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Short Communication

Delineating Two Species *Hoya benguetensis* Schltr. and *Hoya ilagiorum* Kloppenb., Siar & Cajano (Apocynaceae): A Leaf Architectural Approach

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ABSTRACT: Two species of *Hoya* (*Hoya benguetensis* and *Hoya ilagiorum*) under the family Apocynaceae were studied for their leaf architecture in the aim to resolve confusion on its morphological distinctiveness. Results showed that there were similar characters like blade symmetry, position of petiolar attachment, apex shape, margin type, lobation, primary vein category and secondary vein category. However, leaf architecture characteristics delineated the two species evident in seven characters namely blade class, blade shape, base shape, blade area, tertiary vein category, quaternary vein category, and areolation. The study showed that two species were distinct from each other. Moreover, it further supported that leaf architecture is a good tool in delineating and identifying confusing species.

KEY WORDS: *Hoya benguetensis*, *Hoya ilagiorum*, leaf architecture, taxonomy

INTRODUCTION

The genus *Hoya* is generally found in the Southeast Asia and is greatly favored as an ornamental plant (Wanntorp, 2006) consisting of over 500 species recorded by the International Plant Names Index (2012). In differentiating *Hoya* species, the flower morphology such as corona, corolla and pollinarium are significant both qualitative and quantitative (Kloppenburg *et. al.*, 2012). Since the time of Linnaeus, morphological characteristics were given emphasis in taxonomy (Aldhebiani, 2017; Salvaña and Buot, 2014). Reproductive organs such as flowers and fruits are commonly used as the basis for the organization of organisms including determination and recreation of relationships. However, in some instances, these parts are not available; a dilemma botanists usually face (Villareal and Buot 2015).

Land plants exhibits a variety and complex structure of leaf venation. The external appearance of a leaf is formed by the structure and location of various components including size, shape and venation pattern (Torrefiel and Buot, 2017). One of the principal characteristic of numerous venation patterns such as anastomoses are genetically fixed. This makes leaf architecture a good tool in systematic taxonomy. It is even used in paleobotany in the determination of a macrofossil (Roth-Nebelsick, 2001).

Thus, this paper aims to address the dilemma

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of sterility in certain plants and provide identification data using leaf architecture. Despite the potential of leaf architecture, only a few has focused on this aspect (Salvaña and Buot, 2014). Nevertheless, there are a great number of controversial species that has been examined and resolved using leaf architecture such as the venation patterns of the Ranunculaceae (Avita and Rao, 1981); delineation of *H. carandangiana*, *H. bicolensis*, *H. camphorifolia* (Torrefiel and Buot, 2017); leaf architecture with numerical analysis of *H. buotii*, *H. halconensis*, *H. mindorensis* (Jumawan and Buot, 2016); differentiation of certain species of Malvaceae (Laraño and Buot, 2010); and leaf architecture of some Amaranthaceae (Rao and Narmada, 1994).

Regardless of the taxonomic capacity of leaf architecture, this has not been fully explored and examined (Banaticla and Buot, 2004). Hence, the purpose of this study is to present the leaf architectural characters of *Hoya benguetensis* and *Hoya ilagiorum*. Both look similar with slight differences because of its floral morphology, leaf venation, obtuse tip calyces, and vegetative shape which make them look like similar species (Aurigue, 2013) but taxonomically regarded as different and accepted species (Kloppenburg *et al.*, 2010 and Kloppenburg, *et al.*, 2012). This brings confusion to taxonomists and hobbyists, hence this study aims to determine the leaf architecture patterns of both *Hoya* species and validate its current taxonomic status.

MATERIALS AND METHODS

Twenty leaf samples were collected and examined, ten of each *Hoya benguetensis* and *Hoya ilagiorum* from numerous individuals at different environmental conditions. The leaf samples were properly pressed and dried using an oven. Afterwards, all the dried leaves were examined using a stereomicroscope. Using the Manual of Leaf Architecture by the Leaf Architecture Working Group, the leaf architecture of the two species was described through the descriptions and terminologies established. All the points of the leaf were examined for more accurate results. For the measurement, a dial caliper was used in width, length and etc. while a protractor measuring angle of divergence, A dichotomous key to the two Hoya species was constructed. The descriptions for the key were based on the leaf architecture examined.

RESULTS AND DISCUSSION

Leaf Architectural Characteristics of *Hoya benguetensis* Schltr. and *Hoya ilagiorum* Kloppenb., Siar & Cajano

The ten samples used presented various external morphological characters of both species. Table 1 summarized the general leaf architectural characteristics of the two species. The examined leaves exhibited several unifying characters including opposite phyllotaxy, simple leaves, symmetrical, marginal, acuminate apex, entire and unlobed. The blade length, width and area values are quite close to each other.

Table 1. General blade characteristics of *H. benguetensis* and *H. ilagiorum.*

Characters	*Hoya benguetensis*	*Hoya ilagiorum*
Leaf attachment	Opposite	Opposite
Leaf organization	Simple	Simple
Blade length in mm	120-185 (Mean: 149.8)	125-160 (Mean: 141.7)

Table 1. (Continued).

Characters	*Hoya benguetensis*	*Hoya ilagiorum*
Blade width in mm	35-47 (Mean: 41.6)	35-43 (Mean: 37.8)
Blade area mm2	3750-5550 (Mean: 4154)	3167-3848 (Mean: 3564)
Blade class	Mesophyll	Notophyll
L:W ratio	2.78:1- 4.40:1 (Mean: 3.63:1)	3.18:1- 4.57:1 (Mean: 3.78:1)
Blade shape	Elliptical-ovate elliptical	Elliptic to narrow elliptic
Blade symmetry	Symmetrical	Symmetrical
Base shape	Cuneate	Rounded
Position of petiolar attachment	Marginal	Marginal
Apex shape	Acuminate	Acuminate
Margin Type	Entire	Entire
Lobation	Unlobed	Unlobed

In contrast, there were similar characters of the two species based on leaf venation (Table 2) including pinnate, stout and straight primary vein; weak brochidodromous secondary vein, abruptly curved secondary vein, moderate secondary vein thickness and loop-forming branches enclosed by 3° or 4° arches, secondary vein spacing decreasing towards the base, inter-secondary veins absent, acute tertiary vein angle to primary veins, freely ending ultimate veins absent and looped marginal ultimate venation.

Table 2. General venation characters of *H. benguetensis* and *H. ilagiorum*.

Characters	*Hoya benguetensis*	*Hoya ilagiorum*
Primary vein category	Pinnate	Pinnate
Primary vein size	Stout	Stout
Primary vein course	Straight	Straight
Secondary vein category	Weak Brochidodromous	Weak brochidodromous
Secondary vein course	Curved (Abruptly)	Curved (Abruptly)
Thickness	Moderate	Moderate
Behavior of loop-formin g branches	Enclosed by 3° or 4° arches	Enclosed by 3° or 4° arches
Secondary vein spacing	Decreasing toward base	Decreasing toward base
Inter-secondary veins	Absent	Absent
Tertiary vein category	Alternate percurrent	Random reticulate

Table 2. (Continued).

Characters	*Hoya benguetensis*	*Hoya ilagiorum*
Tertiary vein angle to primary veins	Acute	Acute
Tertiary vein angle variability	Uniform	Inconsistent
Quaternary vein category	Regular polygonal reticulate	Alternate percurrent
Areolation	Poorly developed	Poorly developed
F.E.V.S.	Absent	Absent
Marginal Ultimate Venation	Looped	Looped

On the other hand, characters delineating the two species were recorded (Table 3). The leaf blade, shape, apex, base and blade class are useful in distinguishing characters but based on the study of Sri Rahayu *et al* (2010), there are variations of morphological characteristics in leaves and inflorescence of *Hoya* like in the case of *H. multiflora* in different sites. The leaves exhibit various color and shape. This implies that leaf shape, class and blade are plastic characters. Plastic characters changes with the changing environment, making it unreliable as basis of differentiating confusing species. With this, it appears that leaf venation characters are more diagnostic and valid.

Table 3. Leaf architecture characters delineating *H. benguetensis* and *H. ilagiorum*

Characters	*Hoya benguetensis*	*Hoya ilagiorum*
Blade class	Mesophyll	Notophyll
Blade shape	Elliptical-ovate elliptical	Elliptic to narrow elliptic
Base shape	Cuneate	Acute to Rounded
Blade area mm^2	3750-5550 (Mean: 4154)	3167-3848 (Mean: 3564)
Tertiary vein category	Alternate percurrent	Random reticulate
Quaternary vein category	Regular polygonal reticulate	Alternate percurrent
Areolation	Poorly developed	Moderately developed

Based on the leaf venation, there were differences recorded (Figure 1). An evident difference started at the tertiary vein category in which was parallel to the previous study of Torrefiel and Buot (2017). It clearly shows that the characters that strongly delineated the two species were leaf venation characters. Leaf venation such as secondary, tertiary, quaternary veins and areoles are more important in distinguishing species. This means, it is more accurate to rely on venation characters. The results revealed similar outcomes from the previous studies done on *Hoya* species in the Philippines (Baroga and Buot, 2013, Laraño and Buot, 2008, Salvaña and Buot, 2014; Torrefiel and Buot, 2017;

Villareal and Buot, 2015). This strengthens the view that leaf venation characters are genetically fixed (Roth-Nebelsick *et al.*, 2001) and species-specific (Fujita and Mochizuki 2006). Moreover, in the study of Dilcher (1974), the basics and form of leaf venation has substantial association with taxonomy and phylogeny. Also, the study on leaf secondary venation has taxonomic importance in species identification (Sharma, 1991). This is because; the number of veins is constant (Levin, 1929). The leaf venation represented in this study does not only delineate the two species but can also be used to identify species of *Hoya*. Furthermore, this proved the current level of recognition of the two species using the leaf architectural approach based on Hickey (1973).



Figure 1. Illustration of the leaf venation characters of (left) *H. benguetensis* showing 3° vein alternate percurrent, 4° regular polygonal reticulate, and poorly developed areoles; (right) *H. ilagiorum* showing 3° vein random reticulate, 4° vein alternate percurrent and moderately developed areoles.

Key to the Two Species of Hoya

1 Pinnate, apex acuminate, weak brochidodromous.....2

2 3° vein alternate percurrent, 4° vein regular polygonal reticulate.....*H. benguetensis* poorly developed areoles

3 3° vein random reticulate, 4° vein alternate percurrent.....*H. ilagiorum* moderately developed areoles

***Hoya benguetensis* Schltr.: Exsiccata: Tan 6740 (PBDH).** Leaves opposite and simple, marginal petiolar attachment, elliptic to ovate elliptic, symmetrical, cuneate leaf base, acuminate apex, 120-185 mm long, 35 -47 mm wide, 2.78:1-4.40:1 ratio, mesophyll, entire, unlobed. Primary veins pinnate (arcuate), primary vein size stout, primary vein course straight. Secondary veins weak brochidrodromous; secondary vein spacing decreasing towards the base; inter-secondary veins absent, secondary vein course abruptly curved. Tertiary vein alternate percurrent; angle to primary veins acute, angle variability uniform. Quaternary vein regular polygonal reticulate. Areolation poorly developed. Free ending ultimate veins absent. Marginal ultimate venation looped (Figure 2-a).

***Hoya ilagiorum* Kloppenb., Siar & Cajano: Exsiccata: Tan 6741 (PBDH).** Leaves opposite and simple, marginal petiolar attachment, elliptic to narrow elliptic, symmetrical, acute to rounded leaf base, acuminate apex, 125-60 mm long, 35-43 mm wide, 3.18:1-4.57:1 ratio, notophyll, entire, unlobed. Primary veins pinnate (arcuate), primary veins size stout, primary vein course straight. Secondary veins weak brochidodromous; secondary vein spacing decreasing towards the base; inter-secondary veins absent, secondary vein course abruptly curved. Tertiary vein random reticulate; angle to primary veins acute, angle variability uniform. Quaternary vein alternate percurrent. Areolation well-developed. Free ending ultimate veins absent. Marginal ultimate venation looped (Figure 2-b).

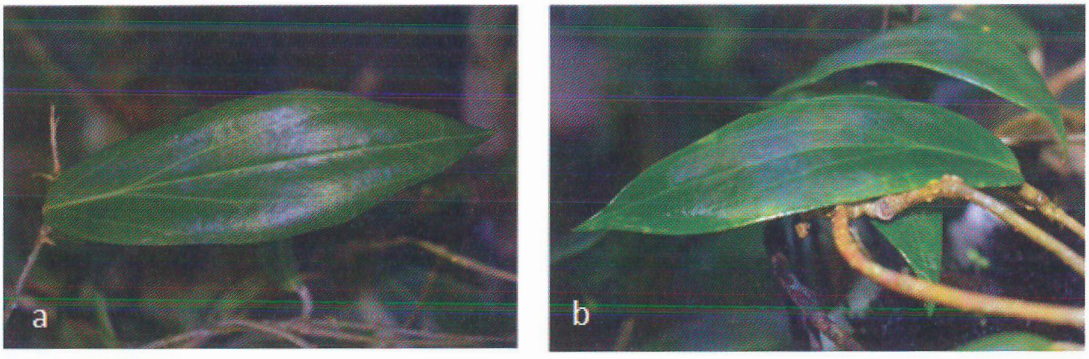


Figure 2. Fresh leaves of the (a) *Hoya benguetensis* and (b) *Hoya ilagiorum* used in the study.

CONCLUSION

Leaf architectural characters of two species of *Hoya* namely, *Hoya benguetensis* and *Hoya ilagiorum* were examined. While some characteristics observed were similar, there were several other characteristics that differentiated the two species. Based on the results of the study, *Hoya benguetensis* and *Hoya ilagiorum* are distinct species refuting existing controversy that both are same species which supports its current taxonomic status. The leaf architectural characters such as tertiary veins, quaternary veins and areoles delineate the two species of *Hoya*. Leaf architecture especially higher leaf venation, is a good tool to delineate and identify confusing species. This method is feasible and less expensive than other methods. There were minimal character variations used in the study, and so other morphological, anatomical characters and molecular analysis should be assessed for future studies whenever possible.

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