The Thailand Natural History Museum Journal 13(2): 77-89, December 2019

Original Article

Resolving Taxonomic Confusion between *Hoya cumingiana* Decne. and *Hoya densifolia* Turcz. (Apocynaceae) using Leaf Architectural Analysis

Alcona Mae P. Baltazar[1, 2*] and Inocencio E. Buot, Jr. [2,3]

[1]Deparment of Biological Sciences, Cavite State University – Main Campus, Indang, Cavite;
[2]Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna. E-mail: apbaltazar@up.edu.ph
[3]Faculty of Management and Development Studies, University of the Philippines Open University, Los Baños, Laguna

ABSTRACT: *Hoya cumingiana* and *Hoya densifolia* are facing taxonomic confusion due to their almost similar foliar and reproductive structural characters. In this study, 10 leaf samples for each species were collected and subjected to leaf architectural analysis. The two *Hoya* species showed dissimilarities in terms of blade shape, apex shape and angle, secondary vein spacing, secondary vein angle, and tertiary vein fabric. Cluster and ordination analyses revealed that *H. cumingiana* and *H. densifolia* are two separate species.

KEY WORDS: *Hoya cumingiana*, *Hoya densifolia*, leaf architecture, leaf venation, taxonomy.

INTRODUCTION

Ornamental interest in the genus *Hoya* is emerging within the last decade and is causing taxonomic proliferation and confusion (Meve, 2002; Rodda, 2012; Villanueva and Buot, 2017). This is a problem in *Hoya* taxonomy since its publication in 1810 by Robert Brown. Numerous confusing species are being lumped together or separated based on their foliar or reproductive morphology but most of these characters are phenotypically unstable. With this in line, the search for an established taxonomic character is a goal for *Hoya* taxonomists.

The primary way of delineating *Hoya* species is through comparison of their morphological characters, but the use of floral characters is most dominant (Kloppenburg, 2001). However, with total dependence on floral parts, a problem may arise due to the availability of *Hoya* flowers in the field (Jumawan and Buot, 2016). This circumstance resulted in the development of methods completely reliant on vegetative characters such as leaf architecture (LAWG, 1999; Ellis *et al*., 2009). This method has been used to delineate morphologically confusing *Hoya* species (Villareal and Buot, 2015; Jumawan and Buot, 2016; Torrefiel and Buot, 2017) and in other plant families (Inamdar and Murthy, 1978; Avita *et al.*, 1981; Mohan and Inamdar, 1982; Rao and Inamdar, 1983; Chaudhari and Inamdar, 1984; Inamdar and Shenoy, 1982; Gupta and

*Corresponding author.
E-mail: apbaltazar@up.edu.ph

Murty, 1988; Todzia and Keating, 1991; Fuller and Hickey, 2005; Millán and Feriz, 2005; Martinez-Cabrera et al., 2007; Rao and Narmada, 2008; Sarma *et al*., 2008).

Hoya cumingiana and *H. densifolia* are two species often confused with each other. *Hoya cumingiana* was published in 1844 and was first described from Philippines as a glabrous, woody shrub with leafy branches and greenish-yellow flowers (Decaisne, 1844; Kloppenburg, 2001). Four years later, a new species was described in Java by Turczaninow and named it as *H. densifolia.* It has been confused with *H. cumingiana* since then. Burton (1992) said that the two species are conspecific but Kloppenburg (2001) noted that its foliage blades are similar to *H. cumingiana* Decaisne but it is petiolate and a dangling plant (Kloppenburg, 2001). They are said to be different as *H. densifolia* has larger, thinner and pointed leaves with a brighter green color than *H. cumingiana*. Their flowers are nearly identical in morphology and scent. They were united by Index Kewensis but through the description in the Prodromus of de Candolle, it seems that the two species are not really similar (Kloppenburg, 2001). The two names are currently accepted in The Plant List.

Although the characters used to delineate them show dissimilarity between the two species, its stability is still in question. Leaf shapes and color can easily change under different sunlight exposure and can lead to wrong identification (Martin *et al*., 2010; Rahayu *et al*., 2010; Medina *et al*., 2016).

This issue on taxonomic confusion will lead to misestimating biodiversity and cause problems for conservationists (Dubois, 2003; Mace, 2004; Costello *et al*., 2015). Hence, new evidence to lessen or eliminate confusion is needed. By separating confusing species, their distinct economic use can also be identified and utilized. In this study, two confusing *Hoya* species were examined using leaf architecture methods.

MATERIALS AND METHODS

Preparation of Plant Materials

The two *Hoya* species were bought from the garden of Mrs. Ann Valenzuela of Robelle Garden Center, Calamba, Laguna, Philippines. The *H. cumingiana* mother plant being propagated in the garden is from Los Baños, Laguna, Philippines while *H. densifolia* is from Calamba, Laguna, Philippines. However, the original location of the plants were not traced and well-documented by the growers and only the identity of plants can be retrieved from them. The gardeners, however, differentiate the two *Hoya* plants by observing the color and shape of the leaves. Ten leaves were obtained from each species and were soaked in 10% sodium hydroxide (NaOH) solution to remove the mesophyll layer and make the veins more visible (Vasco *et al.*, 2014). The soaked leaves were boiled in a water bath until the leaves were yellow in color. To eliminate the excess pigment, they were decolorized using sodium hypochlorite (NaOCl). The leaf samples were carefully placed on a clean paper and were pressed until dry.

Measurement of Leaf Characters

The characters of the cleared and dried leaf samples (Fig. 1) were classified into laminal and venation characters. Laminal characters included leaf attachment, arrangement, blade length, blade width, blade area, blade class, blade shape, margin type, apex shape, base shape and base angle. On the other hand, venation characters included primary vein framework, secondary vein framework, secondary vein spacing, secondary vein angle, presence of intersecondary veins,

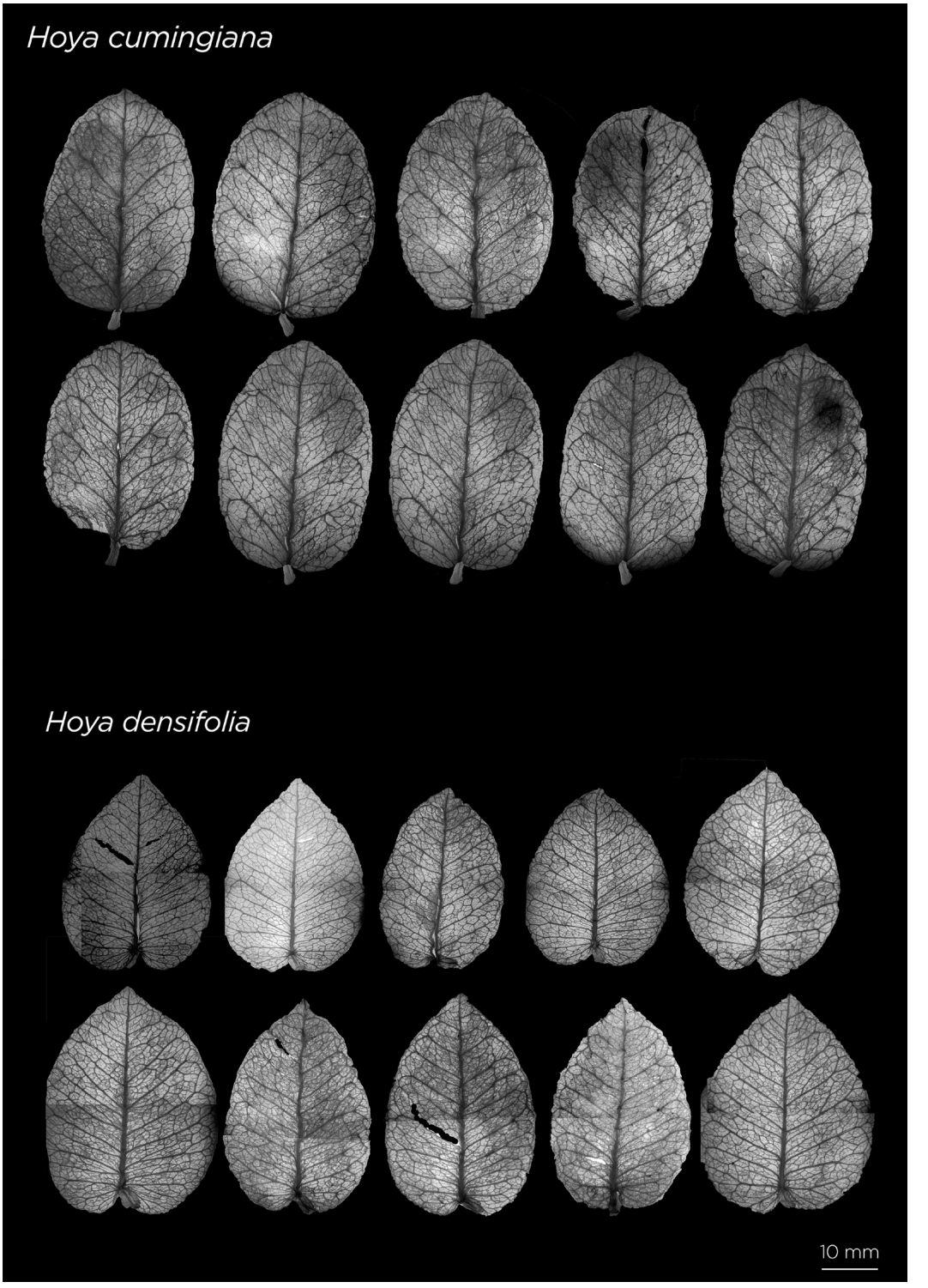

Figure 1. Cleared and dried leaf samples of two *Hoya* species. (Photo credit: A.M.P. Baltazar)

intersecondary distal course, intersecondary vein frequency, tertiary vein fabric, tertiary vein angle, quaternary vein fabric and areolation. Laminal characters were measured using a ruler and protractor while leaf veins (Fig. 2) were observed under a dissecting microscope. All leaf characters were based on Manual of Leaf Architecture by Ellis *et al.*, (2009).

Data Analysis

A total of 690 data sets from 3 *Hoya* species, 23 leaf characters and 10 leaf samples were analyzed. The third plant species is *Hoya carandangiana* which served as out groups to allow the statistical software do cluster and ordination analysis. The data for *H. carandangiana* was retrieved from the work of Torrefiel and Buot (2017). Cluster analysis was done using unweighted pair-group average (UPGMA) and single linkage as algorithms and Eucledian as the distance measure. A dendogram for each algorithm was generated to show the relationships between the two confusing *Hoya* species and the out group. Ordination analysis using Principal Coordinates Analysis (PCoA) was done to illustrate the individual differences of the *Hoya* species. Finally, paired sample test (T-test) was done to measure the significant difference between the leaf length, width, area and number of secondary veins of *H. cumingiana* and *H. densifolia*. PAST (Paleontological Statistical Software) software by Hammer *et al*. (2001) and IBM Statistical Package for the Social Sciences (SPSS) were used for the statistical analysis.

RESULTS AND DISCUSSIONS

Table 1 shows the blade characters of *H. cumingiana* and *H. densifolia*. The leaves of the two *Hoya* species exhibited the same petiolate attachment, decussate arrangement, microphyll blade class, entire margin, cordate base and reflex base angle. However, they have dissimilarities in terms of blade shape, apex shape and apex angle. *Hoya cumingiana* has an oblong blade shape which is exhibited by its widest diameter occupying 1/3 of the leaf's middle part while *H. densifolia* was ovate, having its widest diameter near the leaf base. Also, the apex of *H. cumingiana* was observed to be convex and obtuse because of its margin curving away from the midvein while *H. densifolia* has a straight and acute apex exhibited by its pointed and non-curved tip. This difference in apex shape was already noted by Kloppenburg (2001). Paired sample test shows that the *P* value of leaf length, width and area between the two species are less than 0.05. This implies that the leaf length, width and area of *H. densifolia* were significantly higher than that of *H. cumingiana* (Table 2). However, the shape and size of the leaf are easily affected by the environment, making it an unreliable taxonomic character to support the delineation of the two species. The leaf venation, on the other hand, was recognized to be genetically fixed and is not easily affected by the environment (Roth-Nebelsick *et al.*, 2001; Wenzel *et al.*, 2007; Scarpella *et al*., 2010; Sawchuk *et al*., 2013; Baylis *et al*., 2013; Pahari *et al*., 2014). In Table 3, the venation characters of the two species were presented. Both species have pinnate primary vein, simple brochidodromous secondary veins, weak, reticulating intersecondary veins, inconsistent tertiary vein angle, irregular reticulate quaternary veins and moderately developed areoles. However, *H. cumingiana* and *H. densifolia* differed in terms of secondary vein spacing, secondary vein angle, and tertiary vein fabric. The number of secondary veins in both species exhibits a highly significant difference (Table 2). *Hoya densifolia* has higher number of secondary veins per lamina as compared to *H. cumingiana.* In Table 4, it is shown that the high number of veins of *H. densifolia* is

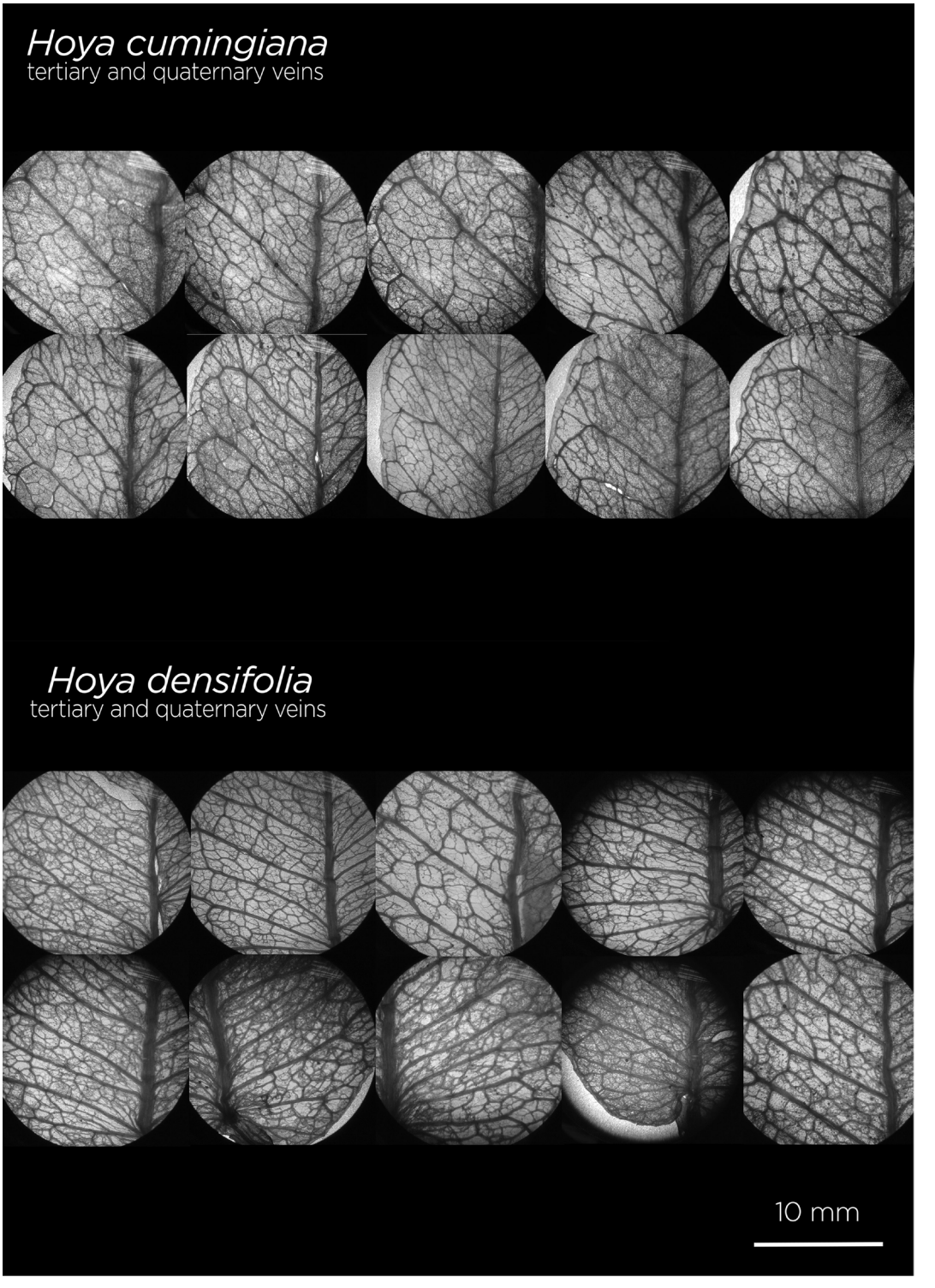

Figure 2. Leaf veins of two *Hoya* species. (Photo credit: A.M.P. Baltazar)

Table 1. General leaf characters of *H. cumingiana* and *H. densifolia*.

Characters	*Hoya cumingiana*	*Hoya densifolia*
Leaf Attachment	Petiolate	Petiolate
Leaf Arrangement	Decussate	Decussate
Blade length (mm)	22−31.5	29−41
Blade width (mm)	20.5−23	22−25
Blade area (mm2)	308−444.67	425.33−683.33
Blade Class	Microphyll	Microphyll
Blade Shape	Oblong	Ovate
Margin Type	Entire	Entire
Apex Shape	Convex	Straight
Apex Angle	Obtuse	Acute
Base Shape	Cordate	Cordate
Base Angle	Reflex	Reflex

Table 2. Paired Samples Test (T-test) for Quantitative Leaf Data.

Leaf Character	Mean	Std. deviation	Sig. (2-tailed)
Length *H. cumingiana* vs. Length *H. densifolia*	-6.43000	5.05746	0.003
Width *H. cumingiana* vs. Width *H. densifolia*	-1.51000	1.53293	0.012
Area *H. cumingiana* vs. Area *H. densifolia*	-129.23300	102.69133	0.003
Secondary Veins *H. cumingiana* vs. Secondary Veins *H. densifolia*	-5.90000	1.96921	0.000

significantly correlated with its larger leaf size. The differences between the two *Hoya* species were summarized in Table 5.

Two dendrograms were generated for cluster analysis using unweighted pair-group average (UPGMA) and single linkage as algorithms (Figures 3 and 4). The horizontal axis represents the distance between clustersand the vertical axis represents the species. In the first dendogram, results showed that *H. cumingiana*, *H. densifolia* and *H. carandangiana* (outgroup) were clustered under different clades. *Hoya cumingiana* and *H. densifolia* were split from *H. carandangiana* at distance between 4.5 and 4.0. Moreover, at distance 3.0 and 2.5, the two confusing species were separated by their dissimilarity in blade and venation characters. The same trend was also exhibited

by the second dendrogram using single linkage algorithm.

Figure 5 shows principal coordinate analysis (PCoA) plot with Eucledian dissimilarity index between *H. cumingiana*, *H. densifolia* and *H. carandangiana* (outgroup). Results showed that each *Hoya* species clustered separately from one another. *H. cumingiana* occupied quadrant 1, *H. densifolia* clustered in quadrant 4 and *H. carandangiana* in quadrant 2. This clearly shows that based on the blade and venation characters, the two confusing *Hoya* species are distinct from each other.

Table 3. General leaf venation characters of *Hoya cumingiana* and *H. densifolia*.

Characters	Hoya cumingiana	Hoya densifolia
Primary vein framework	Pinnate	Pinnate
Secondary Vein Framework	Simple Brochidodromous	Simple Brochidodromous
Secondary Vein Spacing	Decreasing Proximally – Irregular	Irregular
Secondary Vein Angle	Smoothly Decreasing Proximally	Smoothly Increasing Proximally
Number of Veins per lamina	5−7	8−14
Intersecondary Veins	Present	Present
Intersecondary distal course	Reticulating	Reticulating
Intersecondary Vein Frequency	Less than one per intercostal area	Less than one per intercostal area
Tertiary Vein Fabric	Irregular reticulate	Alternate Percurrent
Tertiary Vein Angle	Inconsistent	Inconsistent
Quaternary Vein Fabric	Irregular reticulate	Irregular reticulate
Areolation	Moderately developed	Moderately developed

Table 4. Pearson Correlation of blade area and secondary veins.

		Blade Area	Secondary Veins
Blade Area	Pearson Correlation	1	.790**
	Sig. (2-tailed)		0.000
	N	20	20
Secondary Veins	Pearson Correlation	.790**	1
	Sig. (2-tailed)	0.000	
	N	20	20

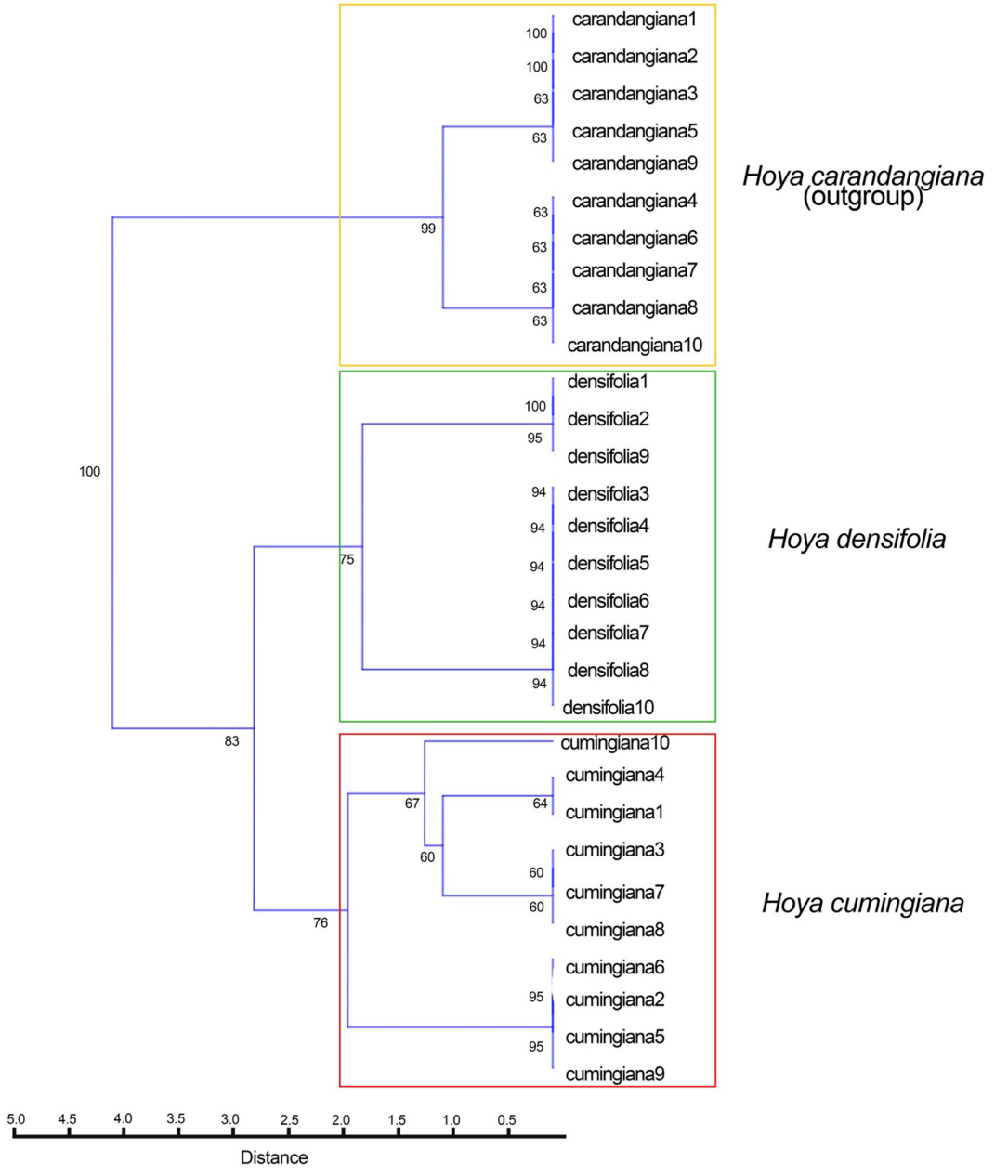

Figure 3. Dendrogram using single linkage (nearest neighbor) clustering approach. Bootstrap value: 1000.

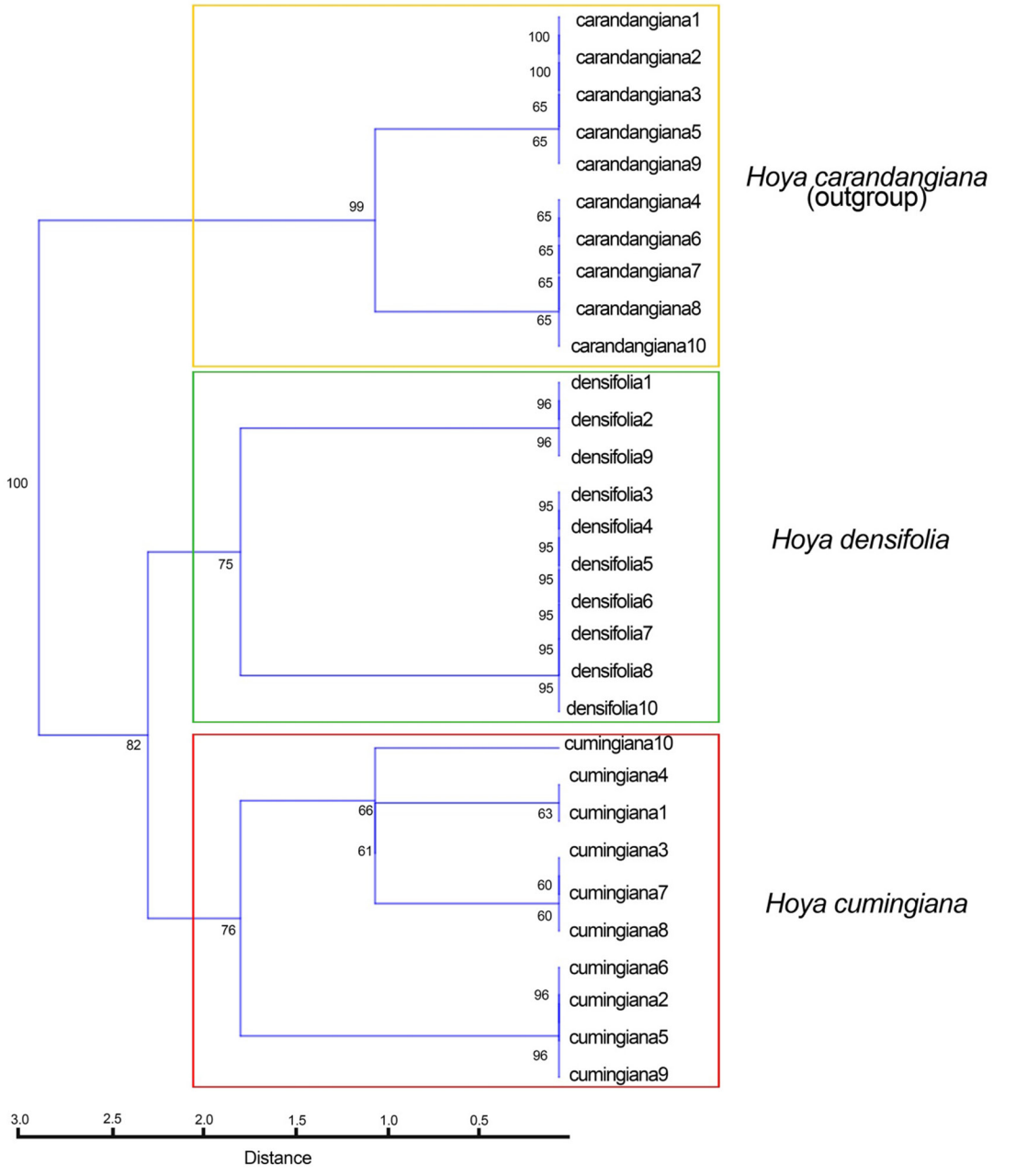

Figure 4. Dendrogram using single linkage (nearest neighbor) clustering approach. Bootstrap value: 1000.

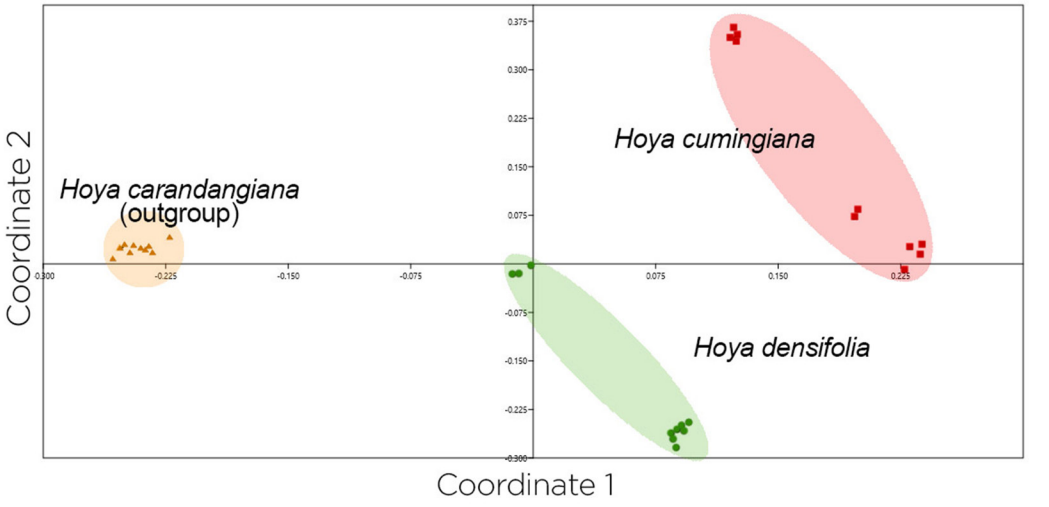

Figure 5. Principal Component Analysis (PCoA) of *Hoya cumingiana* (square), *Hoya densifolia* (circle), *Hoya carandangiana* (triangle).

Table 5. Leaf architecture characters delineating the two *Hoya* species.

Characters	*Hoya cumingiana*	*Hoya densifolia*
Blade length (mm)	22−31.5	29−41
Blade width (mm)	20.5−23	22−25
Blade area (mm^2)	308−444.67	425.33−683.33
Blade Shape	Oblong	Ovate
Apex Shape	Convex	Straight
Apex Angle	Obtuse	Acute
No. of Secondary Veins	5−7	8−14
Secondary Vein Spacing	Decreasing Proximally – Irregular	Irregular
Secondary Vein Angle	Smoothly Decreasing Proximally	Smoothly increasing proximally
Tertiary Vein Fabric	Irregular reticulate	Alternate Percurrent

SUMMARY AND CONCLUSIONS

The two morphologically confusing species, *H. cumingiana* and *H. densifolia* were subjected to leaf architectural analysis to seek a taxonomic character that will delineate them. The two species were separated by their differences in blade shape, apex shape and angle, secondary vein spacing, secondary vein angle and tertiary vein fabric, suggesting that the two are distinct from one another. The use of cluster and ordination analysis

also revealed the relationship between the *Hoya* species.

The use of leaf venation as a taxonomic character is reliable and can be used in the absence of reproductive parts in *Hoya*. However, with the availability of the flowers in cultivation, the use reproductive characters can further provide evidence in the separation of two species. Also, to confirm the taxonomic status of the two species, molecular evidence should be studied in the future.

REFERENCES

Avita, S., N. Rao and J. Inamdar. 1981. Studies on the Leaf Architecture of the Ranunculaceae. *Flora* 171(3): 280−298. doi:10.1016/s0367-2530(17)31271-9

Baranova, M. 1972. Systematic Anatomy of the Leaf Epidermis in the Magnoliaceae and Some Related Families. *Taxon* 21(4): 447. doi:10.2307/1219106

Baylis, T., I, Cierlik, E. Sundberg and J. Mattsson. 2013. Short Internodes/Stylish genes, regulators of auxin biosynthesis, are involved in leaf vein development in *Arabidopsis thaliana. New Phytologist* 197(3): 737−750. doi:10.1111/np *Hoya* 12084

Burton, C.M. 1992. *Hoya densifolia. Hoyan* 13(3) part 2: 17.

Chaudhari, G.S. and J.A. Inamdar. 1984. Leaf architecture of some Acanthaceae. T*he Botanical Magazine Tokyo* 97(4): 469−481. doi:10.1007/bf02489579

Costello, M.J., B. Vanhoorne and W. Appeltans. 2015. Conservation of biodiversity through taxonomy, data publication, and collaborative infrastructures. *Conservation Biology* 29(4): 1094−1099. doi:10.1111/ cobi. 12496

Decaisne, J. 1844. Asclepiadeae. *De Candolle Prodromus Systematis Naturalis Regni Vegetabilis* 8: 636. Downloaded from https://doi.org/10.5962/bhl.title.286 on 1 February 2019.

Dubois, A. 2003. The relationships between taxonomy and conservation biology in the century of extinctions. *Comptes Rendus Biologies* 326: 9−21. doi: 10. 1016/s1631-0691(03)00022-2

Ellis, B., D.C. Dally, L.J. Hickey, K.R. Johnson, J.D. Mitchell, P. Wilf, and S.L. Wing. 2009. *Manual of leaf architecture*. New York: New York Botanical Garden Press.

Fuller, D.Q. and L.J. Hickey. 2005. Systematics and Leaf Architecture of the Gunneraceae. *The Botanical Review* 71(3): 295−353. doi:10.1663/0006-8101(2005)071[0295: salaot]2.0.co;2

Gupta, A.K. and Y.S. Murty. 1988. Leaf architecture in Tamaricaceae. *Proceedings of the Indian Academy of Sciences* 98(6): 471−474 doi: 10.1007/BF03053403

Hammer, Ø., D.A.T. Harper and P.D. Ryan. 2001. *PAST-Palaeontological statistics ver. 3.11.* Downloaded from http://folk. uio.no/ohammer/past/ on 5 July 2018.

Inamdar, J. and G. Murthy. 1978. Leaf Architecture in Some Solanaceae. *Flora* 167(4): 265−272. doi:10.1016/s0367−2530(17)31118-0

Inamdar, J. and K. Shenoy. 1982. Leaf Architecture of *Merremia* Dennst. ex Hall. f. (Convolvulaceae). *Flora* 172(1): 96−104. doi:10.1016/s0367-2530(17) 31315-4

Jumawan, J. and I.E. Buot. 2016. Numerical Taxonomic Analysis in Leaf Architectural Trait of Some *Hoya* R. Br. Species (Apocynaceae) From Philippines. *Bangladesh Journal of Plant Taxon* 23(2): 199−207. Doi: http://dx.doi.org/ 10.3329/bjpt.v23i2.30851

Kloppenburg, R. 2001. *Hoyas* of the World Volume II. A pdf publication devoted to genus *Hoya*.

Leaf Architecture Working Group (LAWG). 1999. Manual of Leaf Architecture -

morphological description and categorization of dicotyledonous and net-veined monocotyledonous angiosperms. *Smithsonian Institution.*

Mace, G.M. 2004. The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society B* 359: 711−719. doi: 10.1098/rstb.2003.1454

Martin, C.E., R.C. Hsu and T. Lin. 2010. Sun/shade adaptations of the photosynthetic apparatus of *Hoya carnosa*, an epiphytic CAM vine, in a subtropical rain forest in northeastern Taiwan. *Acta Physiologiae Plantarum* 32(3): 575−581. Doi:10.1007/s11738-009-0434-9

Martínez-Cabrera, D., T. Terrazas and H. Ochoterena. 2007. Leaf architecture of Hamelieae (Rubiaceae). *Feddes Repertorium* 118(7-8): 286−310. Doi:10.1002/fedr.200711140

Medina, M., V. Amoroso and R. Kloppenburg. 2016. Changes of leaf morphology of *Hoya amorosoa*e from varying light exposure: Its implications to species description and taxonomy. *Journal of Biodiversity and Environmental Sciences* 8(6): 232−237.

Meve, U. 2002. Species Numbers and Progress in Asclepiad Taxonomy. *Kew Bulletin* 57(2): 459. doi:10.2307/4111126

Millán, M. and S. Ferriz. 2005. Leaf architecture of Anacardiaceae. *Revista Mexicana De Biodiversidad* 76(2):137−190.

Mohan, J.S. and J.A. Inamdar. 1982. Leaf architecture of Apocynaceae. *Proceedings of the Indian Academy of Sciences* 91(3): 189−200. Doi: 10.1007/BF03167123

Pahari, S., R.D. Cormark, M.T. Blackshaw, C. Liu, J.L. Erickson and E.A. Schultz. 2014. *Arabidopsis* Unhinged Encodes A VPS51 Homolog And Reveals A Role For The GARP Complex In Leaf Shape and Vein Patterning. *Development* 141: 1894–1905

Rahayu, S., M. Jusuf, Suharsono, C. Kusmana and R. Abdulhadi. 2010. Morphological variation of Hoya multiflora Blume at different habitat type of Bodogol Research Station of Gunung Gede Pangrango Natonal Park, Indonesia. *Biodiversitas, Journal of Biological Diversity* 11(4): 187−193. Doi:10.13057/biodiv/d110404

Rao, N.V. and J.A. Inamdar. 1983. Leaf architectural studies in the Brassicaceae. *The Botanical Magazine Tokyo* 96(1): 15−28. Doi:10.1007/bf02489571

Rao, S.R. and K. Narmada. 1994. Leaf architecture in some Amaranthaceae. *Feddes Repertorium* 105(1-2): 37−44. Doi:10.1002/fedr.4921050109

Rodda, M. 2012. Taxonomy of *Hoya lyi, Hoya yuennanensis* and *Hoya mekongensis* (Apocynaceae – Asclepiadoideae). *Edinburgh Journal of Botany* 69(01): 83−93. Doi:10.1017/s0960428611000412

Roth–Nebelsick, A., D. Uhl, V. Mosbrugger and H. Kerp. 2001. Evolution and Function of Leaf Venation Architecture: A Review. *Annals of Botany* 87(5): 553–566.

Sarma, V., S.S. Rao, and C.H. Beena. 2008. Leaf architecture in relation to taxonomy: Meliaceae. *Feddes Repertorium* 103(7-8): 535−542. Doi:10.1002/fedr.19921030710

Sawchuk, M. G., A. Edgar and E. Scarpella. 2013. Patterning of Leaf Vein Networks by Convergent Auxin Transport Pathways. *PLoS Genetics* 9(2): 1−13. Doi:10.1371/journal.pgen.1003294

Scarpella, E., M. Barkoulas and M. Tsiantis. 2009. Control of Leaf and Vein Development by Auxin. *Cold Spring Harbor Perspectives in Biology* 2(1): 1−17. Doi:10.1101/cshperspect.a001511

Todzia, C. A. and R.C. Keating. 1991. Leaf Architecture of the Chloranthaceae. *Annals of the Missouri Botanical Garden* 78(2): 476. Doi:10.2307/2399575

Torrefiel, J.T. and I.E. Buot. 2017. *Hoya carandangiana*, *Hoya bicolensis*, and *Hoya camphorifolia* (Apocynaceae)

Species Delineation: Insights from Leaf Architecture. *The Thailand Natural History Museum Journal 11*(1): 35−44.

Vasco, A., M. Thadeo, M. Conover and D.C. Daly. 2014. Preparation of samples for leaf architecture studies, a method for mounting cleared leaves. *Applications in Plant Sciences* 2(9): 1−4. http://doi.org/10.3732/apps.1400038

Villanueva, E.C. and I.E. Buot. 2017. An Enumeration of *Hoyas* in Quezon Province, Luzon Island, Philippines. *The Thailand Natural History Museum Journal* 11(1): 23−33.

Villareal, A.M. and I.E. Buot. 2015. Leaf Architecture of *Hoya incrassata* Warb. and *Hoya crassicaulis* Elmer x Kloppenb. (Apocynaceae): Taxonomic Identification and Conservation Concerns. *IAMURE International Journal of Ecology and Conservation* 15(1): 203−213. Doi: 10.7718/ijec.v15i1.1002

Wenzel, C. L., M. Schuetz, Q. Yu,and J. Mattsson. 2007. Dynamics of MONOPTEROS and PIN-FORMED1 expression during leaf vein pattern formation in *Arabidopsis thaliana. The Plant Journal* 49(3): 387−398. Doi:10.1111/j.1365-313x.2006.02977.

Received: 26 June 2018
Accepted:31 December 2019
Published: 31 December 2019