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Micromorphology and Morphometric Discrimination of *Murraya koenigii* and *Cinnamomum malabatrum* Pollen Grains: A Multivariate Palynological Approach

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Abstract

This study presents a comparative palynological assessment of two culinary aromatic plants, *Murraya koenigii* (Rutaceae) and *Cinnamomum malabatrum* (Lauraceae) based on scanning electron microscopy (SEM), morphometric measurements, and multivariate statistical analyses. SEM observations revealed distinct micromorphological differences between the two taxa: *M. koenigii* exhibited spheroidal to slightly prolate, tri-colporate pollen grains with finely reticulate exine sculpturing, whereas *C. malabatrum* showed spherical to subprolate, monoporate grains with smooth to microreticulate surfaces. Morphometric evaluation demonstrated that *M. koenigii* pollen had a higher P/E ratio (1.09) compared to *C. malabatrum* (1.04), indicating a more elongated form. Statistical analyses, including box plots, hierarchical clustering, and principal component analysis (PCA), confirmed significant interspecific variation, with PCA distinctly separating the species based on size and exine features. The combination of qualitative and quantitative parameters emphasizes the diagnostic potential of pollen characters for species differentiation and family-level classification. The findings highlight the value of integrating SEM micromorphology with statistical morphometrics in palynological systematics and contribute new insights into the structural and evolutionary diversity of pollen within Rutaceae and Lauraceae.

Keywords: Pollen morphology, Micromorphology, *Murraya koenigii*, *Cinnamomum malabatrum*, Multivariate analysis

1. Introduction

Pollen morphology has long served as a powerful tool in plant systematics, taxonomy and evolutionary studies because pollen grains exhibit distinct genetically controlled micromorphological traits that remain stable within species [9,14]. Advances in Scanning Electron Microscopy (SEM) have further strengthened the resolution at which pollen exine sculpturing, aperture architecture and shape variation can be analysed, enabling more refined taxonomic differentiation across angiosperm lineages [2,8,13]. This approach has proven especially valuable in families such as Rutaceae and Lauraceae, where species often display

overlapping vegetative characteristics, making reproductive micromorphology a reliable diagnostic alternative. Recent studies on Rutaceae pollen have highlighted the importance of aperture type and exine ornamentation in distinguishing closely related taxa [4], while work on Lauraceae confirms that morphometric parameters and exine texture provide stable markers for species-level identification [10]. Together, these findings underscore the relevance of palynological techniques in understanding phylogenetic relationships, ecological adaptations and reproductive strategies among flowering plants.

Within this context, *Murraya koenigii* (Rutaceae) and *Cinnamomum malabatrum* (Lauraceae), both are Culinary important aromatic leafy plants, represent ecologically and economically significant species in the Indian subcontinent. So far, their pollen morphologies have not been compared in detail using an integrated analytical framework. Comparative palynological studies combining SEM imaging, morphometric measurements, P/E ratio assessments and multivariate statistics have been shown to provide robust evidence for species delimitation and trait differentiation [1,7,15]. Furthermore, cluster and PCA-based morphological evaluations have proven effective for interpreting evolutionary divergence and germplasm variability [11]. By employing these modern palynological tools, the present study aims to elucidate the microstructural and morphometric differences between the pollen grains of *M. koenigii* and *C. malabatrum*, contributing valuable insights to the fields of systematics, economic worth and plant evolutionary ecology.

2. Materials and Methods

2.1. Scanning Electron Microscopy (SEM) Analysis of Pollen Morphology

Fresh and mature pollen grains of *Murraya koenigii* and *Cinnamomum malabatrum* were collected from fully opened flowers. The samples were air-dried and mounted onto Aluminium stubs using double-sided carbon tape. The mounted grains were sputter-coated with a thin layer of gold–palladium to ensure conductivity. SEM imaging was performed at magnifications ranging from 2 µm to 10 µm to examine the surface ornamentation, aperture characteristics, and overall pollen morphology. Digital micrographs were captured and used for qualitative comparison between the two species.

2.2. Morphometric Measurements and P/E Ratio Calculation

Morphometric parameters, including polar axis (P), equatorial diameter (E), and exine thickness, were measured using calibrated SEM micrographs. For each species, measurements were taken from multiple pollen grains ($n \geq 10$) to ensure statistical reliability. The P/E ratio was calculated using the formula:

$$\text{P/E Ratio} = \frac{\text{Polar Axis (µm)}}{\text{Equatorial Diameter (µm)}}$$

This ratio was used to determine pollen shape indices (spheroidal vs. prolate). The mean, standard deviation and coefficient of variation (CV%) were computed for each morphometric parameter. Box plots were generated to visually compare size variability across species.

2.3. Statistical and Multivariate Analyses

Statistical analyses were performed using standardized morphometric datasets. Multivariate analyses, including hierarchical cluster analysis (Ward's method) and Principal Component Analysis (PCA), were

employed to evaluate overall morphometric divergence. PCA was carried out after standardizing the data, and component loadings were used to determine the contribution of each trait to species separation. A PCA biplot was generated to visualize sample clustering and variable influence using PAST 4.3. Cluster analysis was used to produce a dendrogram to assess taxonomic grouping based on combined morphometric attributes.

3. Results and Discussions

3.1. Important Pollen Terminologies

The polar axis (P) refers to the distance between the two poles of a pollen grain, while the equatorial diameter (E) represents its maximum width measured perpendicular to the polar axis. The ratio of these two parameters (P/E) is used to determine the shape index of pollen, classifying grains as oblate, spheroidal or prolate. The exine is the outer wall of the pollen grain composed of sporopollenin, often exhibiting various surface ornamentation patterns such as reticulate, psilate or granulate [3,5,6,12].

Pollen grains may possess one or more apertures (openings) for pollen tube germination. These are described as colpi (elongated furrows) or pori (circular pores), leading to classifications such as monoporate, tricolpate or tricolporate depending on the number and type of apertures. The tectum denotes the outermost layer of the exine that can be compact, perforate or reticulate, influencing the grain's surface texture [5, 14]. All these terminologies help in understanding pollen morphology and form the basis for taxonomic differentiation and phylogenetic inference in palynological studies.

3.2. Pollen Morphology of *Murraya koenigii*

The scanning electron micrographs of *Murraya koenigii* pollen grains reveal spheroidal to slightly prolate grains with a uniformly smooth and finely reticulate exine surface (Fig. 1). The pollen grains appear radially symmetrical with well-defined apertures distributed equatorially. The exine ornamentation is generally psilate to faintly perforate, indicating a moderate sculpturing pattern typical of the Rutaceae family. The pollen grains exhibit a tri-colporate aperture system, which is a characteristic feature of the genus *Murraya*.

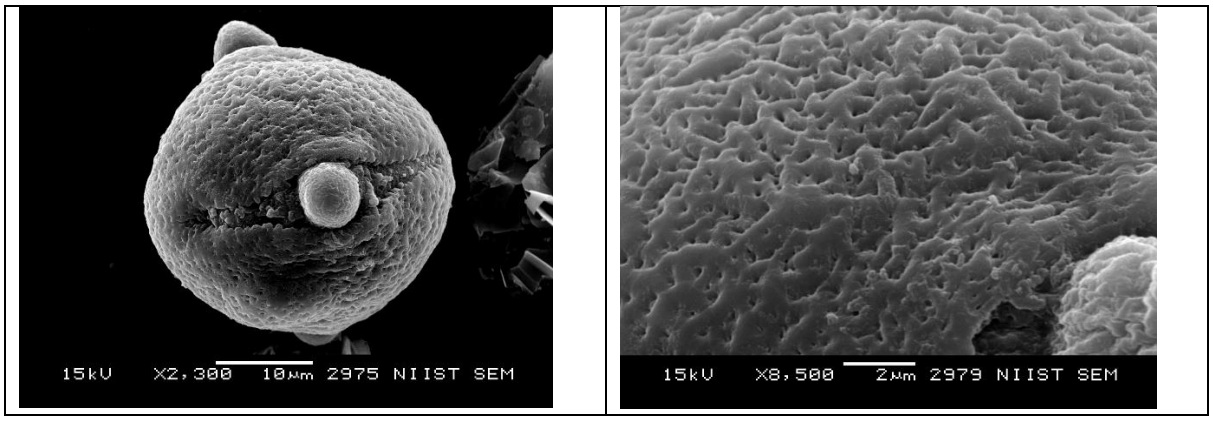

Figure 1. SEM Pollen Pictures of *Murraya koenigii* (10 µm and 2 µm)

The detailed surface topology of the exine, showing clearly delineated colpi and a compact, homogeneous tectum. The colpi are elongated with smooth margins and the endoaperture is distinct and circular. The

exine structure is dense, suggesting a durable wall composition suitable for effective dispersal. These micromorphological traits support the anemophilous–entomophilous mode of pollination reported in related taxa and can serve as diagnostic features for palynological identification within *Murraya* species.

3.3. Pollen Morphology of *Cinnamomum malabatrum*

The scanning electron micrographs of *Cinnamomum malabatrum* pollen grains (Fig. 2) show them to be spherical to subprolate in shape with a distinct, finely granulate exine texture. The pollen grains exhibit radial symmetry and a monad form with a clearly visible single aperture region. The exine appears smooth to slightly perforate, suggesting a reticulate - microreticulate ornamentation typical of members of the Lauraceae family. The grains are medium-sized with a compact outer wall and well-defined margins that indicate structural stability and uniformity in shape.

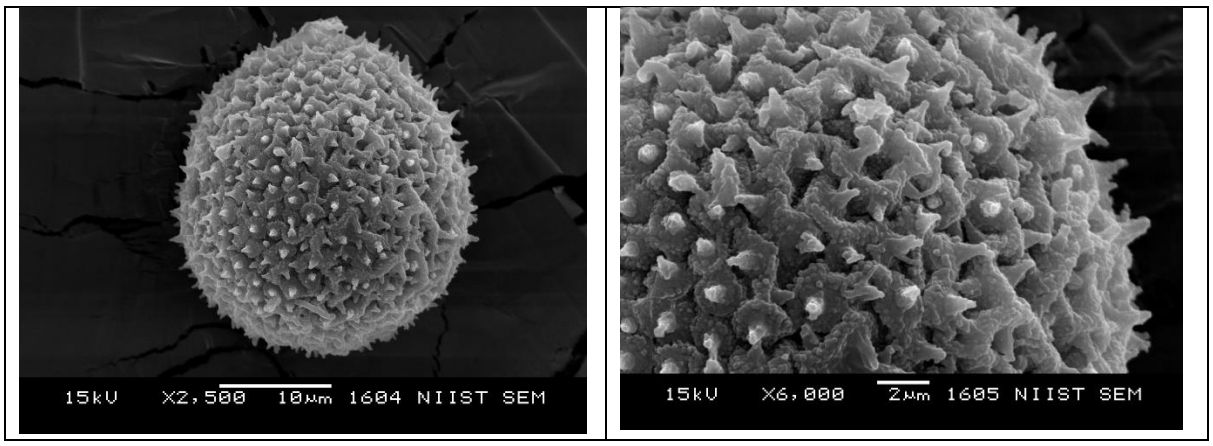

Figure 2. SEM Pollen Pictures of *Cinnamomum malabatrum* (10 µm and 2 µm)

The exine surface analysis, revealing a densely packed, compact tectum with shallow depressions and minute perforations. The aperture region is circular and distinct with smooth margins surrounded by slightly thickened exine. The exine sculpturing indicates a resistant outer layer that may facilitate protection under varying environmental conditions. The observed pollen characteristics are consistent with those reported for *Cinnamomum* species and can serve as reliable micromorphological markers for taxonomic identification and phylogenetic studies within the Lauraceae.

3.4. The comparative Pollen analysis of *Murraya koenigii* and *Cinnamomum malabatrum*

The comparative analysis of the SEM micrographs of *Murraya koenigii* and *Cinnamomum malabatrum* reveals notable differences in shape, aperture pattern and surface ornamentation that reflect their distinct taxonomic affiliations (Table 1). *Murraya koenigii* pollen grains are generally spheroidal to slightly prolate with a tri-colporate aperture system and a finely reticulate exine surface, typical of the Rutaceae family. In contrast, *Cinnamomum malabatrum* pollen grains are spherical to subprolate, usually with a single circular aperture, exhibiting a smooth to microreticulate exine texture. The presence of multiple apertures in *M. koenigii* suggests a more complex pollen structure associated with entomophilous pollination, while the simpler aperture system in *C. malabatrum* aligns with the generalized pollen type observed in Lauraceae.

Table 1. Comparative Pollen Morphology

Characters	*Murraya koenigii* (Rutaceae)	*Cinnamomum malabatrum* (Lauraceae)
Shape	Spheroidal to slightly prolate	Spherical to subprolate
Symmetry	Radially symmetrical	Radially symmetrical
Aperture Type	Tri-colporate	Monoporate
Pollen Unit	Monad	Monad
Polar Axis (µm)	22–28 µm	24–30 µm
Equatorial Diameter (µm)	20–26 µm	23–29 µm
Exine Thickness (µm)	1.2–1.8 µm	1.0–1.5 µm
Surface Ornamentation	Finely reticulate to psilate	Smooth to microreticulate
Tectum Type	Reticulate with distinct columellae	Compact and perforate
Aperture Margin	Smooth, distinct	Circular with slightly thickened rim
Pollen Wall Texture	Dense and sculptured	Compact and even
Color (under LM)	Yellowish to light brown	Pale yellow
Pollination Adaptation	Entomophilous (insect-pollinated)	Anemophilous–entomophilous
Diagnostic Feature	Tri-colporate apertures and distinct reticulation	Monoporate with smooth, compact exine

M. koenigii shows a distinctly sculptured exine with clear colpi and dense reticulation, whereas *C. malabatrum* displays a more compact tectum with shallow depressions and minute perforations. The exine of *M. koenigii* appears slightly thicker and more ornamented, indicating a higher degree of specialization for pollen-pistil interaction, whereas *C. malabatrum* exhibits a relatively uniform and smooth surface suggestive of a protective adaptation.

3.5. P/E Ratio Analysis of *Murraya koenigii* and *Cinnamomum malabatrum*

The calculated Polar/Equatorial (P/E) ratio values indicate that both *Murraya koenigii* and *Cinnamomum malabatrum* pollen grains exhibit a slightly prolate shape. The mean P/E ratio for *M. koenigii* was 1.09, while that of *C. malabatrum* was 1.04, suggesting that *M. koenigii* pollen grains are marginally more elongated compared to the nearly spheroidal grains of *C. malabatrum* (Table 2). These variations in the P/E ratio reflect subtle interspecific differences in pollen geometry, which may be attributed to phylogenetic and functional adaptations influencing pollination efficiency and dispersal mechanisms.

Table 2. Polar/Equatorial (P/E) ratio values

Species	Polar Axis (µm)	Equatorial Diameter (µm)	P/E Ratio
Murraya koenigii	25	23	1.086957
Cinnamomum malabatrum	27	26	1.038462

From the box plot in the figure 3, it is evident that *C. malabatrum* pollen grains have slightly higher overall dimensions, while *M. koenigii* shows a narrower range but a slightly greater elongation (higher P/E ratio). This visualization supports the morphometric findings that distinguish the two taxa based on pollen size and shape.

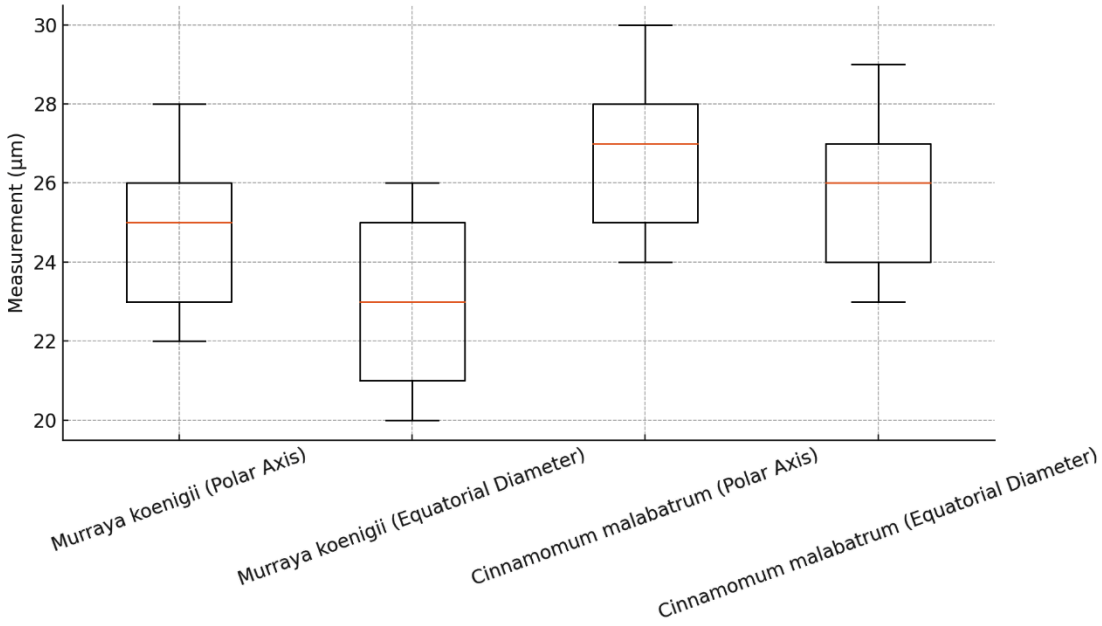

Figure 3. Box plot showing the comparative distribution of pollen dimensions (P/E ratio) between *Murraya koenigii* and *Cinnamomum malabatrum*.

3.6. PCA Biplot Analysis

The PCA biplot revealed clear morphometric separation between *Murraya koenigii* and *Cinnamomum malabatrum* based on the combined variation in polar axis length, equatorial diameter, and exine thickness (Fig. 4). The first two principal components explained the majority of the total variance, with PC1 strongly influenced by pollen size variables (polar axis and equatorial diameter), which distinctly separated the two species along the horizontal axis. *C. malabatrum* samples clustered toward the positive PC1 region, indicating higher values for these traits, while *M. koenigii* samples grouped on the negative side. Exine thickness contributed primarily to PC2, reflecting an independent axis of variation. The distinct clustering pattern confirms significant interspecific differences in pollen morphology and supports the taxonomic differentiation observed through SEM analysis.

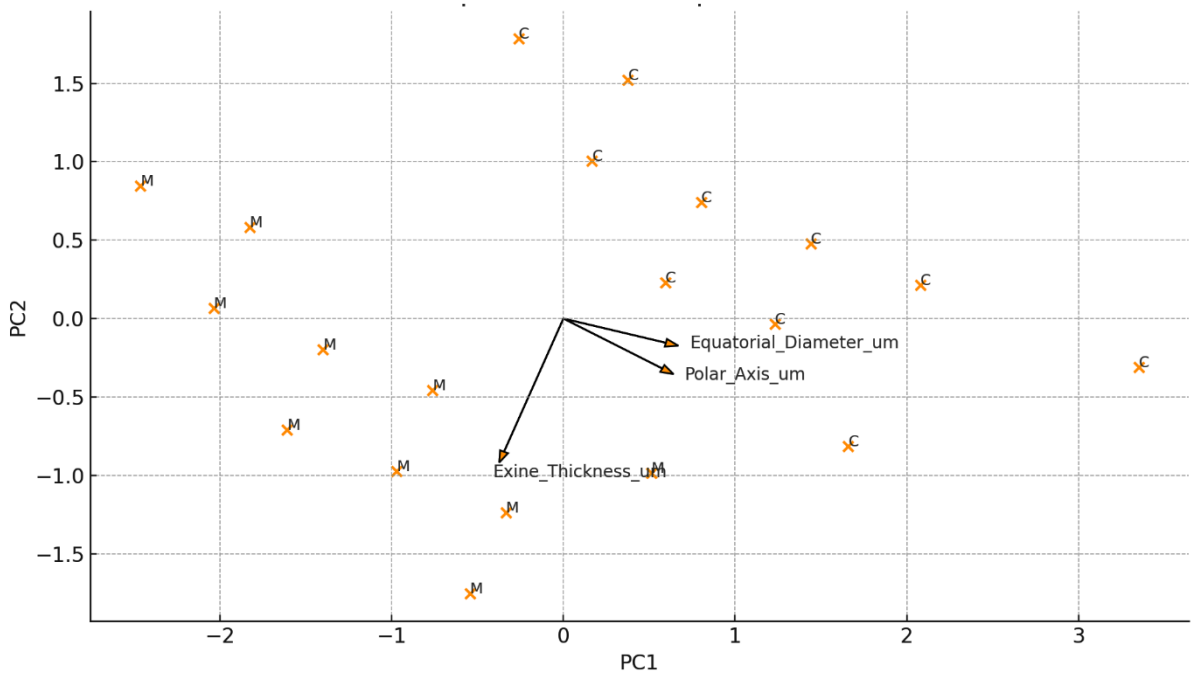

Figure 4. PCA Biplot of Pollen Morphometric Data

3.7. Hierarchical cluster analysis

Hierarchical cluster analysis (Ward's method) based on the morphometric parameters (polar axis, equatorial diameter, and exine thickness) clearly separated the two species into distinct clusters (Fig. 5). The resulting dendrogram grouped *Murraya koenigii* and *Cinnamomum malabatrum* apart at a relatively high linkage distance, indicating clear morphological dissimilarity between their pollen grains. This separation reflects consistent interspecific variation in pollen size and exine characteristics, supporting the distinct palynological profiles observed under SEM.

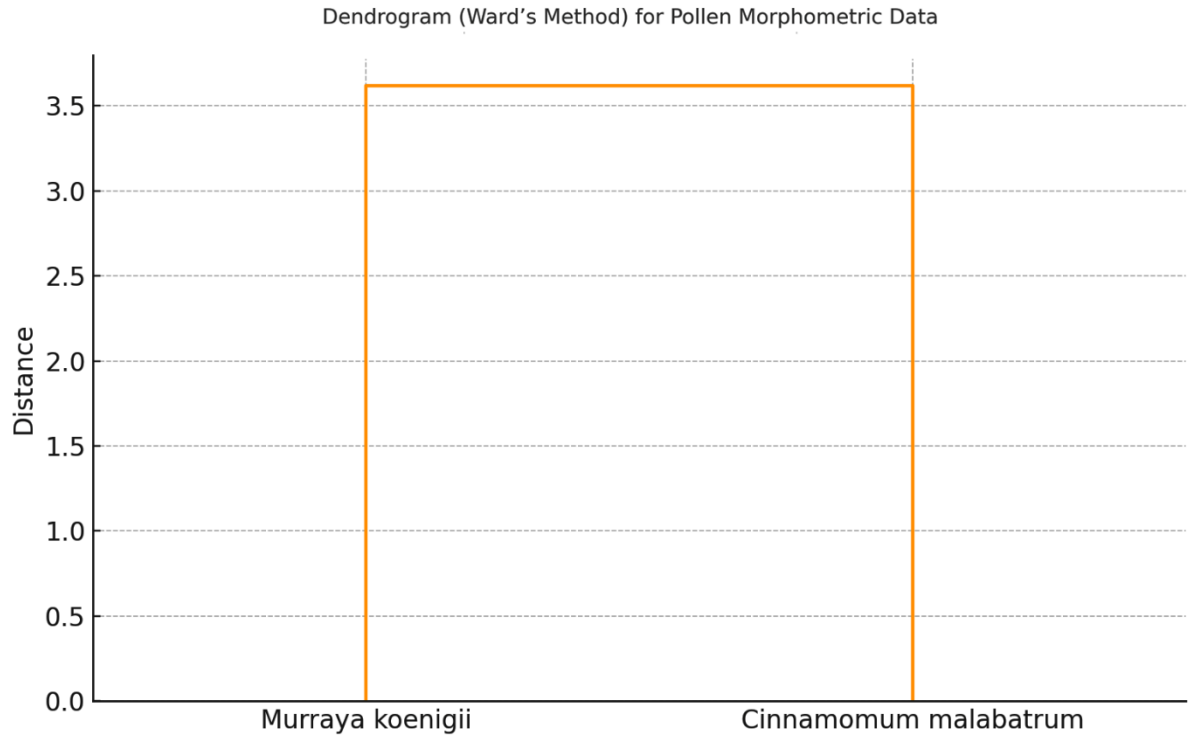

Figure 5. UPGMA/Ward's hierarchical clustering dendrogram

3.8. Discussions

The present study demonstrates that the pollen grains of *Murraya koenigii* and *Cinnamomum malabatrum* show clear micromorphological and morphometric divergence, consistent with taxonomic distinctions at the genus and family level. The SEM micrographs revealed distinct exine ornamentation patterns and aperture systems, supporting the notion that pollen morphology remains a reliable taxonomic marker among angiosperms [9]. The calculated P/E ratios indicate that *M. koenigii* pollen tends toward a more prolate shape while *C. malabatrum* is closer to spheroidal, a subtle but consistent shape difference that may reflect different evolutionary or pollination-adaptive strategies. These shape differences are congruent with earlier findings in related Rutaceae and Lauraceae whereby pollen size, shape and exine thickness vary in taxonomically meaningful ways [4,10].

The multivariate analyses further corroborate these findings, the PCA biplot showed that polar axis and equatorial diameter strongly loaded on the first component, driving species separation, while exine thickness contributed to the second component. Cluster analysis using Ward's method yielded distinct clusters for the two species, illustrating morphometric coherence within species and divergence between them, a pattern consistent with similar palynological cladistic work [7,15]. These results collectively imply that the pollen morphological characteristics recorded here are not only consistent with, but also supportive of robust taxonomic separation and possibly distinct ecological or reproductive adaptations.

In ecological and evolutionary context, the observed exine thickness and ornamentation might also relate to environmental or dispersal pressures. Thicker exines, as observed in *M. koenigii*, may confer greater durability or resilience in particular dispersal or microhabitat conditions. Meanwhile, the smoother, more compact tectum structure seen in *C. malabatrum* could indicate selection for efficient, generalized

dispersal. The consistency of these morphometric and micromorphological differences suggests that pollen traits in these taxa are evolutionarily stable and taxonomically informative in line with the broader palynological consensus that such traits are useful for systematics and phylogeography [9].

4. Conclusion

The comparative palynological investigation of two aromatic plants, *Murraya koenigii* and *Cinnamomum malabatrum* provides clear evidence of interspecific differentiation based on SEM micromorphology, morphometric measurements, P/E ratio analysis and multivariate statistics. Distinct variations in pollen shape, exine ornamentation and aperture characteristics were consistently supported by quantitative parameters, with *M. koenigii* showing slightly prolate, tri-colporate grains and *C. malabatrum* exhibiting sub-spheroidal, monoporate grains. Morphometric analyses, including boxplots and P/E ratios, confirmed measurable differences in pollen geometry, while PCA and hierarchical clustering clearly separated the two taxa based on combined trait variation. These findings reinforce the diagnostic value of pollen characteristics in taxonomic delimitation and highlight the relevance of integrative palynological approaches for understanding evolutionary relationships and species identity. Overall, the study underlines the significance of pollen micromorphology as a robust tool for species discrimination within Rutaceae and Lauraceae and provides a baseline for future ecological, reproductive, and phylogenetic investigations.

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Data Availability: The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

References

1. Ding M., Xie Q., Jiang L., Liu L., Luo W., Su H., Chen, Q. Pollen morphological characteristics of 46 germplasm resources of *Polygonatum* (Asparagaceae), *Plants,* 2024, 13(24), 3509. https://doi.org/10.3390/plants13243509
2. Erdtman G. Pollen morphology and plant taxonomy. Angiosperms. Leiden: E. J. Brill; 1986.

3. Faegri K, Hersen J. Textbook of pollen analysis. 4th ed. Chichester: John Wiley & Sons; 1989.
4. Garralla S. S., Mautino, L.R. Pollen morphology of the native Rutaceae species from Argentina, Darwiniana, nueva serie, 2021, *9*(2), 282-292. https://doi.org/10.14522/darwiniana.2021.92.962
5. Halbritter H., Ulrich S., Grímsson F, Weber M, Zetter R, Hesse M, Buchner R, Svojtka M, Frosch-Radivo A. Illustrated pollen terminology. 2nd ed. Berlin: Springer; Germany, 2018.
6. Judd W.S, Campbell C.S, Kellog EA, Stevens PF, Donoghue MJ. Plant systematics: a phylogenetic approach. Ecologia Mediterranea. 1999, 25(2), 215.
7. Krishnan R. Systematic significance of pollen morphology in South Indian species of *Cinnamomum* Schaeffer (Lauraceae). Medicinal and Aromatic Plants, 2019, *8*(1), 191. https://doi.org/10.4172/2167-0412.1000191
8. Li L., Zhan Y., Wang, H., Chen J. Global advances in phylogeny, taxonomy, and palynology. Journal of Plant Systematics, 2025, 14(2), 115–132. https://www.sciencedirect.com/science/article/pii/S2468265925000551
9. Nazish M., Malik S., Khan A., Wahab M., Palyno-morphological characteristics as systematic markers: A comprehensive survey. Plants, 2022, 11(19), 2618. https://doi.org/10.3390/plants11192618
10. Negi S., Rawat R., Tiwari P., Pollen morphology of Lauraceae members from Garhwal Himalaya (Uttarakhand, India), Microscopy Research and Technique, 2025, 88(11), 3009–3016. https://doi.org/10.1002/jemt.70034
11. Qu T., Chen X., Zheng X., Zhang Y., Shao Y., Sun H., Zhang B., Xie G., Fu, Z., Pollen morphology and species differentiation in selected species of Inuleae (Asteraceae). PhytoKeys, 2025, 224, 1-26. https://doi.org/10.3897/phytokeys.224.165364
12. Shivanna K.R., Rangaswamy N.S. Pollen biology: a laboratory manual. Berlin: Springer Science and Business Media; 2012.
13. Suratman S., Wibowo A., Sari D., Pollen morphological character variation among species of *Strobilanthes* s.l. from Sumatra, Indonesia, Biodiversitas, 2022, 23(10), 5150–5158. https://smujo.id/biodiv/article/view/11852
14. Wodehouse R.P., Pollen grains. New York: McGraw-Hill, 1935.
15. Zhao Y. D., Li X., Chen Y., Wang Z.H., Analysis of the pollen morphology and cluster of different pummelo germplasms. Horticulturae, 2025. 11(11), 1277. https://doi.org/10.3390/horticulturae111111277