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Leaf Epidermal Anatomy of Euphorbiaceae and Phyllanthaceae from the Kaliurang Sector of Mount Merapi, Indonesia

Riska Annisa[1], Dewi S. Amboupe[2*], Muliana GH[2], Jamilatus Sa'diyah[2], Syaidah Fitri[1], Naufal Hafidh Mahdi Sujarwo Putra[1], Wendy A. Mustaqim[3]

[1]Department of Environmental Science, UIN Sultan Aji Muhammad Idris Samarinda, Samarinda, Indonesia;

[2]Department of Biology, Universitas Negeri Makassar, Makassar, Indonesia;

[3]Department of Biology, Universitas Samudra, Aceh, Indonesia.

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*Corresponding Author: **Dewi S. Amboupe,** Universitas Negeri Makassar, Makassar, Indonesia;

Email: dewi.sartika@unm.ac.id

Abstract: Euphorbiaceae and Phyllanthaceae are diverse seed plant families whose classification has been extensively studied, particularly since molecular evidence established Phyllanthaceae as a distinct family. This study investigated leaf epidermal anatomy of species occurring in Central Java, Indonesia, to provide additional evidence for their taxonomic characterization. Twelve species representing six genera were examined, comprising five Euphorbiaceae and seven Phyllanthaceae species, including ten native and two introduced species. Leaf epidermal sections were analyzed based on epidermal cell shape, anticlinal wall patterns, stomatal types, and stomatal distribution. Anomocytic stomata were observed only in *Euphorbia heterophylla* and *Euphorbia hirta*, suggesting their potential as diagnostic characters. Amphistomatic leaves occurred in *E. heterophylla* and *Phyllanthus niruri*, whereas the remaining species were hypostomatic. However, diacytic stomata, epidermal cell shapes, and anticlinal wall patterns overlapped between the two families. Non-metric multidimensional scaling (NMDS) analysis also failed to clearly separate the species according to family classification. These findings indicate that leaf epidermal characters are useful for species-level characterization but do not consistently distinguish Euphorbiaceae from Phyllanthaceae. The study provides baseline anatomical data for tropical Indonesian species and highlights the need for further research incorporating broader anatomical and morphological characters.

Keywords: Anatomy; Classification; *Euphorbiaceae;* Leaf epidermal incision; *Phyllanthaceae.*

Introduction

Euphorbiaceae s.l. (Webster 1994, APG III 2009) is another name for a family of plants that according to APG IV (2016) is now placed in various independent families, namely Euphorbiaceae s.s. and Phyllanthaceae s.s., as well as several much smaller families, i.e. Pandaceae, Peraceae, Picrodendraceae, and Putranjivaceae. Based on data compiled by Christenhusz and Byng (2016), the total Euphorbiaceae s.l. is estimated to be close to 270 genera and 9000 species. Euphorbiaceae s.s. has the largest number of members with 209 genera and 6252 species, followed by Phyllanthaceae s.s. with 57 genera and 2050 species. The Euphorbiaceae and Phyllanthaceae families of plants offer numerous benefits to human life, including uses in medicine, food, vegetables, and fruits. Many species from these families have been cultivated by humans, such as *Phyllanthus androgynus* (L.) Chakrab. & N.P.Balakr. which is commonly known in Indonesia as katuk leaves.

Euphorbiaceae s.l. has undergone substantial taxonomic revision following the increasing application of molecular data in plant systematics. The recognition of Phyllanthaceae as a separate family from Euphorbiaceae s.l. in the APG IV classification (2016) is supported by molecular phylogenetic

evidence, including Wurdack et al. (2004). Molecular studies have also contributed to changes in the circumscription and placement of genera and other taxa formerly included within Euphorbiaceae s.l. (e.g., Pruesapan et al., 2012). Nevertheless, morphological and anatomical characters remain important complementary sources of evidence for species identification and for evaluating diagnostic variation among closely related taxa. Gagliardi et al. (2013), for example, emphasized the importance of morphological and anatomical investigations for understanding the systematics and evolution of Euphorbiaceae s.l. Their study of fruit development also demonstrated that some morphological characters may not correspond consistently with the familial boundaries inferred from molecular evidence. Thus, anatomical studies are valuable not for reassessing the current APG IV classification, but for evaluating the diagnostic utility and patterns of variation of anatomical characters within and between these taxonomic groups.

The flora of Java, including Euphorbiaceae and Phyllanthaceae, was documented extensively by Backer and Bakhuizen van den Brink (1963), at a time when the taxa of Phyllanthaceae were generally treated within Euphorbiaceae s.l. However, detailed regional studies focusing specifically on leaf anatomical characters of these two families remain limited, particularly in volcanic mountain ecosystems such as Mount Merapi. Regional-level studies are important for documenting plant diversity and morphological variation at finer spatial scales and can complement broader floristic treatments. Recent studies of Indonesian flora have likewise highlighted the importance of regional plant documentation for biodiversity assessment, conservation, and sustainable utilization (Hidayat et al., 2025; Tania et al., 2026).

Mount Merapi National Park (MMNP), located in Central Java, represents a dynamic volcanic ecosystem in which recurrent volcanic activity and associated landscape changes may influence vegetation composition and plant distribution. Such environmental dynamics provide an important context for documenting plant diversity and morphological variation. During botanical exploration in MMNP, 12 species belonging to Euphorbiaceae and Phyllanthaceae were collected. The present study focuses on selected paradermal leaf epidermal characters, including epidermal cell shape, anticlinal wall pattern, stomatal type, and stomatal distribution, which have received limited attention for these taxa in the study area. By characterizing these features and evaluating their variation among the sampled species, this study aims to assess their potential diagnostic value for species-level identification and to examine whether the selected epidermal characters show consistent patterns within or between Euphorbiaceae and Phyllanthaceae. The findings are expected to provide baseline anatomical data for these taxa in MMNP and contribute complementary evidence to regional taxonomic and biodiversity studies.

Material and Methods

Specimen Collection and Identification

Specimen collection was conducted along the hiking trail to the summit of Plawangan Hill, Kaliurang, Special Region of Yogyakarta, Indonesia (Figure 1). The collection area was situated within the Plawangan area of Mount Merapi National Park (MMNP), Sleman Regency, Special Region of Yogyakarta. The surveyed sites covered elevations from 900 to 1,291 m above sea level. The geographic coordinates and elevation of each collection point were recorded using a GPS device and are presented together with the corresponding species in Table 1.

An exploratory survey was conducted following the approach described by Rugayah et al. (2004), in which specimens of Euphorbiaceae and Phyllanthaceae encountered along the survey route were collected and documented. Both native and introduced species occurring in the wild were included. Specimens were collected from flowering and vegetative individuals when available. Field observations included species identity, geographic coordinates, elevation, habitat, and phenological condition. Voucher specimens were prepared for taxonomic verification, with the corresponding voucher numbers listed in Table 1.

Species identification was based on

morphological characters and comparison with relevant taxonomic literature, particularly Backer and Bakhuizen van den Brink (1963), Welzen (2003), and Welzen and Pruesapan (2010), supplemented by other relevant taxonomic references. Accepted species names were verified using Plants of the World Online (POWO, 2026). The final nomenclature followed the accepted names provided by POWO.

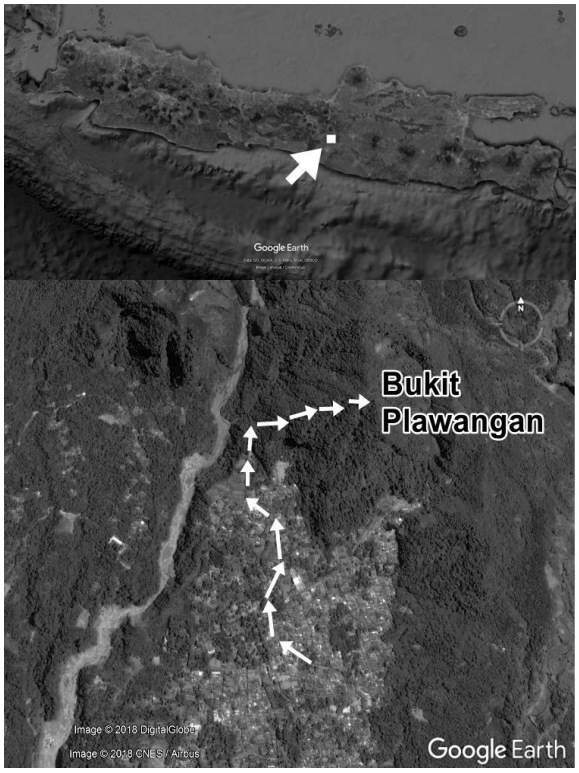

Figure 1. Location of Collection route for *Euphorbiaceae* s.l. specimens in Bukit Plawangan, Merapi National Park (→).

Leaf Epidermal Preparation and Observation

Leaf epidermal studies were conducted to evaluate the diagnostic value of selected epidermal characters among the sampled species of Euphorbiaceae and Phyllanthaceae. Mature and fully expanded leaves were selected from the collected specimens. Leaf samples were preserved in 70% ethanol before anatomical preparation. For epidermal isolation, leaf segments were immersed in nitric acid (HNO_3) to facilitate maceration of the leaf tissues. Following maceration, the samples were thoroughly rinsed with distilled water to remove residual acid. The epidermal tissue was then carefully separated from the remaining leaf tissues using fine forceps and mounted on glass slides using a suitable mounting medium. No additional staining procedure was applied.

The prepared epidermal surfaces were examined under a light microscope, and micrographs were obtained using a camera attached to the microscope. Observations were conducted on mature leaf samples from the collected individuals, with multiple microscopic fields examined for each specimen. Six qualitative leaf epidermal characters were examined: epidermal cell shape, anticlinal wall pattern, stomatal type, stomatal distribution, epidermal cell wall characteristics, and epidermal surface characteristics. The character states and coding criteria are presented in Table 2. Stomatal types were identified according to the classification of Cotthem (1970). Stomatal distribution was determined based on the presence or absence of stomata on the adaxial and abaxial leaf surfaces. Epidermal cell shape and anticlinal wall patterns were characterized based on the observed epidermal morphology.

Quantitative epidermal measurements were also recorded for the Euphorbiaceae species for species-level comparison. The measured characters comprised stomatal size and epidermal cell dimensions. Measurements were obtained from multiple microscopic observations per species and are presented as descriptive measurements in Table and Figures.

Data Coding and NMDS Analysis

The six qualitative epidermal characters were coded according to the character states defined in Table 2. The resulting character matrix was used to evaluate similarities among the 12 sampled species. Before multivariate analysis, taxon names and character states in the matrix were cross-checked against the species list and descriptive data to ensure consistency.

Non-metric multidimensional scaling (NMDS) was performed using PAST version 3.19 (Hammer et al., 2001). Because the variables consisted of qualitative categorical characters, the character matrix was analyzed using Gower distance, which is appropriate for assessing dissimilarities among taxa based on categorical morphological and anatomical characters without treating numerical character codes as continuous measurements. The NMDS analysis was conducted using a two-dimensional

solution, with multiple random starts and a maximum of 100 iterations to obtain a stable ordination configuration. The stress value was used to evaluate the goodness of fit of the ordination.

The resulting NMDS configuration was used to assess patterns of similarity among the sampled species based on the selected epidermal characters and to determine whether these characters produced recognizable patterns corresponding to the current taxonomic affiliation of the species. The analysis was interpreted as an evaluation of the discriminatory value of the selected leaf epidermal characters rather than as a test of the validity of the APG IV classification.

Results and Discussion

Leaf Epidermal Characters

Leaf epidermal characteristics were examined in 12 species representing six genera, comprising seven species of Phyllanthaceae and five species of Euphorbiaceae. The Euphorbiaceae included *Claoxylon longifolium* (Blume) Endl. ex Hassk., *Euphorbia heterophylla* L., *Euphorbia hirta* L., *Homalanthus populneus* (Geiseler) Kuntze, and *Macaranga rhizinoides* (Blume) Müll.Arg. The Phyllanthaceae comprised *Breynia macrantha* (Hassk.) Chakrab. & N.P.Balakr., *Breynia microphylla* (Kurz ex Teijsm. & Binn.) Müll.Arg., and five species of *Phyllanthus*: *P. cernuus* Poir., *P. lutescens* (Blume) Müll.Arg., *P. microcarpus* (Benth.) Müll.Arg., *P. niruri* L., and *P. obliquus* (Willd.) Müll.Arg. Ten species were native to Java and occurred naturally in the study area, whereas *E. heterophylla* and *E. hirta* were introduced species that were found growing in the wild.

Six qualitative leaf epidermal characters showed variation among the examined species, irrespective of familial affiliation (Table 2). The observed characters included epidermal cell shape, anticlinal wall pattern, stomatal type, stomatal distribution, epidermal cell arrangement, and epidermal cell wall pattern. Among the Euphorbiaceae species, quantitative measurements of stomatal size and epidermal cell dimensions were also obtained to provide additional information for species-level comparison. These quantitative characteristics are presented in Table 2 and Figures 2–3.

Variation in epidermal cell characteristics was particularly evident among the examined *Phyllanthus* species. The epidermal cell margins showed differences ranging from straight to notched patterns, consistent with observations previously reported for *Phyllanthus* by Uka et al. (2014). These differences indicate that epidermal cell characteristics may provide useful information for distinguishing species within the genus, although their diagnostic value should be considered together with other morphological and anatomical characters. Overall, the observed variation demonstrates that individual epidermal characters may contribute to species-level characterization, while their ability to distinguish the two families requires evaluation using the combined character dataset.

Multivariate Analysis of Epidermal Characters

The qualitative character states presented in Table 1 were coded according to the criteria defined in Table 2, generating the character matrix presented in Table 3. Before multivariate analysis, the taxon names and character states in the matrix were checked against the species list and character descriptions to ensure consistency. The resulting matrix was used for non-metric multidimensional scaling (NMDS) analysis.

The NMDS ordination did not produce two clearly separated groups corresponding to Euphorbiaceae and Phyllanthaceae based on the six selected epidermal characters (Figure 3). Several species from the two families occupied overlapping positions in the ordination space, indicating similarities in their epidermal character profiles. This pattern suggests that the selected leaf epidermal characters do not consistently reflect the familial boundaries between Euphorbiaceae and Phyllanthaceae in the sampled taxa.

Importantly, the absence of distinct family-level clustering in the NMDS ordination should not be interpreted as evidence against the current classification of Euphorbiaceae and Phyllanthaceae, including the framework adopted by APG IV (2016). Rather, the result indicates that the selected epidermal characters have limited discriminatory power for separating the two families in the present sample. Nevertheless, variation observed among species,

particularly in epidermal cell characteristics and stomatal features, suggests that these characters may have greater value for species-level characterization. Further evaluation using a broader set of anatomical characters, quantitative measurements, and a larger taxonomic sampling is required to determine their diagnostic significance.

Table 1. Characters and values for generating in non-metric multidimensional scaling (MDS)

Character	Criteria
1. Adaxial epidermis cell shape	regular (squared or polygonal) (0); irregular shape (1)
2. Abaxial epidermis cell shape	regular (squared or polygonal) (0); irregular shape (1)
3. Adaxial epidermis Cell Margin shape	faceted (1); corrugated (2); notched (3)
4. Abaxial epidermis Cell Margin shape	faceted (1); corrugated (2); notched (3)
5. Stomata on the adaxial side of the leaf	absent (0); present (1)
6. Stomatal type on the abaxial side	diacytic (0); anomocytic (1)

Table 2. Leaf epidermal characters of *Euphorbiaceae* and *Phyllanthaceae* in Mount Merapi National Park, Central Java, Indonesia

Species	Epidermis Cell Shape		Epidermis Cell Margin Shape		Stomatal Type	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Euphorbiaceae						
C. longifolium	irregular	Irregular	corrugated	corrugated	-	diacytic
E. heterophylla	squared, polygonal	Irregular	notched	corrugated	anomocytic	anomocytic
E. hirta	squared, polygonal	squared, polygonal	faceted	corrugated	-	anomocytic
H. populneus	squared, polygonal	squared, polygonal	notched	notched	-	diacytic
M. rhizinoides	irregular	Irregular	corrugated	corrugated	-	diacytic
Phyllanthaceae						
B. macrantha	irregular	squared, polygonal	corrugated	notched	-	diacytic
B. microphylla	squared, polygonal	squared, polygonal	faceted	faceted	-	diacytic
P. cernuus	squared, polygonal	squared, polygonal	faceted	notched	-	diacytic
P. lutescens	squared, polygonal	squared, polygonal	faceted	notched	-	diacytic
P. microcarpus	squared, polygonal	squared, polygonal	faceted	faceted	-	diacytic
P. niruri	irregular	Irregular	corrugated	notched	diacytic	diacytic
P. obliquus	squared, polygonal	squared, polygonal	faceted	notched	-	diacytic

Table 3. Matrix of leaf epidermal characteristics of *Euphorbiaceae* and Phyllanthaceae in Mount Merapi National Park, Central Java, Indonesia

Family/Species	Epidermis Cell Shape		Epidermis Cell Margin Shape		Adaxial Stomata	Abaxial stomatal type
	Adaxial	Abaxial	Adaxial	Abaxial		
Euphorbiaceae						
C. longifolium	1	1	2	2	0	0
E. heterophylla	0	1	2	2	1	1
E. hirta	0	0	1	2	0	1

Family/Species	Epidermis Cell Shape		Epidermis Cell Margin Shape		Adaxial Stomata	Abaxial stomatal type
	Adaxial	Abaxial	Adaxial	Abaxial		
O. populneus	0	0	2	3	0	0
M. rhizinoides	1	1	3	2	0	0
Phyllanthaceae						
B. macrantha	1	0	3	3	0	0
B. microphylla	0	0	1	1	0	0
G. zeylanicum	0	0	1	3	0	0
P. cernuus	0	0	1	3	0	0
P. lutescens	0	0	1	3	0	0
P. microcarpus	0	0	1	1	0	0
P. niruri	1	1	3	3	1	0

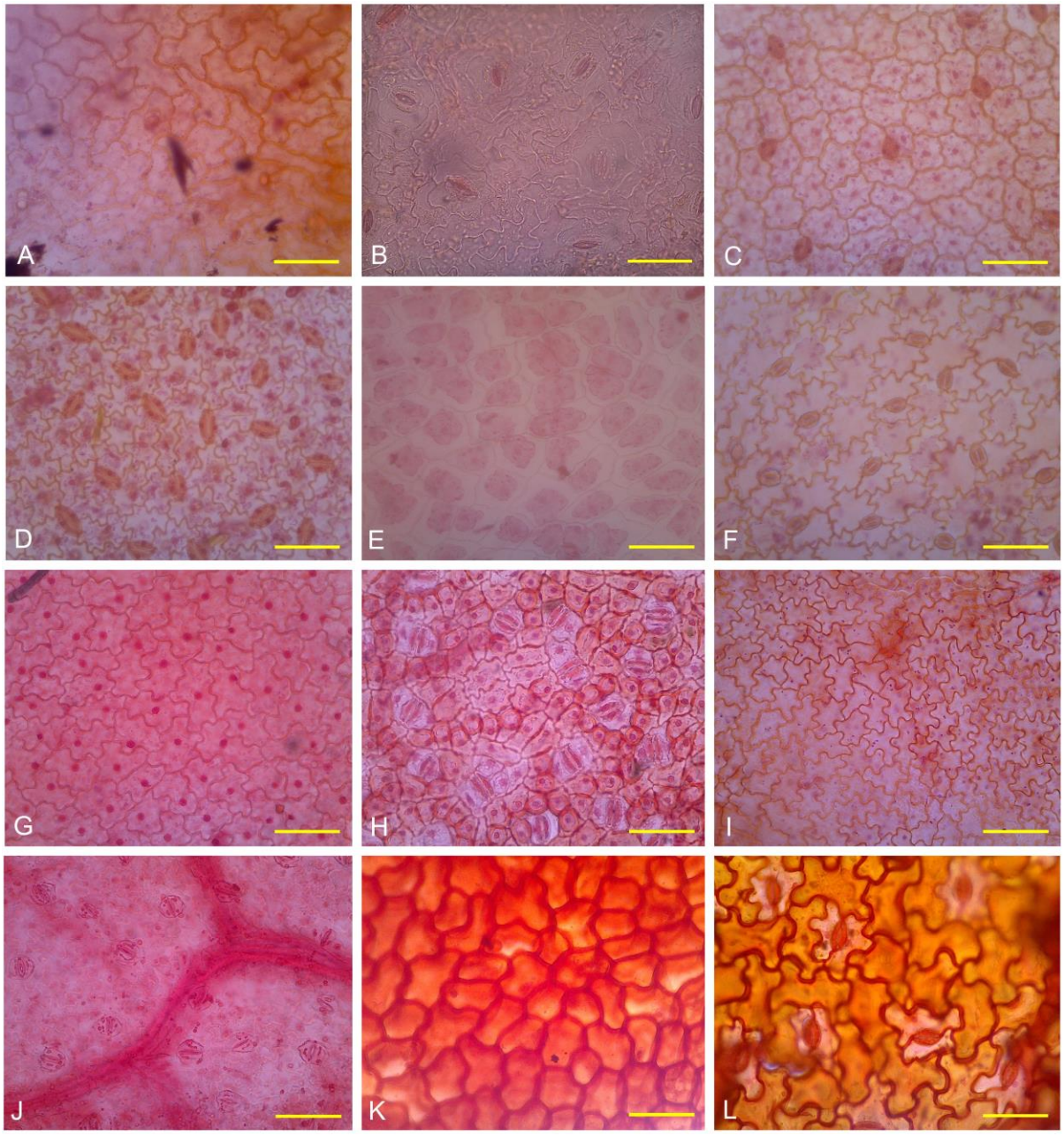

Figure 2. Leaf epidermal characters of some *Euphorbiaceae* (A−B. *C. longifolium*. C−D. *E. heterophylla*.

E–F. *E. hirta*. G–H. *Homalanthus populneus*. I–J. *Macaranga rhizinoides*) and Phyllanthaceae (K–L. *Breynia macrantha*). A, C, E, G, I, K = adaxial; B, D, F, H, J, L = abaxial. Scale: 50 µm.

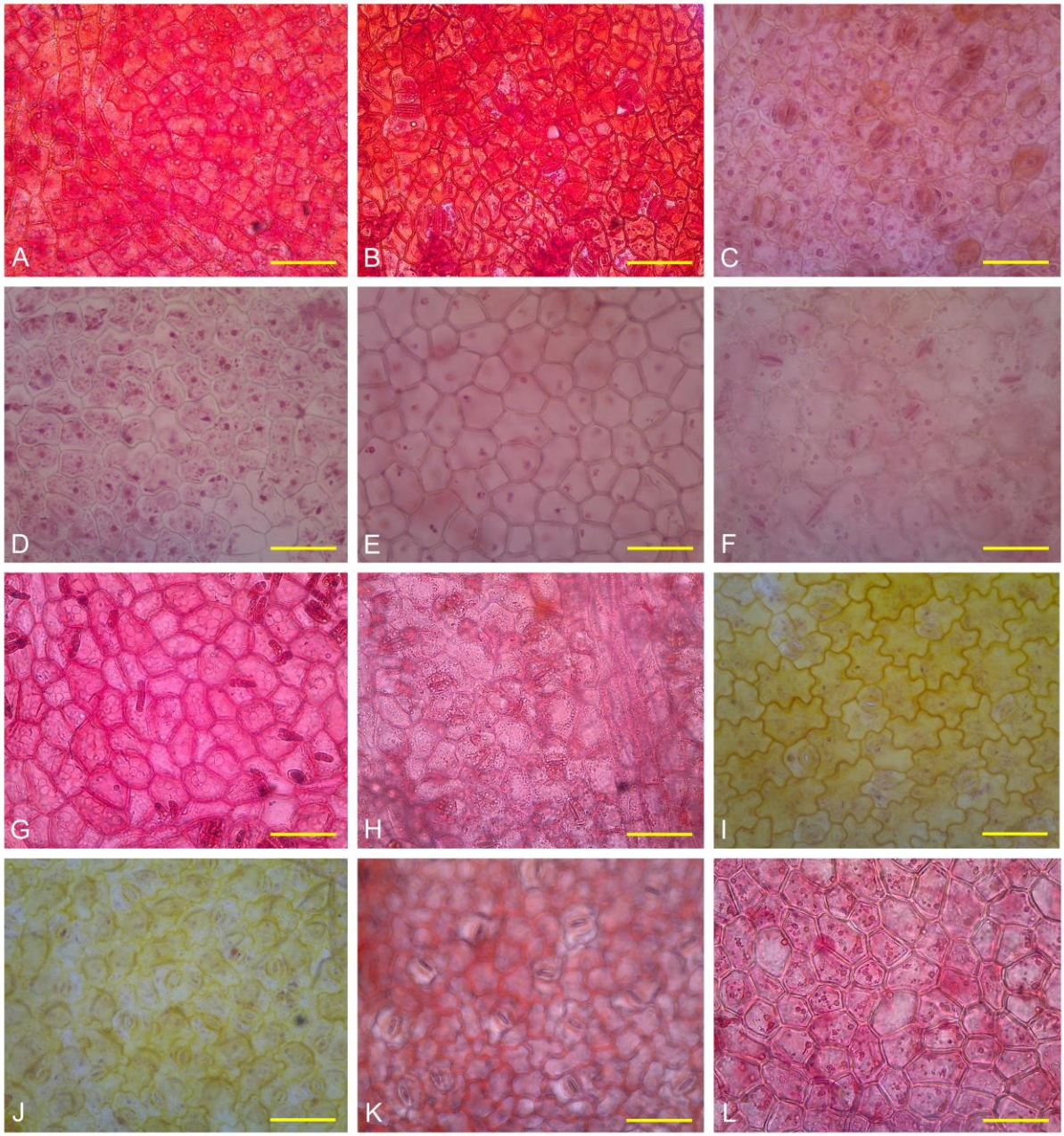

Figure 3. Leaf epidermal characters of Phyllanthaceae (A–B: *Breynia microphylla*. C–D. *Phyllanthus cernuus*. E–F. *Phyllanthus lutescens*. G–H. *Phyllanthus microcarpus*. I–J. *Phyllanthus niruri*. K–L. *Phyllanthus obliquus*). A, C, E, G, I, K = adaxial; B, D, F, H, J, L = abaxial. Scale: 50 µm.

Discussion

Anatomical studies of leaves in species from the families *Phyllanthaceae* and *Euphorbiaceae* reveal significant variations in qualitative leaf epidermal characters such as epidermal cell shape, which can be regular (square or polygonal) or irregular. Some species, such as *P. cernuus* and *P. lutescens*, have regular epidermal cell shapes, whereas others, such as *B. macrantha* and *C. longifolium*, exhibit irregular shapes (Uka et al., 2014).

In addition, the study also found variations in the shape of the epidermal cell margin, which can be faceted (angular), corrugated (wavy), or

notched (indented). This character is important for providing a more detailed description of the leaf epidermal morphology in each species of *Phyllanthus* as also shown by Uka et al. (2014).

The types of stomata observed included diacytic and anomocytic, with stomata on the adaxial surface of the leaves has only been found in two species, i.e. *E. heterophylla* (Euphorbiaceae) and *P. niruri* (Phyllanthaceae). Occurringin both families, the presence of stomata in adaxial leaf surfacescannotbe used to separate thetwo species.

The NMDS analysis based on the six coded leaf epidermal characters showed no clear separation of the sampled species into two groups corresponding to Euphorbiaceae and Phyllanthaceae (Figure 3). The overlapping distribution of species in the ordination suggests that the selected epidermal characters do not consistently discriminate between the two families in the present sample. This result should not be interpreted as evidence against the APG IV classification (2016), which is based primarily on extensive molecular phylogenetic evidence. Rather, it indicates that the selected leaf epidermal characters have limited discriminatory value for assessing familial boundaries, although several characters may remain useful for species-level characterization.

The overlap observed in epidermal characters is particularly evident in features such as epidermal cell shape and margin, as well as stomatal characteristics. Variation in epidermal cell margin among *Phyllanthus* species is consistent with previous observations by Uka et al. (2014), who reported variation in epidermal characteristics within this genus. The present findings therefore support the view that individual leaf epidermal characters should not be used as the sole basis for inferring relationships among higher taxonomic groups. Their diagnostic value is more appropriately evaluated in combination with other morphological, anatomical, and molecular evidence. Although environmental influence or phenotypic plasticity may contribute to variation in leaf anatomical traits, this possibility was not directly tested in the present study and requires comparative investigations involving multiple populations or controlled common-garden experiments.

Phyllanthus niruri is recognized as the type species of *Phyllanthus*, a point of particular taxonomic importance in studies addressing the evolutionary circumscription of the genus (Bouman et al., 2021). Molecular phylogenetic analyses using nuclear and chloroplast markers have provided evidence for relationships among *Phyllanthus* lineages that cannot be inferred reliably from leaf epidermal characters alone. In this context, the placement of *P. niruri* within a molecularly defined clade illustrates the importance of integrating anatomical observations with independent phylogenetic evidence. The present anatomical data therefore provide complementary information rather than an alternative to molecular classification. A multidisciplinary approach combining quantitative leaf anatomy, broader morphological sampling, and molecular phylogenetic markers would be valuable in future studies to determine whether the observed epidermal variation corresponds to evolutionary lineages and to identify characters with stronger taxonomic significance (Moustafa, 2024; Wurdack et al., 2004; Uka et al., 2014).

This study emphasizes the importance of integrating various sources of data to resolve complex taxonomic issues in plant groups with highly variable morphology, such as *Phyllanthaceae* and *Euphorbiaceae*, as well as the need for more modern and comprehensive methodologies (Fayed et al., 2019). This study not only provides important anatomical data on leaves but also reinforces the understanding that modern taxonomic classification must be supported by the integration of anatomical as well as molecular data for greater validity (Kathriarachchi et al., 2005).

Conclusion

Leaf epidermal anatomy provides useful complementary information for characterizing and comparing species of Euphorbiaceae and Phyllanthaceae. In the present study, however, the six qualitative epidermal characters examined in 12 species from a single study area and along a single collection route did not produce clear separation between the two families in the NMDS analysis. Therefore, the observed pattern should be interpreted as evidence of limited discriminatory power of the selected characters within the sampled taxa and should not be

extrapolated to the familial classification as a whole. The findings also represent a single regional sampling and do not include replicated populations across different localities; consequently, they cannot be used to assess the extent of intraspecific variation or environmental effects on epidermal characters. Further studies should incorporate broader taxonomic and geographic sampling, replicated populations and individuals, additional quantitative anatomical traits, and molecular phylogenetic data. Such an integrative approach would provide a stronger basis for evaluating the diagnostic and evolutionary significance of leaf anatomical characters in Euphorbiaceae and Phyllanthaceae.

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