

Comparative analysis of the leaf architectural characters of *Saurauia bontocensis* Merr. and *Saurauia polysperma* (Blanco) Merr. with the application of Digital Image Analysis Software

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Abstract. Daipan BPO, Buot IE Jr., Daipan HMO. 2022. Comparative analysis of the leaf architectural characters of *Saurauia bontocensis* Merr. and *Saurauia polysperma* (Blanco) Merr. with the application of Digital Image Analysis Software. *Biodiversitas* 23: 3869-3875. Major confusion still exists among several authors concerning the taxonomy of the dagwey plant. This species is economically significant to the Ikalahan tribe of Luzon, Philippines. Several papers claimed that the plant is *Saurauia bontocensis*, while others stated that it is *Saurauia subglabra*. Many botanical websites claimed that *S. subglabra* is synonymous with *S. polysperma*, while another article distinguished *S. subglabra* from *S. polysperma*. Thus, to help resolve the taxonomic ambiguities, this paper performed a comparative analysis of the leaf architectural characters of *S. bontocensis* and *S. polysperma* with the aid of digital image analysis software and cluster analysis. Results revealed that the blade characters alone, delineate the two species, particularly, in terms of the laminar size, shape, symmetry, leaf L:W ratio, base and apex angle, and base shape. Additionally, the leaf venation characters delineate the two species, specifically, on the 2° to 5° vein categories, 2° vein angle, 3° vein course, 3° vein angle to 1°, areolation, freely ending ultimate veins, marginal ultimate venation, and leaf rank. Generally, *S. bontocensis* have larger, longer, and wider leaves compared with the *S. polysperma*. The leaf architecture analysis performed in this paper produced a very promising result in differentiating the two *Saurauia* species and could be used to address some of the taxonomic confusion of these species. The study indicated that *S. bontocensis* and *S. polysperma* are two distinct species. Moreover, the use of digital image analysis software was very helpful in the computations of the various blade characters, particularly the digital herbarium exsiccate in the absence of actual leaf samples.

Keywords: Digital Image Analysis, leaf architecture, plant taxonomy, *Saurauia bontocensis*, *Saurauia polysperma*

Abbreviations: CDFP: Co's Digital Flora of the Philippines, IPNI: International Plant Name Index, LAWG: Leaf Architecture Working Group, POWO: Plants of the World Online, FEV: Freely Ending Ultimate Vein

INTRODUCTION

The genus *Saurauia* Willd., which belongs to the family Actinidiaceae, has around 56 known species widely distributed in the Philippines (Pelser et al. 2011). In 2021, a new *Saurauia* species, *Saurauia abbreviata* K.R.Mazo, was discovered in Zamboanga Peninsula, Mindanao Island (Mazo et al. 2021). This clearly indicates that several *Saurauia* species have yet to be discovered in the country. On a negative note, the extent of morphological variation within species has largely remained unknown, restricting progress in *Saurauia* taxonomy. This has made species description challenging and recognizing undescribed taxa difficult particularly in the Malesian Region, where a large diversity of this taxon is still unknown (Conn and Damas 2013; Mazo et al. 2021).

According to the various published papers, two of the most confusing *Saurauia* species are *Saurauia bontocensis* Merr. and *Saurauia polysperma* (Blanco) Merr. These two species are endemic to the Philippines, and mostly found on the island of Luzon. According to the national list of

threatened Philippine plants, *S. bontocensis* is a vulnerable species (DAO 2017-11), which could be due to a high rate of deforestation in its distribution range, the Cordillera Region (Daipan and Franco 2022). It is most commonly found in the provinces of Abra, Benguet, Mountain Province, Kalinga, and Ifugao (Pelser et al. 2011; Malabrigo 2013; Lumbres et al. 2014; Subilla and Baoanan 2021). *Saurauia polysperma*, on the other hand, is mostly found in the provinces of Ilocos Norte, Benguet, La Union, Nueva Viscaya, Pampanga, and Bataan (Pelser et al. 2011). *Saurauia polysperma* is synonymous to *Saurauia subglabra* Merr. and *Gordonia polysperma* Blanco (Pelser et al. 2011; Govaerts et al. 2021; Kew Royal Botanic Gardens Plant of the World Online (POWO)).

Malabrigo (2013), Taguiling (2013) and Lumbres et al. (2014), mentioned the occurrence of *S. bontocensis* in their floristic assessment studies and they were all referring to the deguia / dagwey tree, the commonly used name of the species. This was also observed in the papers of Subilla and Baoanan (2021) and Tacloy et al. (2022) wherein they referred to deguia as the common name of *S. bontocensis*. Other local names for this species include dogdoguay in

Abra; deguay in Bontoc (Merrill 1914); dagway, deguia, dogwe in Ifugao (Balangcod and Balangcod 2009; Taguiling 2013); deguai in Benguet (Lumbres et al. 2014; Tacloy et al. 2022); dagwey (in Ikalahan) Nueva Viscaya (Rice 2002); and dagwey in Kalinga (Malabrigo 2013). However, in the paper of Fabelico (2020) on the phytochemical, antioxidant, cytotoxic, and antimicrobial properties of the endemic dagwey, he used the taxon *S. subglabra*, which is a synonym for *S. polysperma*, to refer to the dagwey tree. The author was referring to the *Saurauia* species' fruit, apparently the *S. subglabra*, which the Ikalahan of Nueva Viscaya uses in their food processing industry. This was supported further by Dolom and Serrano's (2005) article, in which they used the *S. subglabra* to refer to the dagwey fruits used by the Ikalahans in their fruit-processing ventures. Also, Castañeto and Baculanta (1999) and De Luna et al. (2019) both refer to the dagwey plant as the *S. subglabra*. This is contrary to the article published by Subilla and Baoanan (2021), where they used the taxon *S. bontocensis* in reference to the dagwey plant used by the Ikalahan tribe as the most important raw material for the food processing center that was built to enhance their livelihood opportunities. Rice (2002) also used the taxon *S. bontocensis* to refer to the dagwey plant used by the same tribe. Based on these observations, there appears to be a misunderstanding between the *S. bontocensis* and *S. subglabra* (Syn. *S. polysperma*) species when referring to the species used by the Ikalahan tribe, which is economically important and has a high medicinal value. To add to the confusion, Magcale-Macandog et al. (2014) treated the *S. polysperma* and *S. subglabra* as separate species in their floristic composition study in Ifugao. Also, in the International Plant Name Index (IPNI), *S. polysperma* is not indicated as synonym for *S. subglabra*.

To help resolve this taxonomic confusion, a comparative analysis of the leaf architectural characters of *S. bontocensis* and *S. polysperma* with the application of digital image analysis software was carried out. Specifically, this paper compared the shape, size and organization of their leaves including their leaf venation patterns. Several studies in the country have effectively demonstrated the value of using leaf architecture as tools for identifying and classifying plants, as well as in addressing taxonomic confusions (Villareal and Buot 2015; Jumawan and Buot 2016; Torrefiel and Buot 2017; Baltazar and Buot 2019; Paguntalan and Buot 2019; Hernandez et al. 2020; Antonio and Buot 2021; Viacruciz and Buot 2021), and this is among the most valuable morpho-anatomical tools for species delineation (Masungsong et al. 2019).

MATERIALS AND METHODS

Leaf samples

Leaf samples of *S. bontocensis* (A total of ten (10) leaves) were collected from two (2) trees in the forest reservation area at the College of Forestry, Benguet State University (BSU) in La Trinidad, Benguet, Philippines

(Lat: 16°27'22.68"N and Long: 120°35'36.91"E). The samples were only limited to 10 leaves because the authors would like to minimize the effect of leaf removal on the growth and physiological properties of the plant in consideration of its vulnerable status as well as the difficulty of finding the species in the wild. The leaves were photographed using a smartphone and then pressed on a wooden press. In the absence of the actual leaves for the *S. polysperma*, the author utilized the digital photos (ref. DOL146242) from the Co's Digital Flora of the Philippines (CDFP) (Pelser et al. 2011) and the digital herbarium specimen exsiccate No. 2767 by Meyer (1905) from the United State National Museum, Forestry Bureau, Flora of the Philippines, Herbarium of the Bureau of Government Laboratories.

Leaf architectural characters

The description of the shape, size and organization of the leaf, as well as the leaf venation patterns of *S. bontocensis* and *S. polysperma* were based on the *Manual of Leaf Architecture - Morphological Description and Categorization of Dicotyledonous and Net-veined Monocotyledonous Angiosperms* developed by the Leaf Architecture Working Group (LAWG) (1999). The leaf architectural characters included in this paper were the leaf attachment, leaf organization, petiole features, laminar size and shape, laminar symmetry, laminar length/width ratio, base angle, apex angle, base shape, the position of petiolar attachment, apex shape, margin type, and lobation. For the leaf venation patterns, this paper described and/or determined the primary (1°) vein category up to the 5° vein category, agrophic veins, number of basal veins, secondary (2°) vein spacing, 2° vein angle, inter-2° veins, tertiary (3°) vein course, 3° vein angle to 1° vein, 3° vein angle variability, areolation, freely ending ultimate veins (FEVs), highest vein order, marginal ultimate venation, and leaf rank.

Digital image analysis software

This paper used the Digimizer, a digital image analysis software package that allows precise manual measurements as well as automatic object detection with measurements of object characteristics (Schoonjans 2019). The software was used to measure the laminar area, length, width, base, and apex angles (Figure 1). The authors utilized the scale length or ruler attached to the digital leaf samples as a reference to make sure the actual size of the leaf samples from the various digital photos, such as the CDFP data and the digital herbarium specimen exsiccate, are measured. Similarly, the tape measure placed next to the leaves was used as a standard reference to measure the collected leaf samples. Furthermore, in evaluating the venation patterns of the digital leaf samples, the histogram of the image has been stretched, converting it to grayscale and other color spectrums, and sharpening its texture to highlight the vein networks. The trial version of the software can be freely downloaded from the digimizer website.

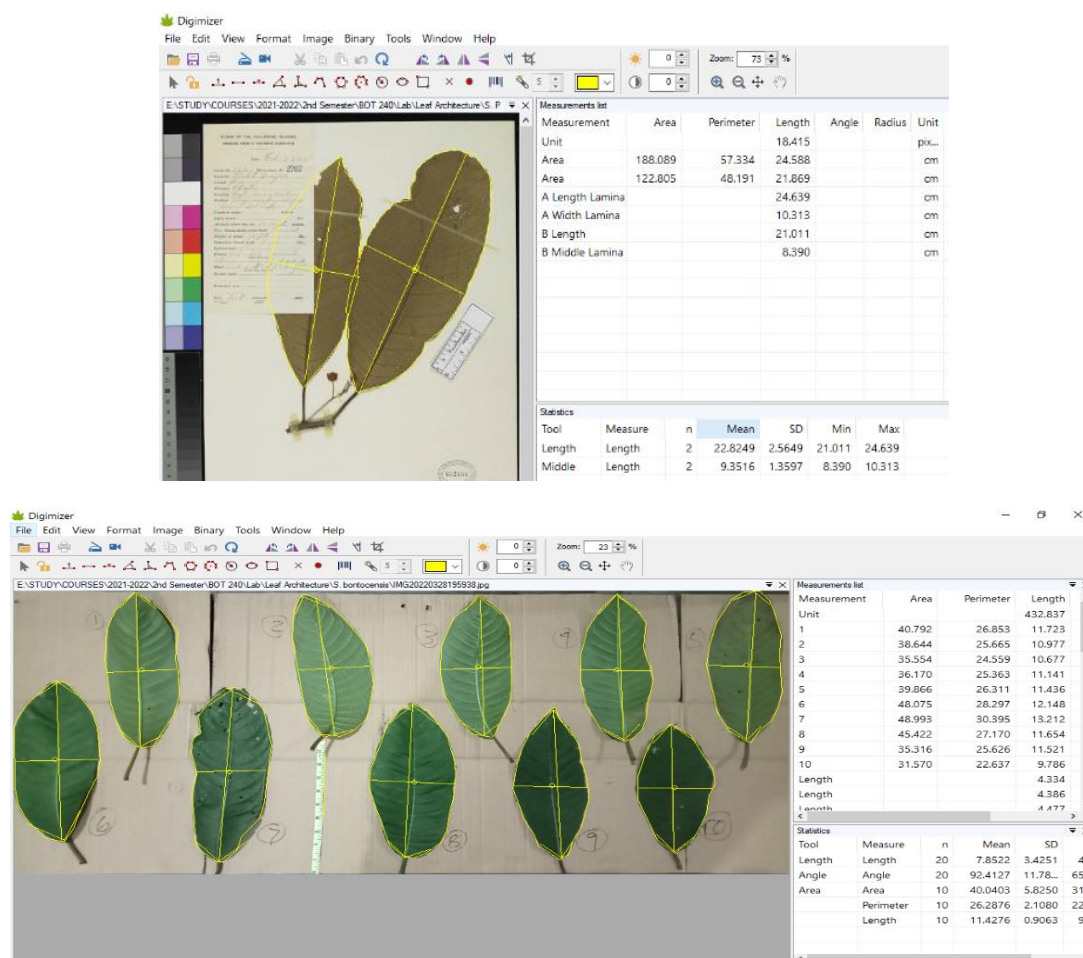


Figure 1. The leaf measurements of (A) *Saurauia polysperma* and (B) *Saurauia bontocensis* leaf samples using the digital image analysis software

Cluster analysis

A cluster analysis was performed to evaluate the differences in leaf area (cm²), leaf length (cm), and leaf width (cm) of *S. bontocensis* and *S. polysperma* species. Specifically, a two-step cluster analysis was performed using the SPSS software. The distance measure used is log-likelihood and the clustering criterion was the Schwarz's Bayesian Criterion. A cluster membership variable was also created in the output. Cluster plots were created using the same software.

RESULTS AND DISCUSSION

Blade characteristics between *S. bontocensis* and *S. polysperma*

Table 1 summarizes the differences and similarities between the two species. Both the *S. bontocensis* and *S. polysperma* had an alternate leaf attachment. Their leaf organization is both simple. The petiole features are both pulvinate. There was a big difference observed in the laminar sizes of these two species. *Saurauia bontocensis* has a macrophyll blade classification, with an average of 25,832 mm² leaf area, while *S. polysperma* has a mesophyll blade classification, with an average of 13,356 mm² leaf area. This suggests that the leaves of *S. bontocensis* is

comparatively larger than that of the *S. polysperma* in terms of their laminar size. The laminar shape of the *S. bontocensis* is oblong, whereas the laminar shape of *S. polysperma* is somewhat elliptical to oblong. Both have symmetrical lamina. The computed laminar ratio average (length:width) for *S. bontocensis* and *S. polysperma* were 2.49:1 and 2.65:1, respectively. The base and apex angle of *S. bontocensis* were classified as obtuse with an average angle of 94.78° and 90.75°, respectively. On the other hand, both the base and apex angle of *S. polysperma* were categorized as acute, with an average angle of 67.75° and 80.39°, respectively. The base of *S. bontocensis* was observed to have a convex or rounded shape, whereas the base of the *S. polysperma* was observed to have a cuneate (straight) shape. Both species have an acuminate apex shape. The position of the petiolar attachment is marginal for both species. Their margin type is both crenate. They are also both unlobed. Lobes are marginal indentations that extend 1/4 or more of the way to the midvein when measured parallel to the lobe's axis of symmetry (LAWG 1999).

Considering the blade characteristics, the two species significantly differ in terms of their laminar size, laminar shape, laminar ratio, base and apex angles, and base shape. *Saurauia bontocensis* has a larger, longer, and wider leaf compared to *S. polysperma*.

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Venation patterns *S. bontocensis* and *S. polysperma*

Based on the extraction and analysis of vein networks using the digital image analysis software (Figure 2), both the two *Saurauia* species have pinnate 1° vein categories. For the 2° vein, *S. bontocensis* was categorized as weak brochidodromous, however some leaves showed a semicraspedodromous characteristics. The 2° vein of *S. polysperma* leaves were also observed to display a weak brochidodromous characteristic or the secondaries joined together in a series of arches. Both the species have simple agrophic veins. Both also have three (3) basal veins. The 2° vein spacing is uniform for the two species. The 2° vein angle for *S. bontocensis* is uniform, whereas it is smoothly increasing toward the base in *S. polysperma*. The two (2) species have both weak intersecondaries. For the 3° vein category, *S. bontocensis* has an opposite percurrent while *S. polysperma* is under the dichotomizing category. Further, the 3° vein course in the former is sinuous while the latter is ramified. The 3° vein angle to 1° was observed to be obtuse in *S. bontocensis* and acute in *S. polysperma*. The 3° vein angle variability is inconsistent for both species. Both the 4° and 5° vein orders in *S. bontocensis* were categorized as regular polygonal reticulate (Figure 3), while *S. polysperma* was categorized as dichotomizing for both vein orders. The areolation in *S. bontocensis* is well developed while *S. polysperma* has a moderately developed areolation. The FEV was not visible in *S. polysperma*. However, in *S. bontocensis*, the FEV has two (2) or more branch as shown in Figure 3. For the marginal ultimate (venation), *S. bontocensis* is looped while *S. polysperma* has incomplete loops. For the leaf rank, *S. bontocensis* was ranked as 4r, while *S. polysperma* was ranked as 2r. Leaf rank is a quantitative representation of the uniformity of the vein system in the leaf, ranging from 1r for the lowest rank or level of organization to 4r for the highest. The rank number is equivalent to the highest order of well-organized veins (LAWG 1999). Table 2 summarizes the differences and similarities of *S. bontocensis* and *S. polysperma* in terms of their leaf venation patterns.

Table 1. General blade characters of the two *Saurauia* species

Characters	<i>Saurauia bontocensis</i>	<i>Saurauia polysperma</i> (Syn. <i>subglabra</i>) (voucher no. 2767, Meyer 1905)
Leaf attachment	Alternate	Alternate
Leaf organization	Simple	Simple
Petiole features	Pulvinate	Pulvinate
Laminar size	Macrophyll	Mesophyll
Laminar area mean (mm ²)	25,832	13,536
Laminar shape	Oblong	Elliptic / oblong
Laminar symmetry	Symmetrical	Symmetrical
Laminar length mean (cm)	29.03	23.04
Laminar width mean (cm)	11.64	8.7
Laminar L:W mean ratio	2.49:1	2.65:1
Base angle	Obtuse (mean 94.78°)	Acute (mean 67.75°)
Apex angle	Obtuse (mean 90.75°)	Acute (mean 80.39°)
Base shape	Convex / rounded	Cuneate (straight)
Position of petiolar attachment	Marginal	Marginal
Apex shape	Acuminate	Acuminate
Margin type	Crenate	Crenate
Lobation	Unlobed	Unlobed

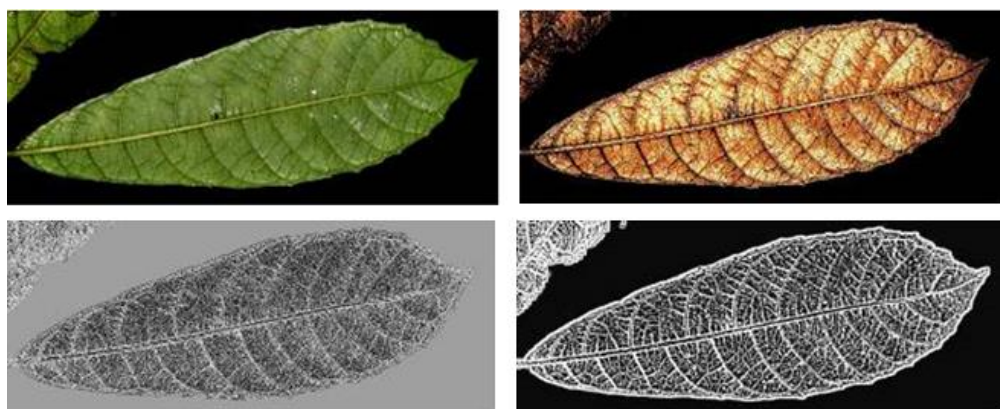


Figure 2. Extraction of the leaf vein network of *Saurauia polysperma* using the digital image analysis software

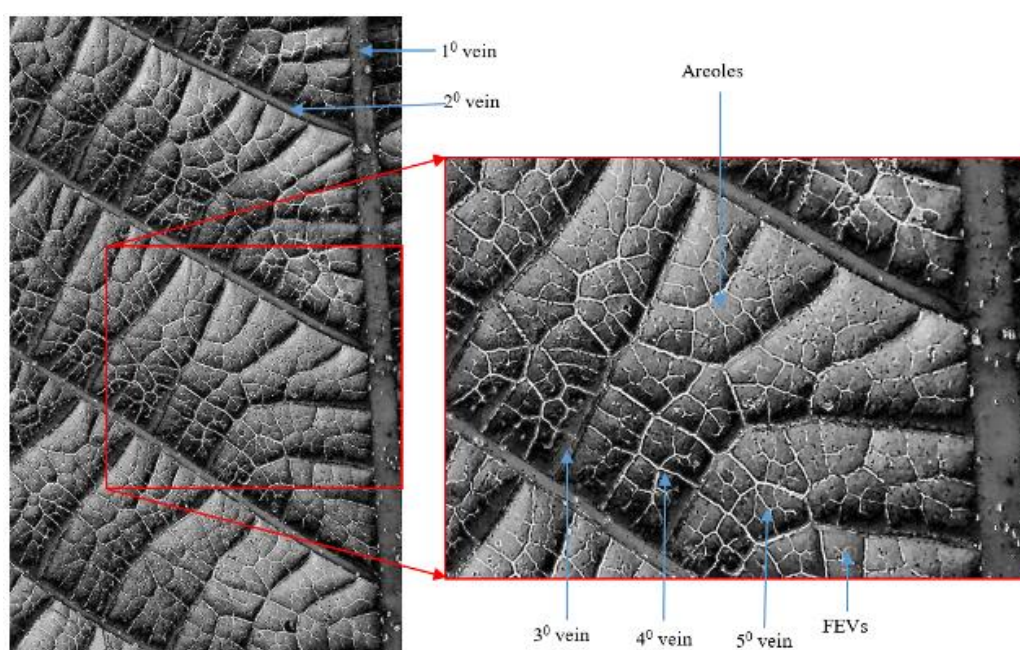


Figure 3. The zoomed-in digital image of *Saurauia bontocensis* shows the different vein orders, areoles, and FEVs. The image was converted to grayscale and highlighted the vein network using the digital image analysis software

Table 2. Leaf venation patterns delineating the two *Saurauia* species

Characters	<i>Saurauia bontocensis</i>	<i>Saurauia polysperma</i>
2° vein category	Weak brochidodromous / semicraspedodromous	Weak brochidodromous
2° vein angle	Uniform	Smoothly increasing toward the base
3° vein category	Opposite percurrent	Dichotomizing
3° vein course	Sinuuous	Ramified
3° (vein) angle to 1°	Obtuse	Acute
4° vein category	Regular polygonal reticulate	Dichotomizing
5° vein category	Regular polygonal reticulate	Dichotomizing
Areolation	Well developed	Moderately developed
F.E.V.S	2 or more branch	Unidentified
Highest order	6	5
Marginal ultimate (venation)	Looped	Incomplete loops
Leaf rank	4r	2r

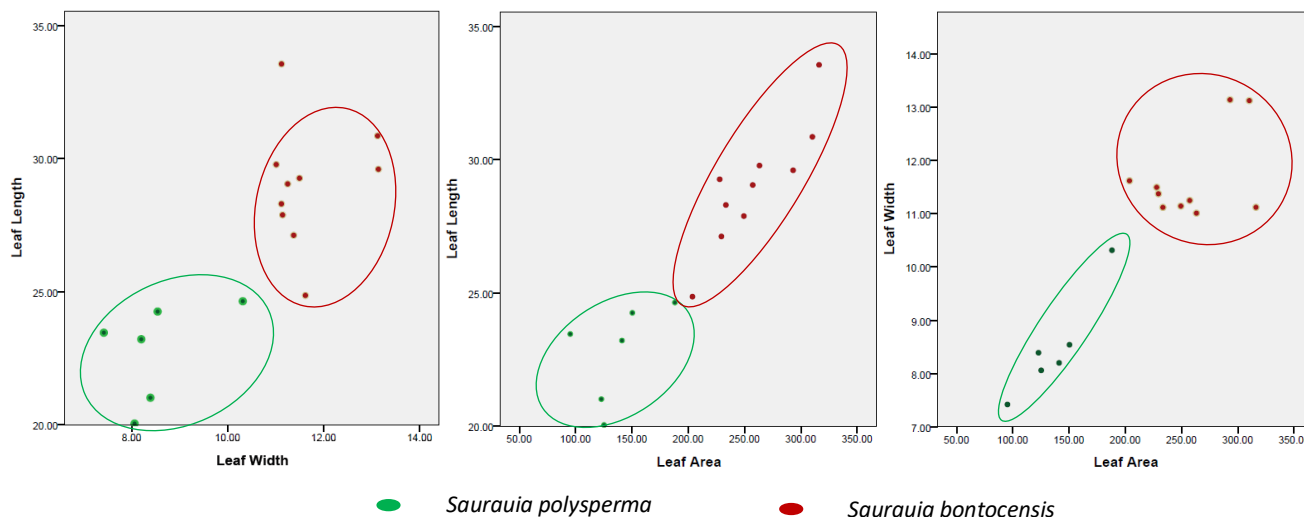


Figure 4. The cluster analysis between the leaf area, length, and width of the *Saurauia bontocensis* and *Saurauia polysperma*

Delineation of the two confusing species

The blade characters delineating the two *Saurauia* species include the laminar size, mean area (mm^2), shape, symmetry, mean length mean (cm), mean width (cm), L:W ratio, base angle, apex angle, and base shape. On the other hand, the leaf venation characters delineating the two species include the 2° vein category, 2° vein angle, 3° vein category, 3° vein course, 3° (vein) angle to 1° , 4° vein category, 5° vein category, areolation, FEVs, highest order, marginal ultimate (venation), and leaf rank. In other similar studies, the delineating characters of two confusing species include the base angle and base shape between *Hoya lacunosa* Blume and *Hoya krohniana* Kloppenb. & Siar, however, there were no significant differences in the venation patterns observed between these two *Hoya* species (Scott and Buot 2022). Also, the delineating leaf characters of the two (2) confusing *Syzygium* species (*Syzygium aqueum* (Burm.f.) Alston and *Syzygium samarangense* (Blume) Merr. & L.M.Perry) observed by Viacruciz and Buot (2021) include the laminar size, apex shape, base shape, areolation, and FEVs. It was also mentioned by Antonio and Buot (2021) that variations can be observed in the number of basal veins and 2° vein angle of divergence of the two infraspecific taxa of *Dioscorea esculenta* (Lour.) Burkill. In the paper of Conda and Buot (2018), they observed that the venation characters delineating the species under the genus *Diplazium* Swartz include the 2° vein angle, 3° vein category, 3° vein angle, 3° vein spacing, and lobation. This only shows the notable application of leaf architecture analysis, particularly the venation patterns and characters, in delineating two confusing species. Overall, the leaf characters that could be used in delineating two or more species based on the result of this paper and other published literature are the laminar size, base shape, base angle, and apex shape for the blade characters, while the 2° vein angle, 3° vein category, 3° vein angle, areolation, FEVs, and lobation for vein characters are seen as the most distinctive features of any two confusing species. The aforementioned papers showed that the leaf architectural characters are very important in

the identification/description of species, resolving some confusion on nomenclature, and confirming the proper taxonomic groupings such as families, sections, among others, of a certain taxon (Buot 2020).

It is evident in the cluster analysis (Figure 4) done in this paper, that *S. bontocensis* had a bigger leaf area (cm^2), longer length (cm) and width (cm) compared with the *S. polysperma* species. The leaf architecture analysis performed in this paper yielded a very promising result in distinguishing the *S. bontocensis* and *S. polysperma* species and could be used to address some of the researchers' identification concerns and confusions in the field, particularly in floral assessment studies.

Digital image analysis in leaf architecture

The use of digital image analysis software to measure the leaf area, laminar length and width, base angles, and apex angles of a digital image, including the digital herbarium specimen, proved to be successful and convenient. Manual measurements may not be as accurate as this method. However, a visible measurement marker on the digital image or herbarium specimen should be present to serve as a point of reference when using the software. The software may provide an incorrect value if these measurement markers are not present. This software is recommended for calculating the aforementioned leaf characteristics in order to support various leaf architectural studies. However, since this software lacks a feature that automatically identifies and classifies vein orders, alternative software or methods for determining and analyzing leaf venation patterns and extracting vein networks may be more appropriate.

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