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Original article

Hoya carandangiana, *Hoya bicolensis*, and *Hoya camphorifolia* (Apocynaceae) Species Delineation: Insights from Leaf Architecture

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ABSTRACT:- Three species of *Hoya*; *H. carandangiana, H. bicolensis, and H. camphorifolia* under the family Apocynaceae, have been studied and re-evaluated using leaf architecture to compare taxonomic markers. The three species seem superficially similar and consequently, controversial. This paper has carefully studied the three species and the results delineate them, proving they are three distinct species. In past decades there have been numerous studies, such as systematic studies and fossil (paleobotanical) studies, which utilized leaf architecture with the aim of resolving certain taxonomic issues. In this present study, leaf architectural characteristics have been utilized to examine the similarities and differences between the three *Hoya* species. Unifying leaf architectural characters were found, exhibited by the three *Hoya* species however; characters such as blade shape, blade class, base shape, apex shape, secondary vein category, tertiary vein category and course were found to delineate them. Cluster analysis was also carried out to illustrate relationships between the three Hoya species by using unweighted pair-group average (UPGMA) and single linkage (nearest neighbor). The results showed that *Hoya carandangiana, Hoya bicolensis* and *Hoya camphorifolia* are three distinct species, demonstrating that leaf architectural characters have the capacity to serve as good taxonomic markers.

KEY WORDS: *Hoya carandangiana*, *Hoya bicolensis, Hoya camphorifolia*, leaf architecture, and taxonomy

INTRODUCTION

Hoya is a genus of more than 500 species of tropical plants in the family Apocynaceae (International Plant Names Index, 2012) commonly known as Dogbane. Asia and the Southeast Asian regions are recognized centers of diversity for this plant, considering that many species are native to this area; many have been recorded and more are currently still being discovered (Rodda and Ang, 2012). However, compared to the other Southeast Asian nations, the Philippines has great species diversity of this plant with more than 80 *Hoya* species currently known (Kloppenburg *et al*., 2012). A major threat for these plants is the relentless deterioration of natural habitats; significantly increasing the number of individuals interested in the collection of this species for study and conservation purposes (Kloppenburg *et al*., 2012).

In plant identification and classification, the reproductive characters are generally given emphases (LAWG, 1999; Alejandro and

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Liede, 2003) considering that these characters are under strong genetic regulation and not easily affected by the changes in the environment. This phenomenon is also true in *Hoya*, in which species circumscription is generally anchored on reproductive structures such as the corolla, corona and pollinarium (Kleijn and van Donkelaar, 2001). Furthermore, these characters were also the bases used to conclude that *Hoya camphorifolia* Warb., *H. bicolensis* Kloppenb., Siar & Cajano, and *H. carandangiana* Kloppenb. & Siar are different species.

The floral morphology and foliage of *Hoya camphorifolia* Warb. and *H. bicolensis* Kloppenb., Siar & Cajano are all very similar but have slight differences, causing confusion. In *Hoya bicolensis*, the ends of the outer corona lobes are acute and not obtuse and the sepals are generally triangular in shape and do not usually touch the corolla sinuses, whereas in *Hoya camphorifolia* Warb. the sepals are generally lanceolate and usually touch the sinuses of the corolla (Kloppenburg, 2015). On the other hand, based on floral morphology *Hoya carandangiana* Kloppenb. & Siar is quite different from *Hoya camphorifolia* Warburg and *Hoya bicolensis* Kloppenb., Siar & Cajano, as it has a pollinarium that is small in diameter compared to the others. However, the overall appearance of its foliage is comparably similar to the two species as mentioned earlier (Kloppenburg, 2015), which has caused confusion in the past. The aim of this paper is to highlight the differences to clear confusion and redefine and correct taxonomic confusion.

This study uses data on leaf architecture, described by Hickey (1973) as "the placement and form of different elements making the outward appearance of leaf structure, which include the leaf shape, leaf size, marginal configuration, gland position and venation pattern". The leaves are predominantly ignored in studies concerning taxonomic and comparative morphology because of a huge lack of a comprehensive, standardized, definite classification of leaf characters. But this dilemma was solved when the work of Hickey (1973) was published. However, since then, leaf characters are still not commonly used as a taxonomic tool, arguing that these characters are believed to exhibit high phenotypic plasticity. However, Roth–Nebelsick *et al*., (2001) have shown that leaf characters with emphasis on leaf venation are actually genetically fixed, supporting its potential as a very good taxonomic tool. But despite its potential as a very good taxonomic tool, still it is largely unexplored (Banactila and Buot, 2004; Salvaña and Buot, 2013; Villareal and Buot, 2015). Studies on the taxonomic usefulness of leaf characters in the Philippines include the study of Banactila and Buot (2004) in the genus *Psychotria*; the study of Salvaña and Buot (2014) on the documentation of leaf characters and delineation of *Hoya coriacea*, *Hoya halconensis* and *Hoya buotii*; and the study of Villareal and Buot (2015) in using Leaf characters for determining whether *Hoya incrassata* and *Hoya crassicaulis* belongs to the same species. Moreover, in some cases leaf characters are of great significance especially in taxonomic studies that utilize sterile plants and fossils without visible reproductive structures (Dilcher, 1974; Hickey and Taylor, 1991).

The aims of this study are; 1) to document the leaf architectural characteristics of *Hoya carandangiana, Hoya bicolensis* and *Hoya camphorifolia*; 2) describe the three *Hoya species* using leaf architectural characters and 3) examine whether the data from these leaf architectural characteristics will support the current species level recognition of these three *Hoya* species that are currently always suspected to be synonyms.

MATERIALS AND METHODS

Ten mature leaf samples were randomly selected for each of the three species, *Hoya carandangiana, Hoya bicolensis* and *Hoya camphorifolia*. The leaf samples of each species were collected from the *Hoya* Accessions, Institute of Plant Breeding, University of the Philippines Los Baños, Laguna, Philippines. The collected leaf samples were pressed and sun dried. Dried materials were used to give more reliable measurements compared to fresh materials, considering that the former eliminates the unstable water concentrations in the assessed plant material which is present in the latter.

The dried leaf samples of each species were examined under a stereomicroscope. Then, the leaf architecture of these three *Hoya* species were described by using the established leaf architecture characters and terminologies defined by Hickey (1973) and that of the Leaf Architecture Working Group (1999). In measuring large measurements such as leaf length and width, a dial caliper was used and for angle measurements, a protractor was used.

The *Hoya* species were described and a dichotomous key to the three species was also constructed using the leaf characters. Moreover, Cluster Analysis was conducted using PAST (Paleontological Statistics version 3.11) by Hammer *et al*., (2016) to determine and analyze significant relationships exhibited by the three *Hoya* species. Several selected characters were used for this analysis and each character state for a certain character was designated with values that correspond to these particular legends: BLS (Blade shape) – 1–3; BC (Blade class) – 1–2; BAS (Base shape) – 1–3; AS (Apex shape) – 1–2; SVC (secondary vein category) – 1–2; TVCT (tertiary vein category) – 1–2; TVCR (tertiary vein course) – 1–2; AR (Areolation) – 1–2. A denrogram to illustrate relationships between the three *Hoya* species was constructed by using Eucledian as the distance measure and unweighted pair-group average (UPGMA) and single linkage (nearest neighbor) as the algorithms, following Salvaňa and Buot (2013).

RESULTS AND DISCUSSION

Leaf Architectural Characters of *H. carandangiana, H. bicolensis* and *H. camphorifolia.*

The leaf samples of the three *Hoya* species that were analyzed are shown in figure 1. Table 1 shows the general blade characteristics while table 2 shows the general venation characteristics of the three *Hoya* species.

As shown in table 1, based on blade characters, the unifying leaf architectural characters exhibited by the three *Hoya* species include, the alternate leaf attachment, simple leaf organization, symmetrical blade symmetry, marginal position of petiolar attachment, entire margin type and unlobed blade. On the other hand based on venation characters, as shown in table 2, the unifying leaf architectural characters exhibited by the three *Hoya* include pinnate (arcuate) primary vein category, secondary vein spacing that is decreasing towards the base, absence of inter–secondary veins, acute tertiary vein angle to primary veins, uniform tertiary vein angle variability, regular polygonal reticulate quaternary vein category, absence of FEVs (freely ending ultimate veins) and looped marginal ultimate venation.

As shown in table 3, the three *Hoya* species were distinct in terms of their respective L: W ratios, blade shape and base shape. In terms of tertiary vein category *Hoya bicolensis* had mixed opposite/ alternate percurrent tertiary veins, *Hoya camphorifolia* had alternate

percurrent and *Hoya carandangiana* had opposite percurrent tertiary veins. Moreover, there were also several characters that were shared by the two species and different from the remaining species.

All leaf samples of *Hoya carandangiana* that were analyzed were microphyll while for *Hoya bicolensis* and *Hoya camphorifolia*, most of the leaf samples were microphyll and some were notophyll. Acute apex was common to *Hoya bicolensis* and *Hoya camphorifolia* while acuminate apex was distinctive to *Hoya carandangiana*. Furthermore, with regards to venation characters, such as for secondary vein category, a weak brochidodromous type was unique to *Hoya carandangiana* while festooned brochidodromous secondary vein category was common to *Hoya bicolensis* and *Hoya camphorifolia*. Tertiary vein course was not applicable for *Hoya camphorifolia* because it is only for opposite percurrent tertiary vein category, accordingly *Hoya carandangiana* and *Hoya bicolensis* (applicable only for its opposite percurrent tertiaries) exhibited a sinous category. In addition, well developed areolation was seen in *Hoya bicolensis* leaf samples while moderately developed areolation was seen in both the leaf samples of *Hoya carandangiana* and *Hoya camphorifolia*.

***Hoya carandangiana* Kloppenb. & Siar:** Leaves opposite and simple, marginal petiolar attachment, ovate to lanceolate, acuminate apex, convex to round base, symmetrical, 44–60 mm long, 16–24 mm wide, 2.33:1–4:1 ratio, microphyll, entire, unlobed. Primary vein pinnate (arcuate). Secondary veins weak brochidodromous; secondary vein spacing, decreasing towards the base; inter–secondary veins absent. Tertiary veins opposite percurrent; sinous; angle to primary veins acute; angle variability uniform. Quaternary veins regular polygonal reticulate. Areolation moderately developed. Freely ending ultimate veins absent. Marginal ultimate venation looped (Figures 1-1, 2-1).

***Hoya bicolensis* Kloppenb., Siar & Cajano:** Leaves opposite and simple, marginal petiolar attachment, elliptic to obovate, acute apex, Cuneate to convex base, symmetrical, 67–102 mm long, 28–49 mm wide, 1.92:1–2.81:1 ratio, microphyll and notophyll, entire, unlobed. Primary vein pinnate (arcuate). Secondary veins festooned brochidodromous; secondary vein spacing decreasing towards the base; inter–secondary veins absent. Tertiary vein mixed opposite/ alternate percurrent; sinous /NA respectively; angle to primary veins acute; angle variability uniform. Quaternary veins regular polygonal reticulate. Areolation well developed. Freely ending ultimate veins absent. Marginal ultimate venation looped (Figs 1-2, 2-2).

***Hoya camphorifolia* Warb.:** Leaves opposite and simple, marginal petiolar attachment, elliptic to ovate, acute apex, convex base, symmetrical, 63–120 mm long, 31–50 wide, 1,322.67–4000 ratio, microphyll and notophyll, entire, unlobed. Primary vein pinnate (arcuate). Secondary veins festooned brochidodromous; secondary vein spacing decreasing towards the base; inter–secondary veins absent. Tertiary vein alternate percurrent; angle to primary veins acute; angle variability uniform. Quaternary veins regular polygonal reticulate. Areolation moderately developed. Freely ending ultimate veins absent. Marginal ultimate venation looped (Figs 1-3, 2-3).

By utilizing leaf architectural characters, *H. carandangiana, H. bicolensis* and H. *camphorifolia* could be delineated into three different species even though the differences were originally not that noticeable. Accordingly, these leaf architectural characters are very significant and should be

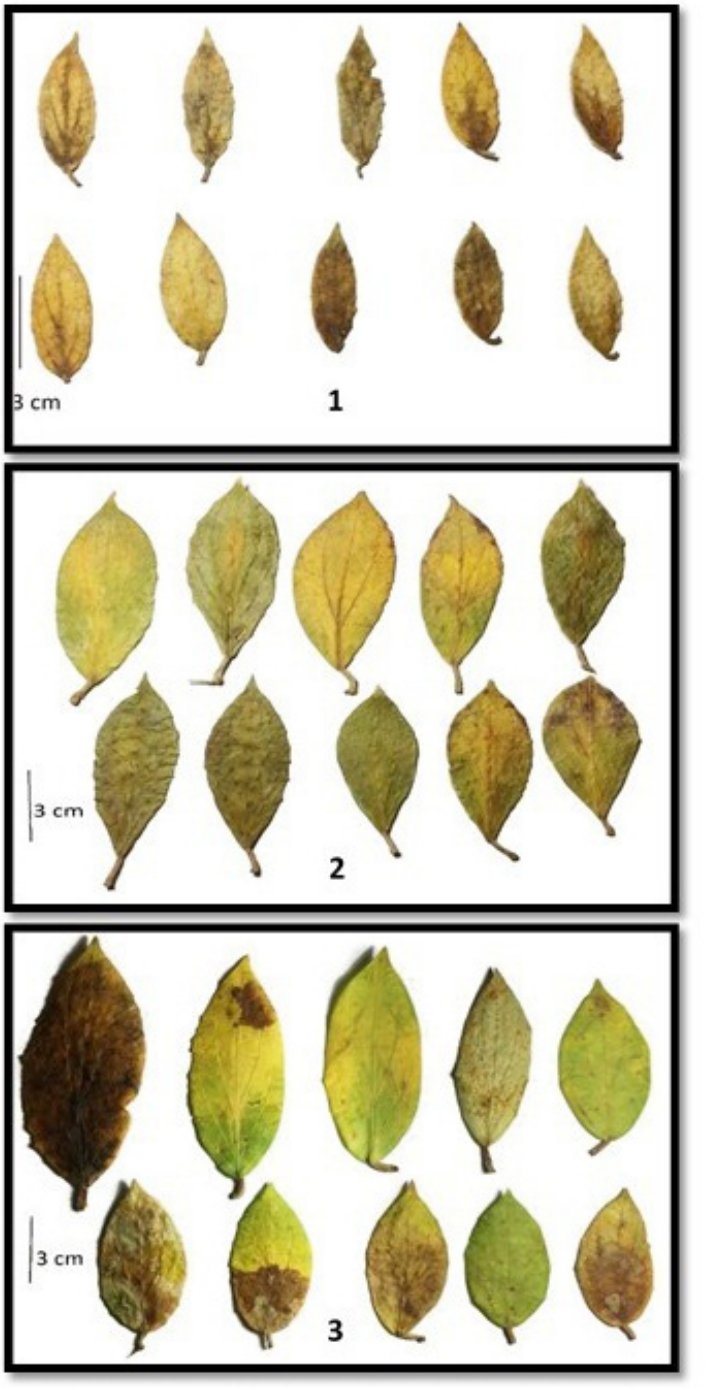

Figure 1. Studied leaf samples, 1) *Hoya carandangiana* 2) *Hoya bicolensis* and 3) *Hoya camphorifolia* (Photo credit : J.T. Torrefied).

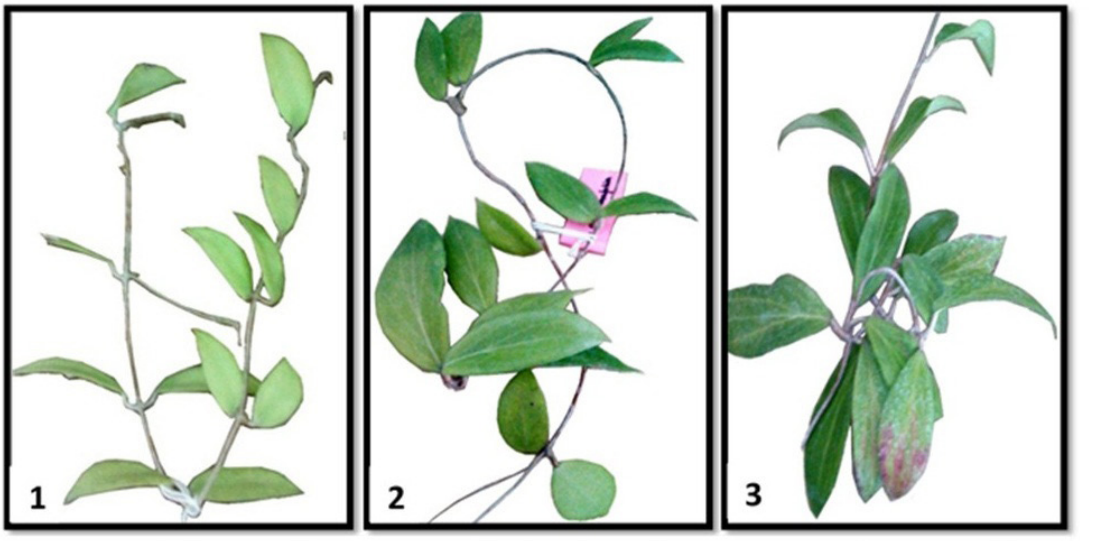

Figure 2. Fresh leaves of the species considered in this study. 1) *Hoya carandangiana* 2) *Hoya bicolensis* and 3) *Hoya camphorifolia* (Photo credit : J.T. Torrefiel)

combined with floral characters of the three *Hoya* species respectively to reinforce and strengthen their respective current taxonomic status as three different species.

The results of this study are parallel to that of two earlier leaf architectural studies in the genus *Hoya* here in the Philippines (Salvaña and Buot, 2013; Villareal and Buot, 2015). The findings of this study support the current species level recognition, of the respective taxa studied, established or based on floral characters. Hence, the present study supports the findings of previous works dating from Hicky (1973) that leaf architectural characters are indeed good taxonomic characters as well.

Dichotomous Key to the Three Hoya Species

1. Blade apex shape acute; secondary vein festooned brochidodromous 2
1. Blade apex shape acuminate; secondary vein weak brochidodromous..... *H. carandangiana*
2. Blade shape elliptic to obovate; base shape cuneate to convex; well-developed areoles (areolation very defined) ..... *H. bicolensis*
2. Blade shape elliptic to ovate; base shape convex; moderately developed areoles (areolation moderately defined)..... *H. camphorifolia*

Cluster Analysis: Cluster analysis (Figs 3 and 4 respectively) using unweighted pair-group average (UPGMA) and single linkage (nearest neighbor) evidently showed that the three Hoyas are distinct species with *H. bicolensis* and *H. camphorifolia* exhibiting more similar characters than with *H. carandangiana* with a similarity level of 0.72. Cluster analysis is a very useful statistical tool that generates a classification of taxa in a hierarchical order based on similarity matrix (Bailey 1994;

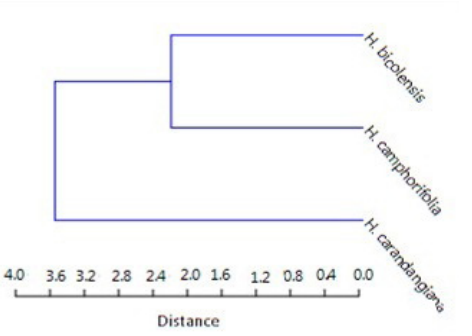

Figure 3. Unweighted pair-group average (UPGMA) clustering approach

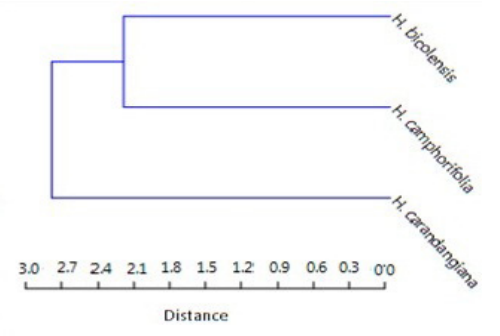

Figure 4. Single linkage (nearest neighbor) clustering approach.

Salvaña and Buot 2013). The UPGMA algorithm generates dendrogram in such a way that the clusters are linked based on the average distance among all members in the two groups (Sokal, and Michener 1958). On the other hand, single linkage (nearest neighbor) algorithm generates dendrogram in such a way that clusters are linked based on the smallest distance among the two groups (Sibson, 1973).

CONCLUSION

Leaf architectural characters, specifically venation and areolation characters, can be used as a very good taxonomic tool in plant taxonomic studies (such as in delineation and classification of taxa). The technique is not costly and difficult. The results demonstrated that the controversial species, *Hoya carandangiana, Hoya bicolensis* and *Hoya camphorifolia* are indeed three distinct species even though the differences were not that noticeable to the eye. It is suggested that these leaf architectural characters should be supplemented with their respective floral characters to highlight delineation between species. Further studies, employing molecular techniques and methods are very much recommended to provide further understanding on the relationships between the three species and other controversial species as well.

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Table 1 General blade characters of *Hoya carandangiana, Hoya bicolensis*, and *Hoya camphorifolia* respectively.

Characters	*Hoya carandangiana*	*Hoya bicolensis*	*Hoya camphorifolia*
Leaf attachment	Opposite	Opposite	Opposite
Leaf organization	Simple	Simple	Simple
Blade length in mm	44–60 (mean: 55.1)	67–102 (mean: 82.1)	63–120 (mean: 82.9)
Blade width in mm	16–24 (mean: 19.4)	28–49 (mean: 34.8)	31–50 (mean: 35.6)
Blade area	469.33–896 (mean: 715.40)	1,288–3, 3332 (mean: 1, 942.27)	1,322.67–4000 (mean: 2,029.80)
Blade class	Microphyll	Microphyll and notophyll	Microphyll and notophyll
L:W Ratio	2.33:1–4:1 (mean: 2.88:1)	1.92:1–2.81:1 (mean: 2.43:1)	1.91:1–3.19:1 (mean: 2.38:1)
Blade shape	Ovate to lanceolate	Elliptic to obovate	Elliptic to ovate
Blade symmetry	Symmetrical	Symmetrical	Symmetrical
Base shape	Convex to round	Cuneate to convex	Convex
Position of petiolar attachment	marginal	Marginal	Marginal
Apex shape	Acuminate	Acute	Acute
Margin type	Entire	Entire	Entire
Lobation	Unlobed	Unlobed	Unlobed

Table 2 General venation characters of *Hoya carandangiana*, *Hoya bicolensis*, and *Hoya camphorifolia* respectively.

Characters	*Hoya carandangiana*	*Hoya bicolensis*	*Hoya camphorifolia*
Primary vein category	Pinnate (Arcuate)	Pinnate (Arcuate)	Pinnate (Arcuate)
Secondary vein category	Weak brochidodromous	Festooned brochidodromous	Festooned brochidodromous
Secondary vein spacing	Decreasing towards the base	Decreasing towards the base	Decreasing towards the base
Inter–secondary veins	Absent	Absent	Absent
Tertiary vein category	opposite percurrent	Mixed opposite/ alternate percurrent	alternate percurrent
Tertiary vein angle to Primary veins	Acute	Acute	Acute
Tertiary vein angle variability	Uniform	Uniform	Uniform
Quaternary vein category	Regular polygonal reticulate	Regular polygonal reticulate	Regular polygonal reticulate

Table 2 (Continue).

Characters	*Hoya carandangiana*	*Hoya bicolensis*	*Hoya camphorifolia*
Areolation	Moderately developed	Well developed	Moderately developed
F.E.V.S (freely ending ultimate veins)	Absent	Absent	Absent
Marginal ultimate venation	Looped	Looped	Looped

*Tertiary vein course is only applicable to opposite percurrent tertiary vein category.

Table 3 The leaf architectural characters delineating the three *Hoya* species.

Characters	*Hoya carandangiana*	*Hoya bicolensis*	*Hoya camphorifolia*
L:W Ratio	2.33:1–4:1 (mean: 2.88:1)	1.92:1–2.81:1 (mean: 2.43:1)	1.91:1–3.19:1 (mean: 2.38:1)
Blade shape	Ovate to lanceolate	Elliptic to Obovate	Elliptic to Ovate
Blade class	Microphyll	Microphyll and notophyll	Microphyll and notophyll
Base shape	Convex to round	Cuneate to convex	Convex
Apex shape	Acuminate	Acute	Acute
Secondary vein category	Weak brochidodromous	Festooned brochidodromous	Festooned brochidodromous
Tertiary vein category	Opposite percurrent	Mixed opposite/ alt percurrent	Alternate percurrent
Tertiary vein course	Sinous	Sinous/NA*	N/A*
Areolation	Moderately developed	Well developed	Moderately developed

*Tertiary vein course is only applicable to opposite percurrent tertiary vein category.