

Palynological Diversity In Selected Medicinal Plant Species Of Rubiaceae From Flora Of India

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Abstract

During the 1990s Rubiaceae turned into a problem area for systematists, chiefly because of the thorough treatment of the family by Robbrecht in 1988. Close to the investigation of macromolecular characters to construe the phylogeny, the palynology of Rubiaceae at long last gotten the consideration it merits. The purpose of this article is to present a cutting-edge analysis of the family's systematic palynology. The scope of variety in dust morphology is wide, and a portion of the dust highlights are not known from other angiosperm taxa; e.g., a looplike or winding example for the place of gaps in pantoaperturate grains. We compiled a generic online database of Rubiaceae's major pollen characters and orbicule presence. This section provides an overview of the variation and character illustrations: pollen size and form, aperture number, position, and type, sexine ornamentation, nexine pattern, and stratification of the sporoderm are all aspects of the dispersal unit. The presence/nonattendance and morphological variety of orbicules at the nonexclusive level is given also. The orderly handiness of dust morphology in Rubiaceae is examined at the (sub)family, ancestral, nonexclusive, and infraspecific levels, utilizing around date transformative speculations for the various genealogies in the family. Also discussed are the advantages and disadvantages of coding pollen characters for cladistic analyses.

Keywords: exine ornamentation; pollen morphology; RET plants; Scanning Electron Microscopy; Western Himalaya, Floristic diversity, Ethnobotanical study, Herbarium, RET Species

INTRODUCTION

Because of Robbrecht's comprehensive treatment of the family in 1988, Rubiaceae became a hotbed for systematists in the 1990s. The palynology of Rubiaceae finally received the recognition it deserved, following the investigation of macromolecular characteristics for determining the phylogeny. The purpose of this article is to present a cutting-edge analysis of the family's systematic palynology. There is a wide range of pollen morphology, and some pollen characteristics are unknown from other angiosperm taxa; for instance, a spiral or loop-like pattern used to position the apertures in pantoaperturate grains. We compiled a generic online database of Rubiaceae's major pollen characters and orbicule presence. This section provides an overview of the variation and character illustrations: pollen size and form, aperture number, position, and type, sexine ornamentation, nexine pattern, and stratification of the sporoderm are all aspects of the dispersal unit. At the generic level, orbicules' presence or absence and morphological variation are also provided. The methodical handiness of dust morphology in Rubiaceae is talked about at the (sub)family, ancestral, conventional, and infraspecific levels, utilizing around date developmental speculations for the various genealogies in the family. The issues and chances of coding dust characters for cladistic examinations are likewise treated.

With more than 13,000 species, the Rubiaceae are one of the largest families of plants. The phylogeny of 534 Rubiaceae taxa from 329 genera with up to five distinct chloroplast regions was estimated using Bayesian analysis in this study. A highly resolved tree with numerous strongly supported nodes was produced as a result. The majority of the 44 tribes included and the three subfamilies (Cinchonoideae, Ixoroideae, and Rubioideae) receive substantial support. A Bayesian method was used to estimate divergence times for clades classified as tribes and subfamilies using a scaled-down data set of 173 Rubiaceae taxa. One fossil for each subfamily and one for the Rubiaceae as a whole (*Faramaea type* pollen, *Scyphiphora* pollen, *Cephalanthus pusillus* fruits, and *Paleorubiaceophyllum eocenicum* leaves) were used as minimum age priors. The assessed ancestry (stem) difference time for Rubiaceae is 90.4 Ma. The subfamilies Cinchonoideae, Ixoroideae, and Rubioideae have estimated lineage divergence times of 73.1 Ma, 84.4 Ma, and 84.4 Ma, respectively. The assessed genealogy dissimilarity times for the clans change somewhere in the range of 86.6 and 14.2 Ma. For each tribe, age estimates, relationships, classification, and distribution in space are presented and discussed. The palynological diversity of highly medicinal Rare, Endangered, and Threatened (RET) plant species found in the Western Himalaya, India, was the subject of this study. Light Microscopy (LM) and Scanning Electron Microscopy (SEM) were used to

examine the pollen morphology of 32 plant species from 29 genera, 23 families, and 18 orders of Angiosperms. According to the most recent version of Angiosperm Phylogeny Group V, the studied plant species' families have been arranged and discussed in the order of their evolutionary progression. The studied pollen characteristics were found to be useful for species-level taxonomic classification. In the concentrated on monocots, the species having a place with request *Zingiberales*, *Asparagales*, and *Liliales* pursued the overall direction of having crude inaperturate to progress monosulcate dust with the exception of *Alismatales* which had inaperturate dust and put in after the requests referenced above in the APG IV grouping framework. In support of previous findings, the evolutionary progression of the eudicot group's pollen aperture from tricolpate to tricolporate and triporate was observed in this study. Except for the *Rhododendron companulatum* (Ericales), which had an evolved tetrad arrangement, all of the examined monocot and eudicot species had monad pollen units, indicating their evolutionary primitiveness. The ongoing review found crude exine ornamentation in Ranunculales to progress in Asterales, Lamiales, Gentianales, and Boraginales and affirms the aftereffects of the past examinations. Understanding the reproductive biology and conservation aspects of these significant high-altitude plant species will be made easier by the pollen morphological data gathered in this study.

PALYNOMORPHS

According to Blackmore (2000), systematic classification of palynomorphs has tremendous potential. They assist with settling convoluted interrelationships among taxa and decide their ordered status, outstandingly with regards to families, subfamilies, clans, genera, species (Ahmad et al., 2010; Wortley et al., 2015; Zhang et al., 2017). Because pollen morphological characteristics like shape, apertural pattern, and exine configuration are conservative features that can be used for the taxonomic assessment of plants, the study of pollen grains is an important area of research (Perveen, 2006; Keshavarzi and others, 2012; Singh and other, 2020). Morphological qualities of dust grains can help in plant scientific classification review on the grounds that numerous dust characteristics are affected by areas of strength for the powers associated with different conceptive cycles, including fertilization, dispersal, and germination (Erdtman, 1952; 1979, Nowicke and Skvarla; Moore and others, 1991). Dust morphology examinations may likewise offer important exploration assets for sensitivity requests (e.g., occasional unfavorably susceptible rhinitis, pollenosis), fertilization science, and honey review (Devender et al., 2016). The study of palynological characters within a broader evolutionary context has a long and distinguished history (Wodehouse, 1935; 1952, Erdtman; 1975, Walker and Doyle; Nowicke and Skvarla, 1979). Morphological consideration of pollen provides a useful alternative support for molecular tree topologies, and morphological characters can assist in understanding evolutionary issues that may be difficult to solve using molecular techniques (Renner et al., 2000). The identification of a monophyletic tricolpate clade within angiosperms (Donoghue and Doyle, 1989), now commonly referred to as the eudicots (Doyle and Hotton, 1991), has brought to light the significance of pollen characteristics in systematics. APG, 1998; APG II, 2003; 2009 APG III). As of late, a few specialists have concentrated on the development of dust characters with regards to phylogeny (Luo et al., 2015; Zhang et al., 2017; Wortley et al., 2015). The Himalayan district's remarkable geology and climatic circumstances make it a rich store of plants, with very nearly 8,000 species, 25.3% of which are native, supporting the existences of millions of individuals (Singh and Hajra, 1996; Negi et al., 2019). The Himalayan region is one of 34 "biological hotspots" due to its high biological and sociocultural diversity (Gautam, 2013). Numerous studies (Bano et al.,) have documented palynological studies on high-altitude plant species. 2012; Ahmad et al., 2013). The connection between vegetation elements and dust in mountain biological systems has been unraveled in a few before studies (Davis, 1984; Tanțău and others, 2014). A study using Light Microscopy on the pollen morphological analysis of 152 Indian plant species belonging to 49 families was conducted in the Indian context (Sharma and Bhat, 2015). Nonetheless, a couple of studies from the Western Himalayan locale have been directed to investigate the ordered significance of dust grain characters (Kayani et al., 2019; Bano and others, 2020; Singh and other, 2020). The Western Himalayan flora almost lacks palynological details. Using Light Microscopy (LM) and Scanning Electron Microscopy (SEM), the pollen morphology of 32 plant species from 29 genera, 23 families, and 18 orders of Angiosperms was examined in this study. The concentrated on plants are high elevation Uncommon, Imperiled, and Undermined (RET) restorative plant species from Western Himalaya, India. Understanding the reproductive biology and conservation aspects of these significant high-altitude plant species will be made easier by the pollen morphological data gathered in this study.

Materials and Methods

We compiled and added all of the contemporary literature to the Seventh Bibliographic Index to the Pollen Morphology of Angiosperms (Tissot & van der Ham, 1994) in order to synthesize the Rubiaceae's pollen morphology. For every sort, we held the latest or the most solid and applicable dust morphological works. From these, subsequent to checking all taxa against the latest orders, we refined the dust morphological characters at the conventional level and put away them in a data set regarding the writing counseled. The dust data set is connected to a deliberate data set holding essential data, for example, species number, dissemination, and the efficient place of every family. You can access the database online at <http://www.kuleuven.ac.be/bio/sys/pollen>. The following quality standards were established for the retained works: 1) There are clear illustrations, preferably SEM and LM photographs; 2) the creators have dependable ordered information on the species explored; (3) There are descriptions of the pollen, or if there aren't, the illustrations can tell you who the main pollen characters are. Geologically arranged palynological commitments (e.g., dust greeneries) or general works on Rubiaceae with sparse notes on dust morphology were consequently frequently precluded, yet reference to them is made in the data set. Genera for which dust information found in writing don't satisfy the quality necessities set above are available in the data set, yet they are not used to set up the dust portrayal of the clans to which they have a place. When

the article's results section contained only descriptions of pollen types and no data on species or generic pollen, it was sometimes difficult to figure out how to convert the raw data into pollen features for genera. A summary cladogram that reflects the Rubiaceae relationships currently accepted is used to evaluate the phylogenetic value of pollen characters at the tribal level. This tree is built with MacClade, and the spine depends on the concentrate by Bremer et al. (1999: *ndhF*, *rbcL*). In this review, three subfamilies were perceived: Cinchonoideae, Ixoroideae, and Rubioideae. Cinchonoideae s.str. is utilized later in this article. likewise Ixoroideae s.l., because these two subfamilies were reduced and expanded in comparison to earlier concepts of the same name proposed by Robbrecht (1988a, 1993). The following articles serve as the foundation for the relationships that are depicted within the three subfamilies in the summary cladogram: Rova and Andersson (1999: *rps16*-intron), as well as Bremer and Manen Piesschaert et al. (*rbcL*, *atpB*, *rbcL*, *rps16*-intron) 2000a: *rps16*-intron), as well as Dessein *rps16*-intron; ITS) for the family Rubioideae; Rova (1999: *rps16*-intron, and *trnL*–F spacer), Razafimandimbison and Bremer (2001: Rova et al. (ITS, *rbcL*, and *trnT*–F) 2002: for the Cinchonoideae s.str., *trnL*–F); as well as Rova *rps16*-intron, *trnL*–F spacer, and Bremer and Thulin's Andreassen et al. (*rbcL*), 1999: Andreassen and Bremer (2000: ITS, *rbcL*) ITS, *rbcL*), Dessein and colleagues 2001b: *rps16*-intron, *rbcL*), and Rova et al. (2002: for the Ixoroideae s.l., *trnL*–F). Unless otherwise noted, the online version of Punt et al.'s terminology is used. 1994), which can be found at <http://www.bio.uu.nl/palaeo/glossary/index.htm>. For shape classes (P/E) we took on the wide meanings of Nilsson and Praglowski (1992); that is, the oblate (0.50–0.75), the suboblate (0.75–0.88), the spheroidal (0.88–1.14), the subprolate (1.14–1.33), and the prolate (0.33–2.00).

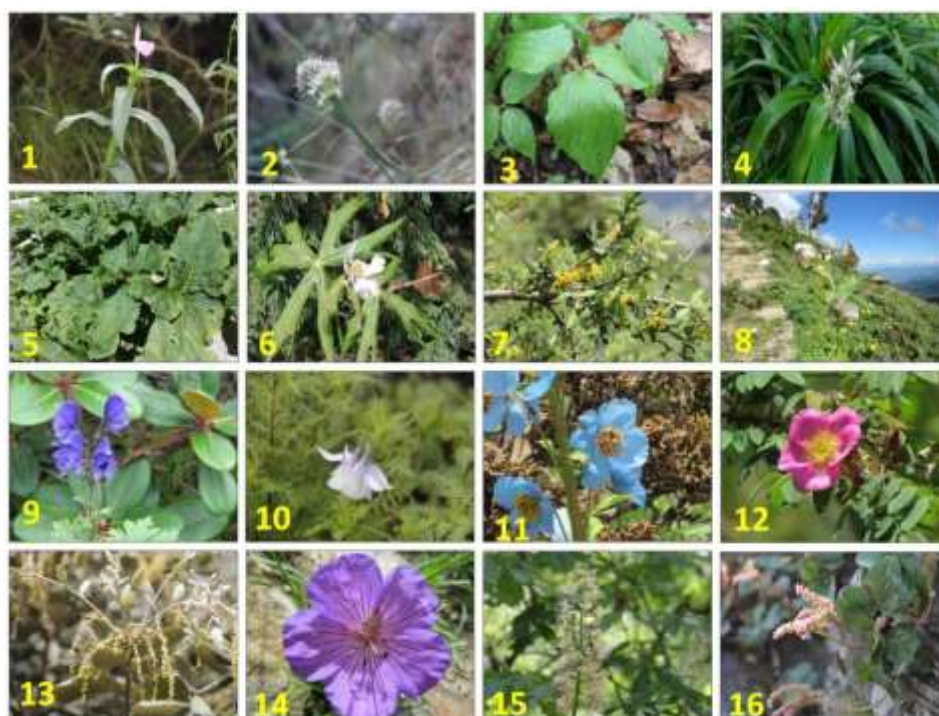
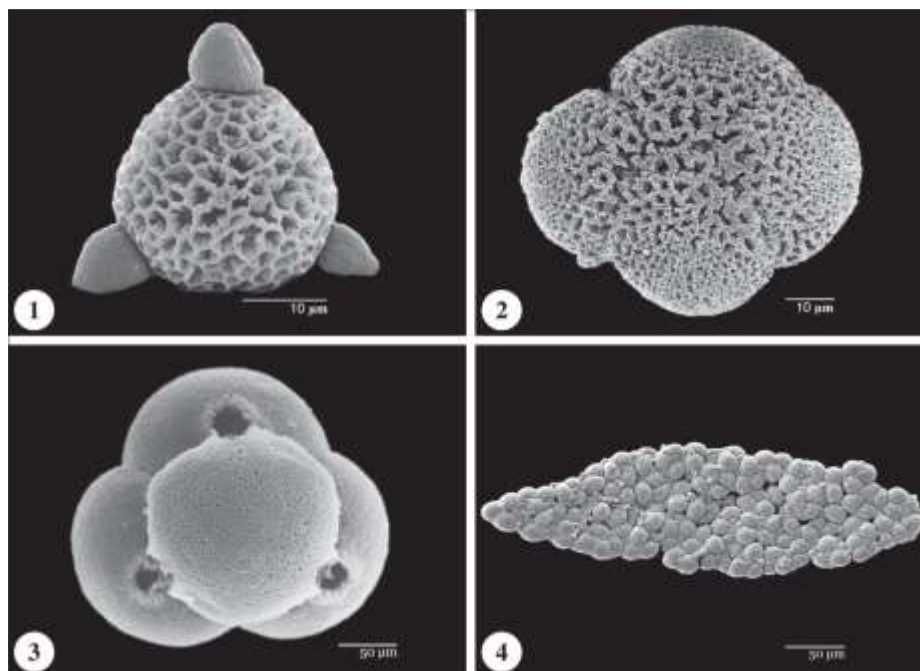




Figure 2. Pictures of the studied RET plant species from Western Himalaya India, 1) *R. purpurea*, 2) *A. stracheyi*, 3) *T. govanianum*, 4) *C. arundinaceum*, 5) *A. propinquum*, 6) *S. hexandrum*, 7) *B. lycium*, 8) *A. heterophyllum*, 9) *A. violaceum*, 10) *A. pubiflora*, 11) *M. aculeata*, 12) *R. webbiana*, 13) *Q. oblongata*, 14) *G. wallichianum*, 15) *A. indica*, 16) *B. ciliata*, 17) *B. stracheyi*, 18) *R. australe*, 19) *R. webbiana*, 20) *O. digyna*, 21) *P. acinosa*, 22) *R. companulatum*, 23) *A. millefolium*, 24) *J. dolomiaea*, 25) *S. obvallata* [Herbarium specimen used for the study, picture not available], 26) *L. fischerii* var. *euodon*, 27) *A. glauca*, 28) *M. longifolia*, 29) *S. hians*, 30) *P. kurroa*, 31) *G. kurroo*, 32) *A. benthamii*

Extensive field visits were conducted during flowering season in Western Himalayan region of India that comprised two union territories of India viz. Jammu and Kashmir, Ladakh and two states i.e. Himachal Pradesh and Uttarakhand (Figure 1). The herbarium specimens of the collected plants were dried, pressed, poisoned with 1% mercuric chloride, and mounted on herbarium sheets following usual herbarium procedures. The collected specimens were identified with the help of regional floras, monographs, research articles (Chowdhery and Wadhwa, 1984; eFloras, 2021; Hooker, 1890; Polunin and Stainton, 1984; Sharma and Kachroo, 1981), and by comparing with authentic specimens available at internationally recognized Janaki Ammal Herbarium (RRLH) at CSIR-IIIM Jammu. Duly identified herbarium voucher specimens were submitted to the Janaki Ammal Herbarium (RRLH). The studied plant families have been arranged and discussed as per the evolutionary sequence of Angiosperm Phylogeny Group IV (APG, 2016). In the present study using light and scanning electron microscopy was conducted on 32 medicinal, rare endangered and threatened plant species that covering 23 families and 29 genera of angiosperms. Pollen grains were analyzed for various quantitative and qualitative characters.

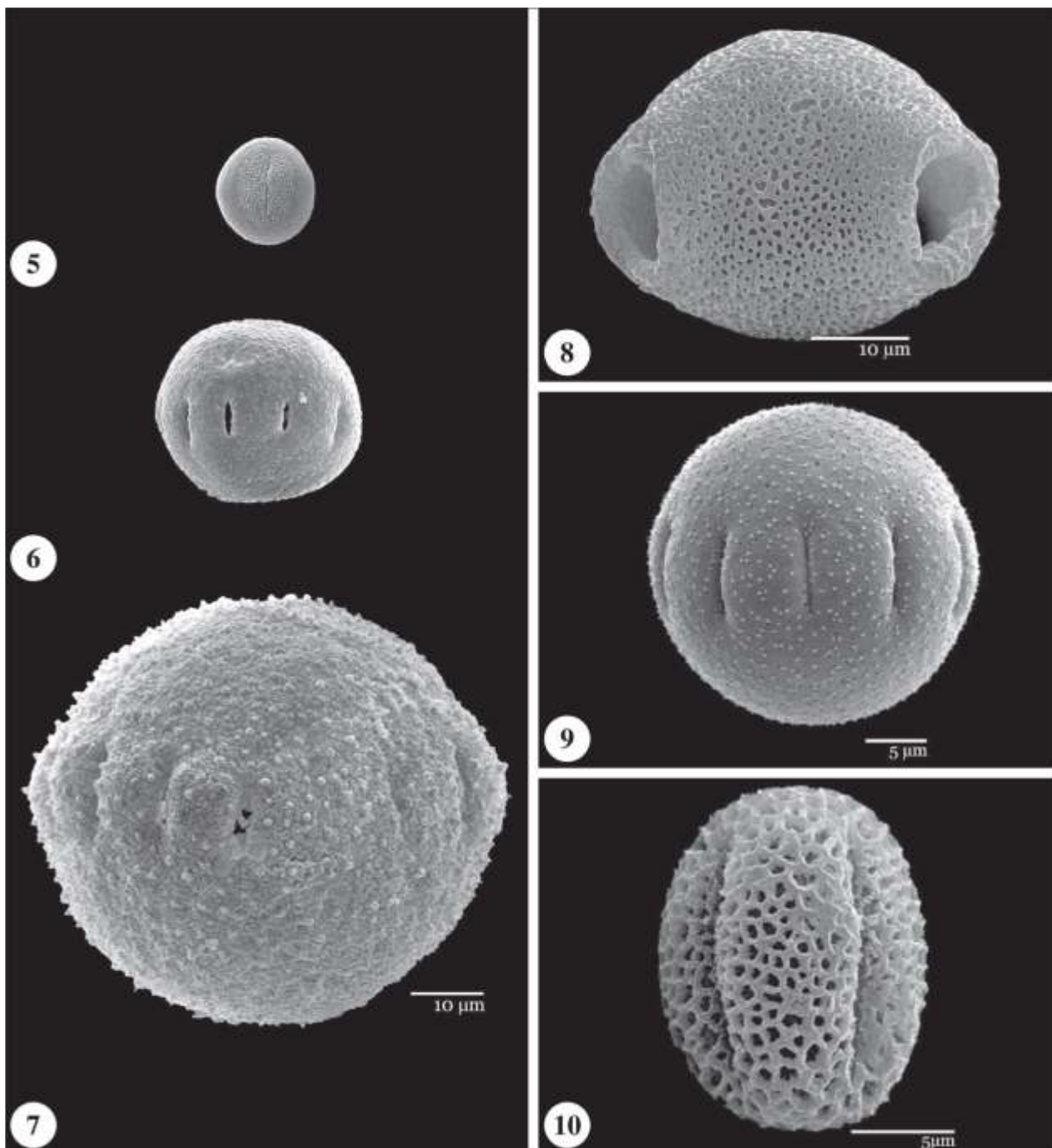


Figs. 1–4. Types of pollen dispersal. 1. Monad of *Psydrax subcordata* (DC.) Bridson, unacetolyzed (F. Lens, unpubl. data). 2. Tetrahedral tetrad with opposite apertures of *Ganguelia gossweileri* (S. Moore) Robbr. (Robbrecht et al., 1996). 3. Uniplanar, rhomboidal tetrad of *Euclinia longiflora* Salisb. (S. Huysmans, unpubl. data). 4. Pollen arranged in massulae, as observed in *Massularia acuminata* (G. Don) Bullock ex Hoyle, unacetolyzed (P. De Block & M. Verhaegen, unpubl. data).

POLLEN MORPHOLOGICAL CHARACTERISTICS OF RUBIACEAE

Dispersal Unit

Rubiaceae pollen grains are generally dispersed as monads (Fig. 1), but permanent tetrads (Figs. 2–3) are present in 13, mainly African, genera of Gardenieae (Robbrecht, 1988a) and in the distant genus *Gleasonia* Standl. in Henriquezieae (Rogers, 1984). *Randia* L. is the only genus known with both monads and tetrads (Persson, 1993), but the generic boundary of this genus is in flux (Gustafsson & Persson, 2002). The occasional presence of tetrads in *Lasianthus* W. Jack as reported by Huang (1972) needs confirmation. Massulae, pollen agglutinated in larger bodies of a few hundred grains and dispersed as a unit (Fig. 4), occur in a single genus of Gardenieae, *Massularia* K. Schum. (Persson, 1993). The observations by Igersheim (1989) of massulae in a few *Psydrax* Gaertn. and *Keetia* E. Philips species need confirmation. The configuration of the permanent tetrads is sometimes variable. *Ganguelia* Robbr., for instance, shows 30% decussate and 70% tetrahedral tetrads in the same specimen; a single rhomboidal uniplanar tetrad has also been observed in that genus (Robbrecht et al., 1996). Persson (1993) described tetrads of 11 genera from the Gardenieae, but he gave no detailed information about configuration; all tetrads shown by him are tetrahedral.



Figs. 5–10. Pollen shape and size. 5. *Spermacoce natalensis*: pollen size 14–19 μm (Dessein et al., 2002a). 6. *Spermacoce chaetocephala* DC.: pollen size 38–52 μm (Dessein et al., 2002a). 7. *Spermacoce ivorensis* Govaerts: pollen size 105–125 μm (Dessein et al., 2002a). 8. Equatorial view of an oblate pollen grain of *Psydrax horizontalis* (K. Schum. & Thonn.) Bridson (Lens et al., 2000). 9. Equatorial view of a spheroidal pollen grain of *Crusea calocephala* DC. (Dessein, 2003). 10. Equatorial view of a prolate pollen grain of *Kohautia grandiflora* DC. (S. Dessein, unpubl. data).

Pollen Size

Size of the pollen after acetolysis (Figs. 5–7) varies from 6 millimeters in the *Danais cernua* Baker (Huysmans, 1998) to 133 millimeters in the *Diodella teres* Small (Dessein, 2003). The majority of species' pollen grains measure between 20 and 40 micrometers. According to Wodehouse (1935), pollen grains of this size are perfectly suited to be released from the anthers, transported by gentle winds, and attached to stigmata and insect vectors. Perception of extremely huge or tiny dust grains may along these lines likewise highlight explicit fertilization disorders. In spite of the fact that dust size is many times stable at the species level, we once in a while saw a significant intraspecific size variety (e.g., the tropical measurement (E) differs from 19 to 41 in *Spermacoce fabiformis* Harwood). This variation can sometimes be explained by infraspecific taxa (for example, Dessein et al., 2002a), polyploidy (e.g., Puff, 1986a), development conditions, bloom/anther size (e.g., Puff, 1986a), or heterostyly (see the part on "Heterostyly and Dust Dimorphism" beneath). The variation is frequently difficult to interpret. In *Spermacoce fabiformis*, for instance, people with bigger dust are much of the time found in drier regions, the very inverse of what one would promptly expect (Dessein et al., 2005b). Size variation

can be significant even within a single person. *K. Schum.*'s *Hedythyrus thamnoides* Bremek., In the same specimen, the polar axis (P) varies from 14 to 30 millimeters, whereas this is the case for all *Ernodea* Sw species. In another, two sizes of pollen were observed (Negrón-Ortiz, 1996). Negrón-Ortiz sorted out that in *Ernodea* huge dust grains are more plentiful than are little dust grains and that reasonability is related with the bigger grains. *Hintonia latiflora* (DC.) pollen also contained two sizes. Bullock, where it appears to be correlated with exine thickness variations (Ochoterena, 2000); in the last option case nothing is had some significant awareness of the practicality of the dust grains. At this time, it is unknown whether Rubiaceae pollen generally exhibits size dimorphism. Mathew and Philip (1983) listed 35 taxa in which they observed significant differences in size, but they did not specify whether these differences were present in just one specimen or in multiple specimens belonging to the same taxon. At the family level, there is no relationship between's bloom size and dust size. Inside specific ancestries, nonetheless, this connection has been noticed (Dessein et al., 2002a; 1984 (Rogers). The way that dust size might be variable inside a solitary animal categories, along with the solid impact of readiness and perception procedures on size and state of dust grains (see Reitsma, 1969), makes examinations of size estimations across various distributions interesting. However, we believe that the size of the pollen can serve as a criterion for identifying certain tribes or genera that are related to one another. The Naucleae and the Rubieae s.l., for instance, the majority of their pollen grains are very small to very small (under 30 m), whereas *Palicourea* Aubl. differs from the majority of the other Psychotrieae by having pollen grains that are extremely large (65–125 m). In eurypalynous gatherings, for example, the Spermacoeae s.str. However, only at the species level is pollen size useful (Dessein, 2003).

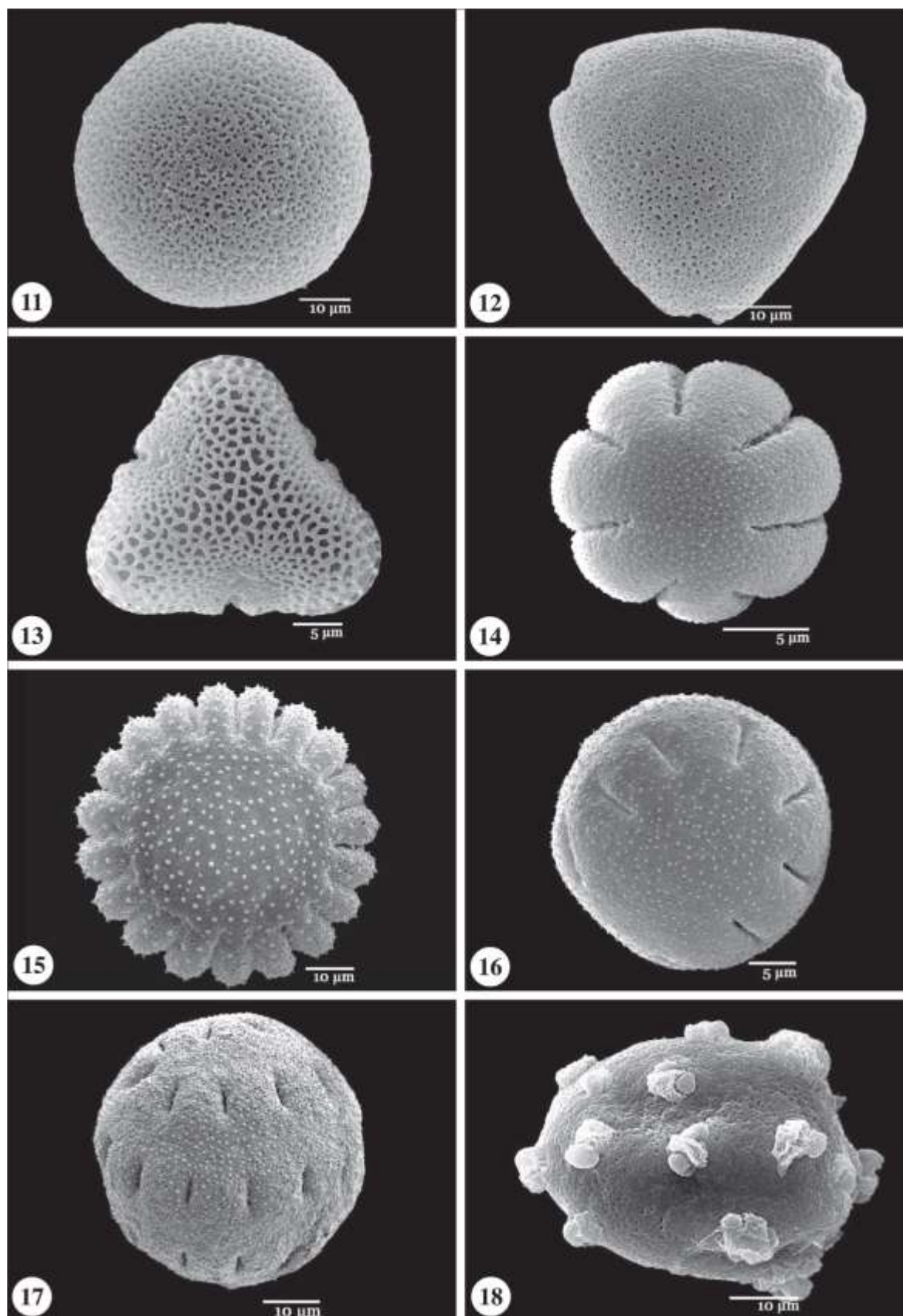
Pollen Shape

In equatorial view (P/E), the shape of the pollen of hydrated grains ranges from oblate to eight) to prolate 10) but typically spherical (Fig. 9). The diagram of hydrated dust grains in polar view (amb) is for the most part roundabout (Fig. 11), frequently somewhat lobed due to depressed colpi (Figs. 14–15). In a small number of species, like *Dictyandra arborescens* Welw. ex Snare. f., a quadrangular shape is commonplace (Robbrecht, 1984), though three-sided shapes (Figs. 12–13) are found, for instance, in the genera *Psydrax* Gaertn and *Tapiphyllum* Robyns. Lens and Co., 2000). The state of the grains relies vigorously upon harmomegathic impacts: The invagination of the ectocolpi by dehydration frequently results in more prolate pollen. The efficient worth of shape characters is problematic in Rubiaceae, in any case, for example, in *Spermacoe stenophylla* F. Muell. and F. Muell's *S. inaperta*, In polar view, pollen is typically oblate and triangular (Dessein et al., 2005b).

Apertures

The plesiomorphic condition of triaperturate pollen grains, which are most prevalent in Rubiaceae (Figs. 12–13). In various genera, tetraaperturate and, all the more once in a while, pentaaperturate dust grains are tracked down notwithstanding the triaperturate ones. Spermacoeae s.str. belongs to the more derived herbaceous groups. and in Rubieae, the majority of grains are pluriaperturate (Figs. 14–17). s.str., Spermacoeae According to Dessein et al., the number of apertures ranges from three to thirty. 2003), whereas the most that has been found in Rubieae to date is 13 (Huysmans et al., 2003). Pluriaperturate pollen grains are also occasionally found in a few other tribes' species or genera, like Theligoneae (*Theligonum* L.) and Spermacoeae s.l. (Baker's *Gomphocalyx*, Cham.'s *Kohautia*, and Schltdl., *Gardenieae* (*Rosenbergiodendron* Fagerl) and *Neanotis* W. H. Lewis (*Phylhydrax* Puff). furthermore, *Randia*). In the paleotropical *Geophila repens* (L.) Johnst. specimens, the openings are essentially as various as 150 (Vanthournout, 2002). *Chassalia* Comm. has been found in some *Antirhea* species (Chaw & Darwin, 1992). ex. Juss. Piesschaert and others, 1999a), *Nertera* Banks and Sol. ex Gaertn. (*Palicourea* Aubl. (Robbrecht, 1982b) *Rudgea* Salisb, *Psychotria* L. (Johansson, 1992), and Robbrecht, 1988a Jung-Mendaçolli, 1984; Piesschaert, not published), There appear to be no openings (Fig. 11). It is muddled whether this dust is truly inaperturate or rather omniaperturate. In the heterostylous types of *Hymenocoleus* Robbr., brevistylous blossoms appear to deliver nonaperturate dust grains, though longistylous blossoms produce 3-5-porate dust grains (Robbrecht, 1977). 16 genera of biaperturate pollen grains have been identified, including *Aidia* Lour. *Atractocarpus* Schltr. & Puttock, 1992). K. Krause, *Coussarea* Aubl. (Puttock, 1992), *Myrmecodia* Jack (Robbrecht, 1988a; Jung-Mendaçolli, 1984), *Schradera* Vahl (Puff et al., 1993a). It could only be used as a diagnostic feature for the monospecific genus *Leucocodon* Gardner (Puff & Buchner, 1998); Both 2-aperturate and 3-aperturate pollen grains can be found in all other genera. *Antirhea* pollen is described as either 1-porate or inaperturate by Chaw and Darwin (1992); D'hondt et al. (2004) report monoaperturate dust grains in *Blepharidium* Standl.; Huang (1972) portrayed dust of *Hedyotis diffusa* Willd. (= *Oldenlandia diffusa* (Willd.) Roxb.) as 1–5 colporate, and pollen grains of 1–3 porate were discovered in *Myrmecodia* by Huxley and Jebb (1993).

It is necessary to verify these Rubiaceae observations of monoaperturate pollen grains and investigate their viability. The quantity of openings might fluctuate significantly inside a family, which is shown for *Spermacoe* L. (Dessein et al., 2002a), in which pollen size and aperture count were found to have a weak positive correlation. The correlation is strong even if the pantoaperturate species are not included in the test. Likewise, inside *Danais* Comm. ex Vent there is by all accounts a relationship between's dust grain size and the quantity of colpi. Taxa with perpetually 3-or 3(- 4)- colporate grains will generally have more modest grain sizes, and species with 4(- 5)- colpi have bigger grains (Buchner and Puff, 1993).



Figs. 11–18. Number of apertures and aperture position. 11. Polar view of a circular, inaperturate pollen grain of *Palicourea petiolaris* H. B. & K. (F. Piesschaert, unpubl. data). 12. Polar view of a triangular, 3-colporate pollen grain of *Tapiphyllum cinerascens* Robyns, with apertures situated at the angles (Lens et al., 2000). 13. Polar view of a triangular 3-colporate pollen grain of *Spermacoce stenophylla* with apertures in the middle of the sides (Dessein et al., 2005b). 14. Polar view of a 7-colpate grain of *Galium mollugo* L. (S. Huysmans, unpubl. data). 15. Polar view of a 19-colporate pollen grain of *Spermacoce dibrachiata* Oliv.; notice the slightly lobed outline due to sunken colpi (Dessein et al., 2002a). 16. A pantoaperturate pollen grain of *Spermacoce octodon*, with the colpi arranged in a looplike pattern similar to the line on a tennis

ball (Dessein et al., 2002a). 17. A pantoaperturate pollen grain of *Spermacoce thymioidea*, with three parallel rows of colpi (Dessein et al., 2002a). 18. A pantoporate grain of *Geophila repens* (K. Es, unpubl. data).

PALYNOLOGICAL CHARACTERS IN RUBIACEAE

Orbicule Characteristics of Rubiaceae

Orbicules are small sporopollenin particles up to 3–4 μm in width that line the inward extraneous and outspread walls of the tapetal cells (Huysmans et al., 1998b, 2000). Subsequently orbicules coat the inward locular surface and are in close contact with the dust grains. Despite having an electron-transparent core, orbicules are always acellular structures. They are frequently, yet not generally, present in Rubiaceae. The principal report of the presence of orbicules in Rubiaceae was by Andronova (1984). She mentioned orbicules in a number of the family's unrelated species. However, this study has received little attention due to the fact that her work was published in Russian. As a result, no Rubiaceae were included in Pacini and Franchi's (1993) list of angiosperm species whose secretory tapetum was found to have orbicules. Orbicules were discovered by Igersheim (1993a) in the monospecific Caribbean genus *Strumpfia* that same year. Since then, the presence of orbicules has been examined in two Rubiaceae subfamilies: Huysmans and others (1997) looked at 14 Cinchonoideae s.str. genera, and Vinckier and coworkers (2000) examined 32 Ixoroideae s.l. genera. Orbicules have additionally been accounted for in a few different genera. All of the available data are compiled into a generic summary in Table II. Rubiaceae contains six different kinds of orbicules (Huysmans et al., 1997; Vinckier and others, 2000): I. Prickly orbicules. Orbicules depend on 3 μm in breadth and are not implanted in tapetal film (albeit uncommon semi-inserted structures do happen). The smooth orbicular wall is punctured by a few to many spinules; there may be one or two perforations. A core exists or does not exist. II. Orbicules with a microrugule (Fig. 48). Orbicules are embedded in the tapetal membrane and have a diameter of less than 2 μm . Although they appear to be made up of several elongated components, their shape is more or less spherical. III. Orbicules that are smooth 49–51). Orbicules rarely embed themselves in the tapetal membrane and rarely measure less than 1.4 μm . There may be aggregates of two or more orbicules. Smooth is the orbicular wall. Orbicules of subtype IIIa are oblate, rounded, and have small wall perforations; subtype IIIb has more leveled, round orbicules with a solitary focal space (donut molded). IV. Orbicules with a wavy fold (Figs. 52–53). Because their surface is folded, orbicules do not have a spherical shape. There may be perforations, but there is no core. They are not embedded in the tapetal membrane, or they are barely there. V. Orbicules that are granular. Orbicules have a 1–2 μm diameter and are flattened and irregular. Small sporopollenine granules can be seen on the surface of the wall, which has several perforations. There is a core, and most of them are compound. The tapetal membrane does not contain them. VI. Orbicules embedded. Orbicules range in size from 0.5–1.6 micrometers and are flattened, regular or irregular in shape. On the surface, there may be small sporopollenin granules and perforations. There are compound orbicules with multiple cores. Electron densities are low at the core-wall interface. The tapetal membrane houses them.

Results

486 pollen morphologically documented genera were identified through our literature review, accounting for approximately 75% of the estimated rubiaceae genera. Pollen data were trustworthy enough to be included in this article for 423 genera (65%), but the following genera were not: *Comm. Antirhea ex juris*, *Aulacocalyx* Hook, *Amarcarpus* Blume *Bobea* Gaudich. f., *Salisb.*, *Bouvardia* Stev. *Callipeltis*, *Canephora* Juss., *Calycosia* A. Gray, *Aiello's* *Ceuthocarpus*, *Rich's* *Chapeliera*, Br., *Coccochondra* *Cosmocalyx* Standl., Benth., *Craterispermum* Benth., *Cremaspora*, *Aiello*, *Deppea*, and *Cubanola* Schltdl. Hook *Didymaea* f., K. Schum. *Dolichometra*, *Duidania* Standl., *Fernelia* Comm., *Feretia* Delile ex Lam., *Baill* of *Flagenium*, *Galiniera* Delile, *Gallienia* Dubard and Dop, *Gillespiea* A. C. Sm., *Heinsen* K. Schum., *Hook*, *Heterophyllaea* f., *Hydnophytum* Jack, *Hypobathrum* Blume, *Hyptianthera* Wight and Arn., *Kraussia* Holdings, & *Machaonia* Head Bonpl., *Hook* of *Morindopsis* f., *Nesohedyotis* (Snare. f) *Bremek* *Oreopolus* Schlegel, *Brem.* *Paraknoxia*, *Pentaloncha* Snare. f., *Phitopsis* Hook, and *Pentanopsis* Rendle. f., *Phuopsis* (Griseb.) Hook. f., *Griseb's* *Pittoniotis*, *Balf* *Placopoda* f., *Pouchetia* A. Rich., *Comm* *Psathura*. ex juris, *Rachicallis* DC, *Pseudonesohedyotis* Tennant, and *Readea* Gillespie, *Rhopalobranchium* Schltr. and *Robynsia* Hutch, K. Krause, *Sarcopygme* Setchell and Christoph., Urb., *Schmidtottia* Urb. *Siemensia*, *Steenisia* Bakh, *Spiradiclis* Blume f., *Stenostomum* C. F. Gilbert, *Korth* *Streblosa*, *Temnocalyx* Robyns, *Striolaria* Ducke, *Tamridaea* Thulin & B. Bremer, *Thecorchus* Bremek, *Aiello's* *Thogsennia*, *Urophyllum* Jack ex Wal., *Klotzsch* *Warszewicz*, *Blume* *Zuccarina*. There were data on orbicules for 94 genera, or 14%. Only about 15% of all Rubiaceae species have been studied, indicating that the documentation of pollen for the majority of genera is far from complete. Furthermore, pollen descriptions can be condensed or lacking in some writings. Occasionally, there were disagreements between pollen data from various publications, mostly regarding shape in equatorial view and size. Incorrect interpretation of the observations is also hindered by divergent usage of pollen terminology. We want to emphasize once more the advantages that Punt et al.'s global glossary has provided (1994).

Discussion

The present study described the pollen micromorphology of 32 Western Himalayan RET medicinally important plants belonging to 23 angiosperm families. The pollen grain has been described using a variety of qualitative and quantitative characteristics in this study. The pollen's shape, size, aperture type, and exine ornamentation all varied significantly between species, according to the findings. These taxa's species delimitation may benefit from the differences in these parameters, demonstrating their potential taxonomic value. The findings of the current study are consistent with those of previous studies (Singh and Dathan, 1980; According to Tomsovic (1997), the architecture of the pollen grains plays a

significant role in the taxonomic descriptions, implications, and taxonomy of angiosperms, which demonstrate the interdependence of plant taxa. Dust grains course of action is the vital consider the developmental line. In accordance with the findings of a previous study (Luo et al.,), the pollen grains were released as monad in all of the monocot species examined. 2015) and suggested that, on the evolutionary scale, the majority of the studied taxa belong to primitive angiosperm. Monads are easiest, while polyads are viewed as most exceptional in the line of advancement (Panicker, 2004). The dust morphology of *R. purpurea* (Zingiberaceae) is accounted for interestingly. However, previous research on other species has examined the pollen characteristics of this species, such as the prolate spherical, large, inaperturate, and long-sized echini on the exine surface (Mangaly and Nayar, 1990; Chen and Xia, 2011; Halbritter and Buchner, 2016). *A. stracheyi* (Amaryllidaceae) pollen morphological characteristics are first described. According to Meerow and Dehgan (1988), the obtained results are consistent with previous research on the family Amaryllidaceae. 2008, Oybak Donmez and Isik; Baser and other, 2019). The monocolpate pollen grains for *T. govanianum* (Melanthiaceae) that were found in these studies (Figures 3, 3b) were also found in earlier studies (Nair and Sharma, 1965; Takahashi, 1983). Contrarily, inaperturate pollen was discovered in other *Trillium* species (Furness et al., As a result, it could be used as a distinguishing characteristic to identify species. *A. propinquum* (Araceae) pollen grains were small, inaperturate, prolate spheroidal, and had an echinate exine. The current study's variation in pollen size and exine ornamentation supports the findings of (Wortley et al., 2015). The dust characters notwithstanding, showed a few distinctions with the past review and were medium measured, aperturate, puncture to fossulate in *Epipremnum pinnatum*, pantoporate in *Alisma orientale*, size enormous, and nonattendance of exine in *Zostera marina* of family Araceae (Luo et al., 2015). This shows the significance of these dust characters in delimiting the class *Arisaema* from the excess genera. According to Muller (1979), the primary functions of angiosperm exine architecture are protection (from adverse atmospheric conditions like desiccation and UV radiation), storage of physiologically active substances, pollen grain clustering, and harmony. In *B. lycium* and *S. hexandrum* (Berberidaceae), dust grains were prolate spheroidal, granulated, psilate, and puncture (Figure 4, 6c and 7c). In *S. hexandrum*, the pollen grains were monoporate, whereas in *B. lycium*, they were tricolpate. On the exine surface of *Sinopodophyllum hexandrum*, Nowicke and Skvarla (1981) discovered gemmae of varying sizes and an aperture resembling a furrow. Additionally, Perveen and Qaiser, (2010) tracked down that dusts of *B. lycium* had a spheroidal shape and sub-psilate exine ornamentation. In this study, the shape, size, and exine ornamentation of the pollen made *A. heterophyllum* distinct from *A. violaceum* (Ranunculaceae). According to Ahmad et al., the pollen characteristics of *A. violaceum*, such as tricolpate, prolate, microechinate, and perforate, are entirely distinct from those of *A. fragrans*. 2018), indicating the significance of these pollen characteristics in Ranunculaceae taxonomy. In *A. pubiflora* (Ranunculaceae) dust grains were medium estimated with tricolpate gap and microechinate puncture ornamentation. Despite the fact that the same taxa were studied by Perveen and Qaiser (2006), who discovered spinulose exine ornamentation on small pollen grains. According to Pérez-Gutiérrez et al., the subprolate, tricolpate, echinate, perforate pollen of *M. aculeata* (Papaveraceae) that is described here has also been found in various genera of this family. 2015; Keshavarzi and others, 2011). Little, monad, tricolporate, prolate, striate dust kept in *R. webbiana* (Rosaceae) in the ongoing examination looks like the aftereffects of the prior investigations on the family Rosaceae (Singh et al., 2020; Hebda and Chinnappa, 1994). Colpate, tuberculate, verrucate, 3-zonocolporate, rugulate dust were recently detailed in the sort *Quercus* (Hayrapetyan and Bruch, 2020). Be that as it may, scabrate and crush dust exine were tracked down in present review and consequently can demonstrate accommodating in distinctive *Q. oblongata* from different types of this sort. The pollen characteristics of *Geranium wallichianum* that Shehata (2008) described—very large, tricolporate, oblate spheroidal, and clavate—were comparable to those described in this study. Moreover, comparative characters have been noticed for the variety geranium (Geraniaceae) (Başer et al., 2016). The first-ever report of a pollen description for *Aesculus indica* (Sapindaceae) is consistent with a previous study on the genus *Aesculus* (Pozhidaev, 1995). Rugulate, reticulate, granular, and smooth patterns have also been observed in the family Sapindaceae's genus *Acer*, in addition to the striate and perforate exine ornamentation described here (Siahkolae et al., 2017). Based on the shape of the pollen and the exine ornamentation, which was spheroidal and perforate in the case of *Bergenia ciliata* and prolate spheroidal and devoid of perforation in the case of *B. stracheyi*, it is simple to distinguish the two studied species of Saxifragaceae. However, in a previous study by Perveen and Qaiser (2009), the exine of *Bergenia ciliata* and *B. stracheyi* was reticulate-rugulate, while the pollen of *B. ciliata* and *B. stracheyi* was subprolate and prolate spheroidal, respectively. The outcomes got for the dust grains of Polygonaceae species showed similitudes and dissimilarities with the past reports. Hong et al. (1998) found that pollens of 30 *Polygonella* and *Polygonum* taxa were prolate to spherical. Yang et al. (Rheum australe and Rheum webbiana were among 40 species of Rheum in the family Polygonaceae that were examined for their sub-spheroidal pollen. Our findings differ from those of Yang et al. 2001), who discovered that the medium-sized and subspherical pollen grains of *R. australe* were distinct from those of *R. webbiana*. The shape of the pollen could be a result of evolution and be used to determine the mode of pollination (Lemmens et al., 2003; Olga and co., 2013). Dust grains of *P. acinosa* were prolate, tricolporate, microechinate, puncture, crush, and psilate, though subprolate dust with reticulate exine ornamentation was noticed already for this species (Nowicke, 1968). According to Simpson and Skvarla (1981), the significance of aperture type in distinguishing the genus *Phytolacca* from *Rivinia* has previously been demonstrated. The observation of medium-sized pollen with a rugulate exine pattern in *Rhododendron companulatum* (Ericaceae) partially supports the previous study (Sarwar and Takahashi, 2013), which found that species other than *R. companulatum* had small pollen. According to Namgay and Sridith (2002), the pollen grains of other *Rhododendron* species were found to be of the oblate and sub-oblate variety and to have gemmae-type exine ornamentation. This characteristic may help differentiate this species from other genus species. In the concentrated on species *Achillea millefolium*, *Jurinea dolomiaea*, *Saussurea obvallata*, *Ligularia fischerii* var. (Miq.) euodon The pollen grains of the

Asteraceae family were oblate-spherical, prolate-spherical, and echinate-perforated on the exine. Just the distinctions were tracked down in the sizes, recurrence, and spinules' thickness among the concentrated on species. As a result, our conclusion is consistent with the findings of 2013). Akyalcin and co. 2011) concentrated on six types of class *Achillea* from Turkey and found that dust grains were for the most part tricolporate with oblatespheroidal, prolate spheroidal, subprolate with echinate microperforate, and echinate-rugulate microperforate exine ornamentation. Khan et al., in contrast to the present study, (2012) discovered in *Achillea millefolium* spheroidal, prolate structures with a tricolporate aperture and an exine thickness of 2.5 m, which was greater than our measurements; nonetheless, the dust grains were of the practically indistinguishable size. According to Bordbar and Mirtadzadin (2015), the pollen characteristics of *Jurinea dolomiaea* were comparable to those that had been previously described. The first-ever report of the *S. obvallata* and *L. fischerii* pollen descriptions. In *Angelica glauca* (Apiaceae) the dust grains were little, tricolporate, prolate, and had rugulate exine ornamentation. No dust information is accessible in the writing to be contrasted and our outcomes. Consequently, these characteristics of pollen may serve as species-specific delimitations. Nonetheless, sub-rhombus, oval, sub-square shape, and equator contracted dust were accounted for in class *Angelica* with the exception of species *A. glauca* from China (Chen et al., 2007). Triporate dust having enormous pipe molded bulges at the equator depicted in the current review for *M. longifolia* (Caprifoliaceae) was like the discoveries of (Blackmore and Cannon, 1983). Similarly, the reticulate characteristics of pollen, such as oblate-spheroidal or prolate-spheroidal hexacolpate; *Salvia hians* (Lamiaceae) perforate ornamentation was consistent with that of other *Salvia* species (Kahraman et al., 2010; Ozler and team, 2011). This demonstrates that the concentrated on dust characters don't play huge part in species ID. For the first time, the ornamental pollen grains of *Picrorhiza kurroa* (Plantaginaceae) are described as being small, tricolporate, subprolate, and psilate exine. Mohsenzadeh and others, (However, 2020) discovered that other species of the genus *Plantago* produced pollen that was medium-sized, prolate-spheroidal to prolate, and pantoporate. As a result, the pollen characteristics that have been reported may be useful in distinguishing this species from others in the same genus. The medium, hexacolpate, reticulate and puncture dust grains in *Gentiana kurroo*, (Gentianaceae) were accounted for without precedent for this review. On the other hand, the only *Gentiana* species that has been studied, *bambuseti*, has been found to contain medium, 3-colporate, and prolate pollen (Hsieh et al., 2007). This suggested that the studied pollen characteristics of *G. kurroa* are useful for identifying *Gentiana* species. In addition, Chassoti and Von Hagen (2008) found that the Gentianaceae family exhibits variation in several morphological characteristics of pollen. According to previous research (Khatamsaz, 2001) on a number of Boraginaceae genera, the pollen characteristics of *A. benthamii* (Boraginaceae) are consistent with those found in this study, indicating that they are not useful for species identification.

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