Describing Leaf Shape Variations of *Caladium* varieties using Landmark-Based Geometric Morphometrics

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Abstract

The interpretation of biological diversity has been greatly enriched by applying geometric morphometrics, an analytical approach that complements traditional morphological studies by providing a quantitative framework for shape analysis. Caladium, renowned for its foliage and patterns, provides a good subject for investigation. An investigation utilizing geometric morphometrics to examine intraspecific variation within Caladium, a species that has been the subject of various experiments. The importance of the intraspecific variation within Caladium opens potential implications for its ecological interactions. The main aim of this study was to describe and quantify the variation in shape within varieties of Caladium. This can help identify and analyze patterns of shape variation. An integrative approach to identify variations of the leaves of Caladium was done using landmark geometric morphometric analysis. The study utilized 540 scanned leaf images, establishing 13 putative homologous landmark points, which were analyzed in the principal component analysis (PCA), canonical variate analysis (CVA), and discriminant analysis (DA). The methods were used to test subtle differences in the leaf shapes of Caladium varieties. Findings showed that geometric morphometrics also differentiated the species significantly based on CVA with significant p values. However, the results of DA exhibited low percentage values. This notes that despite observable differences, there are still common morphological features shared among the varieties indicating the similarities of the leaf shapes of the six identified Caladium varieties. The current findings highlight the applicability of landmark analysis as a possible complement to traditional methods of species identification.

***Keywords**: Caladium*, landmark geometric morphometrics, principal component analysis, Procrustes superimposition

1. Introduction

Geometric morphometrics, a quantitative framework to analyze biological shapes, has improved our understanding of biological diversity (Adams *et al.*, 2004). Based on the mathematical description of form, geometric morphometrics, provides a reliable way to record and compare structures of biological shapes (Zelditch *et al.*, 2012). This method was done to examine the intraspecific variation within *Caladium*, a genus that has captured the attention of hobbyists, botanists, and ecologists. Through geometric morphometrics, the subtle morphological nuances within *Caladium* species are not only determined but also provides understanding of the adaptive significance of its varied forms. Traditionally, morphological studies of *Caladium* bicolor have relied on qualitative traits such as leaf color, shape, and venation patterns (Mayo *et al.*, 1997; Boyce, 2008). However, this study represents a novel approach by integrating quantitative tools, which have not been utilized in previous research.

Shapes encapsulate the outcome of intricate interactions between genetic, developmental, and environmental factors, shaping the phenotypic expression of organisms (Klingenberg, 2011). *Caladium* species are renowned for their vibrant foliage and intriguing patterns; hence, they are good subjects for intraspecific and interspecific variation studies. The importance of unraveling the intraspecific variation within *Caladium* species opens a window into the species' adaptive strategies, response to environmental cues, and potential implications for its ecological interactions. This study aimed to describe and quantify the variation in shape within varieties of *Caladium*. This can help identify and analyze patterns of shape variation.

Geometric morphometrics allows quantitative analysis of form and transforms coordinate data to be subjected to statistical analysis such as principal component analysis (PCA), canonical variate analysis (CVA), and discriminant analysis (DA) collectively, offering a more distinct understanding of morphological diversity. Principal component analysis identifies the primary axes of shape variation, allowing for an objective comparison of morphological traits across different varieties. Canonical variate analysis, on the other hand, maximizes group differences by evaluating shape data and determining the variables that most effectively distinguish varieties. Discriminant analysis then tests the robustness of these group classifications, providing insights into the degree of overlap between morphologically similar varieties.

By using these three tools together, this study fills a research gap, as prior studies on *Caladium* morphology have not applied such a comprehensive geometric morphometric framework. The integration of PCA, CVA, and DA provides a more precise quantification of morphological differences, capturing subtle variations often overlooked in traditional analyses. This approach not only advances the methodology for studying *Caladium* but also sets a foundation for future studies seeking to explore the species' morphological adaptations and phenotypic diversity in greater detail

2. Methodology

2.1 Data Collection

2.1.1 Sampling Sites and Plant Collection

The data and the plant material used in the study were collected in selected locations and households within Malaybalay City, Bukidnon, Philippines (Figure 1). An opportunistic method was employed to account for the maximum possible species. Before collection, permits from the households and locality were obtained. The collection was done from February 2023 to May 2023. Only mature, undamaged leaf specimens were collected from the sampling sites. Samples were placed in plastic bags to prevent dehydration. Specimens were carefully documented and voucher specimens are preserved at the Botanical Gardens and Herbarium Office of Bukidnon State University.

2.1.2 Species Identification

The diagnostic features of the plant samples were cross-checked and verified. Identification of the specimen was done using taxonomic keys from the flora, monographs, and other publications (Madison, 1981; Petersen, 1989; Grayum, 1990; Croat, 1994; Mayo *et al.*, 1997; Govaerts and Frodin, 2002; Boyce, 2008).

2.1.3 Image Acquisition

The adaxial surface images of the leaves were scanned and captured using an image scanner (Epson, L3110, Philippines) with a resolution of 600 dpi x 1,200 dpi. A total of 30 JPEG format images for 30 leaf samples per *Caladium* variety were acquired. A total of 540 leaf images were used in the study.

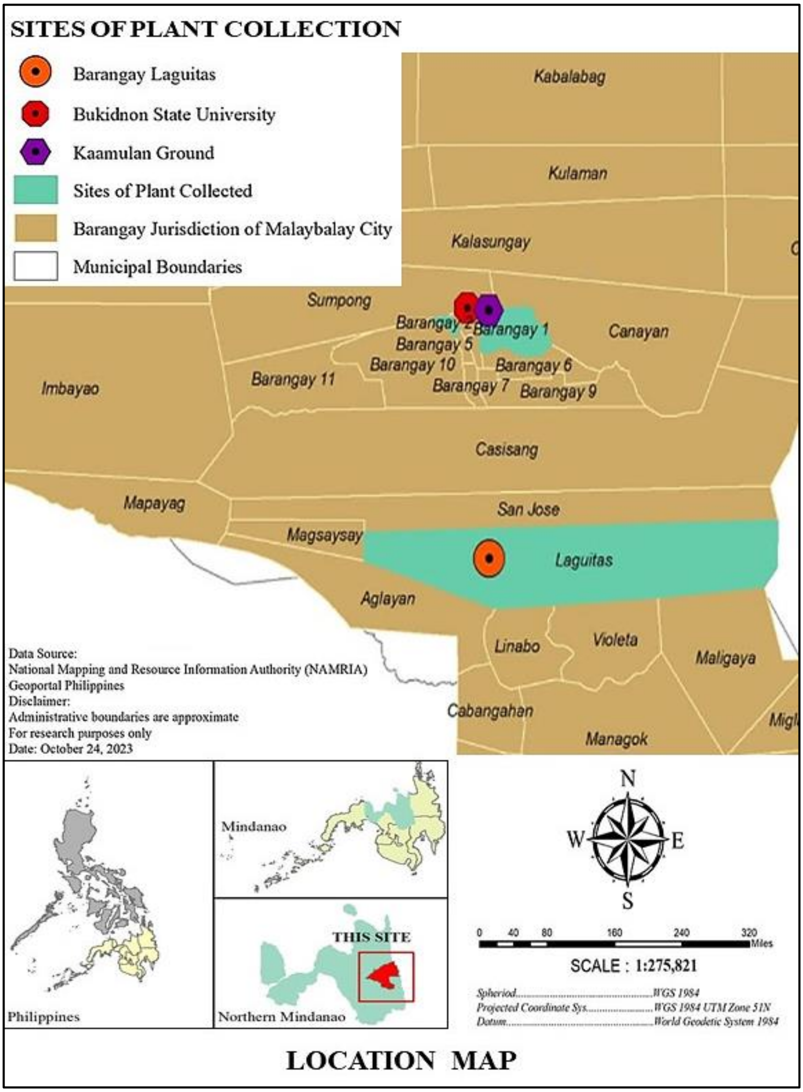


Figure 1. Map of Malaybalay City, Bukidnon and the plant samples collection sites

2.2 Geometric Morphometric Analysis

2.2.1 Landmark Placement

Landmarks were placed on key anatomical features using tpsDig2 software (Rohlf, 2015). Landmark configurations capture the shape variations within each specimen. Thirteen landmark points were selected. The landmarks identified from each leaf sample are shown in Figure 2 and descriptions are tabulated in Table 1.

Table 1. Descriptions of the anatomical landmark points on each sampled *Caladium*

Landmark No.	Description
1	Petiole-Leaf blade midrib junction
2	Midpoint of left and right-hand lobes superior to petiole-leaf blade midrib junction
3, 13	Intersection of the left- and right-hand leaf margin and the first secondary vein of the first basal lobe starting from the petiole
4, 12	Left- and right-hand posterior lobe tips
5, 11	1st pre-eminent secondary vein to the left- and right-hand portions of the leaf blade
6, 10	Intersection of the left- and right-hand leaf margin and the projection of the central secondary vein on the left and right hand of the leaf if there is an odd number of veins on the side; if the number of veins is even, then the landmark point lies intermediate between the projections of the two central secondary veins onto the leaf margin
7, 9	Left- and right-hand curvature of leaf margin from leaf apex
8	Apex of leaf blade

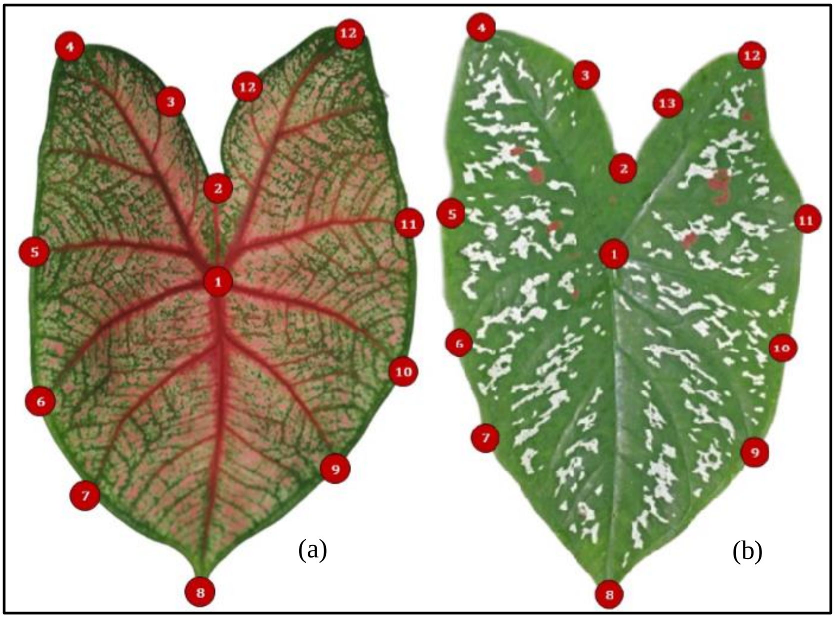
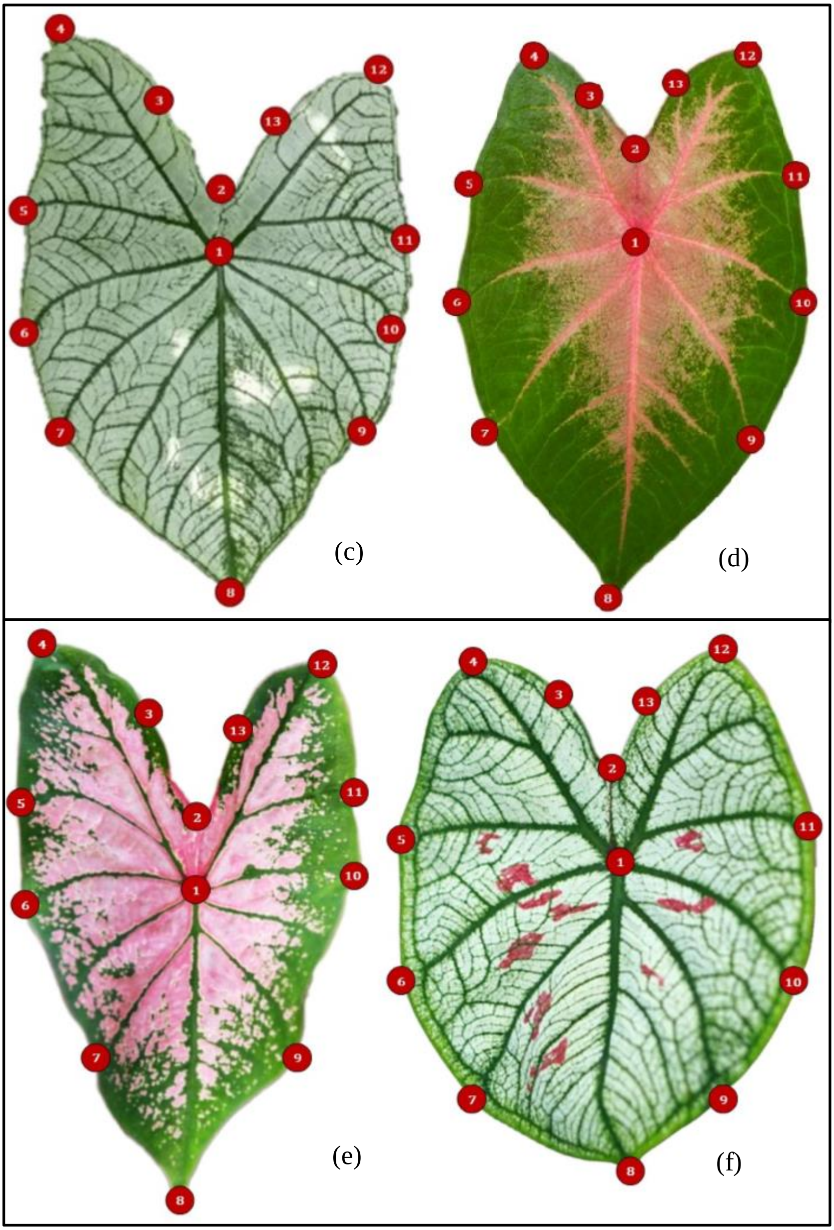


Figure 2. The six (6) varieties of *Caladium* and the selected landmark points: Flatter me (a), Freckles (b), White Christmas (c), Rosebud (d), Celebration (e), and *Candidum* (f)

Figure 2 continued.



2.2.2 Shape Analysis

Geometric morphometric analysis was performed using the Tps software package by Rolhf (2015). From the acquired and digitized images, the software package enabled the processing of the images from landmark point digitization up to data collection and analysis based on the positions of the landmark points of the leaves. The variations observed were derived from landmark points in the adaxial portion of the leaf blade. The software package also enabled the plot of the landmark points along the x and y coordinates and converted it into shape variables. The software performed generalized Procrustes analysis (GPA) which rotates, scales, and superimposes the homologous landmark points from each leaf image (Rolhf, 2015). Relative warps were then generated to describe the shape variations. The scores from relative warps were then subjected to statistical analysis to quantify the major sources of shape differences.

2.2.3 Statistical Analysis

Multivariate statistical tests were conducted to assess the shape differences among groups (Adams and Collyer, 2009). Discriminant function analysis and CVA were conducted to underscore the significance of the differences in the leaf shapes.

3. Results and Discussion

3.1 Consensus Configuration

Figure 3 shows the consensus configuration for each of the *Caladium* leaf samples based on the 13 putative leaf anatomical landmarks. Landmark-based geometric morphometric analysis revealed variations in the six leaf groups as shown in this figure.

Figure 3 exhibits the most visible landmark displacements in the leaf base section (landmarks 7,8, and 9) as well as in the left- and right-hand posterior lobe tips (landmarks 2, 3, 4, and 5). These landmark displacements demonstrated the spatial relationships between the preselected homologous points. These displacements may be recognized as differences in the overall leaf shape indices.

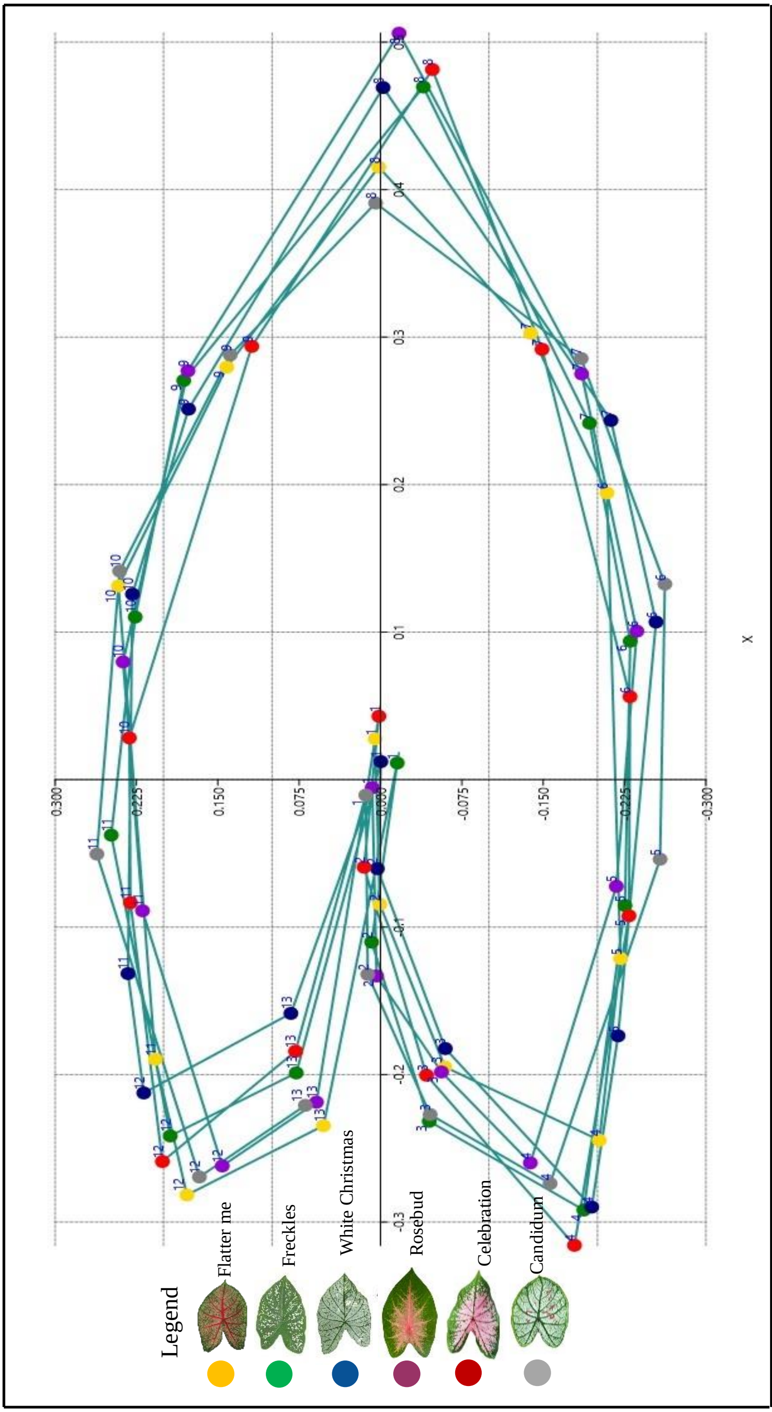


Figure 3. Consensus configuration of the 13 leaf anatomical landmarks of the six *Caladium* leaf groups

The differences are recognized as the descriptions made by Wilfret and Hurner (1982) indicated that *Caladium* varieties and cultivars exhibit a broad variety of differences in the leaves' length, width, and quantity of leaves per plant and from the observations from previous researchers (Madison, 1981; Petersen, 1989; Grayum, 1990; Croat, 1994; Mayo *et al*., 1997; Govaerts and Frodin, 2002; Boyce, 2008) indicating the leaf shape variations among the six varieties of *Caladium* were distinct in form. Freckles displayed broad, ovate leaves marked by evenly spread red spots on a light green background (Boyce, 2008). In contrast, White Christmas and *Candidum* had leaves with more pronounced venation, featuring a heart-shaped base and a pale surface that highlights dark green veins (Mayo *et al*., 1997; Govaerts and Frodin, 2002). The Celebration and Rosebud varieties showcased vibrant hues, with Celebration presenting lobed pink and green leaves, while Rosebud's smaller, rounded foliage exhibits a gentle pink-to-green gradient (Petersen, 1989; Madison, 1981).

3.2 Principal Component Analysis

The GPA results obtained from the Tps software package were subjected to PCA. The study generated a total of 26 principal components. From these components, values with at least 5% variance were considered significant (Lawing and Polly, 2010). This variance provided confidence in establishing differences among the six leaf groups for geometric morphometrics. Figure 4 shows the distribution of datasets of the leaf groups in the space of the first two principal components (PCs).

The PCA's primary goal in this study was to identify the variations in leaf form throughout the entire 540 leaves dataset. Every perpendicular PC symbolizes a unique shape variable. The PC plot shows how the data is projected over the major components. The graph of the first PC presents the separation of the data among the leaf groups as observed in the scatterplot data among different leaf groups along the x-axis. Distinguishing cluster is shown in the confidence ellipses and separation between leaf groups is evident. For the PC2 shape variable, four leaf groups are closely similar in form as they are located in the negative y-axis of the graph which indicates that there are also observable similarities as all of the leaves analyzed have cordate shape forms. Table 2 shows the first 10 eigenvalues of the PCA of pooled leaf shape scores in the six leaf groups. Eigenvalues are the variance of data along each PC axis (Polly, 2004). The eigenvalues show that up to four PCs are needed to express 95% of the total variation.

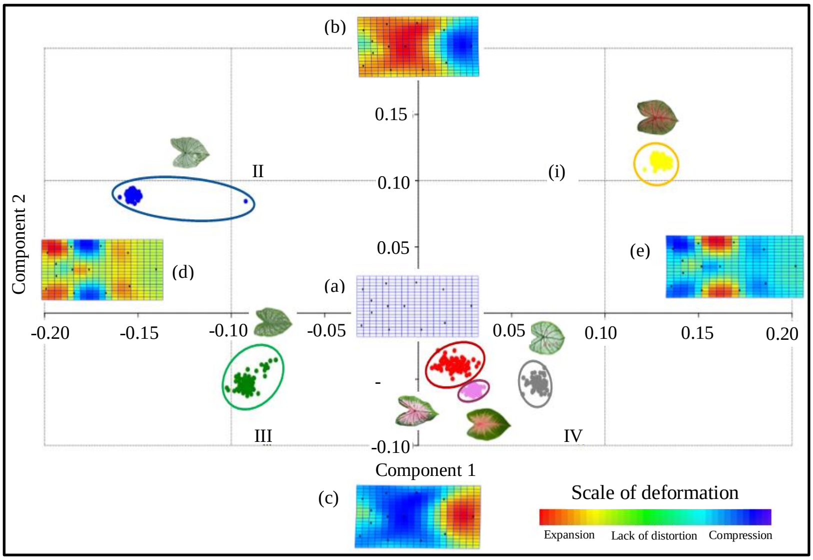


Figure 4. Scatterplots of the pooled data for the six *Caladium* leaf groups in the first and second principal components (PC1, PC2) using PCA of the Procrustes-aligned landmark, showing 95% confidence ellipses and deformation grids as presented by relative warps: computed and generated in PAST ver. 4.12 (Hammer *et al*., 2019): mean shape (a), PC1 maximum + SD (b), PC1 minimum – SD (c), PC2 minimum – SD (d), and PC2 maximum + SD (e).

Table 2. The first 10 eigenvalues from PCA of the pooled data for the leaf shape in the six *Caladium* leaf groups

PC	Eigenvalue	% variance
1	0.00896167	42.35*
2	0.00527739	24.939*
3	0.00426012	20.132*
4	0.00168436	7.9598*
5	0.000684373	3.2342
6	7.27E-05	0.34369
7	5.02E-05	0.23734
8	3.43E-05	0.16231
9	2.78E-05	0.13155
10	2.12E-05	0.10028

**Significant principal components*

The box plot and shape trends (Figure 5) of the first four PCs, represented together 95.38% of the total variance. Morphological descriptions of the variations for the pooled leaf shapes are tabulated in Table 3. These descriptions were based on the significant principal components and the relative warps generated.

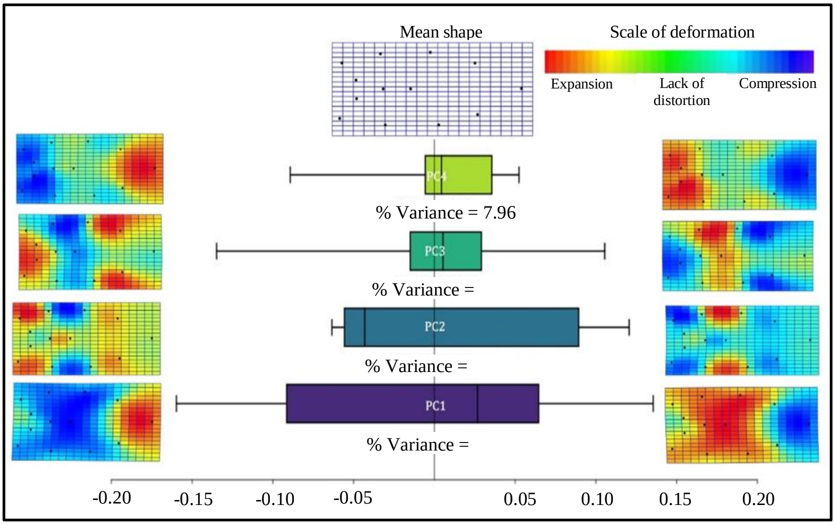


Figure 5. Leaf deformations represented by the first four principal components using PCA of the pooled leaf shape for the six *Caladium* bicolor varieties from relative warps. In the deformation grids, apex of the leaf is to the right. Left-hand figures show the leaf shape corresponding to minimum values of the PC, and the right-hand represent the maximum values; computed and generated in PAST ver. 3.26 (Hammer *et al*., 2019).

Table 3. Descriptive morphological variation of leaf landmarks and percentage variance of the pooled data for the six *Caladium* varieties as explained by significant PCs (relative warps)

PC	% Variance	Maximum score	Minimum score
PC1	42.350	Deformation by compression in the apex of the leaf; expansion in the base to the middle of the leaf with less distortion in the upper half of the leaf and expansion in the leaf apex	Deformation by an expansion in the left- and right-hand posterior lobes up to the midportion of the leaf and compression in the apical portion.
PC2	24.940	Low level of compression in the leaf apex to the midportion of the leaf and the primary vein emanating from the left and right posterior lobes; expansion in the left- and right-hand portions in the margins from base to midportion	Expansion in the leaf apex to the midportion of the leaf and the primary vein emanating from the left and right posterior lobes; compression in the left- and right-hand portions in the margins from base to midportion

Table 3. continued.

PC3	20.130	Compression in the midportion to the leaf apex and base and expansion in the midportion of the leaf	Expansion in the midportion to the leaf apex and base and compression in the midportion of the leaf
PC4	7.960	Lack of distortion in the midportion of the leaf, compression in the leaf apex and expansion in the leaf base	Lack of distortion in the midportion of the leaf, expansion in the leaf apex and compression in the leaf base

3.3 Canonical Variate Analysis

In this study, CVA showed the differences in leaf shapes of the six leaves group mean values. This can be visualized in Figure 6. From the scatter plot of axes one and two, there was an absence of overlap from the ellipses between leaf groups. This suggested leaf shape distinction between the *Caladium* varieties.

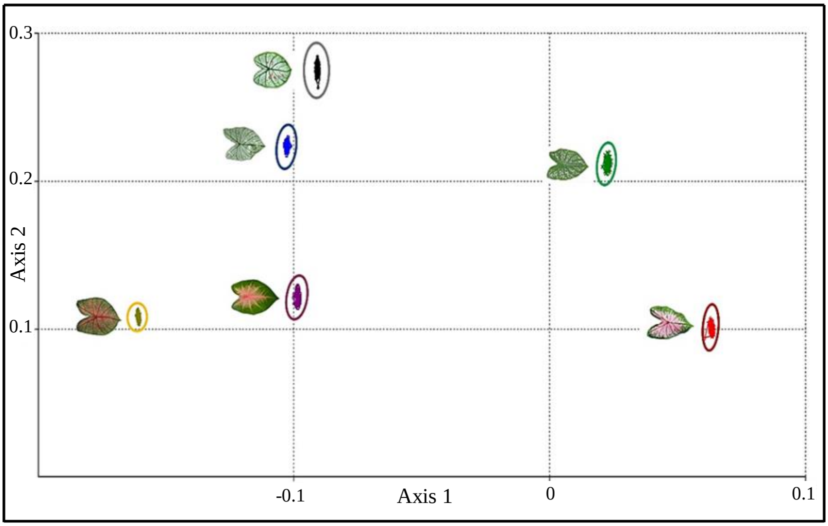


Figure 6. Scatterplots of the pooled data for the six *Caladium* variety leaf groups along the canonical axes (axis 1, axis 2) using CVA from the Procrustes-aligned landmark, showing 95% confidence ellipses; computed and generated in PAST ver. 3.26 (Hammer *et al*., 2019)

Each variety of *Caladium* exhibited distinct characteristics in terms of leaf blade structure and overall form. These traits not only facilitated species identification but also offered valuable clues regarding their ecological adaptations, such as responses to varying light conditions, moisture regulation, and suitability for ornamental use. The morphological diversity observed among the varieties can be linked to adaptive significance, highlighting the complex interaction between phenotype and environment (Madison, 1981; Govaerts and Frodin, 2002; Boyce, 2008). The leaf blade characters, previously described in PCA, consider the morphological differentiation between the leaf groups.

Multivariate analysis of variance (MANOVA) showed a significant difference between the leaf groups (Table 4). Also, the low value of Wilk's lambda signifies discrimination among the leaf groups. The scale ranged from 1 to 0, where 0 means total discrimination and 1 means no discrimination (Todorov and Filzmoser, 2007). CVA axis 1 accounted for a total of 97.59% of the total variation among leaf groups and CVA axis 2 presented 1.159% of the total variation. The plots of the CVA scores indicated a closer similarity for the two varieties (Freckles and Celebration) in the leaf shapes occupying the positive x-axis of the graph. The remaining leaf groups were established in the negative x-axis in the CVA plot (Figure 6).

Table 4. Summary statistics of CVA in landmark-based geometric morphometrics of the pooled data for the six *Caladium* leaf groups

Summary statistics			
Wilks´ lambda:	4.45^{-15}	Pillai trace:	4.982
df1:	130	df1:	130
df2:	2513	df2:	2565
F:	1.58E+04	F:	5381
p:	0.0000^{***}	p:	0.0000^{***}
Eigenvalue 1:	4.991E4	Percent 1:	97.59
Eigenvalue 2:	592.7	Percent 2:	1.159

P-values < 0.05 is *significant; P-values < 0.01 is **significant; P-values < 0.0001 is ***significant.

The positioning of certain leaf groups in the negative x-axis of the CVA plot suggested that these groups shared similar morphological traits that were distinct from those positioned on the positive x-axis. This separation indicated that the traits influencing the negative x-axis were critical in distinguishing these varieties, reflecting specific morphological characteristics that were unique to those leaf groups. The negative positioning may imply that those groups exhibited certain shape features – such as variations in leaf width or lobe prominence – that contributed to their differentiation. This finding provided insights into the adaptive morphological strategies of *Caladium* varieties, as the traits linked to the x-axis may relate to environmental adaptation or genetic factors (Zelditch *et al.*, 2004).

3.4 Discriminant Analysis

The DA in Figure 7 shows the discriminant graph of the scores where the percentage of correctly classified values and the reclassification data are given.

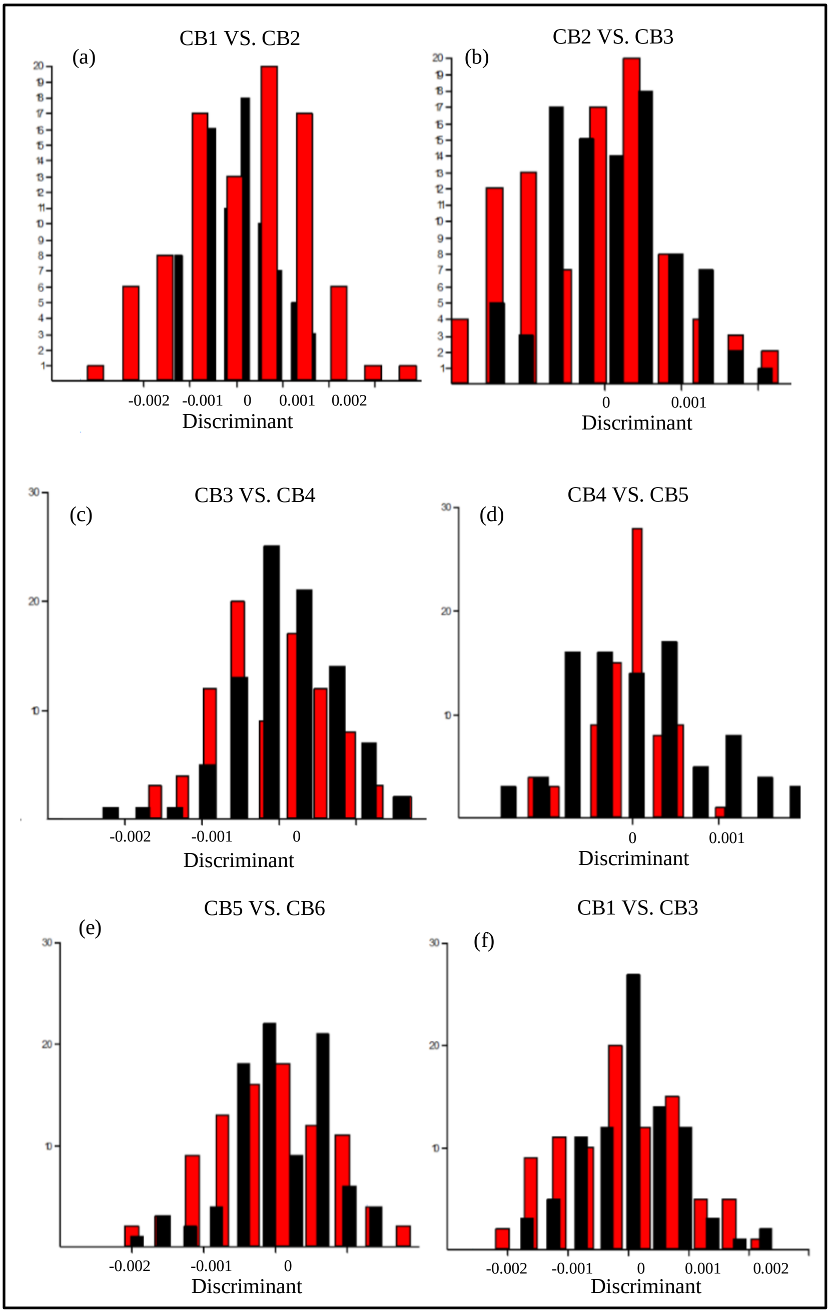
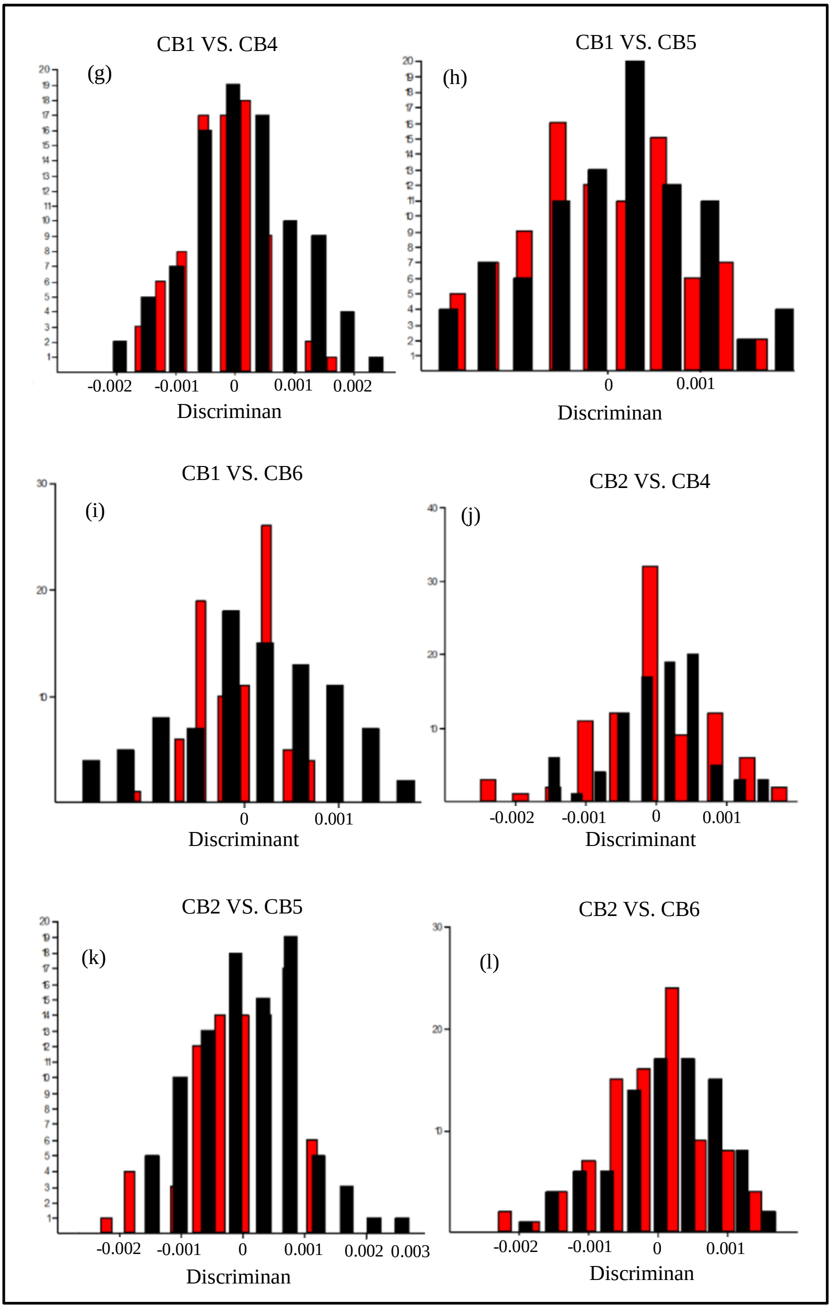


Figure 7. Reclassification graphs of the six *Caladium* varieties from DA of landmark-based geometric morphometrics; computed and generated in PAST ver 3.26 (Hammer *et al.*, 2019). [CB1-Flatter Me, CB2- Freckles, CB3-White Christmas, CB4-Rosebud, CB5-Celebration, CB6-Candidum]

Figure 7 continued.



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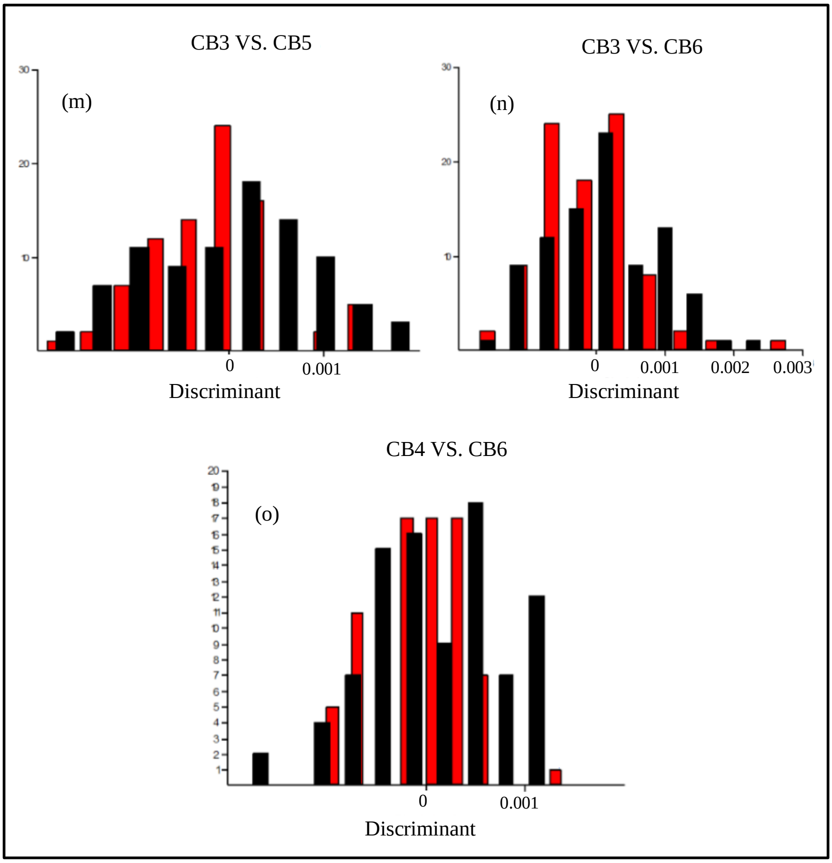

The result of the DA classifies individuals into predetermined groups. According to Poulsen and French (2008), DA is a powerful statistical tool that is used to predict membership in naturally occurring groups. From the results, there were observable overlaps and non-overlaps from the diagrams (Figure 7) indicating similarities between groups. From the reclassification of the species, low percentage values were observed ranging from 47.22-53.33%. This percentage value is lower than the accepted cut-off value of 75% (Tabugo-Rico *et al*., 2017).

This low percentage indicates similarity between the datasets examined. The low values of the discriminant analysis showed relatively low classification accuracy, suggesting that despite observable differences, there are still common morphological features shared among the varieties. The overlaps in the graph presented in Figure 7 may indicate shared adaptive traits or a level of phenotypic plasticity within the species. The non-overlaps presented in the graphs indicate the distinction of the shapes of the six leaf groups.

4. Conclusion and Recommendation

The investigation into *Caladium* varieties created a comprehensive baseline that details the patterns of leaf shape variability within this species. Utilizing Procrustes transformation, shape configurations were aligned and compared to describe and quantify the morphological differences among the *Caladium* species collected. The analysis revealed distinct shape characteristics unique to each variety, signifying that leaf shape variations are specific to particular genetic or environmental factors associated with each group. However, DA showed relatively low classification accuracy, suggesting that despite observable differences, there are still common morphological features shared among the varieties. This overlap may indicate shared adaptive traits or a level of phenotypic plasticity within the species. The application of geometric morphometric techniques successfully captured subtle variations, underscoring the potential of these tools in botanical studies. To fully understand the depth of intraspecific diversity, future research should focus on more extensive sampling and additional morphometric analyses.

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