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Leaf Architectural Study of *Hoya coriacea, Hoya halconensis* and *Hoya buotii* (Apocynaceae)

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Abstract

Leaf architecture characters of the three seemingly similar and hence, controversial species of Hoya, namely H. coriacea, H. halconensis and H. buotii were examined. The use of leaf architecture has been demonstrated in paleobotanical studies and other systematic studies to resolve some taxonomic controversies. These characters were used to analyze the similarities and differences of the three Hoya species. The seven characters, leaf base, margin, form, blade class, primary vein size, variation of angle of divergence and areole development, showed to be distinct from either one or two species. Unweighted Pair Group Method with Arithmetic Averages (UPGMA) and Single Linkage were used for cluster analysis. Both results showed that H. coriacea and H. buotii shared more similar characters than H. halconensis. Elliptic to narrow elliptic form, convex leaf base and stout primary vein size were characteristics common to H. coriacea and H. buotii whereas both H. halconensis and H. buotii had leaves with nearly uniform angle of divergence. Moreover, H. coriacea and H. halconensis have moderately developed areoles while H. buotii had well-developed ones. The study showed that variations in leaf architecture characters can be used to illustrate the relationship among the three species of Hoya.

Keywords: leaf architecture, *Hoya coriacea, Hoya halconensis, Hoya buotii*

Introduction

Genus *Hoya* is considered taxonomically complex brought about by the large number of species that have been published[1]. Approximately, a hundred of species names were used in horticulture[2] and there is an increasing number of individuals collecting and studying this group of plants[3].

Traditionally, morphological parameters have been used for several taxonomic and ecological investigations not only in animals[4-6] but also in plants[7-10]. In the identification and classification of plant taxa, floral characters are given emphasis[11]. This is also true in *Hoya* wherein the bases of delineation of the genus are more focused in the qualitative and quantitative features of the corolla, corona and pollinarium[12]. The use of these characters is also done in separating *H. coriacea, H. halconensis* and *H. buotii*.

Based on the description of Aurigue[13], there is a slight difference on the flower morphology of the three species. The flowers of *Hoya buotii* are more hairy compared to *H. halconensis* and *H. coriacea.* Aurigue[13] also noted three color forms existing in the flower of *H. buotii*, but the one that is considered in this study is the purplish green with light red markings on the corona. The corona of *H. halconensis* has moderate reddish brown inner lobe and greenish outer lobe whereas *H.coriacea* has silky-sericeous-velvety inner surface and papillate outer surface as observed by Aurigue[13].

In this paper we are trying to use leaf architecture data to hopefully supplement the floral information. The use of leaf architecture data has never been optimized despite that they are genetically-fixed and stable especially venation patterns[14]. In fact, several taxonomic investigations and controversies had been conducted and resolved using these characters including: the determination of the differences in the three leaf types of *Sorbus* through the examination of the form and venation[15]; the fundamental differences in the leaf shape of Oak leaves which is suggested by relationship among particular sets of landmark[16]; the determination of infrageneric and interfamilial relationships of Chloranthaceae[17]; the determination of the species of Salicaceae[18]; the relationship of the three subgenera of *Ficus* including *Ficus, Sycomorus* and *Urostigma*[19]; distinguishing some species of family Malvaceae[20]; and the classification of the species of *Camelia* (Theaceae)[21].

There are instances wherein leaf characters are of great importance in taxonomic studies particularly in sterile plants and fossils without reproductive parts[22-23]. However, even though there are already works suggesting the effectiveness of using leaf characters, full taxonomic potential of these remains relatively unexplored[24].

The purpose of this study is to document the leaf architectural characteristics of *Hoya coriacea*, *Hoya halconensis* and *Hoya buotii* and to determine whether these leaf architecture characters strongly support the present recognition of these three distinct species.

Material and Methods

A total of ten mature leaf samples for each of *Hoya coriacea, H. halconensis* and *H. buotii* were collected from the garden of Dr. Inocencio E. Buot Jr. The source of the collection was from the Institute of Plant Breeding: Hoya Accessions, University of the Philippines Los Baños, Laguna, Philippines. All leaf samples were subjected to pressing and drying.

All dried leaves were examined using stereomicroscope. The leaf architecture of the three species was described based on established leaf architecture characters and terminologies of Hickey[25] and Leaf Architecture Working Group[26]. Large measurements such as leaf length, leaf width etc. were measured using dial calliper. Angle of Divergence was measured using a protractor.

A dichotomous key to the 3 species of *Hoya* studied was constructed. Descriptions were based on the characteristic of leaf architecture examined in this study.

Cluster analysis using PAST- PAleontological STatistics ver. 1.64[27] was done to analyze the significant differences among the species. A number of characters were selected and each of its character states was assigned as values corresponding to a legend as follows: F (form)- 1-2; M (Margin)- 1-3; BC (Blade Classs) – 1-2; PVS (primary vein size)- 1-2; ARD (areole development)- 1-2; VAD (variation of angle of divergence- 1-2; LB (leaf base)- 1-2. A dendogram was constructed using Euclidean as distance measure and Unweighted Pair Group Method with Arithmetic Averages or UPGMA Method and Single Linkage or Nearest Neighbor as a linkage method[28].

Results and Discussion

Leaf Architectural Characteristics of *H. coriacea, H. halconensis* and *H. Buotii:* The examined leaves of the three species are shown in figure – 1, 2, and 3. In table – 1, leaf architecture characteristics of *H. coriaceae, H. halconensis* and *H.buotii* are listed. The three species of *Hoya* examined in this study exhibited several unifying features including the unlobed leaf margin; pinnate, camptodromous, straight primary vein; festooned brochidodromous, sinous, secondary veins; moderately acute angle of divergence; inter-secondaries composite; random reticulate tertiary veins; alternate percurrent quaternaries; looping branches are enclosed by 3° and 4° arches; branched veinlets and random areole arrangement.

On the other hand, differences in the three species were also noted in some leaf architectural characters as shown in table - 2. The three species greatly differ in leaf margin, L: W ratio and leaf form. There were also characters common to the two species which differ from the other one. Convex leaf base is a characteristic of *Hoya coriacea* and *H. buotii* whereas *H. halconensis* exhibited rounded leaf base. Moderate primary vein size is exhibited by *H. halconensis* and stout primary vein size is of *H. coriacea* and *H. buotii*. Furthermore, *H. buotii* has a well developed areoles whereas *H. coriacea* and *H. halconensis* has areoles which are moderately developed. In terms of the blade class, both *H. halconensis* and *H. buotii* are under notophyll while *H. coriacea* is under mesophyll. Nearly uniform angle of divergence is recorded in *H. halconensis* and *H. buotii* whereas lower secondaries of *H. coriacea* are more acute than the upper ones.

Figure – 1
Examined leaf samples of *Hoya coriacea*

Figure – 2
Examined leaf samples of *Hoya halconensis*

Table – 1
Leaf architecture characters of the three species of *Hoya*

Characters	***Hoya coriacea* Blume**	***Hoya halconensis* Kloppenburg**	***Hoya buotii* Kloppenburg**
Blade Length (mm)	113- 166	99-136.5	83-137
Blade Width (mm)	34-56	46-60.5	34-49
Blade Area (mm^2)	2561-5674	3102-5220	2130-3990
L:W Ratio	2.6:1- 3.5:1	1.7:1-2.4:1	2:1-3.4:1
Blade Class	Mesophyll	Notophyll	Notophyll
Form	Elliptic to narrow elliptic	Ovate to oblong	Elliptic
Symmetry	Symmetrical	Symmetrical	Symmetrical
Apex	Acuminate	Acuminate	Acuminate
Base	Convex	Rounded	Convex
Margin	Smooth	Entire	Slightly revolute
Lobation	Unlobed	Unlobed	Unlobed
1° vein category	Pinnate	Pinnate	Pinnate
1° vein size	Stout	Moderate	Stout
Course	Straight	Straight	Straight
2° vein category	Festooned Brochidodromous	Festooned Brochidodromous	Festooned Brochidodromous
Relative Thickness	Moderate	Moderate	Moderate
Angle of Divergence	Moderate acute	Moderate acute	Moderate acute
Variation of Angle of Divergence	Lower secondaries more acute than upper ones	Nearly uniform	Nearly uniform
Course	Sinous	Sinous	Sinous
Behavior of Looping Branches	Enclosed by 3° & 4° arches	Enclosed by 3° & 4° arches	Enclosed by 3° & 4° arches
Inter-2° veins	Composite	Composite	Composite
3° vein category	Random reticulate	Random reticulate	Random reticulate
4° vein category	Alternate percurrent	Alternate percurrent	Alternate percurrent
Veinlets	Branched	Branched	Branched
Areole Development	Moderately-developed	Moderately-developed	Well-developed
Areole Arrangement	Random	Random	Random

Table - 2
Characters separating *Hoya coriacea, H. halconensis* and *H. buotii*

Character	***Hoya coriacea* Blume**	***Hoya halconensis* Kloppenberg**	***Hoya buotii***
Margin	Smooth	Entire	Slightly revolute
LWR	2.6:1-3.5:1	1.7:1- 2.4:1	2.4:1-3.4:1
Leaf Base	Convex	Rounded	Convex
Form	Elliptic to narrow elliptic	Ovate to Oblong	Elliptic
Blade Class	Mesophyll	Notophyll	Notophyll
1° vein size	Stout	Moderate	Stout
Variation of Angle of Divergence	Lower secondaries more acute than upper ones	Nearly uniform	Nearly uniform
Areole Development	Moderately- developed	Moderate-developed	Well-developed

There were recorded variations in the size and shape of the three species of *Hoya* in the study. These variations could be physiological in nature as in the study of Zhang et al.[29]. McLellan[30] and Sylvester et al.[31] observed that there are instances that leaves produced on the shoots at the beginning of a season display gradual leaf shape transition that could spell out difference when they mature. For such reasons, the size and the shape of the leaf were considered to yield poor results. This is true in plotting populations wherein there were significant variations in the area and shape of the leaves due to segregation. However, leaf physiognomy characters can work well within single species[32].

Most of the leaves examined in the study were symmetrical. However, there were recorded leaf samples which seemed asymmetrical. This conforms to the result of Torres et al.[33] where there were minor asymmetrical variations in the leaves of 16 species of *Hoya*.

Dichotomous Key to Three Species of *Hoya:* 1. Blade ovate to oblong; 1° vein size moderate, *H. halconensis, 1.* Blade elliptic to narrow elliptic; 1° vein size stout 2. 2. Well-developed areoles; leaf base convex *H. buotii,* 2. Moderately-developed areoles; leaf base convex *H. coriacea.*

***Hoya halconensis* Kloppenburg: Blade** ovate to oblong with acuminate apex and rounded base, symmetrical, 99-136 mm long, 46- 60.5 mm wide, 1.7- 2.4: 1 ratio, notophyll, entire, unlobed. **Venation** camptodromous, pinnate. **1**° stout; straight. **2**° festooned brochidodromous; moderate; sinous; angle of divergence moderately acute and nearly uniform; loop-forming branches enclosed by 3° and 4° arches. **Inter-2° vein** composite. **3**° random reticulate. **Higher vein order** distinct; 4° alternately percurrent ; highest vein order 5°; moderately-developed and random areoles. Marginal ultimate venation looped. Veinlets branched (figure – 4)

Figure – 3
Examined leaf samples of *Hoya buotii*

***Hoya buotii* Kloppenburg : Blade** elliptic with acuminate apex and convex base, symmetrical, 83-137 mm long, 34-49 mm wide, 2-3.4: 1 ratio, notophyll, slightly revolute, unlobed. **Venation** camptodromous, pinnate. **1**° stout; straight. **2**° festooned brochidodromous; moderate; sinous; angle of divergence moderately acute and nearly uniform; loop-forming branches enclosed by 3° and 4° arches. **Inter-2° vein** composite. **3**° random reticulate. **Higher vein order** distinct; 4° alternately percurrent ; highest vein order 5°; 4° and 5° anastomosing to form well-developed, 5 or more- sided and random areoles. **Marginal ultimate venation** looped. **Veinlets** branched (figure – 5)

Figure - 4
***Hoya halconensis* Kloppenberg flower (Photo by: Edward Agdeppa)**

Figure - 5
***Hoya buotii* Kloppenberg flower (Photo by: I.E Buot Jr.)**

***Hoya coriacea* Blume: Blade** elliptic to narrow elliptic with acuminate apex and convex base, symmetrical, 113-166 mm long, 34-56 mm wide, 2.6- 3.5: 1 ratio, mesophyll, smooth, unlobed. **Venation** camptodromous, pinnate. **1**° stout; straight. **2**° festooned brochidodromous; moderate; sinous; angle of divergence moderately acute, lower 2° more acute than upper 2°; loop-forming branches enclosed by 3° and 4° arches **Inter-2**° **veins** composite. **3**°random reticulate. **Higher vein order** distinct; 4° alternately percurrent ; highest vein order 5°; moderately-developed and random areoles. **Marginal ultimate venation** looped. **Veinlets** branched (figure – 6)

Figure - 6
***Hoya coriacea* Blume flower (Photo by: I.E. Buot Jr.)**

There is no doubt that *Hoya halconensis, H. buotii* and *H. coriacea* can be delineated into three distinct species through leaf architecture, though the differences are not that striking.

Cluster Analysis: Qualitative morphological data including leaf base, form, primary vein size, blade class, variation of angle of divergence, areole development of the three species of *Hoya* were subjected to cluster analysis. Figure – 7 shows the dendrograms constructed using Unweighted Pair Group Method using Arithmetic Averages and Single Linkage or Nearest Neighbour Clustering. Both dendrograms clearly illustrated that *H. coriacea* and *H. buotii* shared more similar characters than *H. halconensis* at the similarity level of 0.84. This is also depicted in table - 2.

Cluster analysis is a statistical tool which produces a hierarchical classification of taxa based on similarity matrix[34]. Unweighted Pair Group Method with Arithmetic Averages (UPGMA) and Single Linkage or Nearest Neighbour Clustering are clustering approaches commonly used in the analysis. UPGMA algorithm constructs a dendrogram that reflects the structure present in a pairwise similarity matrix and clusters are joined based on the average distance between the members of a group[35]. Moreover, single linkage clustering constructs a dendrogram which shows the sequence of cluster fusion and the distance at which each fusion takes place and clusters are joined based on the smallest distance between two groups[36]. Apparently, both dendrograms produced by the two approaches depicts the same pattern of clustering of the three species in question. Hammer et al.[27] has stated that if the groupings do not change when tried another algorithm, that grouping should perhaps be trusted. Thus, in terms of leaf architectural characters, *H.coriacea* and *H. buotii* are more related to each other than with *H. halconensis.*

The results of the study indicate that leaf architectural characters are necessary and should be combined with floral characters of the three *Hoya* species to support and strengthen their current status as three distinct and separate species.

Conclusion

Leaf architectural characters of three species of *Hoya* namely *H. coriacea* Blume, *H. halconensis* Kloppenberg and *H. buotii* Kloppenberg, were examined. Description of the leaf architecture characters were given for each species. There were characters common in the three species and only seven were found to be different from any one or two of the species.

Leaf characters, especially the detailed characteristics of the higher vein orders as well as the areoles, can be considered as good taxonomic markers in classifying plant taxa. These can be used in combining or separating several species. In case of the three species of *Hoya* examined in the study, there were a minimal number of variations in the leaf architectural characters that can support their separation. It is suggested therefore to supplement leaf architectural characters with floral characters of *H. coriacea, H. halconensis* and *H. buotii* to stress the fact that the three species are indeed three distinct species as is currently treated. In order to provide further analysis and interpretation of the relationship of the three species as well as other species of *Hoya*, it is recommended to do molecular analysis.

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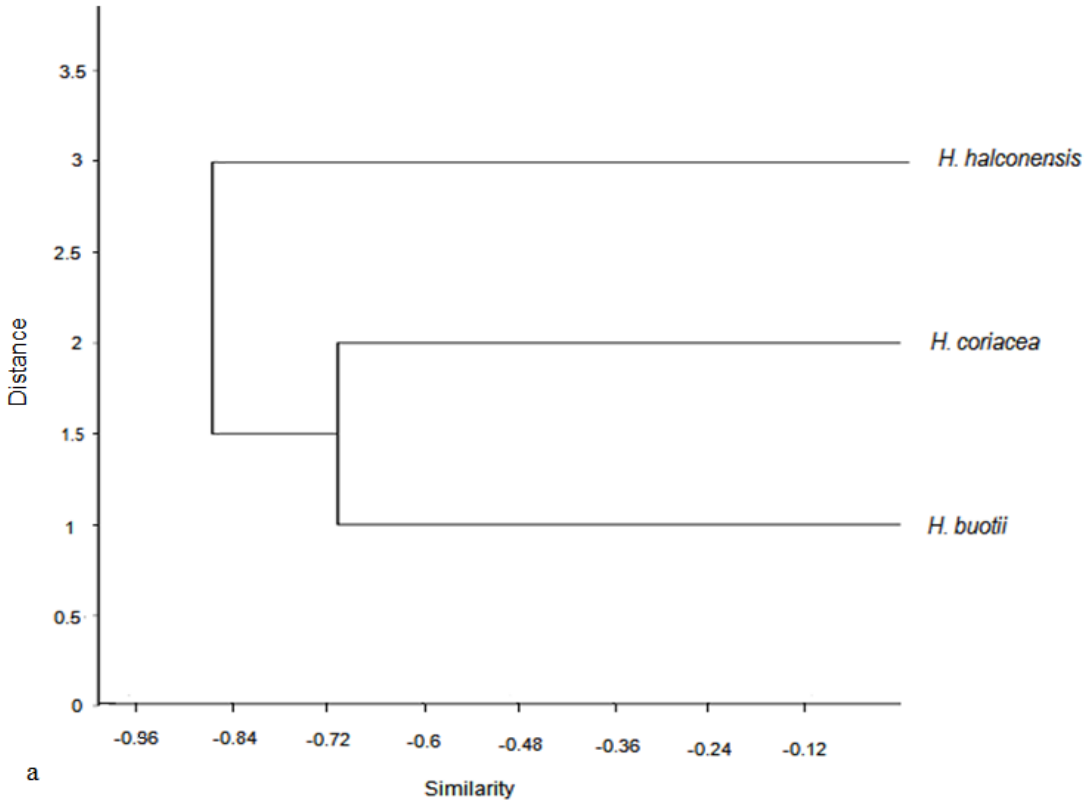
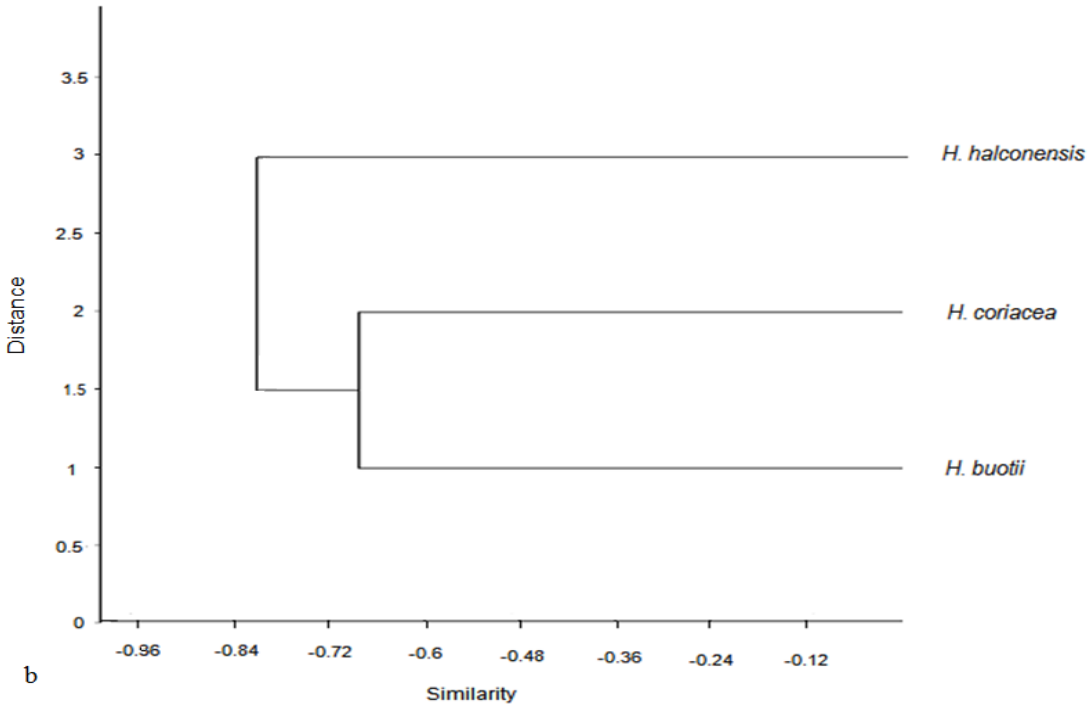


a

(a) Unweighted Pair Group Method with Arithmetic Averages and



b

(b) Single Linkage or Nearest Neighbour Clustering Method

Figure - 7

Dendrogram of the three species of *Hoya* using

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