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Leaf Size Indices and Outline-Based Geomorphometric Analysis of Five Philippine Endemic *Saurauia* Willd. (Actinidiaceae)

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ABSTRACT

Species discrimination among species of *Saurauia* is challenging due to large morphological variation. This study examines the intraspecific variations of the 5 Philippine endemic *Saurauia* species using leaf size indices (LSI) and outline-based geometric morphometrics to facilitate species discrimination. Leaf samples were measured using traditional method, scanned, converted to binary images, subjected to elliptic Fourier Analyses, and quantitatively analyzed using principal component analysis (PCA). The leaf morphology significantly differed among species based on the results of LSI and leaf shape outline analyses. The results showed 7 effective principal components (PCs), which accounted for 94.16% of the total variation. Significant differences were observed in all PCs. Discriminant analysis of the leaf shape outline confirmed the delimitation of species with scores relatively higher than the cut-off value. The tree topology from leaf shape outline, and leaf size indices all exhibited similarity in the clustering at the species level. A key to the species based on leaf morphology is also provided.

1. Introduction

Leaf morphology varies greatly among species and is affected by abiotic factors. In delimiting plant species, understanding leaf variation is extremely important (Wang et al. 2021). Morphological evaluation based on leaves not only gives a specific botanical identity to a species but also reveals interesting features that help understand the range of morphological variation and the responses of plants to varying environmental factors (Ebale et al. 2024; Thakur et al. 2018). The high degree of variation among vegetative characters within trees is evident (Wilde et al. 2023).

Leaf size is one of the most plastic traits of a tree, and variations can be found often among species, genera, individuals within a species, and even within the same individual at different developmental phases or locations on the same tree (Desmond et al. 2021). Despite this very large variation, it is often possible to detect significant associations between a range of environmental variables and leaf size (Ren et al. 2021). In the Philippines, several studies have been conducted using leaf size measurements across different ecosystem types, revealing significant variations that broaden our understanding of the diversity in leaf morphological characteristics among various plant groups and habitats (Aribal et al. 2017; Casilac et al. 2018).

The genus *Saurauia* Willd. is among the most speciose plant taxa, comprising 57 species in the Philippines (Mazo et al. 2021; Olimpos et al. 2024). The striking similarities and minor definable characteristics of the leaf morphology often became the source of dubious and/or erroneous identification of these species. The taxonomic placement of the genus *Saurauia* within the angiosperm has been debated for a long time (Briggs 2011; Cuong et al. 2007; Soejarto 1980). Formerly, this genus was placed under the family Tiliaceae, transferred to Ternstroemiaceae (now Pentaphylacaceae), then to Dilleniaceae, and further elevated to being a family (Saurauiaceae), and later placed under the Actinidiaceae. The latest inclusion of *Saurauia* to Actinidiaceae was mainly due to floral morphology and anatomy (Dickison 1972). Very limited studies on the genus have been published, and significant taxonomic morphological characteristics have not yet been established. Although molecular data facilitate accurate species discrimination, leaf size indices and morphological variations remain crucial for species identification and ecological understanding, particularly for field identification when reproductive structures are absent.

Broadly, the delimitation of *Saurauia* species is challenging and problematic due to the large morphological variation within species (Conn and Damas 2013). Moreover, site factors such as soil, climate, and topography contribute to the variations of these species via phenotypic plasticity, which is of particular concern because failure to account for such influences often leads to taxonomic inaccuracy (Sheth et al. 2020). Using leaf shape outlines via modern geomorphometric analysis is useful in identifying and discriminating species. Here, we utilized outline-based geomorphometric analysis and leaf size indices (LSI) in the 5 endemic *Saurauia* species from the region of Zamboanga Peninsula, southwestern Philippines, namely *S. abbreviata* Mazo, *S. denticulata* C.B. Rob., *S. longipedicellata* Merr., *S. merrillii* Elmer, and *S. zamboangensis* Merr. The present study aims to describe the variations in leaf shape and leaf size indices of the five *Saurauia* species for species discrimination.

2. Materials and Methods

2.1. Location of the Study and Leaf Collection

Leaf samples were collected in the secondary forests of Leon B. Postigo, Zamboanga del Norte (**Fig. 1**). Geographically, it lies between 8°38'4.79" N and 122°56'5.34" E with elevations ranging from 250–750 masl. Thirty fully expanded/mature, healthy, and herbivory-free leaves per species were collected at the first and second branches of the tree to avoid other sources of variation. Specimens were collected from fertile plants for species-level identification. The permission to collect specimens was covered by the Gratuitous Permit No. R-IX-03-2021 was issued by the Department of Environment and Natural Resources (DENR) of Region 9. Voucher specimens were deposited at PNH and CMUH.

2.2. Leaf Size Indices

The leaf length and width were measured manually using a calibrated tape. Leaf area was computed using the formula of Cain and De Castro (1959) as follows:

$$Leaf\ Area = 2/3\ (L \times W) \quad (1)$$

where L is the full length of the leaf, and W is the width of the leaf at its widest portion.

2.3. Leaf Geomorphometric Analyses

Leaf samples were subjected to image scanning using the Canon DCP-T130 scanner (Canon, Japan). The software program SHAPE version 1.3 by Iwata and Ukai (2002) was used for outline-based geometric morphometric analysis. This program is a package with 6 software programs: *ChainCoder*, *ChcViewer*, *PrintComp*, *CHC2NEF*, *NefViewer*, and *PrintPrint* programs. Each scanned image was pooled and arranged horizontally, binarized, and saved as 24 bitmap (BMP) files. The geometry of the leaf shape was obtained by converting leaf images into binary using the *ChainCoder* program. The assigned chain codes were transformed into Elliptic Fourier Coefficients using the first 20 harmonics and were normalized using elliptic Fourier descriptors to reconstruct the leaf outline. The coefficients were imported into the *PrinComp* program to generate effective principal component scores and used for statistical analysis. Finally, the *PrinPrint* program was used to represent graphically the shape variations.

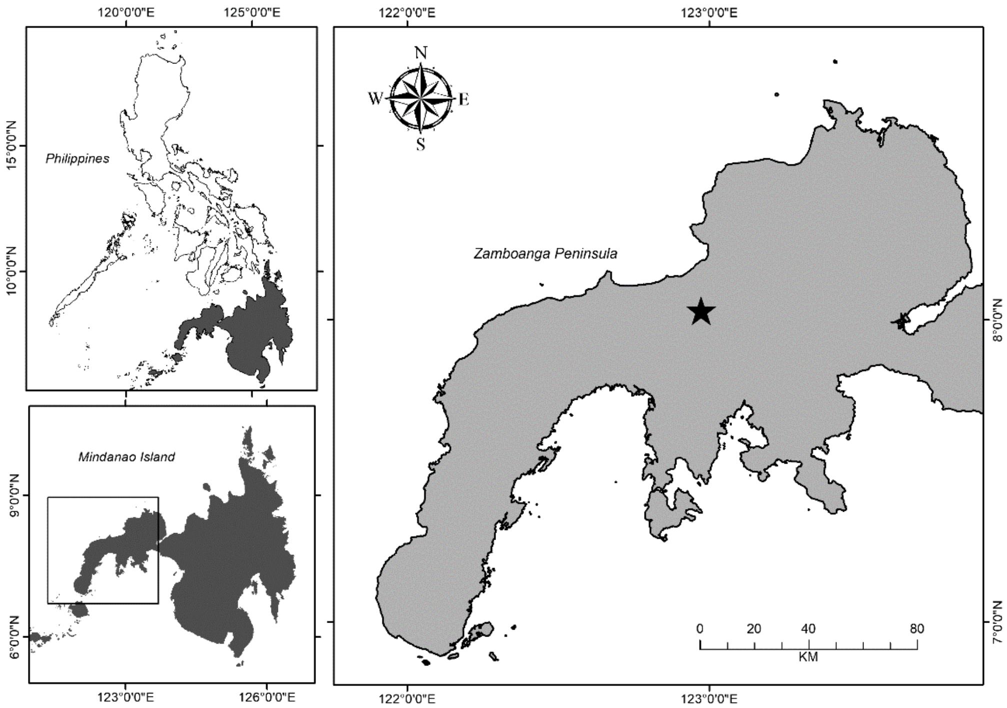

Fig. 1. Map of the Zamboanga Peninsula showing the study site.

2.4. Leaf Geomorphometric Analyses

Data generated from leaf linear measurements were subjected to descriptive statistics. The Principal Component Analysis (PCA) was used for the leaf outline variations. The PCA overviews the linear relationship between objects and variables by summarizing low-dimensional spaces. A discriminant analysis was also used to classify patterns between two species, assuming that all species are linearly separable. Multiple linear discrimination functions representing several hyperplanes in the feature space are created to distinguish the classes (Vaibhaw and Pattnaik 2020). The resulting leaf linear measurements and leaf outline variation scores were used to construct phenograms based on the Euclidean method. All statistical analyses were performed using the Paleontological Statistics (PAST) software version 4.03 (Hammer et al. 2020).

3. Results and Discussion

3.1. Leaf Size Indices

The distribution of leaf size indices from each *Saurauia* species is presented in **Table 1**. A wide distribution of leaf length, width, and leaf area was observed among taxa, resulting in overlapping values from one species to another. Higher values were observed in Saurauia abbreviata and S. denticulata leaf length, while lower values were observed in *S. longipedicellata* and *S. merrillii*. It was observed that *S. abbreviata* had the most leaves with higher leaf width and leaf area, while *S. merrillii* had lower values for the leaf width and leaf area.

Table 1. Summary of quantitative leaf variables of *Saurauia* species

Leaf Characters	Taxa				
	S. abbreviata	***S. denticulata***	***S. longipedicellata***	***S. merrillii***	***S. zamboangensis***
Length (cm)					
Mean ± S.E	26.39 ± 0.56	25.54 ± 0.56	16.22 ± 0.26	17.81 ± 0.83	23.04 ± 0.45
Range	17.50–42.00	22.30–35.60	12.50–25.50	8.30–27.61	14.20–31.80
Std. Dev.	5.27	4.37	2.90	4.54	4.94
Width (cm)					
Mean ± S.E	10.32 ± 0.25	8.32 ± 0.27	6.22 ± 0.27	5.07 ± 0.33	8.1 ± 0.15
Range	6.20–17.10	6.60–13.80	3.90–13.8	2.15–9.68	4.00–10.90
Std. Dev.	2.34	2.11	2.11	1.79	1.63
Area (cm^2)					
Mean ± S.E	186.94 ± 8.26	199.89 ± 8.83	69.44 ± 2.71	64.36 ± 6.97	128.89 ± 4.42
Range	72.33–476.00	98.12–311.88	33.54–178.33	11.87–178.27	41.28–220.48
Std. Dev.	78.39	68.41	29.72	38.18	48.37

Notes: S.E = Standard error; Std. Dev. =Standard deviation.

The Kruskal-Wallis test, a non-parametric form of ANOVA, was performed to determine if leaf length, width, and area differ among *Saurauia* species. The results showed that all variables were highly significant ($p<0.05$) among taxa (**Table 2**).

Table 2. Kruskal-Wallis Test (non-parametric ANOVA) of the leaf linear measurements among *Saurauia* species

Leaf Characters	KW	*p*-value	Remarks
Length	233.1	<0.0001	Highly significant
Width	207.8	<0.0001	Highly significant
Area	230.1	<0.0001	Highly significant

The phenogram based on the leaf size indices shows 2 clusters (**Fig. 2**). Cluster 1 consists of two species, *S. longipedicellata* and *S. merrillii*. On the other hand, cluster 2 includes *S. zamboangensis*, *S. abbreviata*, and *S. denticulata*. The clustering supports the classification of *Saurauia* species with a bootstrap value of 85–100%. The leaf dimensions of *S. longipedicellata* and *S. merrillii* are 12.5–25.5 cm × 3.9–13.8 cm and 8.3–27.6 cm × 2.15–9.7 cm, respectively. The dimensions of the remaining three species range from 12.5–42.0 cm × 4–17.1 cm, which is longer and wider than the former two species.

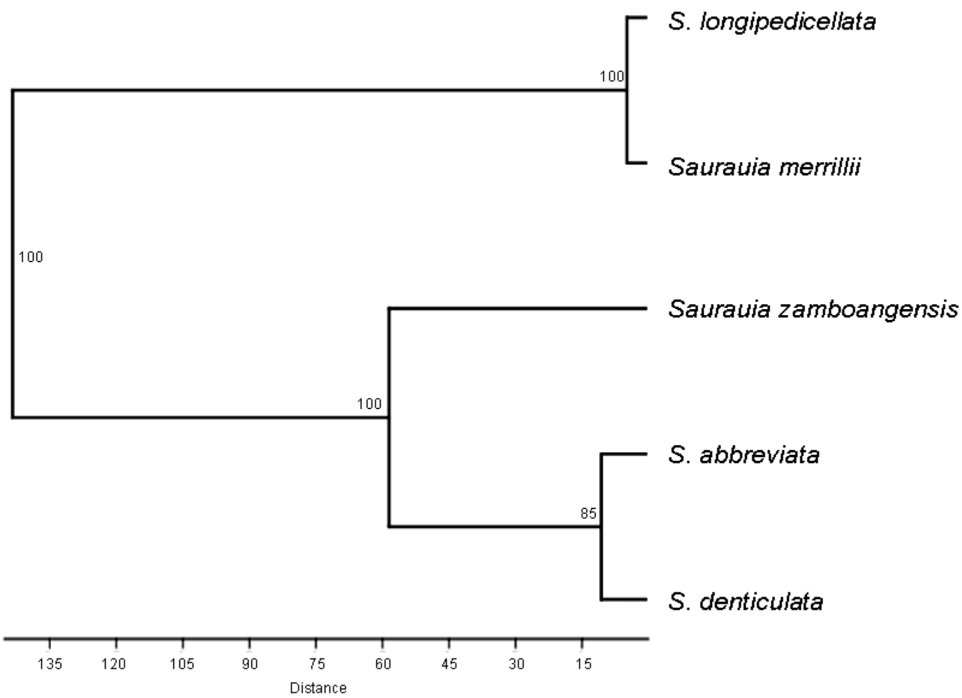

Fig. 2. Relationship of the *Saurauia* species based on LSI.

3.2. Variation in Leaf Morphology

There were 7 effective PCs considered in the analysis based on the results of PCA. The total leaf shape variance accounted for by the 1st 7 PC was 94.16% (**Table 3**). The contribution of each effective PC to the leaf shapes was visualized using the elliptic Fourier descriptors. The reconstructed leaf outlines for the overall shape variation are shown in **Fig. 3**.

Table 3. Significant PCs with corresponding eigenvalues and contribution to the leaf variations among *Saurauia* species

PCs	Eigenvalue (10^{-4})	% Variance	Cumulative Variance (%)
1	9.94	46.51	46.51
2	6.74	31.51	78.02
3	1.07	5.02	83.04
4	0.86	4.04	87.07
5	0.73	3.42	90.49
6	0.47	2.21	92.70
7	0.31	1.45	94.16

The results show that the 1st PC describes the differences in the leaf shape, leaf length-width ratio, leaf apices, bases, and the position of the greatest width among the *Saurauia* species. These changes in the leaf characters among *Saurauia* species constitute 46.51% of the total variance, which can be easily observed in the changes in the leaf symmetry. The 2nd PC comprises 31.51% of total variance, which explains the variation in the proximal and distal portions of the leaf outlines that resulted in elliptic-lanceolate to oblanceolate variations. The 3rd PC refers to the differences in leaf apices contributing to 5.02% of the overall variation. The variation in the basal shape is described by the 4th PC, which is 4.04% of the total variance. The variation on the apex and base angle contributed to 3.42% total variance as reflected by the 5th PC. The 6th PC (2.21%)

explains fine variation in the laminar shape. Subtle variations on the leaf bases and apices are described by the 7th PC (1.45%).

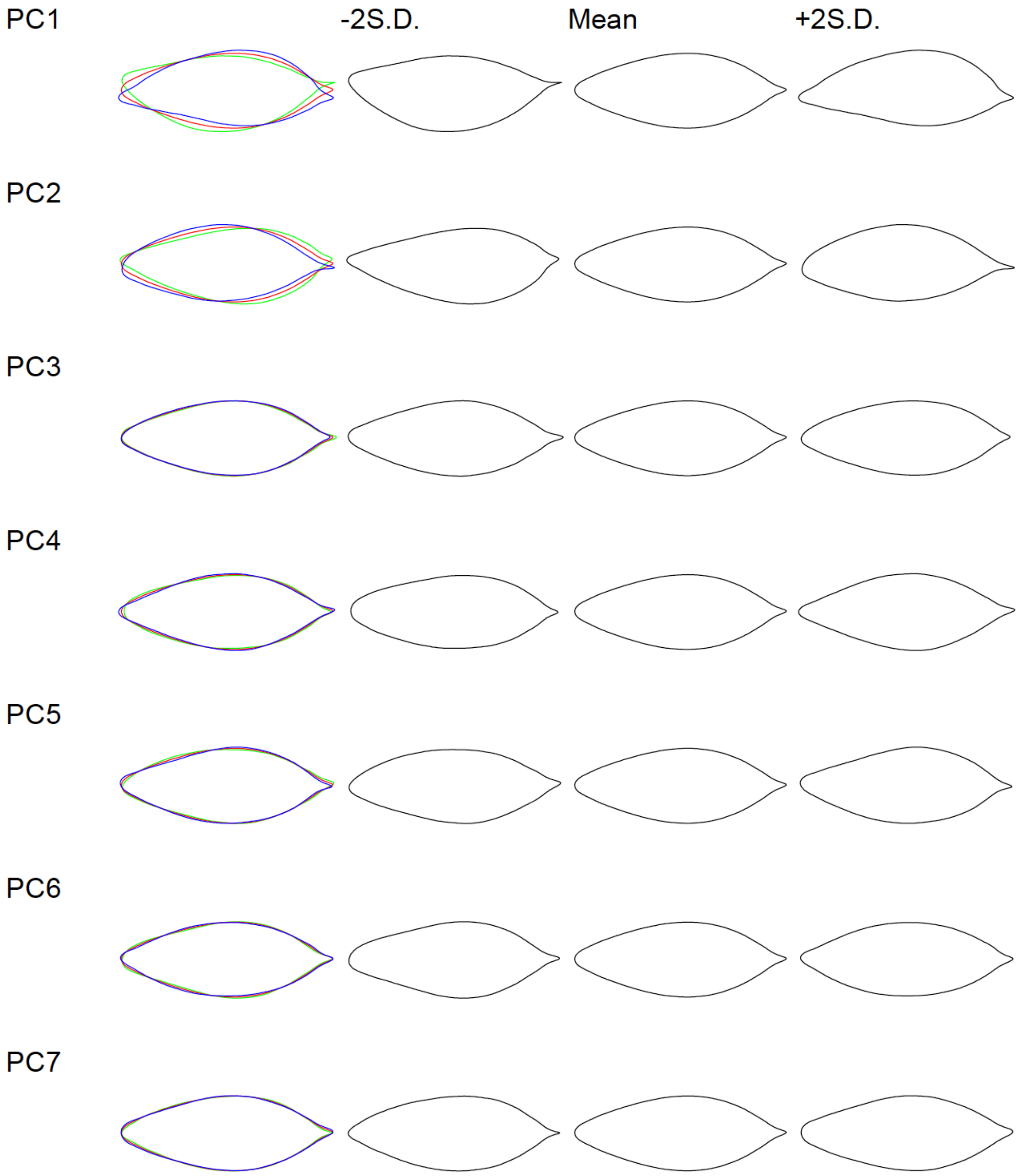

Fig. 3. Leaf shape variations are explained by each PC among *Saurauia* species.

Fig. 4 summarizes the distribution of the first 7 PCs for the overall leaf shape variations among *Saurauia* species. There is a wide range of variations among *Saurauia* species based on the PCs. **Table 4** shows the results of the Kruskal-Wallis test of the first effective PCs. The 7 effective components were highly significant ($p < 0.05$).

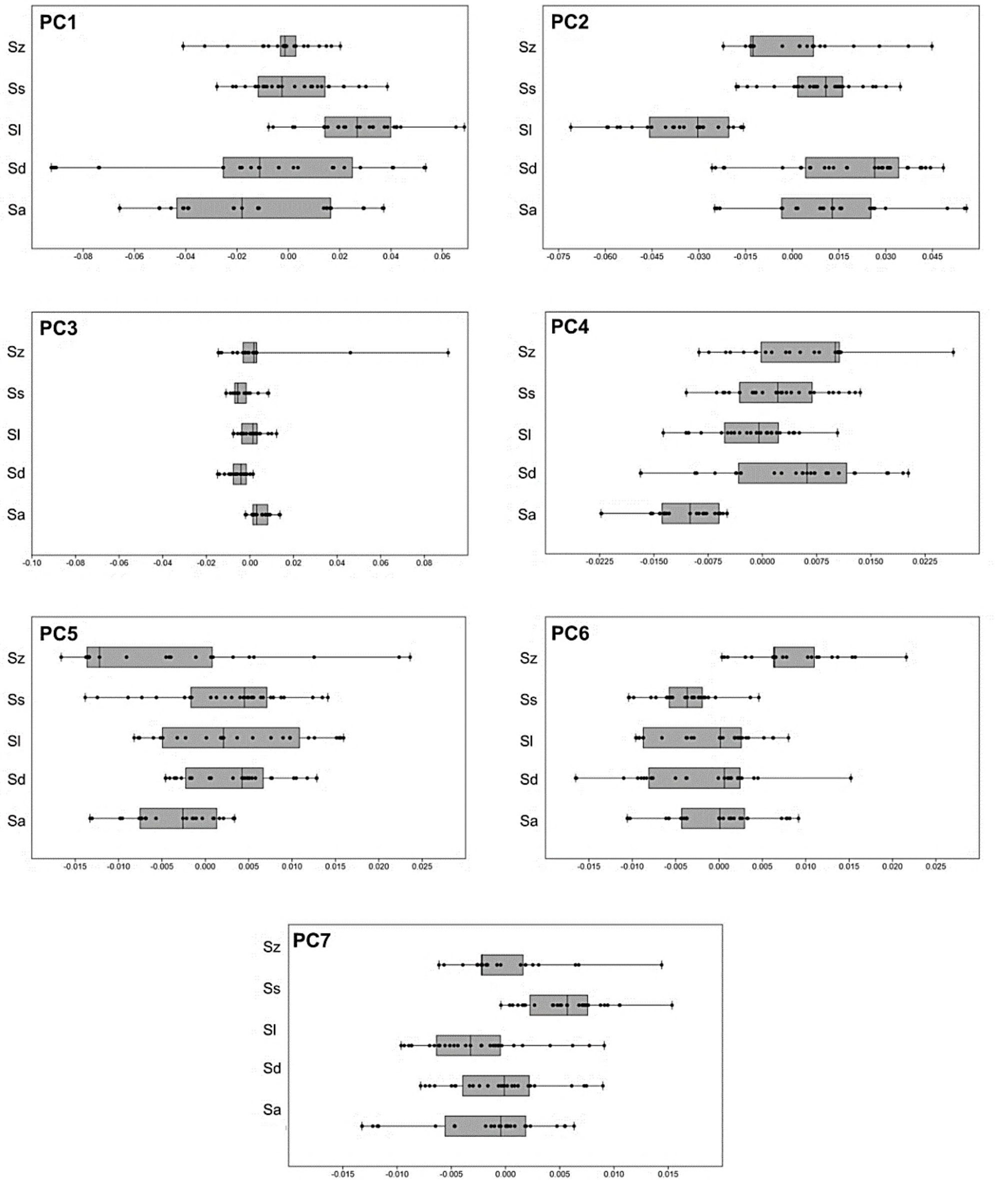

Fig. 1. Summary of the geometric morphometric analysis showing the consensus morphology and the variation in leaf shapes among *Saurauia* species based on the first seven (7) PCs explaining 94.16% of the overall variation. PC1= 46.51%; PC2= 31.51%; PC3= 5.02%; PC4= 4.04%; PC5= 3.42%; PC6= 2.21%; PC7= 1.45%. Legend: Sa= *S. abbreviata*; Sd= *S. denticulata*; Sl= *S. longipedicellata*; Ss= *S. merrillii*; Sz= *S. zamboangensis*.

Table 4. Kruskal-Wallis Test (non-parametric ANOVA) for the significant PC from the leaf outlines of *Saurauia* species

PCs	KW	*p*-value	Remarks
1	33.39	< 0.0001	Highly significant
2	69.81	< 0.0001	Highly significant
3	51.23	< 0.0001	Highly significant
4	63.03	< 0.0001	Highly significant
5	30.59	< 0.0001	Highly significant
6	51.63	< 0.0001	Highly significant
7	44.34	< 0.0001	Highly significant

The ordination plot of PC1 and PC2 revealed that leaf outline variations were distributed in the quadrats of the orthogonal plane (**Fig. 5**), suggesting similarities among the taxa. This variability was displayed by the four *Saurauia* species, which were distributed in four quadrants except for *S. longipedicellata*. Generally, leaves vary significantly in their heritable traits, including shape, size, and asymmetry, due to their environmental interaction (Kozlov and Zverev 2018). Similar results were observed by Jumawan and Bout (2021) on the fluctuating asymmetry of the leaf shape. Detecting variation in leaf asymmetry is extremely important. Thakur et al. (2018) emphasized that morphological evaluation based on leaves not only gives a specific botanical identity to a species but also reveals interesting features vital for understanding the range of morphological variation.

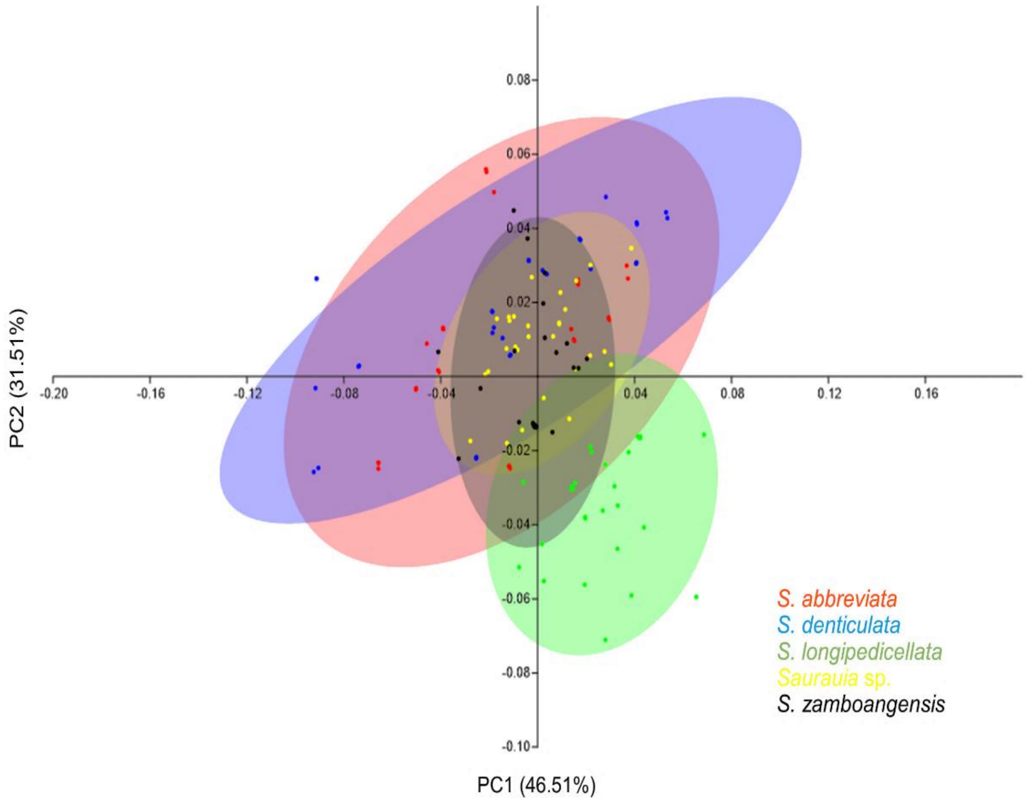

Fig. 5. Ordination plot from PCA of the leaf outline of *Saurauia* species.

Significant differences were observed in all taxa, suggesting that the leaf shape variations distinguish each species. Moreover, the results can be verified by observing the leaf morphology. For example, 96.55% were correctly classified for both *S. abbreviata* and *S. denticulata*. The former has oblanceolate to obovate leaves with sub-oblique, cuneate bases, and cuspidate apices. In contrast, the latter has obovate-elliptic to oblong leaves with subpeltate, subcordate bases and acuminate apex. The discrimination of the leaf shape outline for *S. abbreviata*, *S. longipedicellata*, and *Saurauia merrillii* were 100% classified. The latter two have oblanceolate leaves and acuminate apex, while oblanceolate to obovate and cuspidate in *S. abbreviata*. Moreover, *S. longipedicellata* has a cuneate and sub-oblique base, while subcordate in *S. merrillii*. Similar results were observed in *S. denticulata* and *S. longipedicellata*, *S. merrillii* and *S. longipedicellata*, and *S. merrillii* and *S. zamboangensis* with 100% correct reclassification (**Fig. 6**).

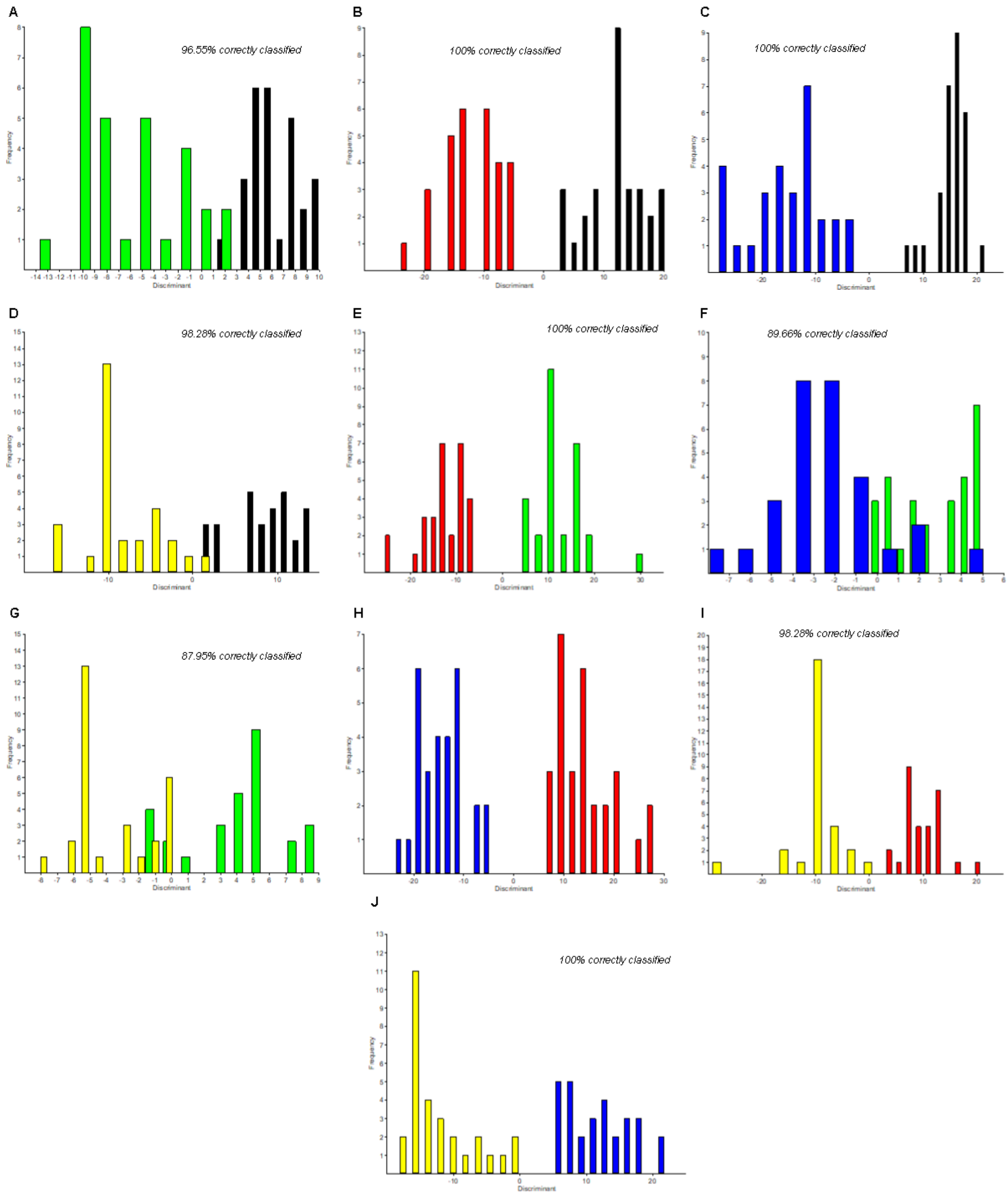

Fig. 2. Discriminant analysis of the *Saurauia* species. The number above in each chart shows the % correctly classified values. Legend: Black bar- *S. abbreviata*; green bar- *S. denticulata*; red bar- *S. longipedicellata*; blue bar- *Saurauia merrillii*; yellow bar- *S. zamboangensis*.

The phenogram of the leaf shape outline supports the separation of *Saurauia* species, forming 2 Clusters (**Fig. 7**). Cluster 1 consists of *S. zamboangensis* and *S. longipedicellata*. In contrast, Cluster 2 includes *S. denticulata*, *S. abbreviata*, and *S. merrillii*. Further results revealed that the small overlap in the reclassification of the taxa is generally attributed to their genetic background. These results were also observed in discrimination between two closely related species (Jumawan and Buot 2021; López-Sampson et al. 2018; Mazo and Aribal 2020).

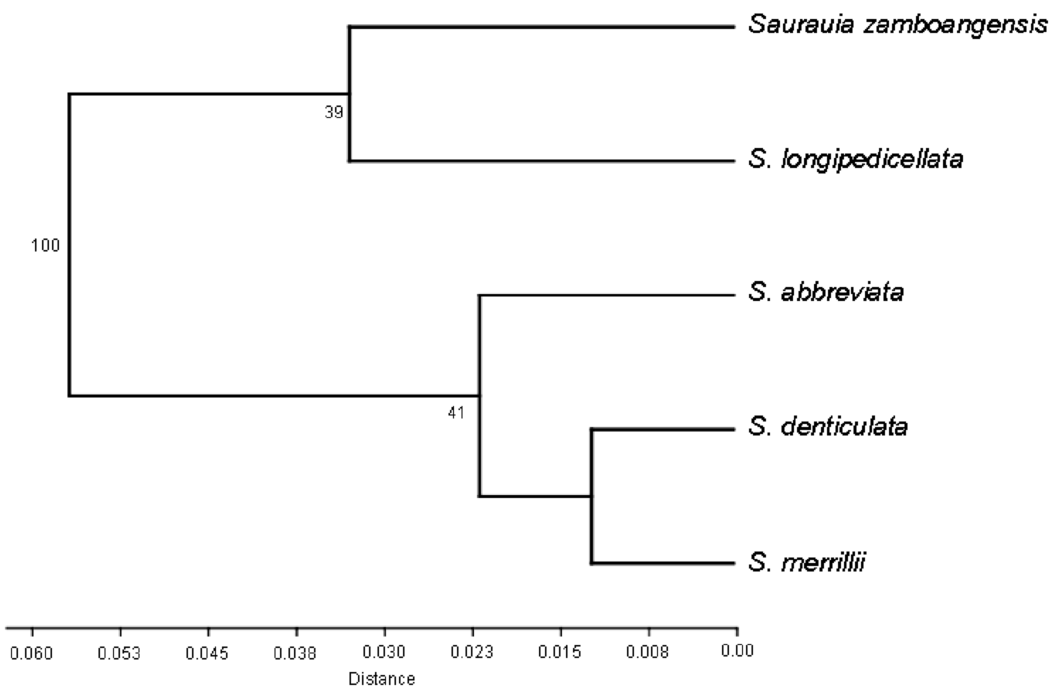

Fig. 7. The relationship of *Saurauia* species based on leaf shape outline contour morphology. Numbers in each node represent the clades' bootstrap values (%) based on 1000 replicates.

Leaf linear measurements, such as length, width, and leaf area, are the classical ways of describing leaves and are also significant in species delineation. Some leaves may look similar to each other but differ in size. For instance, *S. denticulata* and *S. merrillii* have oblanceolate leaf shapes but significantly differ in length. Leaf sizes are greatly varied among taxa. The leaf morphology is influenced by water, light, and gas exchange (Tsukaya 2005). Thus, it is also important to understand the factors affecting leaf size indices. On the other hand, qualitative variables such as leaf base, margin, apex, venation, and leaf indumentum must be considered when differentiating one taxon from another.

The 5 *Saurauia* species collected in Zamboanga del Norte were discriminated up to species level based on leaf morphology, thus supporting the identification of the species. All *Saurauia* species showed diagnostic characters in both leaf shape outline and LSI. For instance, *S. abbreviata* is similar to *S. lanaensis*, a species described by Merrill in 1920 from Lanao del Sur, by having densely setaceous branchlets, abaxially setose leaves with a small, axillary, cymose inflorescence, short setaceous sepals, and short petals (Mazo et al. 2021). However, *S. abbreviata* stands out from longer petioles, a uniformly setose adaxial leaf surface, longer and wider, and more lateral veins. Of the 5 species included in the study, *S. abbreviata* is most similar to *S. denticulata* in leaf shape and size. Notably, *S. abbreviata* differs significantly from *S. denticulata* based on the inflorescence position (axillary vs. cauliflorous). It can be further distinguished from *S. merrillii* in terms of leaf morphology. For example, *S. abbreviata* has leaves that are considerably wider and longer than *S. merrillii*.

A key to the 5 *Saurauia* species in Zamboanga del Norte based on leaf morphology is also provided here.

Key to the 5 *Saurauia* species in Zamboanga del Norte based on leaf characters

1 Lamina oblanceolate to obovate-oblong; base sub-oblique, sub cordate or cuneate …..……..……...…………………2
1 Lamina lanceolate; base acute or cuneate; apex subcaudate or acuminate; 14–32 × 4.0–10.9 cm ……*S. zamboangensis*

2 Leaf apex acuminate; margin denticulate………………………………………………………………..………....3
2 Leaf apex cuspidate; margin entire; lamina oblanceolate-obovate;
base cuneate, suboblique; 17.5–42.0 × 6.2–17.0 cm……………………………………..…..........….........*S. abbreviata*

3 Leaf base cuneate, subcordate; oblique ………………………...…………………………………………………….4
3 Leaf base cuneate, suboblique; lamina oblanceolate, 11.0–25.5 × 4.0–13.8 cm………….……..…....*S. longipedicellata*

4 Leaves 11.0–35.6 × 4.0–13.8 cm; lamina obovate or elliptic-oblong……………………….…………...…..*S. denticulata*
4 Leaves 8.0–27.6 × 2.0–9.7 cm; lamina lanceolate ……………………………..…………………………......*S. merrillii*

4. Conclusions

This study showed a highly significant relationship between leaf length and leaf area among *Saurauia* species. Multivariate analysis revealed the first seven significant principal components, which accounted for a total variance of 94.16%. Their contribution to the leaf shape outline among *Saurauia* species was highly significant. The reclassification accuracy for each species was also above the minimum threshold based on the results of discriminant analysis. Furthermore, this study demonstrated that leaf shapes among *Saurauia* species can be used as diagnostic characters for species discrimination. Compared to leaf size indices, leaf shape is more definitive in differentiating one species from another. This study also confirmed that outline-based geomorphometrics can detect both evident and subtle variations in the leaf shape of *Saurauia* species. However, overlapping of various characters among species was observed; thus, leaf shape can be used for species discrimination, but it is not conclusive in explaining affinities among taxa. Further studies on the leaf morphology of *Saurauia* species could focus on qualitative leaf characters, specifically the type of indumentum and leaf architecture. Information on these traits might be relevant for understanding the evolutionary history of *Saurauia* leaves. Additionally, separate studies on interspecific variations in leaf shape and comparisons across neighboring geographic regions could be of great significance.

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Author Contributions

All authors contributed equally to various aspects of this study, including conceptualization, project administration, methodology, data curation, investigation, formal analysis, validation, supervision, resources, writing – original draft preparation, and writing – review and editing.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation

Not applicable.

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