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Geometric Morphometric Differentiation and Morphology-Based Phylogenetic Analysis of Schismatoglottis Spp. (Araceae) in South Cotabato

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ABSTRACT

Identifying Schismatoglottis plants (Araceae) can be challenging because many species look very similar and differ only in small morphological details. This study aimed to examine shape variation and morphological relationships among three Schismatoglottis morphotypes; lanceolate, oblique, and sagittate, collected from different localities in South Cotabato. A quantitative field-based approach was used. Twenty-seven healthy and fully developed plants were sampled. Their leaves, petioles, stems, and roots were photographed for analysis. Landmarks were digitized from the images, and shape data were standardized using Generalized Procrustes Analysis. Patterns of variation were then examined using Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA). The PCA results showed a high degree of overlap among the morphotypes, which suggests that shape differences occur gradually rather than forming clearly separate groups. In contrast, CVA produced clearer distinctions among the morphotypes, with leaf shape contributing the most to variation. A morphology-based tree constructed from PCA scores revealed two main clusters that reflected overall shape similarity rather than organ type. These findings reveal that geometric morphometrics is useful for detecting subtle morphological differences and can support the differentiation of Schismatoglottis morphotypes in South Cotabato.

INTRODUCTION

Species identification is becoming progressively challenging. For the species of Schismatoglottis (Araceae), a group of understory plants which can be seen in parts of the Philippines, this issue is common. It has high levels of morphological similarities between species and is vulnerable to environmental changes, species hybridisation, and more subtle structural changes which often leads to the misidentification of the species' origin and evolutionary history (Mata-Montero *et al.*, 2016). Specifically, species in South Cotabato exhibit subtle structural differences,

making it difficult to distinguish them using traditional methods of identification and differentiation.

The genus Schismatoglottis is commonly found in parts of Southeast Asia, particularly in Borneo and the Philippines, and is well adapted to riverbanks, limestone areas, and dense forests (Harisin *et al.*, 2023 & Wog *et al.*, 2011). In the Philippines, species like S. prietoi and S. mindanoana demonstrate contrasting traits from vegetative growth in aquatic environments to growing on rocky substrates (Boyce *et al.*, 2015 & Wong *et al.*, 2021). Recently, the chances of identifying an unknown taxa in isolated areas were highlighted by the description of S. minuta from Samar Island, which is characterized by its small structure, viviparous leaves, and unique floral structures (Angeles *et al.*, 2023). However, accurate species identification of plants may still be difficult due to morphological and ecological variations. Wong and Boyce (2024) conducted a comprehensive taxonomic revision of Schismatoglottis and described seven new genera based on molecular and morphological analyses. Cusimano *et al.* (2011) reconstructed a maximum-likelihood phylogeny of Araceae using molecular data from 102 genera and compared it with morphological and anatomical characters across 44 clades, many of which are supported by ecological and geographic patterns. Within S. Calyptrata, six species were identified in Sabah by Sarawak *et al.* (2017) and using internal transcribed spaced (ITS) regions to conduct molecular phylogenetic analysis revealed possible unknown species (Harisin *et al.*, 2023). Recent developments in taxonomic studies involving Schismatoglottis like these show an increase on reliance on molecular instruments to address issues with morphological ambiguity.

Significant morphological flexibility and adaptability is noticeably present in Schismatolgottis, particularly across rainforests in Southeast Asia (Harisin *et al.*, 2023). Its ability to change leaf shape in response to its environment further supports this characteristic of the species. Leaves show the most distinct changes which aids in species identification (Royer *et al.*, 2009 & Ariawan *et al.*, 2020). The increasing interest in quantitative shape is driven by limitations of traditional taxonomy. Considering that leaves are accessible and easily change, its morphology serves a model for addressing phenotypic flexibility and species differentiation. A 2017 study by Klein *et al.* (2017) using geometric morphometrics supported this method's applicability in distinguishing species and genotypes based on leaf shape. Viscosi and Cardini (2011b) likewise

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showed that individual variation in leaf morphology driven by environmental plasticity can be captured using geometric morphometric methods. With leaves as the main feature, Schismatoglottis makes a suitable subject for geometric morphometric analysis (Gimenez *et al.*, 2012).

Schismatoglottis species have slight yet significant morphological traits, pointing to a research gap in understanding their diversity. There have been no localized studies in South Cotabato applying geometric morphometrics to delimit species boundaries of Schismatoglottis. This study aimed to assess morphological variation in selected Schismatoglottis spp. in South Cotabato through geometric morphometrics and morphology-based phylogenetic analyses using PCA and CVA to identify key traits for species delimitation.

Specifically, the study (1) characterized the geometric morphometric variations among Schismatoglottis species in selected sites of South Cotabato; (2) determined patterns of structure variation across and within species using Principal Components Analysis (PCA) and Canonical Variate Analysis (CVA); (3) constructed a morphology-based phylogenetic analysis; and (4) determined the evolutionary relationship of Schismatoglottis based on morphometric pattern and phylogenetic data. Species differentiation was deepened by the goals and ensured the correct species are used for habitat restoration. The role of Schismatoglottis in local biodiversity, fostering education, and community conservation efforts is also greatly emphasized and directly aligns with the goals of SDG 15: Life on Land, providing it with support in achieving its goal.

MATERIALS AND METHODS

Materials

Plant identification in this study was carried out using Seek by iNaturalist and Co's Digital Flora, both widely recognized resources in the Philippines. For field sampling, the researchers used standard collection materials, including gloves, ziplock bags, masking tape, markers, 70% ethyl alcohol, and tissue paper. Specimens were documented with a digital camera, measuring tools, and proper lighting to ensure clear images. To preserve the plants, conventional herbarium practices were followed, using a cardboard plant press, newspaper sheets, labels, picture frames, tie boxes, and naphthalene balls. For image processing and analysis, GIMP 2.10 was used to clean and orient the images, TPSUtil to convert them into TPS format, and TPSDig2 for digitizing landmarks. Geometric morphometric analyses were conducted in MorphoJ, while SPSS and PAST were used to perform statistical tests and validate the data.

Research Design

This study employed a quantitative field research approach to examine shape differences among three Schismatoglottis morphotypes from selected areas in South Cotabato. Fieldwork was conducted systematically from October to December 2025. The morphotype of each plant was considered the independent variable, and shape data from leaves, petioles, roots, and stems were treated as dependent variables. All specimens were processed using standardized methods to maintain consistency. Geometric morphometric techniques, along with statistical analyses, were applied to measure shape variation, identify patterns, and explore the relationships among the morphotypes.

Procedure

Study Site and GIS Mapping

The study was done in South Cotabato, Philippines, specifically in the City of Koronadal, and Municipalities of Tupi and Banga, where Schismatoglottis plants are known to grow. Sampling sites were picked based on confirmed plant presence, suitable conditions, enough plants to collect, and easy access. A stratified random sampling method was used, treating each town as a separate group. Three (3) barangays were randomly chosen from each town, which gave a total of nine (9) sites. During fieldwork, researchers also recorded features of the environment, such as nearby vegetation, soil moisture, and general habitat type, to capture differences between sites. The location of each site was noted using a mobile GPS and later mapped in QGIS. Habitat details were added to the maps to show the surroundings and to illustrate where the plants were found and how the sampling covered the area (Hadika *et al.*, 2020).

Preliminary Identification

Potential Schismatoglottis plants were identified in two (2) steps. First is, Seek by iNaturalist app, which suggested possible species based on photos. These initial identifications were then checked against Co's Digital Flora of the Philippines by comparing key features, with descriptions and reference images. Seek was chosen for its large and reliable database and for quick identification in the field, while verification with trusted sources made sure the identifications were accurate (Florida Museum of Natural History, 2023).

Sample Collection

Samples were collected from several areas in South Cotabato, focusing on three (3) Schismatoglottis morphotypes: lanceolate, sagittate, and oblique. Only mature, healthy plants with complete leaves, strong stems, and active roots were chosen to ensure accurate observations. The morphotype classification was based on clearly defined leaf shapes: narrow and elongated for lanceolate, arrow-shaped with basal lobes for sagittate, and asymmetrical with unequal sides for oblique. Classification was done in the field without bias, relying solely on these morphological features to avoid subjective preferences in the analysis of shape variance by PCA and CVA. A total of 27 specimens were sampled (Kolawole *et al.*, 2016). Each field was sampled over three (3) days, nine (9) samples were collected from each location, and

Figure 1: Selected Sites in South Cotabato: City of Koronadal, Municipality of Tupi, and Municipality of Banga

was carefully uprooted to preserve the roots. Before being put in labeled ziplock bags, each specimen was given a unique field number and kept in moistened tissue paper to keep it hydrated during transit. Before being processed for photographic documentation and herbarium preservation, samples were stored temporarily for no more than six to eight (6-8) hours. (Buck, 2017).

Morphological Identification, Documentation, and Preservation

Each Schismatoglottis specimen's environmental circumstances were documented inside a 1 m² quadrat prior to collection in order to provide each morphotype ecological context (Pedrotti, 2013). While elevation was calculated in QGIS using GPS coordinates obtained on-site, soil moisture was assessed using a portable soil moisture meter that captured volumetric water content (%) (Education Nature Park, 2021). Following collecting, specimens were photographed from above to document the arrangement of the leaves, petioles, roots, and stems. A caliper was used for finer measurements, and a metric ruler was supplied for scale. Each item was laid flat on a foam board and carefully fastened to preserve natural orientation. To guarantee uniform scale and image quality for geometric morphometric analysis, photos were taken using a tripod at a fixed distance of roughly one meter under standard LED lighting (Crampton, 2025). With a few adjustments, specimens were maintained as voucher samples using normal herbarium procedures. Without the use of chemical preservatives, each plant was uniformly compressed between newspaper sheets in a cardboard press until it was completely dry. The collecting site, habitat remarks, collector's name, date of collection, and taxonomic information were all labeled on dried specimens that were placed on picture frames.

Geometric Morphometric Acquisition and Analysis

Imaging was done while the specimens were drying, and geometric morphometric analysis was carried out following specimen preservation. GIMP was used to standardize the orientation of images of leaves, petioles, stems, and roots before TPSUtil was used to convert the images to TPS format. In order to produce two (2) dimensional coordinates that precisely depict organ shape and guarantee consistency between specimens, landmarks were digitized at homologous, easily recognized places on each organ in TPSDig2. Generalized Procrustes Analysis (GPA) was used in MorphoJ to normalize these coordinates in order to eliminate non-shape variance resulting from size, location, and orientation. After identifying the main axes of morphological variation by Principal Component Analysis (PCA), shape variation was analyzed using Canonical Variate Analysis (CVA) to improve morphotype separation and assess classification accuracy. The analyses developed PCA and CVA scatterplots, deformation grids depicting shape changes,

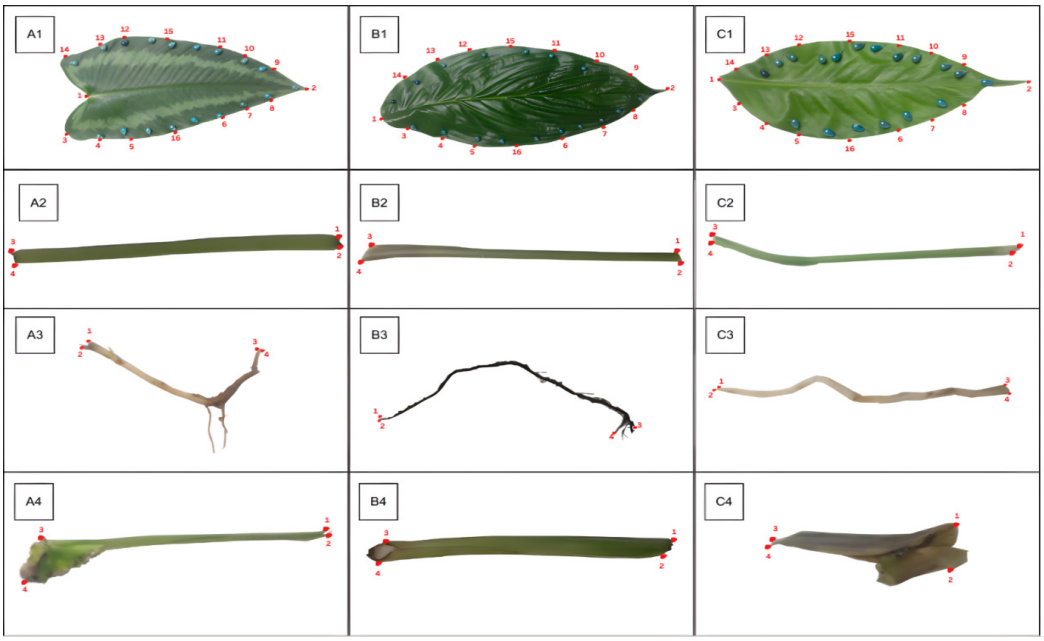

Figure 2: Sample of Morphotypes with Landmarking
Note1. The following letters show landmarking points of each morphotypes: Sagittate (A), Oblique (B), Lanceolate (C)
Note2. The numbers indicate the traits shown in each image: Foliar (1), Petiole (2), Root (3), Stem (4)

and accuracy scores, which provided a quantitative basis for differentiating morphotypes and assessing shape consistency (Freschet *et al.*, 2017; Crampton, 2025; & Klingenberg, 2010).

Morphology-based Phylogenetic Construction and Dendrogram Interpretation

Principal Component (PC) values from MorphoJ were used as quantitative descriptors of form variation across the three morphotypes to create a morphology-based dendrogram in PAST. A distance matrix representing morphological difference was created using these scores, and cluster stability was assessed using bootstrap resampling (1,000 iterations). Branch lengths indicated the degree of morphological divergence among morphotypes, and bootstrap values ≥70% were regarded as strong evidence.

Data Analyses

The three (3) morphotypes of Schismatoglottis spp. were represented by 27 accessions that provided morphological and morphology-based phylogeny data. Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) in MorphoJ2 were used to assess shape variation and group differences. To compare a single independent variable among morphotypes, a one-way ANOVA was conducted in SPSS. Tukey's post-hoc test was then used to pinpoint particular differences. Major shape differences and group separation between morphotypes were shown using PCA and CVA scatterplots. Using relative warp scores and specific coded features, a morphology-based phylogeny was then built in PAST (Hamer *et al.*, 2001), which resulted in a UPGMA dendrogram that displayed morphological grouping patterns and evolutionary links among the morphotypes.

Ethical Considerations

Many standard protocols were followed in conducting this investigation. First, in order to handle the species in a humane and non-invasive manner, the researchers obtained permits from the Department of Environment and Natural Resources (DENR), the City Government of Koronadal, the school principal, and barangay permits from the study site. In order to guarantee a very small environmental impact during the in-field study, the researchers also honored the customs of the study area. In order to maintain scientific integrity, the researchers also maintained openness in their research procedures, data gathering, and reporting.

RESULTS AND DISCUSSION

Geometric Morphometric Variation Among Schismatoglottis Morphotypes

Figure 3 shows that transformation grids in each plant organ varied in shape. The leaf base in Grid A showed obvious lateral displacement and curve, while the tip (right) showed moderate elongation, which indicated localized shape variation in the foliar lamina. Grid B showed the petiole stretching mostly along its length with minimal sideways bending while the stem, shown in Grid D, showed a subtle more tip-focused deformation. This means that this pattern hints the directionally constrained variation, with shape change occurring mostly along length rather than across width. Petiole

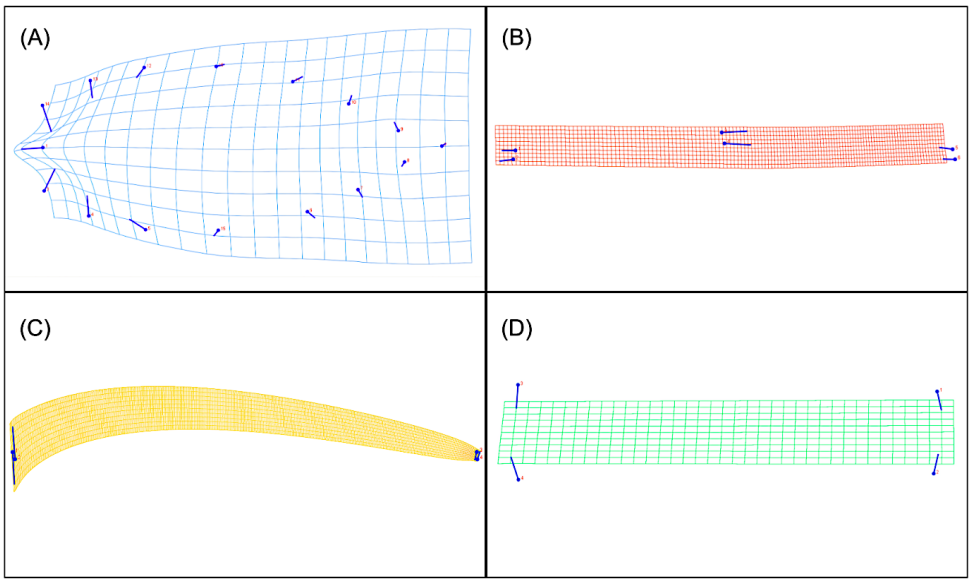

Figure 3: Transformation Grids of Shape Variation Among Morphotypes
Note1. (A) Foliar, (B) Petiole, (C) Root, and (D) Stem

and stem deformation is restricted because their natural growth limits created stability for their structural integrity (Millenaar *et al.*, 2005; Baskin & Jensen, nd; Kembryte *et al.*, 2022). Grid C depicts strong bending and curving in the roots. This further means that the root may change shape more freely and unevenly compared to the other organs. These grids correlate to organ focused growth behavior and developmental restrictions. Functional adaptation, which optimizes leaf shape for light capture and local environmental conditions, may be the cause of foliar curvature (Ahmad et al, 2022). Leaf traits are emphasized not because they are inherently more variable, but because they consistently retain strong ecological and evolutionary signs, making them particularly informative for comparative analyses (Nicotra *et al.*, 2011). Root shape is often highly plastic and environment-responsive, so while it shows strong curvature, it provides supportive but less reliable information for distinguishing morphological differentiation (Jolliffe & Cadima, 2016). Although this focuses on visualizing organ-specific shape changes, when combined with the numerical results from PCA and CVA, it strengthens the biological relevance of the variation by linking quantitative patterns to real morphological differences.

Patterns of Structural Variation Using Principal Components Analysis (PCA) and Canonical Variate Analysis (CVA)

Principal Component Analysis (PCA)

Figure 4 shows the distribution of plant structure

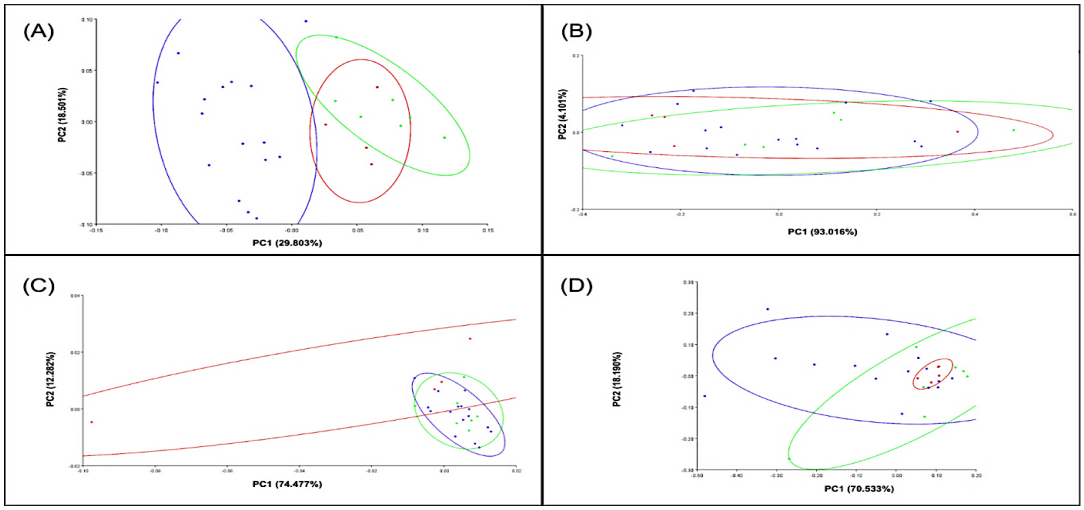

Figure 4: Principal Component Analysis (PCA) of Schismatoglottis Morphotypes based on Structural Traits
Note1. Patterns of shape variation for (A) Foliar, (B) Petiole, (C) Root, (D) Stem structures are shown in the PCA scatterplots. The points represent individual specimens, while ellipses indicate 95% confidence regions.
Note2. Colors denote morphotypes: Lanceolate (Red), Oblique (Green), and Sagittate (Blue).

variation across the first two (2) principal components, as displayed in the PCA scatterplots. The percentages on each axis indicated how much of the total shape variation was explained by that component (Jolliffe, 2021). For foliar traits (A), PC1 (29.803%) and PC2 (18.501%) shared an average proportion of variation, and the partial overlap of confidence ellipses revealed that leaf shape differences existed among groups but were not fully distinct. Petiole traits (B) were dominated by PC1 (93.016%), which indicated that most variations were caused by major tendencies such as length or thickness, which resulted in strong overlap among groups and implies limited differential ability. Root traits (C) showed strong patterns along PC1 (74.477%) with minor effects from PC2 (12.823%), and the tight clustering of points with overlapping ellipses indicated high morphological similarity and restricted root structure across samples (Abdi & Williams, 2010).

In contrast, stem traits (D) showed a more balanced contribution of PC1 (70.533%) and PC2 (18.190%), with reduced overlap among ellipses, which suggested greater structural differentiation. Overall, overlapping ellipses in the scatterplots reflect shared structural form and continuous variation rather than distinct separation, which imply that some structures (especially stems and leaves) relatively contribute to morphotype differentiation than others. Overall, the PCA results show that general morphological traits explain variation among the morphotypes but are not strong enough to clearly separate them. Because of this, a group-focused analysis such as CVA was also performed to identify subtle but consistent differences among the morphotypes.

Canonical Variate Analysis (CVA)

As shown in Figure 5, there was group separation in CVA, where the axes represented the amount of variation caused by each trait. Foliar traits (A) showed that Canonical Variate 1 (85.684%) showed the most differentiation resulting in clearly separated clusters with minimal overlap, which means strong leaf-based variation

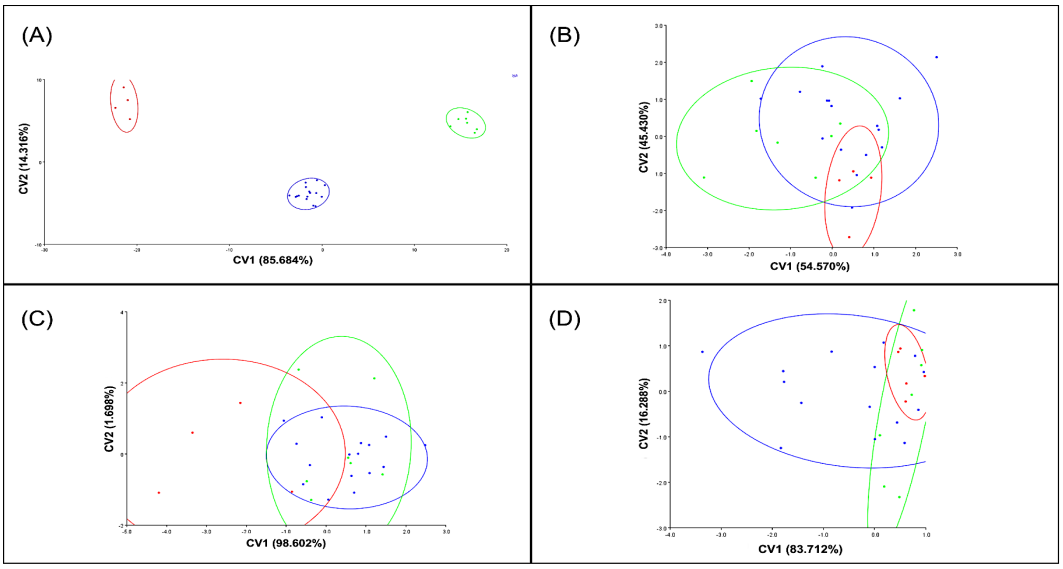

Figure 5: Canonical Variate Analysis (CVA) of Schismatoglottis Morphotypes based on Structural Traits

Note1. CVA Scatterplots show the pattern of shape variation for (A) Foliar, (B) Petiole, (C) Root, and (D) Stem structures. Ellipses represent 95% confidence areas, and points are specimens in each.

Note2. Colors indicate morphotypes: Lanceolate (Red), Oblique (Green) and Sagittate (Blue).

among groups. Petiole traits (B) showed a more balanced contribution of CV1 (54.570%) and CV2 (45.430%), but the wide overlap of confidence ellipses suggested weaker separation and higher within-group variability. Root traits (C) were dominated by CV1 (98.602%) with minor contribution from CV2 (1.398%), and the extensive overlap among groups indicated limited differentiation ability of root traits.

In contrast, stem traits (D) exhibited strong separation along CV1 (83.712%) with reduced overlap among ellipses, suggesting greater effectiveness of stem traits in distinguishing groups. Overall, the overlapping confidence ellipses in CVA indicated that groups shared similar morphological characteristics, which reveals high within-group variability and reduced grouping accuracy when traits barely differ among groups (Jombart *et al.*, 2010). Such overlap suggests that the measured traits occupy a common morphological range and that the differences are continuous rather than having sharp boundaries (Klingenberg & Monteiro, 2005). Similar organ-specific differences in differentiation ability have been reported in plant morphometric studies, where leaves and stems often outperform roots or petioles in distinguishing near-identical groups (Viscosi & Cardini, 2011). These multivariate patterns provide a morphological framework for evaluating whether observed trait differentiation corresponds with evolutionary relationships gathered from the phylogenetic tree.

Morphology-based Phylogenetic Analysis of Schismatoglottis

The dendrogram shows that there were strong and statistically significant patterns of morphological differentiation supported by the 100-value bootstrap at all nodes. This high bootstrap value was the highest level of statistical confidence which indicates that the relationships at each junction are highly reliable. The tree splits into two (2) major clusters: the left and right cluster. The left cluster was primarily made up of stem, leaf, and petiole traits with oblique and lanceolate morphotypes where stems and petioles had the closest relation, and leaf traits gradually join. This means that foliar traits are similar to stem and petiole traits when they share oblique or lanceolate geometry. The right cluster on the other hand was made up of roots, specifically by the sagittate morphotype. Although different plant parts were shown, some similarity was observed between F. Sagittate traits and P. Lanceolate traits. The grouping of lanceolate roots together with sagittate petioles and roots near the terminal branches indicates that root traits tend to cluster closely and are strongly associated with sagittate morphotypes. The distribution of traits across both clusters suggests that similarity is not only determined by organ type but is more strongly influenced by overall geometric shape, where traits with similar form cluster together regardless of their plant part (Weemstra *et al.*, 2016). The intermediate placement of R. Oblique supports this pattern and reflects that underground traits can share similarities with both above- and below-ground morphology (Adams & Collyer, 2017). Overall, the dendrogram reflects a common pattern in morphological clustering, where statistically strong groupings correspond more closely to shared shape characteristics than to strict organ-based separation, (Silva *et al.*, 2012) supporting the assessment of evolutionary relationships of the species.

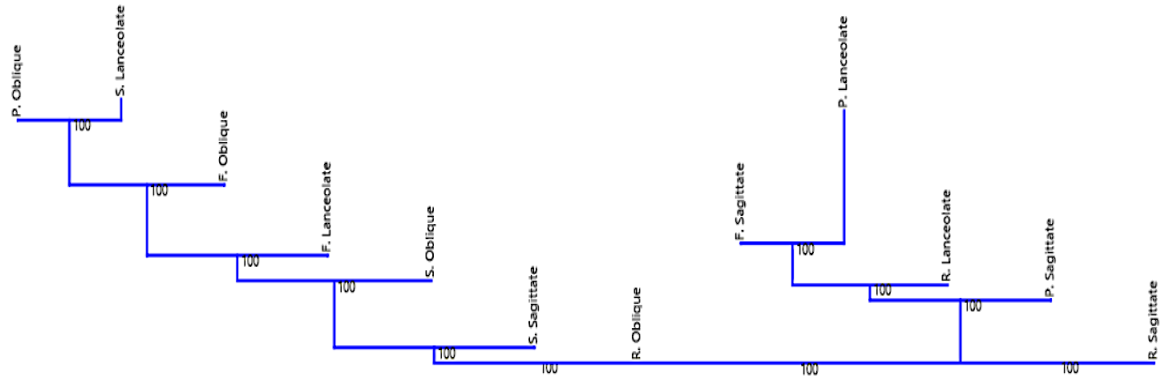

Figure 6: Morphology-based Phylogenetic Relationships Among Schismatoglottis Morphotypes
Note1. The following letters in the labels denote the following traits: (F) Foliar, (P) Petiole, (R) Root, and (S) Stem of each morphotype.

Assessment of the Evolutionary Relationships of Schismatoglottis Based on Morphometric Patterns and Phylogenetic Data

The morphometric and phylogenetic results revealed that evolutionary relationships among Schismatoglottis morphotypes were most clearly reflected in foliar traits, as consistent leaf-shape divergence suggested heritable differentiation among the morphotypes. Leaf morphology is known to retain strong evolutionary and taxonomic signals in Araceae due to its sensitivity to ecological and developmental factors (Silva *et al.*, 2012 & Bessho-Uchara *et al.*, 2025). In contrast, petiole and stem traits show weaker differentiation, likely constrained by functional requirements (Bucksch *et al.*, 2017), while root traits exhibit minimal phylogenetic structure and appear to be shaped primarily by environmental variation rather than shared ancestry. Overall, these patterns support mosaic evolution in Schismatoglottis and indicate that foliar morphometric data provide the most reliable basis for assessing evolutionary relationships, with other vegetative traits serving a secondary, complementary role (Silva *et al.*, 2012 & Bucksch *et al.*, 2017).

CONCLUSION

Patterns of morphological variation are observed in the analyses of three Schismatoglottis morphotypes in South Cotabato. Foliar traits show the most distinct variation, while petiole and stem traits exhibit limited deformation and root traits mainly change in orientation. Scatterplots of PCA analysis illustrate an overlap among the morphotypes, indicating shared characteristics and steady morphological variation. On the other hand, CVA scatterplots reveal clearer separation when group identity is considered. The morphology-based dendrogram forms two (2) main clusters which are not arranged by the plant's organ part, although foliar traits are observed to be more consistently grouped. Partial similarity is seen in petiole traits, while stem and root traits show less consistent clustering. On the whole, foliar traits provide the greatest indicator in distinguishing morphotypes, with petiole, stem, and root traits contributing to additional but less distinct information. These findings support the use of geometric morphometrics with morphology-based phylogenetic analysis in order to improve the understanding of morphological variation and patterns

in biodiversity in the environment.

Recommendation

Based on the conclusion of the study, the researchers recommend the following to improve the methods and interpretations carried out in the study. 1) DNA analysis may help in the future to establish how the various types of Schismatoglottis are related. 2) Collecting more plants from different locations would give a better idea of how they naturally vary, especially in their stems and roots, which are often hard to tell apart. 3) Studying root depth and distribution in each habitat may also give insight into where Schismatoglottis grow. 4) The methods used in this study could be used for other Araceae plants to help with identification, studying biodiversity, and conservation. 5) Using 3D imaging in the future could also make it easier to see small differences in stems, roots, and petioles that are hard to notice in flat pictures.

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