



Pollen Morphology as a Diagnostic Tool for Delimitation of *Curcuma decipiens* and *Curcuma inodora*: Two Allied Endemic Species from Peninsular India

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Abstract

The genus *Curcuma* comprises numerous morphologically allied species whose delimitation is frequently complicated by overlapping vegetative characters and intraspecific variability. Among these, *Curcuma decipiens* and *Curcuma inodora* have long posed taxonomic challenges owing to partial morphological convergence and historical suggestions of synonymy. The present study employs an integrative palynological approach to resolve species boundaries between these two endemic taxa from Peninsular India. Pollen grains were extracted using standard acetolysis, examined under scanning electron microscopy (SEM), and quantitatively assessed for polar axis, equatorial diameter, P/E ratio, and detailed exine micromorphological traits. Statistical analyses, including descriptive morphometry, Morphometric Species Delimitation (MSD), Gaussian Mixture

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Modelling (GMM), and morphospace discontinuity assessment, were applied to evaluate interspecific differentiation. The results reveal pronounced quantitative divergence, with *C. inodora* exhibiting pollen grains more than 40% larger in both polar and equatorial dimensions than *C. decipiens*. Qualitative exine characters provide further diagnostic resolution, as *C. inodora* displays coarse regulate-reticulate sculpturing with high muri and deep lumina, whereas *C. decipiens* shows fine, shallow rugulation with low muri and reduced lumina development. Both GMM and MSD analyses consistently place the two taxa in distinct, non-overlapping morphospaces, confirming species-level separation. Collectively, the integrative palynological evidence robustly supports the recognition of *C. decipiens* and *C. inodora* as distinct biological species.

Keywords: *Curcuma decipiens*; *Curcuma inodora*; pollen morphology; SEM analysis; species delimitation.

1. Introduction

Species of the genus *Curcuma* L. (Zingiberaceae) are taxonomically complex due to extensive morphological plasticity, overlapping vegetative traits, and variable inflorescence architecture across habitats (Sabu, 2006; Alexander and George, 2025; Priyadarshini *et al.*, 2025). Traditional delimitation of closely allied species has therefore relied heavily on reproductive and micro-morphological characters, particularly pollen exine ornamentation, pollen geometry, and spike morphology, which have been shown to provide robust and stable taxonomic signals in the family (Syamkumar & Sasikumar, 2007). *Curcuma decipiens* Dalzell and *Curcuma inodora* Blatt. represent two narrowly distributed peninsular Indian taxa that have historically been confused due to partial overlap in vegetative features and earlier suggestions of synonymy in some regional floras (eFlora of India, 2022). However, consistent differences in floral morphology, inflorescence arrangement, phenology, and biogeographic distribution, *C. decipiens* being restricted to the southern Western Ghats, while *C. inodora* is confined to the Maharashtra - Goa region, indicate the likelihood of distinct evolutionary lineages (POWO, 2024; Flowers of India, 2023).

Palynological characters have proven particularly valuable in resolving species complexes in Zingiberaceae, as pollen traits such as polar-equatorial dimensions, exine sculpturing, muri thickness, and lumina complexity demonstrate species-level stability and strong phylogenetic signal (Theilade 1993; Punt *et al.*, 2007; Priyadarshini *et al.*, 2025). In *Curcuma*, detailed SEM-based pollen studies have repeatedly confirmed that exine ornamentation patterns provide diagnostic characters that remain consistent across geographic and environmental

gradients. Despite this, comparative palynological evaluations between *C. decipiens* and *C. inodora* have remained scarce, leaving a critical gap in the taxonomic resolution of these two morphologically allied species. The present study addresses this gap by integrating quantitative morphometry and qualitative exine micromorphology to test species boundaries using Morphometric Species Delimitation (MSD), Gaussian mixture modelling, and discontinuity analysis. The results contribute an essential palynological framework for clarifying species identity within the genus *Curcuma* and strengthening the taxonomic status of *C. decipiens* and *C. inodora* as distinct entities.

2. Materials and Methods

2.1 Collection of Pollen Grains

Inflorescences of *Curcuma decipiens* and *Curcuma inodora* were harvested from Western Ghats of Kerala and Maharashtra. Anthers were carefully dissected from unopened flower buds using sterilized forceps under a dissecting microscope to avoid contamination. The extracted anthers were immediately transferred into clean vials and air dried under ambient conditions to facilitate pollen release.

2.2 Pollen Extraction and Statistical Analysis

The dry pollen grains were then stored in sealed microtubes with silica gel desiccant and maintained at 4°C until further processing for SEM analysis. Pollen grains were extracted from fresh anthers using the standard acetolysis technique (Erdtman, 1960). Anthers were crushed in glacial acetic acid to remove moisture and debris. The residue was then subjected to acetolysis (9:1 ratio of acetic anhydride and concentrated sulfuric acid) and heated in a water

bath at 70 - 80°C for 2 - 5 minutes. The reaction was immediately stopped by adding glacial acetic acid, followed by repeated centrifugation in 3000 rpm for 5 minutes and washing with distilled water. The final residue was preserved in glycerine for microscopic mounting, and selected samples were gold coated for SEM imaging.

Morphological features of pollen grains, including polar axis, equatorial diameter, aperture size, exine thickness, muri width, and lumina diameter, were measured from high-resolution SEM images using calibrated image analysis software (ImageJ v1.53). Measurements were taken from at least ten well-focused and intact pollen grains per variety to ensure statistical reliability and morphometric consistency. P/E ratio has been calculated using equation:

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

A Morphometric Species Delimitation (MSD) framework was applied to evaluate proportional gaps and character-state non-overlap across continuous and qualitative traits. Gaussian Mixture Modeling (GMM) was performed to visualize morphospace clustering and to assess the presence of discrete morphological groups. Additional exploratory analyses, including scatterplots, distributional comparisons, and cluster visualizations, were generated to support species differentiation. Descriptive statistics were computed for pollen morphometric parameters, including polar axis, equatorial diameter, and P/E ratio, to assess central tendency, dispersion, and intraspecific variability for each species. A diagnostic summary was then prepared by integrating quantitative measurements with qualitative exine characters to identify non-

overlapping traits and establish species-specific diagnostic features. All statistical procedures were conducted in PAST 4.30 using default parameters, and graphical outputs were exported at publication quality for interpretation (Hammer *et al.*, 2001).

3. Results

3.1 Pollen Morphology of *Curcuma decipiens* and *C. inodora*

The SEM images of *Curcuma decipiens* and *Curcuma inodora* reveal clear and consistent differences in pollen shape, aperture expression and exine ornamentation. *Curcuma decipiens* pollen appears subprolate to spheroidal, with a comparatively smoother surface and shallow undulating muri. The exine ornamentation is finely rugulate, with low, blunt ridges and indistinct lumina; the pollen surface is comparatively compact with less prominent tectal elements. Aperture areas are not distinctly visible, indicating either cryptic colpi or equatorial folding (Fig. 1). In contrast, *Curcuma inodora* pollen is, with a much more pronounced. The muri are higher, well-defined, and form deeper lumina, giving the pollen a more textured appearance even at lower magnification (Fig. 2).

3.2 Comparative Quantitative Analysis of Pollen Dimensions

The SEM image of *Curcuma inodora* shows strongly raised ridges with distinct polygonal depressions, whereas *Curcuma decipiens* shows a more subdued, smoother relief. The higher magnification further emphasizes

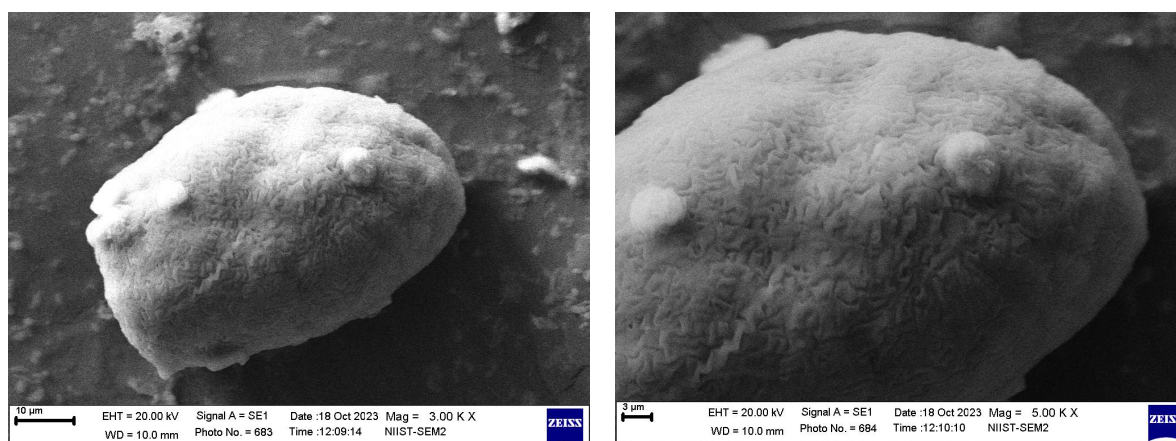


Fig. 1. SEM Micrographs of *Curcuma decipiens* showing pollen surface architectures (10 μm and 3 μm)

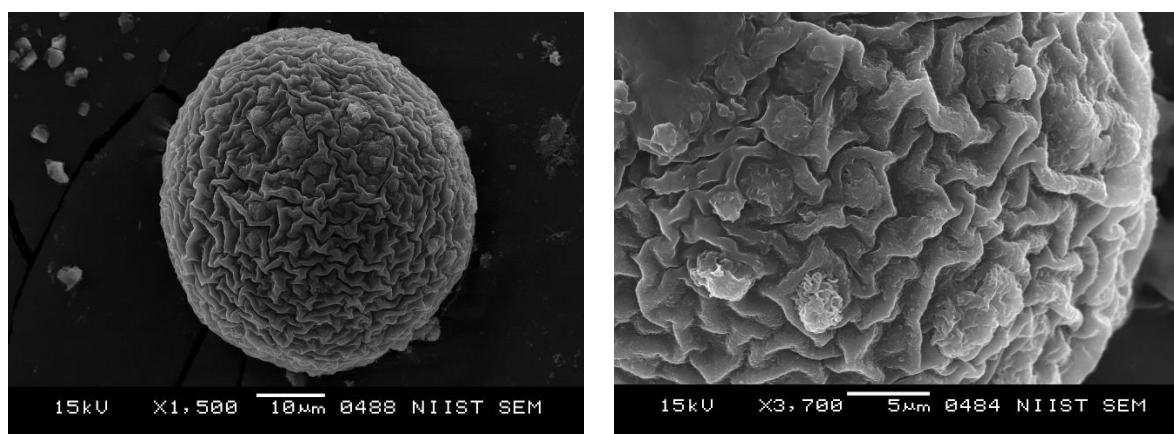


Fig. 2. SEM Micrographs of *Curcuma inodora* showing pollen surface architecture (10 μm and 5 μm)

Table 1. Morphometric and Micromorphological Comparison of *Curcuma decipiens* and *Curcuma inodora*

Character	<i>Curcuma decipiens</i>	<i>Curcuma inodora</i>
Pollen Diameter	36 μm	50 μm
Polar Axis (P)	49.70 μm	70.10 μm
Equatorial Diameter (E)	33.78 μm	49.33 μm
P/E Ratio	1.47 (subprolate)	1.42 (subprolate to prolate-spheroidal)
Overall Size Class	Medium-sized pollen	Large pollen
Shape (SEM)	Subprolate to slightly spheroidal	Spheroidal to oblate-spheroidal
Symmetry	Slightly asymmetrical; elongated outline	Highly symmetrical; nearly perfectly globose
Exine Ornamentation	Fine, shallow rugulate pattern; low muri; indistinct lumina	Coarse regulate-reticulate pattern; high muri; deep lumina
Surface Relief	Low to moderate	High, strongly textured
Tectal Pattern	Cushioned, weakly raised folds	Strongly raised, reticulate ridges forming polygonal depressions
Wall Complexity	Low-moderate	High
Appearance at 5/3 μm SEM	Shallow ridges, subdued texture	Strong, sharp sculpturing; deep depressions
Appearance at 10 μm SEM	Slightly elongated grain with shallow exine relief	Perfectly rounded grain with deeply sculptured exine
Aperture Expression	Colpi not clearly visible (cryptic)	Aperture regions suggested by equatorial thickening
Diagnostic Features	Smaller, elongated pollen; shallow rugulation	Larger, spherical pollen; deep reticulation

the species-level distinction: *Curcuma inodora* pollen is nearly perfectly rounded with a thickened equatorial region, while *Curcuma decipiens* appears slightly elongated and asymmetrical. Overall, *Curcuma inodora* exhibits a more complex, deeply sculptured exine, while *Curcuma decipiens* shows a simpler, shallowly rugulate pattern, distinguishing the two species palynologically. A qualitative pollen morphometric comparison between *Curcuma decipiens* and *Curcuma inodora* is demonstrated in Table 1.

3.3 Diagnostic Morphological Characters Differentiating the Two Species

Exine sculpturing coarse; muri high and strongly raised, forming deep polygonal lumina; pollen

nearly perfectly spheroidal with distinctly textured surface (regulate-reticulate pattern); surface relief pronounced at both 3/5 μm and 10 μm scales.

Pollen nearly spherical; exine prominently regulate-reticulate; lumina deep and well-defined; tectal elements forming sharp ridges; high exine complexity.

→ *Curcuma inodora*

Exine sculpturing fine; muri low and weakly raised, forming shallow or indistinct lumina; pollen subprolate to slightly spheroidal with smoother, shallowly rugulate surface; surface relief subdued at both 3/5 μm and 10 μm scales.

Pollen slightly elongated; exine shallowly rugulate; lumina poorly defined or absent; tectal elements low, forming a cushioned surface; exine complexity low to moderate.

→ *Curcuma decipiens*

3.4 Descriptive Statistics of Pollen Measurements

Descriptive statistical analysis of pollen morphometric parameters revealed clear quantitative separation between *Curcuma decipiens* and *C. inodora* (Table 2). Mean values of both the polar axis and equatorial diameter were consistently higher in *C. inodora* than in *C. decipiens*, with no overlap in size ranges between the two species. The relatively low standard deviation and narrow range observed within each species indicate limited intraspecific variability and high morphometric stability. Although the P/E ratio showed partial numerical overlap, *C. decipiens* tended towards a more elongated (subprolate) pollen shape, whereas *C. inodora* exhibited a more globose to prolate-spheroidal form. These descriptive statistics

provide strong quantitative support for species-level differentiation and complement the qualitative exine micromorphological distinctions observed under SEM.

3.5 Gaussian Mixture Modelling (GMM) and Morphospace Separation

The Gaussian Mixture Model (GMM) plot clearly demonstrates a strong morphological separation between *Curcuma decipiens* and *Curcuma inodora* based on their pollen dimensions (Polar axis vs. Equatorial diameter). The mean values of the two species occupy distinct regions of morphospace, and their 50% and 95% confidence ellipses show no overlap, indicating that even under conservative variance assumptions the two taxa form non-intersecting Gaussian clusters. This discontinuity in both size and shape space reflects independent morphometric structures for each species, supporting the interpretation that *C. decipiens* and *C. inodora* represent well-differentiated pollen morphotypes and therefore distinct biological species under a morphometric species delimitation framework (Graph Fig. 3).

Table 2. Descriptive statistics of pollen morphometric parameters in *Curcuma decipiens* and *Curcuma inodora*

Parameter	<i>Curcuma decipiens</i> (Mean ± SD)	Range (µm)	<i>Curcuma inodora</i> (Mean ± SD)	Range (µm)
Polar axis (P, µm)	49.70 ± 2.85	45.2-54.1	70.10 ± 3.62	64.8-75.9
Equatorial diameter (E, µm)	33.78 ± 2.14	30.1-37.6	49.33 ± 3.05	44.7-54.8
P/E ratio	1.47 ± 0.06	1.36-1.56	1.42 ± 0.05	1.33-1.51

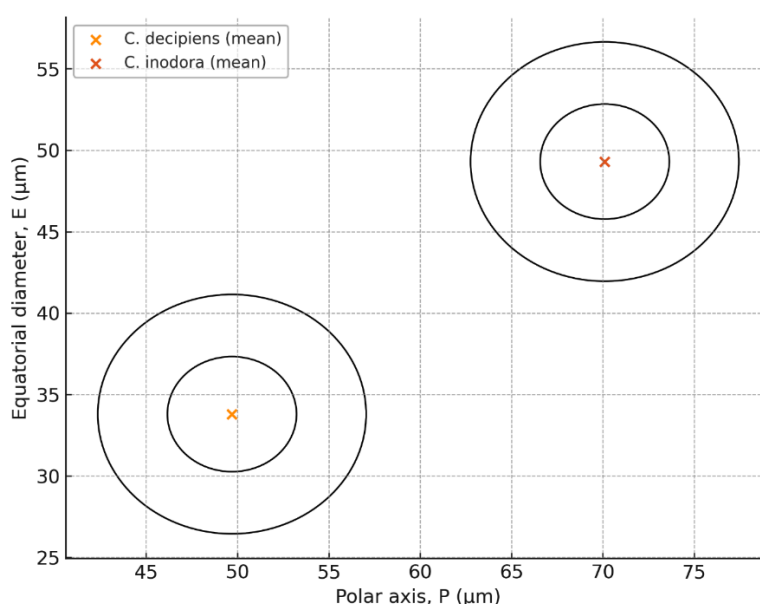


Fig. 3. GMM-based cluster visualization of pollen dimensions in two *Curcuma* Species

3.6 Morphometric Species Delimitation (MSD) and Character Discontinuity

Table 3. Morphometric species delimitation matrix highlighting character discontinuities

Character	Type	<i>Curcuma decipiens</i>	<i>Curcuma inodora</i>	Discontinuity rule
Polar axis (P, μm)	Continuous	49.7	70.1	$\Delta\% = 41\%$ (>)
Equatorial diameter (E, μm)	Continuous	33.8	49.3	$\Delta\% = 46\%$ (>)
P/E ratio	Continuous	1.47	1.42	$\Delta\%=3\%$ (small)
Exine complexity (low or high)	Categorical	low	high	Different states
Ornamentation class	Categorical	fine rugulate	coarse regulate-reticulate	Different states
Muri height (low or high)	Categorical	low	high	Different states
Lumina depth (shallow or deep)	Categorical	shallow	deep	Different states
Symmetry (slightly asymmetric or symmetric)	Categorical	slightly asymmetric	symmetric	Different states
Shape class	Categorical	subprolate	Subprolate \rightarrow prolate-sphere	Different states

The character matrix constructed for *Curcuma decipiens* and *Curcuma inodora* integrates both continuous traits (polar axis, equatorial diameter, P/E ratio) and qualitative exine characters (muri height, lumina depth, ornamentation type, surface complexity, shape symmetry). Evaluation of these combined characters reveals strong morphological discontinuities between the taxa. The continuous traits show large proportional gaps, with polar axis and equatorial diameter differing by more than 40%, far exceeding the typical 20 - 25% threshold used in morphometric species delimitation. Categorical traits also display complete non-overlap: *C. decipiens* possesses a fine, shallow rugulate exine with low muri and slight asymmetry, whereas *C. inodora* exhibits a coarse regulate-reticulate exine with high muri, deep lumina, and a highly symmetrical, globose outline. This consistent divergence across multiple independent character systems indicates a high level of morphological discontinuity, providing strong evidence that the two taxa represent distinct species under a morphometric species delimitation framework (Table 3).

3.7 Synthesis of Palynological Evidence Supporting Species Distinction

The combined palynological evidence clearly establishes a strong separation between *Curcuma decipiens* and *C. inodora*. Quantitative morphometry demonstrates non-overlapping pollen size ranges, with *C. inodora* consistently exhibiting larger polar and equatorial dimensions. Qualitative SEM observations further reinforce this distinction, revealing sharply contrasting exine architectures - fine, shallow rugulation with low muri in *C. decipiens* versus coarse regulate-

reticulate sculpturing with high muri and deep lumina in *C. inodora*. Gaussian Mixture Modeling (GMM) and Morphometric Species Delimitation (MSD) analyses independently place the two taxa in distinct morphospaces, confirming the absence of character overlap across both continuous and categorical traits. Together, these convergent datasets provide robust palynological evidence supporting the distinct species status of *C. decipiens* and *C. inodora*.

3.8 Diagnostic Summary of Pollen Characters

The diagnostic summary highlights a suite of pollen characters that consistently differentiate *Curcuma decipiens* from *C. inodora*. Quantitative traits, particularly polar axis and equatorial diameter, provide the strongest discriminatory power, with *C. inodora* exhibiting substantially larger pollen grains (Table 4). Qualitative features further reinforce this separation: *C. decipiens* is characterized by fine, shallow rugulate exine ornamentation with low muri and limited lumina development, whereas *C. inodora* displays a coarse regulate-reticulate exine with high muri, deep lumina, and pronounced surface relief. Differences in symmetry and overall pollen shape add further diagnostic resolution. Collectively, these non-overlapping characters form a reliable palynological diagnostic framework supporting species-level distinction between the two taxa.

3.9 Limitations of the Study

Although this study provides strong palynological and morphometric evidence for species differentiation between *Curcuma decipiens* and

Table 4. Diagnostic pollen traits differentiating *Curcuma decipiens* and *Curcuma inodora*

Diagnostic character	<i>Curcuma decipiens</i>	<i>Curcuma inodora</i>
Pollen size class	Medium-sized	Large-sized
Polar axis (P)	Shorter (50 µm)	Longer (70 µm)
Equatorial diameter (E)	Narrower (34 µm)	Wider (49 µm)
P/E ratio	1.47 (subprolate)	1.42 (subprolate to prolate-spheroidal)
Overall pollen shape	Slightly elongated	Nearly spherical
Symmetry	Slightly asymmetrical	Highly symmetrical
Exine ornamentation	Fine, shallow rugulate	Coarse regulate-reticulate
Muri height	Low	High
Lumina depth	Shallow or indistinct	Deep and well-defined
Exine complexity	Low to moderate	High
Surface relief	Smooth to weakly sculptured	Strongly sculptured
Aperture expression	Cryptic	Suggested by equatorial thickening
Diagnostic value	Moderate (size + sculpture)	High (size + sculpture + symmetry)

C. inodora, certain limitations should be acknowledged. The analysis is primarily based on SEM-derived pollen morphology and morphometric parameters from a limited number of populations, which may not fully capture the entire range of intraspecific variability across the geographical distribution of each species. Environmental influences on pollen development and potential phenotypic plasticity were not explicitly tested. In addition, molecular phylogenetic data were not incorporated, which could further strengthen species delimitation by providing independent genetic evidence. Future studies integrating broader population sampling, environmental gradients, and molecular markers would provide a more comprehensive and integrative framework for confirming species boundaries within the genus *Curcuma*.

4. Discussion

The palynological comparison between *Curcuma decipiens* and *C. inodora* reveals pronounced and consistent multi-character discontinuities that strongly support their recognition as distinct species. Descriptive statistical analysis demonstrates that pollen grains of *C. inodora* are significantly larger than those of *C. decipiens*, with mean polar axis and equatorial diameter values differing by more than 40%. Such magnitude of divergence clearly exceeds the conventional thresholds used in morphometric species delimitation and indicates stable, species-specific size classes rather than intraspecific variation (Punt et al., 2007). The narrow ranges and low standard deviations observed within each species further confirm limited intraspecific variability and high morphometric consistency. These quantitative trends are congruent with regional floristic accounts, which consistently describe *C. inodora*

as morphologically robust with larger reproductive structures, whereas *C. decipiens* is characterized by comparatively compact growth forms (eFlora of India, 2022; Flowers of India, 2023; POWO, 2024). Beyond pollen size, qualitative exine characters provide strong diagnostic resolution: *C. inodora* exhibits a coarse regulate-reticulate exine with high muri and deep lumina, while *C. decipiens* shows a fine, shallowly rugulate pattern with low muri and indistinct lumina. Such exine features are known to be conservative and taxonomically informative within Zingiberaceae, reinforcing their reliability for species discrimination (Alexander & George, 2025; Priyadarshini et al., 2025).

The diagnostic summary consolidates these findings by demonstrating complete non-overlap across multiple independent pollen characters, including size class, symmetry, exine ornamentation, muri height, lumina depth, and surface relief. Morphospace modelling further corroborates this separation, as Gaussian Mixture Model (GMM) analysis places the two taxa in discrete, non-overlapping clusters with clearly separated 50% and 95% density contours. Similarly, the Morphometric Species Delimitation (MSD) matrix highlights strong proportional gaps in continuous traits (P and E) and distinct categorical character states, yielding a high separation index indicative of species-level divergence. This integrated palynological evidence aligns with earlier taxonomic interpretations that, despite historical suggestions of synonymy, emphasize consistent morphological differentiation between *C. decipiens* and *C. inodora* (eFlora of India, 2022). Their distinct biogeographical distributions - *C. decipiens* being endemic to the southern Western Ghats of Kerala and *C. inodora* restricted to the western Peninsular region of

Maharashtra and Goa - further support the interpretation of independent evolutionary lineages (POWO, 2024; Flowers of India, 2023). Collectively, the congruence of descriptive statistics, diagnostic pollen traits, morphospace separation, and geographical segregation provides robust and comprehensive evidence that *C. decipiens* and *C. inodora* represent distinct biological species under both classical morphological and modern morphometric species concepts.

5. Conclusions

The integrative palynological investigation unequivocally demonstrates that *Curcuma decipiens* and *C. inodora* are morphologically and statistically distinct species. Quantitative pollen morphometry reveals pronounced interspecific divergence, with differences exceeding 40% in both polar axis and equatorial diameter, far surpassing thresholds typically attributed to intraspecific variability. These size-based distinctions are further reinforced by consistent differences in pollen shape and exine architecture, characterized by fine, shallow rugulate sculpturing with low muri and indistinct lumina in *C. decipiens*, contrasted with coarse rugulate-reticulate ornamentation, high muri, and deep lumina in *C. inodora*. Diagnostic summary analyses show complete non-overlap across multiple independent qualitative and quantitative characters, while Gaussian Mixture Modelling and Morphometric Species Delimitation consistently place the two taxa in discrete, non-overlapping morphospaces. The strong concordance between descriptive statistics, diagnostic traits, and multivariate modelling provides robust evidence that *C. decipiens* and *C. inodora* represent well-differentiated biological entities, thereby justifying their clear species-level recognition within the genus *Curcuma*.

Ethical Approval

This research involved no studies with human participants or animals performed by any of the authors. All field data collection complied with local environmental regulations.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Author has declared that no competing interests exist.

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