The Thailand Natural History Museum Journal 14(2), 139-146, 31 December 2020
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http:doi.org/10.14456/thnhmj.2020.6

# Delineation of *Hoya pimenteliana* Kloppenb. and *Hoya cagayanensis* C.M. Burton (Apocynaceae) with Insights from Leaf Architecture

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## ABSTRACT

Leaf architectural characters of *Hoya pimenteliana* and *Hoya cagayanensis* under the family Apocynaceae were examined to resolve the issue concerning the taxonomic identity of these two species. Results showed that laminar size, length and width ratio, base shape, quaternary vein category, free ending veins (FEVs), and areolation were considered as good taxonomic markers as they indicated the distinction between these two species. Cluster analysis also showed the divergence of the two *Hoya* species resulting in evidence of their distinctiveness. Results then indicated that *H. cagayanensis* and *H. pimentilia* were two different species. We also observed the distinction in leaf venation between the two *Hoya* species observing at quaternary vein characters, and this trend was consistent with other confusing *Hoya* species. Generally, the distinction between confusing *Hoya* species surfaced at finer vein characters, usually at tertiary to quaternary vein.

**Keywords**: Apocynaceae, Leaf architecture, venation patterns, *Hoya.*

## INTRODUCTION

Recognized in 1810, *Hoya* or commonly referred to as wax plant belongs to the Dogbane family or Apocynaceae. It is uniquely described as species that have waxy flowers and or leaves and has a milky latex sap common to *Hoya* plants. Also, these species are mostly epiphytes or grows on growing trees or limestone boulders (Aurige, 2013). It is one of the most diverse and one of the largest groups under Apocynaceae as it is estimated to have around 350–450 species and counting (Rodda, 2015). The identification of *Hoya* species largely depends on the traditional taxonomy, in which special attention is given to the reproductive traits (Jumawan and Buot, 2016). *Hoya*'s morphology such as corona, corolla, and pollinarium were significant qualitatively and quantitatively (Kloppenburg *et al.*, 2012). However, reproductive organs are not always available making it more difficult for taxonomist to identify *Hoya* species.

Plant leaves, on the other hand, provide morphological and venation characters that can be used in identification of plant species (Lu *et al.* 2012). Roth – Nebelsick *et al.* (2001) stated that the leaf characters such as venation patterns are genetically fixed. Leaf characters are then good addition. Alongside with leaf architecture, leaf and venation characters are not limited in identification but also can be used in classifying, separating and combining plants species especially those that have confusing taxa (Salvaña and Buot, 2014).

Leaf architecture was first established by Hickey (1973), setting terminologies describing several features on angiosperm leaves. One of the purposes of leaf architecture was to define variations on the leaf features determining if the plant species were an exact taxonomic group. Through time, terminologies in leaf architecture were modified providing more specific characteristics in each category, and additional laminar and vein traits (Pole, 1991; Leaf Architecture Working Group 1999; Ellis *et al.*, 2009). Furthermore, working with confusing species using leaf architecture is recommended since it clarifies distinguished characters and further delineate taxonomic affiliation between treated taxa (Villareal and Buot, 2015).

Two species of *Hoya* namely *H. pimenteliana* and *H. cagayanensis* need further differentiation. Ann Valenzuela (pers. comm), a *Hoya* collector and expert, mentioned the very similar appearance of these two species particularly in terms of their flower bloom

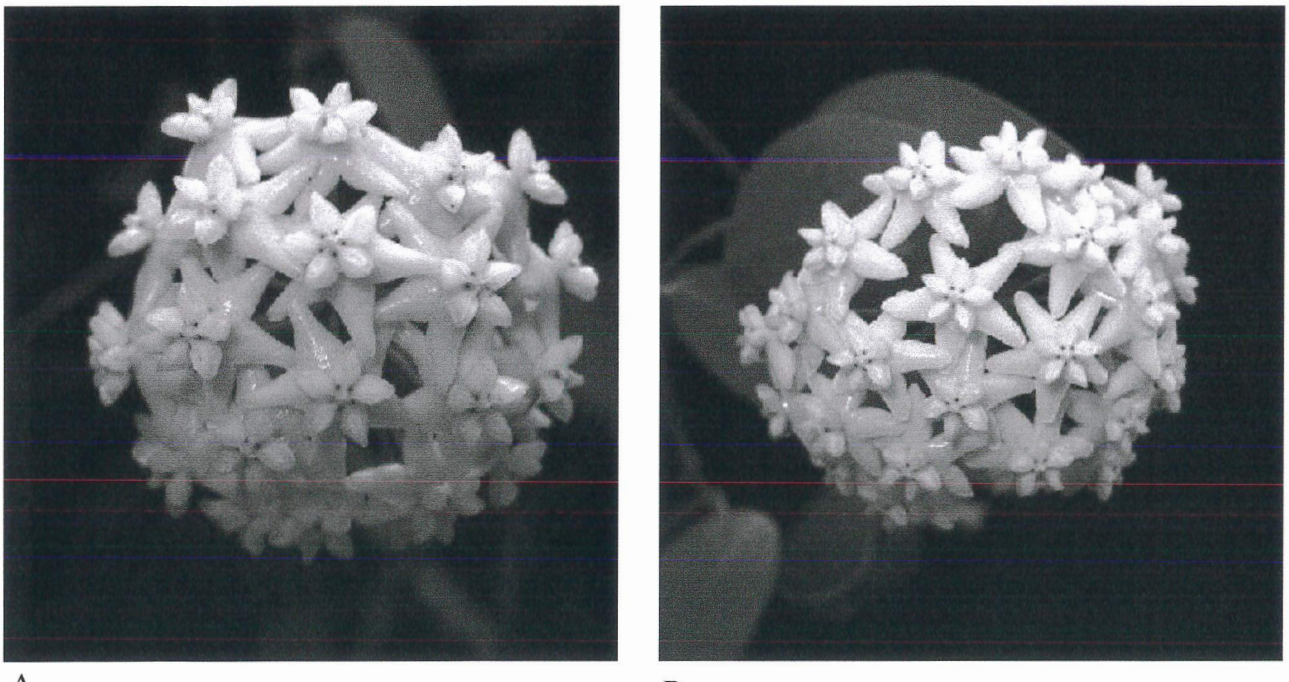
but in Aurige's (2013) list of Philippines *Hoya*, the two species were listed differently. Field observation showed that *Hoya cagayanensis* has white milky sap when cut but *Hoya pimenteliana* does not have this trait. There is a need to further differentiate the two and, in this study, we aim to present the morphological distinctiveness between *Hoya pimenteliana* and *Hoya cagayanensis* using leaf architecture. Yap and Gabriel (2014) emphasized the thorough investigation of the two *Hoya* species to avoid confusion.

## MATERIALS AND METHODS

Thirty mature leaf samples were randomly collected for *H. pimenteliana* from the garden collection of Ann Valenzuela in Los Baños, Laguna, Philippines. The collected leaf samples were properly pressed and dried using an oven. For the *H. cagayanensis*, thirty leaf specimens were loaned from the Plant Systematics Laboratory, Institute of Biological Science, University of the Philippines, Los Baños. *Hoya buotii* was used as the outlier. All specimens were examined using stereomicroscope. Leaf architecture characteristics for each specimen was described by using the set of established leaf architecture terms and descriptions by Hickey (1973), Leaf Architecture Working Group (1999), and Ellis *et al.* (2009). A ruler was used in measuring some characters of the specimens such as blade length, and blade width, while a protractor was used in measuring the angles required. A dichotomous key of the two *Hoya*s was also constructed. Cluster analysis was used using PAST – Paleontogical Statistics version 3.26b to analyze the substantial characters exhibited among the *Hoya* species. The study was conducted from January 2019 to March 2020.

## RESULTS AND DISCUSSION

The floral features and arrangement (Figure 1) of the two *Hoya* species show no significant differences. Both *Hoya* species are the same color (white), have the same arrangement of corolla and corona, and the same clustering of the flowers. In general, floral characters are used in identifying and in the classification of plant species (Salvaña and Buot, 2014). However, the two *Hoya* species cannot be easily distinguished by the naked eye using the floristic characters. On the other hand, the leaf (Figure 2) shows some distinguishable differences. The leaf margin of *H. pimenteliana* was entire while repand or wavy in *H. cagayanesis*. However, this seems to be a plastic character because other individuals of *H. pimenteliana* also showed a repand and wavy margin. Thus, deeper examination of the leaves is needed to further differentiate the two.



A B

**Figure 1.** Inflorescences of *Hoya pimenteliana* (A) and *Hoya cagayanensis* (B). Photo credits: J.R. Sahagun at Co's Digital Flora, https://www.philippineplants.org/Families/Apocynaceae.html.

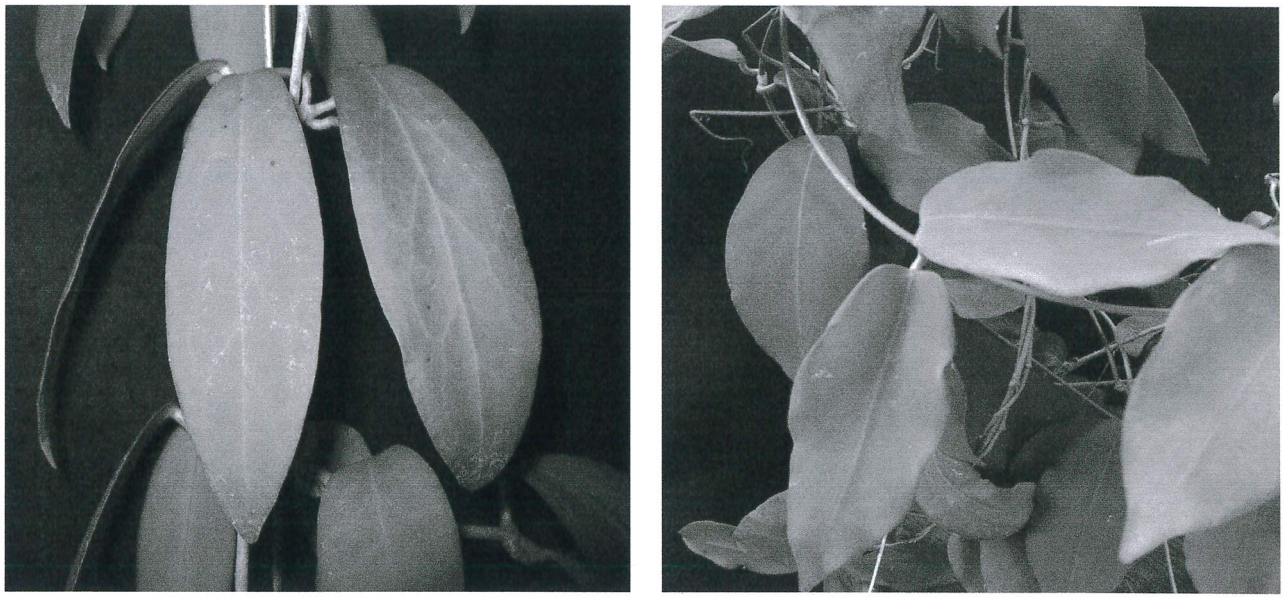


**Figure 2.** Leaves of *Hoya cagayanensis* (left) and *Hoya pimentelina* (right). Photo credits: F.B. Aurige and J.R. Sahagun at Co's Digital Flora, https://www.philippineplants.org/Families/Apocynaceae.html.

**Leaf Architectural Characters of *Hoya pimenteliana* Kloppenb and *H. cagyanensis* C.M. Burton**

The leaf samples used in characterizing *Hoya pimenteliana* and *Hoya cagayanesis* are shown in Figure 3. The leaf blade characters are shown in Table 1 while leaf vein characters are shown in Table 2. The uniting general characters present in both specimens are decussate leaf attachment, simple leaf organization, pulvinate petiole features, elliptic laminar shape, symmetrical laminar symmetry, acute base angle, acute apex angle, marginal position of petiolar attachment, convex apex shape, untoothed margin type, and are unlobed. Furthermore, the uniting vein characters of the two *Hoya* specimens are pinnate primary vein, festooned brachidodromous secondary vein, irregular secondary vein spacing, inconsistent secondary vein angle, weak inter-secondary veins, narrow angle of divergence, random reticulated tertiary vein category, inconsistent tertiary vein angle, irregular reticulated quarternary vein, and looped marginal ultimate.

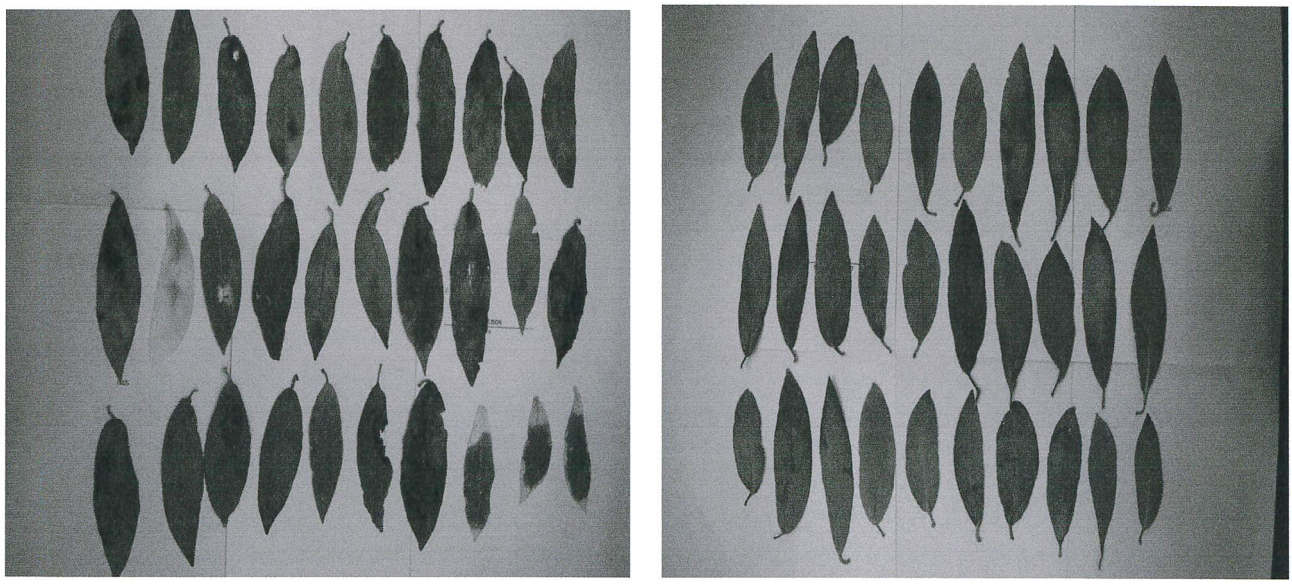


**Figure 3.** Leaf samples of *H. cagayanensis* (left) and *H. pimenteliana* (right).

**Table 1.** General blade characters of *H. pimenteliana, H. cagyanensis* and *H. boutii.*

| Characters | *H. pimenteliana* Exsicc. - Catones and Buot 7038 (PBDH) | *H. cagayanensis* Exsicc. - Paguntalan and Buot 6892 (PBDH) | *H. boutii* Exsicc. - Catones and Bu 7039 (PBDH) |
|---|---|---|---|
| Leaf attachment | decussate | decussate | decussate |
| Leaf organization | Simple | simple | simple |
| Petiole features | pulvinate | pulvinate | pulvinate |
| Blade length (mm) | 82.33 | 81.47 | 93.23 |
| | 64-105 | 62-105 | 71-115 |
| Blade width (mm) | 25.33 | 27.77 | 31.8 |
| | 20-31 | 20-42 | 21-40 |
| Laminar Area ($mm^2$) | 1393.18 | 1522.76 | 2002.64 |
| | 866.67-1963.33 | 866.67-2436.00 | 1050-3066.67 |
| Laminar size | microphyll | microphyll - notophyll | microphyll - notophyll |
| Laminar shape | elliptic | elliptic | elliptic |
| Laminar symmetry | symmetrical | symmetrical | symmetrical |
| L:W ratio (cm) | 3.25:1` | 2.93:1 | 2.93:1 |
| | 2.17:1-4.62:1 | 2.07-3.50:1 | 2.53:1-4.52:1 |
| Base angle | acute | acute | acute |
| Apex angle | acute | acute | acute |
| Base shape | cuneate – convex | convex | convex |
| Position of petiolar attachment | marginal | marginal | marginal |
| Apex shape | convex | convex | acuminate w/ drape tip |
| Margin type | untoothed | untoothed | untoothed |
| Lobation | unlobed | unlobed | unlobed |

On the other hand, some general and vein character differences between the two specimens were also recognized. The laminar size of *H. pimenteliana* was distinguished as microphyll while most specimen of *H. cagayanensis* were microphyll but a few were notophyll. The L:W ratio of *H. pimenteliana* ranges from 2.17–4.62:1 while *H. cagayanensis* ranges from 2.07–3:50:1. Most of the base shape of *H. pimenteliana* were identified as acute but some are counted as cuneate while all samples from *H. cagayanensis* were classified as acute. The quaternary vein category of *H. pimenteliana* was characterized as alternate percurrent while *H. cagayanensis* was irregular reticulate. The areolation of *H. pimenteliana* was well developed while the *H. cagayanensis* was moderately developed. The presence of free ending veins (F.E.Vs) in *H. pimenteliana* was unbranched to two or more branched while *H. cagayanensis* was two or more branched.

**Table 2.** Vein characters of *H. pimenteliana*, *H cagyanensis* and *H. boutii.*

| Characters | ***H. pimenteliana*** Exsicc. - Catones and Buot 7038 (PBDH) | ***H. cagayanensis*** Exsicc. - Paguntalan and Buot 6892 (PBDH) | ***H. boutii*** Exsicc. - Catones and Buot 7039 (PBDH) |
|---|---|---|---|
| 1° vein category | Pinnate | pinnate | pinnate |
| 2° vein category | festooned brachidodromous | festooned brachidodromous | festooned brochidodromous |
| 2° vein spacing | Irregular | irregular | irregular |
| 2° vein angle | Inconsistent | inconsistent | smoothly decreasing toward base |
| Inter-2° veins | weak intersecondaries | weak intersecondaries | weak intersecondaries |
| 2° angle of divergence | Narrow | narrow | moderate |
| 3° vein category | random reticulate | random reticulate | random reticulate |
| 3° vein angle variability | Inconsistent | inconsistent | inconsistent |
| 4° vein category | alternate percurrent | irregular reticulate | alternate percurrent |
| Areolation | well developed | moderately developed | well developed |
| F.E.V.s | unbranched – 2 or more branched | 2 or more branched | unbranched - 1 branched |
| Marginal ultimate | Looped | looped | looped |

We noted that the venation of the two *Hoya* species in the study were similar at the primary to tertiary vein characters but start to deviate at the quaternary vein characters. This shows that the distinction in leaf venation between confusing *Hoya* species are observed at finer vein characters, usually at tertiary or quaternary vein. This trend was also observed in previous studies on *Hoya* by Paguntalan and Buot, (2019), Tan and Buot (2018), Torrefiel and Buot (2017), Villareal and Buot, (2015), Salvaña and Buot (2014), Baroga and Buot (2013), and Laraño and Buot (2008). Several leaf and vein traits noted in the study were also similar with other species of *Hoya* under family Apocynaceae (Mohan and Inamdar, 1982). Our study supports the claim of previous studies (Villareal and Buot, 2015; Ruth- Nebelsik *et al.*, 2001) that leaf characters and venation patterns are useful taxonomic markers in plant identification and classification. It is then highly suggested that leaf architecture be used in delineating *Hoya* species.

**Dichotomous key of the specimens**

1. Apex shape acuminate w/ drape tip; secondary vein angle smoothly decreasing toward base ..... *H. buotii*
1. Apex shape acute; secondary vein angle inconsistent ..... 2
2. Secondary angle of divergence narrow; F.E.V.s unbranched – 2 or more branched..... .... *H. pimenteliana*
2. secondary angle of divergence narrow; F.E.V.s 2 or more branched ..... *H. cagayanensis*

**Leaf Architectural Characters of *Hoya pimenteliana* and *Hoya cagayanensis* with *Hoya buotii* as the outlier**

***H. pimenteliana* Kloppenb.** Leaf decussate, simple, pulvinate, 64–105mm long, 20-31mm wide, 2.17:1–4.62:1 laminar ratio, microphyll, marginal, untoothed, unlobed, primary vein pinnate. Secondary veins festooned brachidodromous; secondary veins spacing irregular; secondary veins angle inconsistent; intersecondary veins weak; secondary veins angle of divergence narrow. Tertiary vein random reticulate; tertiary vein angle variability inconsistent. Quarternary vein alternate percurrent. Areolation well developed. Free ending ultimate veins unbranched to two or more branched. Marginal ultimate venation looped. - Exsicc. - Catones and Buot 7038 (PBDH)

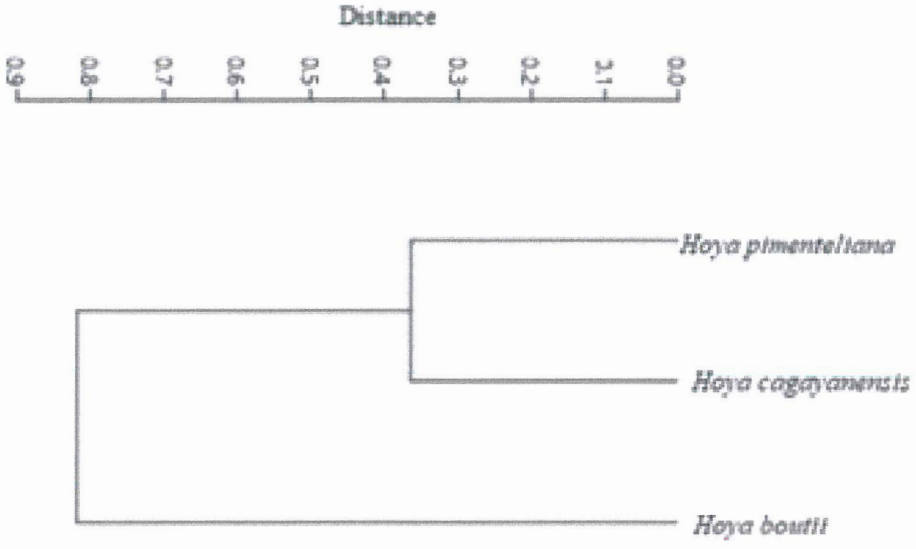



**Figure 4.** Unweighted Pair Group Method Approach.

***H. cagayanensis* C.M. Burton.** Leaf decussate, simple, pulvinate, 62–105mm long, 20–42mm wide, 2.53:1–4.52:1 laminar ratio, microphyll – notophyll, marginal, untoothed, unlobed. Primary vein pinnate. Secondary veins festooned brachidodromous; secondary veins spacing irregular; secondary veins angle inconsistent; intersecondary veins weak; secondary veins angle of divergence narrow. Tertiary vein random reticulate; tertiary vein angle variability inconsistent. Quarternary vein irregular reticulate. Areolation moderately developed. Free ending ultimate veins two or more branched. Marginal ultimate venation looped. Exsicc. - Paguntalan and Buot 6892 (PBDH).

***H. buotii.* Kloppenb.** Leaf decussate, pulvinate, 62–105mm long, 21–40mm, 2.53:1–4.52:1 laminar ratio, microphyll – notophyll, marginal, untoothed, unlobed. Primary vein pinnate. Secondary veins festooned brachidodromous; secondary veins spacing irregular; secondary veins angle smoothly decreasing toward base; intersecondary veins weak; secondary veins angle of divergence moderate. Tertiary vein random reticulate; tertiary vein angle variability inconsistent. Quarternary vein alternate percurrent. Areolation well developed. Free ending ultimate veins unbranched to one branched. Marginal ultimate venation looped. Exsicc. - Catones and Buot 7038 (PBDH).

**Cluster analysis**

Qualitative structural data from leaf and vein characters which were laminar size, blade class, apex shape, base shape, length width ratio, secondary angle of divergence, secondary vein angle, quaternary vein category, areolation, and F.E.V.s were subjected to cluster analysis. Figure 4 shows the dendrogram constructed using Unweighted Pair Group Method Approach (UPGMA). The dendrogram shows that H. pimenteliana and H. cagayanensis share similar at the Gower distance of 95 but remain distinct and different from other, and hence are two separate species.

## CONCLUSION

Leaf architectural characters of *H. pimenteliana* and *H. cagayanensis* were examined. Several leaf and vein characters were seen similar to the two species. Laminar size, length and width ratio, base shape, quaternary vein category, F.E.Vs, and areolation were considered good taxonomic marks as they indicated the distinction

between the two species. Cluster analysis also showed the differences of the two *Hoya* species. The results of the study show that *H. cagayanensis* and *H. pimentilia* are distinct from each other. It is also observed that the distinction in leaf venation between confusing *Hoya* species were observed at finer vein characters, usually at tertiary or quaternary vein. Moreover, this result adds to the growing list of literature that shows leaf architectural characteristics to be a potential and supplementary tool in delineating controversial or confusing species other than molecular.

## ACKNOWLEDGEMENTS

The author would like to thank Mr. Jorge Sahagun and Mr. Fernando B. Aurige for allowing the author to use the images of *Hoya*s retrieved from https://www.philippineplants.org/Families/Apocynaceae.html in this paper.

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Received: 8 July 2020
Accepted: 14 October 2020
Published: 31 December 2020