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Original Article

Micromorphological characterization and electron microscopic study of parasitic species of the family Loranthaceae Juss.

Caracterização micromorfológica e estudo microscópico eletrônico de espécies parasitas da família Loranthaceae Juss.

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Abstract

The Loranthaceae Juss. family includes parasitic species that invade important trees such as fruit trees. In Saudi Arabia, Loranthaceae comprises four genera, which include six species that grow in the western, southwestern, and northern regions: *Tapinanthus globifer* (A.Rich.) Tiegh, *Oncocalyx glabratus* (Engl.) M. G. Gilbert, *Loranthella deflersii* (Tiegh.) S. Blanco & C. E. Wetze, *Phragmanthera austroarabica* A. G. Mill. & J. Nyberg, *Plicosepalus curviflorus* (Benth.ex Oliv.) Tiegh. and *Plicosepalus acaciae* (Zucc.). The species present in the Kingdom of Saudi Arabia have not been the subject of enough studies. This work aims to screen and evaluate the taxonomic importance of the micromorphological traits of leaves and fruits in Loranthaceae species native to Saudi Arabia (SA) using scanning electron microscope (SEM). In this study, cluster dendrogram (CD), principal component analysis (PCA) and analysis of variance (ANOVA) were used to evaluate the ability to discriminate Loranthaceae species using micromorphological characteristics. Most of the micromorphological characteristics of the leaf and fruit surfaces used reflected significant variation between the species of Loranthaceae. The type of stomata, trichome, lenticels, fine relief of the cell wall and wax form were the most taxonomically important characteristics. In addition, the cluster dendrogram of morphological characteristics showed species distribution within branches based on affiliation to subtribes Tapinanthinae and Emelianthinae. To the best of our knowledge, the fruit and leaves of the species under study have never been described using electron microscopy, and this study is considered the first of its kind. It also contributes to solving the classification problems of the family Loranthaceae in general and confirms the importance of the characteristics and methods used as tools for characterizing parasitic species that infect trees and helps to verify their identities. This will help to improve resistance efforts and put effective control plans in place.

Keywords: Loranthaceae, mistletoe, SEM, morphology, taxonomy, identification.

Resumo

A família *Loranthaceae Juss.* inclui espécies parasitas que invadem árvores importantes, como árvores frutíferas. Na Arábia Saudita, *Loranthaceae* compreende quatro gêneros que incluem seis espécies que crescem nas regiões oeste, sudoeste e norte: *Tapinanthus globifer (A.Rich.) Tiegh*, *Oncocalyx glabratus (Engl.) M. G. Gilbert*, *Loranthella deflersii (Tiegh.) S. Blanco & CE Wetze*, *Phragmanthera austroarabica AG Mill. & J. Nyberg*, *Plicosepalus curviflorus (Benth.ex Oliv.) Tiegh.* e *Plicosepalus acaciae (Zucc.)*. Em relação às espécies presentes no Reino da Arábia Saudita observamos a falta de estudos, assim este trabalho tem como objetivo rastrear e avaliar a importância taxonômica das características micromorfológicas de folhas e frutos de espécies de *Loranthaceae* nativas da Arábia Saudita (SA) utilizando microscópio eletrônico de varredura (MEV). Neste estudo, foram utilizados dendograma de cluster (CD), análise de componentes principais (PCA) e análise de variância (ANOVA) para avaliar a capacidade de discriminar espécies de Loranthaceae utilizando características micromorfológicas. A maioria das características micromorfológicas das superfícies foliares e frutíferas utilizadas refletiu variação significativa entre as espécies de *Loranthaceae*. O tipo de estômatos, tricomas, lenticelas, fino relevo da parede celular e forma cerosa foram as características taxonomicamente mais importantes encontradas. Além disso, o dendrograma de agrupamento de características morfológicas mostrou distribuição de espécies dentro de ramos com base na afiliação às subtribos *Tapinanthinae* e Emelianthinae. De acordo com nossos achados, os frutos e folhas das espécies em estudo nunca foram descritos por microscopia eletrônica, e este estudo é considerado o primeiro do gênero. O presente trabalho também contribui para resolver os problemas de classificação da família *Loranthaceae* em geral e confirma a importância das características e métodos utilizados como ferramentas para caracterizar espécies parasitas que infectam árvores e ajuda a verificar suas identidades. Portanto, ajudará a melhorar os esforços de resistência e a implementar planos de controlo eficazes.

Palavras-chave: Loranthaceae, visco, MEV, morfologia, taxonomia, identificação.

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1. Introduction

In general, parasitic plants are plants that obtain all or part of their nutrition from another plant (Ashapkin et al., 2023) without benefiting the host and, in some cases, causing extreme damage to the host. Parasitic plants are categorized as either hemiparasites (photosynthetic parasites) or holoparasites (nonphotosynthetic parasites). Mistletoe leads to reduced growth, productivity and, eventually, death of host trees, particularly during unfavorable weather conditions and if the host plant is only a shrub or a small tree (Menezes et al., 2022).

The two largest groupings of parasitic plants, Orobanchaceae and Santalales, both contain hemi- and holoparasites (Těšitel, 2016). The Loranthaceae family in order Santalales is widespread in tropical and warm temperate settings. Most of them are characterized by growth in important environments such as mangroves (Barlow, 1997). More than 1000 species and 73 genera were discovered from this family (Vidal-Russell and Nickrent, 2008; Alqthanin, 2011; Grímsson et al., 2018). The family is currently divided into five tribes (Psittacantheae, Nuytsieae, Elytrantheae, Gaiadendreae and Lorantheae) and eleven subtribes (Suárez et al., 2021). Species of Loranthaceae have a history of misidentifications because of the overlapping delimitation features between species or differences among individuals of the same species and the lack of adequate knowledge of the taxonomy of the family.

Loranthacese in Saudi Arabia includes six species that grow in the western, southwestern, and northern regions of the Kingdom of Saudi Arabia: *Tapinanthus globifer*, *Oncocalyx glabratus*, *Loranthella deflersii*, *Phragmanthera austroarabica*, *Plicosepalus curviflorus* and *Plicosepalus acacia* (Alqthanin, 2011). The subtribe Emelianthinae includes species *Phragmanthera austroarabica*, while species *Plicosepalus acacia*, *Plicosepalus curviflorus*, *Loranthella deflersii*, *Oncocalyx glabratus*, and *Tapinanthus globifer* belong to subtribe Tapinanthinae. Loranthaceae parasitism affects the quality of fruit in Saudi Arabia (Adesina et al., 2013).

Previous studies have discussed some morphological taxonomic features in Loranthaceae; for example, Mbagwu et al. (2009) screened leaves of five variants of the genus *Viscum* L. (Loranthaceae) and confirmed that the presence of Calcium oxalate crystals within the chloroplasts had a taxonomic characteristic. Waly (2013) conducted an anatomical study of six parasitic Loranthaceae species and mentioned several important taxonomic characteristics of the leaves and stems. The most important characteristics in leaves were the number of midrib and lateral vascular bundles, the form of collenchyma, and the supporting tissue covering the vascular bundles, while the type of axial parenchyma and rays represented the most significant characteristics in the stems. In addition, a comparative morphological study on six Loranthaceae species growing in Saudi Arabia determined the phytochemical properties and effectiveness of these plants as medicinal herbs (Waly et al., 2012). The results revealed the presence of flavonoids and steroids and/or terpenoids as major constituents.

Furthermore, a taxonomic review of the family Loranthaceae in Nigeria was performed by Ibrahim and Ayodele (2013), which mentioned the taxonomic importance of leaf epidermal characteristics. Waly et al. (2012) screened the anatomical leaves and stems of six Loranthaceae species growing naturally in Saudi Arabia: *Plicosepalus acacia, Plicosepalus curviflorus, Phragmanthera austroarabica, Loranthella deflersii*, *Oncocalyx glabratus*, and *Tapinanthus globifer*. The results confirmed that these species had useful taxonomical characteristics, such as the type of leaves mesophyll (isolateral and isobilateral), the number of main vascular bundles, the shape of supporting collenchyma tissue in the leaf, the type of axial parenchyma in the stem, the presence or absence of crystals in the leaves and ray widths in the stems. These characteristics can be used as tools in the identification and understanding of phylogenetic relationships (Table A1).

Although there have been morphological and anatomical studies that included some species of mistletoe, there is still an urgent need for more studies on morphological and especially micromorphological characteristics. In addition, there is a contradiction in some results regarding the importance of these characteristics and their taxonomic ability.

This work focused on Loranthaceae species native to the Kingdom of Saudi Arabia, and the aim was to screen the microcharacteristics of leaves and fruits in Loranthaceae species using SEM and evaluate the taxonomic importance of micromorphological traits in Loranthaceae species.

2. Methods

2.1. Plant material collection and identification

In this research, samples of Loranthaceae species from Saudi Arabia were collected from the field for morphological study. These samples were collected through 2021 and 2022 from six areas of Saudi Arabia, and the locality, latitude, and longitude of each site were noted for each sample that was gathered (Table 1). Samples were identified by experts at Sultan bin Abdulaziz Center for Research and Environmental Studies at King Khaled University, Abha (KKU), based on herbarium specimens and morphologies in the relevant literature. Voucher specimens were prepared and deposited at the Herbaria of Biology Department, Faculty of Applied Science, Umm Al-Qura University, Mecca/KSA (UQU). In addition, this study included herbarium samples of the Loranthaceae family collected from King Khaled University. For the herbarium specimens, significant details were noted, including sample number, the name of the collector, location, and the date of collection.

2.2. Screening micromorphological traits using scanning electron microscopy

The leaf abaxial (AB) and adaxial (AD) surfaces of the species under this study and fruit samples were mounted onto stubs with double-sided adhesive tape, coated for 2 min with gold in a polaron JFC-1100E coating unit, and then examined and photographed with a JEOL JSM-IT200 in Research Laboratories Centre, Faculty of Applied Science at Umm Al-Qura University, and Electron Microscope Unit in Taif University. The quantitative and qualitative features of the epidermal cells of both leaf surfaces (AB and AD) were recorded, including data on leaf surface structures,

Table 1. List of the study samples of Loranthaceae from Saudi Arabia along with information on sample numbers, code, and locations.

No	Scientific Name	Samples code	Locality	Collector	Longitude & Altitude	Voucher
1	*Tapinanthus globifer* (A.Rich.) Tiegh.	Tg	Wadi Alreem	Rahmah Alqthanin	17.9805724°, 42.2380263°	T10011 (KKU)
2	*Oncocalyx glabratus (Engl.)* M. G. Gilbert	Og	Thageef village	Noha Althagafi	20.6197578°, 40.9233393°	G1001 (UQU)
3	*Loranthella deflersii* (Tiegh.) S. Blanco & C. E. Wetze	Ld	Abha – Khamis mushat	Noha Althagafi	18.185097°,42.818443°. 18,299187°,42.497780°.	S1002 (UQU)
4	*Phragmanthera austroarabica* A. G. Mill. & J. Nyberg	Pau	Al-Taef, Gabel Ibrahium Wadi leiah	Noha Althagafi	20.402047°,41.138292°. 18.1913686°,42.8226008°. 18.0602509°,42.7076538°. 18.301805°,42.496372°	P1005 (UQU)
5	*Plicosepalus curviflorus* (Benth.ex Oliv.) Tiegh.	Pc	Wadi leiah	Noha Althagafi	21.218874°,40.557426°. 20.637140°,41.275528°. 18.1913686°,42.8226008°.	N C1008 (UQU)
6	*Plicosepalus acaciae* (Zucc.)	Pa	Tabouk Alola Alwajh	Noha Althagafi	26.5737260°,36.3731150° 26.7392410°,37.1740530°	A1003 (UQU)

both closed and opened stomata, i.e., length and width, as well as the observed surface patterns for fruits (Table 2), following the terminology described in (Barthlott et al., 1998; Özcan, 2002), and measured by ImageJ analysis software (Schneider et al., 2012).

2.3. Statistical analysis

The twenty-eight quantitative micromorphological characteristics (Table 2) were directly used to identify the discrimination power, and the morphometric dataset was used for principal component analysis (PCA) (Sneath and Sokal, 1973). The PCA was performed in XLSTAT version 2023.1.1 Lumivero (2023). Using this analysis helps determine the ability of used characteristics (variables) to clustered of samples based on their similarity. The results of PCA are presented as summary statistics of morphological characteristics and graphs by loading plots and cluster loading for phenotypes to show the relationships between the species.

The matrix of correlation between morphological characteristics was calculated in XLSTAT version 2023.1.1. Lumivero (2023) and was used to construct a heatmap in GraphPad Prism software Version 9.5.1 to make it easier to observe the correlation between morphological characteristics (GraphPad, 2023).

A box plot and one-way analysis of variance (Ashapkin et al., 2023) were performed to analyze the differences between species of Loranthaceae in this study. The application of this analysis indicates if there is a significant variation in micromorphological characteristics between the study species. The significance of the statistics was calculated based on 95 permutations, and the R squared value was calculated. In statistics, R squared (R^2) is the proportion of the variation in the dependent variable that is predictable from the independent variable(s) (Sneath and Sokal, 1973; Glantz et al., 2016). The value ranges from (0.1) to (1). If this ratio is high, it means that the errors in the data are small. A good R squared value is 0.9 or above and shows a high correlation, whereas a value below 0.5 would show a low correlation.

The cluster dendrogram based on morphological characteristics was constructed using the 'factoextra' package Version 1.0.7 Kassambara and Mundt (2017) and RStudio software Version 2.0 (RStudio Development Core Team, 2020) to observe the relationships between loranthaceae species based on morphological characteristics. Samples will distributed into branches and sub-branches based on the distance (dissimilarity) between them.

3. Results

3.1. Descriptive characteristics of epidermis, stomata, and fruit surface

Table A2, and Figures 1-13, represents the characteristics of the epidermis and stomata of the leaves, as well as the fruit surface characteristics of the six species under investigation.

Overall, screening of the micromorphological characteristics of leaves and fruits in this study showed that *P. austroarabica* was distinguished by highly ribbed leaf surfaces covered in circle-shaped lenticels, nonglandular stellate trichomes, and sunken stomata on the AB and AD surfaces (Figure 1). Additionally, it has fruit covered in nonglandular stellate trichomes with central discs (Figure 9). *L. deflersii* is characterized by superficial stomata and the presence of two types of nonglandular stellate trichomes, one with and the other without a central disc, in fruits (Figures 2 and 10). *O. glabratus* had sunken stomata, and the fruit surface was highly ribbed and covered in a small amount of smooth crust (Figures 3 and 11). *P. curvifloru* was distinguished by thick crusts covering the AD and AB leaf surfaces and sunken stomata (Figure 4). *P. acacia* was distinguished by highly ribbed AD leaf surface thick crusts on AD and AB leaf surfaces, with sunken stomata (Figure 5) and fruit surfaces covered by wax granules (Figure 12). *T. globiferus* is distinguished by being covered entirely in platelet crusts, which covers both AD and AB leaf surfaces in addition to raised stomata (Figure 6).

Table 2. Quantitative and qualitative characteristics of epidermal cells and stomatal index characteristics that have been estimated in Loranthaceae species native to the KSA.

Organ	Characteristics	
	Descriptive	**Quantitative**
Leaf	**Abaxial leaf (AB)**	**Abaxial leaf (AB)**
	Epidermal cell shape	Stomatal Pore Length
	Periclinal walls (PW)	Stomatal Pore Width
	Fine relief of the cell wall and wax form	Stomatal Pore Area
	Trichome type	Stomatal Complex Length
	Trichome surface	Stomatal Complex Width
	Trichomes length/µm	Stomatal Complex Area
	Trichomes width/µm	Epidermal Cell Length
	Lenticels type	Epidermal Cell Width
	Lenticels surface	Subsidiary Cell Length
	Lenticels length	Subsidiary Cell Width
	Lenticels width	Subsidiary Cell Area
	Stomata type	Number of Stomata
	Stomata level	Number of Epidermal Cells
	Guard cell surface	Stomatal Index
	Pore shape	
	Adaxial leaf (AD)	**Adaxial leaf (AD)**
	Epidermal cell shape	Stomatal Pore Length
	Periclinal walls (PW)	Stomatal Pore Width
	Fine relief of the cell wall and wax form	Stomatal Pore Area
	Trichome type	Stomatal Complex Length
	Trichome surface	Stomatal Complex Width
	Trichomes length/µm	Stomatal Complex Area
	Trichomes width/µm	Epidermal Cell Length
	Lenticels type	Epidermal Cell Width
	Trichome surface	Subsidiary Cell Length
	Lenticels length	Subsidiary Cell Width
	Lenticels width	Subsidiary Cell Area
	Stomata type	Number of Stomata
	Stomata level	Number of Epidermal Cells
	Guard cell surface	Stomatal Index
	Pore shape	
	Distribution of stomata in (AB)&(AD) surfaces	
Fruit	Fine relief of fruit surface and wax form	
	Trichome type	
	Trichome surface	
	Trichomes length/µm	
	Trichomes width/µm	

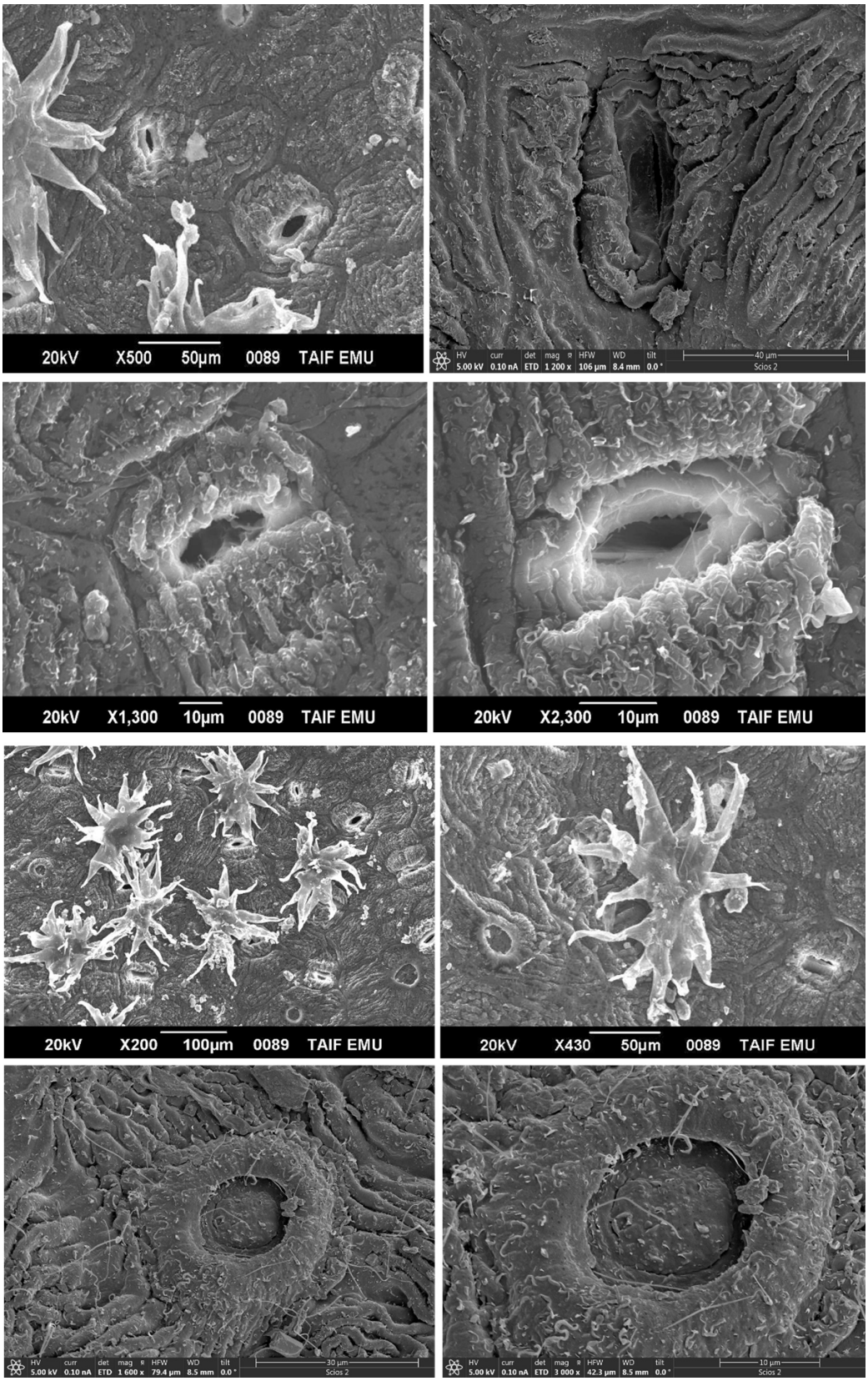

Figure 1. SEM at different magnifications of leaf *Phragmanthera austroarabica* showing highly ribbed cell walls with sunken stomata. Nonglandular stellate trichomes. Circle-shaped lenticels covered in crusts and transitional coiled-rodlets of wax.

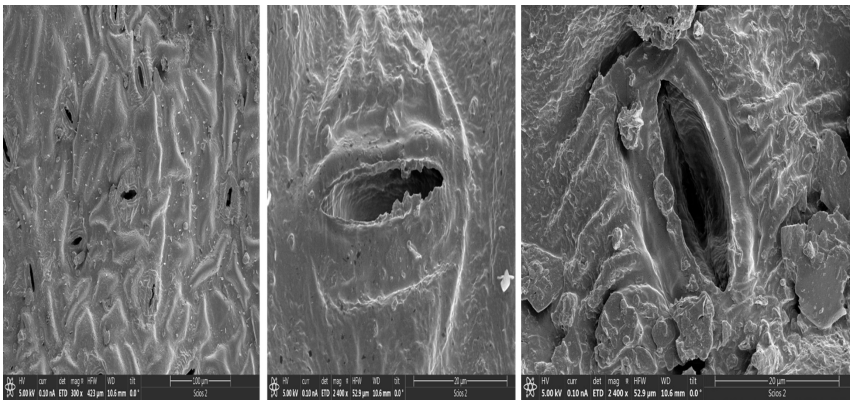

Figure 2. SEM of *Loranthella deflersii* oblong to polygonal epidermal cells, superficial stomata. Stomata covered in granules of wax and crust.

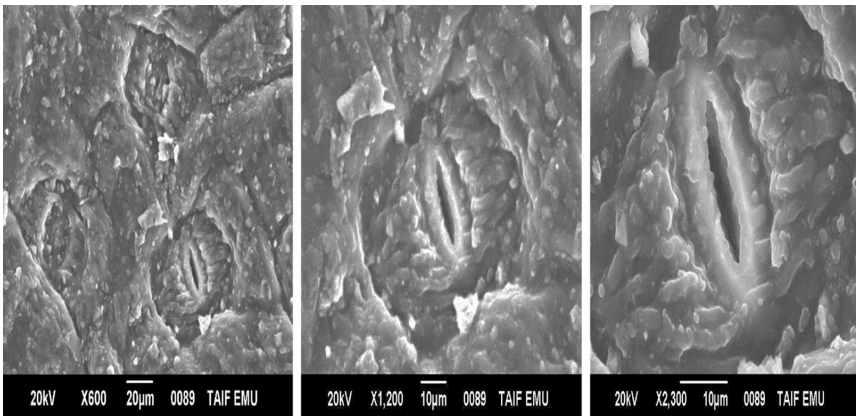

Figure 3. SEM of *Oncocalyx glabratu* oblong epidermis cells covered in granular wax. Stomata Sunken.

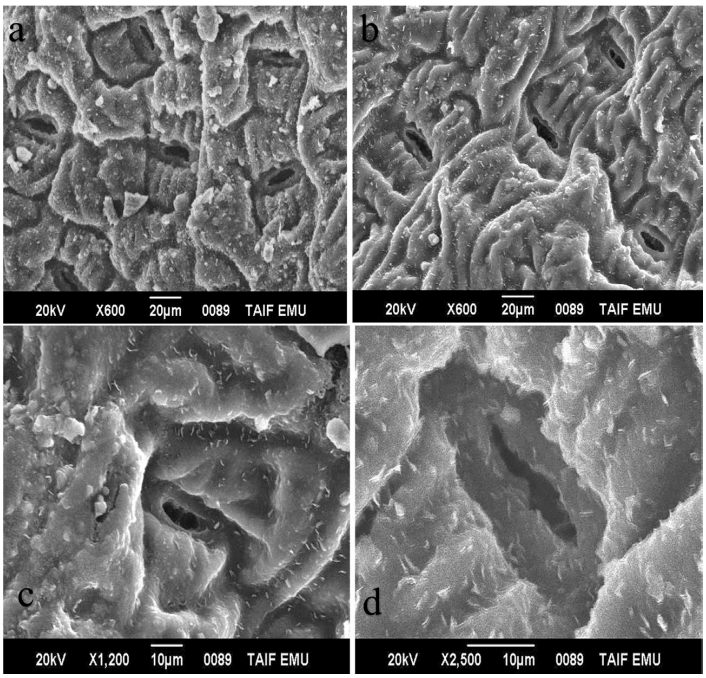

Figure 4. SEM of *Plicosepalus curviflorus*; (a) Moderate ribbed cell wall on the AD surface; (b) Highly ribbed cell wall on the AB surface; (c) Stomata Sunken; (d) Guard cells covered in crusts.

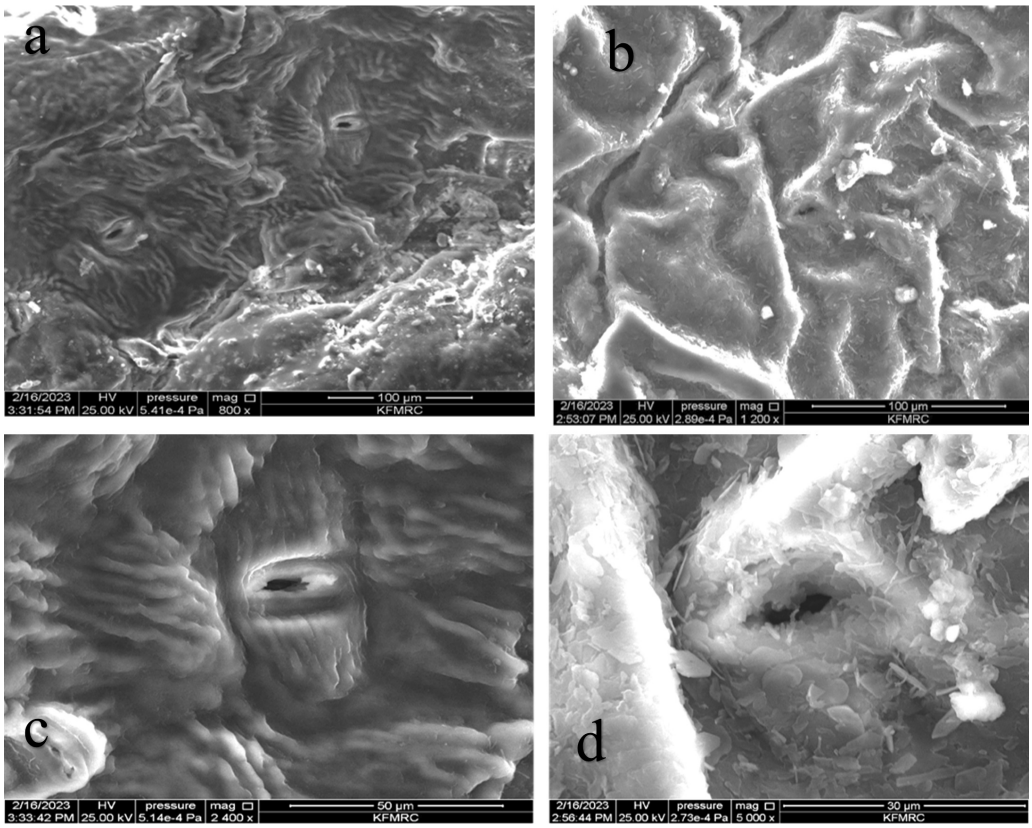

Figure 5. SEM of *Plicosepalus acacia*; (a) Highly ribbed cell wall on AD surface; (b) Moderate ribbed cell wall on the AB; (c) Stomata oval to oblong sunken; (d) Guard cells covered in thick crusts, especially on the AB leaf surface.

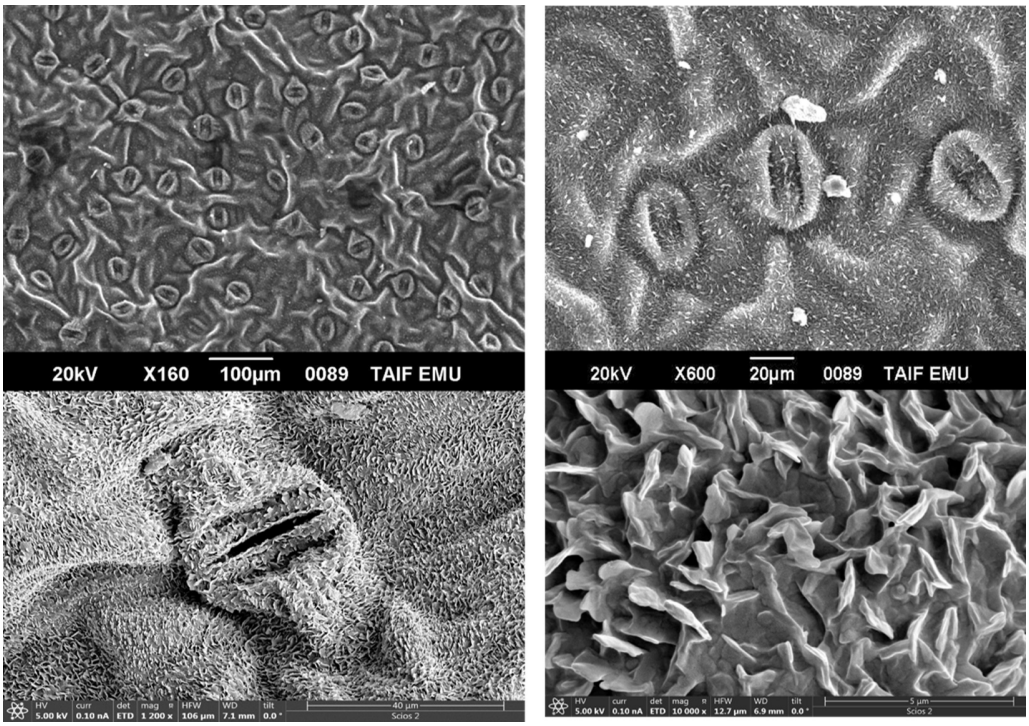

Figure 6. SEM at different magnifications of the leaves of *Tapinanthus globifer*; oblong to bone-shaped epidermal cells. Circular to oblong raised stomata, stomata covered in entire platelets.

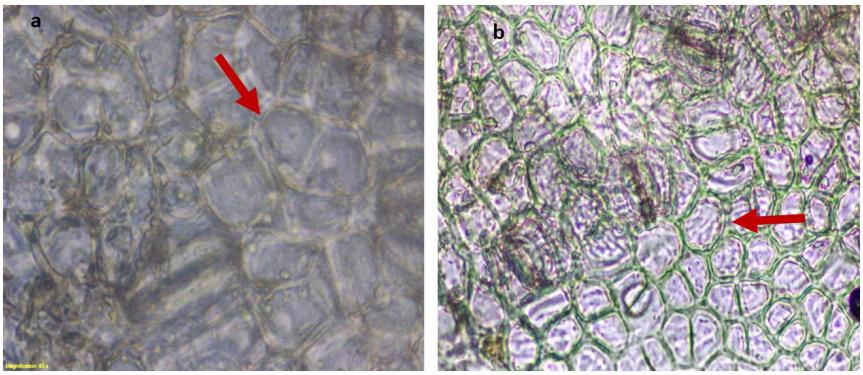

Figure 7. LM in leaves of *Phragmanthera austroarabica* (a and b) showing rounded PW.

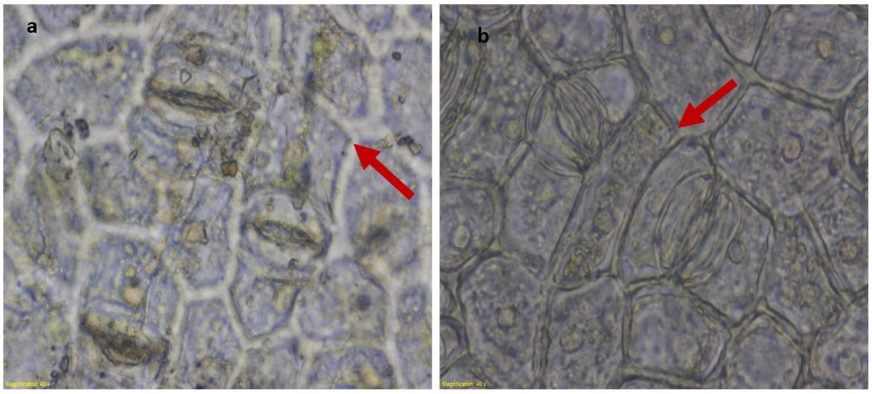

Figure 8. LM in leaves of (a) *Plicosepalus acaciae* and (b) *Plicosepalus curviflorus* showing smooth and angular periclinal walls PW.

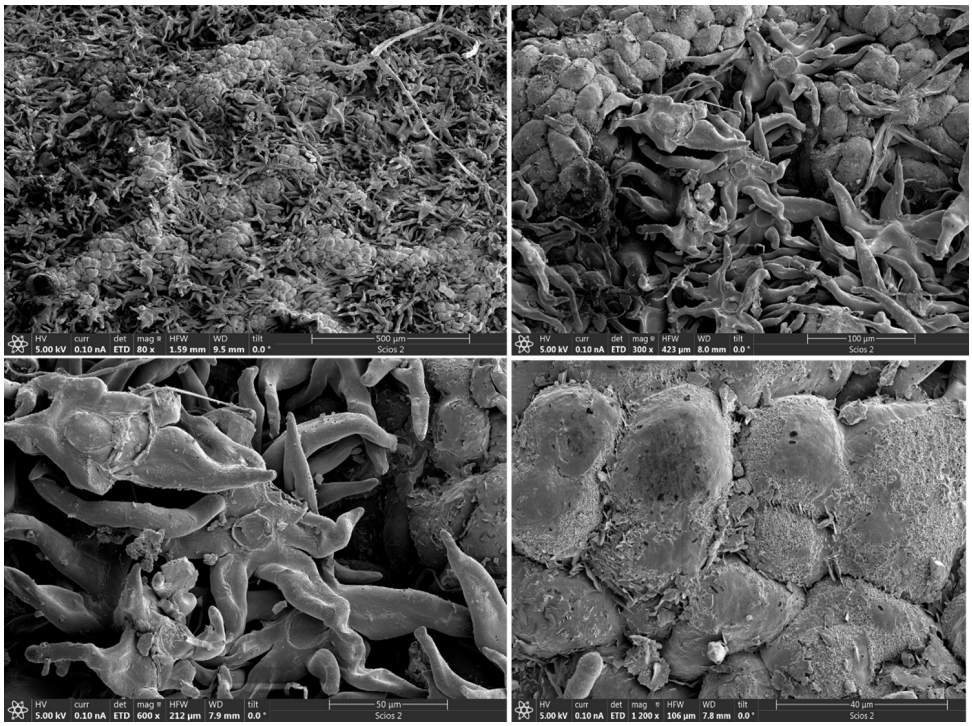

Figure 9. SEM of *Phragmanthera austroarabica* fruit surface covered with nonglandular stellate trichomes and clusters of spherical cells covered by fissured layers of wax and crusts.

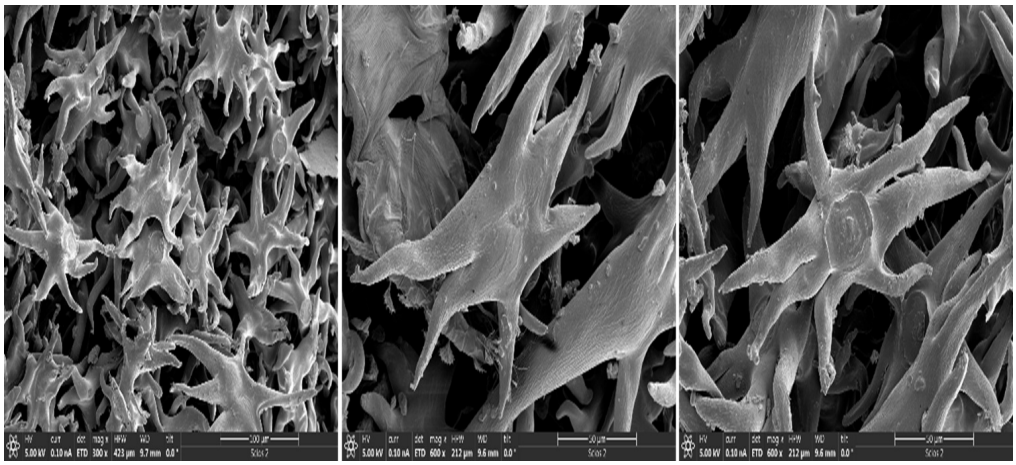

Figure 10. SEM of *Loranthella deflersii* fruit surface covered with two types of nonglandular stellate trichomes with a disc in the center and without a center-disc.

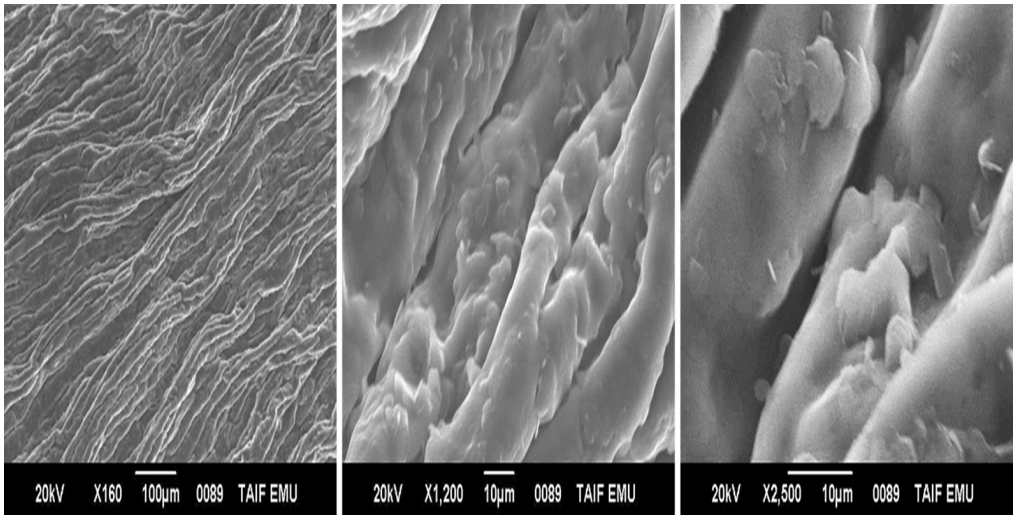

Figure 11. SEM of *Oncocalyx glabratus* fruit surface highly ribbed and covered by smooth and few granules of wax.

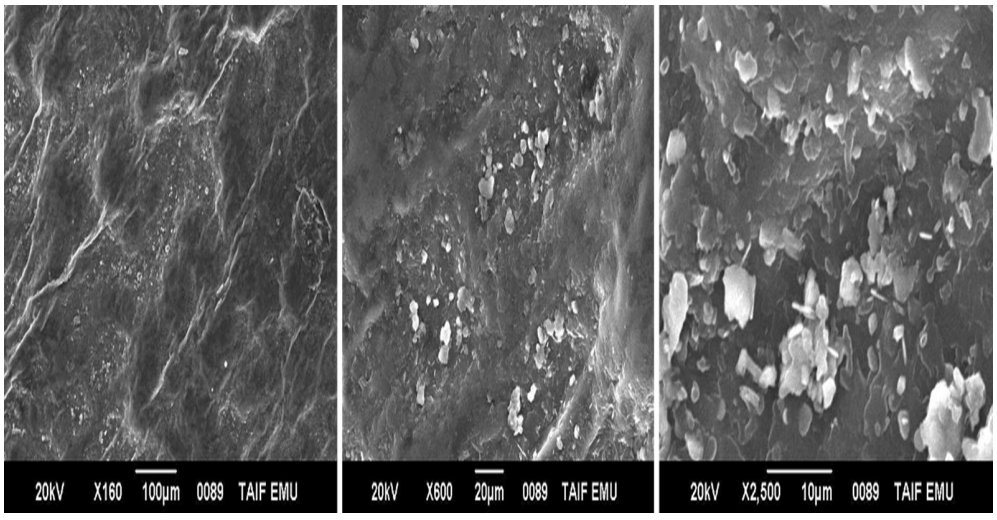

Figure 12. SEM of *Plicosepalus curviflorus* fruit surface covered by fissured layers and few granules of wax.

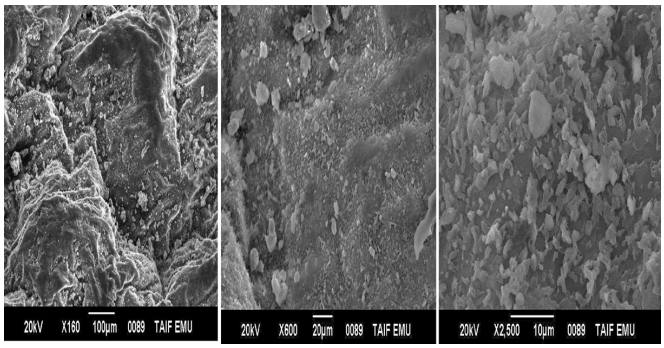

Figure 13. SEM of *Plicosepalus acaciae* fruit surface, less ribbing and densely covered with granules of wax.

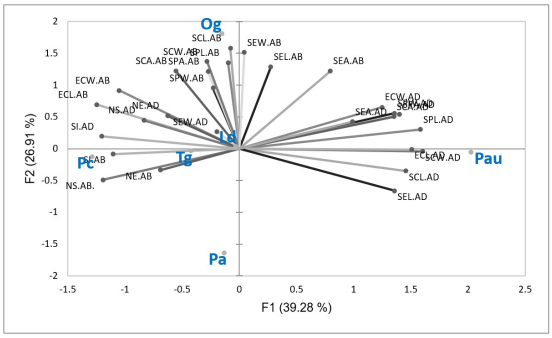

Figure 14. Loading plots and cluster loading for Loranthaceae species of the KSA by the two axes principal components 1/2, based on observation of the micromorphological characteristics of the stomata complex and epidermal cells. The characteristic code is available in Table 4.

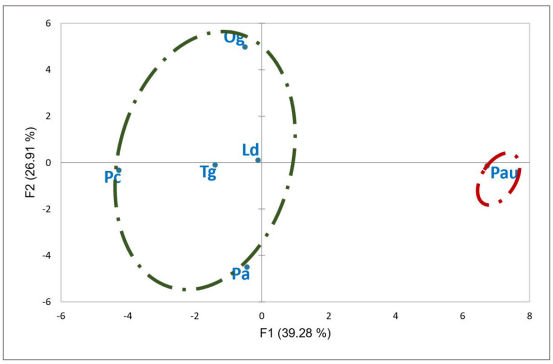

Figure 15. Clusters loading for Loranthaceae species of the KSA, by the two axes principal components 1/2, based on observation of Micro- morphological characteristics of stomata complex and epidermis cell. Sample code available in Table 1. The dotted circle indicates the subtribe. Tapinanthinae (green circle) and subtribe. Emelianthinae (red circle).

3.2. Statistical analysis results

There was obvious variation in the PCA results of the micromorphological characteristics of the epidermis and stomata between the Loranthaceae species under this study. This was reflected in the distribution of samples in different groups based on the results of the PCA and cluster dendrogram.

Table 3 and Figure B1 (Appendix B) show Axis 1 and 2 PCA with high eigenvalues (discrimination power) in PCA (39.276, and 26.915). Features that had the highest positive and negative loading in Axes 1 and 2 are shown in Table 4 and were deduced from loading plot Figure 14 and Figure B2. Stomatal complex width in adaxial leaf surface (AD) and epidermal cell length in AD surface had the highest positive and negative loadings in Axis 1 (0.984 and -0.768). The stomatal complex length width in adaxial leaf surface (AD) and subsidiary cell length (AD) surface had high positive and negative loadings on Axis 2 (0.974 and -0.407).

In accordance with the PCA Axes 1 and 2 results, stomatal complex characteristics that are important in discrimination and capable of separating the samples into groups according to genera and species are “stomatal complex length AD (SCL.AD), Stomatal Complex Width (SCW.AD), Epidermal Cell Length AD (ECL. AD), and Stomatal Pore Length AB” (SPL.AB), which is also evidenced by Figures 14 and 15.

The heatmap in Figure 16 shows the correlation matrix between the micromorphological characteristics of stomata and epidermis, and the most positive correlation was between features of stomatal pore area AD (SPA.AD) and stomatal complex area AD (SCA.AD). The most negative correlation was between stomatal pore length AD (SPL.AD) and stomatal index AD (SI.AD).

The relationships between Loranthaceae species in this study are presented in a cluster dendrogram based on the micromorphological characteristics of the stomatal complex and epidermis (Figure 17). The Lorantheae species were divided into two main clusters.

Table 3. Eigenvalues of Principal Component Analysis based on stomata complex and epidermis cell.

	F1	F2	F3	F4	F5
Eigenvalue	10.997	7.536	4.913	2.746	1.807
Variability (%)	**39.276**	**26.915**	17.548	9.808	6.454
Cumulative %	39.276	66.190	83.738	93.546	100.000

Bold text indicates to axes with high Eigenvalues values.

Table 4. Characteristic loadings in PCA based on stomatal complex and epidermal cell characteristics.

Characters	Code	F1	F2	F3	F4	F5
Stomatal Pore Length Ab	SPL.Ab	-0.061	0.833	-0.494	0.021	-0.242
Stomatal Pore Width Ab	SPW.Ab	-0.141	0.584	-0.237	0.759	-0.081
Stomatal Pore Area Ab	SPA.Ab	-0.168	0.748	-0.328	0.102	0.543
Stomatal Complex Length Ab	SCL.Ab	-0.049	**0.974**	-0.154	0.084	-0.136
Stomatal Complex Width Ab	SCW.Ab	-0.174	0.844	-0.355	0.100	-0.348
Stomatal Complex Area Ab	SCA.Ab	-0.342	0.754	-0.310	0.005	0.468
Epidermal Cell Length Ab	ECL.Ab	-0.**768**	0.428	0.379	-0.056	0.284
Epidermal Cell Width Ab	ECW.Ab	-0.646	0.563	0.429	-0.001	0.285
Subsidiary Cell Length Ab	SEL.Ab	0.169	0.791	-0.003	-0.274	-0.520
Subsidiary Cell Width Ab	SEW.Ab	0.026	0.932	-0.052	0.072	-0.351
Subsidiary Cell Area Ab	SEA.Ab	0.489	0.752	0.302	-0.268	0.180
Number of Stomata Ab	NS.Ab.	-0.733	-0.300	0.469	0.380	-0.098
Number of Epidermal Cells Ab	NE.Ab	-0.425	-0.207	0.050	0.840	-0.262
Stomatal Index Ab	SI.Ab	-0.677	-0.051	0.641	-0.303	-0.187
Stomatal Pore Length Ad	SPL.Ad	0.974	0.187	0.042	0.120	0.031
Stomatal Pore Width Ad	SPW.Ad	0.836	0.342	0.317	-0.089	-0.275
Stomatal Pore Area Ad	SPA.Ad	0.864	0.329	0.274	0.176	0.200
Stomatal Complex Length Ad	SCL.Ad	0.896	-0.217	0.225	0.311	-0.038
Stomatal Complex Width Ad	SCW.Ad	0.**984**	-0.025	0.090	-0.128	0.077
Stomatal Complex Area Ad	SCA.Ad	0.831	0.308	0.304	0.217	0.273
Epidermal Cell Length Ad	ECL.Ad	0.926	-0.007	0.326	-0.143	-0.127
Epidermal Cell Width Ad	ECW.Ad	0.767	0.403	0.334	-0.371	0.010
Subsidiary Cell Length Ad	SEL.Ad	0.835	-0.**407**	0.295	0.155	-0.162
Subsidiary Cell Width Ad	SEW.Ad	-0.120	0.163	0.759	0.613	-0.079
Subsidiary Cell Area Ad	SEA.Ad	0.608	0.259	0.526	0.427	0.323
Number of Stomata Ad	NS.Ad	-0.514	0.275	0.804	-0.089	-0.068
Number of Epidermal Cells Ad	NE.Ad	-0.386	0.319	0.829	-0.181	-0.170
Stomatal Index Ad	SI.Ad	-0.740	0.120	0.619	-0.226	0.059

Bold text indicates to high positive and negative values in PCA axes 1 and 2.

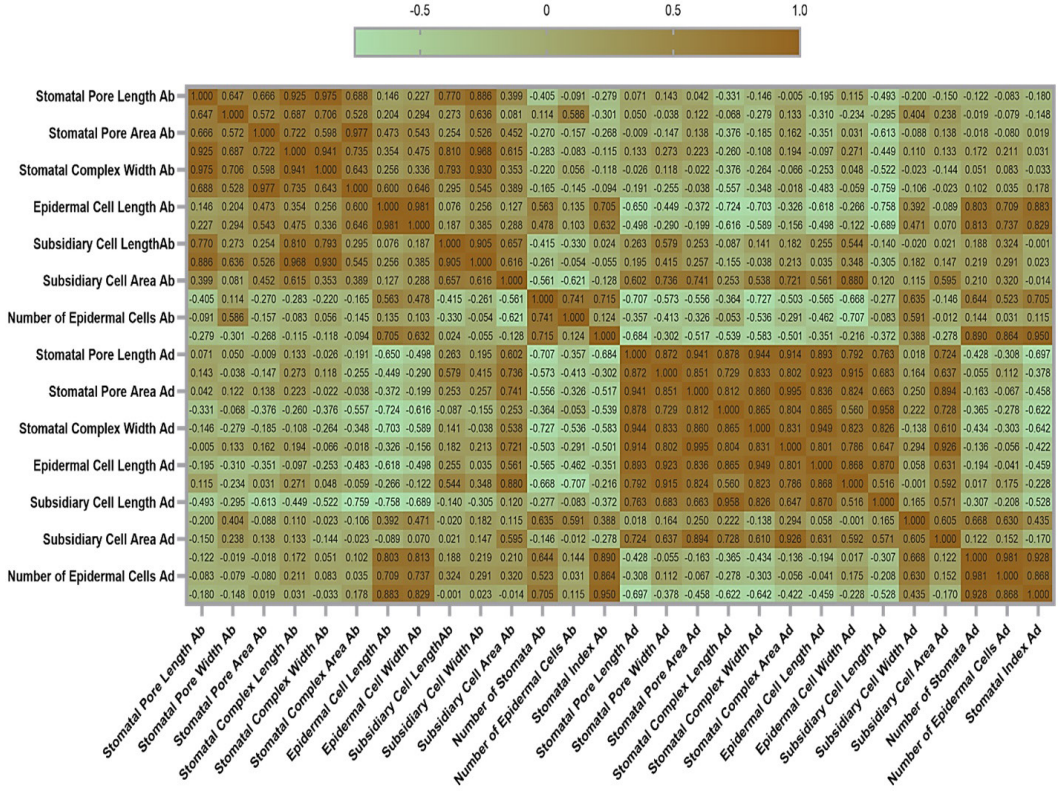

Figure 16. Heatmap of the correlation matrix between the morphological characteristics of the stomatal complex and epidermal cells evaluated in the current study. Dark brown indicates a high positive correlation. Light green indicates a high negative correlation.

The first cluster contained *Phragmanthera austroarabica* (Pau) species and represented subtribe Emelianthinae. The second main cluster divided into two subclusters, first one included *Plicosepalus acacia* (Pa), while the second subclusters included species *Plicosepalus curviflorus* (Pc), *Tapinanthus globifer* (Tg), *Loranthella deflersii* (Od), and *Oncocalyx glabratus* (Og). all species in the second main cluster are belongs to subtribe Tapinanthinae.

The results of ANOVA (Table 5) showed that most of the micromorphological characteristics of the epidermis and stomata reflect high variation between Loranthaceae species, particularly stomatal complex length, stomatal complex width, stomatal complex area, epidermal cell length, epidermal cell width, subsidiary cell length, subsidiary cell width, subsidiary cell area, stomatal pore width, and stomatal pore area, with P value <0.0001 and R squared= 0.770 to 0.960. The characteristic of stomatal pore length represented moderate variation between species, P value= 0.007 and R squared= 0.645. However, features such as the number of stomata, number of epidermal cells, and stomatal index were less significant in the variance between species according to ANOVA.

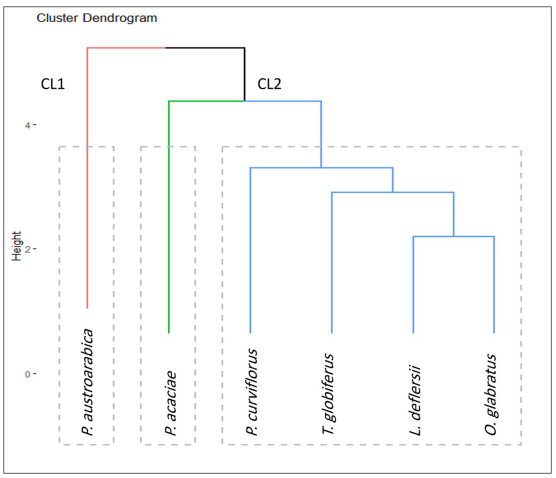

Figure 17. Cluster dendrogram for Loranthaceae species local to the KSA based on observations of the morphological characteristics of stomatal complexes and epidermal cells. sample code available in Table 1.

Oncocalyx glabratus had the largest values recorded for the subsidiary cell length, subsidiary cell width (Figures 18a, b), and stomatal complex length, and stomatal complex traits on the AD leaf surface, which is clear in the results of the boxplot shown in Figure B3, with mean=53.151, 67.252, 61.772, and 37.578, maximum= 60.032, 70.836, 67.025, and 46.866, minimum value= 42.569, 63.032, 55.085, and 30.961, standard deviation SD= 5.972, 3.431, 4.630, and 5.617, respectively, Table A3 in Appendix A.

Phragmanthera austroarabica presented the highest variation in epidermal cell length, and subsidiary cell area (Figures 18c, d), stomatal complex area, epidermal cell width, and stomatal pore area (Figure B4) characteristics on the leaf AD surface, with means of 112.291, 86.828, 115.031, 78.396, and 51.896, respectively. Maximum= 132.467, 94.675, 135.91, 89.317, and 82.465, minimum value= 95.532, 80.474, 95.632, 69.63, and 42. SD= 13.364, 5.186, 14.903, 9.064, and 15.612, respectively, Table A3 in the Appendix A.

Table 5. ANOVA results showing P values and R squared values for stomatal complex and epidermal cell characteristics.

Characteristics	P value	R squared
Stomatal Pore Length	0.007	0.645
Stomatal Pore Width	0.000	0.770
Stomatal Pore Area	0.000	0.949
Stomatal Complex Length	<0.0001	0.886
Stomatal Complex Width	<0.0001	0.871
Stomatal Complex Area	<0.0001	0.860
Epidermal Cell Length	<0.0001	0.924
Epidermal Cell Width	<0.0001	0.781
Subsidiary Cell Length	<0.0001	0.927
Subsidiary Cell Width	<0.0001	0.837
Subsidiary Cell Area	<0.0001	0.960
Number of Stomata	0.307	0.740
Number of Epidermal Cells	0.937	0.391
Stomatal Index	1.798	0.643

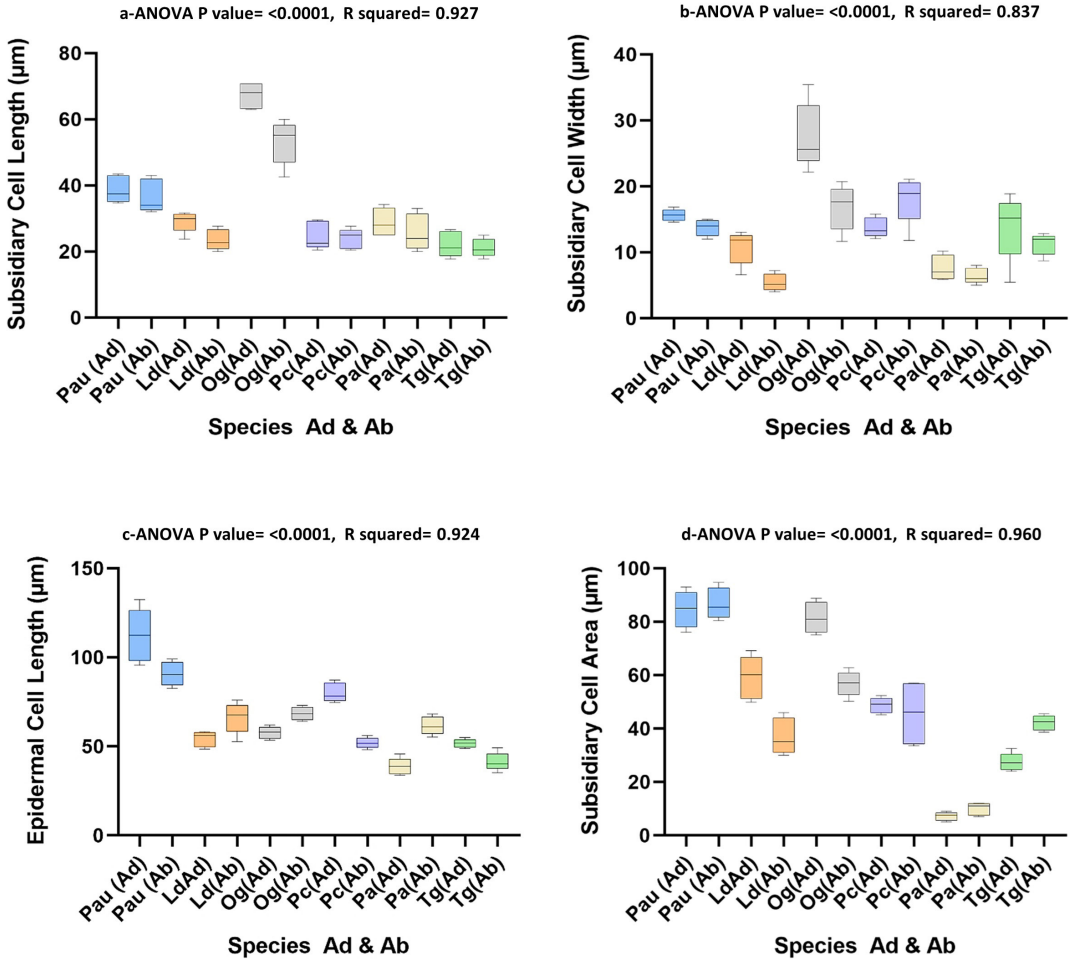

Figure 18. Boxplots and ANOVA values, a,b; *O. glabratus* (Og) presented the highest variation in subsidiary cell length, and subsidiary cell width (µm) characteristics. c,d; *P. austroarabica* (Pau)presented the highest variation in epidermal cell length, and subsidiary cell area (µm), characteristics, evaluated within Loranthaceae native to the KSA. Abaxial leaf (AB). Adaxial leaf (AD).

4. Discussion

The main objective of this study was to contribute to the species designation and help overcome taxonomic problems of the family Loranthaceae by identifying and revealing those native to Saudi Arabia.

In the current study, investigation of stomatal and epidermal cell characteristics using light and SEM showed clear variation between the species of the Loranthaceae family, specifically in the shape of epidermal cells, periclinal wall (PWs), fine relief of cell walls, wax form, stomatal level, guard cell surface, and stomatal pore shape. In addition, according to the PCA and ANOVA, quantitative characteristics of stomata have taxonomic importance and help to separate them into the following categories: stomatal complex length, stomatal complex width, stomatal complex area, epidermal cell length, epidermal cell width, subsidiary cell length, subsidiary cell width, and subsidiary cell area. the characteristics used in this study of the leaf surface and stomata gave significant results in distinguishing between the species under study. This is consistent with the results of previous studies, Ibrahim and Ayodele (2013) mentioned that all Loranthaceae species that have been studied in Nigeria had distinct characteristics, such as amiphstomatic leaf type, polygonal cell shape, straight to curved anticlinal wall, and pericytic stoma types, so these micromorphological leaf characteristics contributed to the classification of some study species. thus, these features could be used as a taxonomic tool to identify Loranthaceae species.

The same observations have been recorded in many taxonomic studies, emphasizing the importance of the anatomical characteristics of the leaf in many plant families. For example, Gul et al. (2019) recorded that the micromorphology of trichome types, epidermal cells and stomatal complexes may be systematically important, especially for the discrimination and identification of complex and problematic taxa in Lamiaceae. Previous studies have indicated the importance of micromorphological stem stomatal traits and leaf epidermis to distinguish between complex taxonomic species in the family Caryophyllaceae (Ullah et al., 2018; Chandra and Rawat, 2018; El-Banhawy et al., 2021).

Furthermore, Ullah et al. (2021) studied Scrophulariaceae in Pakistan, which consists of taxonomically complex genera and species, based on qualitative anatomical features, such as epidermal cell shape, epidermal cell cover, anticlinal wall, trichome type, stomatal type, and stomatal position, in addition to quantitative characteristics on both the AD and AB surfaces of the leaf epidermis, such as the length and width of the leaf epidermal cell, stomata, stomatal pore, subsidiary cell, and trichomes. They mentioned that both the qualitative and quantitative characteristics provide baseline information to differentiate the studied taxa.

In the samples of the current study, *Phragmanthera austroarabica* was distinguished by the presence of stellate nonglandular trichomes and circle-shaped lenticels on the AB and AD surfaces of the leaf. Ohikhena et al. (2017) screened AB and AD leaf surfaces in *Phragmanthera capitata* and found stellate trichomes and oval-shaped lenticels arranged in parallel on the abaxial midrib of the leaf. We could conclude that the lenticels shape can be a taxonomic feature at the species level.

This study on leaves and fruits using SEM revealed remarkable diversity and variation among the species under study in the fine relief of leaf and fruit surfaces and wax forms. Different types of wax structures were identified in the samples, including thick or few layers of crusts, entire platelets, fissured layers, granular wax, and transitional coiled-rodlets. The current results confirm what was mentioned in previous research (Shavvon et al., 2012) on two species, *Loranthus europaeus* and *L. grewingkii*, of Loranthaceae of Flora Iran. They investigated the anatomical characteristics of the stem, leaf, petiole, and fruit of these species using SEM. They identified 3 types of wax crystalloid structures on the leaf surface, including irregular glandular structures, platelets in *L. grewingkii*, and smooth structures in *L. europaeus*. They mentioned that the crystalloid structures of the wax allow differentiation of both species. Özcan (2002) mentioned that the micromorphological surface features of leaves and fruits could be a possible tool in species delimitation. We can conclude that the fine characteristics wax crystalloid of leaves and fruits in loranthaceae family are represented a well taxonomic tools for distinguishing between species.

This study contributed effectively to distinguishing the species in the family Loranthaceae in general and describing the species found in Saudi Arabia in particular. It added an accurate description of the fine morphological characteristics of the characteristics of the surface of the fruit, stomata, and leaf with statistical evidence, which contributes significantly to distinguishing between species and solving complex taxonomic problems and contributing to Accurate identification of species. We noticed a significant development in the fine morphological characteristics of the surface of the leaves and fruits of the species. We recommend using these characteristics as taxonomic evidence in future studies of species belongs to this parasitic family.

5. Conclusion

The main objective of this study was to identify and revealing those native to Saudi Arabia and contribute to the species designation and help overcome taxonomic issues in the family Loranthaceae. In the current study, most of the descriptive and quantitative characteristics of the stomatal complex and epidermal cells showed clear variation between the species of the Loranthaceae family native to the KSA.

Furthermore, we observed evolution and great variation in the fine characteristics of leaf and fruit surfaces, as well as wax forms, trichomes and lenticel types. we recommended to used micro-morphological traits as a tool for identifying Loranthaceae species.

The results of the current study will greatly help in identifying and classified parasitic species. We recommended focusing on understanding this family in terms of the species, hosts and the impact or damage that it can cause. As well we suggested to future studies focused deeper into the relationships between Loranthaceae species and their hosts.

The results of such studies will be useful in diagnosing the species that infect trees and will help facilitate resistance efforts, where detecting parasitic species is the most important step for effective resistance.

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Supplementary Material

Supplementary material accompanies this paper.

Appendix A

Table A1: Morphological traits of 6 species of Loranthaceae in Saudi Arabia (Waly et al., 2012, 2013).

Table A2: Results of screening qualitative characteristics of epidermal cells, stomatal index, and fruit surface in Loranthaceae spp. using scanning electron microscopy.

Table A3: Results of evaluated quantitative characteristics of epidermal cells and stomatal index characteristics in abaxial leaves (ABs) and adaxial leaves (ADs) of Loranthaceae species native to the KSA.

Appendix B

Fig. B1: Principal component analysis eigenvalues for Loranthaceae species in the KSA based on observations of the micromorphological characteristics of stomatal complexes and epidermal cells.

Fig. B2: Loading plots of the two axes principal components 1 and 2 for local Loranthaceae species in the KSA based on observations of the micromorphological characteristics of stomatal complexes and epidermal cells.

Fig. B3: Boxplots and ANOVA values, O. glabratus (Og) presented the highest variation in stomatal complex length, and stomatal complex width (µm) characteristics evaluated within Loranthaceae native to the KSA. Abaxial leaf (AB). Adaxial leaf (AD).

Fig. B4: Boxplots and ANOVA values, P. austroarabica (Pau) presented the highest variation in stomatal complex area, epidermal cell width, stomatal pore area (µm) characteristics evaluated within Loranthaceae native to the KSA. Abaxial leaf (AB). Adaxial leaf (AD).

Fig. B5: Boxplots and ANOVA values for stomatal pore width (µm) characteristics evaluated within Loranthaceae native to the KSA. Abaxial leaf (AB). Adaxial leaf (AD).

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