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# DIVERSITY IN POLLEN GRAIN CHARACTERISTICS AND ITS IMPORTANCE IN DISTINGUISHING LORANTHACEAE Juss. SPECIES GROWN IN SAUDI ARABIA

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**Abstract**
Members of the Loranthaceae family are considered parasitic, and mistletoe negatively affects the growth and productivity of host trees. Loranthaceae has several complicated taxonomic issues. Six species of Loranthaceae grow naturally in Saudi Arabia, but these species have not been well studied. The aim of this work was to evaluate the importance of morpho-palynological characters as a taxonomic tool in the identification of Loranthaceae species growing in Saudi Arabia. The discriminatory power of these characteristics was evaluated using different statistical analysis methods. The descriptive characteristics of pollen polar and equatorial views and colpus type are useful for describing the species, and the presence of three colpus patterns was detected among the study samples: syn-(3)-, demisyn-(3)-, and zono-(3)-colpate. The quantitative characteristics, specifically, the equatorial diameter and P/E ratio, are the most useful and can be used to classify species within clusters based on their affiliation with genera, species, and subtribes Emelianthinae and Tapinanthinae. This study confirmed that pollen grain characteristics can be used as a taxonomical tool to identify Loranthaceae species. This information will aid in determining parasitic species that infect trees and could be useful for strengthening efforts in weed management.

**Keywords:** Mistletoe. Morphology. Morpho-palynology. Parasitic. Taxonomy.

## 1. Introduction

The nutrition of parasitic plants depends entirely or partly on host plants (Teixeira-Costa and Davis 2021; Ashapkin et al. 2023), but these plants do not benefit the host and, in some cases, cause extreme damage to the host. Parasitic plants can reduce growth and productivity and may cause host tree death (Biyon et al. 2022a; Biyon et al. 2022b; Menezes et al. 2022).

The Loranthaceae family, which includes most of the mistletoe (parasitic) genera (Wilson and Calvin 2006a: Wilson and Calvin 2006b; Su et al. 2021) and contains 73 genera and more than 1000 species, is widespread in tropical and warm temperate areas (Barlow 1997; Liu et al. 2018). Loranthaceae is divided into five tribes (Psittacantheae, Nuytsieae, Elytrantheae, Gaiadendreae and Lorantheae) and approximately eleven subtribes (Suárez et al. 2021). The relationships among species within the genus are unclear, and the family has many complex taxonomic issues (Nickrent et al. 2021).

There are six species of Loranthaceae in Saudi Arabia (Abulfaith and Emara 1988): *Tapinanthus globiferus* (A.Rich.), *Oncocalyx glabratus* (Engl.), *Loranthella deflersii* (Tiegh.) S. Blanco & C. E. Wetze, *Phragmanthera austroarabica* A.G.Mill. & J.A.Nyberg, *Plicosepalus curviflorus* (Benth. ex Oliv.) Tiegh., and

*Plicosepalus acaciae* (Zucc.) (Alqthanin, 2011). These species grow naturally in the northern and southern parts of the western region of the kingdom (Al-Robai 2023).

Currently, the taxonomy of Loranthaceae is mostly based on flower characteristics and, in some cases, is poorly supported by molecular evidence and contradicts that based on pollen morphological evidence. Grímsson et al. (2018) reviewed pollen grains in 35 genera of Loranthaceae. This study, along with molecular phylogenetic available sequence data from the National Centre for Biotechnology Information (NCBI), was useful for detecting the distribution pattern of the Loranthaceae family and assessing its palaeophytogeography. However, species such as *Tapinanthus globiferus, Oncocalyx glabratus,* and *Phragmanthera austroarabica* growing in Saudi Arabia were not included in this study.

Many of the species recorded in the family Loranthaceae have not been the subject of adequate taxonomic studies, especially pollen grain studies, and the species growing in the Kingdom of Saudi Arabia have not received adequate examination or morphological studies and are causing severe damage. The aim of this work was to evaluate the taxonomic importance of the morpho-palynological characteristics of pollen grains from Loranthaceae species native to Saudi Arabia.

## 2. Material and Methods

### Plant materials

Fresh flowers of Loranthaceae species native to Saudi Arabia were collected during field trips in 2021 and 2022 from six areas in the northern and southwestern regions of the Kingdom of Saudi Arabia. Information on the samples, the names of the regions, the dates of collection, and the locations (longitudes and altitudes) are available in Table 1. The identity of the samples was confirmed by experts at King Khalid University.

### Screening of the macromorphological traits of pollen grains via light microscopy (LM)

Pollen grains were extracted from flower samples by pressing on the stamens. Then, the pollen samples were stained with a 1% safranin solution, and the grains were mounted in glycerol-gelatine. The prepared slides were viewed under a Nikon Eclipse 400 light microscope (Nikon Instruments Inc/USA). Approximately nine characteristics, including 4 quantitative (polar diameter, equatorial diameter, P/E ratio, and exine thickness) and 5 qualitative (polar view, equatorial view, pollen size, colpus type, and exine type) traits, were measured in the pollen samples, and the characteristics are shown in Tables 2 and 4. Five replicates were examined for each species. The macromorphological characteristics were examined following the methods described by (Punt et al. 2007; Grímsson et al. 2018) and measured by ImageJ analysis software (Schneider et al. 2012).

### Data statistical analysis

The taxonomic importance of four quantitative pollen grain characteristics was evaluated. The collected quantitative data of the pollen grains were subjected to principal component analysis (PCA) (Sneath and Sokal 1973), which was conducted in the XLSTAT V.1.1 Lumivero (2023) program. This approach clusters samples into groups based on similarity, which aid in separating and distinguishing between samples.

The correlation matrix of morphological characteristics was calculated in XLSTAT V.1.1 Lumivero (2023) and presented in the form of a heatmap to facilitate comparisons between values and infer whether the characteristics were negatively or positively related. The heatmap was generated in GraphPad Prism software V. 9.5.1.

The taxonomic relationships among species in this study were evaluated using a cluster dendrogram constructed in the ‘factoextra’ package V. 1.0.7 (Kassambara and Mundt 2017) in RStudio software V. 2.0 (RStudio Core Team 2020).

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

| Scientific name | Sample code | Locality | Date of collection | Longitude & altitude |
|---|---|---|---|---|
| *Tapinanthus globiferus* (A.Rich.) | Tg | Wadi Alreem | 22 Mar 2020 | 17.9805724°, 42.2380263° |
| *Oncocalyx glabratus* (Engl.) | Og | Thageef village | 7 Oct 2021 | 20.6197578°, 40.9233393° |
| *Loranthella deflersii* (Tiegh.) S. Blanco & C. E. Wetze | Ld | Abha – Khamis mushat | 2 Oct 2021 | 18.185097°,42.818443°. 18,299187°,42.497780°. |
| *Phragmanthera austroarabica* A.G.Mill. & J.A.Nyberg | Pau | Al-Taef, Gabel Ibrahium Wadi leiah | 20 Mar 2021 | 20.402047°,41.138292°. 18.1913686°,42.8226008°. 18.0602509°,42.7076538°. 18.301805°,42.496372° |
| *Plicosepalus curviflorus* (Benth. ex Oliv.) Tiegh. | Pc | Wadi leiah | 1 Oct 2021 | 21.218874°,40.557426°. 20.637140°,41.275528°. 18.1913686°,42.8226008°. |
| *Plicosepalus acaciae* (Zucc.) | Pa | Tabouk Alola Alwajh | 10 Oct 2020 | 26.5737260°,36.3731150° 26.7392410°,37.1740530° |

Furthermore, one-way analysis of variance (ANOVA) and a box plot (Ashapkin et al. 2023) were performed and constructed in GraphPad Prism software V. 9.5.1 based on 95 permutations. The R-squared and P-values were calculated via ANOVA to detect traits that have taxonomic value in distinguishing between species (Glantz et al. 2016). The R-squared value ranges from 0.1 to 1; R-squared values ≥ 0.9 indicate a strong correlation, whereas values ≤ 0.5 indicate a weak correlation. A high value of R means less error in the data, and vice versa.

## 3. Results

### Descriptive characteristics of pollen grains

Pollen grains in *Phragmanthera austroarabica* (Figure 1A) oblate, concave in polar view, elliptic in equatorial view, equatorial apices T-shaped, broadly obcordate, medium size, colpus type syn-(3)-colpate, Table 2. Polar diameter (PD) 12-16 µm, equatorial diameter (ED) 19-26 µm, P/E ratio 0.592-0.684 µm, and exine thickness (EX) 1-1.4 µm.

*Loranthella deflersii* (Figure 1B) Pollen oblate, straight to concave-triangular in polar view, emarginate in equatorial view, equatorial apices obcordate; small pollen size, and zono-(3)-colpate. Polar diameter 9-18 µm, equatorial diameter 20-28 µm, P/E ratio 0.450-0.643 µm, and exine thickness 0.9-1.3 µm. *Oncocalyx glabratus* (Figure 1C) pollen oblate, convex-triangular in polar view, elliptic in equatorial view, subrhombic in equatorial view, equatorial apices broadly obcordate; medium size, colpus type zono-(3). Polar diameter 9-15 µm, equatorial diameter 18-25 µm, P/E ratio 0.500-0.600 µm, and exine thickness 0.8-1.2 µm.

*Plicosepalus curviflorus* (Figures (1D) Pollen oblate, trilobate to concave-triangular in polar view, elliptic in equatorial view, lobes very narrow, equatorial apices obcordate; pollen small, syn-(3)-colpate. Polar diameter 8-15 µm in LM, equatorial diameter 15-20 µm, P/E ratio 0.533-0.718 µm, and exine thickness 0.8-1.2 µm. *Plicosepalus acacia* (Figures 1E) pollen oblate, trilobate to straight-triangular in polar view, elliptic in equatorial view, lobes very narrow, equatorial apices obcordate, interapertural areas (mesocolpia) sunken; small in size and syn-(3)-colpate. Polar diameter 13-18 µm in LM, equatorial diameter 16-23 µm, P/E ratio 0.722-0.875 µm, and exine thickness 1-1.5 µm.

*Tapinanthus globiferus* (Figure 1F) pollen, oblate, trilobate in polar view, elliptic to (sub)rhombic in equatorial view, equatorial apices T-shaped, pollen small, demisyn-(3)-colpate. Polar diameter 14-20 µm in LM, equatorial diameter 28-35 µm, P/E ratio 0.483-0.581 µm, and exine thickness 0.9-1.3 µm.

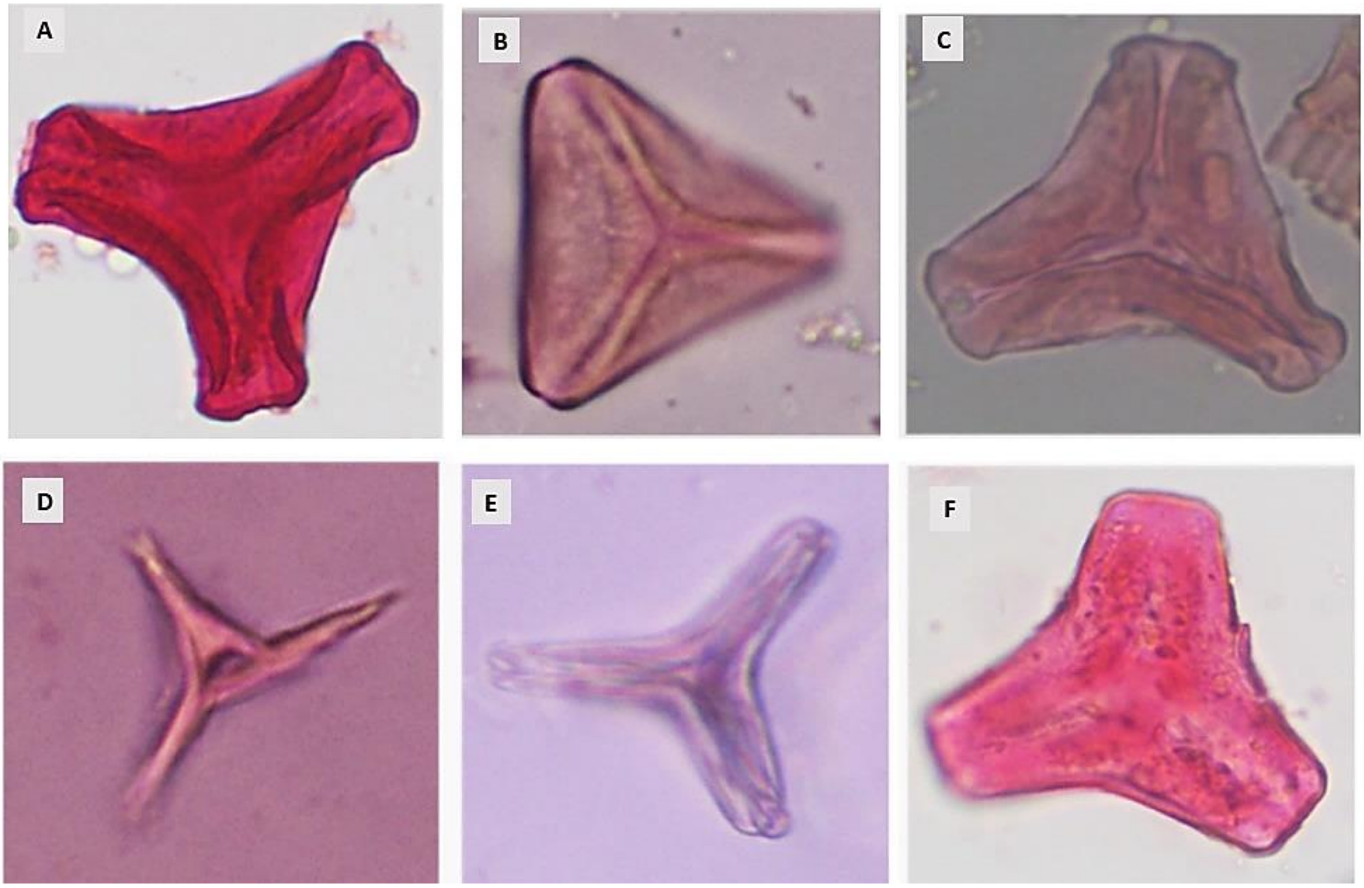



**Figure 1.** LM polar view of pollen grains in A – *Phragmanthera austroarabica*; B – *Loranthella deflersii;* C – *Oncocalyx glabratus;* D – *Plicosepalus curviflorus;* E – *Plicosepalus acacia;* F – *Tapinanthus globiferus.*

**Table 2.** Descriptive characteristics of pollen grains of Loranthaceae species native to the KSA obtained using light microscopy (LM).

| Characters | | *P. austroarabica* | *L. deflersii* | *O. glabratus* | *P. curviflorus* | *P. acacia* | *T. globiferus* |
|---|---|---|---|---|---|---|---|
| Polar view | PV | Oblate, concave | Oblate, straight to concave-triangular | Oblate, convex-triangular | Oblate, trilobate to concave-triangular | Oblate, trilobate to straight-triangular | Oblate, trilobate |
| Equatorial view | EV | Elliptic, T-shaped | Emarginate | Elliptic, subrhombic | Elliptic | Elliptic | Elliptic |
| Pollen size | PS | Medium | Medium | Medium | Small | Small | Small |
| Colpus type | CT | Syn-(3)-colpate. | Zono-(3) | Zono-(3) | Syn-(3)-colpate | Syn-(3)-colpate. | Demisyn-(3)-colpate |
| Exine | E | Thick | Thick | Thick | Thick | Thick | Thick |

## Evaluation of quantitative characteristics

*Principal component analysis (PCA)*

Table 3 and Figure 2 show Axes 1 and 2 in the PCA, which explain a large amount of eigenvalue variability (69.969% and 27.771%, respectively). The features that had the highest positive and negative loadings on Axes 1 and 2 are shown in Table 4, as well as the deduced loading plots in Figures 3 and 5. The polar diameter, equatorial diameter, and P/E ratio loadings are on Axis 1 (0.937, 0.960, and 0.960, respectively). The exine characteristic had the highest positive loading on Axis 2 (0.954), while equatorial diameter and P/E ratio had negative loadings on Axis 2 (-0.261). The PCA results showed that the pollen grain characteristics polar diameter, equatorial diameter, P/E ratio, and exine (Figure 5) were important for discriminating between Loranthaceae species in the present study. These features were efficient in separating samples into groups according to species and genus (Figures 4 and 5).

**Table 3.** Eigenvalues of principal component analysis based on pollen characteristics.

| | F1 | F2 | F3 |
|---|---|---|---|
| Eigenvalue | 2.799 | 1.111 | 0.090 |
| Variability (%) | *69.969* | *27.771* | 2.260 |
| Cumulative % | 69.969 | 97.740 | 100.000 |

The italics indicate high positive and negative values on PCA Axes 1 and 2. F: Factorial axis.

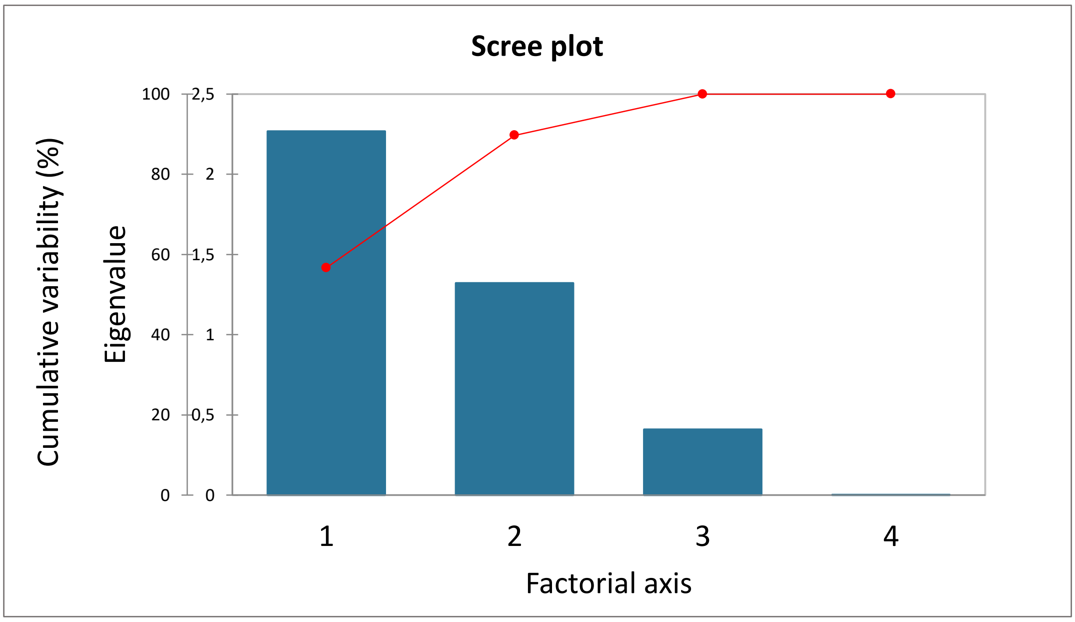



**Figure 2.** Principal component analysis eigenvalues of Loranthaceae pollen grain characteristics showing the most effective factor axes for separating species.

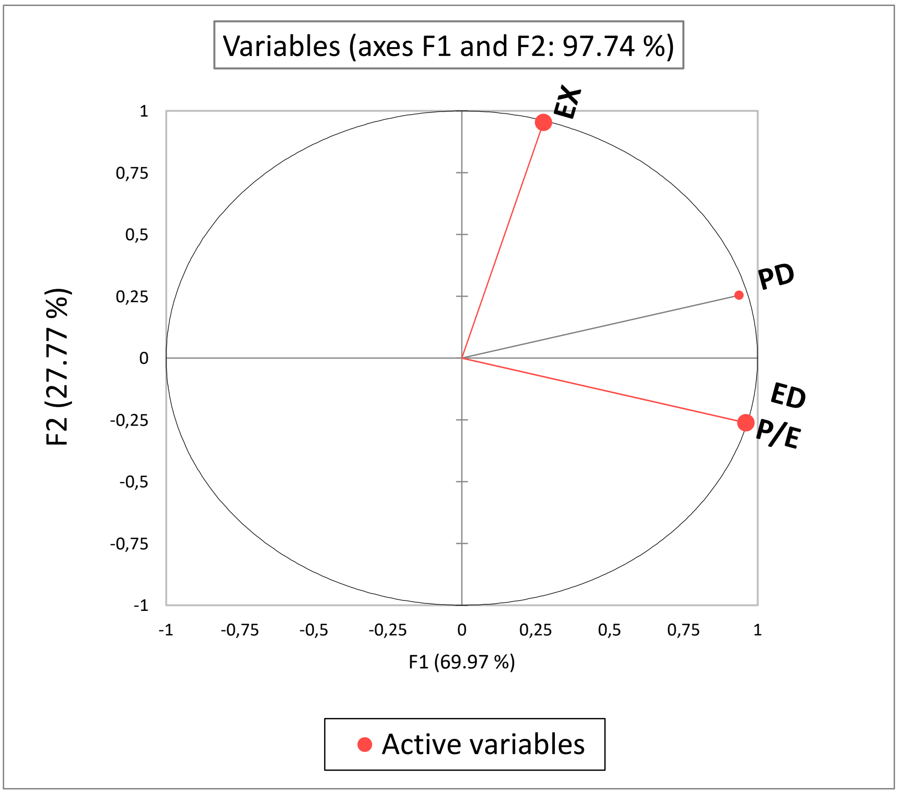



**Figure 3.** Loading plots of the two axes of principal components 1 and 2 and pollen grain characteristics of Loranthaceae species. The character code is available in Table 4.

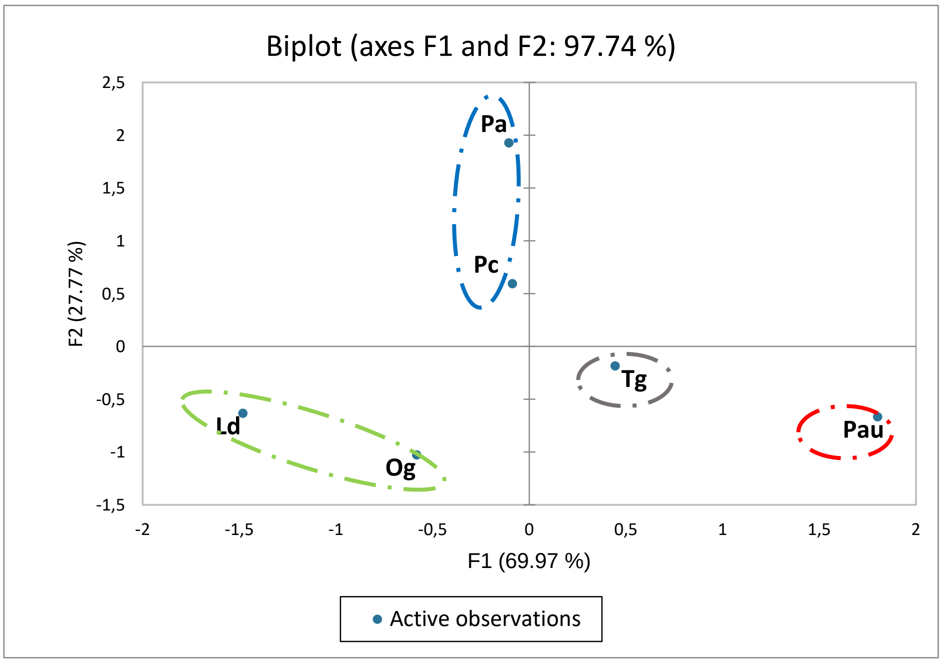



**Figure 4.** Cluster loading of the two principal component Axes 1/2, based on pollen grain characteristics in Loranthaceae species. The separation of samples into groups according to genus and species is shown. The sample code is available in Table 1.

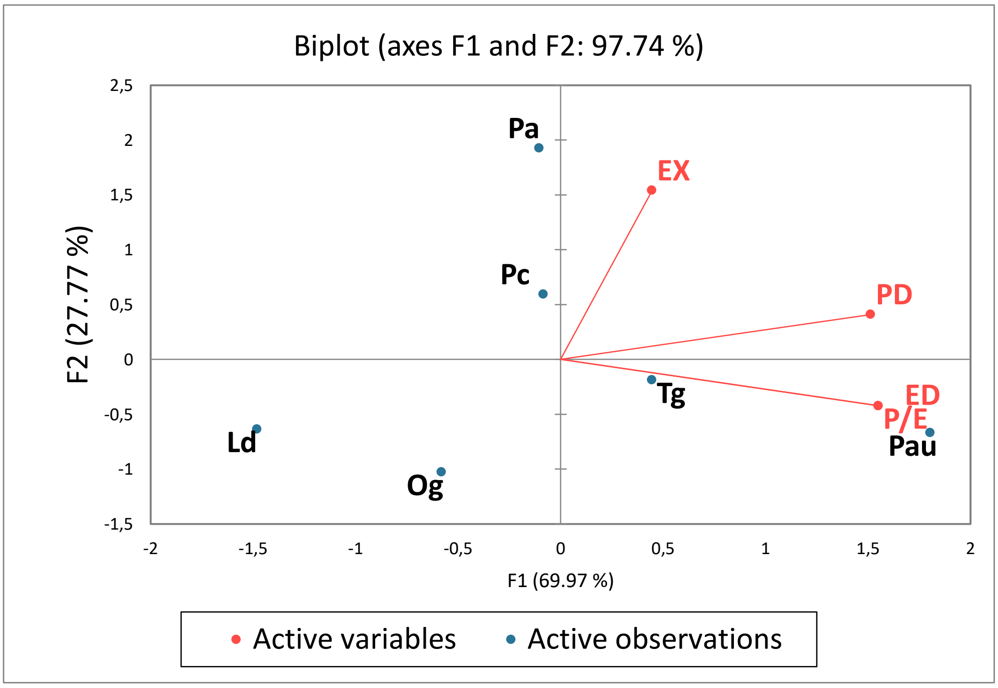



**Figure 5.** Loading plots and cluster loadings of the two principal component axes 1/2 and pollen grain characteristics of Loranthaceae species.

**Table 4.** Characteristic loadings in PCA based on pollen grain characteristics.

| Character | Character code | F1 | F2 | F3 |
|---|---|---|---|---|
| Polar diameter | PD | *0.937* | 0.254 | 0.238 |
| Equatorial diameter | ED | *0.960* | *-0.261* | -0.099 |
| P/E ratio | P/E | *0.960* | *-0.261* | -0.099 |
| Exine | EX | 0.276 | *0.954* | -0.118 |

The italics indicate high positive and negative values on PCA Axes 1 and 2. F: Factorial axis.

*Heatmap*

The heatmap (Figure 6) shows the correlations between pollen grain characteristics. The most positive correlations were observed between polar diameter, equatorial diameter, and P/E ratio. However, the most negative correlations were observed between exine and the other characteristics.

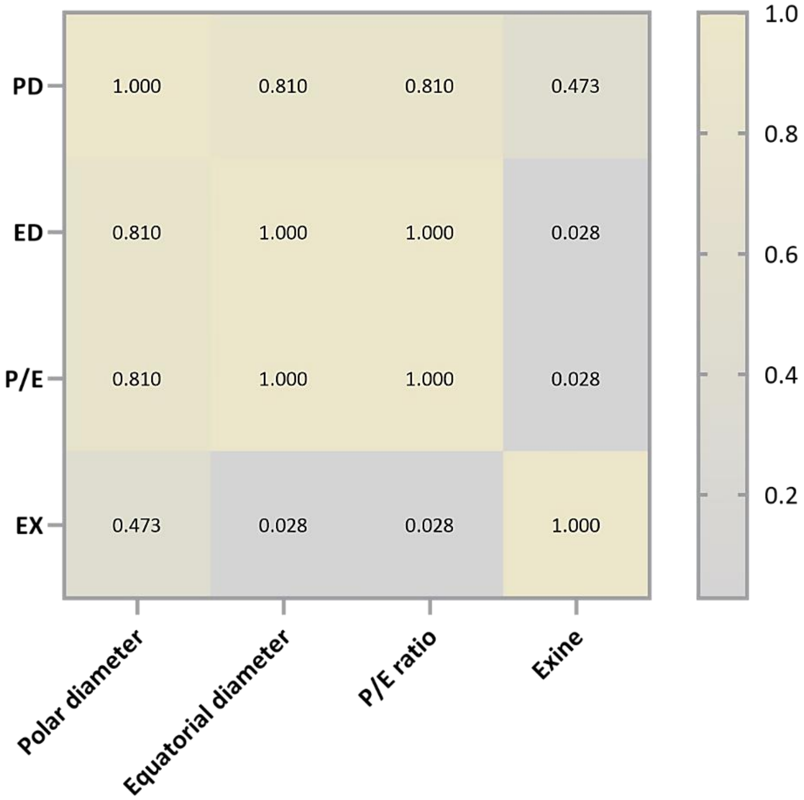



**Figure 6.** Heatmap of the correlations between the pollen grain characteristics. A beige colour indicates a strong positive correlation. A grey colour indicates a strong negative correlation.

*Cluster dendrogram*

The taxonomic relationships between Loranthaceae species based on pollen grain characteristics are presented in a cluster dendrogram (Figure 7). The study species were divided into two main clusters. The first cluster contained *Phragmanthera austroarabica* (Pau). The second main cluster was divided into two subclusters. The first subcluster included *Tapinanthus globiferus* (Tg). *Oncocalyx glabratus* (Og), *Loranthella deflersii* (Ld), *Plicosepalus curviflorus* (Pc), and *Plicosepalus acacia* (Pa) were included within the second subcluster. Species in the first main cluster belonged to the subtribe Emelianthinae, and all species in the second main cluster belonged to the subtribe Tapinanthinae.

*ANOVA and Boxplots*

The ANOVA and boxplot results presented in Table 5 show that the equatorial diameter and P/E ratio of the pollen grains showed the largest amount of variation between species (P-value <0.0001) and R-squared value (R-squared= 0.7275 and 0.7364), respectively. The polar diameter and exine characteristics were less variable according to ANOVA, with P-values of 0.0176 and 0.1207 and R-squared values of 0.417 and 0.290, respectively.

*Tapinanthus globiferus* exhibited markedly larger equatorial diameter than the other species, as shown in Figure (8-a), with a mean of 30.825, maximum of 35, minimum of 28, and SD of 2.474 (Table 6). Additionally, *Plicosepalus acaciae* had the highest P/E ratio (Figure 8-b), with a mean of 0.787, maximum of 0.875, and minimum of 0.722 (SD= 0.049) (Table 6).

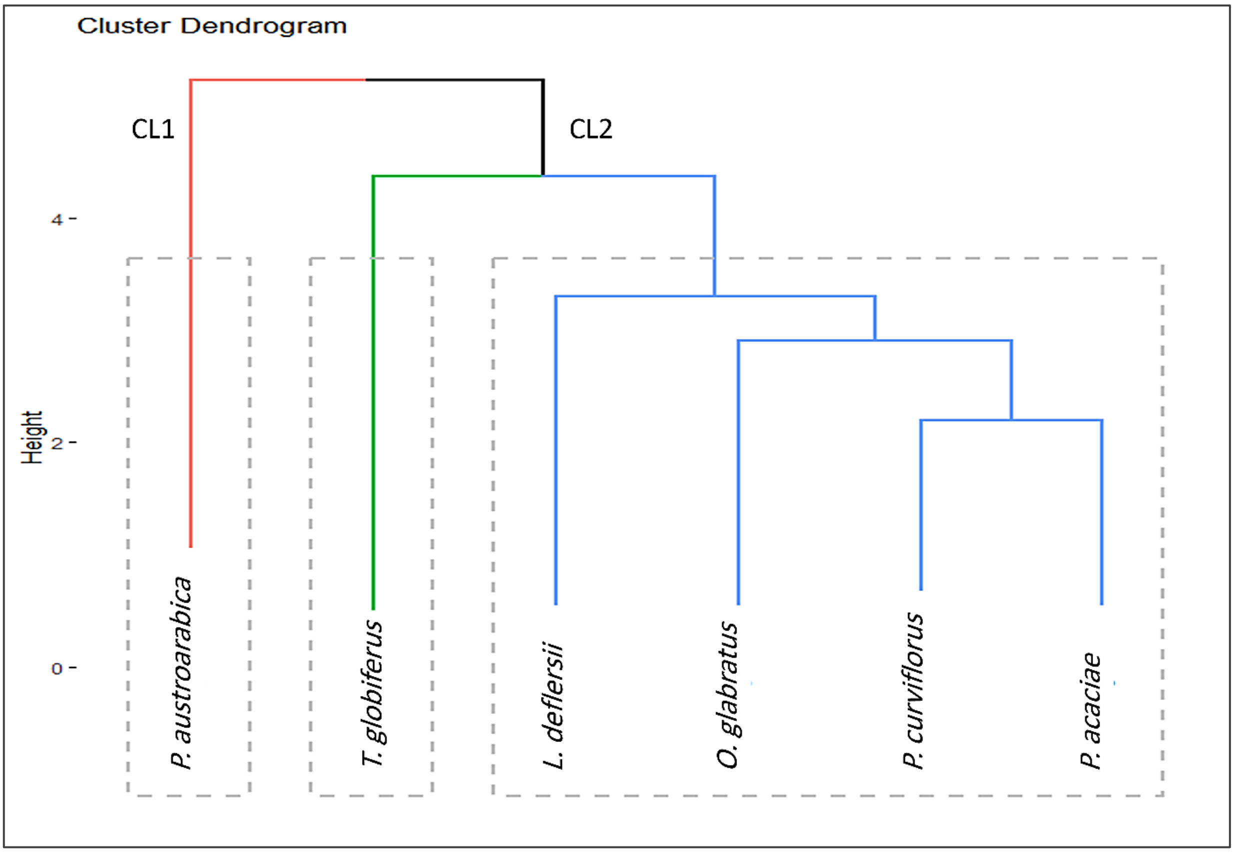



**Figure 7.** Cluster dendrogram of the pollen grain characteristics of Loranthaceae species, classifying the samples into two main groups according to the subtribes Emelianthinae and Tapinanthinae.

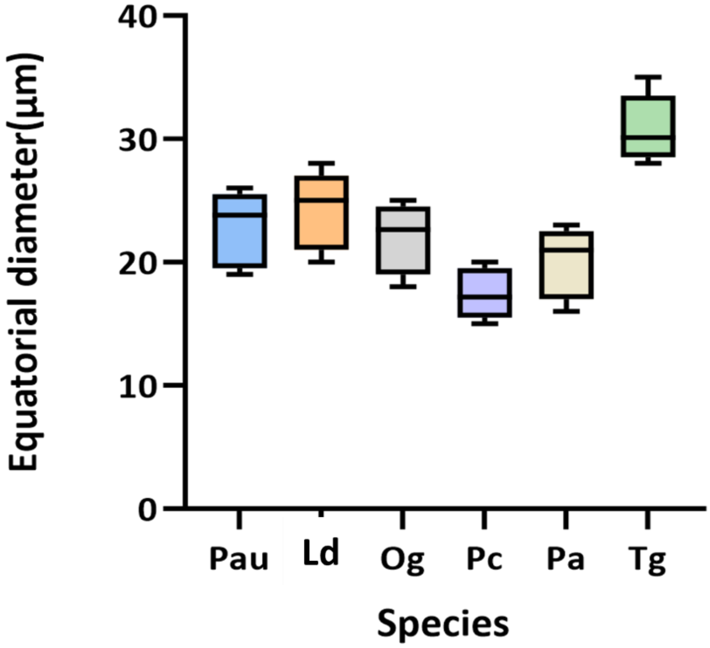
**Table 5.** ANOVA results (P-value and R-squared) for pollen grain characteristics.

| Character | P-value | R-squared |
|---|---|---|
| Polar diameter | 0.0176 | 0.417 |
| Equatorial diameter | <0.0001 | 0.7275 |
| P/E ratio | <0.0001 | 0.7364 |
| Exine | 0.1207 | 0.2904 |

**Table 6.** Results of evaluated quantitative pollen grain characteristics of Loranthaceae species native to the KSA.

| | Pau | | | | | Ld | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Characteristics | MAX | MIN | MEAN | MEDIAN | SD | MAX | MIN | MEAN | MEDIAN | SD |
| Polar diameter | 16 | 12 | 14.023 | 14.117 | 1.415 | 18 | 9 | 13.645 | 14.225 | 3.269 |
| Equatorial diameter | 26 | 19 | 22.766 | 23.83 | 2.772 | 28 | 20 | 24.2 | 25 | 2.857 |
| P/E ratio | 0.684 | 0.592 | 0.618 | 0.6 | 0.034 | 0.643 | 0.45 | 0.555 | 0.569 | 0.072 |
| Exine | 1.4 | 1 | 1.2 | 1.2 | 0.141 | 1.3 | 0.9 | 1.1 | 1.1 | 0.141 |
| | Og | | | | | Pc | | | | |
| Characteristics | MAX | MIN | MEAN | MEDIAN | SD | MAX | MIN | MEAN | MEDIAN | SD |
| Polar diameter | 15 | 9 | 12.1476 | 12.738 | 2.022 | 14 | 8 | 11.467 | 12.335 | 2.177 |
| Equatorial diameter | 25 | 18 | 21.9308 | 22.654 | 2.585 | 20 | 15 | 17.4376 | 17.188 | 1.848 |
| P/E ratio | 0.6 | 0.5 | 0.551 | 0.55 | 0.032 | 0.718 | 0.533 | 0.652 | 0.684 | 0.067 |
| Exine | 1.2 | 0.8 | 1 | 1 | 0.141 | 1.2 | 0.8 | 1 | 1 | 0.141 |
| | Pa | | | | | Tg | | | | |
| Characteristics | MAX | MIN | MEAN | MEDIAN | SD | MAX | MIN | MEAN | MEDIAN | SD |
| Polar diameter | 18 | 13 | 15.687 | 16.435 | 1.881 | 20 | 14 | 16.9022 | 17.511 | 2.155 |
| Equatorial diameter | 23 | 16 | 19.996 | 20.98 | 2.606 | 35 | 28 | 30.8246 | 30.123 | 2.474 |
| P/E ratio | 0.875 | 0.722 | 0.787 | 0.783 | 0.049 | 0.581 | 0.483 | 0.547 | 0.563 | 0.035 |
| Exine | 1.5 | 1 | 1.26 | 1.3 | 0.185 | 1.3 | 0.9 | 1.1 | 1.1 | 0.141 |

A – ANOVA P value= <0.0001, R-squared= 0.728




B – ANOVA P value= <0.0001,  R-squared= 0.736

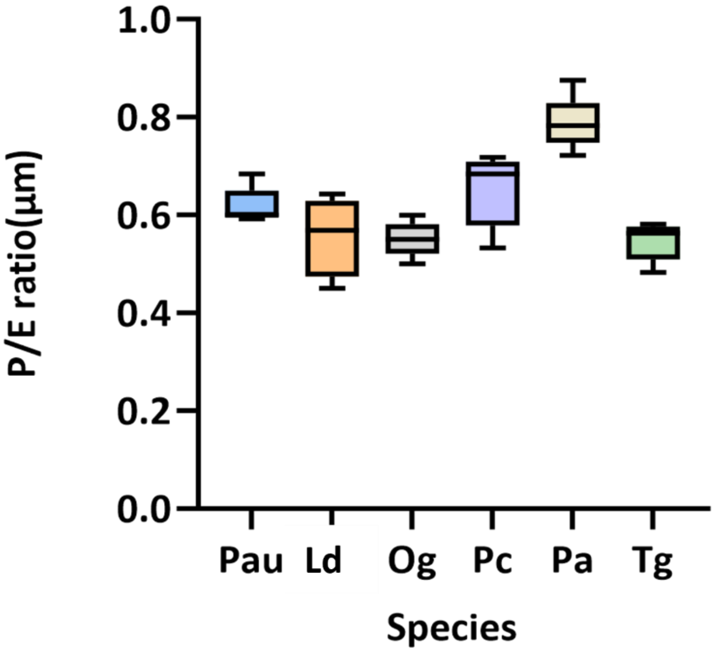



**Figure 8.** Boxplots and ANOVA values of characteristics that showed the greatest amount of variation and distinguished between species; A – Equatorial diameter and B – P/E ratio (µm) of pollen grains evaluated in Loranthaceae.

**4. Discussion**

According to the results of the current study, the qualitative characteristics that play important roles in discriminating between the examined species are polar and equatorial views and different patterns of colpi. Grímsson et al. (2018) screened fossil Loranthaceae pollen and determined pollen features to be a taxonomic indicator; they recommended that increased effort be made to include and differentiate pollen in palaeontological research.

Grímsson et al. (2017) classified the pollen of tribe Lorantheae under Section B as follows: "Small to medium-sized, oblate, trilobate to triangular in polar view, elliptic in equatorial view, or emarginate, equatorial apices truncated or with more or less protruding ‘lips’ (obcordate, T-shaped), basically syn-(3)-colpate, in some lineages the colpi are demicolpate". The species under study belong to two subtribes of Lorantheae, namely, Emelianthinae (*Phragmanthera austroarabica*) and Tapinanthinae (*Tapinanthus globiferus*, *Oncocalyx glabratus*, *Loranthella deflersii*, *Plicosepalus curviflorus* and *Plicosepalus acacia*). Pollen grains of the species *P. austroarabica, L. deflersii,* and *O. glabratus* were medium in size, while those of the species *P. curviflorus, P. acacia,* and *T. globiferus* were small. The most positive correlations were observed between polar diameter, equatorial diameter, and P/E ratio. We found that the descriptive characteristics of pollen in the species growing in Saudi Arabia correspond to those in Section B to some extent (Grímsson et al. 2017). Thus, our results largely support those of previous studies (Grímsson et al. 2017) on the Loranthaceae classification utility of pollen grains. However, in general, the size of the pollen grains of *O. glabratus, P. curviflorus,* and *P. acacia* was smaller than that mentioned by Grímsson et al. (2017).

Furthermore, we detected three colpus patterns among the study samples: syn-(3)-colpate in the species *P. austroarabica, P. curviflorus,* and *P. acacia*; zono-(3)-colpate in *L. deflersii* and *O. glabratus*); and demisyn-(3)-colpate in *T. globiferus*.

In accordance with the PCA results, Axes 1 and Axes 2 had high eigenvalue variability and were able to cluster samples in groups according to genus and species. Polar diameter, equatorial diameter, and P/E ratio had the highest positive loadings and separated the samples along Axis 1. The exine characteristic had the highest positive loading and separated the samples along Axis 2. Thus, the quantitative pollen grain characteristics that can help distinguish between species and divide study samples of Loranthaceae into groups according to species and genus include polar and equatorial diameter, P/E ratio, and exine characteristics.

The cluster dendrogram classified the taxonomic relationships and showed that the pollen grain characteristics used in the present study could divide the species into two main groups according to the subtribes Tapinanthinae and Emelianthinae. ANOVA and boxplots showed that the characteristics identified in this study, specifically the equatorial diameter and P/E ratio, reflected the most variation and best distinguished between species; thus, these parameters could be used as taxonomic tools. These results agree with those obtained by Ibrahim and Ayodele (2017), who confirmed that pollen grain traits are useful taxonomic tools for determining variation among the Loranthaceae family.

Recent studies have confirmed the taxonomic importance of pollen grains and their utility in discriminating among closely related or taxonomically complex species and inferring evolutionary relationships (Bapir and Galalaey 2023; Peng et al. 2023; Yildiz 2023). Furthermore, (Khan et al. 2020) screened pollen from mimosaceous species via LM and confirmed the discrimination power and strength of morpho-palynological characteristics for identifying closely related taxa. Many studies have used LM and confirmed that pollen characteristics, such as symmetry, size, shape, pore ornamentation, and exine characteristics, are important for distinguishing closely related species in Amaranthaceae (Al-Qahtani 2023).

Compared to the 73 genera and 1000 species (Liu et al. 2018) recorded within the Loranthaceae family, the number of species in which pollen characteristics have been recorded (35 genera) is low (Grímsson et al. 2018). According to the results of this study, we recommend using the macromorphological characteristics of pollen grains as taxonomical tools to identify Loranthaceae species in future studies. The results of such studies are useful for determining the macromorphological characteristics of parasitic plant species and will thus facilitate their control.

## 5. Conclusions

This study confirmed that pollen grain characteristics can be used as a taxonomical tool to identify Loranthaceae species. This information will aid in determining parasitic species that infect trees and could be useful for strengthening efforts in weed management.

**Authors' Contributions:** AL-JUHANI, W.: conception and design, acquisition of data, analysis and interpretation of data, drafting of the article, and critical review of important intellectual content; AL THAGAFI, N.T.: conception and design, acquisition of data, analysis and interpretation of data, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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