

Leaf architectural analysis of taxonomically ambiguous *Hoya lacunosa* Blume and *Hoya krohniana* Kloppenb. & Siar

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Abstract. Scott HC, Buot JR IE. 2022. Leaf architectural analysis of taxonomically ambiguous *Hoya lacunosa* Blume and *Hoya krohniana* Kloppenb. & Siar. *Biodiversitas* 23: 2055-2065. The horticulturally important *Hoya* R.Br. species *Hoya lacunosa* Blume and *Hoya krohniana* Kloppenb. & Siar are often mistaken for each other because of their generally similar inflorescence morphology. Based on the hypothesis that leaf venation patterns are genetically fixed, leaf architectural analysis was done to determine the difference between these two taxonomically confusing species. Thirty fully expanded leaves were obtained per species from mature plants of *H. lacunosa*, *H. krohniana* and an outgroup, *H. pubicorolla*. Laminar and venation characters were analyzed using standard leaf architecture protocols. Results show that the main distinction lies in their laminar characters, particularly the base angle and base shape. *H. lacunosa* samples showed acute to obtuse base angles with cuneate and convex leaf bases, while *H. krohniana* were found to have obtuse reflex base angles with convex, cordate and rounded leaf bases. Analysis of venation characters shows no considerable difference between the patterns seen in *H. lacunosa* and the patterns observed in *H. krohniana*. Further investigation of higher vein orders is recommended. Initial comparison of *H. lacunosa* pollinaria with the photos of *H. krohniana* pollinaria from its type description show strikingly similar morphology; for this reason, we also recommend floral morphology comparisons, particularly pollinaria morphology to further establish the similarity and delineation of *H. lacunosa* and *H. krohniana*.

Keywords: Leaf architecture, numerical taxonomy, *Hoya* genus

INTRODUCTION

Leaf architecture describes the placement and form of elements that express leaf structure, "including venation pattern, marginal configuration, leaf shape, and gland position". Leaf fingerprint helps identify the plant species based on anatomy, morphology, and chemical aspects (Baltazar and Buot 2019). Most dicots have stable patterns of leaf architecture, making this method of description a useful tool in taxonomic studies (Hickey 1973). Leaf architecture has been primarily used by paleobotanists, whose main study materials are fossilized leaf remnants, like leaf impressions and compressions. A review of the literature on leaf architecture shows an increasing range of applications of this method (Vasco et al. 2014). Roth-Nebelsick (2001) has found that in general, the leaf venation pattern of a species is genetically fixed, providing the basis for using the leaf venation as a taxonomic tool. Some applications include Laraño and Buot (2010) on Malvaceae, and Masungsong et al. in 2019 on *Cucumis* species, among others. A comprehensive review of the utility of leaf architecture for resolving plant taxa controversies was published by Buot (2020).

As far as plant taxa controversies go, the genus *Hoya* R.Br. stands out in the Philippines (Baltazar and Buot 2019). The recent spike in the discovery and naming of new *Hoya* species in the country has brought to light the need to evaluate the genus and conduct a critical revision (Juhonewe and Rodda 2017). The number of Philippine

Hoya species currently published has increased considerably, from 109 in 2013 (Aurigue) to 207 in 2021 (Co's Digital Flora of the Philippines, Pelsner et al. 2021). A portion of this substantial increase can be attributed to the online publication *Hoya New* by Green and Kloppenburg (Cabactulan et al. 2019). No genus revision has been published as of this writing; the need for this revision persists amid the recent increase in the popularity of urban gardening, which gives even more public significance to identifying plants properly. In the interim, several studies have utilized leaf architecture analysis to offer supplemental information on the delineation of some controversial *Hoya* species, such as *H. incrassata* vs. *H. crassicaulis* (Villareal and Buot 2015), *H. buotii* vs. *H. halconensis* (Jumawan and Buot 2016), among *H. carandangiana*, *H. bicolensis*, and *H. camphorifolia* (Torrefiel and Buot 2017), *H. merrillii* vs. *H. quinquenervia* (Paguntalan and Buot 2019), to name a few.

Two horticulturally important *Hoya* species that often elicit confusion are *Hoya lacunosa* Blume and *Hoya krohniana* Kloppenb. & Siar. The inflorescences are reported to be similar in general morphology, while Kloppenburg (2009) has cited the difference in pedicel lengths between the two species in his publication of *H. krohniana*. Growers and collectors anecdotally indicate the difference in leaf shape and size when trading these two species, citing lanceolate leaves for *H. lacunosa*, and consistently cordate leaves for *H. krohniana*, earning it the trade name "heart-shaped lacunosa" prior to the publication

of the taxon. However, leaf shape in *Hoya* has exhibited a wide range of variability (Medina et al. 2016). Thus, in order to expand our understanding of the distinction between these two species, this study aims to: (i) Identify the leaf architectural characters of *H. lacunosa* Blume and *H. krohniana* Kloppenb. & Siar; (ii) Analyze these characters in order to offer information on numerical taxonomy for the delineation between these two species.

MATERIALS AND METHODS

Study Species

Hoya lacunosa Blume is an epiphytic plant with a decumbent climbing habit. It has delicate, sweet-scented white or yellow flowers. The name derives from its having leaves that are convex between the secondary veins, which in Latin is *lacuna*. It is widely distributed in Southeast Asia, and is native to Thailand, Peninsular Malaysia, Singapore, Sumatra, Java, Borneo (Kalimantan), the Philippines (Lamb and Rodda 2016), New Guinea and Papua New Guinea (Aurigue 2013). The taxon was first published in 1826 by Carl Ludwig Blume in *Bijdragen tot de flora van Nederlandsch Indië* (POWO, 2021). The species is notably variable (Aurigue 2013), and its flowers are easily confused with *Hoya nabawanensis* (Lamb and Rodda 2016) and *H. krohniana* (Kloppenb. & Siar 2009); another related species is *Hoya mirabilis* (Kidyoo 2012).

Hoya krohniana Kloppenb. & Siar is described in its first publication in 2009 as being similar to *H. lacunosa* in most respects, but different in having longer pedicel length and larger corolla. Its habit in the wild is not described in the publication; the type locality is similarly omitted, with the type source cited as “thought to be from the Philippines” and having come from a certain Cindy Krohn, of unspecified location, who provided the plant material and photos to the authors in 2006. The taxon was published in *Fraterna*, the official bulletin of the International Hoya Association (Kloppenb. & Siar 2009). It is accepted by Govaerts et al. as shown for this taxon record on Plants of the World Online (POWO, 2021) and the International Plant Names Index (IPNI, 2022), but reported as “ambiguous” in World Flora Online (WFO, 2021).

The morphological variability of *H. lacunosa* is evidenced by the differences in observed characteristics that have been published for the taxon over the years. Three relevant sets of these observations are listed in Table 1, along with the corresponding character states indicated in the type description of *H. krohniana* by Kloppenb. & Siar (2009). Descriptions of *H. lacunosa* by Kloppenb.

have been taken from his 2004 Monograph of Malaysian *Hoya* species, in which he translates the type description for *H. lacunosa* and adds a separate 1848 description by Blume. Kidyoo (2012), in publishing the related species *H. mirabilis*, described *H. lacunosa* from the type and additional spirit material from their own collection. Description from Aurigue (2013) is taken from *A Collection of Philippine Hoyas and their Culture* ISBN 978-971-20-0554-1; Lamb and Rodda (2016) descriptions are from *A Guide to Hoyas of Borneo* ISBN 978-983-812-168-2.



Figure 1. *Hoya krohniana* inflorescence



Figure 2. *Hoya lacunosa* inflorescence

Table 1. Comparison of characters documented for *Hoya lacunosa* and *Hoya krohniiana*

Characters	<i>Hoya lacunosa</i> Blume				<i>Hoya krohniiana</i> Kloppenb. & Siar type description, 2009
	Blume (1826, 1848) as cited by Kloppenb. (2004)	Kidyoo (2012)	Aurigue (2013)	Lamb & Rodda (2016)	
Leaf shape	“Ovate or ovate-lanceolate, acuminate, with the base rounded”	Ovate, elliptic, lanceolate, oblanceolate Base cuneate, obtuse, rounded Apex acute to acuminate	Ovate to narrowly elliptic	Narrowly lanceolate to elliptic	Cordate
Leaf size	“1-1.5 inches, 8-10 lines wide”	3-7 cm x 1-2.5 cm	Approx. 7.5 cm x 2 cm	(1.5)2-5(9) cm x 0.7-2.5 cm	“Small”
Leaf Venation	“Veinless”, but noted by Kloppenb. as pinnate, with side nerves about 60° to the midrib	3-5 pairs of lateral nerves, at right angles to the midrib	-	Pinnate with raised secondary veins (Lamb and Rodda, 2016)	-
Leaf surface and other characteristics	“Fleshy above the base minute paired glands, the superior blade without veins and traversed a little with pronounced dips (lacunose) shiny, below deeply convex veinless and whitish”	Margin faintly recurved Abaxial surface glabrous	Hairless, with uneven surfaces on the deep green upper surface	Glabrous, dark green above, lighter green underneath. Petioles are round, 0.5-2 cm long	Deep green, opposite foliage
Inflorescence	-	-	Umbel 2.8 cm, flat with approx. 20 flowers	Pendulous flat to slightly concave, 2-3.5 cm in diameter with 20-35 flowers; peduncle 2-8 cm, glabrous or sparsely pubescent	-
Corolla	revolute	Rotate, adaxially pubescent Lobes ovate, revolute with acute apex	-	3.5 - 6 mm in diameter, white or cream, pubescent outside, with revolute lobes	Revolute, surface pubescent except for the apex, which is glabrous; Apex to center 6 mm
Corona	-	Coronal scales elliptic, outer angle acute and upcurved	-	White or light yellow, darker in the center with rounded outer lobes and acute, raised inner lobes; base surrounded by a broad skirt	Glabrous, shiny; corona skirt extends downward (centrally) around this column, skirt is continuous with scalloped areas between the lobes.
Fruit	-	-	-	Fusiform 4-5 cm x c. 8mm	-

Collection and sampling of plant materials

Plants were acquired from local *Hoya* collectors who obtained them from reputable *Hoya* growers in the Philippines. Sampling was aimed at collecting specimens that were traded under the names *H. lacunosa* and *H. krohniana*, regardless of whether the plants were in flowering stage or not, since these two species are distinguished in trade based on leaf shape, and sold in common practice without the need for verified identification. An outgroup was included; for this, the distinctly different *Hoya pubicorolla* Kloppenb., G.Mend. and Ferreras was sampled. Individual plant sources are cited in the Acknowledgments.

Thirty fully expanded leaves from each plant were processed for leaf clearing, which was done by boiling the specimens in sodium hydroxide solution. The duration of boiling varied per species, relative to the size of the leaves, with the largest leaves in the outgroup *H. pubicorolla* taking the longest. The epidermal cuticle was carefully removed from the adaxial surfaces of the leaves, the fragile mesophyll and abaxial cuticle were left intact in order not to disturb the venation structure. The prepared leaves were then left to dry flat at room temperature between sheets of plastic film weighed down by wood panels. Once dried, each leaf was placed in individual sleeves of oriented polypropylene and assigned a unique code. Thirty *H. lacunosa* samples were labeled LAC01-LAC30, *H. krohniana* samples were labeled KRH01-KRH30, and *H. pubicorolla* samples were labeled PBC01-PBC30.

Measurement of leaf aspects and venation characters

General leaf and vein characteristics were identified based on the terminology and recommendations detailed in the Manual of Leaf Architecture by Ellis et al. (2009). Two categories of characters were identified, (1) laminar and (2) venation characters.

The linear aspects were measured using a digital caliper. A circular protractor was used to measure angles. The laminar characters that were observed and measured were: laminar length, laminar width, laminar area, blade class, laminar ratio, laminar shape, laminar symmetry, base angle, base shape, apex angle, and apex shape.

Given the small size of the specimens, accuracy in identifying the venation characters was improved by placing the leaves on a backlit, transparent acrylic sheet and photographing each leaf; after which, observation was done using enlarged prints of the photographs. A dissecting microscope was used to photograph the smaller venation patterns. Venation characters described were: primary vein framework, major secondary vein framework, interior secondaries, minor secondary course, perimarginal veins, major secondary spacing, variation of major secondary angle to midvein, major secondary attachment to midvein, intersecondary veins proximal course, intersecondary vein length, intersecondary vein distal course, and intersecondary vein frequency.

Statistical analysis

Measurements were tabulated and formatted for analysis in R (v2.12 R package, 2021). The laminar and

venation characteristics of the leaf samples were used to cluster *H. lacunosa* and *H. krohniana*. An outgroup species, *H. pubicorolla*, was included to help establish the degree of similarity between the two species. Gower's coefficient of dissimilarity (*cluster* v2.12 R package) (Gower, 1971) was used to create the distance matrix between samples followed by agglomerative hierarchical clustering (*stats* v4.1.2 R package) using single, average, and complete linkage methods. To decide which among the three linkage methods best describe the clustering, cophenetic correlation coefficient (*stats* v4.1.2 R package) was used. Distance-based redundancy analysis (*vegan* v2.5-7 R package) was done after doing principal coordinate analysis (*ape* v5.5 R package) to identify which leaf characteristics had a major influence on the clustering. To identify which species had the higher intraspecies variation, deviation from the mode (*qualvar* v0.2.0 R package) (Wilcox 1973) and mean of the log₁₀-transformed variance was used for the qualitative and quantitative leaf characteristics, respectively. The mean of the first two axes of the principal coordinate analysis was used to calculate the centroid of each species. Five samples farthest from the centroid (Euclidean distance) of each species were identified to be the samples most distant from the consensus of each species characteristics.

RESULTS AND DISCUSSION

Summarized in Table 2 is the range of values and character states observed in the specimens for this study. *Hoya lacunosa* specimens were fully expanded leaves collected from a garden in Mabini, Batangas, Philippines. *Hoya krohniana* specimens were fully expanded leaves collected from a garden in Kalayaan, Laguna, Philippines. The sources for both plants are reputable collectors (see Acknowledgements). Both plants generally exhibited the distinct traits commonly used in trade to distinguish them from other *Hoya* species, i.e. consistently cordate leaves for *H. krohniana* and generally elliptic to lanceolate leaves for *H. lacunosa*.

Venation was characterized only up to the secondary vein order since some samples exhibited potentially disturbed tertiary vein fabric from having the epidermal cuticle removed. The mesophyll of all samples was thick enough to obscure the view of finer venation patterns, but too delicate to be cleared without disturbance.

Laminar characters common to all species and showed no variability among samples were: leaf attachment, leaf arrangement, leaf organization, medial symmetry, and margin type. Laminar characters that showed variation were: laminar width, laminar length, laminar area, blade class, laminar shape, apex angle, apex shape, base angle, and base shape. The range of values is reported in Table 2.

Consistent with characteristics used in trade, numerical qualifiers from Ellis et al. (2009) put *H. lacunosa* leaves as generally elliptic; many samples were narrowly elliptic but none were found to be lanceolate, i.e. no sample had its widest laminar point occurring in the proximal two-fifths of the laminar length. Similarly consistent with the identifying

characteristics used in its trade, *H. krohniana* leaves were generally more ovate to elliptic, with leaf base shapes observed to be convex, cordate or rounded; none of the *H. lacunosa* samples had a cordate base shape. Cluster analysis of the actual laminar observations is illustrated in Figure 3, showing the extent of dissimilarity between the samples. As demonstrated by the dendrogram (Fig. 3),

there is a distinct difference in the leaf shape characteristics of *H. lacunosa* and *H. krohniana* samples. Any similarities may be attributed to unifying genus characters and the possibility of phylogenetic relatedness between the two species, accounting for the taxonomic ambiguity that prompted this study.

Table 2. General laminar characters as measured and observed by the authors in the study specimens of *Hoya lacunosa*, *Hoya krohniana* and *Hoya pubicorolla*. Character differences between the main study species are highlighted

Laminar characters	<i>Hoya lacunosa</i>	<i>Hoya krohniana</i>	<i>Hoya pubicorolla</i>
Leaf attachment	Petiolate	Petiolate	Petiolate
Leaf arrangement	Opposite	Opposite	Opposite
Leaf organization	Simple	Simple	Simple
Laminar length	27.86 to 74.36 mm	16.47 to 30.18 mm	59.7 to 113.56 mm
Laminar width	9.59 to 20.58 mm	13.68 to 20.50 mm	21.91 to 37.62 mm
Laminar area	225.36 to 926.34 mm ²	184.47 - 421.32 mm ²	1,022.21 to 3,204
Blade class / Laminar size	Microphyll	Nanophyll to microphyll	Microphyll to notophyll
Laminar ratio	6.2:1 to 1.9:1	1.9:1 to 1.1:1	2:1 to 4.4:1
Laminar shape	Narrowly elliptic to elliptic	Elliptic to ovate	Elliptic to ovate
Medial symmetry	Symmetrical	Symmetrical	Symmetrical
Base symmetry	Symmetrical	Symmetrical	Symmetrical
Margin type	Entire	Entire	Entire
Special margin features	None	None	None
Apex angle	Acute	Acute	Acute
Apex shape	Acuminate	Acuminate	Acuminate
Base angle	Acute to obtuse	Obtuse to reflex	Obtuse to reflex
Base shape	Cuneate, convex	Convex, cordate, rounded	Convex, cordate, rounded
Surface texture	Adaxial surface of the lamina as well as the petiole are sparsely puberulent; entire abaxial surface sparsely puberulent, more sparse than the adaxial surface	Adaxial surface of the lamina as well as the petiole are sparsely puberulent, abaxial surface sparsely puberulent on the margin and base	Glabrous

Table 3. General venation characters as measured and observed by the authors in the study specimens of *Hoya lacunosa*, *Hoya krohniana* and *Hoya pubicorolla*. Character differences between the main study species are highlighted

Venation characters	<i>Hoya lacunosa</i>	<i>Hoya krohniana</i>	<i>Hoya pubicorolla</i>
Primary Vein Framework	Pinnate	Pinnate	Pinnate
Major Secondary Vein Framework	Brochidodromous	Brochidodromous	Festooned brochidodromous
Interior Secondaries	Absent	Absent	Absent
Minor Secondary Course	Absent	Absent	Absent
Perimarginal Veins	Intramarginal	Intramarginal	Absent
Major Secondary Spacing	Irregular	Irregular	Irregular
Variation of Major Secondary Angle to Midvein	Uniform, smoothly decreasing proximally, inconsistent	Uniform, smoothly decreasing proximally, smoothly increasing proximally, inconsistent	Uniform, smoothly decreasing proximally, inconsistent
Major Secondary Attachment to Midvein	Excurrent, deflected	Excurrent, deflected	Decurrent
Intersecondary Veins Proximal Course	Parallel, perpendicular	Parallel, perpendicular	Parallel, perpendicular
Intersecondary Vein length	Variable - full range seen	Variable - full range seen	Variable - full range seen
Intersecondary Vein Distal Course	Ramifying	Ramifying	Ramifying, perpendicular to a subadjacent major secondary
Intersecondary Vein Frequency	Variable - full range seen	Variable - full range seen	<1 to 1

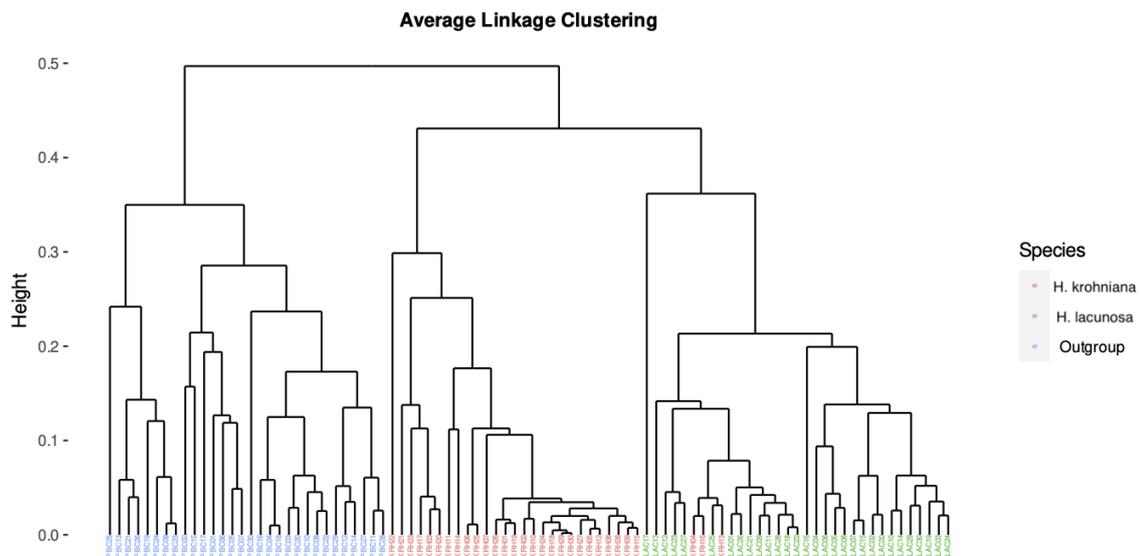


Figure 3. Cluster analysis of *H. lacunosa*, *H. krohniana* and outgroup based on laminar characters

Venation characters that were common to all species and showed no variability among samples were: primary vein framework, major secondary vein framework, interior secondaries, minor secondary course, perimarginal veins, major secondary spacing, and intersecondary vein distal course. Venation characters that showed a range of differences were: variation of major secondary angle to midvein, major secondary attachment to midvein, intersecondary veins proximal course, intersecondary vein length, intersecondary vein frequency. The latter set of characters may appear to have the same range in Table 3, but the actual frequency of each observation varies. The

variability is demonstrated in a cluster analysis shown in Figure 4. However, unlike the analysis for the laminar characters, the venation patterns show a more ambiguous picture of the degree of dissimilarity between *H. lacunosa* and *H. krohniana* samples. Given that the leaf venation pattern of a species is genetically fixed (Roth-Nebelsick 2001), the ambiguity found in the results may support a degree of similarity between the two species that may indicate they are not distinct enough from each other to be considered two separate species. This possibility will need to be further explored using floral morphology comparisons and molecular studies.

The laminar observations present a different degree of similarity compared to the results using venation patterns. Analysis combining these two sets of characters, shown in Figure 5, shows that the two species appear to be generally distinct. To better understand which of the characters observed exerted the most influence on distinction or similarity, a distance-based redundancy analysis was conducted.

The scatter plot for laminar traits (Fig. 6) demonstrates that the observations for leaf base shape and base angle are what distinguish *H. krohniana* from *H. lacunosa*, which is consistent with trade practices around the two species. The scatter plot for venation traits (Fig. 7) shows far less distinction between the two species, supporting the cluster analysis results (Fig. 4) that the venation patterns of the two species are not remarkably different from each other.

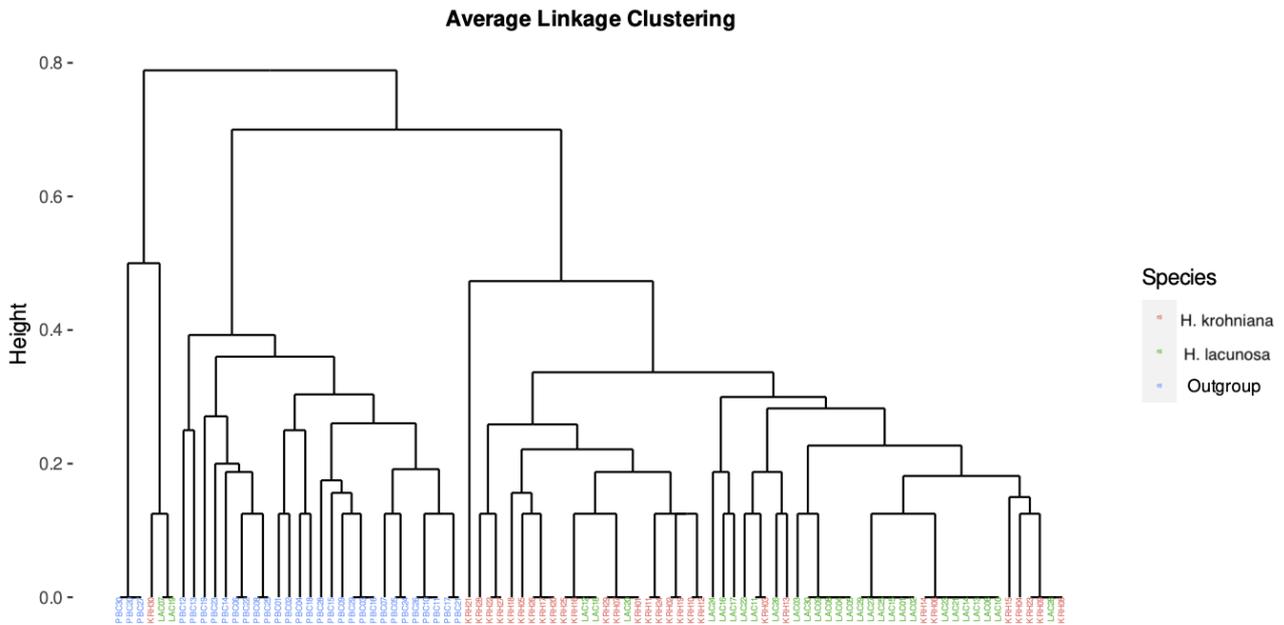


Figure 4. Cluster analysis of *H. lacunosa*, *H. krohniana* and outgroup based on venation characters

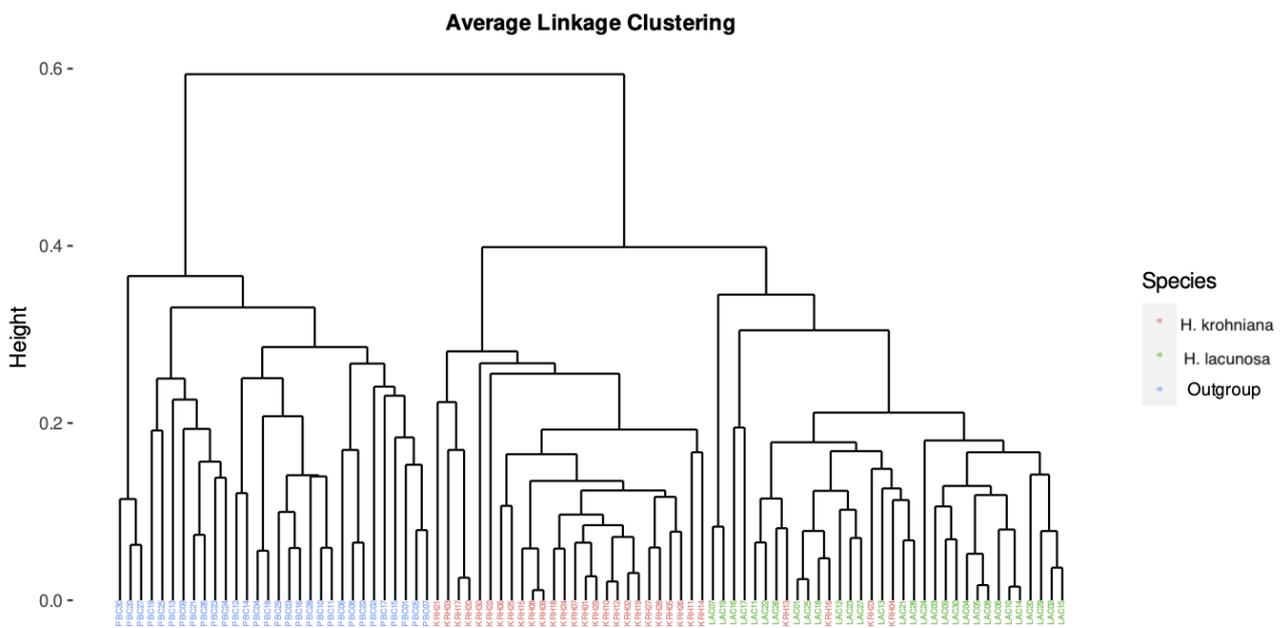


Figure 5. Average linkage clustering for combined laminar and venation characters of *H. lacunosa*, *H. krohniana* and outgroup

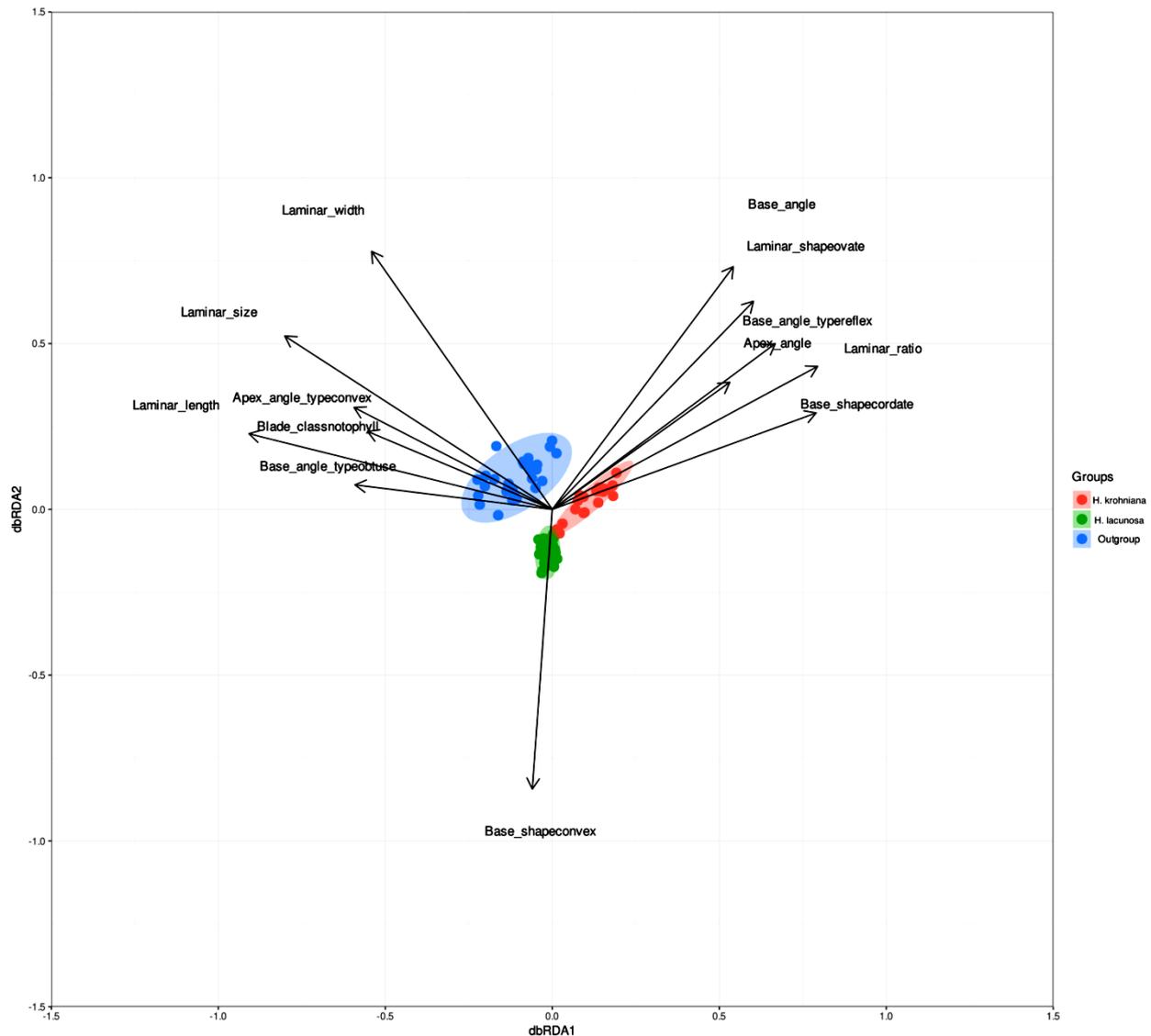


Figure 6. Distance-based redundancy plot for laminar characters of *H. lacunosa*, *H. krohniana* and outgroup

Several web and mobile applications for plant identification have been developed to utilize image-based analyses which largely factor in leaf shape for species recognition (Wäldchen and Mäder 2017). However, from the authors' experience as of this writing, the results given by many of these applications when used to identify *Hoya* plants have been less than satisfactory. While leaf shape is considered a generally reliable basis for plant identification (e.g. Laga et al. 2012; Mouine et al. 2013; Zhao et al. 2015), the variability of leaf morphology in *Hoya* species is a long-standing and well-supported observation. Examples of such observations are statements by Kleijn and van Donkelaar (2001) that some *Hoya* species are "simply indistinguishable" until they flower; in Forster and Liddle's (1991) combinations of subspecies under the *Hoya australis* complex; and the need for numerical taxonomy by

Kidyue et al. (2005) to delineate species of the *Hoya parasitica* complex, to mention a few. Floral morphology is considered a more reliable means of delineation of *Hoya* species, as supported by findings by Widiarsih et al. (2012) confirming that reproductive characters gave a more significant contribution to determining the genetic diversity among *Hoya mindorensis* accessions, and in the findings of Medina et al. (2016) that the phenoplasticity of *Hoya* vegetative structures require the use of reproductive structures for a more definitive way of describing *Hoya* species. To utilize leaf shape characters in the delineation in *Hoya* species, it may be used in combination with leaf venation and peduncle shape (Kleijn and van Donkelaar 2001), but to increase the reliability of identification, floral characters are best included, particularly pollinaria morphology (Baltazar and Buot 2019).

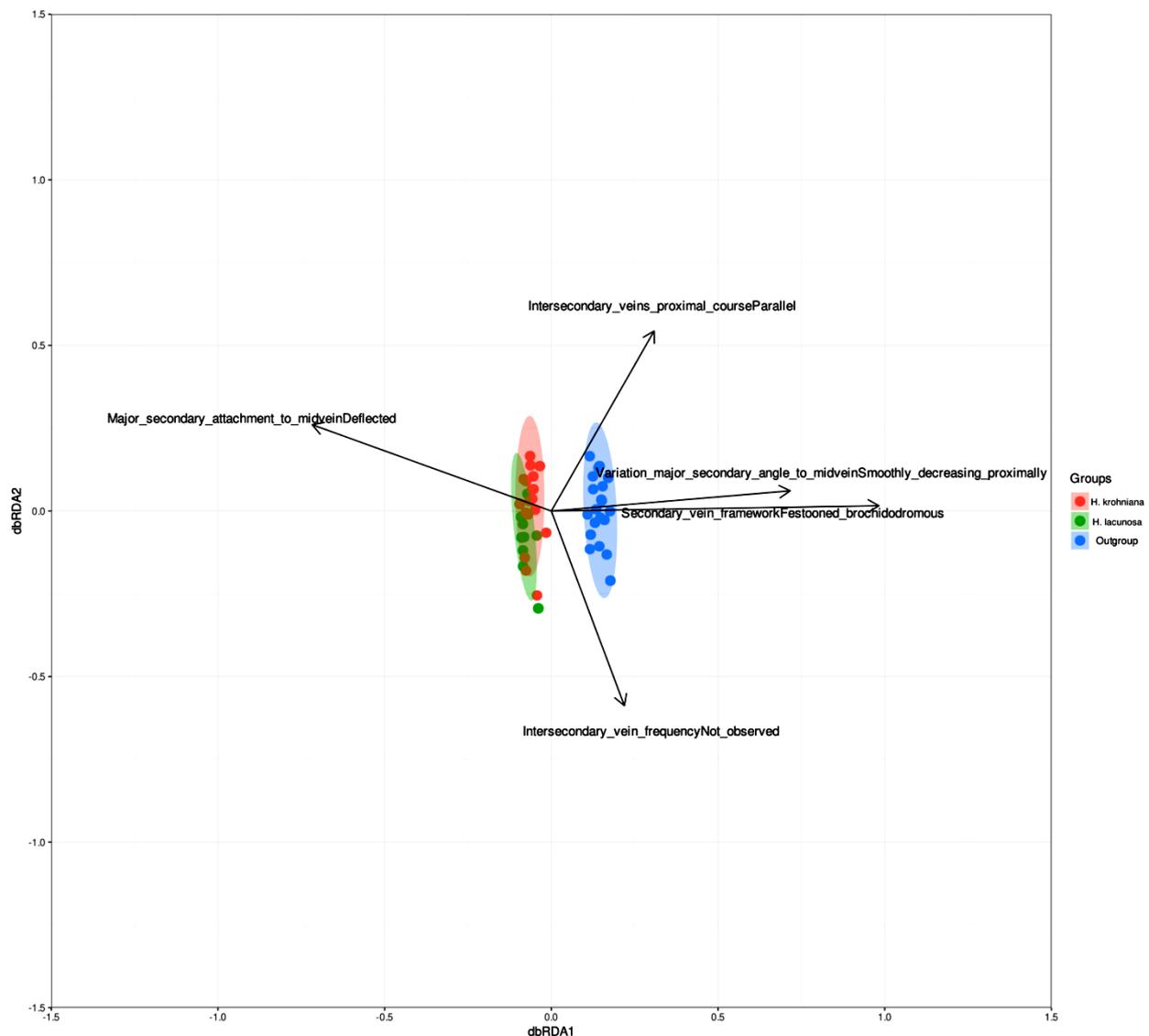


Figure 7. Distance-based redundancy plot for venation characters of *H. lacunosa*, *H. krohniana* and outgroup

Preliminary examination of *H. lacunosa* pollinaria shows a remarkable similarity to the pollinarium photograph presented in Kloppenburg and Siar's 2009 publication of *H. krohniana*. The caudicle and pollinia shapes are similar, as well as the proportions of the caudicle wings and retinaculum relative to the whole structure. Further sampling and measurements are needed to establish the degree of this similarity, but these initial observations, when combined with the results of the leaf architectural analysis, contribute to the ambiguity between the two taxa. *H. krohniana* may be another form of *H. lacunosa*, or a geographically separated subspecies. However, to consider the latter possibility brings up another concern around the taxonomy of *H. krohniana*.

The publication of *H. krohniana* in the magazine *Fraterna* 22:4 lacks specific geographical information on the provenance of the type specimen, therefore the type locality is not known for this species. It is currently

recognized as being endemic to the Philippines. However, in his article publishing the taxon, Kloppenburg offers the possibility that a number of herbarium sheets for *H. lacunosa* seem to have the leaf shape and size of *H. krohniana*. The sheets named appear in his monograph of Malaysian *Hoya* Species III (2004) under the entry for *H. lacunosa* and according to the details provided there, none of the sheets mentioned were collected from the Philippines. If these herbarium sheets are indeed misidentified, the implication is that *H. krohniana* may not even be endemic to the Philippines.

The only firm vegetative point of delineation that stands is the leaf base shape: no *H. lacunosa* sample was found to have a cordate base, yet there is an overlap of leaf base characters observed. Both species exhibit obtuse base angles and convex base shapes.

In conclusion, given that the character set that shows distinction is the laminar characters, we are unable to

establish the two species as distinctly different because leaf morphology is known to be variable in Hoyas (Medina et al., 2016). Venation characters show no considerable difference between the patterns seen in *H. lacunosa* from the patterns observed in *H. krohniana*. However, the venation data set in this study alone may not be sufficient to fully support the similarity. An improved leaf clearing protocol that specifically suits small, succulent Hoya leaves, which preferably need more time for the thick mesophyll to naturally desiccate and lose pigmentation under an intact epidermal cuticle, will help make it possible to have more granular observations of higher vein orders. Initial comparison of *H. lacunosa* pollinaria with the photos of *H. krohniana* pollinaria from its type description show strikingly similar morphology. Hence we also recommend conducting floral morphology comparisons to better establish the delineation between these taxa. Further, we posit that increasing the number of plant individuals to be sampled to include several plants of the same species that have been grown in nonidentical conditions will increase the chances of capturing the range of variability in observable leaf shapes for each of these species. Cultivating the two species under uniform conditions may similarly help identify their differences.

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REFERENCES

- Aurigue FB. 2013. A Collection of Philippine Hoyas and Their Culture. Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD). Department of Science and Technology (DOST).
- Baltazar AMP, Buot Jr IE. 2019. Controversies on Hoya R.Br. taxonomy. *Thail Nat Hist Mus J* 13 (1): 59-68.
- Baltazar AMP, Buot Jr IE. 2019. Short Communication: Leaf architectural analysis of taxonomically confusing coffee species: *Coffea liberica* and *Coffea liberica* var. *dewevrei*. *Biodiversitas* 20 (6): 1560-1567. DOI: 10.13057/biodiv/d200611.
- Buot Jr IE. 2020. Leaf architecture as a promising tool in confirming identity of confusing plant taxa. *J Nat Stud* 19 (1): 134-143.
- Cabactulan DD, Aurigue F, Pimentel R, Leon MDD, Sahagun J, Rodda M. 2019. Hoya of the Philippines, Part II: *Hoya pulchra* (Apocynaceae), a New Species from Southern Philippines. *Annales Botanici Fennici* 56 (1-3): 49. DOI: 10.5735/085.056.0109.
- Ellis B, Daly DC, Hickey LJ, Mitchell JD, Johnson KR, Wilf P, Wing SL. 2009. *Manual of Leaf Architecture* (Illustrated ed.). Comstock Publishing Associates.
- Forster PI, Liddle DJ. 1991. Variation in *Hoya Australis* R. Br. Ex Traill (Asclepiadaceae). *Austrobaileya* 3 (3): 503-521. <http://www.jstor.org/stable/41738789>.
- Govaerts R, Nic Lughadha E, Black N, Turner R, Paton A. 2021. The World Checklist of Vascular Plants, a continuously updated resource for exploring global plant diversity. *Sci Data* 8 (1): 1-10. DOI: 10.1038/s41597-021-00997-6.
- Gower JC. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857-874. DOI: 10.2307/2528823.
- Hickey LJ. 1973. Classification of the architecture of Dicotyledonous Leaves. *Am J Bot* 60 (1): 17-33. DOI: 10.1002/j.1537-2197.1973.tb10192.x.
- Juhonewe NS, Rodda M. 2017. Contribution to a revision of Hoya (Apocynaceae: Asclepiadoideae) of Papuaia. Part I: ten new species, one new subspecies and one new combination. *Gard Bull Singap* 69 (1): 97-147. DOI: 10.26492/gbs69(1).2017-07.
- Jumawan JH, Buot IE. 2016. Numerical taxonomic analysis in leaf architectural traits of some *Hoya* R.Br. species (Apocynaceae) from Philippines. *Bangladesh J Plant Taxon* 23 (2): 199-207. DOI: 10.3329/bjpt.v23i2.30851.
- Kidyoo M. 2012. *Hoya mirabilis* Kidyoo, a new species of Hoya (Asclepiadaceae) from Western Thailand. *Trop Nat Hist* 12 (1): 21-28.
- Kidyue M, Boonkerd T, Thaitong O, Seelanan T. 2005. Numerical taxonomy of the *Hoya parasitica* (Asclepiadaceae) complex in Thailand. *Nat Hist J Chulalongkorn Univ* 5 (2): 47-59.
- Kloppenbun RD, Siar S. 2009. *Hoya krohniana* Kloppenburg & Siar 2009. *Fraterna* 22 (4).
- Laga H, Kurtek S, Srivastava A, Golzarian M, Miklavcic SJ. 2012. A Riemannian Elastic Metric for shape-based plant leaf classification. 2012 International Conference on Digital Image Computing Techniques and Applications (DICTA). 3-5 December 2012. DOI: 10.1109/dicta.2012.6411702.
- Laraño AAP, Buot Jr IE. 2010. Leaf architecture of selected species of Malvaceae *sensu* APF and its taxonomic significance. *Philippine J Syst Biol* 4 (21): e54 DOI: 10.3860/pjsb.v4i0.1563.
- Leaf Architecture Working Group. 1999. *Manual of Leaf Architecture*. Leaf Architecture Working Group, Department of Paleobiology, Smithsonian Institution.
- Masungsong LA, Belarmino MM, Buot JR IE. 2019. Delineation of the selected Cucumis L. species and accessions using leaf architecture characters. *Biodiversitas* 20 (3): 629-635. DOI: 10.13057/biodiv/d200303.
- Medina MN, Amoroso V, Kloppenburg R. 2016. Changes of leaf morphology of *Hoya amorosae* from varying light exposure: Its implications to species description and taxonomy. *J Biodivers Environ Sci* 8 (6): 232-237.
- Mouine S, Yahiaoui I, Verroust-Blondet A. 2013. Combining Leaf Salient Points and Leaf Contour Descriptions for Plant Species Recognition. In: Kamel M, Campilho A (eds). *Image Analysis and Recognition*. ICAR 2013. Lecture Notes in Computer Science, vol 7950. Springer, Berlin, Heidelberg. DOI: 10.1007/978-3-642-39094-4_24.
- Paguntalan DP, Buot IE. 2019. Short Communication: Investigation of leaf architectural patterns: Implications in delineating taxonomically controversial *Hoya merrillii* Schlechter and *Hoya quinquenervia* Warburg. *Biodiversitas* 20 (3): 833-839. DOI: 10.13057/biodiv/d200329.
- Rahayu S, Rodda M. 2019. Hoya of Sumatra, an updated checklist, three new species, and a new subspecies. *Eur J Taxon* 508. DOI: 10.5852/ejt.2019.508.
- Roth-Nebelsick A. 2001. Evolution and Function of Leaf Venation Architecture: A Review. *Ann Bot* 87 (5): 553-566. DOI: 10.1006/anbo.2001.1391.
- Torrefiel J, Buot IE Jr. 2017. *Hoya carandangiana*, *Hoya bicolorensis*, and *Hoya camphorifolia* (Apocynaceae) species delineation: Insights from leaf architecture. *Thail Nat Hist Museum J* 11 (1): 35-44.
- Vasco A, Thadeo M, Conover M, Daly DC. 2014. Preparation of samples for leaf architecture studies, a method for mounting cleared leaves. *Appl. Plant Sci* 2 (9): 1400038. DOI: 10.3732/apps.1400038.
- Villareal AMM, Buot Jr IE. 2015. Leaf architecture of *Hoya incrassata* Warb. and *Hoya crassicaulis* Elmer x Kloppenb. (Apocynaceae): Taxonomic identification and conservation concerns. *IAMURE Intl J Ecol Conserva* 15 (1): 203-213. DOI: 10.7718/ijec.v15i1.1002.
- Wäldchen J, Mäder P. 2017. Plant species identification using computer vision techniques: A systematic literature review. *Arch Comput Methods Eng* 25 (2): 507-543. DOI: 10.1007/s11831-016-9206-z.
- Widiarsih S, Siar S, Lalusin A, Carandang J, Borromeo T. 2012. Genetic diversity assessment in vegetative and reproductive characters of *Hoya mindorensis* Schlechter. *Philippine J Crop Sci (PJCS)* 37 (3): 23-29.

- Wilcox AR. 1973. Indices of qualitative variation and political measurement. *West Political Q* 26 (2): 325-343. DOI: 10.2307/446831.
- World Flora Online. 2021. *Hoya krohmiana* Kloppenb. & Siar. <http://www.worldfloraonline.org/taxon/wfo-0000746580>.
- Zhao C, Chan SS, Cham WK, Chu L. 2015. Plant identification using leaf shapes-A pattern counting approach. *Pattern Recogn* 48 (10): 3203-3215. DOI: 10.1016/j.patcog.2015.04.004.