The Thailand Natural History Museum Journal 14(2), 113-125, 31 December 2020
©2020 by National Science Museum, Thailand
http:doi.org/10.14456/thnhmj.2020.3

# Leaf Architecture of Representative *Hopea* and *Shorea* Species (Dipterocarpaceae): Implication on Current Taxonomic Classification

**Christine Dawn Obemio[1, 2,*] and Inocencio Buot, Jr.[2]**

[1]*Science Department, Mindanao State University-General Santos City (MSU-GSC) Fatima, General Santos City 9500 Philippines*

[2]*Plant Biology Division, Institute of Biological Sciences, University of the Philippines Los Baños (UPLB), College, Laguna 4030 Philippines*

*Corresponding Author: cgobemio@up.edu.ph

## ABSTRACT

Leaf architecture of representative *Hopea* and *Shorea* species was evaluated. Details on its morphological characteristics: venation, shape, angle, size, among others were determined from representative herbarium vouchers in the Plant Biology Division, Institute of Biological Science and leaf samples from College of Forestry and Natural Resources of the University of the Philippines, Los Baños, Laguna. Analysis through numerical taxonomic parameters was further undertaken to elucidate relationships among representative *Hopea* and *Shorea* species. Results showed four component axes accounting for approximately 94% of the cumulative variance, with axis 1, 2 and 3 accounting for >80% variability. From such axes, distinct clusters were noted in *H. quisumbingiana*, *H. philippinensis*, *S. polysperma*, and *S. malibato* suggesting their recognizably different morphology with reference to leaf ratio, apex and base angles, length, width and number of secondary veins. Moreover, parallels or close association have been observed between *S. guiso* and *S. almon* and that of *H. mindanensis* and *H. plagata* for the same characters as they appear to lie closely within the axis. Qualitative traits as leaf rank, venation and areolation appear to largely influence the observed variation in *Hopea* and *Shorea* groups. However, trees generated for both qualitative and quantitative morphological features showed clusters of both groups intermingling, suggesting the possibility of these groups to be the same with one subsumed in the other. Hence, deeper resolution of taxonomic relationship is warranted.

**Keywords:** cluster analysis, dipterocarps, principal component analysis.

## INTRODUCTION

The primary goal of systematics is to provide relevant evidence that can be useful in classifying and describing the taxa of interest (Celadiña *et al.*, 2012) through morphological, biochemical to molecular analyses. Leaf architecture is a traditional morpho-anatomical method that has been used in characterizing plant species both in the past and recent systematic studies (Fuller and Hickey, 2005). Many works have also shown that leaf morphological characters are very good diagnostic markers in the classification of plants, primarily its leaf venation patterns (Banaticla and Buot, 2004; Laraño and Buot, 2009; Baroga and Buot, 2014; Pulan and Buot, 2014). In fact, venation is said to be a genetically fixed vegetative character in plants (Roth-Nebelsick *et al.*, 2001) and may be useful in delineating variation among plant species being investigated. Although leaf architecture may often be underutilized, several studies have shown its usefulness in the resolution of taxonomic controversies (Loufty *et al.*, 2005; Swaminathan *et al.*, 2012).

Among those that warrant resolution are the *Hopea* Roxb. and *Shorea* Roxb. ex C.F. Gaertn groups (Gamage *et al.*, 2005; Kamiya *et al.*, 2005; Talip, 2008). *Hopea* and *Shorea* species belong to Dipterocarpaceae, a dominant and economically important tree family in tropical rainforests. Their overwhelming presence in some of the forest formations became the basis for calling the vegetation zones dipterocarp forests (Appanah and Turnbull, 1998). They are utilized as source of quality timber used in many domestic and industrial purposes. However, populations of dipterocarps are declining

due to timber extraction, shifting cultivation, and the establishment of commercial plantations (Daisuke *et al.*, 2013) placing them in threatened conservation status based on the Red List Assessments of the International Union for the Conservation of Nature (IUCN version 2016-3).

Ashton (1982) described the key morphological differences of *Hopea* and *Shorea* with *Hopea* having two long and three short fruit calyx wings, while that of Shorea is three long and two short wings on the fruit. Yet, further studies presented uncertainties in the delimitation of these groups. Yulita *et al.* (2005) described that the taxonomic limits of the genera are problematic as inferred from recent molecular phylogenies where *Hopea* was nested within the *Shorea* group, rendering *Shorea* paraphyletic. Talip (2008) also revealed overlapping characters of these closely allied genera *Hopea* and *Shorea* based on their pollen morphology. Hence, more studies are yet to settle the delineation between these two genera.

The use of molecular analysis to resolve uncertainty in phylogenetic relationships of Dipterocarps has been recommended because it is not influenced by the environment or the developmental changes in plant hence may provide greater resolution (Rath *et al.*, 1998; Yulita *et al.*, 2005). However, this approach aside from the fact that it is very expensive, may still provide insufficient evidence compared to the broad-range morphological considerations of traditional taxonomy (Lipscomb *et al.*, 2003). Hence, assessment of morphological traits is still practical and convenient method to supplement molecular data making classification robust and more reliable.

A previous leaf architectural study on *Shorea* in the Philippines by Pulan and Buot (2014) highlighted unique morphological characteristics in ten Philippine *Shorea* species, while that in *Hopea* is yet to be assessed; hence, this study was conceptualized. This investigation seeks to aid in the resolution of relationships between Philippine *Hopea* and *Shorea* on the basis of its leaf morphological characteristics. It aimed to assess leaf morphological traits of five *Hopea* species (*Hopea basilanica* Foxw., *Hopea mindanensis* Foxw., *Hopea philippinensis* Dyer, *Hopea plagata* S. Vidal and *Hopea quisumbingiana* H.G.Gut) and five *Shorea* species (*Shorea almon* Foxw., *Shorea astylosa* Foxw., *Shorea guiso* Blume, *Shorea malibato* Foxw. and *Shorea polysperma* Merr.) and infer their association through numerical taxonomy.

## MATERIALS AND METHODS

### Plant samples

*Hopea* and *Shorea* species were acquired from the nursery of College of Forestry and Natural Resources (CFNR) and the PBD Herbarium in UPLB. Ten (10) leaf samples were randomly taken from different branches of the identified plant. Five species of *Hopea* (*Hopea basilanica*, *Hopea mindanensis*, *Hopea philippinensis*, *Hopea plagata*, and *Hopea quisumbingiana*) and *Shorea* (*Shorea almon*, *Shorea astylosa*, *Shorea guiso*, *Shorea malibato*, and *Shorea polysperma*) were evaluated for their leaf architectural characteristics (Figures 1A and 1B).

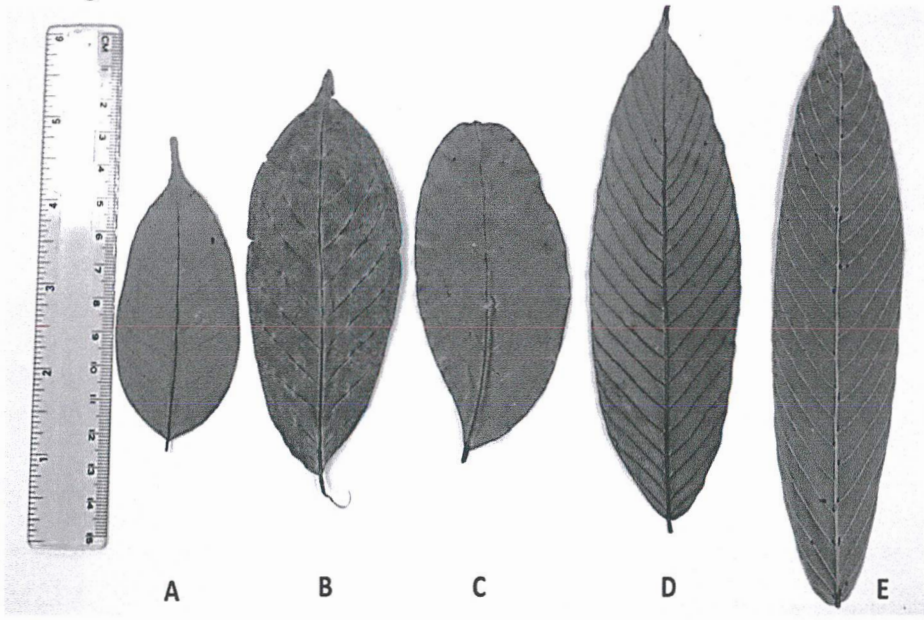



**Figure 1A:** Representative Leaf samples of *Hopea* species (A. *H. quisumbingiana*, B. *H. plagata*, C. *H. basilinica*, D. *H. mindanensis*, E. *H. philippinensis*).

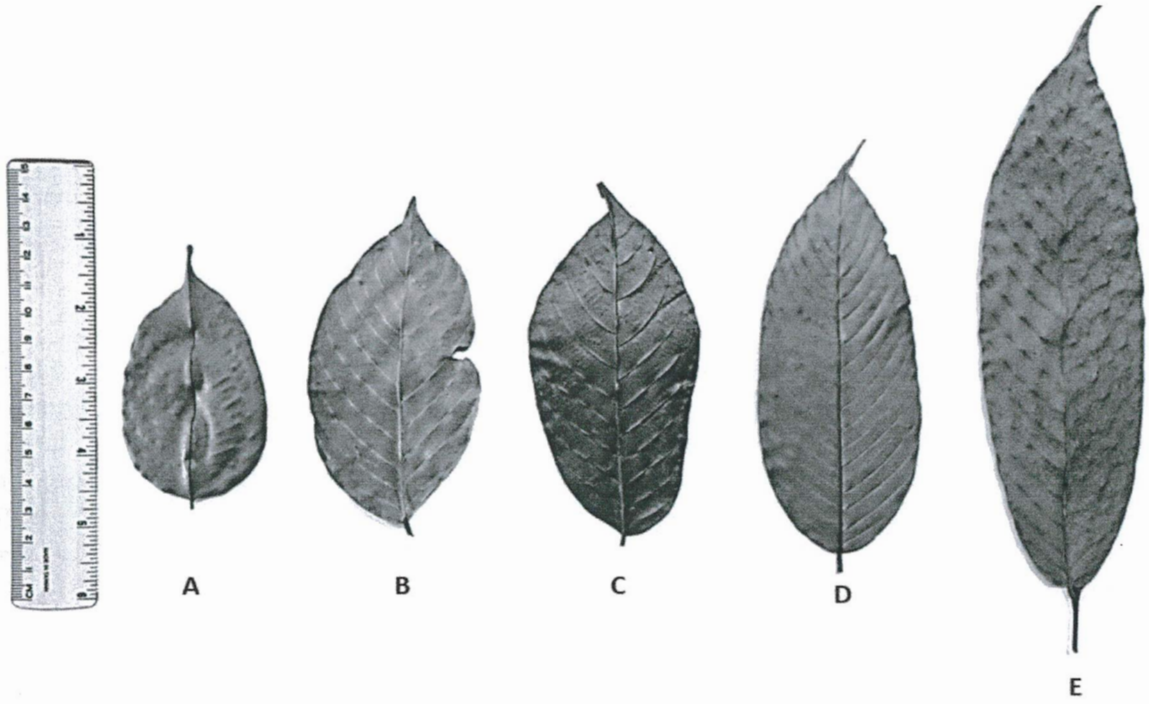



**Figure 1B:** Representative Leaf samples of *Shorea* species (A. *S. almon*, B. *S. astylosa*, C. *S. guiso*, D. *S. malibato*, E. *S. polysperma*).

**Table 1.** 20 Leaf Morphometric Characters Observed in the Study

| | |
|---|---|
| **L** | Lamina length |
| **W** | Lamina width |
| **LW** | Left Laminar Width |
| **RW** | Right Laminar Width |
| **LA** | Leaf Area (LxWx2/3) |
| **LR** | Leaf Ratio (L/W) |
| **LSV** | Number of Left Secondary Veins |
| **RSV** | Number of Right Secondary Veins |
| **BA** | Base Angle |
| **BS** | Base Shape |
| **LS** | Laminar Size |
| **LSym** | Laminar Symmetry |
| **BC** | Blade Class |
| **AA** | Apex Angle |
| **2VC** | Secondary Vein Category |
| **3VC** | Tertiary Vein Category |
| **3VCrz** | Tertiary Vein Course |
| **4VC** | 4th Vein Category |
| **Areo** | Areolation |
| **LRnk** | Leaf Rank |

*Leaf character selection and measurements*

A total of 20 morphological traits, representative of shape, size, angle and venation characters were evaluated. Their respective description and illustration are presented in Table 1. These morphometric characters were based from the Leaf Architecture Manual developed by the Leaf Architecture Working Group in 1990. Some modifications were also employed to provide additional parameters (*i.e.* number of secondary veins, left and right laminar width) following the method of Jumawan and Buot, 2016. Qualitative data from previous leaf architectural study of *Shorea* species (Pulan and Buot, 2014) was further utilized and compared with the data on *Hopea* species evaluated in this study (Figure 2).

**Data Analysis**

Twenty (20) leaf morpho-anatomical traits were evaluated across five *Hopea* and five *Shorea* species, each with 10 leaf replicates. This produced a total of 2000 data points from 200 data sets examined in each species. Quantitative data of *Hopea* and *Shorea* representative species were subjected to numerical taxonomic analysis (univariate and multivariate) while qualitative data were treated through comparative phylogenetic analysis through Mesquite version 3.31. (build 859).

Univariate and multivariate analysis were done through Paleontological Statistics (PAST) software package version 3.14. Univariate data presents the minimum and maximum values, the mean and standard deviation for each of the morphological traits. Data were transformed to qualify for normal distribution test through two-way ANOVA. Box plot was generated to evaluate the distribution of univariate data across the evaluated morphological traits.

Multivariate analysis was undertaken through ordination and clustering. Principal Component Analysis (PCA) was employed to search for patterns in the data highlighting leaf architectural similarities and differences. Components in PCA that presented an eigenvalue of at least 1 (Kaiser's Rule) or described at least 80% of the variance were utilized in evaluating data. Further cluster analysis was done using Unweighted Pair-Group Method with Arithmetic Mean (UPGMA), Euclidean distance to generate a dendogram showing inferred relationship among Hopea and Shorea groups.

**RESULTS**

**Qualitative leaf morphological traits**

It was observed that *Hopea* and *Shorea* species represented in this investigation showed similarities in some traits and variations in others. Apex shape (acuminate) was shown to be the morphological trait

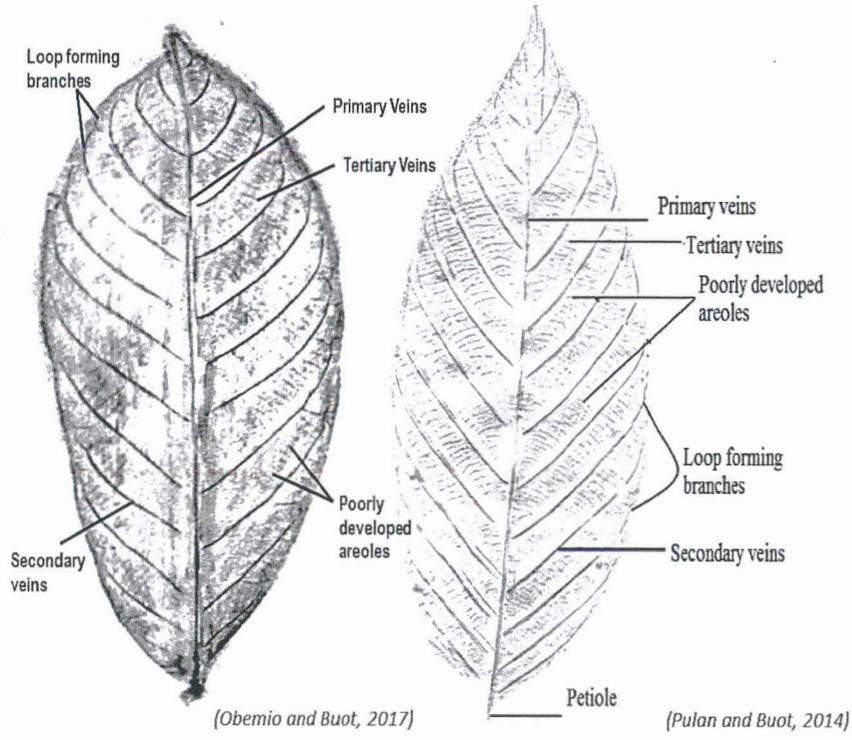



**Figure 2.** Typical venation pattern in A. *Hopea* and B. *Shorea* species showing primary, secondary and tertiary arrangements and loop forming branches of 2° venation (a pattern common in both groups).

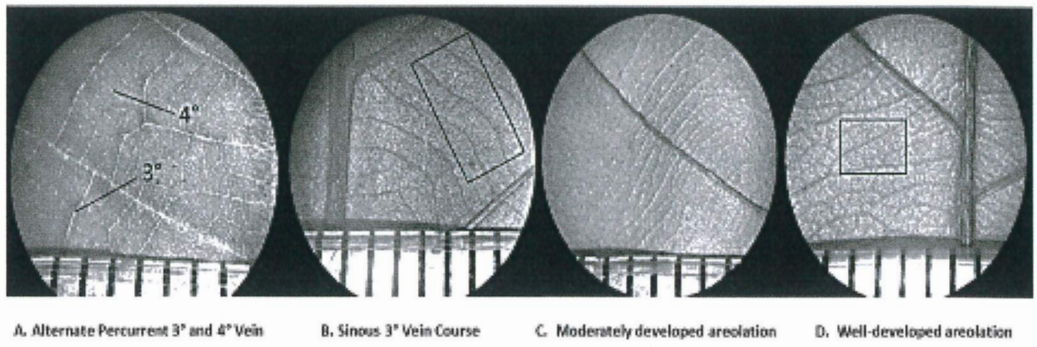



**Figure 3.** Illustration of venation and areolation leaf architectural characteristics (magnification 20x).

common in majority of *Hopea* and *Shorea* species examined. Asymmetry in leaf bases, the 2° vein categories were also the same in most of Shorea and Hopea representatives (brochidodromous). Venation (3rd–4th) and areolation (poor to well-developed) patterns on the other hand were found to vary within these groups. Figures 2 and 3 illustrate some of the qualitative traits evaluated in this study.

Among the *Hopea* species, *H. quisumbingiana* presented distinctive leaf shape (oblong), base shape (rounded) and 3° vein category (ramified) from the rest of the *Hopea* species observed in this study. In the *Shorea* group, *S. polysperma* also stood out in having a well-developed areolation and regular polygonal reticulation. Leaf rank was also evident to be highest in this *Shorea* species with *S. polysperma* having its 4th vein order still organized while the rest had intact 3rd vein (*S. almon*, *S. guiso*, *S. malibato*) and 2nd vein (*S. astylosa*) orders (Table 2).

Mesquite analysis of non-phylogenetic morphometrics (Figure 4) presented three (3) clusters for the qualitative leaf morphometrics. It showed the distinctiveness of *Hopea quisumbingiana* and *Shorea polysperma* from the rest. Three *Hopea* species (*H. philippinensis*, *H. plagata*, *H. mindanensis*), were found nested with other *Shorea* species in the bigger cluster. Meanwhile, *Hopea basilinica* was further found to be associated with *Shorea astylosa*. As for *Shorea*, all species evaluated, except for *S. polysperma*, could be found in a big cluster along with the rest of the species with *S. malibato*, *S. guiso* and *S. almon* in a monophyletic position.

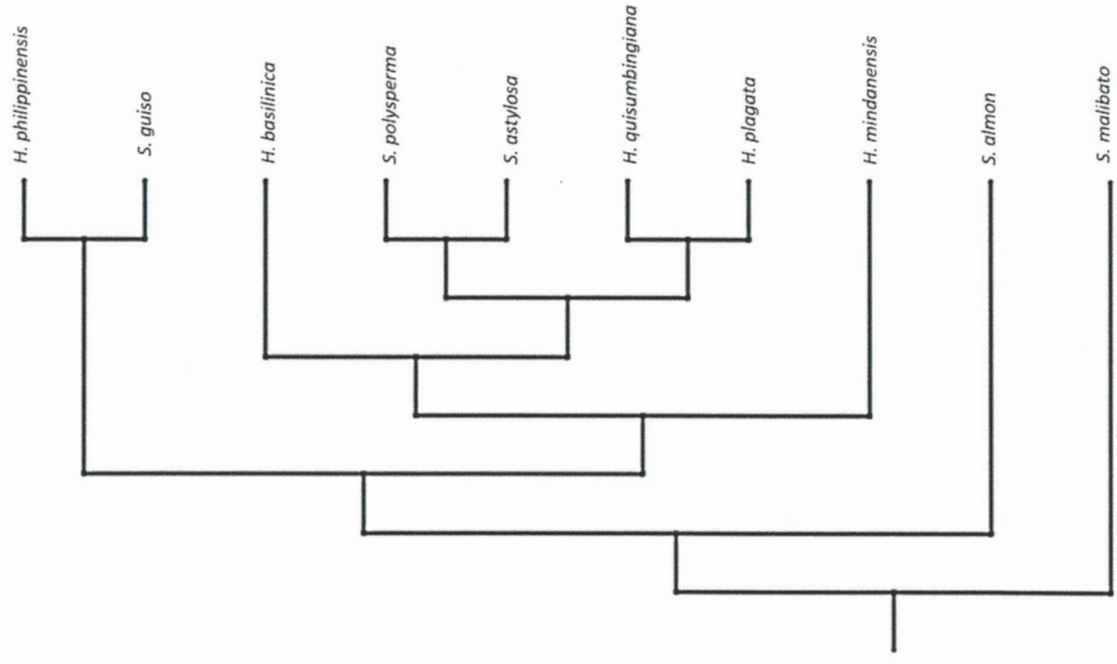



**Figure 4.** Comparative phylogeny generated from qualitative traits of *Hopea* and *Shorea* leaves through Mesquite Analysis.

**Table 2.** Qualitative Characteristics of *Hopea* and *Shorea* Representatives

| Species | LSym | BS* | 2VC | 3VC | **3VCrz** | **4VC*** | **Areo*** | **LRnk** |
|---|---|---|---|---|---|---|---|---|
| *Hopea basilinica* | assymetrical apex and base | ovate | brochidodromous | mixed opposite and alternate | sinuous | dichotomizing | moderate | 2 |
| *Hopea mindanensis* | assymetrical base | ovate | brochidodromous | opposite pecurrent | sinuous | dichotomizing | poor | 2 |
| *Hopea philippinensis* | assymetrical base | elliptic | brochidodromous | alternate pecurrent | sinuous | opposite pecurrent | poor | 3 |
| *Hopea plagata* | assymetrical base | ovate | brochidodromous weak | mixed opposite and alternate | straight | dichotomizing | moderate | 2 |
| *Hopea quisumbingiana* | assymetrical apex | oblong | brochidodromous weak | random reticulate | ramified | dichotomizing | moderate | 2 |
| *Shorea almon* | Symmetrical | oblong | brochidodromous | mixed opposite and alternate | sinuous | opposite pecurrent | poor | 3 |
| *Shorea astylosa* | assymetrical base | elliptic | brochidodromous | mixed opposite and alternate | sinuous | dichotomizing | moderate | 2 |
| *Shorea guiso* | Symmetrical | ovate | brochidodromous | opposite pecurrent | sinuous | opposite pecurrent | poor | 3 |
| *Shorea malibato* | Assymetrical | ovate | brochidodromous | alternate pecurrent | sinuous | dichotomizing | poor | 3 |
| *Shorea polysperma* | Symmetrical | elliptic | brochidodromous | regular polygonal reticulate | sinuous | alternate pecurrent | well-developed | 4 |

** Data for Shorea species were taken from Pulan and Buot, 2014*

## Quantitative Analysis of Taxonomic Characters

Univariate analyses of the morphological traits through descriptive statistics and two-way ANOVA for data transformed are shown in Tables 3a and 3b and Table 4. Data showed that leaf area, apex and base angles width and length were the most variable among the measured leaf characters in all species examined as evident in their respective standard deviations. The least variable were leaf ratio (LR) and left and right widths (LW, RW). Boxplot (Figure 5) illustrates the distribution of data for each of the morphological character evaluated. It can be observed that the distribution between *Hopea* and *Shorea* do not vary greatly. Moreover, quantitative features such as, leaf area, apex and base angle have wider range distribution while the length, width, number of secondary veins and width of left and right leaf portions and leaf ratio presented the least distribution. Univariate test through ANOVA further discloses no significant difference in the evaluated morphological data.

**Table 3a.** Univariate Analysis of Leaf Architectural Features in Selected *Shorea* Species

| Species | Statistics | L | W | LW | RW | LA | LR | L2V | R2V | BA | AA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Shorea almon* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 | 10 |
| | Min | 8.3 | 4.7 | 2.3 | 2.4 | 30.43 | 1.5 | 12 | 13 | 80 | 40 |
| | Max | 11.8 | 7.5 | 3.8 | 3.7 | 58 | 2.3 | 18 | 17 | 110 | 95 |
| | Mean | 9.97 | 5.73 | 2.84 | 2.89 | 38.32 | 1.765 | 15 | 14.9 | 93 | 73 |
| | Std Dev | 1.252 | 0.842 | 0.455 | 0.390 | 9.090 | 0.284 | 1.633 | 1.449 | 9.487 | 14.636 |
| *Shorea astylosa* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Min | 11.5 | 3.7 | 2 | 1.7 | 33.3 | 2.4 | 16 | 13 | 54 | 20 |
| | Max | 16.8 | 5.8 | 2.9 | 2.8 | 63.84 | 3.65 | 19 | 19 | 85 | 46 |
| | Mean | 14.67 | 5 | 2.5 | 2.43 | 49.266 | 2.959 | 16.8 | 15.7 | 63.2 | 29.4 |
| | Std Dev | 1.601 | 0.625 | 0.302 | 0.323 | 10.334 | 0.345 | 1.033 | 1.567 | 10.633 | 7.834 |
| *Shorea guiso* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Min | 12.2 | 4.7 | 2.3 | 2.3 | 39.17 | 2.33 | 13 | 12 | 70 | 41 |
| | Max | 17.2 | 6 | 2.9 | 3 | 68.8 | 2.87 | 15 | 16 | 90 | 49 |
| | Mean | 13.85 | 5.23 | 2.57 | 2.57 | 48.597 | 2.647 | 13.9 | 14.5 | 77.3 | 45.4 |
| | Std Dev | 1.600 | 0.403 | 0.200 | 0.221 | 9.200 | 0.189 | 0.568 | 1.269 | 6.717 | 2.875 |
| *Shorea malibato* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Min | 8.9 | 3.7 | 1.9 | 1.8 | 21.95 | 2.41 | 10 | 10 | 67 | 38 |
| | Max | 13 | 5.1 | 2.7 | 2.4 | 43.86 | 2.8 | 12 | 13 | 85 | 52 |
| | Mean | 11.42 | 4.3 | 2.14 | 2.11 | 33.006 | 2.657 | 11.1 | 11.4 | 75.2 | 43 |
| | Std Dev | 1.200 | 0.422 | 0.237 | 0.202 | 6.465 | 0.136 | 0.738 | 1.075 | 5.903 | 4.190 |
| *Shorea polysperma* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Min | 11.5 | 4.8 | 2.3 | 2.4 | 36.8 | 2.4 | 11 | 10 | 61 | 42 |
| | Max | 19.2 | 6.7 | 3.3 | 3.5 | 81.4 | 3.32 | 16 | 15 | 80 | 70 |
| | Mean | 17.15 | 5.94 | 2.88 | 2.97 | 68.507 | 2.888 | 12.4 | 13 | 67.5 | 52.4 |
| | Std Dev | 2.2392 | 0.6204 | 0.2898 | 0.3498 | 13.7770 | 0.2824 | 1.7127 | 1.4142 | 5.3177 | 9.3119 |

**Table 3b.** Univariate Summary Statistics of Leaf Architectural Features in Selected *Hopea* Species

| Species | | L | W | LW | RW | LA | LR | L2V | R2V | BA | AA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Hopea basilinica* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Min | 7.4 | 3.7 | 1.7 | 1.9 | 18.25 | 1.72 | 8 | 8 | 70 | 45 |
| | Max | 9.6 | 5 | 2.5 | 2.5 | 31.67 | 2.03 | 13 | 13 | 90 | 60 |
| | Mean | 8.61 | 4.49 | 2.22 | 2.23 | 25.961 | 1.923 | 10.6 | 10.2 | 83.5 | 56.1 |
| | Std Dev | 0.789 | 0.458 | 0.262 | 0.189 | 4.745 | 0.097 | 1.838 | 1.751 | 7.091 | 4.508 |
| *Hopea mindanensis* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Min | 8 | 4.1 | 2 | 2 | 21.87 | 1.95 | 13 | 13 | 60 | 35 |
| | Max | 15.8 | 5.6 | 2.8 | 2.8 | 57.87 | 3.27 | 20 | 19 | 85 | 52 |
| | Mean | 13.05 | 4.91 | 2.41 | 2.38 | 43.36 | 2.642 | 16.1 | 16.1 | 71.1 | 42.1 |
| | Std Dev | 2.612 | 0.495 | 0.242 | 0.274 | 12.092 | 0.372 | 2.378 | 1.969 | 8.452 | 5.607 |
| *Hopea philippinensis* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Min | 14.5 | 3.8 | 1.8 | 2 | 36.73 | 2.94 | 17 | 17 | 47 | 34 |
| | Max | 25 | 7.3 | 3.7 | 3.5 | 121.67 | 4 | 21 | 21 | 80 | 57 |
| | Mean | 21.09 | 5.85 | 2.88 | 2.84 | 84.94 | 3.625 | 18.9 | 18.9 | 65.2 | 43.6 |
| | Std Dev | 4.165 | 1.203 | 0.632 | 0.544 | 30.087 | 0.321 | 1.370 | 1.101 | 10.009 | 8.758 |
| *Hopea plagata* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Min | 145 | 47 | 25 | 20 | 36.73 | 2.94 | 17 | 17 | 47 | 34 |
| | Max | 250 | 79 | 42 | 35 | 121.67 | 4 | 21 | 21 | 80 | 57 |
| | Mean | 210.9 | 63 | 31.8 | 28.4 | 84.94 | 3.625 | 18.9 | 18.9 | 65.2 | 43.6 |
| | Std Dev | 13.17 | 10.3 | 5.94 | 5.44 | 30.09 | 0.32 | 1.37 | 1.1 | 10.01 | 8.75 |
| *Hopea quisumbinguina* | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Min | 7.4 | 3.7 | 1.7 | 1.9 | 18.25 | 1.72 | 8 | 8 | 70 | 45 |
| | Max | 9.6 | 5 | 2.5 | 2.5 | 31.67 | 2.03 | 13 | 13 | 90 | 60 |
| | Mean | 8.61 | 4.49 | 2.22 | 2.23 | 25.961 | 1.923 | 10.6 | 10.2 | 83.5 | 56.1 |
| | Std Dev | 0.789 | 0.458 | 0.262 | 0.189 | 4.745 | 0.097 | 1.838 | 1.751 | 7.091 | 4.508 |

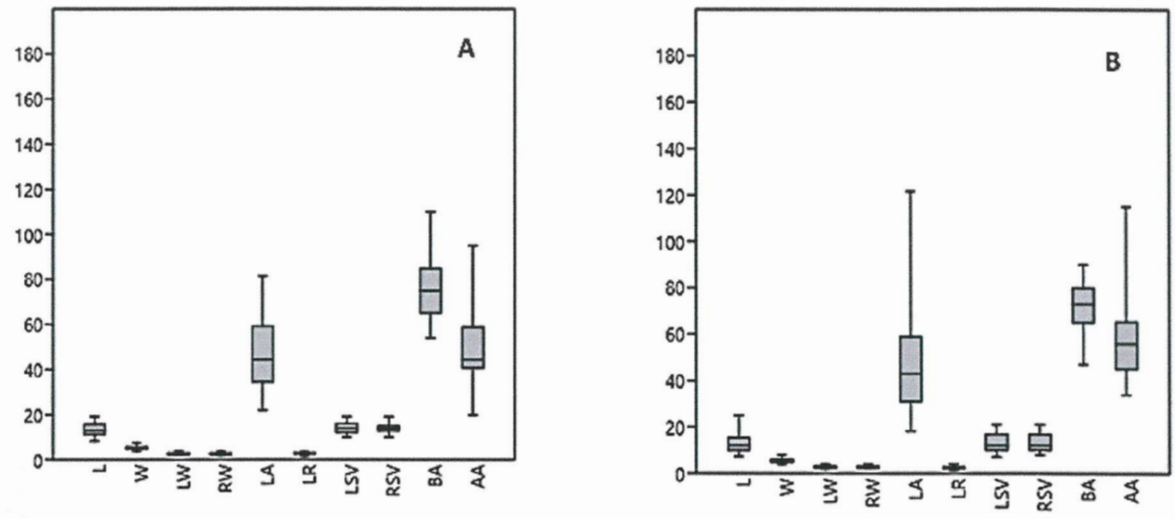



**Figure 5.** Boxplot showing the distribution of quantitative parameters (including outliers) evaluated for (A) *Shorea* and (B) *Hopea* species.

**Table 4.** Two-way ANOVA accounting for the Sources of Variation in Leaf Morphometrics of *Hopea* and Shorea

| | Sum of sqrs | df | Mean square | F | p (same) |
|---|---|---|---|---|---|
| Species: | 575.057 | 9 | 63.8952 | 0.9594 | 0.4796 |
| Characters: | 60820 | 9 | 6757.78 | 101.5 | 2.583E-40 |
| Error: | 5394.64 | 81 | 66.6005 | | |
| Total: | 66789.7 | 99 | | | |

**Table 5.** PCA eingenvalues of each component axis and its accounted variances

| Axis | Eigenvalue | % Variance | Cumulative Variance |
|---|---|---|---|
| 1 | 4.56715 | 45.671 | 45.671 |
| 2 | 3.2849 | 32.849 | 78.520 |
| 3 | 1.04786 | 10.479 | 88.999 |
| 4 | 0.483753 | 4.8375 | 93.837 |
| 5 | 0.425248 | 4.2525 | 98.089 |

Multivariate analysis using ordination through Principal Component Analysis (PCA) showed three component axes accounting for approximately 94% of the cumulative variance, with axis 1 and 2 explaining > 75% variability (Table 5). Based on Kaiser's rule, three (3) components comprised the > 80% variance. Moreover, highest positive values in Axis 1 and 2 were attributed to the scores of *S. polysperma*, *S. astylosa*, *S. almon*, *S. guiso*, *H. philippinensis*, *H. basilinica* and *H. plagata*. This shows the influence of the measurements from these species in accounting for the > 75% variability. PC 1 accounts for length and leaf area while PC 2 shows the influence of base and apex angles. On the other hand, Axis 3 account for the number of secondary veins in left and right portions and base angle.

Loading plots in PCA showed the correlation of components (Figure 6). Positive coefficients presented the traits that had greater influence on the variation among the groups and species. For principal component 1 (PC1) which explains roughly 46% and the highest of the variation, Length (L), Width (W), Left and Right Width (LW, RW), Leaf Ratio (LR), Leaf Area (LA), Left and Right Secondary Vein Orders (LSVO, RSVO)

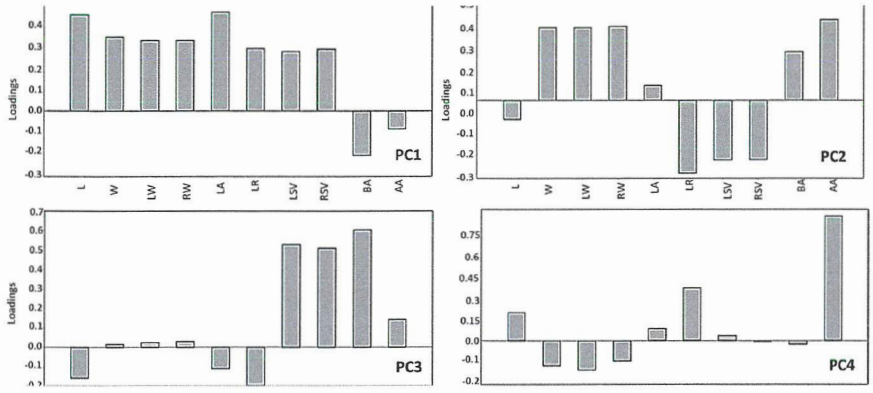



**Figure 6.** Loading plots of the four component axes presenting the factors (leaf characteristics) affecting variability in *Hopea* and *Shorea* groups.

showed positive coefficients. The second component (PC2) displays high positive coefficients for Width (W), Left and Right Width (LW, RW), Base Angle (BA) and Apex Angle (AA) that along with others contribute an additional variation of approximately 33%. The third component, (PC3), presented about 10% additional variation, Width, Left and Right Secondary Vein Orders (LSVO, RSVO) and Base Angle (BA) obtained high positive values, while the fourth component (PC4) showed Width and Leaf Area to account for another 5% of the variation.Scatter plot diagram presents the distribution of variables along the axes, the morphological characters that influence the variation of *Hopea* and *Shorea* (Figures 7 and 8) and the association of each species. In the PC1 and PC2 (Figure 7), Quadrant 1 is occupied largely by *H. basilinica*, *H.*

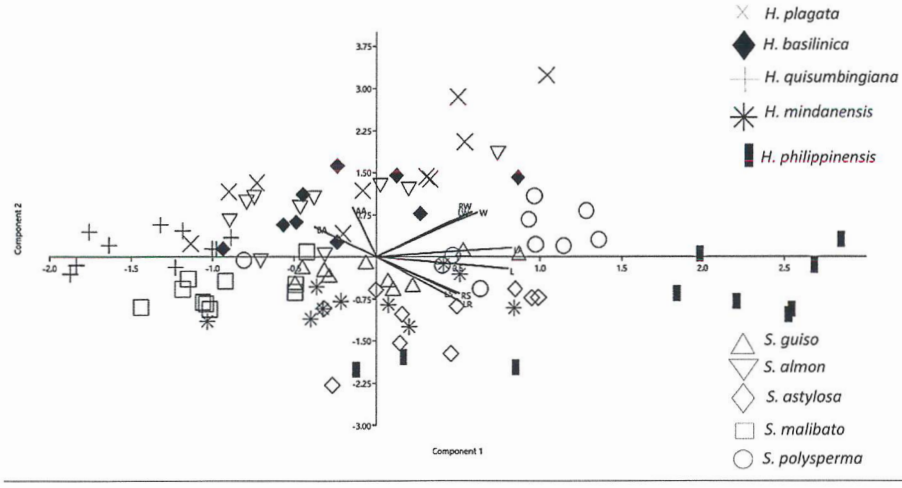



**Figure 7.** Principal Components (PC) 1 and 2 Data ordination of the quantitative morpho-anatomical traits of *Hopea* and *Shorea* species.

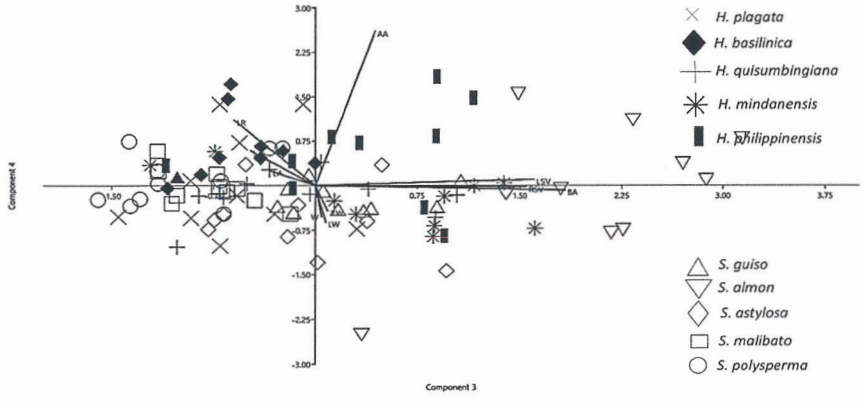



**Figure 8.** Principal Components (PC) 3 and 4 Data ordination of the quantitative morpho-anatomical traits of *Hopea* and *Shorea* species.

*quisumbingiana* and *S. almon* defined by increasing apex and base angles. *H. basilinica* showed the highest apex angles, while *H. plagata* had the highest base angle. *H. quisumbiangana* presented distinct position in the axis, intermediate of *H. basilinica* and *S. almon*. In quadrant 2, it is evident that *S. polysperma* and *H. plagata* localize along width gradient and leaf area with *S. polysperma* having the greatest leaf area and left and right width measurements from the midrib, while *H. plagata* projects highest width. On the other

hand, cluster analysis (Figure 9) determined using Euclidean Similarity Coefficient, showed six clusters at approximately 32 Euclidean distance with cophenetic correlation of about 0.80. Cluster 1 is occupied by *H. philippinensis* characterized by high length and leaf area. The second cluster presents *S. astylosa* marked by high leaf ratio and number of secondary veins. Meanwhile, the next aggregate is the biggest with

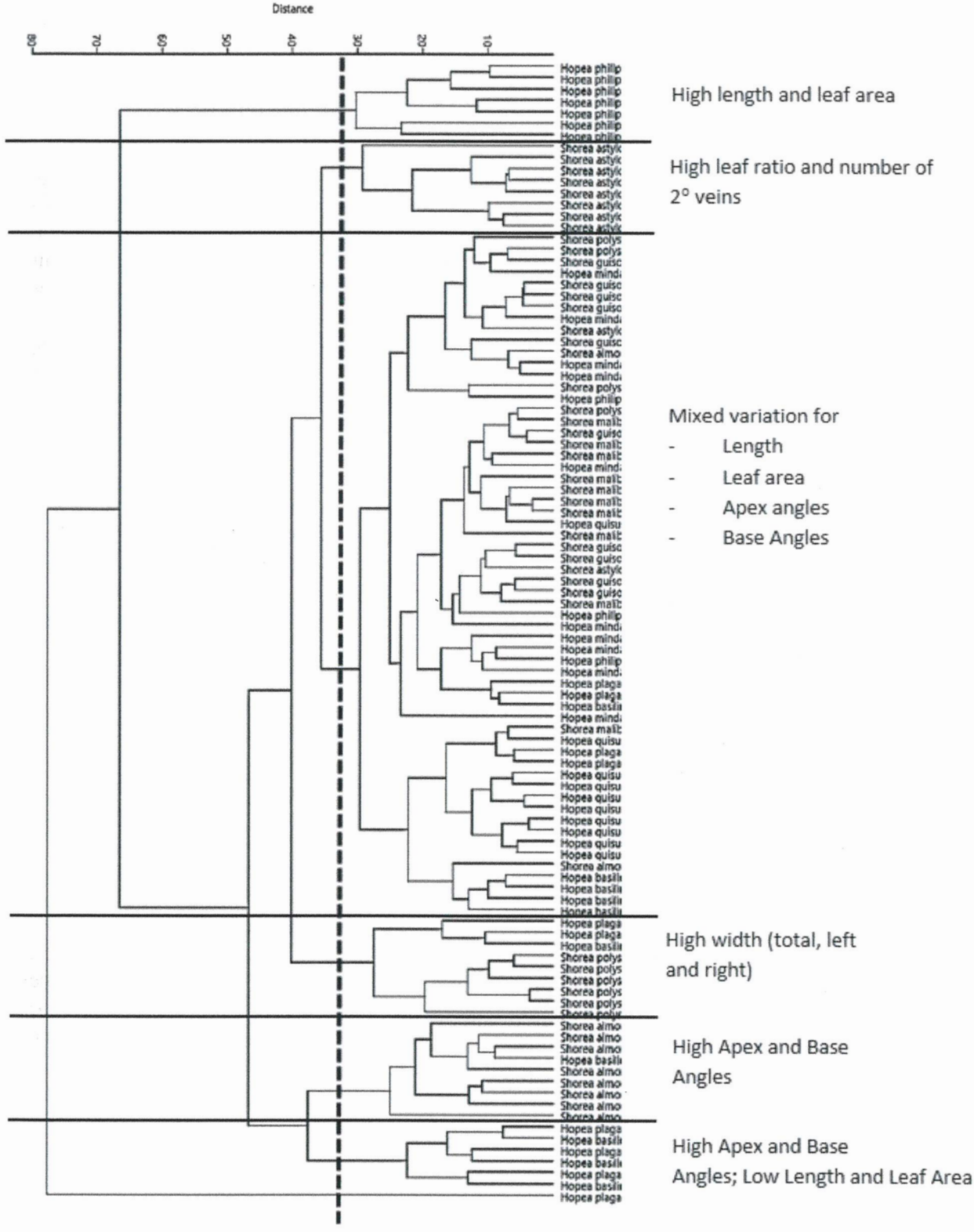



**Figure 9**. Dendogram inferred from Paired Group-UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and Euclidean Similarity Index showing clusters of *Hopea* and *Shorea* and their respective distinguishing leaf architectural traits.

several *Shorea* and *Hopea* species nested. This cluster is defined by variation in length, width, leaf area and angles. For example, *S. polysperma*, *S. guiso* and *H. mindanensis* are characterized by high leaf area, length and width, while the neighboring mix of *S. malibato*, *S. guiso*, *S. astylosa*, *S. polysperma*, *H. philippinensis*, *H. basilinica*, *H. plagata* and *H. mindanensis* cluster around lower width patterns, and leaf area and intermediate length. Another sub-group within this cluster show *H. quisumbingiana* and *H. plagata* in decreasing pattern of length, leaf area and angles. The 4th cluster presents high width and left and right width patterns in *H plagata*, *H. basilinica* and *S. polysperma*. The 5th and 6th clusters form under one bigger cluster showing the association of *S. almon* with *H. plagata* and *H. basilinica*, defined by high apex and base angles, with the latter having least length and leaf area.

## DISCUSSION

Data exploration of the leaf morphometrics of *Hopea* and *Shorea* representatives presented variability and resemblances determined through descriptive, univariate and multivariate analysis. Qualitative descriptions present the role of venation (patterns, vein orders, vein course) and areolation in determining groups and species. For example, the 3rd vein course in *Shorea* is sinuous while *Hopea* shows opposite/alternate or a mixture of both. Meanwhile, areolation in *Shorea* representatives present moderate form except in *S. polysperma* a well-developed areolation is observed (Pulan and Buot, 2014). It can be further gleaned from the data that the 3rd and 4th vein categories, areolation as well as blade class and base shape can be useful qualitative characters in describing the *Hopea* and *Shorea* species since it showed distinctive traits in the species examined. This has also been emphasized in the works of Pulan and Buot (2014) on Shorea, Salvaña and Buot (2014) and Villareal and Buot (2015) on *Hoya*, Kpadehyea and Buot (2014) on Philippine *Mussaenda* and Celadiña *et al.* (2012) on Philippine *Cinnamomum*, among others.

Looking into the qualitative traits evaluated in this study, venation (higher order, > 2°) and areolation would appear to be good markers for delineating groups and species. The contribution of areolation in the leaf morphometrics has been observed in other plant species of *Shorea* (Pulan and Buot, 2014), Hoya (Salvaña and Buot, 2014; Villareal and Buot, 2015), Philippine Mussaenda (Kpadehyea and Buot, 2014) and Philippine Cinnamomum (Celadiña *et al.*, 2012).

The variations as inferred from univariate analysis showed no significant variation in the leaf morphometric data. This was further supported by multivariate analysis where ordination and cluster analysis showed overlapping association of some *Hopea* and *Shorea* in terms of their leaf architectural characteristics. Interestingly, the evaluation of the qualitative data was in agreement for most part with that of the quantitative supporting the distinctiveness of some *Hopea* (*H. philippinensis* and *H. quisumbingiana*) and *Shorea* (*S. polysperma*) and the parallels or close association of the rest. This intermingling within the dendrogram may be indicative of their close association and possible merging.

The results of this investigation appear to be in concord with previous molecular (Kamiya *et al.*, 2005; Yulita *et al.*, 2005) and morphological (Talip, 2008) findings, where close association or overlapping of characters between *Hopea* and *Shorea* was observed. Gamage *et al.* (2006) presented the possibility that these two genera have not yet reached the generic level of divergence though they may have projected some different morphological characters.

Many of the *Hopea* and *Shorea* species in the Philippines are already Critically Endangered, Endangered or Vulnerable. Being classified in this list indicates that there is continuing decline in its population, reduction of area of occupancy due to habitat fragmentation and an observed decrease in number of mature individuals (IUCN Red List, 2016-3). Kamiya *et al.* (2005) considered these threats as forest fragmentation and recolonization caused by climate fluctuations to be influential in producing patterns and levels of intraspecific variation, species differentiation, and interspecific hybridization. This makes the taxonomic identities of member species unstable, and in the long run may challenge systematic classifications. Hence, while the need for further resolution on taxonomic relationships is evident, it is also deemed that a rigorous conservation of these species be pursued.

This study has shown the applicability of leaf architecture in detecting variation among *Hopea* and *Shorea* species. Leaf features of these species showed considerable variation as shown by some *Hopea* and *Shorea* species as well as distinct similarities or overlapping morphological features in others. Our findings support earlier inferences of the association of both groups, one possibly subsumed with the other. In light of this, we further suggest the utilization of more morphological characteristics (*i.e.* vegetative or reproductive parts) and species from both groups to be consolidated along with leaf architecture in order to generate deeper resolution of taxonomic relationship.

## ACKNOWLEDGMENTS

The authors are grateful for the generous support of the Plant Systematics Laboratory, Plant Biology Division of the University of the Philippines Los Baños, Laguna. Due recognition is also expressed for Mr. Dennis Pulan who helped in the collection and identification of *Hopea* species.

## REFERENCES

Appanah, S. and J. Turnbull. 1998. *A Review of Dipterocarps: Taxonomy. Ecology and Silviculture.* Bogor, Indonesia, CIFOR. DOI:10.17528/cifor/000463 pp. 1–4.

Ashton, P.S. 1982. Dipterocarpaceae. *Flora Malesiana* 9: 237–552.

Banaticla, M.C.N. and I.E. BuotJr. 2004. Leaf architecture of ten Philippine *Psychotria* species (Rubiaceae). *The Philippine Scientist* 41: 74–90.

Baroga, J.B. and I.E. Buot, Jr. 2014. Leaf architecture of ten species of Philippine Terminalia Linn. (Combretaceae). *International Research Journal of Biological Sciences* 3(3): 83–88.

Celadiña, D.A., I.E. Buot Jr., D.A. Madulid, T.T. Evangelista and D. Tandang. 2012. Leaf architecture of selected Philippine *Cinnamomum* Schaeff. (Lauraceae) Species. *The Thailand Natural History Museum Journal* 6(2): 89–111.

Daisuke, H., K.Tanaka, K.J. Jawa, N. Ikuo and S. Katsutoshi. 2013. Rehabilitation of degraded tropical rainforest using dipterocarp trees in Sarawak, Malaysia. *International Journal of Forestry Research* 2013: 1 http://dx.doi.org/10.1155/2013/683017

Fuller D. and L. Hickey. 2005. Systematics and leaf architecture of the Gunneraceae. *Bot Rev.* 71(3): 295–353.

Gamage, D.T., M.P. Silva, N. Inomata, T. Yamazaki and A.E. Szmidt. 2006. Comprehensive molecular phylogeny of the sub-family Dipterocarpoideae (Dipterocarpaceae) based on chloroplast DNA sequences. *Genes and Genetic Systems* 81: 1–12.

Jumawan, J.H. and I.E. Buot Jr. 2016. Numerical taxonomic analysis in leaf architectural traits of some *Hoya* R. BR. species (Apocynaceae) from Philippines. *Bangladesh Journal of Plant Taxonomy* 23(2): 199–207.

Kamiya, K., K. Harada, H. Tachida and P.S. Ashton. 2005. Phylogeny of PgiC gene in *Shorea* and its closely related genera (Dipterocarpaceae), the dominant trees in Southeast Asian tropical rain forests. *American Journal of Botany* 92(5): 775–788.

Kpadehyea, J.T. and I.E. Buot Jr. 2014. Leaf architecture of two species and nine infraspecific taxa of the Philippine *Mussaenda* Linn. (Rubiaceae): conservation concerns. *International Research Journal of Biological Sciences* 3(10): 13–21.

Laraño, A.A. and I.E. Buot Jr. 2010. Leaf architecture of selected species of Malvaceaesensu APG and its taxonomic significance. *Philippine Journal of Systematic Biology* 4: 21–54.

Leaf Architecture Working Group. 1999. *Manual of Leaf Architecture – morphological description and categorization of dicotyledonous and net-veined monocotyledonous angiosperms.* Washington, DC. pp. 10—57.

Lipscomb, D., N. Platnick and Q.D. Wheeler, 2003. The intellectual content of taxonomy: a comment on DNA taxonomy. *Trends in Ecology and Evolution* 18(2): 65–66.

Loutfy, M.H.A., E.A.K Karakish, S.F. Khalifa and E.R.A. Mira. 2005. Numerical taxonomic evaluation of leaf architecture of some species of genus Ficus L. *International Journal of Agriculture and Biology* 7(3): 352–357.

Pulan, D.E. and I.E. Buot Jr. 2014. Leaf architecture of Philippine *Shorea* species (Dipterocarpaceae). *International Research Journal of Biological Sciences* 3(5): 19–26.

Rath, P., G. Rajaseger, C.J. Goh, and P. Kumar. 1998. Phylogenetic analysis of dipterocarps using random amplified polymorphic DNA markers. *Annals of Botany* 82(1): 61–65.

Roth-Nebelsick A., D. Uhl, V. Morsbrugger and H. Kerp. 2001. Evolution and function of leaf venation architecture: a review. *Annals of Botany* 87: 553–566.

Salvaña, F.R.P. and I.E. Buot, Jr 2014. Leaf architectural study of *Hoya coriacea*, *Hoya halconensis* and *Hoya buotii* (Apocynaceae). *International Research Journal of Biological Sciences* 3(3): 37–44.

Swaminathan, C., R. Vijendra Rao and S. Shashikala. 2012. Preliminary evaluation of variations in anatomical properties of *Meliadubia* Cav. Wood. *International Research Journal of Biological Sciences* 1(4): 1–6.

Talip, N. 2008. Systematic significance of pollen morphology of *Shorea*, *Hopea*, *Parashorea* and *Neobalanocarpus* (Dipterocarpaceae) in Malaysia. *Sains Malaysiana* 37(2): 169–176.

Villareal, A.M.M. and I.E. Buot Jr. 2015. Leaf architecture of *Hoya incrassate* Warb. and *Hoya crassicaulis* (Apocynaceae): taxonomic identification and conservation concerns. IAMURE *International Journal of Ecology and Conservation* 15: 203–213.

Yulita, K., R. Bayer and J. West. 2005. Molecular phylogenetic study of *Hopea* and *Shorea* (Dipterocarpaceae): Evidence from trnL-trnF and

internal transcribed spacer regions. *Plant Species Biology* 20: 167–182.

Received: 10 September 2019
Accepted: 13 April 2020
Published: 31 December 2020