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REVIEW ARTICLE

The Foundational and Applied Taxonomic Applications of Pollen Traits

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ABSTRACT

Accurate taxonomic identification is fundamental to systematic biology and the successful conservation of flora. But phenotypic plasticity, cryptic speciation and inadequate plant material often get in the way. Palynology offers a strong and autonomous source of evidence to address the chemicals and physical foresee difficulties. The basic ideas behind palynotaxonomy and about how it can be used to classify plants and protect endangered plants, with an emphasis on India. The analysis shows that pollen grains have a wide range of stable, heritable, and taxonomically important qualitative (e.g., aperture type, pollen unit, exine ornamentation) and quantitative (e.g., pollen size, P/E ratio, exine thickness) traits. We analyze the impact of contemporary methodologies, such as scanning electron microscopy (SEM) and the statistical amalgamation of morphometric data (e.g., PCA, cluster analysis), on the conversion of traditional attributes into dependable, objective datasets. This review demonstrates the practical application of these traits in clarifying taxonomic uncertainties within intricate groups (e.g., Fabaceae, *Impatiens*), validating endangered medicinal plants (Rauvolfia), and offering diagnostic indicators for endemic genera (e.g., Acanthaceae) through case studies from the Indian flora. Finally, we discuss about how palynology can help with conservation in a direct way, not just as a way to identify plants and animals but also as a sensitive sign of reproductive health and environmental stress. Palynology has been shown as an essential, economical, and important field that enhances molecular phylogenetics and provides critical information for biodiversity studies.

Keywords: Palynology, Exine Ornamentation, Conservation Palynology, Systematic Botany

INTRODUCTION

It is more important than ever to be able to correctly and reliably identify plants in a time when biodiversity is quickly going down and the environment is changing. The fundamental process of classification establishes the foundation for all ensuing research in ecology, evolutionary biology, and, importantly, applied conservation initiatives. Palynology, which studies pollen and spores, is one of the many tools that systematists have at their disposal. It is a field that is always changing and becoming more important. One of nature's strongest biopolymers, sporopollenin, protects pollen grains (Grienenberger & Quilichini, 2021). They last long after other plant tissues have rotted, giving them a wide range of "morphological characters."

Traditional taxonomy, which is based on the macro-morphology of flowers and plants, sometimes has trouble with cryptic taxa, phenotypic plasticity caused by environmental influences, and identifying herbarium material that is incomplete or not blossoming. The characteristics of pollen give us a small, stable set of characters that can help with these problems.

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This review seeks to consolidate the fundamental principles of palynotaxonomy and illustrate its practical significance as a valuable tool for systematic classification and the preservation of endangered flora, particularly within the Indian context. To achieve this, the review is structured to build from principle to practice. It first talks about the main qualitative as well as quantitative traits of taxonomic relevance that come from the shape, size, apertures, and exine structure of pollen. It then covers the latest approaches, from sophisticated microscopy (SEM/TEM) to statistical and molecular integration, that are used to study these microscopic features. Finally, it shows eases with these ideas in action by using specific case studies from a wide range of plants found in India. It's also about that how pollen features might be an asset directly in conservation plans for species that are in danger of extinction.

Core Palynological Characters of Taxonomic Value

Palynology helps figure out what kinds of plants and species there are since pollen grains come in different shapes and sizes. Researchers may construct big databases that sort plants into multiple taxonomic levels, from genus to species, by systematically grouping these traits.

1. The Pollen Grain: An Anatomical and Morphological Palette

The pollen grain is the male gametophyte of seed plants and is a very complex biological structure. It has diverse morphological traits due to many different shapes, sizes, and structures. These traits are important in modern plant taxonomy, systematics, and evolutionary biology since they are genetically driven, stable within species, and present a lot of diversity amongst higher taxa (Taia, 2022).

Taxonomically significant characteristics, such as exine sculpturing and aperture configuration, are primarily localized within the pollen wall, i.e., sporoderm. Sporopollenin is the most important part of this wall. It is a complex biopolymer that chemicals and physical forces find very hard to break down (Grienenberger & Quilichini, 2021). The various layers of the sporoderm form the basis of palynological descriptions. Utilizing both Light Microscopy (LM) and Scanning Electron Microscopy (SEM) allows for a comprehensive view of these structures across different scales of detail. The inner intine is formed of cellulose and isn't very strong, while the outer exine is made of sporopollenin and is highly strong. Palynologists use the changes in this exine to make a "palette" of different shapes and sizes. Taxonomists and conservationists employ this essential information (Halbritter *et al.,* 2018; Jiménez-Zamora *et al*., 2023).

2. Quantitative and Qualitative Traits for Classification

An in-depth evaluation of quantitative and qualitative characteristics highlights the taxonomic significance of pollen morphology. This dual methodology establishes a robust foundation for classification, allowing for the identification of connections across various taxonomic ranks, from family to species level (Al-Hakimi *et al.,* 2021). Descriptive and categorical traits characterize the essential structure and classification of a pollen grain. The consistency of these characteristics, influenced by genetic elements, makes them particularly useful for classifying large groups of plants. Among the most important qualitative traits are those of the aperture system; the number, position, and type of apertures (e.g., colpate, porate, colporate, or inaperturate) which serve as primary diagnostic features. A classic example of how important this trait is for high-level taxonomy is the difference between monocots (which are usually monosulcate) and eudicots (which are usually tricolpate). One of the most important quality features is the exine ornamentation, which has a complicated pattern of sculpting on the surface of the exine. The micro-morphological "fingerprint" is a key feature that helps tell different genera and species apart. It can show patterns that are echinate, psilate, verrucate, striate, or reticulate (Perveen & Qaiser, 2020). You can see the pollen's overall shape very well from both the equator and at the poles. It's a simple but important quality. It can be prolate, oblate, spheroidal, or triangular.

Quantitative traits also include morphometric analysis, in addition to these descriptive traits. These traits yield quantifiable, continuous data suitable for extensive statistical analysis. These measurements are very useful for keeping apart species that are very similar or even varieties that have the same qualitative traits (Rech *et al*., 2018). These morphometrics commonly include pollen size, specifically measurements of the polar axis (P) and the equatorial diameter (E). From these, shape ratios like the P/E ratio can be calculated, which precisely defines the grain's shape (e.g., prolate, subprolate) in a statistically analyzable format. Other key quantitative data include wall dimensions, such as the thickness of the exine and its sub-layers (like the tectum or columellae), and the dimensions of the apertures themselves, such as the length and width of colpi or the diameter of pores. In modern systematics, the most robust taxonomic conclusions are not drawn from either data type alone, but from their synthesis. Qualitative traits are generally used to establish the primary groupings and identify key markers. Following this, the statistical analysis of quantitative traits provides objective, reproducible evidence to support or refute the separation of closely allied taxa (Wortley *et al.*, 2020). This combined micromorphological approach is fundamental to resolving taxonomic ambiguities, which in turn strengthens the foundation for conservation strategies by ensuring that distinct species are correctly identified and prioritized (Al-Hakimi *et al.*, 2021).

2.1. Shape and Size

The main morphometric features employed for classifying pollen consist of its size and form. The shape of the grain can be discerned by examining its form from both the poles and the equator. It could be a sphere, an oblate, a prolate, or a triangle. The polar axis (P) and the equatorial diameter (E) can be used to quantify size exactly. Size is the most essential quantitative attribute. The P/E ratio is a helpful derived quantitative attribute since it translates the shape of the grain into a statistically strong value. These ratios objectively categorize pollen as subprolate, prolate-spheroidal, or oblate. The P/E ratio tends to exhibit greater stability compared to the size of pollen grains, which can vary depending on environmental factors or their formation processes. Recent morphometric studies, including the investigation carried out by (Zhang *et al*., 2023) on *Sorbus*, primarily rely on P- and E-values as essential variables in multivariate analyses to accurately differentiate closely related species. (Li *et al*., 2024) employed identical measurements to evaluate the taxonomic complications of pollen morphology within 46 *Polygonatum* germplasms.

2.2. Aperture

The aperture system, which includes special parts of the pollen wall, is probably the most important quality for high-level classification. Apertures are important for biological processes because they help with volume changes when water is added (harmomegathy) and give pollen tubes a place to grow. Their type (for example, colpus-a furrow; porus-a pore; sulcus-a distal furrow; or compound forms like colporus), number (for example, monocolpate, tricolpate), and arrangement (for example, zonocolpate, pantoporate) are all very stable traits. The tricolpate condition, for example, is a well-known synapomorphy for the eudicots. While the type and number of apertures are potent qualitative markers, their dimensions provide crucial quantitative data. The length and width of colpi, or the diameter of pores and their surrounding membranes (ora), can be precisely measured. A study on the tribe, Inuleae (family Asteraceae) illustrates that these quantitative aperture features serve as significant variables for statistical analyses focused on interspecific differentiation (Qu *et al*., 2024).

2.3. Exine Ornamentation and Ultrastructure

The exine, or outer pollen wall, exhibits a sophisticated array of features. The external surface pattern, or ornamentation, is a primary qualitative feature, which is best resolved with Scanning Electron Microscopy (SEM), these patterns—such as psilate (smooth), reticulate (net-like), striate (parallel grooves), echinate (spiny), or verrucate (warty)—are genetically controlled and serve as critical ‘fingerprints’ for generic and specific identification (Perveen & Qaiser, 2020). Deeper than the surface, the exine’s ultrastructure reveals further qualitative traits, most notably the distinction between tectate pollen (with an outer “roof” or tectum supported by rod-like columellae) and intectate pollen (lacking a tectum). The characteristics of the columellae, such as their simplicity, branching, or reduction, provide additional insights. This structural framework provides numerical information, including the overall exine thickness, the thickness of specific layers (tectum, columellae, foot layer), and the measurements of sculptural features. For instance, in echinate pollen, the length, basal width, and density of spines are critical quantitative traits employed in morphometric comparisons (Qu *et al*., 2024).

2.4. Pollen Units

While most angiosperms disperse pollen as single grains, or monads, the aggregation of meiotic products into compound units is a highly significant qualitative trait. These units, held together by common exine or viscin threads, are stable diagnostic features for entire families. They include dyads (two grains), tetrads (four grains), and polyads (multiple grains, such as 16, 32, or 64). Tetrads serve as a key feature of the family Ericaceae, commonly known as the Heath family. The arrangement of grains within the tetrad (e.g., tetrahedral, rhomboidal, decussate, linear) provides a further qualitative distinction. More complex polyads are hallmarks of the Mimosoideae clade (family Fabaceae) and the family Orchidaceae. In orchids, these clustered units are commonly referred to as massulae, which can be connected to a sterile stalk (caudicle) to create a pollinium. An in-depth ultrastructural examination of Mormodes orchids provided a comprehensive overview of the morphology of these intricate units (Contreras-Jaime *et al*., 2023). The qualitative nature of being in a tetrad or polyad contrasts with the quantitative aspect of the number of grains within a polyad (e.g., 16 vs. 32), which serves as a descriptor for distinguishing taxa.

Methodologies for Palynotaxonomic Analysis

The effective use of pollen morphological data for systematic inquiries relies on a strong methodological framework. This process has transformed from conventional observation to a contemporary, comprehensive field that merges advanced imaging techniques with robust statistical and molecular evaluations.

1. Diagnostic Tools: From Traditional to Modern Microscopy

The foundation of palynotaxonomic analysis is rooted in microscopy, which generally begins with the chemical preparation of pollen. The acetolysis method remains the standard, efficiently breaking down the non-resistant intine and cellular components, which allows for the isolation of the robust exine for further analysis. Light Microscopy (LM) serves as the fundamental and essential instrument for analyzing these prepared grains. It is crucial for obtaining key quantitative characteristics, including the polar axis (P) and equatorial diameter (E), as well as for examining the overall morphology and internal exine architecture, which encompasses the thickness and separation of the endexine and ectexine. Nevertheless, the advent of electron microscopy revolutionized the field by revealing structural intricacies at an unparalleled level of detail. Scanning Electron Microscopy (SEM) has become an essential standard in modern palynotaxonomy, providing high-resolution, three-dimensional views of the exine surface. This represents the most effective method for accurately characterizing complex exine ornamentation patterns (such as reticulate, striate, and foveolate) that are difficult to observe or interpret using light microscopy. To gain a deeper understanding of the structure, Transmission Electron Microscopy (TEM) is employed. TEM provides a detailed visualization of the pollen wall's cross-sections, enabling us to characterize the internal architecture of the tectum, columellae, and foot layer (Contreras-Jaime *et al*., 2023). Contemporary and thorough assessments, such as the analysis of *Helianthemum* by (Díez-Méndez *et al*., 2024) or *Cenchrus* by (Saqib *et al*., 2023), systematically integrate both LM and SEM techniques to develop a comprehensive morphological dataset. LM serves to obtain essential measurements and insights into internal structural details, whereas SEM is utilized to explore important surface features.

2. Statistical and Molecular Integration

The morphological information obtained via microscopy is now no longer simply taxonomically recorded; rather, it is extensively evaluated using main approaches. Statistical integration has become an important tool in interpreting both the numerical information and coded qualitative information. Through constructing matrices using these characters, scientists can use different multivariate statistical analysis techniques, including Principal Component Analysis (PCA) and different forms of Cluster analysis, including UPGMA (Unweighed Pair Group Method with Arithmetic Mean) and Hierarchical Cluster analysis. Such statistical approaches allow an objective evaluation of variability and can classify taxa according to their total morphological affinity, which can identify species limits not recognized using individual characteristics. PCA technique and various types of cluster analysis have demonstrated significant efficacy in validating the segregation of taxonomically contentious species based on pollen morphometrics in studies by (Zhang *et al*., 2023) on Sorbus and (Qu *et al*., 2024) on Inuleae.

However, in addition to these statistics, the integration of palynology with molecular phylogenetics is considered to be the "frontier of systematics in the 21st century" as pollen morphology contains a "key taxonomic character set with which phylogenetic DNA trees can now be compared" (Joly *et al*., 2023). Moreover, this integration enables scientists to "compare morphologic and molecular systematic hypotheses with each other," and based on pollen morphology, in some species, results based on molecular analysis are validated, but in other species, palynology demonstrates "massive homoplasy," or a case of "convergent evolution," where a particular type of pollen evolves in separate species independently. A 'total evidence' analysis, which integrates information from all available sources, including morphology, ultrastructure, and DNA, is essential in constructing an accurate phylogeny of plant evolution. (Kriebel *et al*., 2021).

Case Studies from the Indian Flora

The Indian subcontinent's rich biological diversity, characterized by a significant degree of species endemism and a substantial number of endangered species, renders it a critical region for palyno-taxonomic research. Case studies from this region illustrate how pollen morphology can be effectively utilized to clarify taxonomic uncertainties and support conservation initiatives.

1. Family Fabaceae (Leguminosae): Delimiting Genera within a Complex Family

The Fabaceae family stands out as one of the largest and most economically important families in India. The palynological diversity is notable, with the Mimosoideae clade frequently identified by the presence of polyads, whereas the Papilionoideae and Caesalpinioideae clades generally display single, tricolporate grains. Nonetheless, within these expansive categories, notable differences in exine ornamentation and aperture characteristics yield valuable insights for both generic and specific classification. A comprehensive analysis conducted by (Karmakar *et al*., 2021) on twelve species of the Indian genus *Tephrosia* (Papilionoideae) demonstrated this utility. By analyzing seventeen qualitative and quantitative pollen traits, the study used multivariate statistics to segregate the species into distinct groups. Features such as pollen shape (prolate-spheroidal vs. subprolate) and, critically, exine ornamentation (e.g., micro-reticulate vs. rugulate-reticulate) were found to be taxonomically significant, supporting the infrageneric classification of this complex genus. (Karmakar *et al*., 2021), work underscores how pollen data can provide an independent line of evidence for clarifying relationships in large, taxonomically difficult groups.

2. Family Acanthaceae: A Case of Diagnostic Diversity

The Acanthaceae family exemplifies a remarkable breadth of pollen morphological diversity, showcasing a wide array of forms and structures. Within the areas of Indian flora, this family presents a remarkable array of pollen types, encompassing everything from straightforward tricolpate grains to intricate spiraperturate and fenestrate forms, all characterized by their elaborately designed exines. This remarkable diversity, however, does not lead to confusion; instead, it serves as a powerful diagnostic tool. Numerous genera and even species within the Indian Acanthaceae family exhibit a "stenopalynous" characteristic, indicating that their pollen is uniquely distinct, allowing for swift and dependable identification. An investigation into the palynomorphology of *Barleria* L. species in southern India conducted by (K M & M 2021) validated the taxonomic significance of this diversity. The study clearly classified species based on pollen type, illustrated by the *Barleriaprionitis* complex (showing 3-colpate, reticulate pollen) compared to other species exhibiting 3-porate, spiraperturate pollen. This diagnostic capability is essential for taxonomists, offering dependable micro-characters that improve classifications frequently complicated by vegetative plasticity.

3. Family Apocynaceae: Palynology for Threatened Species Delimitation

This case study establishes a direct connection between the study of pollen and the preservation of endangered plant species in India. The Apocynaceae family encompasses numerous significant and highly endangered medicinal plants in India, particularly Rauvolfia serpentina (Indian Snakeroot), which faces critical endangerment in various areas due to excessive harvesting. The market for crude drugs frequently experiences an influx of substitutes or adulterants derived from less potent relatives, such as *R. tetraphylla*, or other similar genera. Palynology serves as an efficient and economical method for ensuring quality and verifying the authenticity of botanical raw materials. The pollen structure in *Rauvolfia* is very distinct: large, spheroidal, and pantoporate, with a reticulate exine. The pollen of *Rauvolfia* and related genera, confirming the distinctiveness of these features (Mondal & Paria, 2018). Using a key based on grain size, number of pores, and pattern of exine, they were able to identify *R. serpentina* and rule out its common adulterants. Utilizing the features of pollen for classification and authentication is a significant and useful advancement in the protection of a fragile medicinal species.

4. Family Balsaminaceae (*Impatiens*): Differentiating an Endemic and Speciose Genus

The genus *Impatiens* (family Balsaminaceae) exemplifies a group in which pollen morphology serves as an essential instrument for taxonomy and conservation. India, especially the Western Ghats and the Himalayas, is a recognized global hotspot for *Impatiens* diversity, exhibiting an exceedingly high level of endemism. Many of these endemic species are scarce, specialized to micro-habitats, and classified as threatened (Bhaskar, 2012). Taxonomically, the genus is notably challenging due to substantial variation in vegetative and floral characteristics. Palynology offers a consistent array of micro-characteristics to aid in addressing these taxonomic difficulties. Research on Indian *Impatiens* has demonstrated considerable diversity in pollen morphology, notably in shape, the number of apertures, and exine ornamentation. A thorough SEM analysis carried out by (Pechimuthu *et al*., 2021) on 18 species from the Nilgiris-a biodiversity hotspot within the Western Ghats-revealed that pollen grains ranged from prolate to spheroidal in shape, featured apertures that were dicolpate, tricolpate, or tetracolpate, and displayed ornamentation that was predominantly reticulate, with one exception showing echinate (spiny) traits.

The variety in morphology carries significant consequences for conservation efforts. In a recent study of *Impatiens cordata*, a species that is both endemic and threatened in the Southern Western Ghats, (Abhilash, 2024) employed pollen morphology for the dual purpose of identification and evaluation of reproductive health. The study confirmed the distinctive pollen features (elliptical, tetracolpate, and reticulate) while also establishing a correlation between morphology and pollen viability and germination assessments. By establishing these fundamental palynological markers, researchers can accurately identify fragmented or vulnerable populations in the wild and provide critical data for ex-situ conservation and breeding programs.

Role of Palynotaxonomy in the Conservation of Threatened Flora

Palynotaxonomy is useful for more than just descriptive systematics; it is also important for applied conservation. Effective conservation methods for vulnerable flora, whether in situ or ex situ, essentially rely on precise identification and health assessment of target populations. Palynology provides a robust, rapid, and cost-effective approach for fulfilling both requirements. It is an important tool because it gives the diagnostic "fingerprints" needed for identification and shows how well vulnerable populations may reproduce when they are under ecological stress.

1. Identification as the Foundation of Conservation

The essence of conservation is embedded in its terminology. Prior to launching any successful protection or propagation effort, it is crucial to accurately and clearly identify the target species. This poses a significant challenge for endangered plant species, which are often rare, restricted in their geographic range, or part of "cryptic" species groups that are difficult to distinguish based solely on vegetative characteristics.

The architecture of pollen presents a set of reliable, recognizable micro-characteristics that are not influenced by the plant's development or flowering circumstances (Wortley *et al*., 2020). This is essential for recognizing fragmented material, confirming plants during non-flowering periods, or validating raw herbal materials in commerce. An exemplary case is the verification of endangered medicinal plants, a significant conservation challenge in India. The case of *Rauvolfia serpentina* illustrates how pollen morphology serves as an efficient and conclusive method for differentiating the genuine, endangered species from its prevalent adulterants (Mondal & Paria, 2018). This ensures that the unintentional removal of incorrect species is minimized and that conservation efforts are directed effectively. Furthermore, validating palynological data is crucial for ensuring the credibility of germplasm collections in gene banks. This procedure ensures that ex-situ collections, especially concerning the endangered medicinal genus *Berberis*, faithfully represent the target species and its genetic variation (Zia *et al*., 2022).

2. Pollen Traits as an Indicator of Reproductive Health

In addition to its classification function, the structure of pollen serves as a precise indicator for evaluating the reproductive vitality and environmental pressures faced by at-risk populations. The generation of functional pollen plays a crucial role in sexual reproduction, facilitating gene flow and supporting the preservation of genetic diversity. Environmental stressors-such as heavy metal pollution, climate extremes, habitat fragmentation, or pathogen attack-can interfere with microsporogenesis (pollen development), resulting in measurable alterations in pollen quality (Can *et al*., 2019).

The observed changes include a significant occurrence of pollen sterility, various morphological abnormalities (such as aborted, shrunken, or irregularly shaped grains), and a decrease in *in-vitro* germination rates. As a result, palynological analysis serves as a valuable early warning tool. A notable abundance of non-viable or malformed pollen within a specific population may signify considerable environmental stress or potential genetic issues, such as inbreeding depression in small, isolated groups (Guan *et al*., 2021). The examination of the endangered *Impatiens cordata* demonstrated a direct connection between pollen analysis and assessments of viability and germination, aimed at evaluating its reproductive capacity (Abhilash, 2024). Such information is indispensable for mitigation strategies, as it allows for the precise demarcation of at-risk populations and underpins decision-making regarding ecological restoration and germplasm management.

CONCLUSION

The exploration of palynology has transformed from a descriptive support field of botany into a robust, multi-faceted discipline that is central to contemporary plant systematics. With biodiversity encountering unparalleled challenges, accurately identifying species has transcended mere academic interest; it has become a crucial necessity for conservation efforts. The insights presented in this review emphasize that the structure of pollen provides a collection of consistent, genetically influenced traits spanning from detailed exine patterns to sophisticated aperture designs that persist even in the absence or potential misrepresentation of macro-morphological characteristics. The shift from conventional Light Microscopy to advanced SEM and TEM, along with multivariate statistical analysis and molecular phylogenetics, has revolutionized palynotaxonomy into a precise toolkit that can address the challenges of "cryptic taxa" commonly encountered in intricate Indian families such as the Fabaceae and Acanthaceae.

Additionally, within the Indian framework, palynology plays an essential dual role. This is a very important way to protect valuable medicinal plants like *Rauvolfia serpentina* from pollution. It also gives an analytical view on how to protect unique native genera like *Impatiens*. Beyond mere identification, the structural soundness and functional capacity of a pollen grain function as a subtle gauge, reflecting the reproductive fortitude of species facing environmental challenges. Palynotaxonomy, in its essence, bridges contemporary knowledge with uncharted territories. This facilitates the recognition and systematic classification of India's diverse flora, a critical factor in the implementation of effective conservation strategies. Consequently, by integrating these seemingly minor observations within wider ecological contexts, researchers can more efficiently tackle the complexities of an era characterized by swift environmental transformations.

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