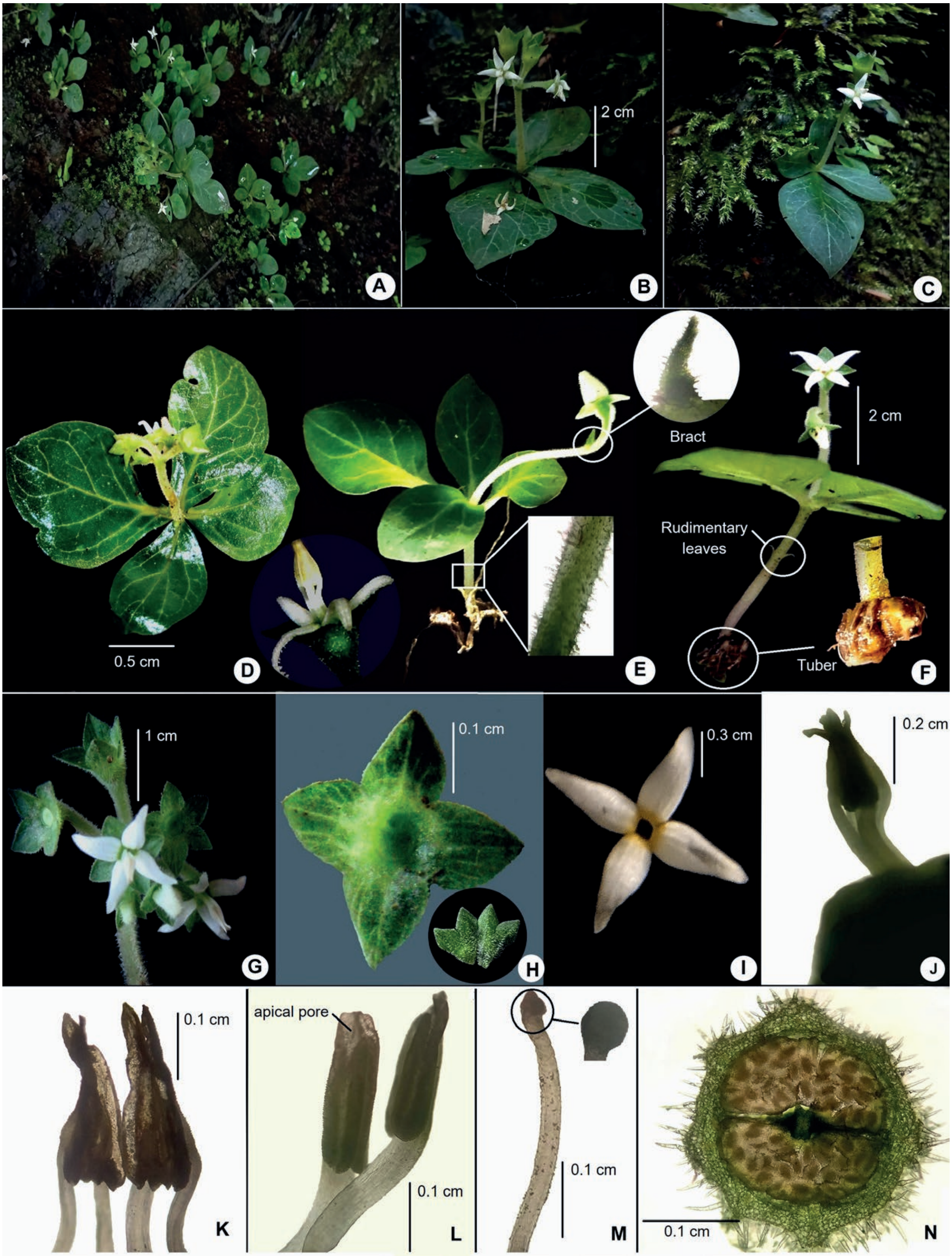

Figure 2. *Argostemma kamjongense* A–C. Epilithic habit D. Anisophyllous leaves in 2 pairs with single flower in inset E. Habit with single flower showing pubescent stem & bract in inset F. Rudimentary leaves & tubers G. 6–flowered inflorescence H. Calyx I. Corolla, yellowish at base J–K. Agglutinated anthers L. Anther with poricidal dehiscence M. Filiform style with capitate stigma N. 2–locular ovary showing seeds.

Species significance and uses

The species remain isolated and confined beyond human detection. Also, because of their miniature size and unfriendly rough terrain habitat, these plants remain unnoticed and unexplored. According to Sridith, the genus *Argostemma* can act as a potential ecological indicator and as an indicator of phytogeographic patterns (Sridith 2007). This unique attribute is mainly because *Argostemma* usually grows only in intact or undisturbed forests and can never be relocated in secondary or disturbed habitats. During the course of the study, it was observed that this species has specific requirements like constant moisture availability, moderate temperature (15–20°C) and has narrow limit of tolerance for sunlight. These factors limits their distribution and therefore can act as a measure of existing environmental conditions. Hence, *Argostemma* can be a great ecological indicator.

DISCUSSION AND CONCLUSION

The new species, *Argostemma kamjongense* of Rubiaceae is collected from an isolated mountainous terrain growing as epilithic in between the wet crevices of rocks and boulders near a waterfall. In general, most of the *Argostemma* usually grows near margin of streams and waterfalls. From the type locality, we also collected a few specimens of *A. verticillatum* growing sparsely along the rocky walls adjoining the waterfall, which is also recorded for the first time from Manipur State. We also assessed around 20 other locations with waterfalls within the type locality and areas adjoining the district. In many of these sites, we could collect only *A. verticillatum*. As such, the new species is placed under the Critically Endangered (CR) category B2ab (i, ii, iii), D of IUCN based on area of occupancy (<10 km^2), location number (1), limited extent of occurrence and limited number of mature individuals (<50).

To survive in the rough hostile environment, the new species has manifested certain ecological adaptations which are highlighted below:

1. Presence of tubers which are perennial in nature. This feature boosts the survival rate of the plant which has an epilithic habit by providing nutrients during unfavorable conditions (nutrient deprivation, temporary dehydration, desiccation of photosynthetic parts etc.).
2. Anthers coherent into a cone at young stage (agglutination), becoming free and widely opening towards

Table 1. Morphological comparison between *A. kamjongense, A. verticillatum* and *A. sarmentosum.*

Characters	*A. kamjongense*	*A. verticillatum*	*A. sarmentosum*
Habit and Habitat	Epilithic, 4–6 cm tall, erect, tuberous	Epilithic, 2–7 cm tall, erect, tuberous	Epilithic, 10–20 cm tall, creeping at base with filiform runners
Leaves	Ovate to elliptic, 0.5–2.5 cm long, 3–4 lateral leaf veins on each side	Ovate, elliptic, elliptic–oblong or obovate, 2–7 cm long, 4–7 lateral leaf veins on each side	Broadly ovate, rounded or elliptic, 2–10 cm long, 5–7 lateral leaf veins on each side
Numbers of flowers per inflorescence	1–8 flowered	2–several flowered	6–10 flowered
Peduncle	1.5–3 cm, pubescent	1–3 cm, glabrous	3–6 cm, pubescent
Bracts	1–2, non–foliaceous	Groups of 4 or basally fused, non-foliaceous	More than 2, foliaceous
Flowers	4–merous	5–merous	4–merous
Calyx	4 lobes, ovate, pubescent, up to 4 mm long	5 lobes, ovate–acute, glabrous, up to 2 mm long	4 lobes, ovate–oblong, strigose, up to l0 mm long
Corolla	4 lobes, lanceolate–acute, white, yellowish at base, slightly reflexed, uncoiled tip, outer upper lobe pubescent, inner lobe glabrous	5 lobes, ovate–acuminate, white, greenish at base, strongly reflexed, coiled tip, glabrous both side	4 lobes, lanceolate–acute, white, greenish at base, rarely reflexed, outer lobe pubescent
Stamens & Nature of agglutination	4 stamens, filaments free, anthers coherent, connivent	5 stamens, filaments fused around the middle to form a short tube, anthers free but connivent	4 stamens, filaments free, curved, rarely approaching to form connivent anthers
Style	Not exserted, enclosed within anther cone	Exserted	Exserted
Geographical Distribution	Yeasom Hills (Manipur, India)	Bhutan, Myanmar, Nepal, Thailand, Laos, Vietnam, Northeast India	Himalayas, Bhutan, Pakistan, Northeast India (Sikkim, Meghalaya)

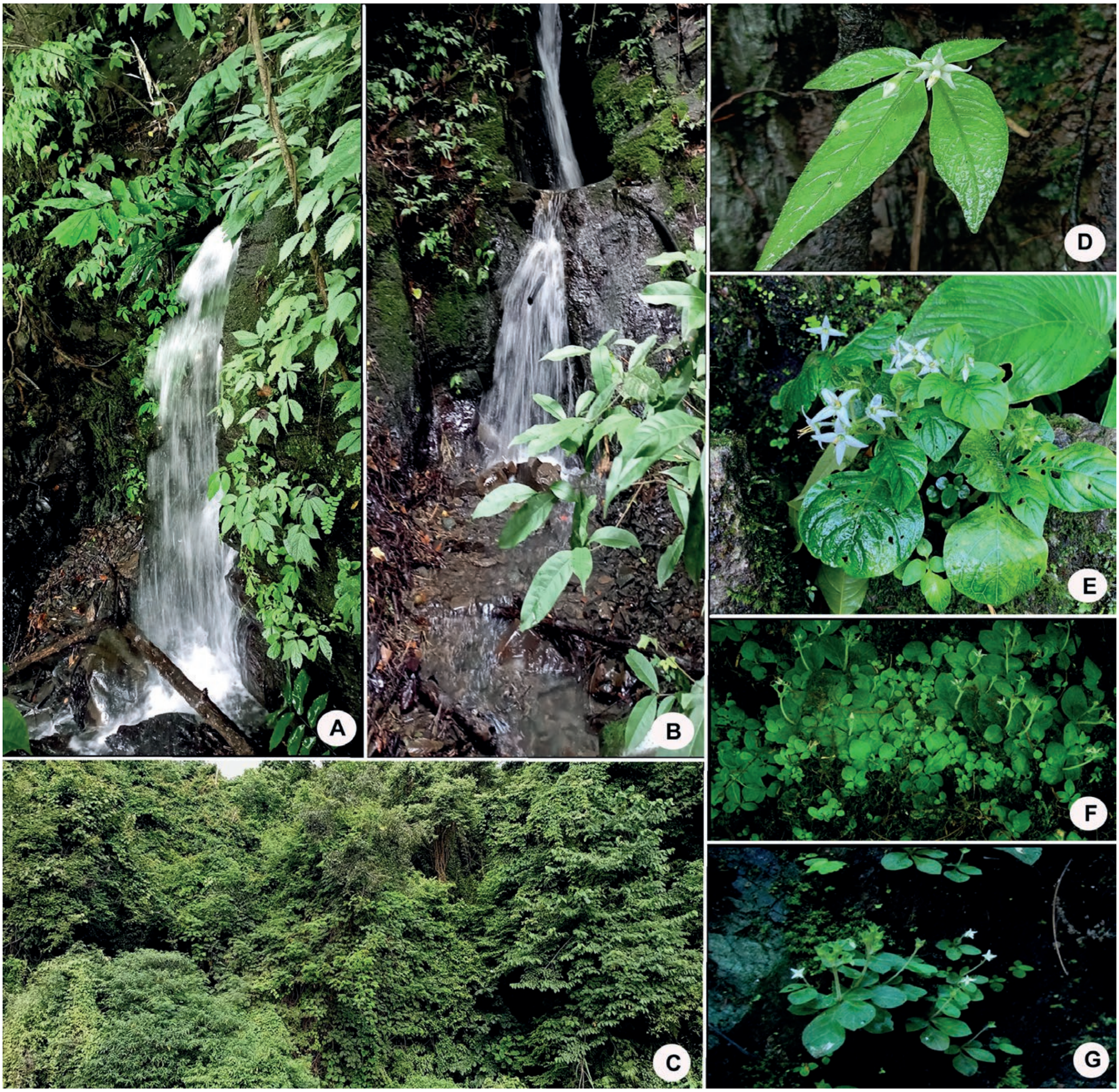

Figure 3. A–C. Habitat showing waterfalls and forest canopy D. *A. verticillatum* from Kamjong District, Manipur E. *A. sarmentosum* from Meghalaya F–G. A population of *A. kamjongense* growing among mosses under low light intensity.

maturity. This ensures protection of the young developing pollens. Filaments are robust, which served as a reliable platform for buzz pollination. Interestingly, dehiscence takes place from apical pores, and effectively translates the mechanism of insect pollination. Agglutinations and synandry syndrome in *Argostemma* also help in insect pollination (Puff et al. 1995).

3. As in most of the species under *Argostemma*, the fruit is protected by a persistent calyx and opens by an apical operculum. Seeds are extremely small in size and enable them to easily lodge inside the narrow rock crevices and are protected from being washed away by the water drops hitting against the rocky walls. They will grow again when soil moisture and temperature conditions are appropriate for germination.
4. Manifestation of sciophytic adaptations like growing in top thin layer humus deposited places (forest

floor, rock crevices, mossy boulders, bogs etc.), slow growth, small sized non showy flowers, little tolerance to bright sunlight, anisophylly and thin leaves and extensive vegetative growth.

Few factors also attributed to its habitation in a small or isolated geographical area. Morphologically, *A. kamjongense* is very small in size compared to other well flourished species of the genus. As in most of the epilithic sciophytes, the vegetative growth phase is comparatively longer than the fruiting phase. Other factors like nature of seed (minute, low yield of around 15–25 seeds per locule, absence of appendages/parts related to seed dispersal), limited number of flowers (1–8) per inflorescence, reduced rudimentary leaves decreasing photosynthetic yield, thin–layered cuticle etc. also contributed to their small sized population. The species has narrow range of tolerance to sunlight and temperature above 25°C. Many streams and waterfalls in Manipur are perennial as the State receives an annual rainfall of around 1500–2000 mm. The phenological period of this plant coincide with the monsoon season. As a result, there are high chances of viable seeds being washed off/ carried away by the flowing water currents from the site. When landed in exposed alien environment, the seeds will not germinate if it does not meet the optimum conditions. The occurrence of this new species in Yeasom Hills also indicates that the forest is presently undisturbed by anthropogenic activities as *Argostemma* are ecological indicators and can only survive in undisturbed primary environment. They are very sensitive to micro–climatic changes and any disturbance in ecological balance will soon cause the population to die (Sridith and Puff 2000). Changes in the existing abiotic factors (sunlight, temperature, precipitation, minerals, soil), local land–use pattern (jhum, terrace farming, clearing of forest for firewood), landslides etc. will be a major concern for keeping the forest intact and for shaping the biodiversity.

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AUTHOR CONTRIBUTIONS

BSS & DW conducted repeated field surveys in various parts of the State & studied the taxonomic parameters. SDY & SDK analyzed, identify the specimen, preparation of photo plates and did extensive literature survey. All the authors finally read and approved the manuscript.

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A new species of *Begonia* section *Baryandra* from Zamboanga Peninsula, Southwestern Philippines

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Abstract. A new species of *Begonia* sect. *Baryandra*, *B. sebodensis,* from Zamboanga del Norte, southwestern Philippines is described and illustrated. *Begonia sebodensis* is morphologically similar to *B. anisoptera* in obliquely ovate to widely ovate leaves, four-tepaled staminate and pistillate flowers and three locular ovary but differs in glabrescent stipules, sericeous petioles, leaf margins distantly serrate and ciliate, many-flowered inflorescences, capsule wings subequal and flat. Following IUCN criteria, we propose *B. sebodensis* as Endangered (EN).

Keywords: *Begonia anisoptera,* endemic, lithophyte, taxonomy, Zamboanga del Norte.

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INTRODUCTION

With at least 2116 species, *Begonia* L. (Begoniaceae) is considered the sixth-largest genus of flowering plants (Hughes et al. 2015–; Moonlight et al. 2018). In the Philippines, 163 species are recognized and distributed in 3 sections, namely; *B.* sect. *Petermannia*, *B.* sect. *Baryandra* A. de Candolle (1859: 122) and *B.* sect. *Platycentrum* (Amoroso et al. 2023; Hughes et al. 2015–; Mazo and Rubite 2022; Mazo et al. 2022; Rubite et al. 2022).

The region of Zamboanga Peninsula, southwestern Mindanao is one of the botanically unexplored areas in the Philippines. Based on herbarium records and literature, 17 species and one subspecies of *Begonia* were recorded in Zamboanga Peninsula (Naive et al. 2022; Mazo et al. 2022). *Begonia* sect. *Baryandra* is represented by a single species in the region, *Begonia anisoptera* Merr., and all the remaining taxa belong to *Begonia* sect. *Petermannia*.

During a botanical exploration in the municipality of President Manuel A. Roxas (PMAR), Zamboanga del Norte (Fig. 1), a rhizomatous *Begonia* with 4-tepaled staminate and pistillate flowers, and 3-locular ovary was documented. These characters indicate that it is a member of the *B.* sect. *Baryan-*

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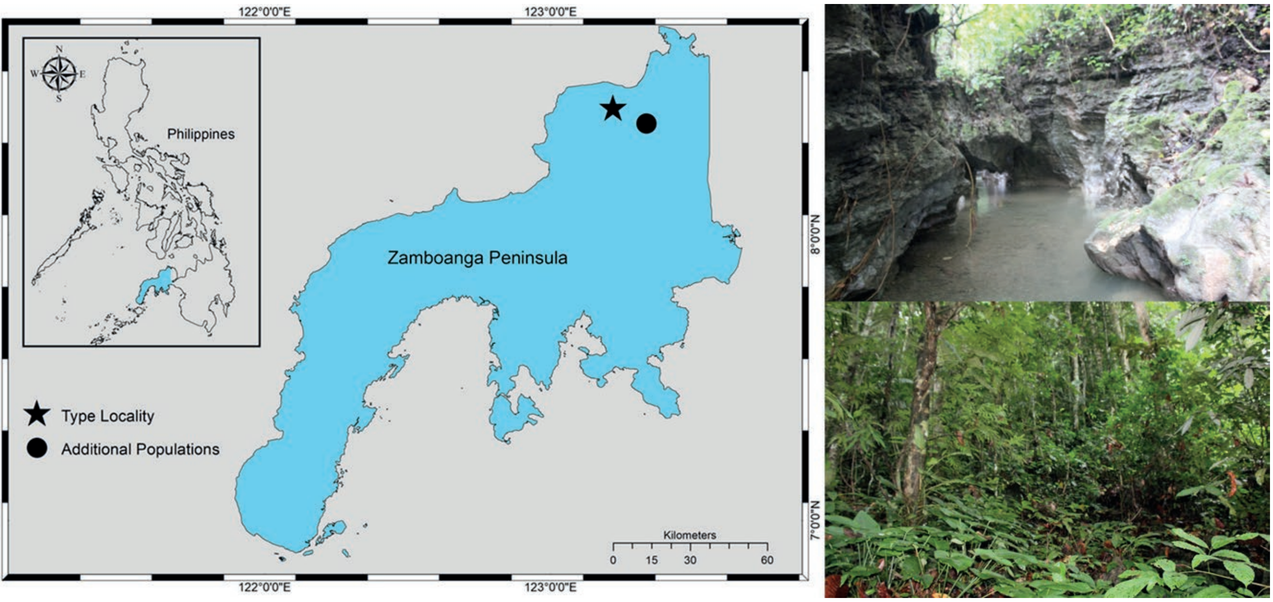

Figure 1. Map showing the distribution (left) and habitat (right) of *Begonia sebodensis* in Zamboanga Peninsula.

dra. Based on a detailed examination of morphological characters of the collected material and closely related species, we concluded that it is new to science, which is hereby named *Begonia sebodensis* Mazo & Rubite (Fig. 2) and described as the second representative of the *B.* sect. *Baryandra* in Zamboanga Peninsula, Philippines.

MATERIALS & METHODS

Fruiting and flowering materials of the *B. sebodensis* were collected in June 2023. The descriptions were based on both living and dried collections vouchered under Wildlife Gratuitous Permit (GP) No. IX-2023-11 issued by the Department of Environment and Natural Resources (DENR) Region 9. Protologues, herbarium specimens, and other relevant literature were examined for morphological comparisons. *B. sebodensis* closely resembles *B. anisoptera* (Fig. 3) the only *Begonia* sect. *Baryandra* representative in the region. The conservation status was assessed following the International Union for Conservation of Nature (IUCN) criteria (IUCN Standards and Petitions Subcommittee 2022).

TAXONOMIC TREATMENT

Begonia sebodensis Mazo & Rubite, **sp. nov. (**Figure 2**);** Sect. Baryandra

Type: Philippines, Mindanao, Zamboanga del Norte, municipality of President Manuel A. Roxas, barangay Sebod, 8°22'12.40"N, 123°11'39.13"E, 350 m. a.s.l., June 4, 2023*, K.R.F. Mazo 113* (holotype PNH).

Diagnosis

Similar to *Begonia anisoptera* in having obliquely ovate to widely ovate leaves, 4-tepaled staminate and pistillate flowers and three winged capsules. However, the new species can be easily distinguished in having stipules glabrescent (vs. densely hirsute), petioles 16.5–28.0 cm long, indumentum reddish-maroon, appressed (vs. 4–12 cm long, hairs brown and spreading), larger leaves 8–17 × 7.0–11.5 cm, distantly serrate (vs. 6–12 × 4–8 cm, entire), inflorescence cymosely branching 5–7 times (vs. 2–3 times), oblanceolate inner staminate and pistillate tepals (vs. obovate to cuneiform) with rounded apex (vs. truncate to slightly retuse), capsule recurved or pendulous (vs. splash cup), wings subequal and flat (vs. strongly unequal, abaxial wing cucullate, lateral wings curved).

Description

Herbaceous, lithophytic, rhizomatous, perennial, monoecious. Rhizome creeping up to 13 cm long, 9–15 mm thick, puberulent, reddish to maroon, prominent petiolar scars, internodes 10–23 mm long. Stipules persistent, triangular 11.5–13.5 × 6–8 mm, reddish, adaxially glabrescent, abaxially glabrous, herbaceous, strongly keeled with reddish brown hairs fused at the keel, margin slightly revolute, apex aristate (3.5 mm long). Leaves alternate; *petiole* terete, 16.5–28.0 cm long, 5–7 mm

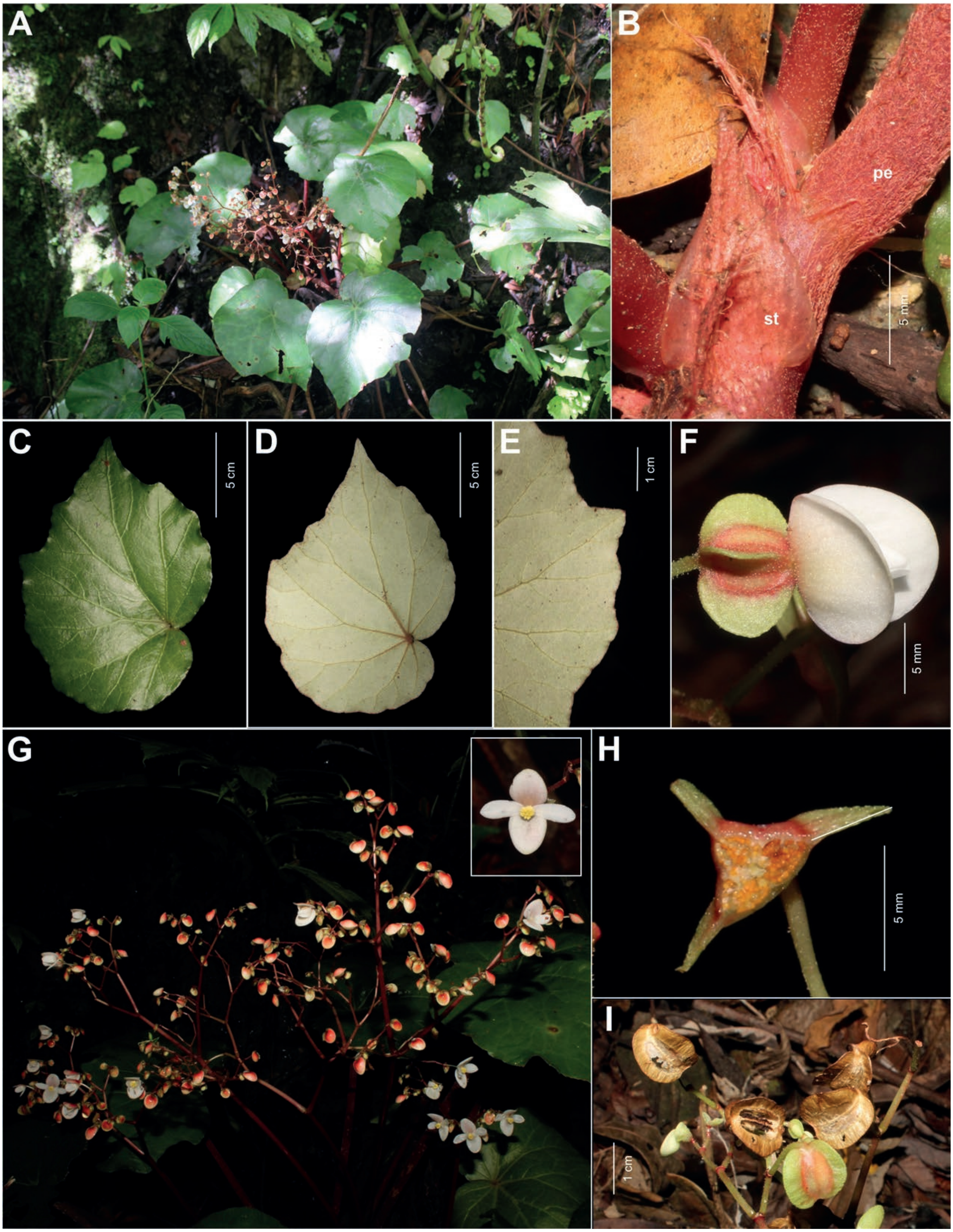

Figure 2. *Begonia sebodensis* Mazo & Rubite. A. Habit; B. Stipules (st) and portion of the petioles (pe); C. Leaf adaxial surface; D. Leaf abaxial surface; E. Leaf margin; F. Pistillate flower, side view showing the ovary; G. Inflorescences, inset: front view of the staminate flower; H. Cross-section of the ovary; I. Capsules and fruit. All from *K.R.F. Mazo 113*.

Figure 3. *Begonia anisoptera* Merr. A. Habit; B. Petiole showing white and spreading hairs; C. Stipule; D. Fruit showing strongly unequal wings, abaxial wing cucullate.

thick, maroon, sericeous; *leaf blade* asymmetric, succulent, oblique, ovate to widely ovate, (8–)13–17 × (7–)9.5–11.5 cm, broad side width 6.5–8.3 cm, basal lobes cordate, sinus overlapping, margin distantly serrate, slightly undulate, ciliate (hairs 0.6 mm), apex acuminate; adaxially green, glabrous, abaxially ivory, lanate, reddish hirsute trichomes on veins (hairs 0.5 mm long); venation palmate, 7–8 primary veins, abaxially raised, branching dichotomously, tertiary veins reticulate. Inflorescence axillary, erect, bisexual, protandrous, cymosely branching 5–7 times; peduncle 16–35 cm long, red, sparsely puberulent in newly develop and turning glabrous. Bracts caducous, widely ovate, boat-shaped, 6–7 × 5.3–5.6 mm, pale green, glabrous, margin entire, apex rounded. Staminate flower pedicel 7–11 mm long, pale green to red, puberulent, tepals 4; outer 2, widely ovate, 9.5–11.0 × 8.5–9.0 mm, pinkish to white, adaxial surface sparsely puberulent to glabrous, venation distinct, apex rounded; inner 2 oblanceolate, 8–9 × 3.5–4.0 mm, white, glabrous both surfaces; androecium actinomorphic, 3.5–4.5 mm in diameter; stamens yellow, 35–40, filaments shortly fused at the base; anthers obovate, *ca.* 1 mm long, apex retuse. Pistillate flower pedicel 2.5–5.5 mm long, pale green to red, puberulent, tepals 4; outer 2 suborbicular, 9.5–10.5 × 8.5–10.0 mm, pinkish to white, adaxially glabrous, abaxially sparsely puberulent to glabrous, venation distinct, apex rounded; inner 2 elliptic to oblanceolate, 8–8.5 × 3.8–4.5 mm, white, glabrous both surfaces, apex rounded; *ovary* trigonous-ellipsoid, 4.5–5.5 × 3.0–3.5 mm, green to red, sparsely puberulent to glabrous; *wings* 3, subequal, flat, 7.8–8.2 × 3.0–3.6 mm, proximally cordate, distally rounded to truncated at an angle, margin entire; ovary 3-locular, placenta bilamellate; *styles* 3, 4.8–5.0 mm long, fused at the base at 1.5 mm; *stigma* spirally twisted and papillose all around. Capsule recurved or pendulous 10.0–11.5 × 8.0–12.6 mm, wings subequal, flat 1.7–11.0 mm long, 1.5–3.5 mm wide, truncate to slightly retuse proximally, rounded to truncate distally, dehiscing along the attachment with the wings.

Etymology

The specific epithet derived from the locality where the new species was collected.

Phenology

Observed flowering and fruiting from March to June.

Distribution and ecology

Begonia sebodensis is endemic to Zamboanga Peninsula and is currently known only from the municipalities of President Manuel A. Roxas, and Katipunan, Zamboanga del Norte (Fig. 1). It grows on rocks and vertical cliffs in shady areas at elevations of 200–500 meters above sea level. In the type locality, the plant associated with *B. sebodensis* includes *Homalomena philippinensis* Engl. (Araceae), *Monophyllaea merrilliana* Kraenzl. (Gesneriaceae), and species of *Ficus* L. (Moraceae), *Elatostema* J.R.Forst. & G.Forst. (Urticaceae), and *Calamus* L. (Arecaceae).

Proposed conservation assessment

Begonia sebodensis is only known from two barangays of two different municipalities in Zamboanga del Norte: Barangay Sebod, President Manuel A. Roxas, and Barangay Miatan, Katipunan. In the two barangays, total of 10 to 15 subpopulations were recorded with less than 100 mature individuals. The type locality is near a waterfalls which is being developed as a tourist destination, vegetation in the area is being cleared and planted with ornamental plants, the falls is frequently visited by local tourists. The two barangays are near farm lots and charcoal making was also observed. These barangays where the *B. sebodensis* were recorded are currently not protected under the country's National Integrated Protected Areas System by the Department of Environment and Natural Resources. Following IUCN red list and criteria (IUCN 2022), *Begonia sebodensis* is hereby proposed as Endangered [EN, D].

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Systematic studies on some West African species of the Tribe Bauhinieae (Cercidioideae)

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Abstract. The tribe Bauhinieae is the largest and most taxonomically complex group within the subfamily Cercidoideae. They possess the most distinguishable morphological features but are the most variable group. Here, we explore the phylogenetic relationship of the tribe Bauhinieae using morphological, anatomical and molecular data (ITS, rbcL, trnL-F, ITS+rbcL+trnL-F). Relationships inferred from morphological, anatomical and molecular data revealed congruent result, a non-monophyletic *Bauhinia* and *Piliostigma* group. The leaf epidermal shape in all *Bauhinia* species examined are polygonal with straight cell walls except *B. tomentosa*, which has an undulating cell wall. Stomatal types observed vary between the two genera studied. *Bauhinia* species has paracytic stomata while *Piliostigma* species exhibited hemiparacytic stomata. Dendrogram generated revealed the interrelationship between the species at a distance value of 80. Bayesian analysis revealed a high resolution of species and posterior probability. The strict consensus tree for all the tested gene regions revealed a polyphyletic *Bauhina* divided into three major clades. The *Piliostigma* group exhibited a paraphyletic and polyphyletic relationship within the *Bauhinia* group at high support values. *B tomentosa* exhibited a closer relationship with *Piliostigma* species. These results support the proposition to divide members of the large *Bauhinia* s.s group into subclades. This study has attempted to elucidate the unresolved species and genus level taxonomy of the tribe Bauhinieae. However, more variable gene regions in addition to broader species sampling should be considered for further phylogenetic patterns of this taxon.

Keywords: *Bauhinia*, *Piliostigma*, ITS, *rbcL*, *trnL-F*, molecular phylogeny, plant anatomy.

INTRODUCTION

The plant family Fabaceae is the third largest angiosperm family only after Orchidaceae and Asteraceae. They vary in habit from herbs to shrubs, vines, lianas, and trees, with an extremely high diversity of 651 living genera and 19,500 species across different habitats of the world (Wang et al. 2014). The family was formerly divided into three subfamilies, Mimosideae, Ceasalpinioideae and Papilionoideae. Upon recent reclassification, the family is now divided into 6 sub-families (LPWG 2017): a recircumscribed Caesalpinioideae DC., Cercidoideae Legume Phylogeny Working Group (stat. nov.), Detarioideae Burmeist., Dialioideae Legume Phylogeny Working Group (stat. nov.), Duparquetioideae Legume Phylogeny Working Group (stat. nov.), and Papilionoideae DC. Since then, studies are ongoing to revise and ratify the classification of the new subfamilies (Estrella et al. 2018). Amongst the sub family Cercidoideae, the taxonomic and phylogenetic relationships among members of the tribe Bauhinieae has remain challenging and been the subject of recent studies (Zhang 1995; Sinou et al. 2020).

The tribe Bauhinieae possess the most distinguishable morphological features but are the most variable group among the cercidoideae (Meng et al. 2014), owing to their bilobate, bifoliolate, or unifoliolate pulvinate leaves with basal actinodromous or acrodromous venations. They exhibit seeds with a crescent-shaped hilum and an aril-lobed funiculus (Wunderlin et al. 1987; Sinou et al. 2020); leaves are mostly simple (entire to bilobed) or bifoliolate with zygomorphic flowers. Specifically, these species exhibit wide-ranging distribution and eclectic morphological variability (Hao et al. 2003; Sinou et al. 2009). Members of the tribe Bauhinieae are disjunctly distributed in tropical and sub-tropical regions of Africa such as Sudan, Ivory Coast, South Africa, Algeria and even in Eastern and South-Western Nigeria. They are found in nearly all ecosystems, including forests (Amazonian, Atlantic, gallery forests), savannas (cerrados, campos rupestres) and caatinga (dry deciduous forest of the semi-arid Brazilian Northeast). The formerly recognized tribe Cercioideae (now subfamily Cercidioideae) was divided into the subtribes Cercidiinae and Bauhiniinae (now elevated to tribal rank). Across West African countries, the former comprises the genera *Cercis*, *Adenolobus* and *Griffonia* while the subtribe Bauhiniinae contains the genera *Bauhinia*, *Barklya*, *Brenierea*, *Gigasiphon*, *Lysiphyllum*, *Phanera*, *Piliostigma* and *Tylosema* (Wunderlin et al. 1987), out of which *Bauhinia* and *Piliostigma* are the only West African species. Many members are of huge economic import (Burkhil 2000), usually cultivated as ornamental trees worldwide and known to be medicinally significant. Species can be used for prevention of tumours, are antihaemorrhagic, control levels of glucose in the blood, and used for the treatment of constipation and other gastro-intestinal infections (Larsen and Larsen 1991; Duarte-Almeida et al. 2015).

According to LPWG (2017), the taxonomic history and classification of the *Bauhinia* group in its broadest circumscription comprising about 300 to 350 species is likewise complex and particularly difficult to delimit. It is the largest and most taxonomically complex group within the subfamily Cercidoideae (Wunderlin et al. 1987). Within the Bauhinieae, an unresolved species- and genus-level taxonomy has hindered the understanding of the taxonomic significance of the varied morphological and anatomical features and been the subject of a number of regional studies (Wunderlin et al. 1987; Lewis and Forest 2005; Queiroz 2006; Vaz 2010; Wunderlin 2011). Until date, no comprehensive species-level overview has been published. Furthermore, previous analyses using morphological and molecular data suggest contrasting relationships with complicated and poorly resolved evolutionary relationships in this lineage (Bruneau et al. 2001; Sinou et al. 2009). The tribe is currently the subject of much phylogenetic research and combining both molecular and anatomical examination (Banks et al. 2014) will provide useful information on the diagnostic characters at generic and infrageneric taxonomic level. All of the previous phylogenetic studies have concluded that the *Bauhinia* group is non-monophyletic and represents an artificial grouping that could be divided into several genera (Bandyopadhyay and Ghoshal 2015; Mackinder and Clark 2014; Trethowan et al. 2015; Clark et al. 2017). However, Sinou et al. (2020) reported that the subtribe Bauhinieae is weakly supported as monophyletic based on plastid and duplicated nuclear gene sequences. The study portrayed a superfluous taxonomic relationship in the Cercidoideae. Hence, using a single and multi-tiered datasets of three different gene regions in addition to both micro and macro-morphological data, this study present a systematic studies of the tribe Bauhinieae so as to further elucidate on the body of knowledge surrounding this taxon group.

MATERIALS AND METHODS

Plant material

Twenty-two samples of *Bauhinia* and *Piliostigma* species representing 7 species were collected from selected sites in Nigeria in addition to the outgroup species *Detarium macrocarpum* Harms. The outgroup taxon was selected based on results of previous studies, which indicate members of the Detaroidieae is sister to the subfamily Cercidioideae (LPWG 2017). Additional sequences used were downloaded for GenBank. Collected samples were identified and authenticated at the University of Lagos Herbarium (LUH). The voucher number and other information about samples are given in Table 1.

Morphology

A morphologically description of the species was done using their qualitative and quantitative characteristics. Observed qualitative characters include leaf apex,

Table 1. Details about the source of the plant samples used for the study.

S/N	Plant species	Locality	GPS location	Collector's name	Collector's number	GenBank number (ITS)	GenBank number (rbcL)	GenBank number (trnL-F)
1	Bauhinia monandra Kurz.	Kamuku National Park, Kaduna State	10°47'49"N 6°18'20"E	Dr. Aramide Igbari	LUH 9663	KX057835	KX119264	KX268152
2	*Bauhinia tomentosa* L.	Kainji National Park, Niger State	9°59'56"N 4°17'10"E	Dr. Aramide Igbari	LUH 9664	KX057838	KX119268	KX268155
3	*Bauhinia rufescens* Lam.	Yankari Game reserve National Park, Kastina State	9°45'24"N 10°30'34"E	Mr. Daramola	LUH 5124	KX057837	KX119266	KX268154
4	*Bauhinia purpurea* L.	Ahmadu Bello University, Zaria, Kaduna State	11°15'12"N 7°64'46"E	Dr. Aramide Igbari	LUH 9675	KX057836	KX119265	KX268153
5	*Bauhinia vahlii* Wight & Arn.	Ahmadu Bello University, Zaria, Kaduna State	11°15'12"N 7°64'46"E	Dr. Aramide Igbari	LUH 9664	-	KX119267	KX268137
6	*Piliostigma thonningii* (Schum.) Milne-Redh.	Kainji National Park, Niger State	9°59'56"N 4°17'10"E	Dr. Aramide Igbari	LUH 8518	-	KX119320	KX268205
7	*Piliostigma reticulatum* (DC.) Hochst	Yankari Game reserve National Park, Kastina State	9°45'24"N 10°30'34"E	Dr. Aramide Igbari	LUH 9684	KX057894	KX119319	KX268204

leaf base, leaf venation, leaf shape, leaf margin while stem length, petiole length, leaf length, leaf are some of the quantitative characteristics recorded.

Anatomy

Dried specimens from median portion of the leaves near the midrib were carefully cut, and soaked in concentrated nitric acid inside McCartney bottles for about 2-6 hours to macerate the mesophyll and bleach the leaf portions. Tissues disintegration was noticed by bubbles and the epidermal layers were separated and transferred into petri dishes containing water for cleansing and then separated with forceps. Separated strips of adaxial and abaxial surfaces of the leaves were stained with safranin following standard protocols and viewed under the microscope following Ogundipe et al. (2009); Onuminya et al. (2020). The diagnostic features of the adaxial and abaxial surface of the leaves were photographed using Motic image plus version 2.0 mm with MC camera mounted on an Olympus compound light microscope at a magnification of 9600. In addition, the number, length and width of the stomata, and epidermal cells were recorded using a calibrated micrometer eyepiece.

Statistical analysis

The descriptive statistics of the mean, standard deviation, standard error, minimum and maximum value were calculated for all variables. The Stomata Index (S.I) was calculated using the formula of Metcalfe and Chalke (1979):

$$({}^{S}/_{S+E}) \times 100$$

Where, S denotes the number of stomata per unit area and E is the number of epidermal cells of the same area.

In addition, the sequential, hierarchical and nested (SAHN) clustering analysis was done using PAST V4.0 software package on both anatomical and morphological characters. Dendrograms were generated based on Nei genetic distances following Sneath and Sokal (1973).

DNA extraction and amplification

Total genomic DNA was isolated from approximately 0.0300 g of silica-gel dried and 0.0180 g of herbarium plant material following a modified 2X CTAB protocol of Doyle and Doyle (1987). Herbarium samples were precipitated for one week while silica dried for 1hr. Extracted DNA was stored at -20°C prior subsequent use. Polymerase chain reaction (PCR) was performed in 50 µl reaction mixtures containing 25 µl biomix, 1 µl BSA, 2 µl DMSO, 1.75 µl of 10 µM of each primer, 17.5 µl of millipore H2O and 1 µl of 30-50 ng template DNA. Primers according to Sun et al. (1994), Olmstead et al. (1992) and Taberlet et al. (1991) were used for ITS, rbcL and trnL-F regions respective-

Table 2. Amplification profiles.

Region	Initial denaturing Temp./time	Denaturation Temp./time	Annealing Temp./time	Extension Temp./time	Final extension Temp./time	No. of cycles
ITS	97°C/2:00	97°C/1:00	55°C/0:45	72°C/0:45	72°C/7:00	30
matK	94°C/5:00	94°C/0:40	48°C/0:40	72°C/0:40	72°C/7:00	30
trnL-F	94°C/2:00	94°C/1:00	55°C/1:00	72°C/2:00	72°C/10:00	30

ly. PCR profiles run for each region are given on Table 2. Amplifications were run on a Veriti® 96 well thermal cycler. Each PCR product was run on 1% agarose gel stained in ethidium bromide and successful amplified products were sent to Source Bioscience (UK) for bidirectional sequencing using the same primer used in PCR.

Phylogenetic analysis

Chromatographic traces and contiguous alignments were edited using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan). Any uncertain base positions, generally located close to the priming sites, and regions of uncertain alignment were excluded from the phylogenetic analysis. Sequences were aligned and edited in Bioedit (Hall 1999). Informative insertion/deletion events (indels) were identified and coded as binary characters, and gaps were treated as missing data. All three regions were analyzed separately. Less than 1% of the data were scored as missing. A Bayesian analysis (Ronquist et al. 2012) was carried out by first determining the optimal substitution model using MrModeltest v2.3 (Nylander 2004) and the Akaike information criterion. The general reversible model with a gamma shape (GTR+G) was selected for the nuclear ITS region, Hasegawa–Kishono–Yano with a proportion of invariable sites and gamma shape (HKY+I+G) for rbcL region and Hasegawa–Kishono–Yano with a gamma shape (HKY+G) for trnL-F region. Four discrete states were used for the gamma substitution. The data were therefore partitioned into two for the Bayesian analysis and the correct substitution model as specified by MrModeltest was specified for each partition. The partitions were unlinked so that each parameter could be specified separately. Analysis was run for 75,000,000 generations with sampling every 75,000 generations. The first 18,000,000 samples trees were discarded as 'burn in' while the remaining trees were used to build a 50% majority rule consensus tree with posterior probability for nodes.

RESULTS

Morphological and anatomical studies

The qualitative and quantitative foliar morphological characters of the species (Fig. 1) are presented in Tables 3 and 4. All of the species examined have a bifoliate leaf shape except *Bauhinia purpurea* that has orbiculate leaves. Although, there were variations in lobe division of each leaf, their leaf base ranges between cordate and subcordate with entire leaf margin. A palmate reticulate leaf venation, leathery and glabrous leaf surface distinguishes members of *Philiostigma* from *Bauhinia* morphologically. Anatomically, there are variations in the epidermal cells of the species examined; all epidermal cells are polygonal with straight, curved, wavy or undulating anticlinal wall patterns. The two *Piliostigma* species has distinct anticlinal wall patterns (Tables 5 and 6). The stomata shape for *Bauhinia* species are paracytic while *Piliostigma* species possess hemiparacytic stomata. Observed foliar trichomes were mostly non-glandular (*Piliostigma* species and *B. vahlii* and *B. refuscens*), conical and unicellular (*Bauhinia purpurea*) and glandular, unicellular (*Bauhinia tomentosa*). Trichomes were present on both adaxial and abaxial leaf surfaces of the species (Fig. 2). Cluster analysis based on distance matrix revealed similarities and differences among the species. Dendrogram generated from both morphological and anatomical data obtained showed the interrelationship between the species studied at a distance value of 80 (Fig. 3). It revealed the closeness similarity of species to each other based on the examined features. *B. rufescens* showed to be closest to *B. vahlii*, *P. reticulatum* closest to *B. monandra* while *B. purpurea* is closest to *P. thonnongii*.

Molecular studies

The strict consensus tree for all the tested gene regions revealed a polyphyletic *Bauhina* group divided into three major clades. In the ITS gene tree, *B. tomentosa* is clustered with *Piliostigma reticulata* as well as

Figure 1. A = *Bauhinia monandra*, B- *Bauhinia tomentosa*; C = *Bauhinia rufescens*; D = *Bauhinia purpurea*; E = *Bauhinia vahlii*; F=*Piliostigma thonningii*; G = *Piliostigma reticulatum*.

Table 3. Qualitative foliar morphological characteristics of the selected species of the tribe Bauhinieae.

Species	Leaf shape	Leaf apex	Leaf margin	Leaf base	Venation	Leaf surface
Bauhinia monandra	Bifoliate and folded in the centre	Rounded and split up to 1/3 leaf length	Entire	Sub cordate	Palmate	Glabrous
Bauhinia tomentosa	Bifoliate and elliptic	Acuminate and split up to ½ leaf length	Entire	Sub cordate	Palmate	Glabrous
Bauhinia rufescens	Bifoliate	Cordate rounded, and split up to ¾ leaf length	Entire	Sub cordate	Palmate	Glabrous
Bauhinia purpurea	Orbiculate	Emarginate	Cleft, lobed	Cordate	Palmate	Glabrous
Bauhinia vahlii	Bifoliate	Apiculate	Entire	Cordate	Palmate	Hairy
Piliostigma thonningii	Bifoliate	Acuminate and split up to 1/8 leaf length	Entire	Cordate	Palmate Reticulate	Leathery and finely pubescent beneath
Piliostigma reticulatum	Bifoliate	Rounded to cuneate	Entire	Cordate	Palmate reticulate	Leathery and glabrous beneath

Table 4. Quantitative foliar morphological characteristics of the selected species of the tribe Bauhinieae Min (Mean ± S.E) Max.

Species	Stem length (cm)	Leaf length (cm)	Leaf width (cm)	Leaf blade (cm)	Petiole length (cm)
Bauhinia monandra	21.6 (23.1 ± 0.8) 25.4	8.2 (8.6 ± 0.2) 9.1	8.9 (9.6 ± 0.3) 10.2	29.8 (32.9 ± 0.8) 34.4	3.6 (3.8± 0.1) 4.0
Bauhinia tomentosa	39.9 (41.8 ± 0.8) 44.2	4.8 (5.3 ± 0.3) 6.5	4.9 (5.4 ± 0.2) 5.9	16.9 (18.8 ± 0.9) 21.8	1.7 (3.8 ± 0.1) 2.1
Bauhinia rufescens	39.9 (43.4 ± 0.9) 45.4	1.5 (1.6 ± 0.1) 1.8	1.9 (2.1 ± 0.1) 2.3	3.9 (4.5 ± 0.3) 5.7	0.6 (0.8 ± 0.1) 1.1
Bauhinia purpurea	47.2 (47.6± 0.7) 50.1	11.9 (13.1 ± 0.3) 13.8	10.8 (12.6 ± 0.7) 15.1	39.6 (42.2 ± 0.8) 44.7	3.5 (3.8 ± 0.1) 4.2
Bauhinia vahlii	38.4 (40.8 ± 0.9) 43.1	5.9 (6.5 ± 0.2) 7.1	10.9 (11.6 ± 0.3)12.3	17.9 (18.8 ± 0.3) 19.6	2.8 (3.1 ± 0.1) 3.5
Piliostigma thonningii	36.8(38.5 ± 0.7)40.5	10.5 (10.8 ± 0.1) 11.2	11.9 (12.6 ± 0.2) 13.2	33.7 (36.6 ± 0.9) 38.7	3.6 (3.9 ± 0.1) 4.2
Piliostigma reticulatum	39.4 (40.3 ± 0.3) 41.1	5.9 (6.9 ± 0.4) 8.1	8.9 (11.5 ± 0.8) 13.2	19.1 (20.6 ± 0.5) 22.1	4.9 (5.9 ± 0.5) 7.3

Table 5. Qualitative Foliar Anatomical characteristics of the selected species of the tribe Bauhinieae.

Species	Cell wall Shape		Anticlinal Wall shape		Stomata type		Trichome type	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Bauhinia monandra	Polygonal	Polygonal	Straight	Straight	Paracytic	None	Glandular	Glandular
Bauhinia tomentosa	Polygonal	Polygonal	Curved	Curved	Paracytic	None	Glandular, Unicellular	Glandular, Unicellular
Bauhinia rufescens	Polygonal	Polygonal	Straight	Straight	Paracytic	None	Non glandular	Non glandular
Bauhinia purpurea	Polygonal	Polygonal	Straight	Straight	Paracytic	None	Conical and unicellular	Conical and unicellular
Bauhinia vahlii	Polygonal	Polygonal	Straight	Straight	Paracytic	Paracytic	Non glandular	Non glandular
Piliostigma thonningii	Polygonal	Polygonal	Wavy	Wavy	Hemiparacytic	None	Non glandular	Non glandular
Piliostigma reticulatum	Polygonal	Polygonal	Undulating	Undulating	Hemiparacytic	None	Non glandular	Non glandular

with other *Piliostigma* species. (Fig. 4). The strict consensus *rbcL* gene tree (Fig. 5) also revealed a polyphyletic *Bauhinia* group; however, *Piliostigma* species were distributed in a different clade but with *B. rufescens* and *B. tomentosa*. In the *trnL-F* consensus gene tree, *Piliostigma* species were clustered among the three clades of *Bauhina* species, exhibiting closest relationships with *B. rufescens, B. tomentosa* and *B. blakeana* (Fig. 6). The two *P. thonningii* samples were nested in two different *Bauhinia* clades. The phylogram for the concatenated matrix exhibited a similar tree topology to the *trnL-F* gene tree. *Bauhinia* group is polyphyletic while *Piliostigma* species were clustered within the three Bauhinia clades (Fig. 7) also suggesting *B tomentosa* exhibited a closer relation-

Table 6. Quantitative Foliar Anatomical characteristics of the selected species of the tribe Bauhinieae.

Species	Epidermal cell number Min.(mean±S.E)max	Epidermal cell length (μm) Min.(mean±S.E)max	Epidermal cell width (μm) Min.(mean±S.E)max	Epidermal cell wall thickness (μm) Min.(mean±S.E)max
B. monandra				
Adaxial	100.00(103.80±0.91)110.00	10.00(12.50±0.60)15.00	10.00(11.40±0.34)13.00	1.00(1.20±0.13)2.00
Abaxial	60.00(72.70±3.03)88.00	12.00(17.30±0.97)20.00	14.00(16.60±0.52)18.00	1.00(1.50±0.17)2.00
B. tomentosa				
Adaxial	100.00(105.70±1.26)112.00	12.00(18.90±1.06)24.00	11.00(12.80±0.53)16.00	1.00(1.00±0.00)1.00
Abaxial	102.00(116.80±2.98)130.00	11.00(14.10±0.90)18.00	10.00(11.80±0.61)14.00	1.00(1.00±0.00)1.00
B. rufescens				
Adaxial	40.00(53.60±2.38)62.00	56.00(87.20±7.42)140.00	56.00(79.80±3.98)96.00	11.00(17.80±1.31)22.00
Abaxial	40.00(60.30±3.61)76.00	29.00(62.20±4.58)76.00	32.00(47.00±3.29)68.00	7.00(14.00±2.12)25.00
B. purpurea				
Adaxial	98.00(108.70±2.71)120.00	7.00(9.40±0.82)16.00	7.00(8.50±0.56)13.00	1.00(1.60±0.16)2.00
Abaxial	98.00(103.40±1.10)110.00	16.00(20.10±0.75)24.00	11.00(13.40±0.69)16.00	1.00(1.40±0.16)2.00
B. vahlii				
Adaxial	7.00(9.80±0.59)12.00	50.00(60.80±3.00)77.00	38.00(47.50±2.58)57.00	6.00(8.00±0.47)10.00
Abaxial	6.00(9.50±0.79)12.00	52.00(64.20±3.17)80.00	31.00(50.10±6.53)89.00	7.00(9.50±0.5)11.00
P. thonningii				
Adaxial	91.00(102.70±1.66)110.00	16.00(18.90±0.69)22.00	11.00(13.40±0.54)15.00	1.00(1.50±0.17)2.00
Abaxial	72.00(88.6±3.58)107.00	12.00(13.70±0.42)16.00	11.00(13.00±0.56)16.00	1.00(1.00±0.00)1.00
P. reticulatum				
Adaxial	93.00(104.70±1.96)120.00	18.00(19.80±0.79)32.00	11.10(14.70±0.65)17.50	1.30(1.90±0.27)4.80
Abaxial	78.00(66.8±5.58)107.00	13.00(15.70±0.84)17.90	14.00(12.50±0.56)16.00	1.51(1.40±0.31)1.70

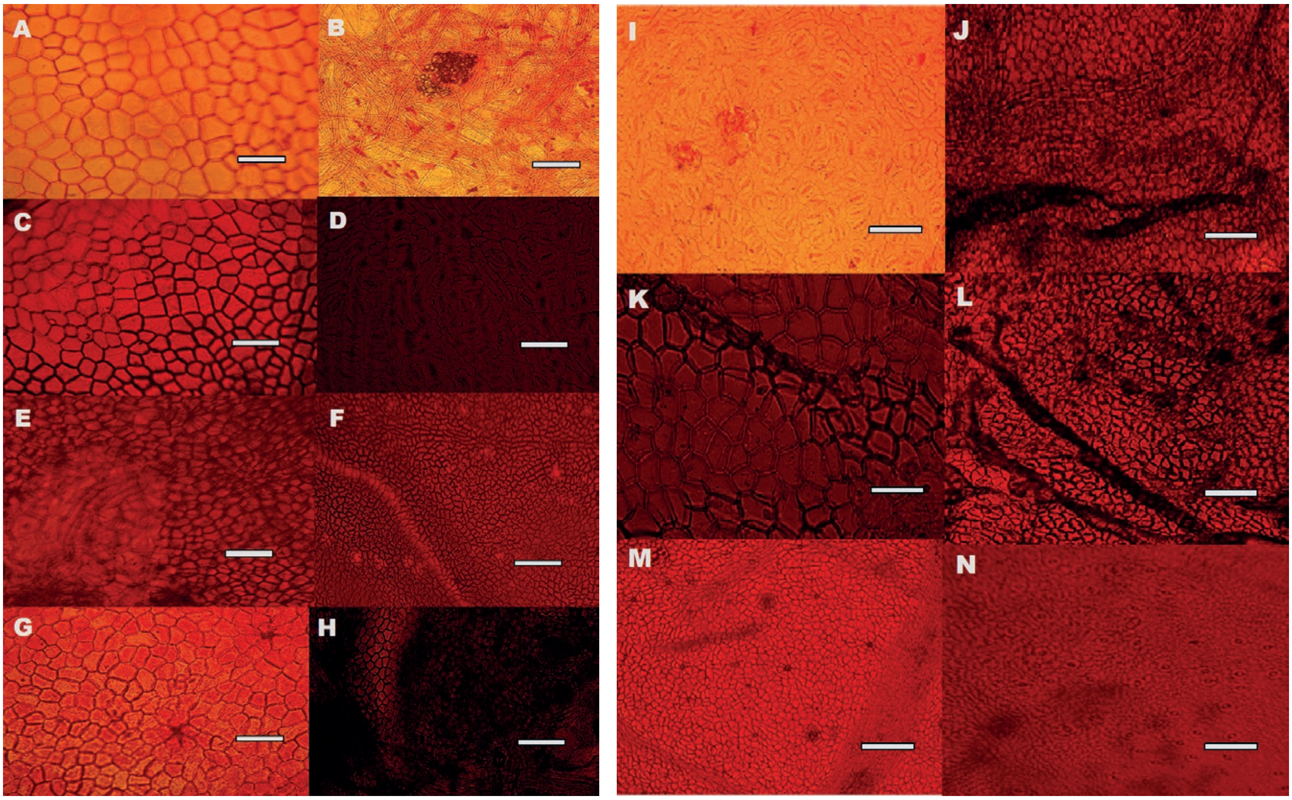

Figure 2. Leaf epidermal of members of the Bauhininieae: adaxial on the left, abaxial on the right, A,B- *Bauhinia monandra*, C,D- *B. tomentosa*, E, F- *B. rufescens*, G, H- *B. purpurea*, I,J- *B. vahlii*, K,L- *Piliostigma thonningii*, M, N- *P. reticulatum* Scale bars: 50μm.

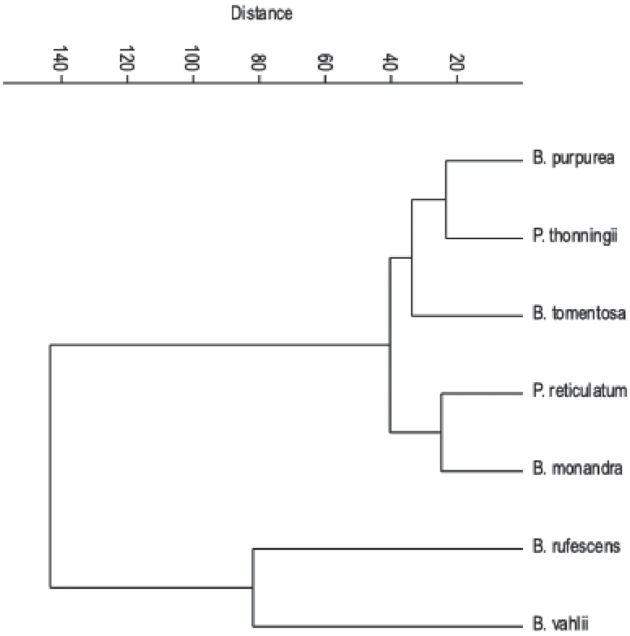

Figure 3. UPGMA similarity tree showing relationships amongst members of the tribe Bauhinieae studied based on combined morphological and anatomical data.

ship with *Piliostigma* species. The robustness of most clades were at high bayesian inference of >9 indicating a higher resolution of species cluster at distinct node with a high posterior probability.

DISCUSSION AND CONCLUSIONS

The systematic studies of the tribe Bauhinieae was elucidated based on morphological and molecular data in order to unravel the relationship among members of this group. Both anatomical and morphological data were analyzed in addition to molecular data using two chloroplast regions (*rbcL*, *trnL-F*) and the nuclear ITS region. Results from both data revealed a polyphyletic *Bauhinia* and *Piliostigma* group, some *Bauhinia* species were clustered among *Piliostigma* species.

Morphologically, members of the genus *Bauhinia* generally possess bilobate leaves with glabrous surface; amongst all species examined, only *B. vahlii* possess hairy leaves supporting descriptions provided by Elbanna et al. (2016). The present study showed that the epidermal shape in all *Bauhinia* species are polygonal and the cell walls are straight except *B. tomentosa*, which has an undulating cell wall supporting Duarte-Almeida et al. *(*2015). Vaz and Tozzi (2005) confirmed the stomatal types observed among the two genera studied. According to Carpenter and Smith (1975), variations in stomatal frequencies have taxonomic importance at a generic level. After the quantitative investigations of stomatal frequency and index of the species examined, there was a remarkable variation between the two genera showing that these characters were significant at the genus level supporting of Patil and Patil (1987), Ogundipe at al. (2009), Onuminya et al. (2020).

Carlquist (1961) emphasizes the contribution of stomatal size variation in delimiting species within a genus. Major variations in stomatal frequencies of *B. monandra* and *B. tomentosa* are also notable; the distribution of stomata is likewise specific in *B. purpurea*, with amphistomatic stomata, while other studied species exhibited hypostomatic stomata supporting Metcalfe and Chalk (1979) and Albert and Sharma (2013). *B. vahlii* is characteristically distinct in its leaf margins and veins. Some dissimilarities were observed in the trichome index of the species studied. *Bauhinia* species possess both long and short hairs, but with variations in size and morphology of the hair, this corroborates a proposed hypothesis of Pereira et al. (2018). Trichomes observed are mainly unicellular, long, and tapers to a pointed tip except *B. tomentosa* whose hairs are nonglandular, while both *P. thonningii* and *P. reticulatum* lack trichomes as illustrated by Bannerje et al. (2002). These results confirms the importance of trichomes in taxonomic studies. Hence, based on the observed foliar morphological and anatomical features, a diagnostic key is proposed as below:

1a. Leaf bifoliate, palmate veination with paracytic stomata...... ....***Bauhinia*** L.

2a. Polygonal cell wall with straight anticlinal cell wall 3

2b. Polygonal cell wall with curved anticlinal cell wall ....***B. tomentosa***

3a. Leaf surface glabrous, each leaf lobe rounded and split up to 1/3 of leaf length..***B. monandra***

3b. Leaf surface glabrous, leaf lobe cordate or rounded and split up to 3/4 of leaf length...............................***B. rufescens***

4a. Leaf apex emarginate with conical and unicellular trichomes..***B. purpurea***

4b. Leaf apex apiculate with nonglandular trichomes. ***B. vahlii***

1b. Leaf bifoliate, palmate reticulate venation with hemiparacytic stomata.. ***Piliostigma*** Hochst.

6a. Leaf finely pubescent beneath, and leaf apex acuminate .... ***P. thonningii***

6b. Leaf glabrous beneath and leaf apex rounded to cuneate..... .... ***P. reticulatum***

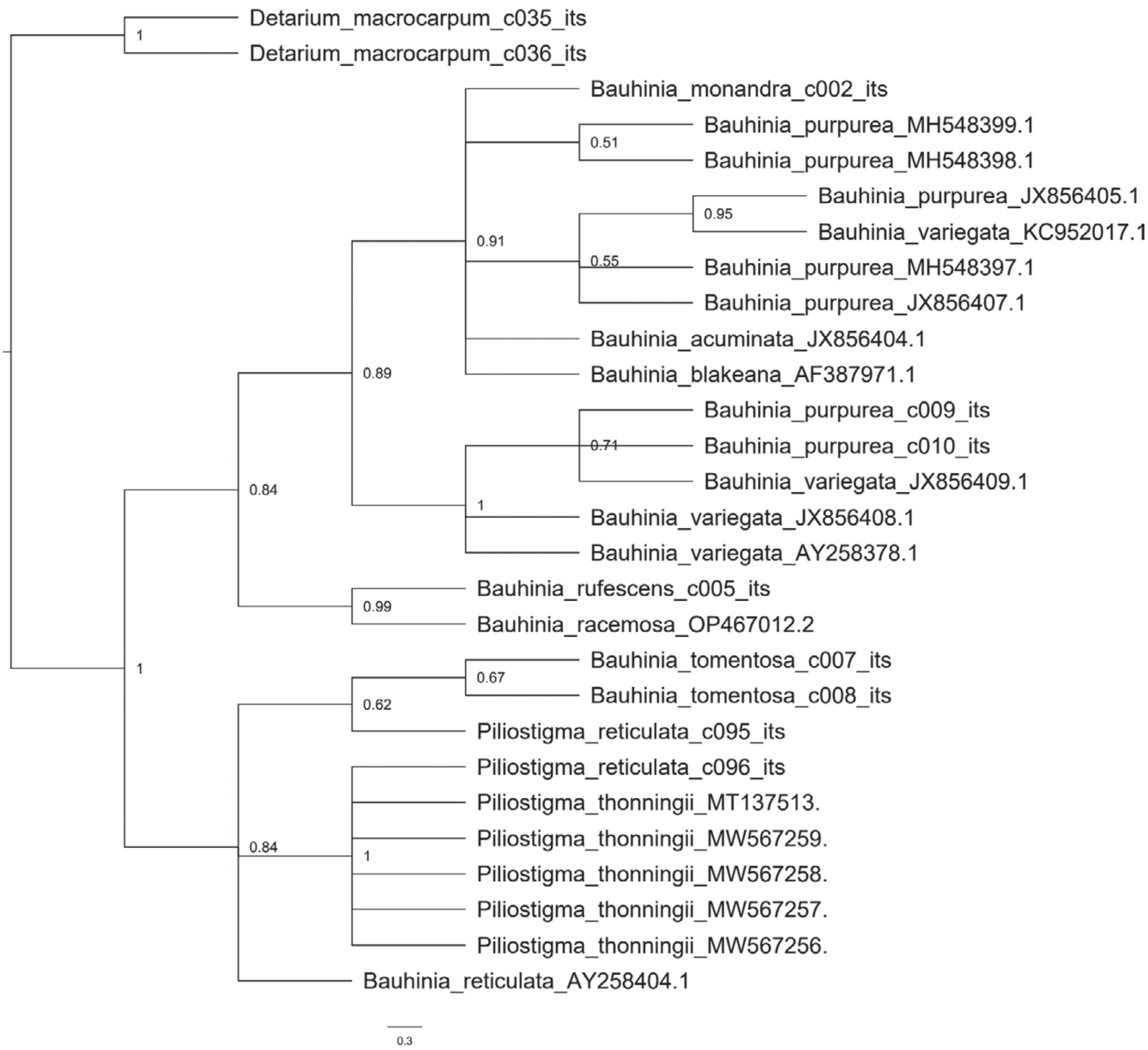

Figure 4. Phylogram inferred by Bayesian analysis (ITS), numbers at node indicate posterior probability value.

The phylogenetic pattern of the Bauhinieae has always been controversial. In this study, based on three gene regions, we explored the generic patterns of this taxon. In previous studies, *Piliostigma* group has been debated to be monophyletic (Hao et al. 2003; Sinuou, 2020), this submission contradicts our findings. The ITS and concatenated matrix phylo-tree exhibited a polyphyletic relationship with the *Bauhinia* ss group. Although, *Piliostigma* species has distinct morphological features, but a polyphyletic group was observed from both morphological and anatomical data. Hence, employing morphological, anatomical and molecular data *Piliostigma* species exhibits a close relationship with *B. rufescens*, *B. tomentosa* and *B. purpurea*. This supports the reports of some authors who propose *Piliostigma* as a section of *Bauhinia*, rather than as a separate genus (Bentham 1865; Wunderlin et al. 1987; Zhang 1995; Hao et al. 2003).

In addition, results from this study observed a complex phylogenetic pattern among the *Bauhinia* s.s. group and these results support previous works and proposition to divide members of this large group into subclades. In this study, the *Bauhina* species were divided into 3 subclades at weak to strong bayesian inference. The dendrogram using morphological and anatomical

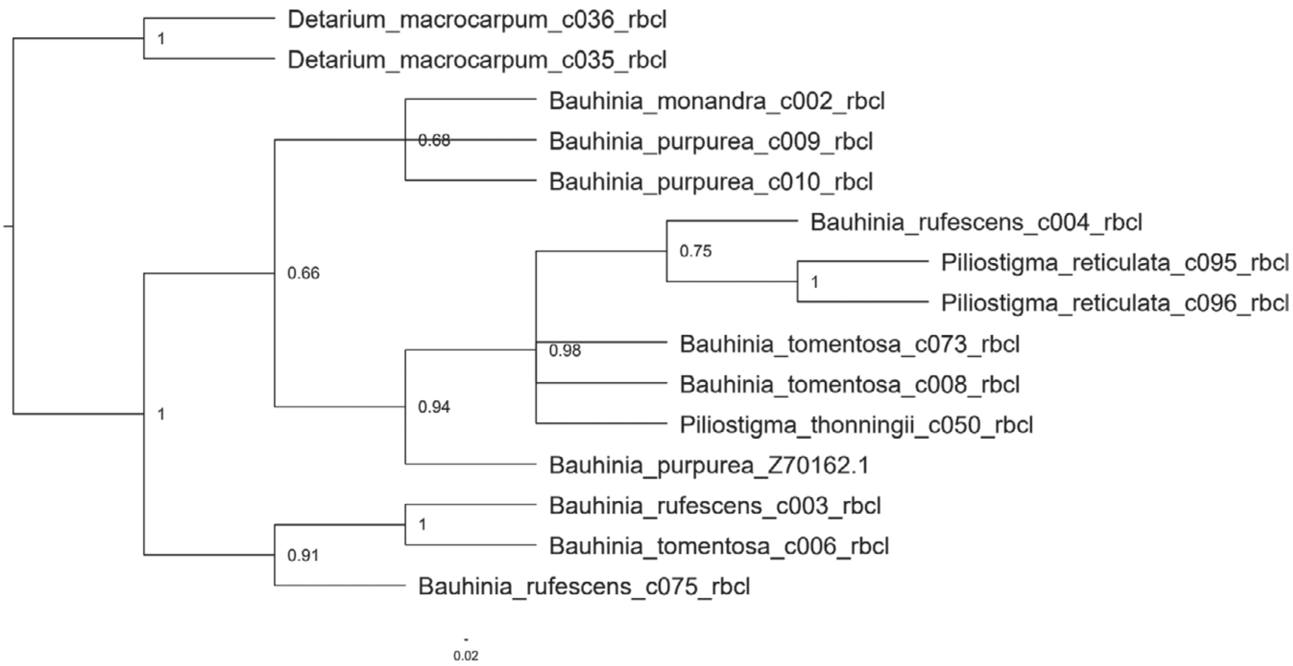

Figure 5. Phylogram inferred by Bayesian analysis (*rbcL*), numbers at node indicates posterior probability value.

data also presented 3 clades; *B. rufescens* and *B. vahlii*; *B. tomentosa* and *B. purpurea* and *B. monandra* groups. The ITS phylo-tree presented two clades comprising clade 1: *B. purpurea*, *B. monandra, B. acuminata, B. variegata* and *B. blakeana;* clade 2: *B. rufescens* while the rbcL phylo-tree exhibited clades comprising *B. purpurea*, *B. monandra* and *B. rufescens*, *B. tomentosa* groups. A similar complex topology was observed for trnL-F phylo-tree and the concatenated matrix phylo-tree. This corroborates Sinou et al. (2020) that posited that the subtribe Bauhinieae is weakly supported as monophyletic. Although Sinou et al. (2020) made a proposition for a geographical distribution of species into groups, suggesting species from each region to be grouped into a clade. Within the West African members of the tribe Bauhinieae, results from this study revealed a polyphyletic relationship. This could probably be as a result of the limited sampling of this taxon as well as the poor performance of some species during amplication of the selected gene regions. Similarly, it was observed that some species were phylogenetically divergent in relationship with members of other species e.g. *P. thonningii*, *B. pupurea* and *B. variegata*, this could be as a result of different localities of sampling or misrepresentation of samples. A powerful solution would likely be found in a denser sampling and highly variable character selection for better species resolution.

In summary, the phylogeny based on both chloroplast and nuclear DNA as well as morphological and anatomical data confirms the polyphyly of Bauhinieae. Our results show that similarities in the morphological and anatomical structures of members of this taxon were due to some evolutionary processes and this has posed a complexity in their classification. Furthermore, the monophyly of the *Piliostigma* group exhibited a paraphyletic and polyphyletic relationship with the *Bauhinia* group at high support values. The relationship among the West African *Bauhinia* species is polyphyletic and remain unresolved. This study has attempted to elucidate the unresolved species- and genus-level taxonomy of the tribe Bauhinieae. However, more variable gene regions in addition to broader species sampling should be considered for further phylogenetic patterns of this taxon.

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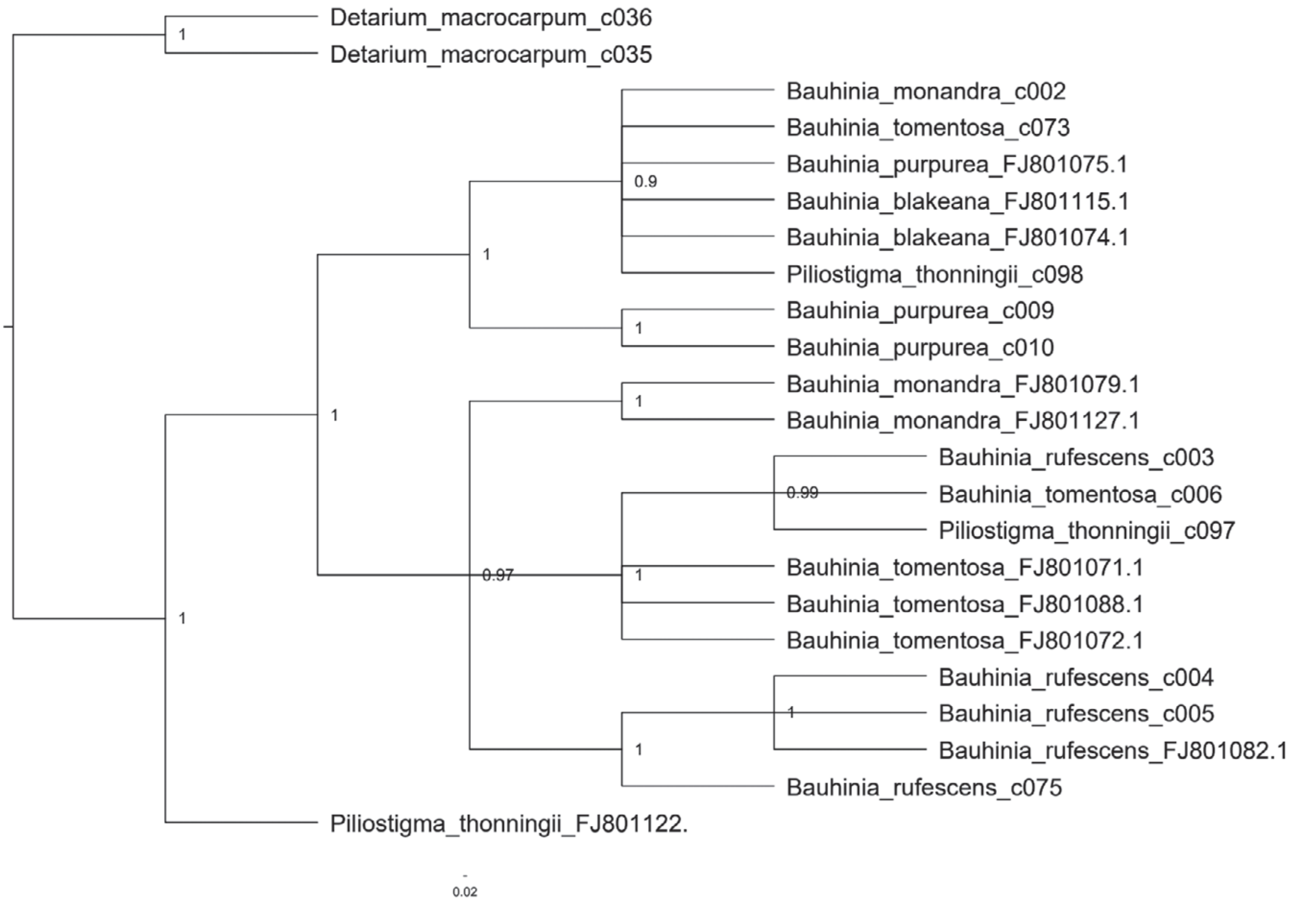

Figure 6. Phylogram inferred by Bayesian analysis (*trnL-F*), numbers at node indicates posterior probability value.

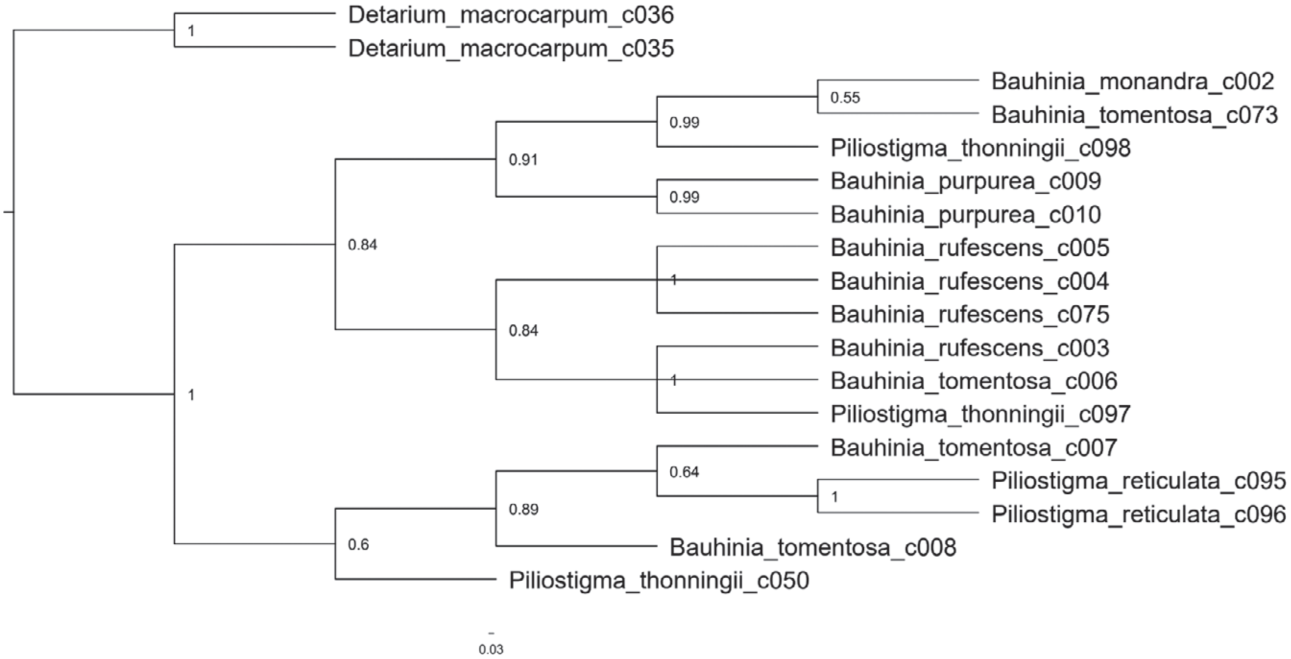

Figure 7. Phylogram inferred by Bayesian analysis (ITS+*rbcL*+*trnL-F*), numbers at node indicates posterior probability value.

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Three new species and a new record of *Monstera* Adans. sect. *Marcgraviopsis* Madison (Araceae: Monsteroideae: Monstereae) from the Caribbean watershed in Costa Rica and Panama

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Abstract. Three new species from *Monstera* sect. *Marcgraviopsis*, *M. caribaea* M.Cedeño, O.Ortiz & A.Hay, *M. lamersiana* M.Cedeño & A.Hay and *M. panamensis* M.Cedeño & O.Ortiz, are described, and new record of *M. guzmanjacobiae* Díaz Jim. et al. for Costa Rica is reported here. The new species are compared with the most similar described species with shingling juveniles, and illustrated from living plants.

Keywords: Araceae, Central America, conservation, *Monstera*, taxonomy.

INTRODUCTION

The genus *Monstera* has been considered one of the most taxonomically difficult Neotropical groups within the Araceae, despite its relatively small size compared to the giants *Anthurium* and *Philodendron* whose species are estimated to number in the thousands (Grayum 2003; Boyce and Croat, 2011 onwards; Cedeño-Fonseca et al. 2022). It is represented by approximately 52 species in Central America, which are distributed in wet tropical forested regions at low to medium elevations, from 0–2300 m above sea level (Grayum 2003; Cedeño-Fonseca et al. 2021; Croat et al. in progress). Nearly all are appressed-climbing hemi-epiphytes or nomadic vines (Zotz 2013; Sperotto et al. 2020).

Monstera Adans. has been divided into four formal sections (Madison 1977): section *Echinospadix* Madison (1 sp.), section *Marcgraviopsis* Madison (up till now 11 spp.), section *Tornelia* Madison (3 spp.) and section *Monstera* (37 spp.) (Cedeño-Fonseca et al. 2020, 2022). Work on fuller systematic understanding of this genus is now much progressed but still ongoing, and, although a valuable preliminary molecular phylogenetic analysis of the Monstereae has been made (Zuluaga et al. 2019), indicating among other things that Madison's sections in *Monstera* are partly unnatural, a complete molecular phylogenetic analysis is not yet available to provide the appropriate level of certainty for a revised infrageneric classification to be developed (Cedeño-Fonseca et al. 2022). We therefore persist with Madison's sections for now.

Section *Marcgraviopsis* is characterized by the earliest climbing phase having the leaf blades themselves closely appressed to the substrate (often termed 'shingle plants') with petioles that are less than half as long as the blades (Madison 1997). The great majority of the species in this section are Central American, and a mere four of them also occur in South America: the widespread *Monstera spruceana* (Schott) Engl. and *M. dubia* (Kunth) Engl. & K.Krause along the northern Andes and Amazonia, and *M. pittieri* Engl. and *M. filamentosa* Croat & Grayum reaching only into the Department of Chocó in northwestern Colombia close to Panama.

Nevertheless, we note here, in passing, that species of the exclusively South American, almost entirely Amazonian *Monstera subpinnata* Engl.—*M. barrieri* Croat, Moonen & Poncy complex also have appressed shingling juveniles, but of somewhat distinct morphology from the juveniles of sect. *Marcgraviopsis* [the most obvious, but not the sole difference being the shingling blades held obliquely upward, versus the more usual obliquely downward posture of sect. *Marcgraviopsis* shingling blades]. These species have never been placed in sect. *Marcgraviopsis*, we presume because, in spite of their radical and abrupt differentiation from the adult form, the heteroblastic juveniles have not been noted until relatively recently, and appear to be unrepresented in herbarium collections. The infrageneric placement of this complex also awaits fuller resolution of phylogenetic relationships within the genus, and more detailed description of their heteroblastic phases will form part of a forthcoming commentary on morphological diversity in *Monstera* (Cedeño-Fonseca et al., in prep.).

Despite the recent taxonomic revision of *Monstera* for Costa Rica by Cedeño-Fonseca et al. (2022) and for Central America as a whole by Croat et al. (in progress), new species of the genus are still being documented along the Cordillera Volcanica Central in Costa Rica and the Cordillera de Talamanca between Costa Rica and Panama. High intraspecific variation within populations and the fragmentary nature of herbarium samples hampers the establishment of evident morphological characters needed for the accurate identification of all species (Grayum 2003; Cedeño-Fonseca et al. 2022). Furthermore, populations encountered in the field only in the sterile state have impeded fuller understanding of species limits. Documentation in the natural state of morphological characteristics of seedlings, juveniles, and adult fertile plants has been essential.

Fieldwork conducted between 2015 and 2021 aimed to document *Monstera* in different regions in Mexico, Costa Rica and Panama, as part of the the first author's master's thesis (Cedeño-Fonseca 2019). However, some populations of the genus found in the Caribbean of Costa Rica and Panama could not be identified or included within the range of variation of species already described for Central America. With further observations, we consider that three such taxa represent undescribed species from the Caribbean watershed between Costa Rica and Panama, and a fourth is a new record for Costa Rica of a species recently described from Mexico (Díaz-Jiménez et al. 2020).

We therefore describe and illustrate these three new species belonging to the sect. *Marcgraviopsis* based on morphological evidence, and newly record *Monstera guzmanjacobiae* Díaz Jim. et al., from the same section, for Costa Rica.

MATERIAL AND METHODS

To compare morphological characters, living plants and herbarium specimens were evaluated. Besides the literature analysis from Central and South America, the following herbaria were consulted (acronyms follow Thiers continuously updated): B, CHIP, COL, CR, CUVC, HEM, HLDG, HUAZ, JAUM, JVR, LSCR, MA, MEXU, MO, NY, PMA, SEL, SCZ, UCH, UJUAT, USJ, XAL, as well as images and type specimens accessible on-line at COL, EAP, MEXU, and JSTOR Global Plants (2022).

Photographs of living plants were taken with a Nikon COOLPIX P530 and also mobile phones with integrated high-resolution cameras, such as Huawei Y7 and Huawei p20. Measurements were made on herbarium specimens and living plants in the nature. Due to the high demand for aroid species as ornamental plants, and a rapidly growing black market that endangers native populations (even in protected areas), coordinates are here omitted from all specimen citations, and no distribution maps are provided. Stated life zones follow the terminology of Holdridge et al. (1971).

TAXONOMY

Monstera caribaea M.Cedeño, O.Ortiz & A.Hay, **sp. nov.** (Figure 1).

Type: Costa Rica, Provincia Limón, Cantón Talamanca, distrito Cahuita, alrededores de Puerto Viejo, 20 m, 30 Jan 2019, *M. Cedeño & A. Hay 1615* (holotype USJ!).

Diagnosis

Monstera caribaea has similarities to *M. pittieri*, with which it grows sympatrically, but differs in having larger leaves on adult plants 20–35 × 15–21 cm (vs. 13–18 × 5–10 cm in *M. pittieri*), margins entire or pinnatilobate (vs. entire margins never pinnatilobate), erect inflorescences on ascending and/or pendulous stems (vs. pendulous inflorescences on pendulous stems), peduncle 4.0–5.0 cm long (vs. 1.0–4.0 cm long), spadix 12–13 × 2.5–3.5 cm (vs. 4.0–9.0 × 1.5–3.5 cm), styles not separated from adjacent ones (vs. the styles slightly separated from adjacent ones), and the style shorter than the ovary (vs. style longer than the ovary).

Description

Robust nomadic vine, of appressed-climbing and sometimes later also pendent habit. Seedlings: filiform. Juvenile plants: root climbers; stems green, smooth, flattened; internodes 2.0–8.0 cm long, 3.0–6.0 mm diam.; petiole not visible (covered by blade), dark green, smooth, 2.0–4.0 cm long; blades obovate, cordate at base, obtuse at apex, coriaceous, 4.0–11 × 5.0–9.0 cm, appressed to the phorophyte; fenestrations absent. Adult plants: root climbers; stem light or dark green, smooth; internodes 3.0–13 cm long, 1.0–1.5 mm diam.; anchor roots beige; feeder roots brown; petiole dark green, smooth, striated at the base, 20–25 cm long, sheathed to the base of the geniculum; petiole sheath semi-persistent; geniculum smooth or striated, slightly terete, 2.0–3.0 cm long; blades lanceolate, rounded or subcordate at base, apex acuminated, thinly coriaceous, 20–35 × 15–21 cm, midrib grooved above, convex below; primary lateral veins 9.0–11 per side, submerged on upper surface, prominent on lower surface; secondary venation parallel, reticulate towards margin; collecting vein slightly visible; fenestrations present or absent, when present, only one side of blade and narrow; margins entire or pinnatifid, due to tearing of the fenestrations that extend to the margin, often only one side is pinnatifid. Inflorescences: on both ascending attached and/or free hanging stems; peduncle smooth, 4.0–5.0 cm long; spathe obtuse or mucronate, in developing inflorescences light green, colour at anthesis unknown; spadix white during development, in male anthesis cream, 12–13 × 2.5–3.5 cm, the basal region of sterile flowers narrowed towards the peduncle; basal sterile flowers 1.5–3.0 mm long; fertile flowers 5.0–7.0 mm long; stamens not seen; ovary rectangular in longitudinal section and ribbed, 3.0–4.0 × 1.5–2.0 mm; style compressed and hexagonal, 1.5–2.0 × 1.5–3.0 mm; stigma linear; berries with stylar layer after anthesis yellowish green, mature stylar cap unknown; pulp unknown; seeds unknown.

Etymology

The species epithet refers to the Caribbean coast, where the new species was first discovered.

Distribution and habitat

This species is endemic to the southern Caribbean slopes of Costa Rica and western Caribbean slope of Panama, at 20–25 m elevation. It occurs in *Tropical wet forest* life zones, in open areas.

Phenology

Flowering time is unknown but fruiting was recorded in January and March.

Notes

The species is a member of sect. *Marcgraviopsis*. It differs from other species of the section by its appressed-climbing and pendent habit, highly variable blades with the presence of lobes and perforations, but sometimes restricted to only one side of the blade, and the erect inflorescence with short peduncle <5 cm long. The hanging stems of *Monstera caribaea* that connect to the ground are similar to the adult individuals of *M. pittieri*, mainly in that some have perforations in only one margin of the blade.

Additional specimens examined (paratypes).

PANAMA. Provincia Veraguas: distrito Santa Fe, corregimiento San Fe, 18 Mar 2021, 25 m, *M. Cedeño, O. Ortiz & J.E. Jiménez 2351* (PMA); Veraguas: carretera hacia Calovebora, cerca del Río Luis, 194 m, 28 Sep 2019, *O. Ortiz & M. Cedeño 3938* (PMA).

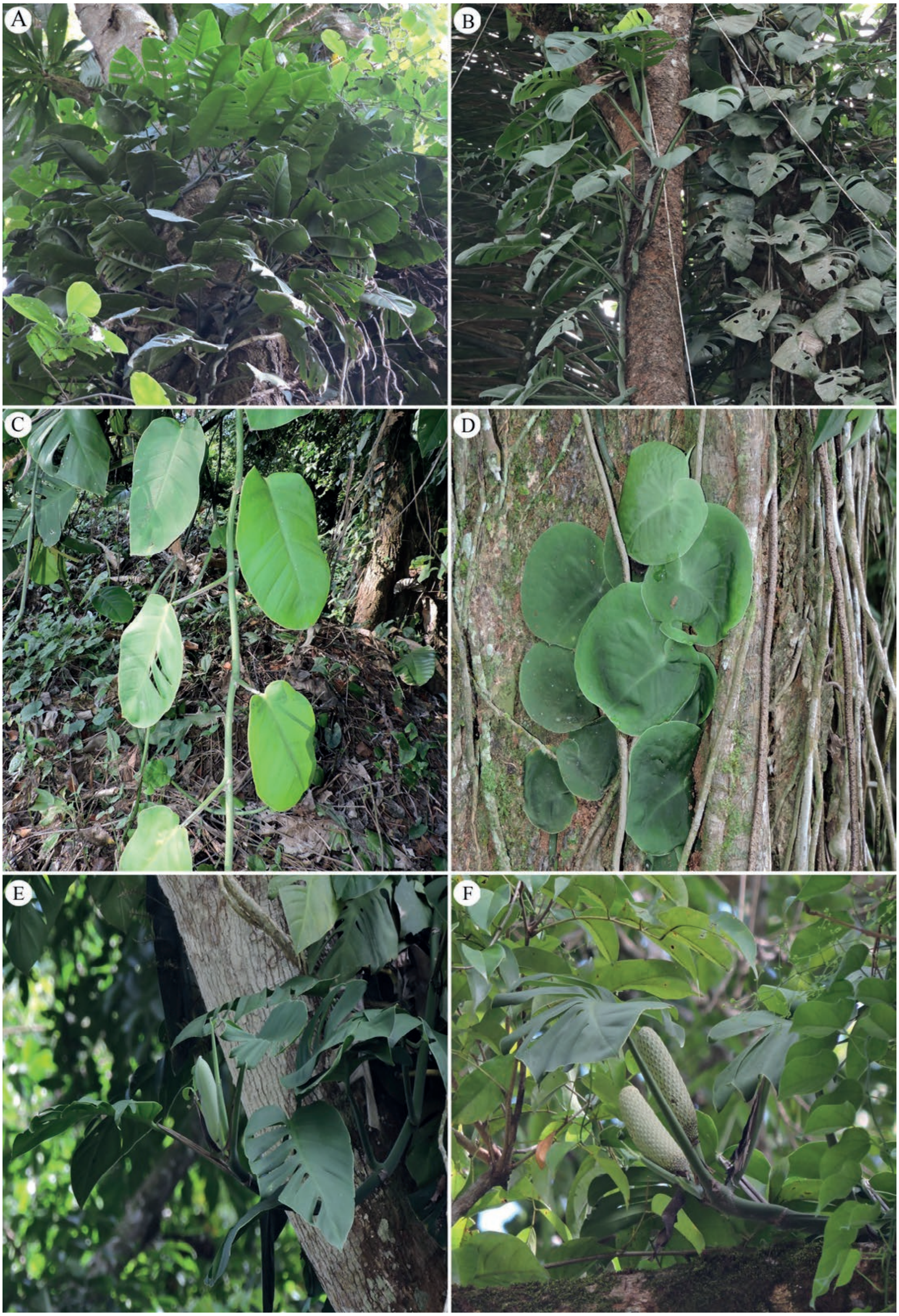

Figure 1. *Monstera caribaea*. **A.** Adult plant, leaf-blades perforated and with 2–5 lobes per side. **B.** Adult plant, the leaf-blade entire and with 2 or 5 lobes per side. **C.** Hanging stem leaf-blade with 2 or 3 perforations on one side. **D.** Juvenile plant appressed to the host tree. **E.** Hanging stem with erect inflorescence in development. **F.** Climbing stem with infructescence in development. Photos by Marco Cedeño-Fonseca. *M. Cedeño & A. Hay 1615* (USJ).

Monstera lamersiana M.Cedeño & A.Hay, **sp. nov.** (Figure 2).

Type: Costa Rica, Provincia Alajuela, Cantón San Carlos, distrito Florencia, Reserva del Hotel Talajari, 43 m, 18 Jan 2021, *M. Cedeño & J. Mark Hughes 2206* (holotype USJ!; isotype MO!).

Diagnosis

Monstera lamersiana is similar to *M. filamentosa*, from which it differs vegetatively (in adult plants) by having a shorter petiole (15–25 cm vs. 25–55 cm in *M. filamentosa*), petiole green, (vs. cream or pink), and smaller leaf blade (34–38 cm × 28–33 cm vs. 35–105 cm × 25–40 cm), and the ripe infructescence on a hanging peduncle (vs. ripe infructescence on an erect peduncle).

Description

Nomadic vine, appressed-climbing. Seedlings: filiform. Juvenile plants: root climbers; stems smooth, dark green, flattened; internodes 3.5–9 cm long × 0.5–1 mm diameter; blades appressed to the phorophyte with visible petiole, dark green, smooth, 2–7 cm long, sheathed to blade base, petiole wings persistent or deciduous; blade lanceolate, cordate at the base, apex acuminate, thinly coriaceous, 7–12 × 3.5–7 cm, slightly flattened to the phorophyte, without fenestrations. Adult plants: root climbers; stem brown to light brown or beige, dorsoventrally compressed, smooth, with longitudinal striations; internodes 3–14 cm long × 1.5–2.5 mm in diameter; anchor roots; dark brown; petiole grooved at the base, adaxially flattened, dark green, 15–25 cm long, sheathed to the base of the geniculum; petiole sheath deciduous, with fibrous residues close to the apex; geniculum smooth, slightly terete, 3–4 cm long; blades oblong-ovate, rounded or subcordate at base, apex obtuse, thinly coriaceous, 34–38 × 28–33 cm, midrib flat on upper surface, convex below; primary lateral veins 9–11 per side, submerged on upper surface, prominent on lower surface; secondary veins pinnate and reticulate; collective vein not visible, lamina completely entire with or without fenestrations near midrib, and then perforations oval or triangular, or deeply pinnatifid, with 5–8 lobes per side, 2.5–4 cm broad, with 1–2 veins per lobe, connected by filaments. Inflorescences: on ascending adherent stems, solitary; peduncle smooth, 4–10 cm long; spathe dark green during development, unknown in anthesis; spadix white during development, in anthesis unknown; basal sterile flowers unknown; fertile flowers unknown; anthers unknown; ovary unknown; style compressed and hexagonal, 1–2 × 3–4 mm; stigma linear; infructescence ovoid-cylindric 6.0–8.0 × 3.0–4.0 cm on a pendent peduncle; berries with a green stylar cap during development and when mature; pulp white; seeds yellowish, 4–5 mm long.

Etymology

This species is named in honour of Gijsbertus Lamers, who has generously supported exploration of both *Monstera* and *Philodendron* in Central America.

Distribution and habitat

This species is endemic to Costa Rica. It is currently known only in the region of Muelle de San Carlos, at elevations of ca. 40–300 m, in the Caribbean slope. It occurs in *Tropical wet forest* life zones, in primary forests.

Phenology

Flowering time is unknown but fruiting was recorded in January.

Notes

The species is a member of sect. *Marcgraviopsis*. It differs from the other species of the section by its internodes 3–14 cm long with longitudinal striations, its smooth dark green petiole striated at the base and adaxially flattened; its deciduous petiole sheath, and almost terete geniculum; the lanceolate leaf blade, rounded or subcordate at the base, with the apex obtuse; and the ripe infructescence on a hanging peduncle with the stylar layer green and the pulp white. *Monstera lamersiana* usually has pinnatilobate leaves, with the basal lobes connected to each other by filaments. Another characteristic of *M. lamersina* is that some individuals have the leaf blade with an entire margin and without perforations. These occur sympatrically with populations of individuals with pinnatilobed and fenestrate blades.

Additional specimens examined (paratypes).

COSTA RICA. Provincia Alajuela: Cantón San Carlos, distrito Florencia, 31 Jan 2019, 43 m, *M. Cedeño & A. Hay 1615* (USJ); Cantón San Carlos, 9 km north of Ciudad Quesa, on road to La Florencia; in patch of forest along Rio Peje on property of Jose Corrales, 3 Jun 1986, 300 m, *B. Hammel & G. de Nevers 15309* (MO).

Monstera panamensis M.Cedeño & O.Ortiz, **sp. nov.** (Figure 3).

Type: Panama, Coclé: Parque Nacional Omar Torrijos Herrera. El Copé, carretera que va hacia La Rica, 771

Figure 2. *Monstera lamersiana*. **A.** Adult plant, leaf-blades pinnatilobed with 5–8 lobes per side. **B.** Adult plant, leaf-blades entire without perforations. **C.** Juvenile plant appressed to the host tree. **D.** Adult plant, leaf-blades pinnatilobed with 5–8 lobes per side with pendulous infructescence in development. Photos by Marco Cedeño-Fonseca. *M. Cedeño & J. Mark Hughes 2206* (USJ).

m, 30 Sep 2019, *O.O. Ortiz & M. Cedeño 3950* (holotype PMA!; isotype MO!).

Diagnosis

Monstera panamensis is most easily confused with *M. molinae* and *M. spruceana*, but it differs from *M. molinae* in having larger leaves on adult plants (20–45 cm to long vs. 10–30 cm long), pinnatilobed and with perforations (vs. completely pinnatilobed without perforations), and the style hexagonal, distally slender and cylindrical, and strongly projecting, (vs. a distally square, cylindrical or hexagonal projecting style). It differs from *Monstera spruceana* in having inflorescences on free hanging stems (vs. on attached ascending stems), the petiole completely smooth (vs. smooth or warty), the petiole sheath deciduous without fibrous remnants (vs. deciduous with fibrous frag-

Figure 3. *Monstera panamensis*. **A.** Adult plant, leaf-blades pinnatilobed with 2–6 lobes per side. **B.** Adult plant with hanging stem, leaf-blades pinnatilobed with 2–6 lobes per side, erect infructescence in development. **C.** Infructescence in development with style strongly projected and distally cylindrical. **D.** Fruit with style strongly projected and distally cylindrical. Photos by Marco Cedeño-Fonseca. *O.O. Ortiz & M. Cedeño 3950* (PMA).

ments), and style hexagonal and strongly projecting and cylindrical distally (vs. style hexagonal and not raised).

Description

Robust nomadic vine, appressed-climbing and pendent habit. Seedlings: filiform. Juvenile plants: root climbers; stems dark green, smooth, cylindrical; internodes 2–6 cm long, 0.5–10 mm diam.; petiole not visible, dark green, smooth, 3–5 cm long; blades obovate, subcordate at base, acuminate at apex, coriaceous, 5–12 × 6–10 cm, appressed to the phorophyte; fenestrations present, usually 1 that breaks at the margin. Adult plants: root climbers with free lateral branches; stems dark-green or brown, cylindrical, smooth; internodes 3–15 cm long, 2–4 cm diam.; anchor roots light brown; feeder roots dark brown; petiole light-green, smooth, 17–30 cm long, sheathed to base of the geniculum or to base of the blade; petiole sheath deciduous; geniculum smooth, sunken adaxially, convex abaxially, 2–4 cm long; blades lanceolate or ovate to sub-orbicular, obtuse or truncate at base, obtuse to slightly acuminate at apex, coriaceous, 20–45 × 17–20 cm, decurrent on the geniculum; midrib ribbed adaxially, convex abaxially; primary lateral veins 7–10 per side, sunken adaxially, prominent abaxially; collective veins not visible; fenestrations present in transition to adult plants and in some adult leaf blades; margins pinnatilobed, 2–6 lobes per side, 1.5–5 cm wide, 1–2 veins per lobe. Inflorescences: in hanging stems, 1 solitary at flowering time, arranged in the axils of the leaves; peduncle smooth, up to 6 cm long; spathe unknown in development and anthesis; spadix unknown during development, unknown at anthesis, 13–15 cm long, 3–4 cm diam.; basal sterile flowers 5–7 mm long; fertile flowers 7–10 mm long; stamens unknown; anthers unknown; ovary rectangular in longitudinal section, ribbed, more slender than style, 4–6 × 2–3 mm; style hexagonal and strongly projected, slender and cylindrical distally, 4–5 × 3–4 mm, stigma linear; berries with a green stylar cap during development, mature stylar cap unknown; pulp unknown; seeds unknown.

Etymology

The epithet is drawn from the Republic of Panama, and alludes to the species' origin.

Distribution and habitat

This species is endemic to Panama, at elevations of 0–850 m, on the Caribbean and Pacific slope. It occurs in *Tropical wet forest* and *Premontane rain forest* life zones, in primary forest.

Phenology

Flowering time is unknown but fruiting was recorded in September and November.

Notes

The species is a member of section *Marcgraviopsis*. It is distinguished by its lengthy internodes up to 15 cm long, the pinnatilobed adult leaf blade, the smooth petiole, sheathed up to the base of the geniculum, short peduncles (<6 cm), long spadices (14–15 cm), and a hexagonal, strongly projecting, and distally cylindrical style.

J.P Folsom 6207 (MO) was long confused with *M. molinae*. However, due to differences that could be observed in the flowers in the herbarium sample, fieldwork was carried out to examine the populations in their natural state, with the result that this collection is here redetermined as *M. panamensis*. The majority of populations of *M. panamensis* from the Caribbean lowlands have not been seen with reproductive structures.

Additional specimens examined (paratypes)

PANAMA. Provincia Coclé: Area surrounding Rivera Sawmill, 7 km north of El Cope; Forgotten Hill; 5 Nov 1977, 650-850 m, *J.P. Folsom 6207* (MO); Parque Nacional Omar Torrijos Herrera, El Copé, carretera que va hacia La Rica, 771 m, 30 Sep 2019, *O. O. Ortiz & M. Cedeño 3949* (PMA). **Provincia Veraguas:** distrito Santa Fe, corregimiento San Fe, Camino al pueblo Calovebora, 18 Mar 2021, 25 m, *M. Cedeño, O. Ortiz & J.E. Jimenez 2353* (PMA); corregimiento Calovebora, Camino de regreso al pueblo Cucuyo, 22 Nov 2022, 120 m, *M. Cedeño, O. Ortiz & N. Köster & R. Dapena 2802* (PMA).

New record

Monstera guzmanjacobiae Díaz Jim., M.Cedeño, Zuluaga & Aguilar-Rodr., Phytotaxa 437(1): 41. 2020.

Type: Mexico, Veracruz: Municipio Catemaco, La Palma, Selva alta perennifolia, 18º 33' 21'' N, 95º 03' 35'' W, 56 m, 31 May 2014, *Pedro Díaz Jiménez & Valeria Guzmán Jacob 1305* (holotype XAL!; isotypes, MEXU!, UJAT!).

The species is characterized by its habit with appressed-climbing and pendent shoots, terete stems, light-green, smooth, dorsiventrally compressed petioles each with a projecting ligule up to 3.0 cm long, adult leaf blades with 1–4 fenestrations often only on one side, or without perforations, and flowers with a conical pyramidal style (Díaz-Jiménez et al. 2020). *Monstera guzmanjacobiae* was described as endemic from Los Tuxtlas (Veracruz, Mexico) and it has never been collected in other localities apart from the municipality of Catemaco (Díaz-Jiménez et al. 2020). Plants from Mexico and Costa Rica display rather wide ecological amplitude, generally growing on the edge and interior of the forest, as well as in abandoned areas with secondary vegetation. (Fig. 4).

At present, *Monstera guzmanjacobiae* is only known to occur in Mexico and Costa Rica. This disjunct geographic distribution may be related to the relative lack of sampling of the Araceae in general and *Monstera* in par-

Figure 4. *Monstera guzmanjacobiae*. **A.** Adult plant ascending on a tree and with hanging stems. **B.** Hanging stem with erect infructescence in development (note: divided upper left leaf is of *M. filamentosa*). **C.** Developing infructescences on ascending stem. **D.** Developing infructescences with pyramidal and conical style. Photos by Marco Cedeño-Fonseca. *M. Cedeño & J.M. Hughes 2205* (USJ).

ticular in Belize, El Salvador, Honduras, Guatemala, and Nicaragua. A similar situation is found in *M. tacanaensis* Matuda, which is distributed in the Tacana Volcano, Chiapas Mexico and in Guatemala, and then jumps to Costa Rica and Panama (Cedeño-Fonseca et al. 2020).

Additional specimen examined

COSTA RICA. Provincia Heredia: Cantón Sarapiquí, Distrito Horquetas, Camino sobre bosques aislados en potreros, 202 m, 17 Jan 2021, *M. Cedeño & J. Mark Hughes 2205* (USJ).

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Two new species of *Ooia* (Araceae) from Sabah, Malaysian Borneo

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Abstract. Two new species of *Ooia* (Araceae) from Sabah, Malaysia, *Ooia sayapensis* Kartini and *Ooia ulusenagangensis* Kartini are described as new species. Both are distinguished from the only current Sabahan species, *Ooia kinabaluensis* (Bogner) S.Y. Wong & P.C. Boyce by the presence of pistillodes at the pistillate flower zone.

Keywords: Araceae, *Ooia*, Sabah, Malaysia Borneo, rheophytes.

INTRODUCTION

Wong and Boyce (2010) defined *Ooia* by the spadix axis remaining fresh through to fruit maturation and the spent flowers shedding at the post-anthesis. The group is monophyletic (Boyce and Wong 2016), accepting 10 species with the *Ooia kinabaluensis* (Bogner) S.Y.Wong & P.C.Boyce is the only species recorded from Sabah and Brunei.

The spathes of the genus *Ooia* S.Y.Wong & P.C.Boyce are remarkably similar in external appearance. However, the spadix at anthesis is critically crucial as the diagnostic characteristics in this genus (Wong and Boyce 2010). Thus, the present presence of about 5-9 spirals of pistillodes at the base of the pistillate flower zone in the two species of Sabahan's *Ooia* to be described here apparently represents an undescribed taxonomic novelty compared to the very few pistillodes in *O. kinabaluensis*.

Recognition of these two species, namely *O. sayapensis* and *O. ulusenagangensis* will takes *Ooia* to three species in Sabah and 12 species on Borneo.

Ooia sayapensis Kartini, **sp. nov.** (Figure 1).

Type: Malaysia, Sabah, Sayap, Kinabalu UNESCO Global Geopark, 6°09.775'N 116°33.935'E at 938 m asl, 14 April 2019, *Kartini BORH 2710* (holotype BORH!)

Diagnosis

Ooia sayapensis are differentiated from *O. kinabaluensis* by having pistilloides with about 5–9 spirals almost equalling 1/4 of the spadix length,

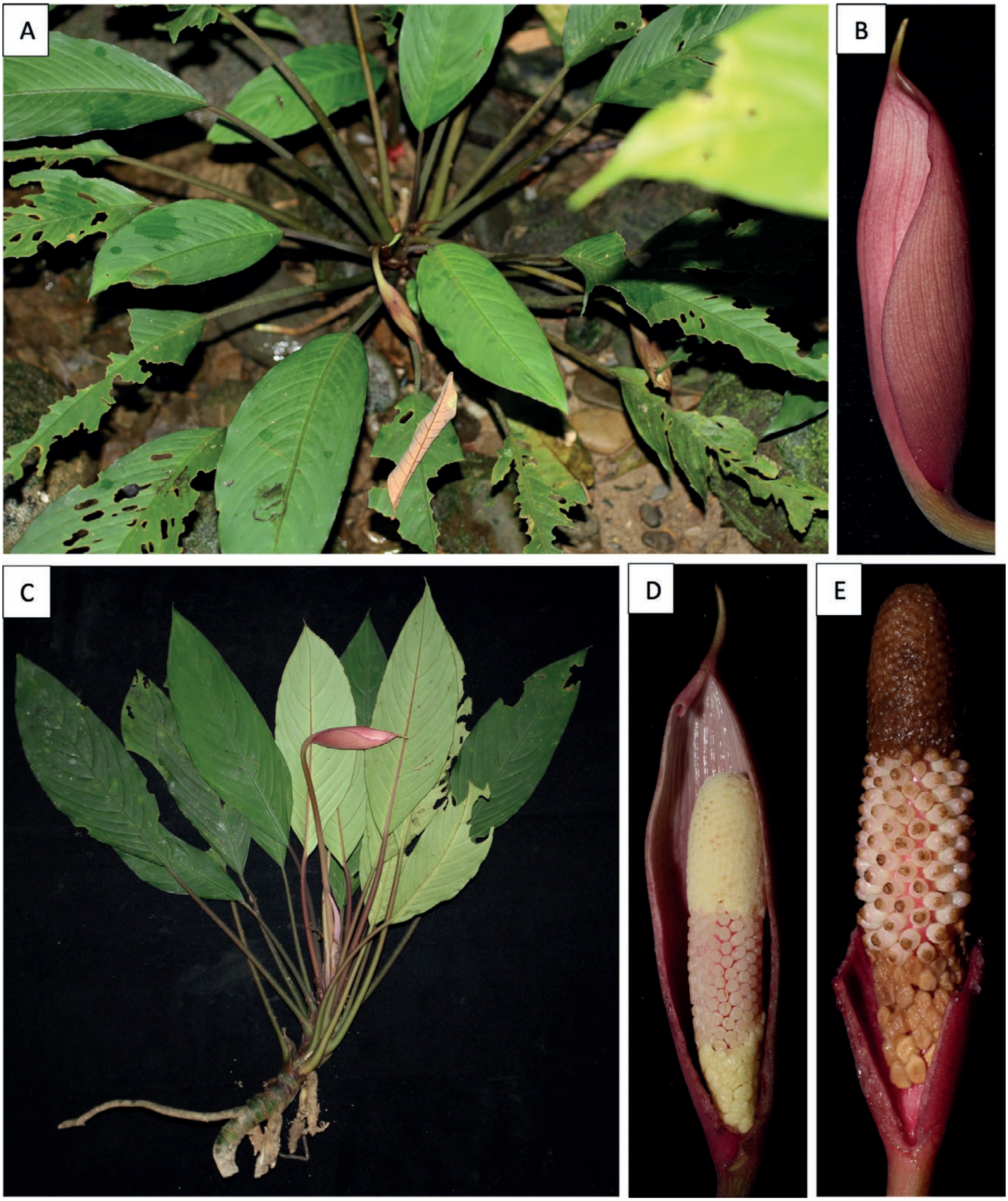

Figure 1. *Ooia sayapensis* – A: flowering plant in habitat; B: Inflorescence; C: whole plant; D: inflorescence at onset of pistillate anthesis (spathe artificially removed); E: inflorescence at later stage of anthesis – All from Kartini BORH 2710 – All photographs by Kartini Saibeh.

and by the pink pistil (versus very few pistilloides and white pistil). Inflorescences smelling of isoamyl acetate (fruity pear odour) at pistillate anthesis is a characteristic shared with *O. kinabaluensis*.

Description

Medium-sized rheophytic herb c. 10–30 cm tall. Stem rather elongated sometimes branched rhizomes, c. 15-20 cm long, internodes to c. 1.5 cm long, c. 1.5–2 cm diam., greenish brown to reddish brown; rooting along length, strongly adhesive, c. 3–5 mm thick. Leaves up to 15 together, loosely clustered at shoot tips and also distributed along stem; petiole 8–15 cm long, c. 3 mm diam., sheathing at the extreme base, the wings extended into a triangular somewhat marcescent ligular portion up to c. 1–1.5 cm; blade weakly coriaceous, elliptic to ovate, c. 10–18 cm long × c. 4–6 cm wide, base cuneate, apex acute to shortly acuminate c. 2 mm, blades dark green adaxially, abaxially light green; midrib robust, adaxially less prominent, abaxially prominent; primary lateral veins 8 on each side, alternating with interprimaries, diverging at 30–40°, often somewhat reddish brown; secondary venation fine, adaxially more or less obscure; tertiary venation adaxially obscure, abaxially forming a faint tessellate reticulum. Inflorescence solitary with prophylls, with up to four produced in sequence each interspersed by a foliage leaf; fragrance weakly isoamyl acetate; peduncle erect, exceeding petioles, c. 16 cm long, slender, pale green to reddish brown. Spathe subcylindric, c. 3 cm long, reddish, persistent, apiculate for c. 2–4 mm, nodding on an erect peduncle at anthesis. Spadix c. 2.5 cm long, stoutly cylindrical, sessile, obliquely adnate to the spathe at the base; pistillodes present, confined to a robust zone below the female zone of up to 5–9 oblique spirals, slightly exceeding height of pistils, irregularly polygonal flat-topped, milky white, c. 1.1 mm diam.; pistillate flower zone slightly obconic, c.1 cm long, c. 8 mm diam., pinkish; pistils laxly arranged, c. 1 mm high, c. 0.8 mm diam.; stigma sessile, discoid, pale orange; interstice absent; staminate flower zone c. 1.2 cm long, bullet-shaped, apically obtuse; stamens minutely pubescent. Fruits completely contained within the persistent spathe; berry sub-cylinder c. 2 mm height, c. 1 mm diam., flat stigmatic remnant on top, greenish.

Etymology

From Sayap + *ensis*, a suffix denoting the place of origin.

Distribution

Recorded only from Sayap substation, located at the north of the Kinabalu UNESCO Global Geopark in Kota Belud.

Ecology

Rheophytic on shaded Quaternary sandstones, riverine boulders under wet upper hill forest between 900-1100 m above sea level.

Notes

Ooia sayapensis occurs consistently on shaded water-splashed boulders. This medium size rheopyhtic herb have elongated sometimes branched rhizome-like stem and rooting along its length.

Ooia ulusenagangensis Kartini, **sp. nov.** (Figure 2).

Type: Malaysia, Sabah, Ulu Senagang, Crocker Range National Park, 5°22.00'N 116°01.656'E, 539 m asl, 17 April 2019, *Kartini BORH 2711* (holotype BORH!).

Diagnosis

The presence of several rows of pistilloides at the base of pistillate flowers zone is a characteristic shared with *O. sayapensis*. *Ooia ulusenagangensis* has a densely arranged glassy white pistil, which is differentiated from *O. sayapensis* (laxly arranged pink pistil) and *O. kinabaluensis* (laxly arranged white pistil), as shown in Figure 3. The inflorescences smelling of isoamyl acetate at pistillate anthesis is very weak in *O. ulusenagangensis* compared to the strong smell in *O. kinabaluensis*.

Description

Medium-sized rheophytic herb 10–30 cm tall. Stem condensed, c. 5 cm long, c. 1.5 cm diam., greenish; roots strongly adhesive, 3–5 mm thick. Leaves up to 15 together; petiole 9–11 cm long, c.3 mm diam., sheathing at the extreme base, the wings extended into a triangular somewhat persistent ligular portion up to 1/3 of the petiole length; blade weakly coriaceous, lanceolate to oblong lanceolate, c. 13 cm long × c. 4 cm wide, base cuneate, apex acute, sometimes acuminate c. 3 mm, blades light green adaxially, abaxially paler; midrib robust, abaxially prominent; primary lateral veins 8–12 on each side, alternating with interprimaries, diverging at 30°- 40°, often somewhat reddish brown; secondary venation fine, adaxially more or less obscure; tertiary venation adaxially obscure, abaxially obsure or forming a very faint tessellate reticulum (most easily seen in dry material). Inflorescences solitary with prophylls; no detectable distinct fragrance or very weak fruity odour, peduncle erect, exceeding petioles, c. 16 cm long, slender, pale green to reddish brown. Spathe subcylindric, c. 4 cm long, reddish, persistent, apiculate for c. 7 mm; nodding on an erect peduncle at

Figure 2. *Ooia ulusenagangensis* – A: flowering plant in habitat; B: Inflorescence; C: inflorescence at onset of pistillate anthesis (spathe artificially removed); D: whole plant; E: inflorescence at later stage of anthesis – All from Kartini BORH 2711. – All photographs by Kartini Saibeh.

Figure 3. Spadices of *Ooia* compared – A: *O. sayapensis* Kartini; B: *O. ulusenagangensis* Kartini; C: *O. kinabaluensis* (Bogner) S.Y. Wong & P.C. Boyce – All photographs by Kartini Saibeh.

anthesis. Spadix c. 2.5 cm long, stoutly cylindrical, sessile, obliquely adnate to the spathe at the base; pistillodes present, confined to a robust zone below the female zone of up to 5-9 oblique spirals, slightly exceeding height of pistils, irregularly polygonal flat-topped, milky white, c. 1 mm diam.; pistillate flower zone slightly obconic, 6–8 mm long, c. 5 mm diam., glassy white; pistils densely arranged, c. 1 mm high, c. 1 mm diam.; stigma sessile, discoid; interstice absent; staminate flower zone c. 1 cm long, bullet-shaped, apically obtuse; stamens minutely pubescent. Fruits completely contained within the persistent spathe; berry sub-cylinder c. 2 mm height, c. 1 mm diam., flat stigmatic remnant on top, creamy.

Etymology

From Ulu Senagang + *ensis*, a suffix denoting the place of origin.

Distribution

Recorded only from Ulu Senagang substation, located at the western end of the Crocker Range National Park in Keningau.

Ecology

Rheophytic on exposed Oligocene sandstone, riverine boulders between 400-600 m asl. The surrounding vegetation consists of disturbed hill dipterocarp forest.

Notes

Ooia ulusenagangensis occurs on exposed bare rocks along river of open canopy forest and is frequently exposed to dry conditions at times of low water. A somewhat persistent petiolar ligular about 3 cm long was observed and easily seen in the living collection.

Key to the species of *Ooia* of Sabahan species

1a. Pistillodes absent or very few at the base of spadix.............. ....**O. kinabaluensis**

1b. Pistillodes present in several spirals at the base of spadix.... 2

2a. Pistils lax, pinkish; Sayap, Kota Belud............**O. sayapensis**

2b. Pistil dense, glassy white; Ulu Senagang, Keningau ....**O. ulusenagangensis**

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Schismatoglottideae (Araceae) of Borneo LXXVI — Two new *Burttianthus* species from Sarawak

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Abstract. Two new species of *Burttianthus* are described from Sarawak: *B. rejangicus* from the Rejang and Balleh rivers of the western Rejang Basin, and *B. solus* from the Similajau Basin, Bintulu. Both novelties are illustrated from living plants and the spadices of eight of the nine *Burttianthus* species are compared in accompanying figures.

Keywords: Araceae, *Burttianthus*, Malaysia, Kapit Division, Bintulu Division, Borneo.

INTRODUCTION

Burttianthus S.Y.Wong, S.L.Low & P.C.Boyce (Low et al. 2018) is a genus of seven species, two further herein described, of obligate Steenisian rheophytes (Boyce and Wong 2019) restricted to N Borneo, occurring from the Tatau Basin of Bintulu, through Brunei (although apparently absent from the eastern part of the country), to Lawas wedged between the eastern portion of Brunei and Sabah. The species are divisible into three groups. Five described species, including the type of the genus, *B. caulescens* (M.Hotta) S.Y.Wong & P.C.Boyce, plus the two here proposed, have numerous glabrous staminate florets arranged in longitudinally aligned pairs, with the horns inside the lip of the anther cavity when the rim is thick, to more or less marginal when rim is thin, and an absent or vestigial appendix. Another group, with three species including *Burttianthus veluntandrus* (S.Y.Wong, S.L.Low & P.C.Boyce) S.Y.Wong & P.C.Boyce, has at most two spirals of pubescent staminate florets of similar structure to the preceding, and an appendix accounting for up to half the spadix. The third group, comprising *B. longipedunculatus* (M.Hotta) S.Y.Wong & P.C.Boyce and *B. purseglovei* (Furtado) S.Y.Wong & P.C.Boyce, have anthers with the rims very thin, and long thecae horns exserted far from the anther cavity, with the numerous glabrous staminate florets arranged in longitudinally aligned pairs, and the spadix fertile to the tip.

Burttianthus is unusual among obligate schismatoglottid rheophytes in that species are frequently co-occur, as for example at Bukit Kana in the western Tau Range where *B. caulescens* and *B. longipedunculatus* and *B. purseglovei* occur, although in different ecologies, and at Mulu where *B. hansenii* (Bogner) S.Y.Wong & P.C.Boyce and *B. purseglovei* are respectively on upper hillforest sandstones and on lowland shales. A further peculiarity of the genus is the seemingly relatively widespread nature of some of the species — *B. caulescens* and *B. purseglovei* apparently occur almost throughout the lowland range of the genus, in stark contrast to many other taxa in the tribe in which species' distributions are often extremely restricted. However, these apparently widespread distributions may equally be owing to imprecise taxonomy. Certainly, this appears to be the situation with *B. caulescens* wherein the problem is exacerbated by inadequate field observations and overreliance on poor quality of herbarium material, resulting in several cryptic species having been obscured.

Here we describe two new species that until now have remained undetected within a too widely circumscribed *B. caulescens*.

Geology in this paper is specified based on Hutchinson (1989, 2005) and Tate (2001).

Burttianthus rejangicus S.Y.Wong, M.Lo & P.C.Boyce, **sp. nov.**

Type: Malaysia. Sarawak, Kapit, Kapit town, Sungai Seranau, 2°02'00.7"N 112°57'01.8"E, 66 m asl. 29 Dec 2021, *Wong Sin Yeng & P.C.Boyce AR-5317* (holotype SAR!; isotype SAR - spirit!). (Figures 1, 2, 3 & 5C).

Diagnosis

Burttianthus rejangicus is unique by the fusiform pistillate floret zone narrower than the staminate floret zone, the almost inflated rims to the stamens, and by the dull pale green stigmas with a central depression.

Description

Small obligate Steenisian rheophytes to 20 cm tall, but usually about half this. Stem initially congested with numerous leaves in a terminal tuft, older plants with stems sub-decumbent and rooting from the lower parts with the active portion erect, 1–6 cm long, 3–4 mm in diam.; petiole ca 2.5 cm long, ca 1.5 mm in diam., adaxially canaliculate, sheathing at extreme base; petiolar sheath with wings extended into a very narrowly triangular ligular portion 2 cm long soon drying dark red-brown and then marcescent; blade thinly coriaceous, adaxially dark green, paler abaxially, elliptic to elliptic-lanceolate, 2.5–7 cm long × ca 1 cm wide, base narrowly cuneate, apex acute, apiculate for ca 1 mm, margin somewhat thickened and smooth to conspicuously undulate; midrib adaxially and abaxially prominent, primary lateral veins indistinguishable from interprimary venation, diverging at 20–35° and running to a more or less thick marginal vein; secondary venation adaxially and abaxially very faint to completely obscure; tertiary venation mostly completely obscure in living material, forming a faint tessellate reticulum in dry material. Inflorescence solitary; peduncle stout, exceeding petioles, ca 2 cm long × 1.5 mm in diam., terete, pale green; spathe more or less ovoid with apex recurved, not constricted, ca 4 cm long and apically beaked to ca 5 mm; lower part campanulate, green, persistent, upper part gaping at anthesis, glistening white with the dorsal median middle part stained green, caducous during or just following staminate anthesis, apical beak medium

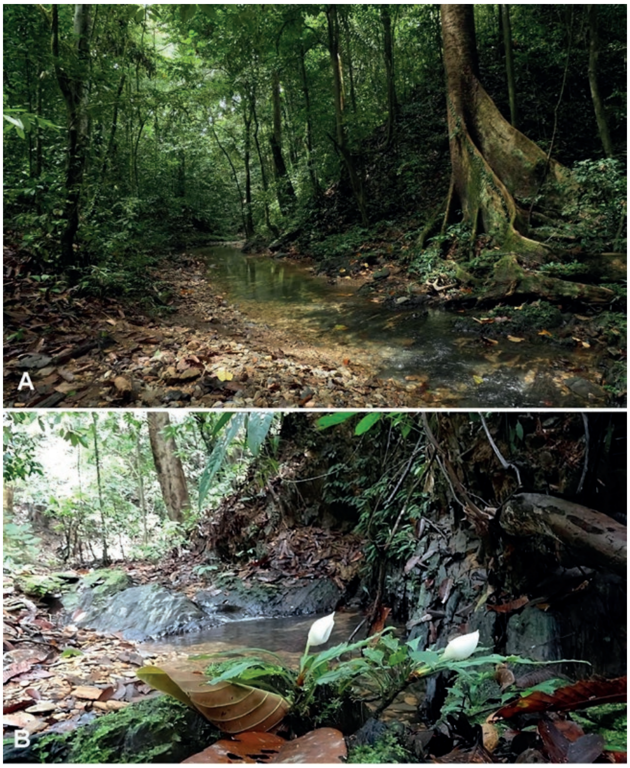

Figure 1. *Burttianthus rejangicus* S.Y.Wong, M. Lo & P.C.Boyce. A. Habitat. B. Plants flowering in habitat. Photos: Mike Lo.

Figure 2. *Burttianthus rejangicus* S.Y.Wong, M. Lo & P.C.Boyce. A. Bloom at pistillate anthesis. B. Bloom at pistillate anthesis, nearside spathe artificially removed. Photos: P.C.Boyce.

Figure 3. *Burttianthus rejangicus* S.Y.Wong, M. Lo & P.C.Boyce. A. Bloom at onset of staminate anthesis, spathe limb beginning to shed. B. Bloom at late staminate antheses, spathe limb fallen to leave the persistent lower spathe. Photos: P.C.Boyce.

green. Spadix stoutly cylindrical, ca 3.5 cm long, ca 1 cm in diam.; pistillate floret zone ca 7 mm long, fusiform, narrower than remainder of spadix, comprised of 5–6 crowded spirals of sub-globose pistils ca 1.5 mm in diam.; stigma sessile, discoid, centrally impressed, slightly wider than ovary, closely appressed to the neighbouring stigmas, dull medium green; interpistillar staminodes confined to a row along spathe/spadix adnation, rhomboid-topped, very shortly stalked, medium yellow, ca 0.9 mm in diam., very slightly shorter than pistillate florets; sterile interstice furnished with a single row of much-reduced sterile stamens, these clavate with the tops narrowly hollowed out, pale yellow; staminate floret zone ca 2.5 cm long, composed of many crowded spirals of fertile glabrous stamens arranged in longitudinally aligned pairs with each staminate floret interpreted as being composed of two stamens, these slightly obliquely facing one another with the deeply excavated thecae together on inner (with respect to stamen pairs) side of anther, stamens ellipsoid to ellipsoid-oblong from above, ca 1 mm long × 2 mm wide; thecae separated by a ridge forming a septum in the cavity, the outer margins of each stamen thickened and inrolled, very shortly horned, the horns inside the lip of anther cavity and pointing laterally, glossy pale yellow; appendix ca 1 cm long, comprised of individualized sterile stamens and patches for diminutive sterile florets. Fruiting spathe thick-walled, obconic fruits and seeds not observed.

Etymology

From Rejang, plus Greek *-ikos* [*-icos*], indicating belonging to.

Distribution

Known only from the Rejang and Balleh rivers of the western Rejang basin where the small populations are widely scattered.

Ecology

Exposed shales in the flood zone along forest streams under lowland perhumid forest, very occasion-

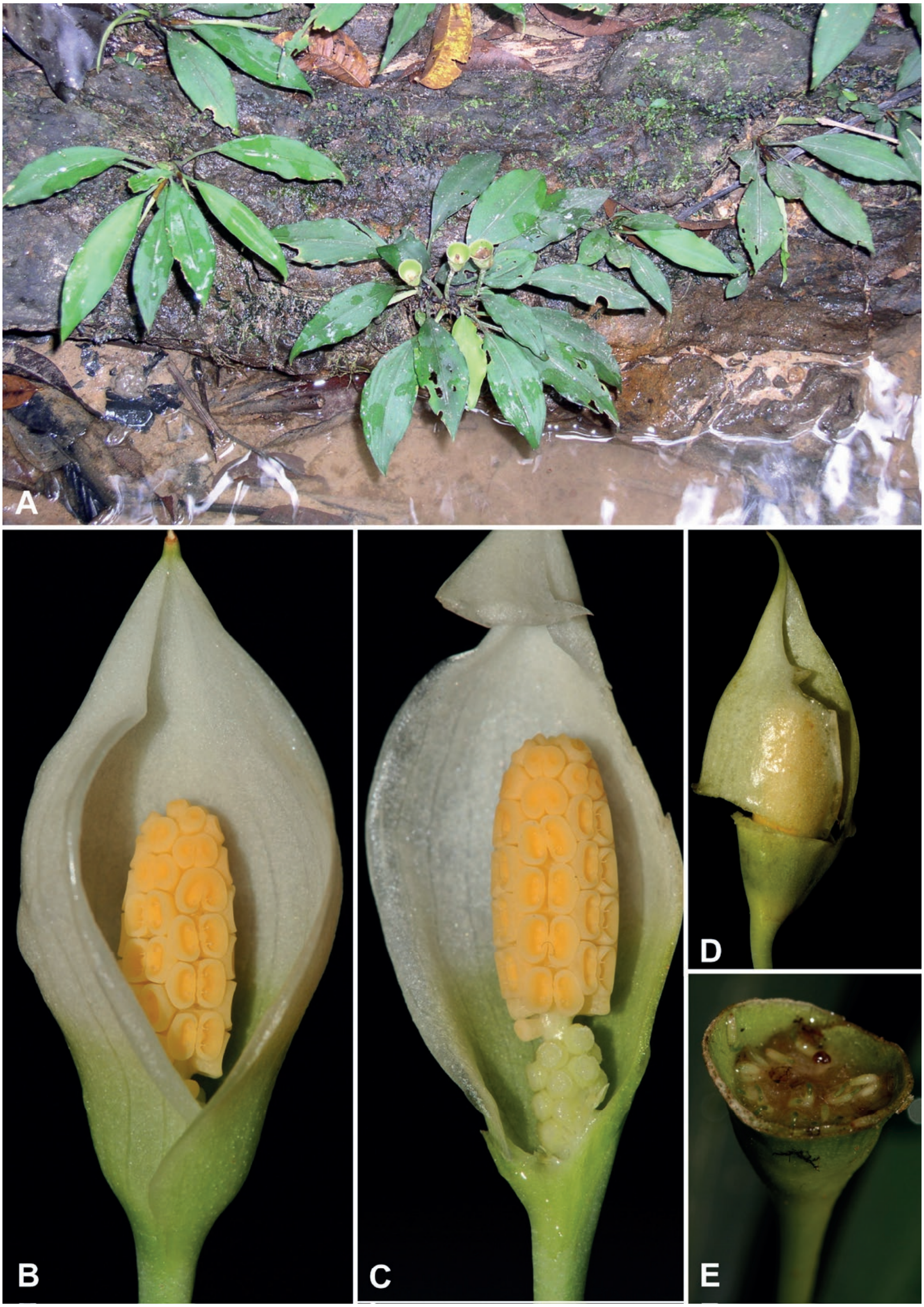

Figure 4. *Burttianthus solus* S.Y.Wong, M. Lo & P.C.Boyce. A. Plants in habitat. B. Bloom during pistillate anthesis. C. Bloom at pistillate anthesis, nearside spathe artificially removed. D. Bloom at onset of staminate anthesis, spathe limb beginning to shed. E. Lower persistent splash-cup spathe with the fruits decomposing and seed beginning to germinate. Photos: P.C.Boyce.

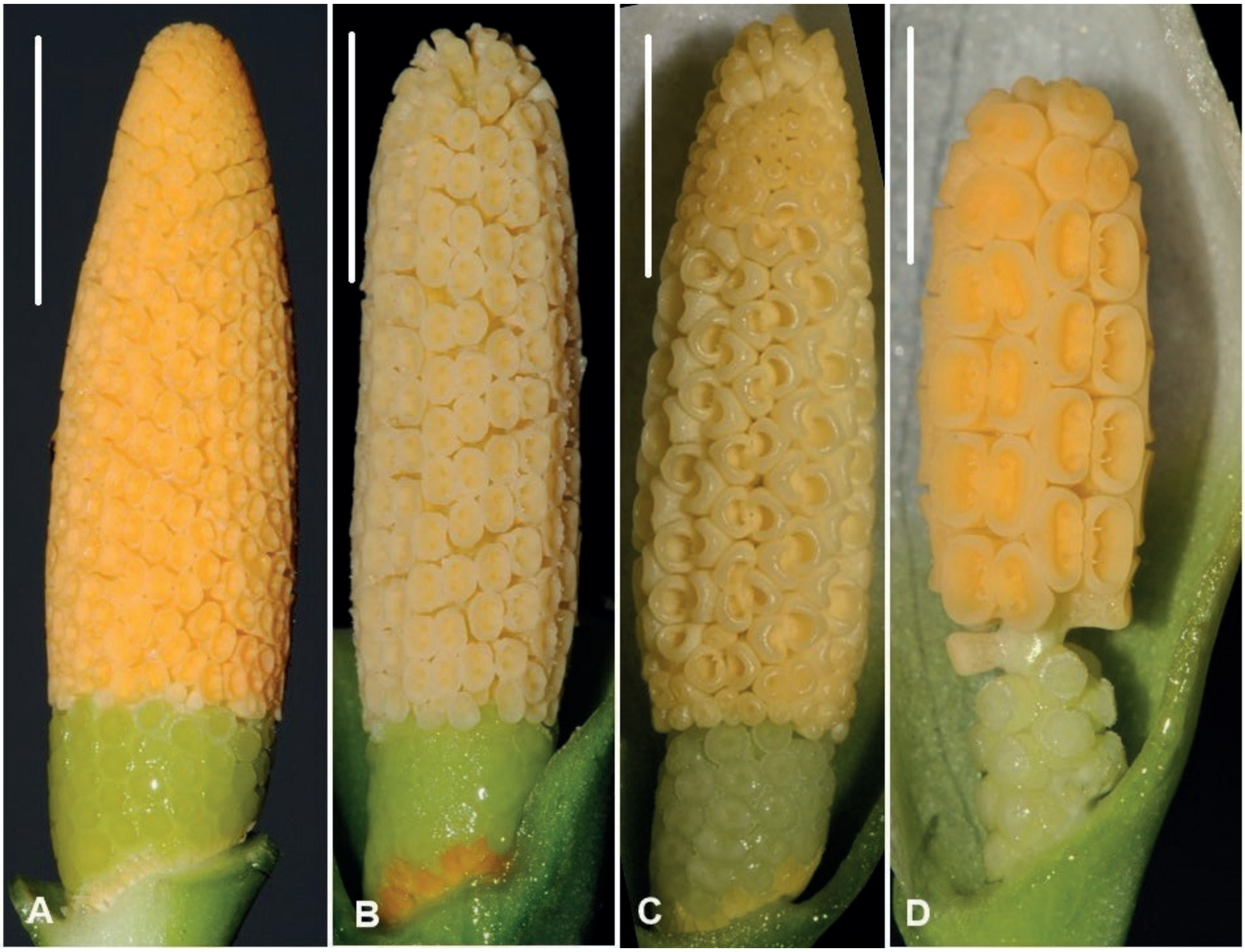

Figure 5. Spadix comparisons. A. *Burttianthus caulescens*. B. *Burttianthus hansenii*. C. *Burttianthus rejangicus*. D. *Burttianthus solus*. Scale bar = 1 cm. Photos: P.C.Boyce.

ally occurring in sheltered parts of the banks of larger rivers.

Notes

In the absence of provenance and without examining the spadix, plants of *B. rejangicus* are virtually indistinguishable from *B. caulescens* (Fig. 5A) and *B. hansenii* (Fig. 5B). A similar situation exists with the long-confounded *B. longipedunculatus* (Fig. 6A) and *B. purseglovei* (Fig. 6B).

Additional specimens examined (paratypes)

MALAYSIA. Sarawak. Kapit, Pelagus Rapids, Woodpecker Trail, 2°11'15.1"N 113°03'29.01"E, 70 m asl., 14 Mar 2005, *P.C.Boyce, Jeland ak Kisai & Jepom ak Tisai AR-1036* (SAR); Kapit, Belaga, km 10 Bakun - Bintulu-Miri road junction, 2°50'51.7"N 114°01'57.6"E, 182 m asl., 11 Oct 2005, *P.C.Boyce, Jeland ak Kisai & Jepom ak Tisai AR- 1395* (SAR); Kapit, Pelagus Rapids, Woodpecker Trail, 2°11'15.1"N 113°03'29.01"E, 70 m asl, 1 Dec 2004, *Jeland ak Kisai AR-779 (SAR)*; Kapit, Belaga, Bakun Dam, Sungai Linau, 2°31.950'N 114°13.161'E, 299 m asl., 2 Oct 2017, *Mike Lo AR-2825* (SAR); Kapit, Batang Balleh, Nanga Putai, 2°01'0.0"N 113°01'0.0"E, 20 m asl, 29 May 2013, *Kazuya Nakamoto AR- 4162* (SAR).

Burttianthus solus S.Y.Wong, M.Lo & P.C.Boyce, **sp. nov.**

Type: Malaysia. Sarawak, Bintulu, Similajau N.P., Batu Anchau trail, 3°21'21.8"N 113°09'41.0"E, 36 m asl., 1 Sep 2012, *Mike Lo AR-4033* (holotype SAR!; isotype SAR - spirit!). (Figures 4 & 5D).

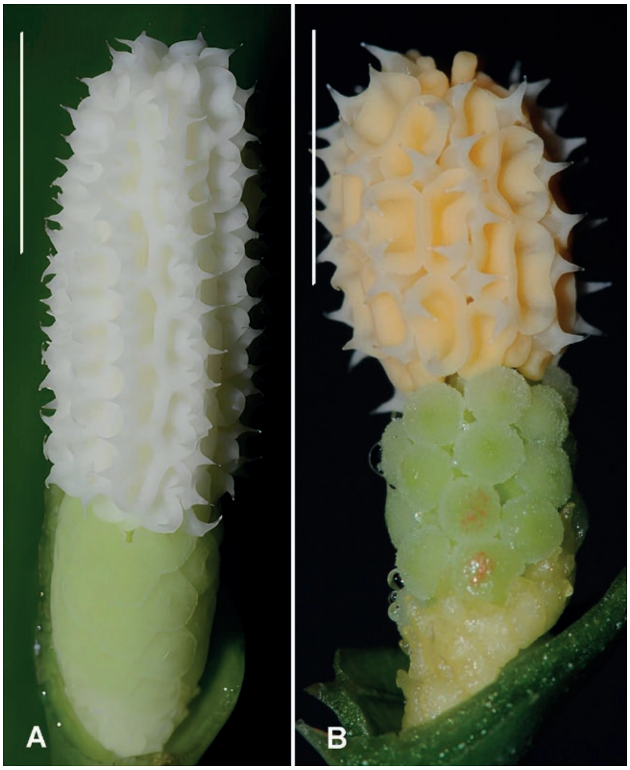

Figure 6. Spadix comparisons A. *Burttianthus longipedunculatus*. C. *Burttianthus purseglovei*. Scale bar = 1 cm. Photo A: Mike Lo; B: P.C.Boyce.

Diagnosis

Burttianthus solus is distinguished from all other species by the pistillate and staminate zones of florets separated by a naked interstice, and further by the lax (not congested) pistillate florets.

Description

Small obligate Steenisian rheophytes to 10 cm tall. Stem short, 1–5 cm long, 3–4 mm in diam. Leaves few together, tufted; petiole ca 2.5 cm long, ca 1.5 mm in diam., adaxially canaliculate, sheathing at extreme base; petiolar sheath with wings extended into a very narrowly triangular ligular portion 1.5 cm long drying dark brown and then soon marcescent; blade coriaceous, adaxially medium green, paler abaxially, elliptic to elliptic-lanceolate, 2.5–4 cm long × ca 1 cm wide, base narrowly cuneate, apex acute, apiculate for ca 1 mm, margin somewhat thickened and slightly undulate; midrib abaxially very prominent, adaxially prominent, primary lateral veins indistinguishable from interprimary venation, diverging at 20–35° and running to a more or less thick marginal vein; secondary venation adaxially and abaxially very faint to completely obscure; tertiary venation mostly completely obscure in living material, forming a faint tessellate reticulum in dry material. Inflorescence solitary; peduncle stout, exceeding petioles, ca 3 cm long × 1 mm in diam., terete, pale green; spathe more or less ovoid with a recurved apex, not constricted, ca 4 cm long and apically beaked to ca 5 mm mm; lower part campanulate, green, persistent, upper part gaping at anthesis, glistening white with the dorsal median middle part stained green, caducous during or just following staminate anthesis, apical beak medium green. Spadix stoutly cylindrical, ca 3 cm long, ca 1 cm in diam.; pistillate flower zone ca 8 mm long, cylindrical, about half the width of the remainder of spadix, comprised of ca 3 spirals of pistils; pistils lax, sub-oblong globose, ca 1.5 mm in diam.; stigma sessile, discoid, slightly wider than ovary, individual stigmas not contiguous to others, greenish white; interpistillar staminodes absent; sterile interstice ca 2.5 mm long, very pale green, naked except for one or maybe two solitary sterile stamens, these narrowly clavate with truncate tops; staminate floret zone ca 2 cm long, composed of ca 6 spirals of fertile flowers arranged in longitudinally aligned pairs; staminate flowers glabrous, crowded, each composed of two excavated stamens, truncate, the deeply excavated parts slightly obliquely facing and each with thecae together on inner (with respect to stamen pairs) side of anther, ellipsoid to ellipsoid-oblong from above, ca 1 mm long × 2 mm wide; thecae separated by a ridge forming a septum in cavity, the outer margins of each stamen thickened and inrolled, very shortly horned, with horns inside lip of anther cavity, medium yellow with the rims paler;

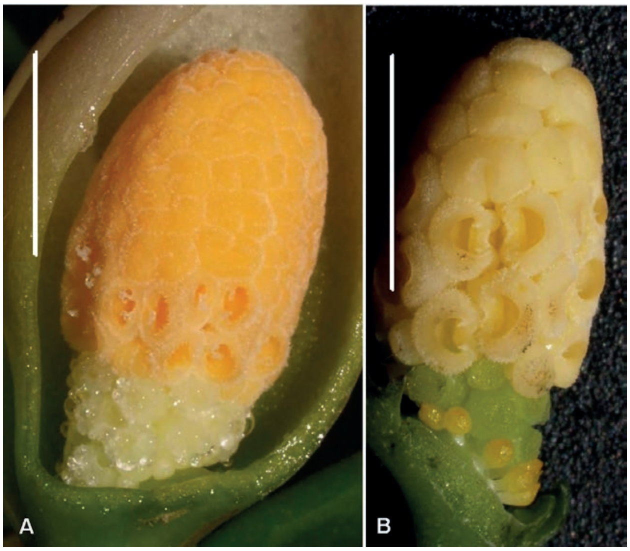

Figure 7. Spadix comparisons. A. *Burttianthus spissus*. B. *Burttianthus velutandrus*. Scale bar = 5 mm. Photos: P.C.Boyce.

appendix absent. Fruiting spathe thick-walled, obconic fruits oblong-cylindric, truncate, ca. 2 mm long; seeds ellipsoid with a well-developed hooked micropylar extension ca 1.5 mm long.

Etymology

Latin, *sōlus* (feminine *sōla*, neuter *sōlum*); alone, sole, only, by oneself with no others around – in allusion to the separation of the pistillate and staminate zones, and the laxly arranged pistillate florets.

Distribution

Northern Bintulu where it is known from three very restricted populations in the Similajau Basin. At the Type locality *B. solus* occurs mixed with (but is very much rarer than) *B. velutandrus* (Fig. 7 B). *Burttianthus solus* is more abundant at the other two localities, but both these sites are threatened by housing developments. The Air Terjun Baloi population occurs intermixed with the locally endemic *Schismatoglottis heterodoxa* S.Y.Wong (Wong 2012).

Ecology

Exposed Oligocene sandstone boulders and waterfalls in the flood zone of small streams under rather open humid lowland forest.

Notes

As with the preceding *Burttianthus rejangicus* it is necessary to examine the spadix of *B. solus* to distinguish it from *B. caulescens* (Fig. 5A) and *B. hansenii* (Fig. 5B).

Additional specimens examined (paratypes)

MALAYSIA. Sarawak, Bintulu, Kidurong, Air Terjun Kidurong, 3°13’34.5”N 113°04’27.0”E, 75 m asl., 1 Oct 2019, *Wong Sin Yeng, A. Hay & P.C.Boyce AR-4193* (SAR). Sarawak, Bintulu, Air Terjun Baloi, 3°08’34.5”N 113°04’17.2”E, 50 m asl., 9 Aug 2013, *Wong Sin Yeng & P.C.Boyce AR-4198* (SAR).

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Notes on the lectotypification of *Crotalaria nana* Burm.f. (Leguminosae: Papilionoideae)

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Abstract. *Crotalaria nana* Burm.f., is lectotypified using a G-PREL collection after reviewing earlier typifications.

Keywords: *Crotalaria*, Fabaceae, nomenclature, Papilionoideae, typification.

INTRODUCTION

The genus *Crotalaria* L. is represented globally with 702 species displaying its maximum diversity in Africa and Madagascar (Le Roux et al. 2013). In India, the genus is represented by 102 species, 3 subspecies, 19 varieties, and 2 forms (Ansari and Chauhan 2020). After the comprehensive taxonomic study of this genus in India (Ansari 2008), recently Ansari and Chauhan (2020) published an annotated, photographed checklist of *Crotalaria* in the country, where remaining lectotypification was presented. However, due to various reasons, Turner (2021) Lectotypified and Neotypified 17 names under the genus *Crotalaria* L., thus superseding many of the earlier typifications.

During the phytochemical and reproductive biological studies on *Crotalaria* L. in India, with special emphasis on Pyrrolizidine alkaloids, we have collected several accessions of *Crotalaria nana* Burm.f. and *Crotalaria nana* var. *umbellata* (Wight ex Wight) Trimen from various parts of the Southern Western Ghats. While going through the literature, we found some discrepancies in the lectotypification of *Crotalaria nana* Burm.f., which are discussed below. The lectotypes are selected based on Art. 9.3 and 9.12 of the Shenzen Code (Turland et al. 2018). Herbarium acronyms follow Thiers (2016).

Nicolaas Laurens Burman (1734–1793), son of Johannes Burman (1706–1779), was a Dutch physician and a botanist at Amsterdam who described *Crotalaria nana* Burm. f. in 1768. In the protologue, he described *Crotalaria nana* as having "*foliis, simplicibus, oblongis, subsessilibus, glabris, pedunculis lateralibus trifloris, Crotalaria minor benghalensis, flore luteo, Crotalaria mal-*

abarica Garcin. Herb., habitat in India. " From the diagnosis, it is clear that he saw the generative specimen of *Crotalaria nana* with yellow flowers, which are three in number per inflorescence. He might also have seen the collections of Laurent Garcin, a Dutch army physician who travelled in Flanders, Spain, and Portugal and made three trips to the East Indies, India, Ceylon, Arabia, and Persia—between 1720 and 1729. The original collections of Garcin are destroyed, but several sets of specimens collected by Garcin are at G (in the Burman herbarium, and currently cited under the acronym G-PREL in the herbarium of the Conservatory and Botanic Garden of Geneva) and at L (Stafleu and Cowan 1976).

Merrill (1921), who was a pioneer in reviewing the names published in N.L. Burman's *Flora Indica* (1768), cited tab. 48. f.2 "Habitat in India" after *Crotalaria nana* Burm.f. He reviewed 10 names in Leguminosae published in *Flora Indica* without studying the original material either collected by N. L. Burmann or others, at the pre-Linnean collection of the Geneva Herbarium (G-PREL).

Niyomdham (1978) also gave an indication about the type material under the distribution data, however abstained from typification. Adema (2006) while providing notes on Malesian Fabaceae, incorporated a taxonomic key only to *C. nana* and not tried to designate a type. Ansari (2008) cited tab. 48. Fig. 2 of protologue! as the type and not used "designated here" or *hic designatus* or equivalent for effective lectotypification. So this cannot be taken as an inadvertent lectotypification. Later, Ninakaew et al. (2017) cited Walker *s.n.* (K000591116!) as the holotype. Further Ansari & Chauhan (2020) cited it in a different way as Lectotype: tab. 48. fig. 2. of protologue! India; *Walker s.n.* (K000591116!), designated here. Here also, tab 48. fig. 2. of protologue lacks an explicit statement "designated here", or *hic designatus* or an equivalent. Burman filius neither studied a Walker specimen nor gave any indication through the protologue proposed in the publication.

After studying the virtual specimens available at K (K000591116, duplicates at E, M) that have been cited by Ansari and Chauhan (2020) and Ninakaew et al. (2017) as types, we confirmed the identity as *Crotalaria umbellata* (Wight ex Wight) Trimen, (not *Crotalaria umbellata* (Wight ex Wight) Ansari as has been cited in many literature). *Crotalaria umbellata* is characterised by short inflorescences, 4-9 flowers, caducous bracts and glabrous keel margin. The base of the stem has numerous branches. Eventhough *Crotalaria nana* shows profuse branching from the base of the stem, the inflorescence bears just 1-3 flowers, persistent bracts and puberulous keel border (Ninakaew et al. 2017; Ansari 2008). The persistent nature of the bracts and 2-3 flowers are clearly illustrated in the tab. 48 f.2. (See Burman 1768).

According to Stafleu and Cowan (1976), the specimens of N.L. Burman are incorporated in the herbarium of the Conservatory of Geneva, most specifically in the pre-Linnean collection cited as G-PREL, and contain most of the types. Some of the type specimens are also available at L, and M. The Thunberg Herbarium (UPS) contains "Burman" (father and son) material from the Cape that was gathered by Herman, Oldenland, Hartog, and other Burman correspondents.

While going through the historical collections at G-PREL we have found an annotated specimen (G00812546) with the handwriting of Burman filius that closely matches the protologue. The specimen bears, solitary flowers (see the second branch from left to right of Figure 1.) as well as 3 flowers, as mentioned in the protologue. Hence, it is here designated as the lectotype of *Crotalaria nana* Burm.f. A duplicate of the same is also available at Munich herbarium (M0219504), which is here designated as the isolectotype.

The M specimens also bears 1–3 flowers, per inflorescence. In the lower-bottom corner of G-PREL specimen, somebody (May be Augustin Pyramus de Candolle who directly studied it (v.s. in h. Deless.) as evidenced through the notes in his *Prodromus* Volume 2. page no.127.1825) wrote the identity as *Crotalaria biflora* Linn. This might be a later writing when compared with the former elaborate one at the middle right hand side, where N.L. Burman doubted the specimen as *Crotalaria triflora* (because of three flowers per inflorescence) of Linnaeus Sp.Pl. 2: 715. No.5. This reflects his correspondence and involvement with Linnean materials. Currently, *Crotalaria triflora* L. is considered as a synonym of *Rafnia triflora* Thunb. (see POWO 2023).

NOMENCLATURE

Crotalaria nana Burm. f. Fl. Indica 156. 1768.

Type: s.l., s.d., *Anonymous s.n.* (G-PREL!, barcode G00812546!, lectotype designated here; India, s.d., *Anonymous s.n.* (M! barcode M0219504!, isolectotype). Figure 1.

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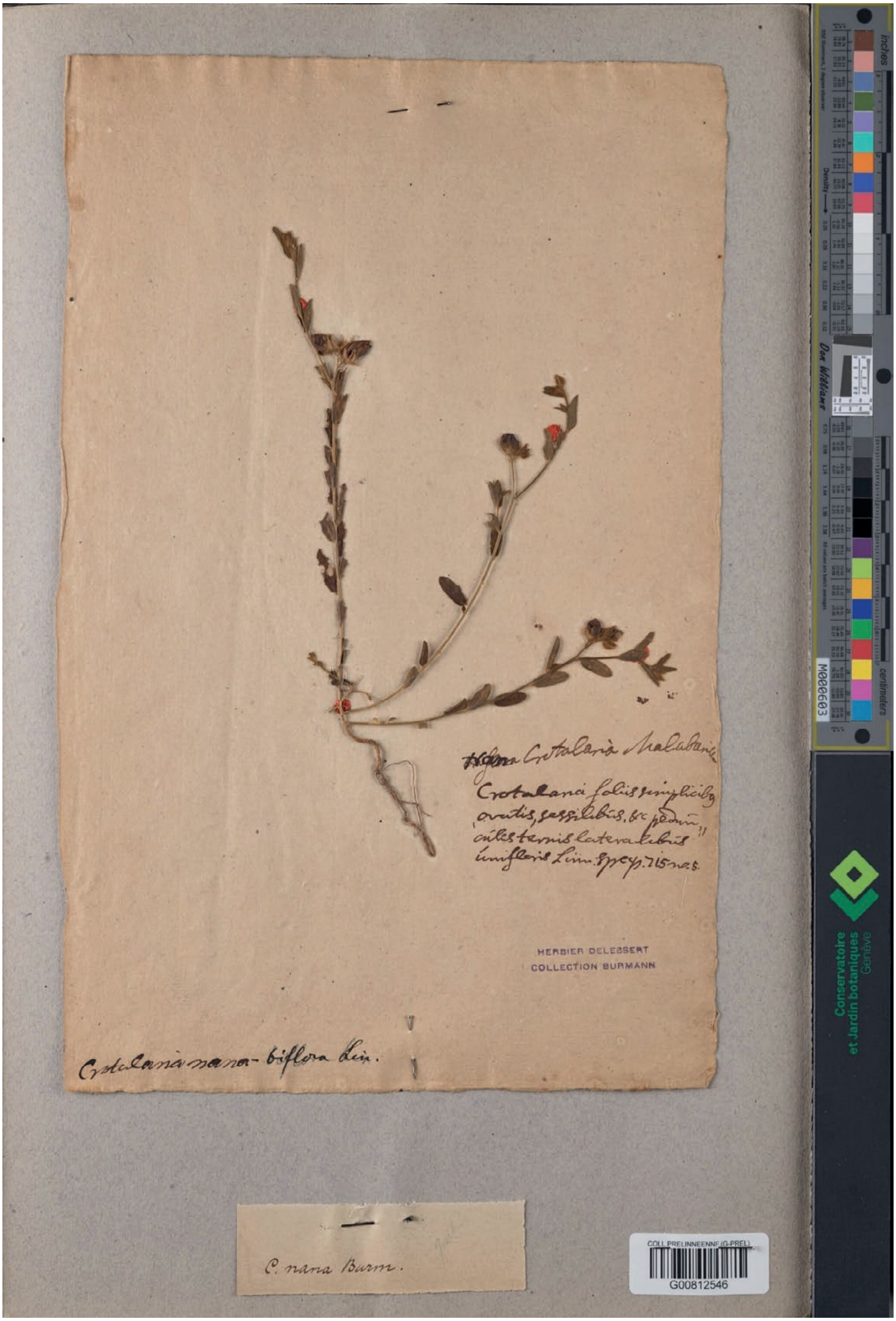

Figure 1. Lectotype of *Crotalaria nana* Burm.f. (G00812546). @ Conservatoire & Jardin botaniques de la Ville de Genève.

Herbaria (G-PREL & M) for providing digitised images of types, that helped to sort the problem.

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Applications of leaf architecture characters in delineating selected species and infraspecific taxa of Genus *Cucumis* L. (Cucurbitaceae)

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Abstract. Leaf venation characters of twelve (12) *Cucumis* taxa, comprised of eight species, three subspecies, and three varieties were examined to determine patterns of leaf architectural characters and delineate these accessions at the species, subspecies, and variety level. The leaf architecture of twenty-seven accessions representing six taxa were examined and described in this study. Additionally, data on the leaf architecture of another six taxa (represented by 57 accessions) published by Masungsong et al. (2022) were incorporated in the analysis. Results revealed that the twelve (12) taxa of *Cucumis* primarily varied in blade class, apex angle, primary vein size, secondary vein spacing, tertiary vein angle to primary vein, and areole development. These variations were found consistent with *Cucumis* species examined in previous studies. A dichotomous key was constructed for the twelve *Cucumis* taxa using leaf characters derived. Cluster analysis (based on UPGMA) formed two major clusters at 0.130 Gower distance. Cluster I includes *Cucumis melo* subsp. *melo, C. pustulatus, C. melo* var. *flexuosus, C. melo* subsp. *agrestis, and C, melo* var. *texanus* which exhibited secondary vein spacing that increases towards the base. while Cluster II is composed of the remaining *Cucumis* taxa, all displaying irregular secondary vein spacing. Furthermore, subclusters were formed based on tertiary vein angle in relation to primary vein, blade class, apex angle, and primary vein size. Results showed that utilizing leaf architecture is an effective technique to describe, characterize and delineate closely related taxa with many similar characters.

Keywords: *Cucumis*, *Cucurbitaceae*, leaf architecture, leaf venation, species delineation.

INTRODUCTION

Plant reproductive structures (flowers and fruits) are the most common and widely used taxonomic features for the identification and classification of plant species. However, these structures can be challenging to investigate due to their seasonality (da Silva et al. 2015). The use of plant vegetative charac-

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ters such as the leaves are similarly important since they are present throughout the plant's life span (Buot 2020; Masungsong et al. 2019a). Leaf architecture is one of the most valuable tools that is being utilized in many recent taxonomic studies (Buot 2020). It is very significant in describing, classifying, and identifying taxa with confusing characters regardless of its often-debated high phenotypic plasticity. Although some leaf features like shape and size may be plastic, venation patterns are genetically fixed according to Roth-Nebelsick et al. (2001) and Huiet et al. (2018).

Leaf architectural characters have been proven useful in delineating species of Philippine *Cinnamomum* Schaeff. (Celadiña et al. 2012), *Camellia* L. (Lu H et al., 2012), *Terminalia* L. (Baroga 2014), *Saxifraga* Tourn. ex L. (Zhang 2015), *Bauhinia* Plum. ex L. (Lin et al. 2015), *Diplazium* Sw. (Conda et al., 2017), *Adiantum* L. (Huiet et al., 2018), *Dioscorea* Plum. ex L. (Antonio and Buot 2021), and *Hoya* R. Br. (Salvaña and Buot 2014; Villareal and Buot 2015; Jumawan and Buot 2016; Torrefiel and Buot, 2017; Tan and Buot 2018; Baltazar and Buot, 2019; Paguntalan and Buot 2019). These researches revealed that leaf architecture has enormous potential as a tool for solving taxonomic problems.

The genus *Cucumis* comprises a variety of cultivated species classified as food crops. *Cucumis sativus* and *C. melo* are just two of the many economically important members of the genus. Several leaf architectural studies on *Cucumis* have been conducted and contributed to the identification, classification, and delineation of the genus (Rao and Rao 2015; Masungsong et al. 2019a; Masungsong et al. 2019b; Averion-Masungsong and Buot 2020; Masungsong et al. 2022).

Nevertheless, a significant number of *Cucumis* species remain taxonomically problematic in terms of identification and classification due to overlapping general morphology. Currently, there are seventeen (17) *Cucumis* species that are conserved in the Hortanova Farm and Research Center (HFRC) gene bank of Eastwest Seed Company Inc. Identification of these species has increasingly become a challenge when reproductive structures are absent.

This study assessed the diversity of leaf architectural characters from various accessions of twelve (12) *Cucumis* taxa, including eight (8) species, three (3) subspecies, and three (3) varieties. It analyzed the leaf architecture of six (6) taxa that were not included in the previous *Cucumis* leaf architecture studies of Masungsong et al. (2019a), Masungsong et al. (2019b), Averion-Masungsong and Buot (2020); and Masungsong et al. (2022). By combining the current research results with that of previous studies, the accessions were classified and the species, subspecies, and varieties of *Cucumis* were delineated based on leaf architecture.

MATERIALS AND METHODS

Leaf sample collection

Twenty-seven (27) accessions representing six (6) *Cucumis* taxa with previously unexamined leaf architecture (Table 1), were systematically planted at the HRFC, East West Seed Company Inc. screen house, in Lipa City, Batangas Philippines. Three (3) leaves from three (3) standing crops, were collected from each accession. A total of two hundred forty-three (243) leaves were collected, pressed, dried, and observed under a dissecting microscope. Herbarium specimens were prepared from each accession, then deposited at the Plant Biology Division Herbarium (PBDH), Institute of Biological Sciences, University of the Philippines, Los Baños.

Secondary data

In the work of Masungsong et.al. (2022), fifty-seven (57) accessions representing six (6) taxa of *Cucumis* were examined. The leaf architecture descriptions of *C. melo* subsp. *agrestis* (Naudin) Pangalo, *C. melo* var. *flexuosus* (L.) Naudin, *C. melo* var. *texanus* Naudin, *C. sativus* L., *C. sativus* var. *hardwickii* (Royle) Gabaev, and *C. zambianus* Widrl, J.H.Kirkbr., Ghebret. & K.R.Reitsma were reported and therefore will not be elaborated on in this paper. Nevertheless, the data on these six taxa will be incorporated in the analysis to provide a more comprehensive coverage of the genus *Cucumis.*

Leaf venation analysis

Twelve (12) general leaf- and eleven (11) venation characters used in previous *Cucumis* studies were also observed and measured in this study. This is to ensure consistent classification for all the *Cucumis* taxa and accessions used. The leaf venation characters were based from Hickey's (1973), LAWG's (1999), and Elli's et al. (2009) leaf architectural descriptors. Leaf architecture data generated from this study were combined with data from the study of Masungsong et al. (2022) to construct a dichotomous key to twelve *Cucumis* taxa. The combined data was also used to perform cluster analysis based on the Unweighted Pair Group Method using Averages (UPGMA) and Gower distance correlation, using the PAleontological Statistics (4.04) software.

Table 1. *Cucumis* taxa and accessions examined in the study.

Accession number	Accession name	Scientific name	Country of origin
GB-00250	PI 203974	*C. africanus* L.f.	South Africa
GB-00253	PI 299571 (563)	*C. africanus* L.f.	South Africa
GB-00518	Ames 23562 (JEWM 064)	*C. africanus* L.f.	South Africa
GB-00525	PI 542126 (JT48)	*C. africanus* L.f.	Zimbabwe
GB-00526	PI 542127 (JT53)	*C. africanus* L.f.	Botswana
GB-00446	140836	*C. dipsaceus* Ehrenb. ex Spach	Zimbabwe
GB-00576	PI 236468	*C. dipsaceus* Ehrenb. ex Spach	Ethiopia
.GB-00577	PI 390450 (Bolsa de toro)	*C. dipsaceus* Ehrenb. ex Spach	Ecuador
GB-00578	PI 441993 (IVT 0163)	*C. dipsaceus* Ehrenb. ex Spach	The Netherlands
GB-00579	PI 504553	*C. dipsaceus* Ehrenb. ex Spach	Kenya
GB-00257	PI/ 273192 (19077)	*C. ficifolius* A.Rich.	South Africa
GB-00258	PI 299570 (416)	*C. ficifolius* A.Rich.	South Africa
GB-00259	PI 299572 (605)	*C. ficifolius* A.Rich.	South Africa
GB-00488	140890	*C. ficifolius* A.Rich.	France
GB-01396	WMR 29	*C. melo* subsp. *melo* L.	Iran
GB-01397	Cantaloup d'Alger	*C. melo* subsp. *melo* L.	Iran
GB-01398	Santon	*C. melo* subsp. *melo* L.	Iran
GB-01399	Naples	*C. melo* subsp. *melo* L.	Iran
GB-01400	Bale	*C. melo* subsp. *melo* L.	Guatemala
GB-00411	PI 203977	*C. myriocarpus* subsp. *myriocarpus* Naudin	South Africa
GB-00412	PI 409732 (2602)	*C. myriocarpus* subsp. *myriocarpus* Naudin	South Africa
GB-00413	PI 532627 (79)	*C. myriocarpus* subsp. *myriocarpus* Naudin	Zimbabwe
GB-00414	PI 532628 (85)	*C. myriocarpus* subsp. *myriocarpus* Naudin	Zimbabwe
GB-00415	PI 542137 (517)	*C. myriocarpus* subsp. *myriocarpus* Naudin	Botswana
GB-00642	PI 343699 (No. 1)	*C. pustulatus* Naudin ex Hook.f.	Nigeria
GB-00643	PI 343700 (No. 2)	*C. pustulatus* Naudin ex Hook.f.	Nigeria
GB-00644	PI 343701 (No. 3)	*C. pustulatus* Naudin ex Hook.f.	Nigeria

RESULT AND DISCUSSIONS

General leaf characters and venation characters of Cucumis species

Based on the data gathered from a total of eighty-four (84) accessions (27 from this study and 57 from the study of Masungsong et al. (2022), the twelve (12) *Cucumis* taxa six (6) from this study and six (6) from Masungsong et al. (2022) share many common leaf architecture traits (Table 2 and Masungsong et al. (2022). In fact, all twelve taxa displayed the same traits in nine (9) out of the twelve (12) general leaf characters and eleven (7) out of the eleven (11) leaf venation characters.

The unifying features of the leaves of these *Cucumis* taxa are: simple leaf organization, orbiculate and symmetrical lamina, convex apex, lobate base with wide obtuse angle, serrated leaf margins, spinose tooth apex, palmate lobation, actinodromous (i.e., arising from the same point of origin) suprabasal primary veins that are straight-branched, craspedodromous (i.e., pinnately arranged and terminating at the leaf margins) secondary veins with two-pair acute basal secondaries, alternate percurrent tertiary veins, regular, polygonal and reticulate quaternary veins, and looped marginal ultimate venation. These shared features can be used to distinguish the leaves of *Cucumis* species from other genera.

Variations of leaf characters were observed in their blade class and apex angle. Among the 12 taxa, only *C. ficifolius* exhibited a microphyll to notophyll blade class while other species have mesophyll to macrophyll blade class (*C. zambianus, C. melo* var. *flexuosus, C. sativus* var. *hardwickii,* and *C. melo* subsp. *agrestis*), mesophyll blade class (*C. sativus, C. pustulatus, C. melo* subsp. *melo* and *C. melo* var. *texanus*) and strictly notophyll blade class (*C. dipsaceus, C. africanus,* and *C. myriocarpus* subsp. *myriocarpus).* Blade class has been used to delineate species, infraspecific taxa, and accessions. Similar to the study of Masungsong et al. (2019b) it differentiated *C. anguria* from *C. anguria* var. *longaculeatus*. However,

Table 2. Leaf architecture traits of six species of *Cucumis* based on twenty-seven (27) accessions.

Characters	Species					
	C. pustulatus	*C. dipsaceus*	*C. ficifolius*	*C. melo ssp. melo*	*C. africanus*	*C. myriocarpus* ssp. *myriocarpus*
General leaf characters						
Leaf organization	Simple	Simple	Simple	Simple	Simple	Simple
Blade shape	Orbiculate	Orbiculate	Orbiculate	Orbiculate	Orbiculate	Orbiculate
Blade symmetry	Symmetrical	Symmetrical	Symmetrical	Symmetrical	Symmetrical	Symmetrical
Length: Width ratio	1.00 – 1.25	0.97 – 1.16	0.85 – 1.26	0.88 – 1.11	0.97 – 1.60	0.95 – 1.53
Blade class	Mesophyll	Notophyll	Microphyll to mesophyll	Mesophyll	Notophyll	Notophyll
Apex shape	Convex	Convex	Convex	Convex	Convex	Convex
Apex angle	Odd-lobed obtuse	Odd-lobed obtuse	Odd-lobed obtuse	Odd-lobed obtuse	Odd-lobed acute	Odd-lobed acute
Base shape	Lobate	Lobate	Lobate	Lobate	Lobate	Lobate
Base angle	Wide obtuse	Wide obtuse	Wide obtuse	Wide obtuse	Wide obtuse	Wide obtuse
Margin	Serrate	Serrate	Serrate	Serrate	Serrate	Serrate
Tooth apex	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose
Lobation	Palmately lobed	Palmately lobed	Palmately lobed	Palmately lobed	Palmately lobed	Palmately lobed
Venation						
Primary vein						
Category	Actinodromous suprabasal	Actinodromous suprabasal	Actinodromous suprabasal	Actinodromous suprabasal	Actinodromous suprabasal	Actinodromous suprabasal
Size	weak	moderate	Weak to moderate	weak	moderate	Weak to stout
Course	Straight branched	Straight branched	Straight branched	Straight branched	Straight branched	Straight branched
Secondary vein						
Category	Craspedodromous	Craspedodromous	Craspedodromous	Craspedodromous	Craspedodromous	Craspedodromous
Spacing	Increasing towards base	Irregular spacing	Irregular spacing	Increasing towards base	Irregular spacing	Irregular spacing
Angle category	Two pair acute basal secondaries	Two pair acute basal secondaries	Two pair acute basal secondaries	Two pair acute basal secondaries	Two pair acute basal secondaries	Two pair acute basal secondaries
Tertiary vein						
Category	Alternate percurrent	Alternate percurrent	Alternate percurrent	Alternate percurrent	Alternate percurrent	Alternate percurrent
Angle to primary	obtuse	obtuse	obtuse	obtuse	obtuse	obtuse
Quaternary vein category	Regular polygonal reticulate	Regular polygonal reticulate	Regular polygonal reticulate	Regular polygonal reticulate	Regular polygonal reticulate	Regular polygonal reticulate
Marginal ultimate venation	Looped	Looped	Looped	Looped	Looped	Looped
Areole development	Well developed	Well developed	Well developed	Well developed	Well developed	Well developed

two accessions of *C. anguria* displayed a different blade class clustering them with *C. anguria* var. *longaculeatus* implying the close relationship of the two aforementioned varieties. Some leaf architecture studies were also able to delineate species using blade class (Baroga and Buot 2014; Kpadehyea and Buot 2014; Tan and Buot 2018; Antonio and Buot 2021). Moreso, blade class was used as an identification tool for *Glycine max* (L.) Merr. accessions (Chen and Nelson 2004). However, in this study blade class alone is not enough to distinguish species as we observed different types of blade class in *C. melo* subspecies and varieties, as well as in *C. sativus* varieties.

All the *Cucumis* taxa exhibited odd-lobed and obtuse apex angles with the exception of *C. melo* var. *flexuosus* (obtuse apex angle), *C. melo* var. *texanus* (obtuse to odd-lobed obtuse apex angle), and *C. sativus* and *C. sativus* var. *hardwickii* (odd-lobed acute apex angle). Similar with the result of this study, diversity of apex angle is also observed by Masungsong et al. (2019a, 2019b) in other *Cucumis* species. The apex angle of odd-lobed leaves can represent the degree of lobation in *Cucumis* (Nandyal et al. 2013).

Variations were observed in their primary vein size, secondary vein spacing, tertiary vein angle in relation to primary vein category, and areolation. In terms of primary vein size, the *Cucumis* accessions can be grouped into four: (1) those having weak primary vein size (*C. melo* subsp. *agrestis*, *C. melo* var. *texanus*, *C. melo* var. *flexuosus*, *C. zambianus*, *C. sativus*, *C. sativus* var. *hardwickii*, *C. pustulatus*, and *C. melo* ssp. melo), (2) moderate primary vein size (*C. dipsaceus* and *C. africanus*), (3) weak to moderate primary vein size (*C. ficifolius*), and (4) weak to stout primary vein size (*C. myriocarpus* subsp. *myriocarpus*). Hence, primary vein size can be used to delineate *C. ficifolius* and *C. myriocarpus* subsp. *myriocarpus* from the rest of the taxa.

Studies have shown that primary vein size and other primary vein characteristics can be used in classifying species, as demonstrated in *Podocarpus* L. Her. ex Pers. (Salvaña et al. 2018) and *Anthurium* Schott subsections (Mantovani et al. 2009). Two groups were formed on the basis of secondary vein spacing. *Cucumis pustulatus* and all the subspecies and varieties of *C. melo* have increasing spacing towards the base secondary vein while other species have irregular secondary vein spacing. These two character states were also observed in other *Cucumis* species (Masungsong et al. 2019b). Just like apex angle, secondary vein spacing is associated to lobation. The extent of lobation is inversely proportional to leaf surface area. Lobation is important in discerning specific vein patterns in the leaf and it can also reflect evolutionary relationships between taxa (Yang et al. 2022).

Most of the *Cucumis* taxa display obtuse tertiary vein angle to primary vein while *C. melo* subsp. *agrestis*, *C. melo* subsp. *texanus*, *C. melo* subsp. *flexuosus* and *C. zambianus* have an acute tertiary vein angle to primary vein. This character delineated *C. metuliferus* from *C. myriocarpus* as observed in the study of Masungsong et al. (2019a, 2019b). Nelson and Dengler (1997) also indicated that intra- and interspecific variations can be seen in higher vein orders like tertiary veins. For the areole development, *C. zambianus* accessions can be distinguished as they have moderately developed areolation while the other taxa have well-developed areolation. Areolation was also cited by Kpadehyea and Buot (2014) as one of the most useful leaf characters in differentiating species and infraspecific taxa of *Mussaenda* Burm. ex L. in the Philippines.

A dichotomous key was produced, using the leaf architecture descriptions of the examined taxa. The usefulness of leaf architecture, especially venation characters in delineating and describing *Cucumis* species is apparent. Similar findings were reported by Celadiña et al. (2012) in Philippine *Cinnamomum* species, Conda et al. (2017) in selected *Diplazium* species, Salvaña and Buot (2014) in *Hoya* species, and Pulan and Buot (2014) in Philippine *Shorea* Roxb. ex C.F. Gaertn. species.

Key to twelve taxa of *Cucumis* L. based on leaf architecture

1. 2° vein craspedodromous with irregular spacing................2

2. Convex apex shape, odd lobed obtuse apex angle..............3

2. Convex apex shape, odd lobed acute apex angle................7

3. 3° vein alternate percurrent with obtuse 3° vein angle to 1° 4

3. 3° vein alternate percurrent with acute 3° vein angle to 1° .. ..***C. zambianus***

4. 1° vein actinodromous suprabasal, with moderate vein size 5

4. 1° vein actinodromous suprabasal, with weak to moderate or weak to stout vein size...6

5. Palmately lobed, notophyll with 0.97 to 1.16 L:W ratio........ ....***C. dipsaceus***

5. Palmately lobed, notophyll with 0.97 to 1.60 L:W ratio........ .... ***C. africanus***

6. Weak to moderate 1° vein size ....***C. ficifolius***

6. Weak to stout 1° vein size .... ***C. myriocarpus*** subsp. ***myriocarpus***

7. Well-developed areolation, macrophyll with 0.95 to 1.17 L:W ratio ....***C. sativus***

7. Well-developed areolation, macrophyll with 1.03 to 1.12 L:W ratio .... ***C. sativus*** var. ***hardwickii***

1. 2° vein craspedodromous with increasing towards the base spacing..8

8. Convex apex shape with odd lobed obtuse apex angle 9

8. Convex apex shape with obtuse apex angle.......................10

9. 3° vein alternate percurrent with obtuse 3° vein angle to 1° 11

9. 3° vein alternate percurrent with acute 3° vein angle to 1° .. .. ***C. melo*** subsp. ***agrestis***

10. 4° vein regular polygonal reticulate, mesophyll with 0.97 to 1.03 L:W ratio....................................... ***C. melo*** var. ***texanus***

10. 4° vein regular polygonal reticulate, mesophyll with 0.99 to 1.06 L:W ratio.....................................***C. melo*** var. ***flexuosus***

11. Symmetrical, orbiculate, mesophyll with 1.00 to 1.25 L:W ratio..***C. pustulatus***

11. Symmetrical, orbiculate, mesophyll with 0.88 to 1.11 L/W ratio.. ***C. melo*** subsp. ***melo***

Species and infraspecific delineation of Cucumis based on leaf architecture

Cluster analysis classified the eighty-four (84) accessions into two major clusters. The UPGMA dendrogram with a cophenetic correlation of 0.9031 and Gower distance of 0.130 is shown in Figure 1. Cluster I includes all accessions of *C. melo* var. *flexuosus*, *C. melo* var. *texanus, C. pustulatus*, *C. melo* ssp. *melo* and *C. melo* subsp. *agrestis* characterized by secondary vein spacing increasing towards the base. On the other hand, Cluster II is composed of all accessions of *C. sativus*, *C. sativus* var. *hardwickii*, *C. africanus*, *C. ficifolius*, *C. myriocarpus* subsp. *myriocarpus* and *C. zambianus* with irregular secondary vein spacing. This result is congruent with the results of the previous study (Masungsong et al. 2019a 2019b) wherein all the studied *Cucumis* species and accessions were primarily delineated on the basis of secondary vein spacing. Thus, secondary vein spacing can be used as a delineating character of *Cucumis* species and infraspecific taxa.

The regularity in the spacing of leaf veins is a remarkable trait, especially in recently evolved monocots and dicots wherein reticulate venation is apparent despite the differences in leaf shape. On account of its regularity, it is a stable character that can be used in distinguishing taxonomic groups. There are observed differences in the uniformity of the veins which can either be primary, secondary, tertiary, or higher vein orders (Nelson and Dengler 1997). The spacing indicates the strength of the support on the blade. Leaves with palmate lobation need shorter spacing of secondary veins to support the lobed part since it is relatively distant from the midvein and petiole. Increasing spacing of secondary veins towards the base implies that the petiole provides support to the blade.

In the first cluster, *C. melo* subsp. *melo* and *C. pustulatus* formed a separate sub-cluster due to the similarity in tertiary vein angle in relation to primary vein which is obtuse. *Cucumis melo* var. *flexuosus* (accessions 601 and 603) and *C. melo* subsp. *agrestis* (accessions 510, 503, 466, and 487) formed another sub-cluster as blade class for these accessions were similar (macrophyll blade class). Blade class is mainly based on the leaf surface area, with macrophyll leaves having a larger surface area than microphyll leaves. Blade classes can be used as a tool in identifying the different accessions of *Cucumis*. This character has been proven to be useful in the classification of *Glycine max* accessions (Chen and Nelson 2004). The set of leaf characters used in this study was able to separate all the subspecific taxa of *C. melo* from the rest of the species, except for *C. pustulatus*. The sub-clusters correspond to the different subspecies and varieties of *C. melo*. As seen in Figure 1, accessions of *C. melo* subsp. *melo* and *C. pustulatus* are very similar to each other. It might be worth re-examining the two accessions of *C. pustulatus* to check for possible misclassification or mislabeling of the plant materials used.

In the second cluster, *C. zambianus* accessions formed a separate sub-cluster for having an acute tertiary vein angle in relation to primary vein and apex angle separates accessions of this species into two. *Cucumis myriocarpus* subsp. *myriocarpus* accessions 412 and 414 were separated from the other accessions of the species for having an acute tertiary vein angle in relation to primary vein. Accessions of *C. africanus* (250, 253, 518, 525, 526), *C. dipsaceus* (446, 576, 577, 578, 579), *C. ficifolius* (488, 257, 258, 259), *C. myriocarpus* subsp. *myriocarpus* (411, 413, 415) formed a separate sub-cluster due to similarity in apex angle (odd lobed obtuse). Sub-cluster formed by *C. sativus* and *C. sativus* var. *hardwickii* accessions was supported by similarity in primary vein size which is weak. While clustering of accessions from the same species was clearly observed in *C. zambianus* and *C. sativus* (with the two varieties joining in one subcluster), *C. africanus* and *C. dipsaceus* appear to have very similar leaf character traits. Some accessions of different species were observed to form a group with other species. *Cucumis myriocarpus* subsp. *myriocarpus* accessions 411, 413, and 415 were separated from accessions 412 and 414 due to tertiary vein angle in relation to primary vein. This implies that, within the same species, the tertiary vein angle in relation to primary vein may differ which can cause a range of character states general to all accession of the species. It can be indicated, based on the result that *C. myriocarpus* subsp. *myriocarpus* accessions have acute to obtuse tertiary vein angle in relation to primary vein. In terms of blade class, *C. myriocarpus* subsp. *myriocarpus* accession 411 also separated from other accessions of the species. Similar to tertiary vein angle in relation to primary vein, it can also be indicated that *C. myriocarpus* subsp. *myriocarpus* accessions have a range of blade classes from notophyll to mesophyll. Similarly, *C. ficifolius* accession 488 and 258 separated from accession 257 and 259 for exhibiting notophyll blade class and weak primary vein size. This result also implies that there is a range of character states for the aforementioned leaf

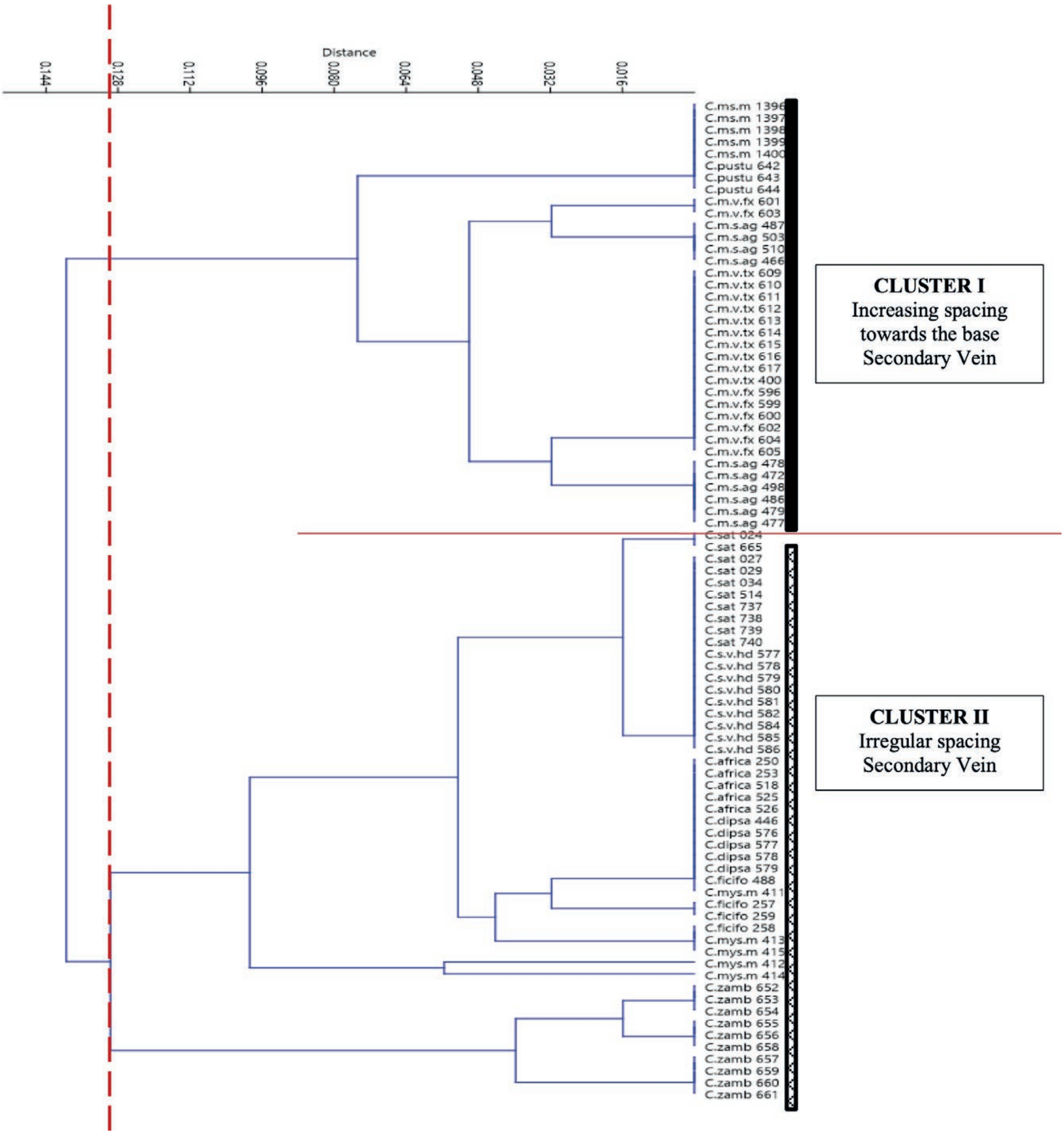

Figure 1. Unweighted Pair Group Method using Averages (UPGMA) dendrogram based on Gower distance (0.130), of eighty-four accessions representing twelve taxa of *Cucumis*: two major clusters formed based on secondary vein spacing; Cluster I: *Cucumis* species accessions with increasing spacing towards the base secondary vein (▮) and Cluster II: *Cucumis* species accessions with irregular spacing secondary vein (▨).

architectural characters of *C. ficifolius*. As evidenced in the dendrogram, this cluster analysis results where some accessions of *Cucumis* species grouped with other accessions of other *Cucumis* species were attributed to their leaf characters' similarities and differences therefore can be considered as a range of character states for the different *Cucumis* species and accessions. Nevertheless, similarities of leaf architectural characters of this species support assigning these accessions under the same species.

The distinct leaf characters obtained, the dichotomous key constructed, and the results of the cluster analysis for the 12 *Cucumis* taxa examined proved the reliability of leaf architecture as a tool in identifying and classifying species within the genus and at a certain level, subspecies and varieties within the same species.

CONCLUSION

The *Cucumis* accessions used in this study can be classified at the species- and infra-specific levels using leaf architecture, especially venation patterns. This approach can be successfully used to identify *Cucumis* species and accessions, as well as other morphologically similar species. In order to solve the issue of resource and space conservation, it can also act as a guide for gene banks, seed firms, and repositories where various accessions of species are preserved thus, aids in planning and management.

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Exploring leaf architecture in varieties of *Hibiscus rosa-sinensis* L. (Malvaceae)

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Abstract. Leaf architecture has been successfully used in the taxonomic and systematic studies of many plant families, genera, and species but little employed at the variety level. The present study demonstrated how leaf architecture can be equally important for plant varieties as well. The leaf architecture of four *Hibiscus rosa-sinensis* L. varieties namely 'Reddy or Not', 'Nay Isa', 'Lolay', and 'Saskia De Lang' from the Institute of Plant Breeding was studied. A total of 150 leaf specimens were digitized, examined, measured, and described. The semicraspedodromous pattern of the secondary veins and the angle between the 3° to 1° vein were characters found to have taxonomic significance in describing *H. rosa-sinensis* species while the angle of the 2° vein was important for delineating *H. rosa-sinensis* varieties. The resulting dendrogram from the cluster analysis grouped all the *H. rosa-sinensis* from the outgroup and further created three subclusters that were closely related.

Keywords: leaf architecture, leaf morphology, venation patterns, *Hibiscus rosa-sinensis*, varieties.

INTRODUCTION

Leaf architecture has been recognized as a promising tool in naming and classifying plant taxa, especially in the absence of reproductive parts such as flowers and fruits (Bhat 1995; Fuller and Hickey 2005; Pacheco-Trejo et al. 2009; Laraño and Buot 2010; Lu et al. 2012; Masungsong et al. 2019; Buot 2020). The leaf provides important diagnostic features such as venation patterns, shape, and size that can help describe and establish taxonomic relationships. Through a detailed examination and documentation of leaves, even taxonomic confusion in the identity can be resolved (Baltazar and Buot 2019; Buot 2020).

Hibiscus rosa-sinensis L. (Malvaceae) is a widely cultivated ornamental plant in the tropical and subtropical regions of the world. Prized mainly for its large, showy, and colorful flowers, many breeders have successfully produced hybrids that are coming in and out of the plant market. In the Philippines, the Institute of Plant Breeding (IPB) at the University of the Philippines Los Baños has collectively released more than 40 hybrids since 1995

(Magdalita and Pimentel 2013; Magdalita and San Pascual 2021). But this is only a small part of the more than 24,000 cultivars listed in the database of the International Hibiscus Society (2023), many not officially named.

Over the years, most leaf architecture studies have focused mainly on the family, genus, and species levels. For instance, Laraño and Buot (2010) studied the leaf architecture of the Malvaceae family to present pieces of evidence of its circumscription in the Angiosperm Phylogeny Group (2003). Similarly, Bhat (1995) employed leaf architectural analysis within the genus *Hibiscus* as represented by 13 species including the most famed *Hibiscus rosa-sinensis*. We currently have little information about the significance of leaf architecture at a varietal level. Therefore, this study was conducted to fill this research gap by examining the leaf architecture of *Hibiscus rosa-sinensis* varieties.

MATERIALS AND METHODS

Four varieties of *Hibiscus rosa-sinensis* namely 'Lolay', 'Nay Isa', 'Reddy or Not', and 'Saskia De Lang' were selected for this study based on the availability of stock plants at the Institute of Plant Breeding, the University of the Philippines Los Baños. The species *Hibiscus mutabilis* L. was also included as an outgroup.

A cumulative total of 150 fresh and mature leaf samples were collected, pressed, and dried with 30 samples gathered from each kind. Digital images of the specimens were produced using MicroTek ObjectScan 1600 at the maximum resolution of 1600 dpi. Their leaf architecture was described following the manual of the Leaf Architecture Working Group (Hickey 1973; LAWG 1999; Ellis et al. 2009). ImageJ software was utilized to measure areas and angles. Data collected were analyzed using Multivariate Hierarchal Cluster Analysis in Past4. Algorithm was set to Unweighted Pair-Group Method with Arithmetic Means (UPGMA) and Euclidean distance was applied as the Similarity Index; Boot N = 10,000.

RESULTS AND DISCUSSION

The 30 collected leaf samples for each kind shared overall similar laminar features. There were only nuanced variations on the sizes and colors of the blade that were directly observed both in the fresh and dried states.

Leaves of the four *Hibiscus rosa-sinensis* varieties (Fig. 1) were generally ovate, symmetrical, serrated, and unlobed. Size can be categorized into microphyll, mesophyll, and notophyll; the base angle is either wide obtuse or obtuse; the base shape is cordate, cuneate, or convex; the apex shape is either acute or acuminate; and the apex angle can be obtuse or acute. Petiolar insertion is found at the margin (Table 1).

In terms of venation patterns, results show that the 1° vein category, 2° vein spacing, inter-2° veins, 3° vein category and course, areolation, marginal ultimate venation, and freely ending ultimate veins based on Hickey's descriptors (1973) were found similar within and among species (Table 1). Hence, these qualitative characters cannot be considered of taxonomic significance to the members of the genus and among *H. rosa-sinensis* varieties. However, it can probably have taxonomic importance when classifying higher taxa rankings.

The basal actinodromous pattern, well-developed areoles, and an incomplete marginal venation were consistent in all *Hibiscus* species as reported by Bhat (1995). The findings of the present study also indicated that these patterns remain unchanged among H. rosa-sinensis varieties. Hence, it confirms that these characters were significant in delineating the genus with other members of the family Malvaceae.

On the other hand, the category of the 2° veins in all four varieties displayed a semicraspedodromous pattern in which one of the branches terminate at the margin and the other joined the super-adjacent 2° veins. Similarly, in *Mangifera indica*, the category of the 2° vein which was camptodromous was consistent in all 30 varieties (Sharma et al. 2016). The pattern of the 2° veins can be used as a robust taxonomic criterion for delimiting species, although further validation is necessary to ascertain its consistency across varieties of other plant species.

In terms of numerical data (Table 2), *Hibiscus rosa-sinensis* had 5 primary veins and the highest vein order was identified up to 4 orders. The L: W ratio was reported in full range values for all varieties. Areole area was found smallest in *H. mutabilis* followed by 'Reddy or Not', 'Nay Isa', 'Saskia De Lang', and the largest 'Lolay'. With regards to the 2° vein angle, 'Saskia De Lang' had the largest while the outgroup species had the smallest measured angle. Lastly, the angle of the 3° veins relative to the 1° vein was found smallest at 'Lolay' followed by 'Nay Isa', 'Saskia De Lang', 'Reddy or Not', and *H. mutabilis* respectively.

Although many neglected leaf characters as a basis for taxonomic delineation due to views that these exhibit high phenotypic plasticity (Medina et al. 2016; Buot 2020), venation patterns are likely determined by genetic factors and are consistent (Roth-Nebelsick et al. 2001). Succeeding studies also vouched the findings of Roth-

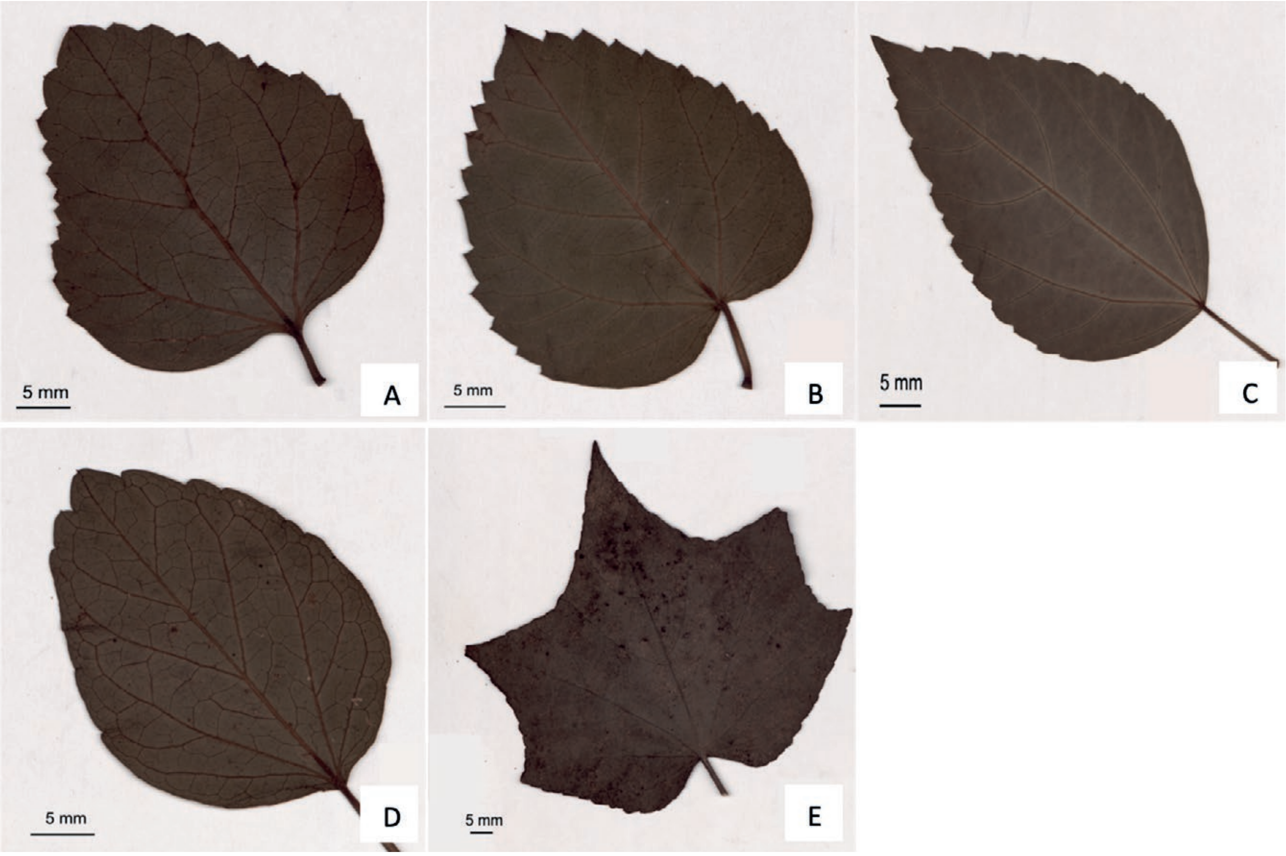

Figure 1. Photograph showing the leaf architecture details of A. *Hibiscus rosa-sinensis* 'Lolay' B. *Hibiscus rosa-sinensis* 'Nay Isa' C. *Hibiscus rosa-sinensis* 'Reddy or Not' D. *Hibiscus rosa-sinensis* 'Saskia De Lang' and E. *Hibiscus mutabilis* (outgroup).

Nebelsick et al. (2001) that leaf architecture patterns are genetically fixed (Huiet et al. 2018; Baltazar and Buot 2019; Tan and Buot 2020). In fact, accurately extracting leaf shape and venation patterns digitally for plant identification and botanical research is of interest in the field of computer vision (Cao et al. 2017).

Moreover, qualitative morphological characters of leaves cannot be solely relied upon as criteria for taxonomic and phylogenetic evaluation due to differing opinions on which characters must be considered significant (Bhat 1995; Buot 2020). It also tends to generalize characteristics that could probably have significant value when actual measurements were used in the analysis. Hence, the measured quantitative data on L: W ratio, highest vein order, no. of primary veins, 2° vein angle, the angle between the 3° and 1° vein, and areole area were the basis for the cluster analysis of this study.

The resulting dendrogram from the hierarchal cluster analysis differentiated all the *Hibiscus rosa-sinensis* species with the outgroup *Hibiscus mutabilis* at 19 Euclidean distances with bootstrap values = 100 (Fig. 2). The angle between the 3° vein in relation to the 1° vein proved to be a valuable character in depicting *H. rosa-sinensis* from the outgroup. The findings aligned with other plant genera such as *Cucumis* (Masungsong et al. 2019), *Ficus* (Loutfy et al. 2005), and *Terminalia* (Baroga and Buot 2014) where in the same character contributed to species delineation.

On the other hand, the 2° vein angle grouped the four varieties into 3 subclusters. 'Reddy or Not', a cross between *H. rosa-sinensis* and *H. schizopetalus*, separated from the IPB varieties at 9 Euclidean distances with 73 bootstrap values. The said variety was a known rootstock hybrid from Australia (International Hibiscus Society 2023). The three-way cross hybrid 'Saskia De Lang' from the IPB Diplomat Series released in honor of the Netherlands Ambassador to the Philippines (Afable 2022) formed the second subcluster with 2 other IPB varieties at 5 Euclidean distances with 100 bootstrap values. Lastly, 'Nay Isa' and 'Lolay' both from the Centennial Series were sister taxa. Therefore, the 2° angle is an important character for the taxonomical investiga-

Table 1. Qualitative leaf features of *Hibiscus rosa-sinensis* varieties.

No	Taxonomic character	*H. rosa-sinensis* 'Lolay'	*H. rosa-sinensis* 'Nay Isa'	*H. rosa-sinensis* 'Reddy or Not'	*H. rosa-sinensis* 'Saskia De Lang'	*H. mutabilis*
1	Leaf size	notophyll	notophyll	mesophyll	microphyll	mesophyll
2	Leaf shape	ovate	ovate	ovate	ovate	ovate
3	Leaf symmetry	symmetrical	symmetrical	symmetrical	symmetrical	symmetrical
4	Base angle	wide obtuse	wide obtuse	obtuse	obtuse	wide obtuse
5	Base shape	cordate	cordate	cuneate	convex	cordate
6	Apex angle	obtuse	obtuse	acute	obtuse	odd-lobed obtuse
7	Apex shape	acute	acuminate	acuminate	acute	acuminate
8	Petiole position	marginal	marginal	marginal	marginal	marginal
9	Margin Type	serrate	serrate	serrate	serrate	serrate
10	Lobation	unlobed	unlobed	unlobed	unlobed	Palmately lobed
11	1° vein category	basal actino-dromous	basal actino-dromous	basal actino-dromous	basal actino-dromous	basal actino-dromous
12	2° vein category	semicraspe-dodromous	semicraspe-dodromous	semicraspe-dodromous	semicraspe-dodromous	craspe-dodromous
13	2° vein spacing	increasing to base	increasing to base	increasing to base	increasing to base	increasing to base
14	Inter-2° veins	strong	strong	strong	strong	strong
15	3° vein category	alternate percurrent	alternate percurrent	alternate percurrent	alternate percurrent	alternate percurrent
16	3° vein course	sinuous	sinuous	sinuous	sinuous	sinuous
17	Areolation	well-developed	well-developed	well-developed	well-developed	well-developed
18	Marginal ultimate venation	incomplete; toothed	incomplete; toothed	incomplete; toothed	incomplete; toothed	incomplete; toothed
19	Free-ending veins	absent	absent	absent	absent	absent

Table 2. Numerical data on venation patterns of *Hibiscus rosa-sinensis* varieties.

Name of Taxa	L: W Ratio	Highest Vein Order	No. of 1° vein	2° vein angle	Angle between 3° to 1° veins	Areole Area (mm^2)
H. rosa-sinensis 'Lolay'	1:1	4	5	51.75	37.01	8.55
H. rosa-sinensis 'Nay Isa'	7:6	4	5	52.04	38.39	5.45
H. rosa-sinensis 'Reddy or Not'	2:1	4	5	57.22	41.67	2.67
H. rosa-sinensis 'Saskia De Lang'	13:9	4	5	46.98	39.67	7.33
H. mutabilis	11:12	5	7	64.00	53.00	1.28

tions of *H. rosa-sinensis* varieties. Leaf characters such as highest vein order, number of primary veins, areole area, and L: W ratio were deemed insignificant.

CONCLUSIONS

This paper demonstrated the significance of leaf architecture in delineating different *H. rosa-sinensis* varieties namely 'Lolay', 'Nay Isa', 'Saskia De Lang', and 'Reddy or Not'. The findings confirmed that leaf architecture is a tool helpful not just in higher taxa but also in classifying varieties, especially in the absence of reproductive structures. The 2° vein angle was an important taxonomic character for the delineation of *Hibiscus rosa-sinensis* varieties while the 2° vein category and the 3° vein angle in relation to the 1° vein were both important for the species. It is suggested to further explore leaf architecture in varieties of other plant species.

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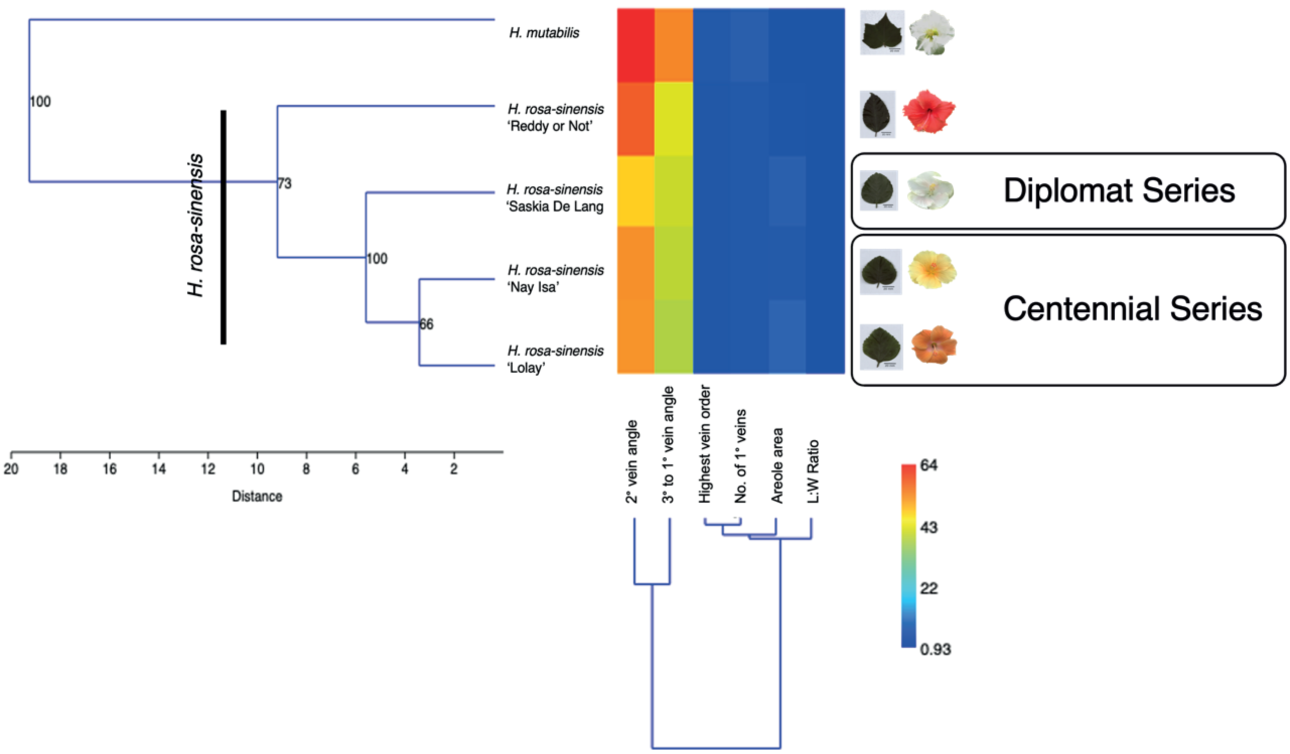

Figure 2. Dendrogram showing clustering of *Hibiscus rosa-sinensis* varieties using Unweighted Pair-Group Method with Arithmetic Means (UPGMA) with Euclidean Distance as the Similarity Index; Bootstrap values = 10,000.

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Announcement

Montgomery Botanical Center and *Centro Studi Erbario Tropicale*, *Università di Firenze* develop research and academic ties in Tropical Botany

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Dr. Riccardo M. Baldini, Editor in Chief of Webbia, and researcher at the Centro Studi Erbario Tropicale of the University of Florence, Italy, was invited by Montgomery Botanical Center (MBC), Coral Gables, Miami-Dade, Florida to deliver a talk entitled "*The naturalistic explorations in the Caribbean and Neotropics: Understanding Plant Taxonomy of the New World*." The lecture was very well attended, and took place on June 15 at MBC. The venue was also part of the undergraduate course "Biodiversity on the Caribbean Islands" that Prof. Francisco-Ortega delivers regularly in Florida International University (FIU). Montgomery Botanical Center is the premier botanic garden in palm and cycad research and conservation of the United States. This garden was established by Col. Robert Montgomery, who was also the founding and first president of Fairchild Tropical Botanic Garden. Dr. Baldini's visit spanned between June 12 and 17, and plans were outlined for future initiatives between MBC, FIU, and Dr. Baldini centered in plant exploration and botanical history of palms and cycads as well as graduate student teaching.

This visit was jointly sponsored by MBC, and it is anticipated future additional academic developments with the participation of the Graduate Program of Florida International University and the International Center for Tropical Botany to which Prof. Francisco-Ortega is formally affiliated to.

Participants in R.M. Baldini's lecture at Montgomery Botanical Center, Miami, FL, USA.

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Index of New Taxa

Edited by Riccardo M. Baldini (Editor in Chief)

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