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Research Article

Palynological diversity of highly medicinal rare, endangered, and threatened plants from Western Himalaya, India

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Abstract

The present study investigated the palynological diversity of highly medicinal Rare, Endangered, and Threatened (RET) plant species dwelling in higher altitudes of Western Himalaya, India. The pollen morphology of 32 plant species covering 29 genera, 23 families, and 18 orders of Angiosperms was analyzed by Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The families of the studied plant species have been arranged and discussed following the evolutionary sequence as per the updated version of Angiosperm Phylogeny Group IV. The studied pollen characters were found to be helpful in the delimitation of taxa at the species level. In the studied monocots, the species belonging to order Zingiberales, Asparagales, and Liliales followed the general trend of having primitive inaperturate to advance monosulcate pollen except for Alismatales which had inaperturate pollen and placed after the orders mentioned above in the APG IV classification system. The pollen aperture of the eudicot group in the present study followed the evolutionary pattern from tricolpate to tricolporate and triporate, which corroborated previous reports. All the investigated species of both monocot and eudicot groups had monad pollen units suggesting their primitiveness on the evolutionary scale except for the *Rhododendron companulatum* (Ericales), having evolutionarily advanced tetrad arrangement. The current study found primitive exine ornamentation in Ranunculales to advance in Asterales, Lamiales, Gentianales, and Boraginales and confirms the results of the previous studies. The data on the pollen morphological features of the studied species generated in the present study will help understand these important high-altitude plant species' reproductive biology and conservation aspects.

Keywords: exine ornamentation; pollen morphology; RET plants; Scanning Electron Microscopy; Western Himalaya

Introduction

Palynomorphs have remarkable potential in systematic classification (Blackmore, 2000). They help resolve complicated interrelationships between taxa and determine their taxonomic status, notably in the

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context of families, subfamilies, tribes, genera, species (Ahmad *et al*., 2010; Wortley *et al*., 2015; Zhang *et al*., 2017). The study of pollen grains is an important area of research because the pollen morphological characters such as the shape, apertural pattern, and exine configuration are conservative features, which can be used for the taxonomic assessment of the plants (Perveen, 2006; Keshavarzi *et al*., 2012; Singh *et al*., 2020). Morphological characteristics of pollen grains can help in plant taxonomy studies because many pollen traits are influenced by the strong selective forces involved in various reproductive processes, including pollination, dispersal, and germination (Erdtman, 1952; Nowicke and Skvarla, 1979; Moore *et al*., 1991). Pollen morphology experiments may also offer valuable research resources for allergy inquiries (e.g., seasonal allergic rhinitis, pollenosis), pollination biology, and honey study (Devender *et al*., 2016).

There is a long and illustrious history of studying palynological characters in a broad evolutionary context (Wodehouse, 1935; Erdtman, 1952; Walker and Doyle, 1975; Nowicke and Skvarla, 1979). Morphological characters can help understand evolutionary problems, which may not be easy to solve by molecular techniques, and morphological consideration of pollen is useful alternative support for molecular tree topologies (Renner *et al*., 2000). The importance of pollen characters in systematics has been highlighted through the recognition of a monophyletic tricolpate clade within angiosperms (Donoghue and Doyle, 1989), now widely known as the eudicots (Doyle and Hotton, 1991; APG, 1998; APG II, 2003; APG III, 2009). Recently, some workers have studied the evolution of pollen characters in the context of phylogeny (Luo *et al*., 2015; Zhang *et al*., 2017; Wortley *et al*., 2015).

The Himalayan region's unique topography and climatic conditions make it a rich repository of plants, with almost 8,000 species, 25.3% of which are indigenous, sustaining the lives of millions of people (Singh and Hajra, 1996; Negi *et al*., 2019). Due to its high biological and socio-cultural diversity, the Himalayan region has been identified as one of 34 "biological hotspots" (Gautam, 2013). Palynological studies on the plant species of high altitudinal regions have been recorded in several studies (Bano *et al*., 2012; Ahmad *et al*., 2013). The relationship between vegetation dynamics and pollen in mountain ecosystems has been deciphered in some earlier studies (Davis, 1984; Tanțău *et al*., 2014). From the Indian context, a study on pollen morphological analysis of 152 plant species from India belonging to 49 families was conducted using Light Microscopy (Sharma and Bhat, 2015). However, only a few studies from the Western Himalayan region have been conducted to analyze the taxonomic importance of pollen grain characters (Kayani *et al*., 2019; Bano *et al*., 2020; Singh *et al*., 2020). The palynological details of the Western Himalayan flora are almost absent. In the present study, the pollen morphology of 32 plant species covering 29 genera, 23 families, and 18 orders of Angiosperms was analyzed using Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The studied plants are high altitude Rare, Endangered, and Threatened (RET) medicinal plant species from Western Himalaya, India. The data on the pollen morphological features of the studied species generated in the present study will help understand these important high-altitude plant species' reproductive biology and conservation aspects.

Materials and Methods

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).

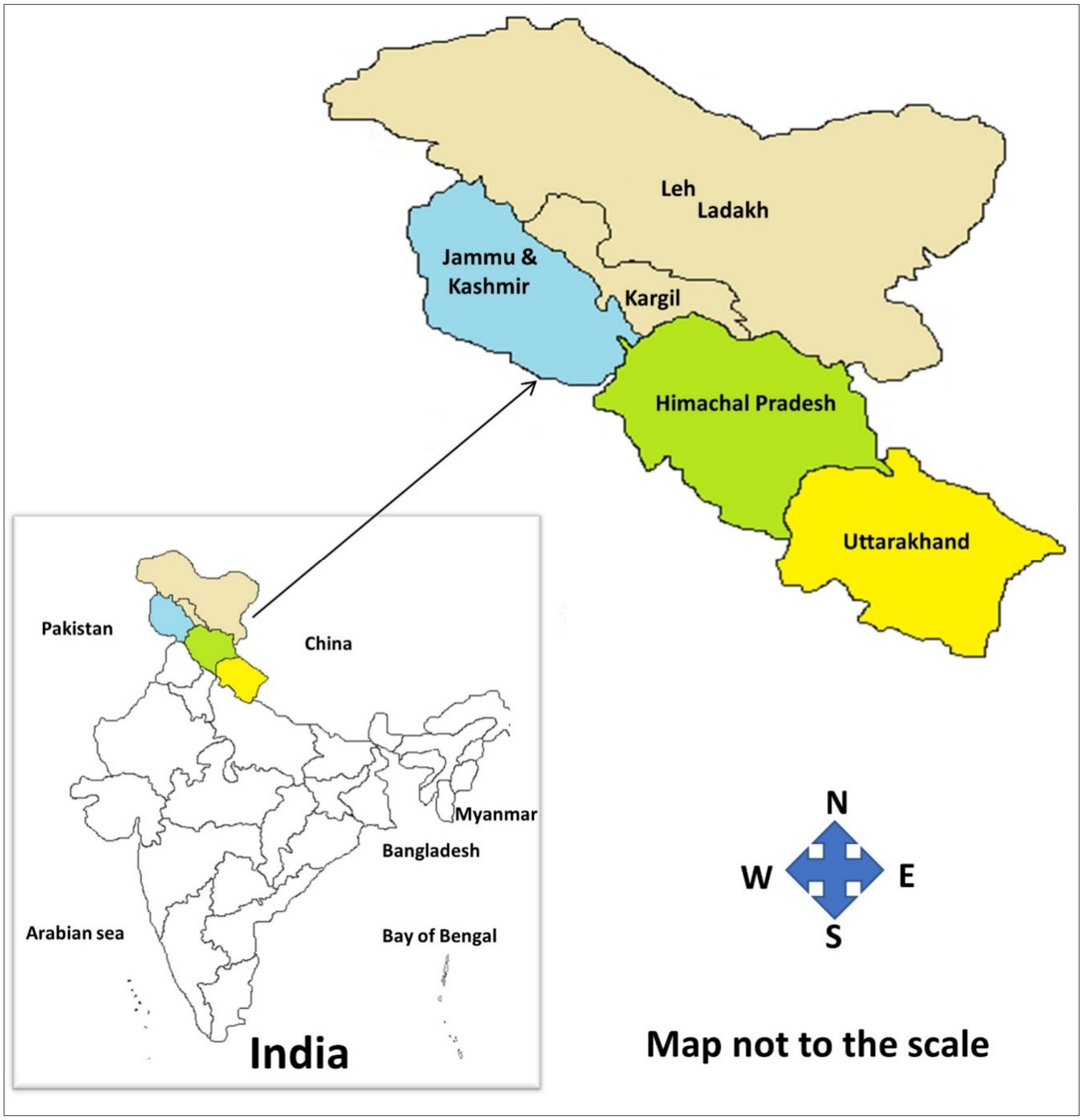

Figure 1. Map of the study area

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 (Table 1 and Figure 2). Pollen grains were analyzed for various quantitative and qualitative characters (Table 2).

Table 1. Details of the studied RET plant species from Western Himalaya, India

Order	Family/Botanical name/Herbarium no. (RRLH–)	Vernacular name	IUCN (Source)	Distribution with Altitudinal range (Source)	Locational details of the studied specimens			
					Localities, District, State	Altitude (m asl)	Latitude	Longitude
Zingiberales	**Zingiberaceae** *Roscoea purpurea* Sm./ **25292**	Kakoli	Common (Gaur, 1999)	Himalayan region of India, Pakistan, Bhutan, and Tibet, 1500–3300 m asl (Balkrishna, 2012)	Gangnani, Uttarakhand	2199	30°56.14ʹ	78°41.08ʹ
Asparagales	**Amaryllidaceae** *Allium stracheyi* Baker/**23772**	Farn	Endangered (Mohan *et al*., 2019)	Western Himalaya in India, Nepal and Pakistan, 2500–3625 m asl (Singh and Sanjappa, 2006; Rawat *et al*., 2016; Tiwari *et al*., 2014)	Saroli top, Udhampur, J&K	2699	33°02.25ʹ	075°23.54ʹ
Liliales	**Melanthiaceae** *Trillium govanianum* Wall. ex D.Don/**23761**	Nagchatri	Endangered (Sharma *et al*., 2018)	The Himalayas in India, Nepal, China, Bhutan; 2500–3800 m asl (Sharma *et al*., 2018)	Chattergalla top, Doda, J&K	2945	32°54.28	075°36.36
	Liliaceae *Chlorophytum arundinaceum* Baker/**23776**	Safed Musli	Endangered (Narasimham and Ravuru, 2003)	The Himalayas in India, Cultivated in India (Chopra *et al*., 1956; WOI, 2000)	CSIR–IIIM, Jammu, J&K (Cultivated)	322	32°43.89ʹ	037°51.03
Alismatales	**Araceae** *Arisaema propinquum* Schott/**23740**	Sap di Makah	NA	The Himalayas from Kashmir to Southeast Tibet; 2400–3600 m asl (Mir *et al*., 2019)	Sarthal valley, Kathua, J&K	2360	32°50.13ʹ	075°46.46ʹ
Ranunculales	**Berberidaceae** *Sinopodophyllum hexandrum* (Royle) T.S. Ying/**23738**	Ban Kakdi	Endangered (Pant and Pant, 2011)	Inner ranges of the Himalaya from Kashmir to Sikkim; 1800–4000 m asl (Gaur, 1999; Khare, 2007)	Chattergalla, Doda, J&K	3125	32°52.69ʹ	075°43.65ʹ
	Berberis lycium Royle/**23743**	Kamblu	Endangered (Pant and Pant, 2011)	Northwest Himalaya; up to 2000 m asl (Gaur, 1999, Khare, 2007)	Sarthal valley, Kathua, J&K	2360	32°50.13ʹ	075°46.46ʹ
	Ranunculaceae *Aconitum heterophyllum* Wall. ex Royle/**23833**	Patis	Critically endangered (Pant and Pant, 2011)	Found in northwestern Himalaya cultivated in Himachal Pradesh; 2000–4000 m asl (Gaur, 1999, Khare, 2007)	Near Kailash Kund, Doda, J&K	3895	32°52.49ʹ	075°41.24ʹ
	Aconitum violaceum Jacquem. ex Stapf./**23832**	Atis	Vulnerable (Pant and Pant, 2011)	Alpine zone of the Himalaya from Gilgit to Kumaon; 3600–4800 m asl (Gaur, 1999, Khare, 2007)	Near Kailash Kund, Doda, J&K	3890	32°52.43ʹ	075°41.23ʹ
	Aquilegia pubiflora Wall. ex Royle/**23759**	Jora	NA	The Himalayas from Pakistan, India to West Nepal; 2500–3400 m asl (Kumar *et al*., 2018)	Seoz dhar, Udhampur, J&K	2360	32°53.82ʹ	075°34.07ʹ
	Papaveraceae *Meconopsis aculeata* Royle/**23755**	Neel Kanth	Endangered (Pant and Pant, 2011)	Western Himalaya from Kashmir to Kumaon; 3300–4500 m asl (Gaur, 1999, Khare, 2007)	Seoz top, Doda, J&K	3220	32°54.66ʹ	075°36.70ʹ
Rosales	**Rosaceae** *Rosa webbiana* Wall ex Royle/**2775**	Gulab	NA	NA Sub-tropical areas to higher altitudes of Ladakh Himalayas: 792-4504 m asl (Singh *et al*., 2020)	Ligri, Paddar, J&K	2775	33°18.03ʹ	075°01.44ʹ
Fagales	**Fagaceae** *Quercus oblongata* D.Don Syn. *Quercus leucotrichophora* A.Camus ex Bahadur/**25293**	Banj	NA	NA	Sohal, Paddar, J&K	1955	33°15.807ʹ	076°07.716ʹ
Geraniales	**Geraniaceae** *Geranium wallichianum* D.Don ex Sweet/**23756**	Ratanjot	Critically endangered (Malik *et al*., 2011)	Nepal, Pakistan, Afghanistan, Bhutan, Indian Himalayan region; 2500–4000 m asl (Vikram, 2014; Shaheen *et al*., 2017)	Mid Seoz dhar, Udhampur, J&K	2945	32°54.28ʹ	075°36.36ʹ

Sapindales	**Sapindaceae** *Aesculus indica* (Wall. ex Cambess.) Hook. / **25294**	Van khodi	NA	NA	Lalpura, Lolab, Kupwara, J&K	1789	30°56.14'	78°41.08'
Saxifragales	**Saxifragaceae** *Bergenia ciliata* (Haw.) Sternb/**23736**	Zakhme–e–hayat	NA	Temperate Himalaya from Kashmir to Bhutan; 900–3000 m asl (Gaur, 1999, Khare, 2007)	Dhera top, Udhampur, J&K	3015	32°59.41'	075°24.97'
	Bergenia stracheyi (Hook. f. & Thomson) Engl./**23737**	Kadgotar	Vulnerable (Pant and Pant, 2011)	Western Himalaya from Afghanistan to Uttarakhand; 3300–4500 m asl (Gaur, 1999, Khare, 2007)	Chattergalla, Doda, J&K	3336	32°52.69'	075°43.36'
Caryophyllales	**Polygonaceae** *Rheum australe* D.Don/**23773**	Choekri	Endangered (Pant and Pant, 2011)	Subalpine Himalaya from Kashmir to Sikkim; 3300–5200 m asl (Gaur, 1999, Khare, 2007)	Gangotri, Uttarkashi, Uttarakhand	3061	30°59.60'	078°56.54'
	Rheum webbianum Royle/**23733**	Revandchini	NA	China, India, Pakistan, and Nepal; 2,400–4,300 m asl (Rashid *et al*., 2014)	Seoz top, Doda, J&K	3488	32°53.21'	075°39.57'
	Oxyria digyana (L.) Hill/**23764**	Churboo	NA	Higher reaches of the Himalayan region from Kashmir to Sikkim; 2400–5000 m asl (Farooq and Saggoo, 2014)	Chattergalla, Doda J&K	3184	32°52.65'	75°43.596'
	Phytolaccaceae *Phytolacca acinosa* Roxb.**/23741**	Metha Kaffal	Vulnerable (Malik *et al*., 2011)	Western Himalaya; 1600–2700 m asl (Devi *et al*., 2019)	Sarthal valley, Kathua, J&K	2360	32°50.13'	075°46.46'
Ericales	**Ericaceae** *Rhododendron companulatum* D.Don/**24026**	Inga	Vulnerable (Pant and Pant, 2011)	NA	Shroth dhar, Paddar, J&K	3115	33°13.75	76°07.52
Asterales	**Asteraceae** *Achillea millefolium* L./**23734**	Dannd peerah di jari	NA	Western Himalaya from Kashmir to Kumaon; 900–300 m asl (Gaur, 1999, Khare, 2007)	Mid Seoz, Udhampur, J&K	2950	32°54.29'	075°36.84'
	Jurinea dolomiaea Boiss./**23758**	Dhoop	Endangered (Pant and Pant, 2011)	Northwestern Himalaya of India, Pakistan to east Nepal; 3000–4300 m asl (Chauhan, 1999)	Seoz top, Doda, J&K	3475	32°53.15'	075°39.46'
	Saussurea obvallata (DC.) Edgew/**21745**	Braham kamal	Vulnerable (Pant and Pant, 2011)	Indian Himalayan region; 3800–4800 m asl (Kirtikar and Basu, 1984)	Hemkund, Chamoli, Uttarakhand	4200	30°41.93 '	079°36.90'
	Ligularia fischerii var. *euodon* (Miq.) Kitam./**23749**	Khalar	NA	The Himalayas from Pakistan, India to Bhutan and China; 2100–3600 m asl (Mir *et al*., 2019)	Seoz dhar, Udhampur, J&K	3500	32°53.25'	075°39.69'
Apiales	**Apiaceae** *Angelica glauca* Edgew./**25382**	Choru	Endangered (Pant and Pant, 2011)	Jammu & Kashmir, Himachal Pradesh, and Uttarakhand; 3000–3700 m asl (Singh. *et al*., 2020)	Solang valley, HP	2513	32°19.166'	077°09.170'
Dipsacales	**Caprifoliaceae** *Morina longifolia* Wall. ex Dc/**23754**	Kandayri	NA	Common in alpine meadows; 3000–4600 m asl (Gaur, 1999, Khare, 2007)	Mid Seoz, Udhampur, J&K	2951	32°54.28'	075°36.86'
Lamiales	**Lamiaceae** *Salvia hians* Royle ex Benth/**23750**	Kalijari	NA	Central Himalayan region of Uttarakhand, Jammu, and Kashmir, Himachal Pradesh; 2800–3800 m asl (Melkani *et al*., 2011)	Top Seoz, Udhampur, J&K	3475	32°53.15'	075°39.46'
	Plantaginaceae *Picrorhiza kurro*a Royle ex Benth/**23751**	Kutki	Endangered (Pant and Pant, 2011)	Alpine Himalaya from Kashmir to Sikkim; 3300–4300 m asl (Gaur, 1999, Khare, 2007)	Kailash Kund, Doda, J&K	3905	32°52.44'	075°41.22'
Gentianales	**Gentianaceae** *Gentiana kurroo* Royle/**25383**	Neel Kanth	Critically endangered	Pakistan, Western Himalayan region of Uttarakhand, Jammu, and Kashmir, Himachal	Chicham, Lahaul spiti, HP	4131	32°20.470	077°58.946

			(Pant and Pant, 2011)	Pradesh; 2000–300 m asl (Shabir *et al*., 2017)				
Boraginales	**Boraginaceae** *Arnebia benthamii* (Wall. ex G.Don) I.M. Johnst. /**23753**	Ratanjot	Critically endangered (Pant and Pant, 2011)	Subalpine to alpine Himalaya; 3000–3900 m asl (Manjkhola *et al*., 2003)	Seoz top, Kailash Kund, Doda, J&K	3552	32°53.28ˊ	075°39.60ˊ

Figure 2. Pictures of the studied RET plant species from Western Himalaya India, 1) *R. purpurea*,2) *A. strachyei*, 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. webbianum*, 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*

Table 2 List of characters considered for analysis of pollen

S. No.	Pollen characters
1	Length of the polar axis (P)
2	Length of the equatorial axis (E)
3	Polar axis/equatorial axis ratio (P/E)
4	Pollen unit (PU)
5	Pollen shape (PSh)
6	Pollen size (PSz)
7	Exine thickness (Ex)
8	Exine ornamentation (Or)
9	Aperture number (AN)
10	Aperture type (AT)
11	Aperture condition (AC)

Collection and storage of pollen material

Several randomly selected inflorescences or flowers for each studied species were collected, labeled, and stored in vials containing Carnoy's fixative and then transferred to 70% ethyl alcohol after 24 h and stored at 4 °C for further analysis. Efforts were made to collect all the flowers on the stage just before anthesis to overcome the contamination of other pollen grains. Also, associated information about altitude and geo-coordinates for each collected plant sample was recorded in the field (Table 1). In case of *Saussurea obvallata* (DC.) Edgew, pollen grains were obtained from the herbarium specimen available at Janaki Ammal Herbarium (RRLH).

Pollen sample preparation

All pollen samples were acetolysed according to Erdtman's method (Erdtman 1952) with minor modifications. Finally, pollen material is mounted on a glass slide with glycerin for Light microscopic observations. The observations and measurements were carried out on acetolysed pollen grains with a Light microscope, LEICA DM 750, with an associated camera (LEICA ICC50 E). For Scanning Electron Microscopy (SEM), acetolysed pollen grains were mounted on 12.5mm diameter stubs then coated in sputter coater with approximately 25nm gold-palladium and observed under SEM model JEOL JSM-IT300. A total of 960 pollen grains have been studied, with 30 pollens of each species. The list of characters (qualitative and quantitative) observed in the present study is mentioned in Table 2. The size and shape class of pollen grains given by (Erdtman, 1952) were followed. The pollen terminology, Halbritter *et al*. (2018), Punt *et al*. (2007), Hesse *et al*. (2009) and PalDat (2020) was used for pollen description. Statistical analysis was performed using excel 2007.

Results

The pollen morphology of 32 important RET plants from Western Himalaya, India, covering 23 angiosperm families, belonging to 29 genera of 23 families (four monocotyledon and 14 dicotyledon orders) was studied (Table 1). The high range of variability in quantitative and qualitative pollen characters was observed in the present study (Tables 3 and 4).

Table 3 Details of the observed pollen characters of the studied RET plant species from Western Himalaya, India

S. No.	Species	Pollen Unit	Pollen size (PSz)[a]	Aperture no. (AN)	Aperture type (AT)	Aperture Conditions (AC)	Pollen shape (PSh)	Exine Ornamentation (Or)
1	*Roscoea purpurea*	Monad	Large	0	No aperture	Inaperturate	Prolate spheroidal	Echinate
2	*Allium stracheyi*	Monad	Medium	1	Sulcus	Sulcate, Monosulcate	Prolate	Striate-rugulate, perforate
3	*Trillium govanianum*	Monad	Large	1	Colpus	Colpate, Monocolpate	Prolate	Gemmate
4	*Chlorophytum arundinaceum*	Monad	Medium	1	Sulcus	Sulcate, Monosulcate	Prolate	Microreticulate
5	*Arisaema propinquum*	Monad	Small	0	No aperture	Inaperturate	Prolate-spheroidal	Echinate
6	*Sinopodophyllum hexandrum*	Monad	Medium	1	Porus	Porate, Monoporate	Prolate-spheroidal	Granulate
7	*Berberis lycium*	Monad	Medium	3	Colpus	Colpate, Tricolpate	Prolate-spheroidal	Psilate, perforate
8	*Aconitum heterophyllum*	Monad	Small	3	Colporus	Colporate, Tricolporate	Subprolate	Microechinate
9	*Aconitum violaceum*	Monad	Medium	3	Colporus	Colporate, Tricolporate	Prolate	Microechinate, perforate
10	*Aquilegia pubiflora*	Monad	Medium	3	Colpus	Colpate, Tricolpate	Prolate	microechinate, perforate
11	*Meconopsis aculeata*	Monad	Small	3	Colpate	Colpate, Tricolpate	Subprolate	Echinate, perforate
12	*Rosa webbiana*	Monad	Small	3	Colporus	Colporate, Tricolporate	Prolate	Striate,
13	*Quercus oblongata*	Monad	Small	3	Colpus	Colpate Tricolpate	Subprolate	Scabrate, granulate
14	*Geranium wallichianum*	Monad	Very Large	3	Colporus	Colporate, Tricolporate	Oblate-spheroidal	Clavate
15	*Aesculus indica*	Monad	Small	3	Colpus	Colpate Tricolpate	Prolate	Striate, perforate
16	*Bergenia ciliata*	Monad	Medium	3	Colporus	Colporate, Tricolporate	Spheroidal	Rugulate, perforate
17	*Bergenia stracheyi*	Monad	Small	3	Colporus	Colporate, Tricolporate	Prolate-spheroidal	Rugulate,
18	*Rheum australe*	Monad	Medium	3	Colpus	Colpate, Tricolpate	Prolate-spheroidal	Perforate, microechinate
19	*Rheum webbianum*	Monad	Medium	3	Colpus	Colpate, Tricolpate	Prolate-spheroidal	Perforate, microechinate
20	*Oxyria digyana*	Monad	Small	3	Colpus	Colpate, Tricolpate	Oblate-spheroidal	Microechinate, perforate
21	*Phytolacca acinosa*	Monad	Medium	3	Colporus	Colporate, Tricolporate	Prolate-spheroidal	Microechinate, perforate
22	*Rhododendron companulatum*	Tetrad	Medium	3	Colporus	Colporate, Tricolpate	Prolate -spheroidal	Verrucate
23	*Achillea millefolium*	Monad	Medium	3	Colporus	Colporate, Tricolporate	Prolate-spheroidal	Echinate, microreticulate
24	*Jurinea dolomiaea*	Monad	Medium	3	Colporus	Colporate, Tricolporate	Prolate-spheroidal	Echinate, perforate
25	*Saussurea obvallata*	Monad	Medium	3	Colporus	Colporate, Tricolporate	Oblate-spheroidal	Echinate, perforate
26	*Ligularia fischerii*	Monad	Medium	3	Colporus	Colporate, Tricolporate	Prolate-spheroidal	Echinate, perforate
27	*Angelica glauca*	Monad	Small	3	Colporus	Colporate, Tricolporate	Prolate	Rugulate
28	*Morina longifolia*	Monad	Very Large	3	Porus	Porate, Triporate	Prolate	Verrucate, perforate
29	*Salvia hians*	Monad	Medium	6	Colpus	Colpate, Hexacolpate	Prolate-spheroidal	Bireticulate, reticulate
30	*Picrorhiza kurroa*	Monad	Small	3	Colporus	Colporate, Tricolporate	Subprolate	Psilate
31	*Gentiana kurroo*	Monad	Medium	6	Colpus	Colpate, Hexacolpate	Subprolate	Reticulate, perforate
32	*Arnebia benthamii*	Monad	Medium	5	Colpus	Colpate, Pentacolpate	Prolate	Psilate, microechinate

[a]Small= 10–25 µm, Medium= 25–50 µm, Large= 50–100 µm, very large= 100–200 µm (Erdtman, 1945).

Table 4. Quantitative pollen characters of the studied species

S. No.	Species studied	P (µm) (±SE)		E (µm) (±SE)		P/E (µm) (±SE)		Ex (µm) (±SE)	
		Min-max	Mean ± SE	Min-max	Mean ± SE	Min-max	Mean ± SE	Min-max	Mean ± SE
1	*Roscoea purpurea*	44.64-97.04	64.08 ± 5.42	38.98-91.25	60.76 ± 6.23	0.92-1.33	1.08 ± 0.03	1.5-2.00	1.70 ± 0.05
2	*Allium stracheyi*	30.57-39.21	34.75 ± 0.65	17.45-23.82	20.26 ± 0.53	1.54-1.98	1.72 ± 0.03	0.36-2.82	1.41 ± 0.26
3	*Trillium govanianum*	44.3-80.84	60.02 ± 3.32	23.11-40.56	32.55 ± 1.62	1.57-2.26	1.85 ± 0.06	0.54-2.20	1.27 ± 0.17
4	*Chlorophytum arundinaceum*	44.27-51.47	47.72 ± 0.49	21.03-34.04	27.45 ± 0.92	1.30-2.30	1.77 ± 0.06	1.37-2.02	1.66 ± 0.07
5	*Arisaema propinquum*	13.55-19.60	16.54 ±0.53	13.38-19.62	14.47 ± 0.59	0.95-1.06	1.01 ± 0.01	1.47-1.87	1.65 ± 0.03
6	*Sinopodophyllum hexandrum*	33.57-55.99	46.08 ± 1.10	23.11-60.94	42.77 ± 2.66	0.85-1.51	1.11 ± 0.05	1.32-2.53	1.88 ± 0.13
7	*Berberis lycium*	24.08-38.26	31.49 ± 1.27	23.04-37.09	29.66 ± 1.30	0.93-1.47	1.07 ± 0.03	1.09-4.11	2.54 ± 0.33
8	*Aconitum heterophyllum*	18.28-26.45	21.17 ± 0.57	11.45-25.82	17.99 ± 0.95	0.96-1.70	1.21 ± 0.06	0.81-1.21	0.96 ± 0.04
9	*Aconitum violaceum*	26.60-43.66	33.75 ± 1.45	17.92-33.45	25.14 ± 1.06	0.96-1.71	1.36 ± 0.04	1.30-2.70	1.81 ± 0.12
10	*Aquilegia pubiflora*	20.66-31.03	25.02 ± 0.82	12.92-31.03	22.28 ± 1.38	1.03-1.93	1.45 ± 0.10	0.95-1.77	1.17 ± 0.06
11	*Meconopsis aculeata*	21.41-29.16	24.10 ± 0.67	15.19-29.22	21.51 ± 1.09	0.92-1.50	1.15 ± 0.05	1.09-2.82	1.96 ± 0.17
12	*Rosa webbiana*	19.65-28.13	24.82 ± 0.70	12.71-20.01	17.49 ± 0.59	1.32-1.54	1.42 ± 0.02	1.02-1.08	1.43 ± 0.05
13	*Quercus oblongata*	19.12-30.36	24.15 ± 1.20	12.62-29.21	20.74 ± 2.02	1.00-1.82	1.22 ± 0.08	1.01-1.06	1.35 ± 0.05
14	*Geranium wallichianum*	76.76-131.77	109.56 ± 5.31	76.62-131.72	111.23 ± 5.19	0.89-1.08	0.98 ± 0.01	0.69-0.87	0.79 ± 0.01
15	*Aesculus indica*	20.96-31.39	20.52 ± 7.05	12.48-21.51	13.00 ± 4.19	1.28-1.67	1.48 ± 0.04	0.05-1.02	0.81 ± 0.06
16	*Bergenia ciliata*	18.87-32.79	26.96 ± 1.06	19.4-33.49	27.01 ± 1.13	0.84-1.09	1.00 ± 0.01	1.21-2.20	1.70 ± 0.12
17	*Bergenia stracheyi*	17.78-28.91	23.89 ± 0.94	13.46-32.12	22.34 ± 1.30	0.90-1.44	1.09 ± 0.04	1.35-2.11	1.70 ± 0.08
18	*Rheum australe*	20.69-27.38	28.82 ± 0.54	16.92-26.93	23.67 ± 0.68	0.88-1.31	1.01 ± 0.02	0.94-1.51	1.28 ± 0.05
19	*Rheum webbianum*	18.01-36.19	29.36 ± 1.37	13.6-35.59	27.65 ± 1.75	0.95-2.02	1.10 ± 0.07	1.71-2.40	2.01 ± 0.07
20	*Oxyria digyana*	18.28-22.69	19.72 ± 0.29	17.43-23.96	19.83 ± 0.47	0.91-1.15	0.10 ± 0.01	0.70-1.20	0.97 ± 0.05
21	*Phytolacca acinosa*	22.4-39.93	32.45 ± 1.79	18.25-42.26	31.10 ± 1.93	0.91-1.22	1.05 ± 0.02	1.33-3.39	2.54 ± 0.20
22	*Rhododendron companulatum*	48.03-64.36	52.65 ± 1.23	42.81-58.44	48.31 ± 1.28	0.98-1.27	1.09 ± 0.02	1.25-2.01	1.53 ± 0.08
23	*Achillea millefolium*	20.56-33.96	25.67 ± 0.98	17.26-31.61	24.43 ± 1.15	0.93-1.38	1.06 ± 0.03	0.31-0.59	0.47 ± 0.02
24	*Jurinea dolomiaea*	45.45-55.44	49.49 ± 0.84	43.14-54.61	47..19 ± 0.92	0.96-1.13	1.05 ± 0.01	0.42-0.81	0.56 ± 0.03
25	*Saussurea obvallata*	32.25-56.07	42.56 ± 1.93	32.21-53.93	42.79 ± 1.96	0.89-1.08	0.91 ± 0.02	2.68-5.63	4.11 ± 0.30
26	*Ligularia fischerii*	26.56-42.70	35.10 ± 1.22	28.14-41.49	33.76 ± 0.93	0.94-1.16	1.04 ± 0.02	1.46-2.67	1.99 ± 0.10
27	*Angelica glauca*	21.48-30.60	24.95 ± 0.82	8.91-13.46	10.70 ± 0.35	1.84-2.70	2.34 ± 0.07	1.01-1.06	1.40 ± 0.04
28	*Morina longifolia*	113.2-178.78	157.67 ± 4.33	80.13-120.1	98.14 ± 3.83	1.00-1.99	1.65 ± 0.09	4.36-6.68	5.70 ± 0.22
28	*Salvia hians*	27.91-66.60	44.11 ± 2.75	29.81-50.67	41.28 ± 1.74	0.87-1.52	1.06 ± 0.04	1.89-3.54	2.47 ± 0.19
29	*Picrorhiza kurroa*	18.07-30.54	24.73 ± 1.25	16.74-27.46	20.53 ± 0.68	0.93-1.46	1.20 ± 0.05	1.62-2.43	1.98 ± 0.08
31	*Gentiana kurroo*	25.51-31.35	29.37 ± 0.65	17.16-29.31	25.14 ± 1.14	1.05-1.48	1.18 ± 0.03	1.4-1.8	1.63 ± 0.03
32	*Arnebia benthamii*	15.49-36.32	28.99 ± 1.40	12.19-30.15	16.17 ± 1.13	0.51-2.47	1.89 ± 0.11	1.09-1.68	1.31 ± 0.05

The light micrographs and scanning electron micrographs of the pollen grains of the studied medicinal plant species are presented in Figures 3 to 9. The families of the studied plant species in the table followed the evolutionary sequence as per Angiosperm Phylogeny Group IV classification.

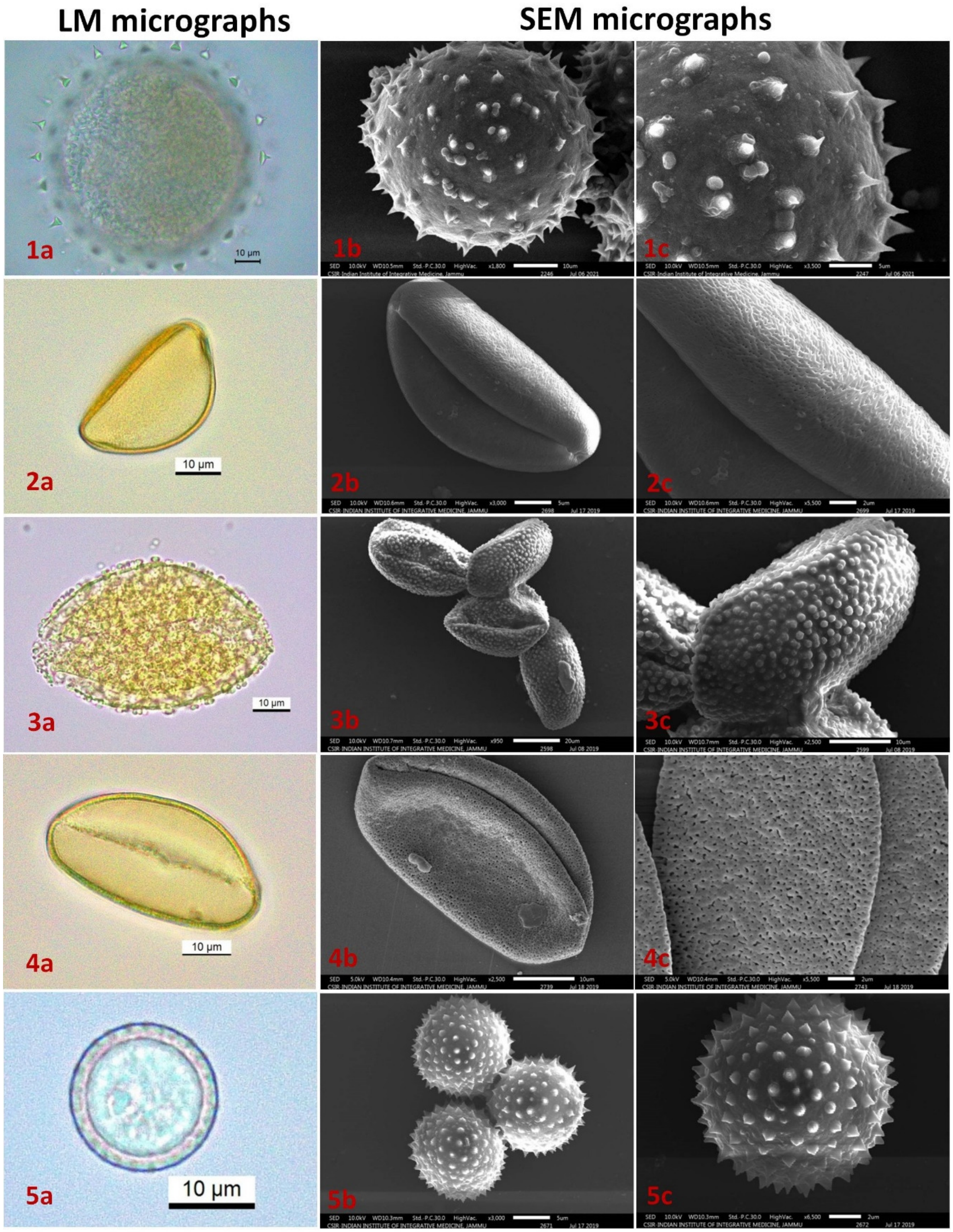

Figure 3. LM (first column, 1a–5a) and SEM (second column, 1b–5b and third column, 1c–5c) depicting outline, apertures and exine sculpturing of pollens grains. 1a) *R. purpurea,* 1b) Polar view showing sparse echini, 1c) showing pointed echini and in between this ornamentation is psilate; 2a) *A. stracheyi,* 2b) equatorial view showing sulcus, 2c) striate-rugulate, perforate ornamentation; 3a) *T. govanianum,* 3b) pollens in equatorial & polar view, 3c) gemmate ornamentation; 4a) *C. arundinaceum,* 4b) equatorial view shows sulcus, 4c) perforations on exine; 5a) *A. propinquum,* 5b) monads in polar view, 5c) details of echinate ornamentation

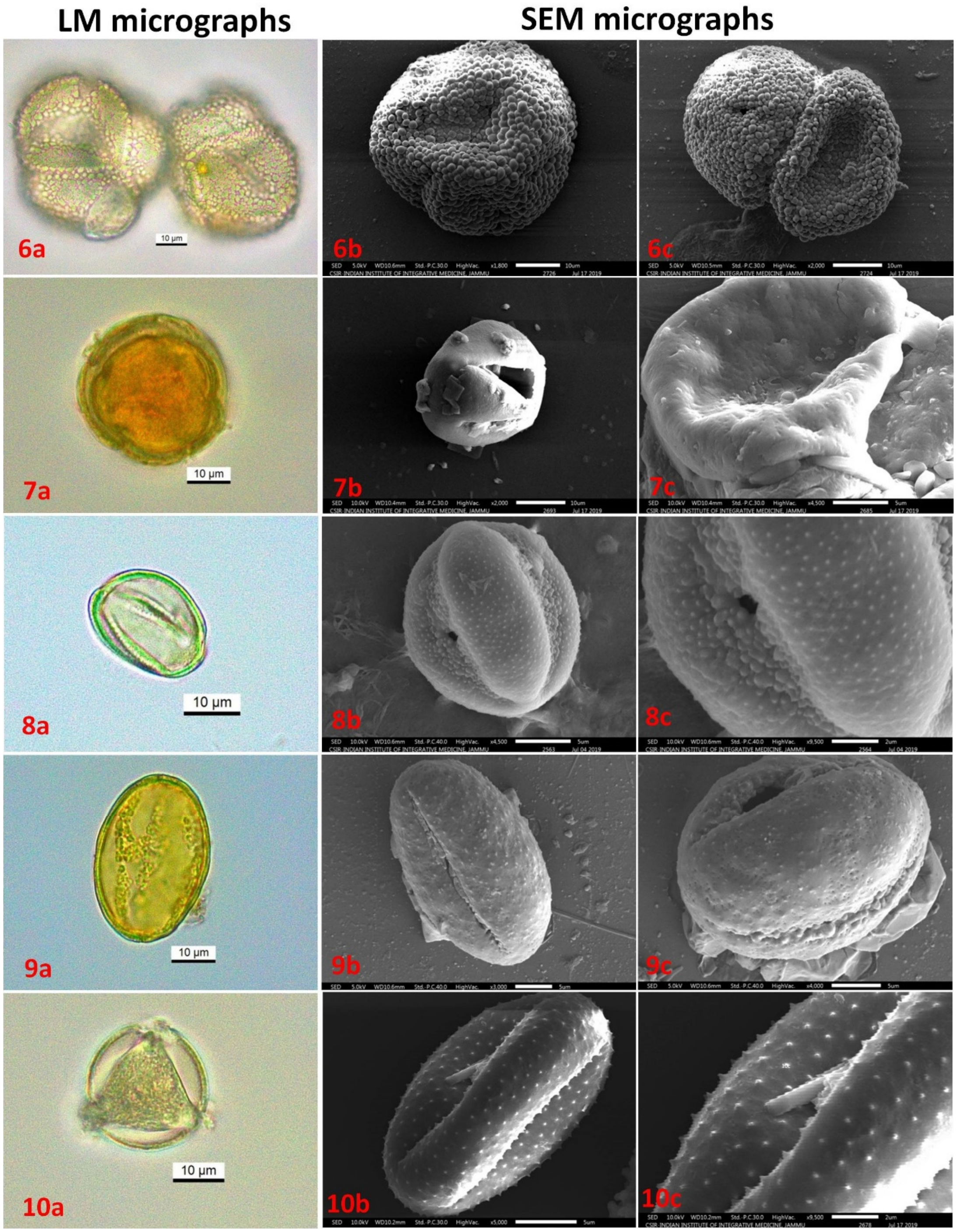

Figure 4. LM (first column, 6a–10a) and SEM (second column, 6b–10b and third column, 6c–10c) depicting outline, apertures and exine sculpturing of pollens grains. 6a) *S. hexandrum,* 6b) equatorial view shows grooves due to acetolysis, 6c) single small pore and details of exine sculpturing; 7a) *B. lycium,* 7b) monad in polar view showing large ectoaperture 7c) smooth exine; 8a) *A. heterophyllum,* 8b) equatorial view showing circular pore and colpus, 8c) showing pore and ornamented colpus membrane; 9a) *A. violaceum,* 9b) monad in equatorial view, 9c) showing ectoaperture and rough aperture membrane; 10a) *A. pubiflora,* 10b) equatorial view showing colpus, 10c) microechinate ornamentation

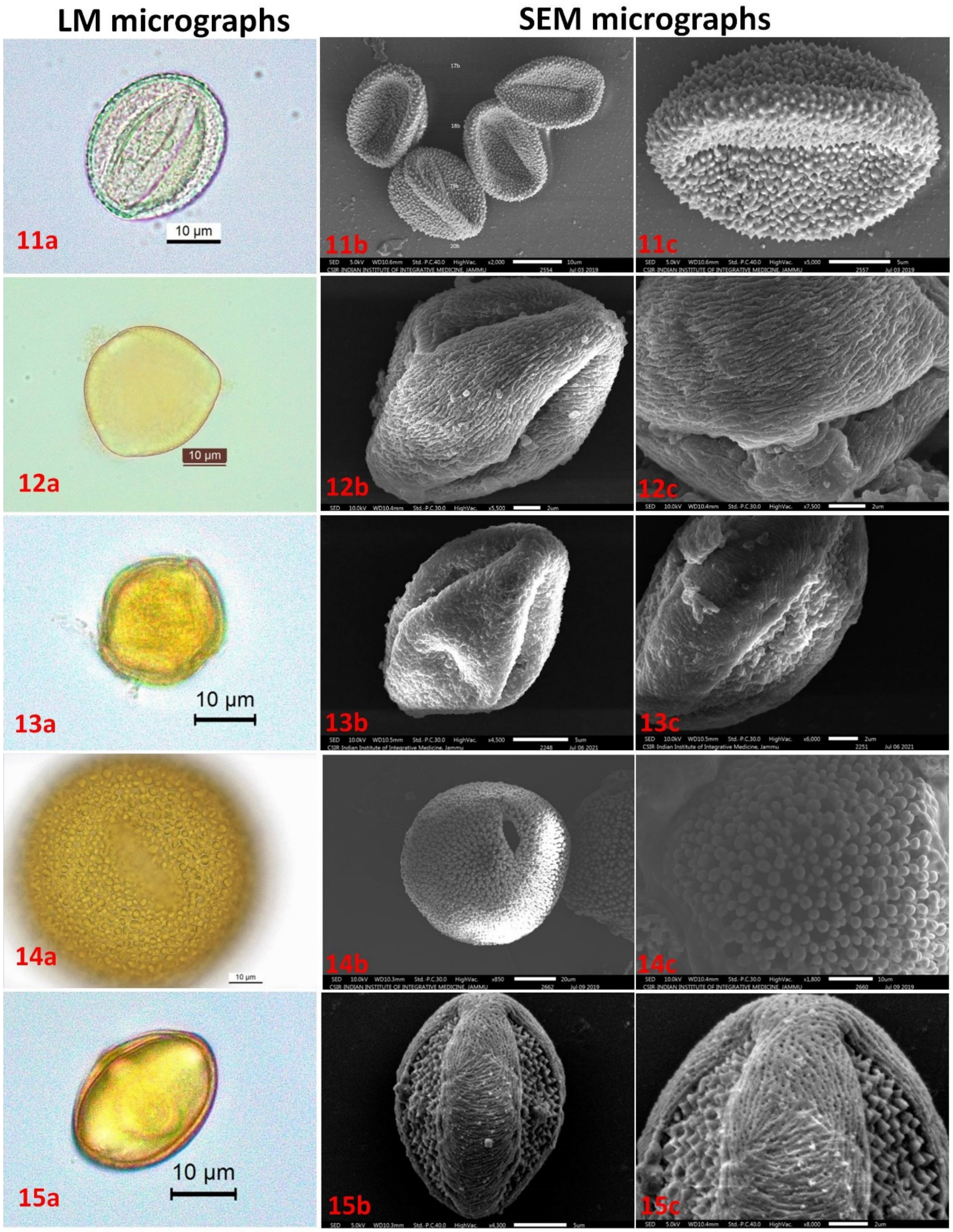

Figure 5. LM (first column, 11a–15a) and SEM (second column, 11b–15b and third column, 11c–15c) depicting outline, apertures and exine sculpturing of pollens grains. 11a) *M. aculeata*, 11b) monads in equatorial view, shapes distorted due to acetolysis, 11c) details of echinate exine surface; 12a) *R. webbiana*, 12b) monad showing apertures, 12c) striate ornamentation; 13a) *Q. oblongata,* 13b) polar view showing apertures, 13c) showing wider ectocolpus with rough ornamentation; 14a) *G. wallichianum,* 14b) monad showing ectoaperture, 14c) Details of clavate ornamentation; 15a) *A. indica*, 15b) equatorial view showing echinate colpus, 15c) details of ornamentation showing perforations

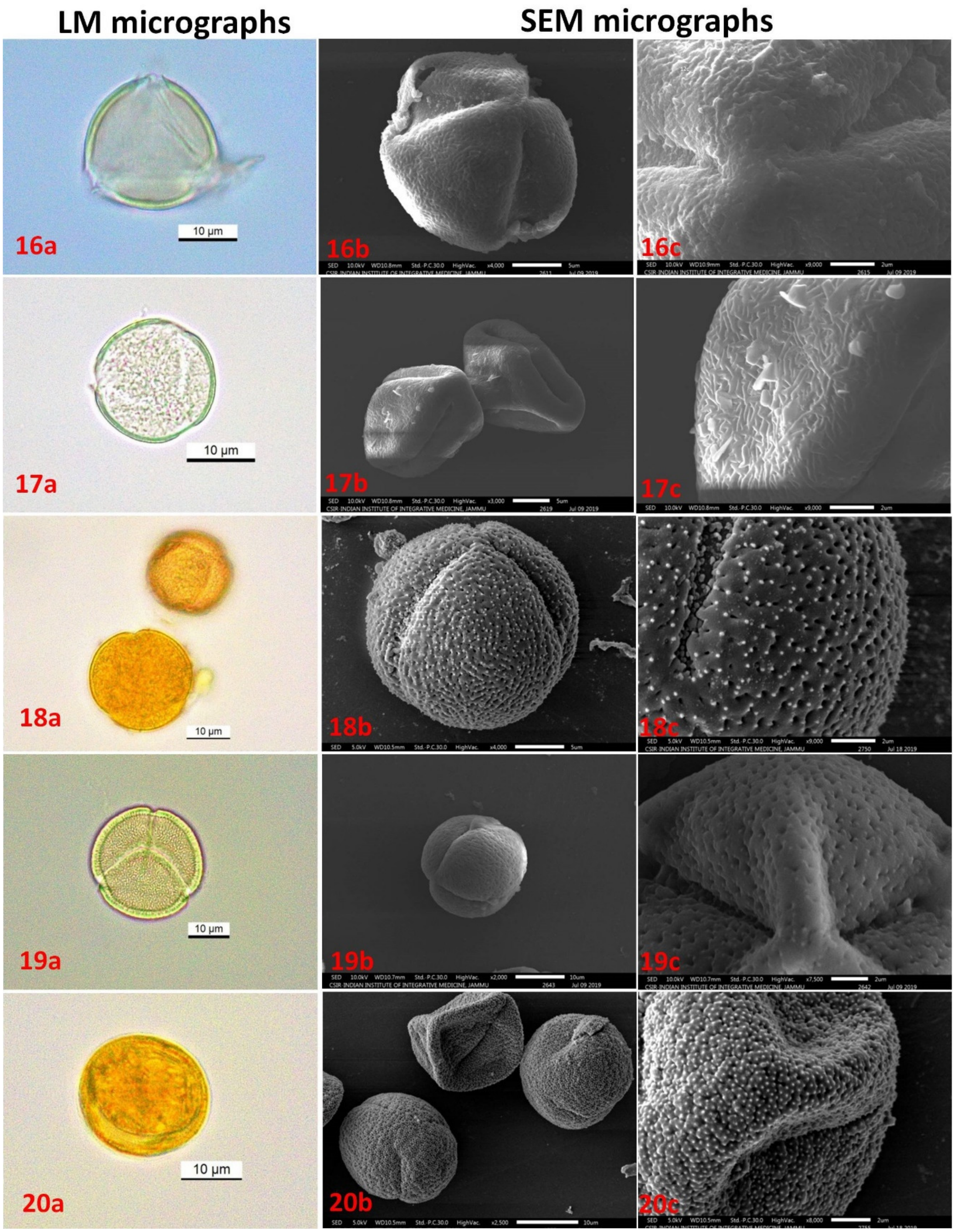

Figure 6. LM (first column, 16a–20a) and SEM (second column, 16b–20b and third column, 16c–20c) depicting outline, apertures and exine sculpturing of pollens grains. 16a) *B. ciliata,* 16b) polar view showing colpus, 16c) polar view showing aperture with obtuse ends; 17a) *B. stracheyi*, 17b) monads showing apertures, 17c) details of rugulate ornamentation; 18a) *R. austral,* 18b) equatorial view showing apertures, 18c) showing perforation & granules on colpus membrane; 19a) *R. webbianum*, 19b) monad in equatorial view showing apertures, 19c) polar view showing sharp and fishtail like ends; 20a) *O. digyana,* 20b) monads in polar view showing apertures, 20c) details of exine showing perforations

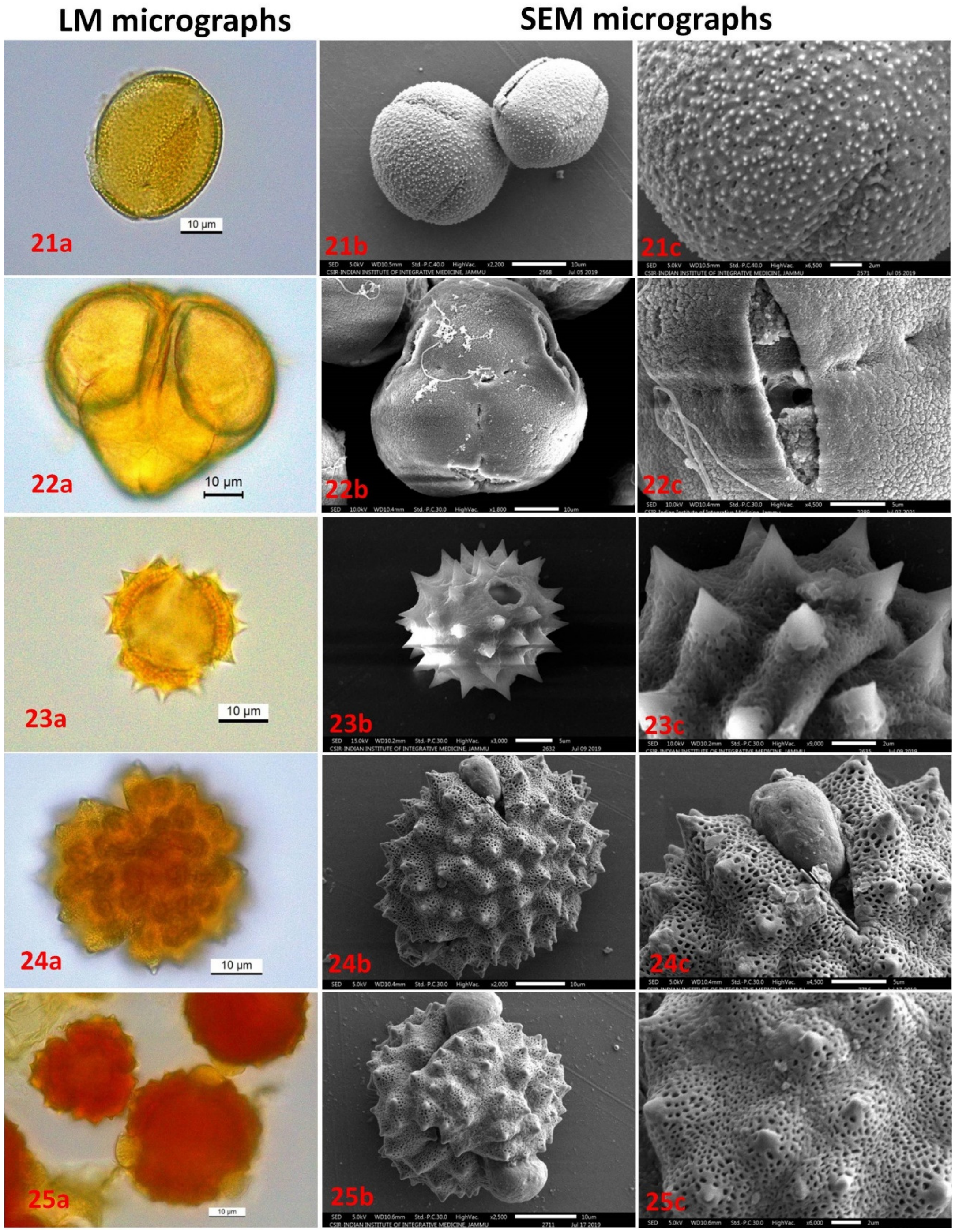

Figure 7. LM (first column, 21a–25a) and SEM (second column, 21b–25b and third column, 21c–25c) depicting outline, apertures and exine sculpturing of pollens grains. 21a) *P. acinosa*, 21b) monads in polar view showing apertures, 21c) showing ornamented aperture membrane and perforations; 22a) *R. campanulatum,* 22b) Tetrad in equatorial view, 22c) showing boat shaped colpus and rugulate exine ornamentation; 23a) *A. millefolium,* 23b) circular in polar view showing circular pore, 23c) details of exine showing sharp spines & perforations; 24a) *J. dolomiaea*, 24b) equatorial view, 24c) aperture with sharp ends and prominent perforations; 25a) *S. obvallata*, 25b) monad in equatorial view showing bulging out material from apertures, 25c) showing perforations and sparsely distributed reduced echinii

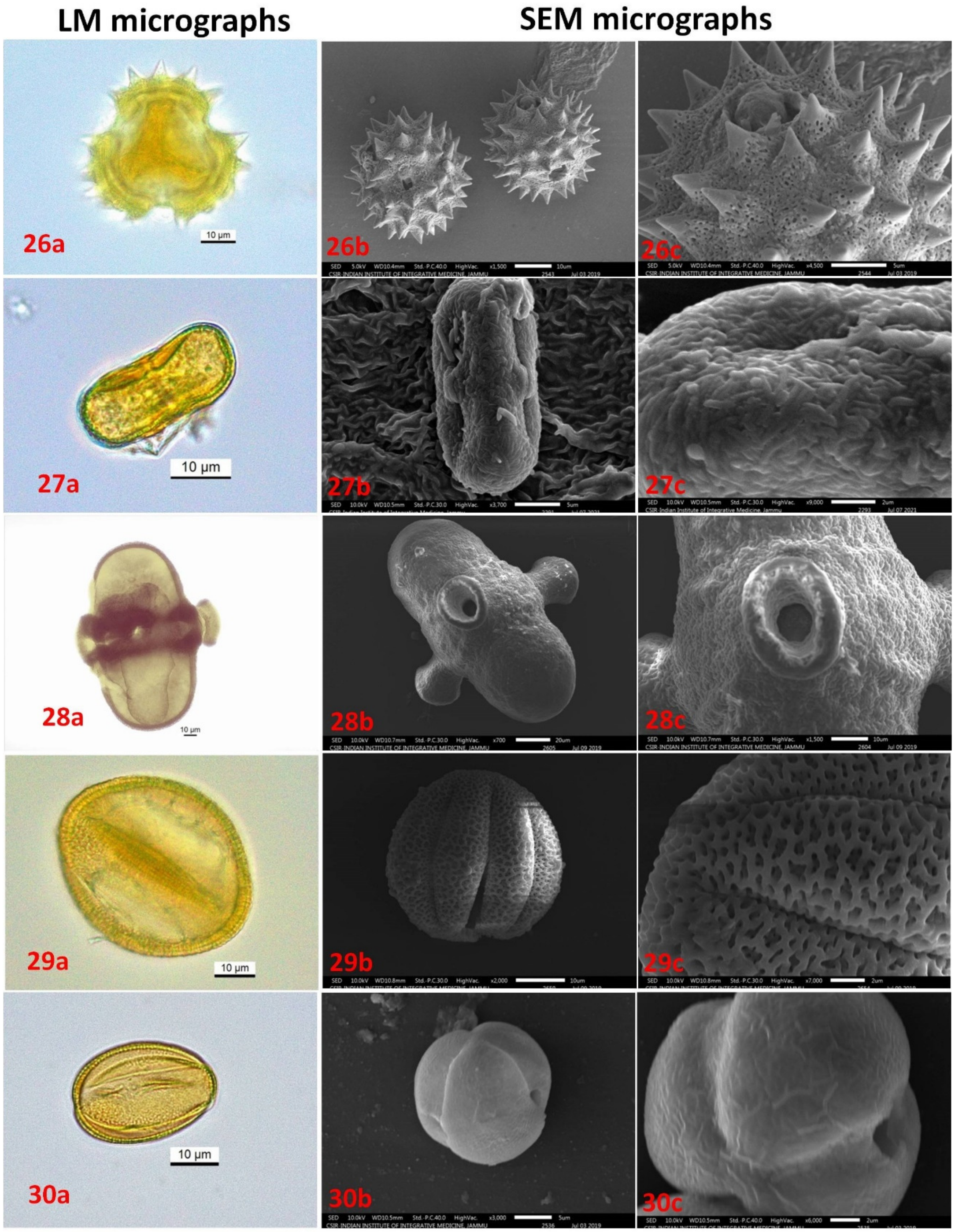

Figure 8. LM (first column, 26a–30a) and SEM (second column, 26b–30b and third column, 26c–30c) depicting outline, apertures and exine sculpturing of pollens grains. 26a) *L. fischerii,* 26b) monads circular showing Colporus, 26c) details of exine showing dense echinii, perforation & circular aperture; 27a) *A. glauca,* 27b) Equatorial view showing apertures, 27c) rugulate ornamentation; 28a) *M. longifolia,* 28b) spindle-shaped monad with conspicuous annulus, 28c) details of verrucate exine showing perforations and aperture protrude upward hollow inside with circular pore; 29a) *S. hians,* 29b) polar view showing colpus, 29c) colpus showing bireticulation; 30a) *P. kurroa*, 30b) polar view showing colpus & pores, 30c) details of psilate exine showing smooth ectocolpus membrane having pore

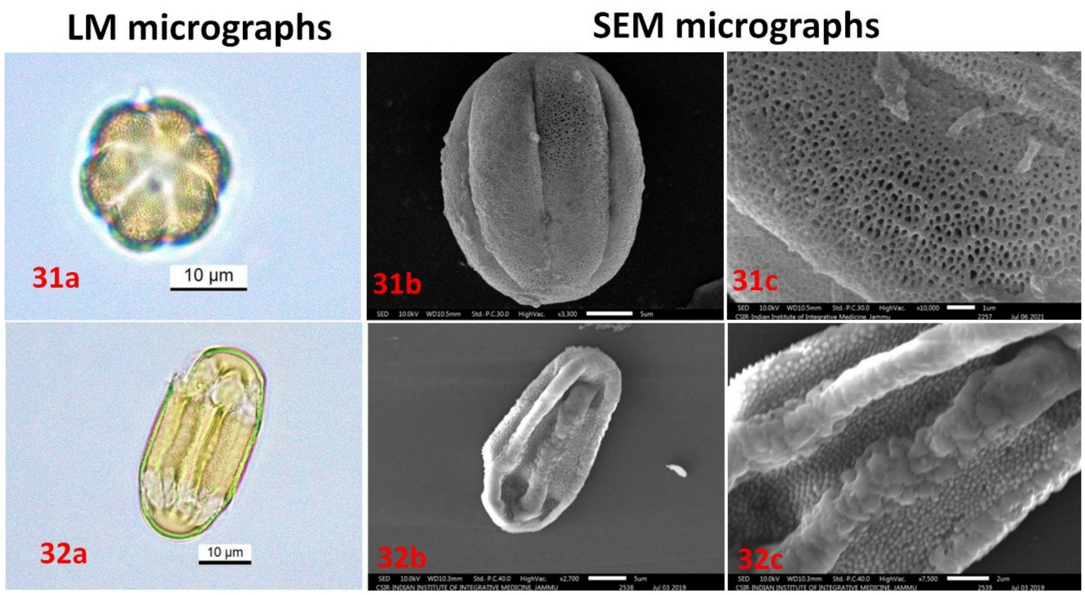

Figure 9. LM (first column, 31a–32a) and SEM (second column, 31b–32b and third column, 31c–32c) depicting outline, apertures and exine sculpturing of pollens grains. 31a) *G. kurroo,* 31b) equatorial view showing colpus, 31c) details of exine ornamentation showing reticulation; 32a) *A. benthamii*, 32b) distorted shape pollen due to acetolysis, showing pentacolpate monad in polar view, 32c) showing ornamented aperture membrane

Shape and size

From monocot group, pollen grains of five species viz., *Roscoea purpurea* Sm, *Allium stracheyi* Baker, *Trillium govanianum* Wall. ex D.Don, *Chlorophytum arundinaceum* Baker, and *Arisaema propinquum* Schott belonging to four orders Zingiberales, Asparagales, Liliales, and Alismatales, were studied (Table 1). The pollen grains were released as monads in all the studied monocot species (Table 3). The pollen grains were prolate spheroidal shaped in *R. purpurea* and *A. propinquum,* whereas prolate-shaped pollen grains were found in *A. stracheyi, T. govanianum,* and *C. arundinaceum*. Pollen grains were small in *A. propinquum*, medium in *A. stracheyi, T. govanianum,* and *C. arundinaceum,* and large in *R. purpurea* (Table 3).

In the eudicot group, pollen grains of twenty-seven species belonging to fourteen orders (Table 1) were investigated. The pollen grains were sub-prolate in *Aconitum heterophyllum* Wall. ex Royle, *Meconopsis aculeata* Royle, *Quercus oblongata* D.Don, *Picrorhiza kurroa* Royle ex Benth, and *Gentiana kurroo* Royle, whereas prolate shaped pollen was found in *Aconitum violaceum* Jacquem. ex Stapf, *Aquilegia pubiflora* Wall. ex Royle, *Rosa webbiana* Wall ex Royle, *Aesculus indica* (Wall. ex Cambess.) Hook, *Angelica glauca* Edgew, *Morina longifolia* Wall. ex Dc, Arnebia *benthamii* (Wall. ex G. Don) I.M. Johnst. In *Sinopodophyllum hexandrum* (Royle) T.S. Ying, *Berberis lycium* Royle, *Bergenia stracheyi* (Hook. f. & Thomson) Engl., *Rheum australe* D.Don, *Rheum webbianum* Royle, *Phytolacca acinosa* Roxb, *Rhododendron companulatum* D.Don, *Achillea millefolium* L., *Jurinea dolomiaea* Boiss., *Ligularia fischerii* var. *euodon* (Miq.) Kitam., and *Salvia hians* Royle ex Benth; the pollen grains were prolate spheroidal whereas oblate-spheroidal in *Geranium wallichianum* D.Don ex Sweet, *Oxyria digyana* (L.) Hill, *Saussurea obvallata* (DC.) Edgew, and spheroidal in *Bergenia ciliata* (Haw.) Sternb.

The pollen grains were released as monad in all the studied dicot species except *R. companulatum* of Ericaceae (Ericales), in which the pollen grains were released as a tetrad. Pollen grains of three sizes, i.e., small, medium, and very large, were reported in the dicot group. In Ranunculales, pollen grains of the species belonging to the family Ranunculaceae were small and medium, whereas species of Berberidaceae and Papaveraceae family were medium and small, respectively (Table 3). In order Rosales and Fagales, the investigated species possessed small pollen grain, whereas very large-sized pollen grains were found in *G. wallichianum* of Geraniaceae (Geraniales). The pollen of *A. indica* (Sapindaceae; Sapindales) was small, while species of Saxifragales, i.e., *B. ciliata, B. stracheyi,* possessed medium pollen. In the investigated species of order Caryophyllales, pollen grains were small and medium in Polygonaceae and medium in *Phytolacca acinosa* (Phytolaccaceae). Medium pollen grains were observed in the species belonging to orders Ericales, Asterales, and Apiales (Table 3). The pollen grains in *M. longifolia* (Dipsacales; Caprifoliaceae) were very large, while medium (Lamiaceae) and small (Plantaginaceae) pollen grains were found in the studied species of Lamiales. In *G. kurroa* (Gentianales; Gentianaceae) and *A. benthamii,* small and medium pollen grains were found, respectively.

Aperture

In the monocot group, inaperturate, monocolpate, monosulcate, inaperturate pollen grains were recorded (Table 3). Inaperturate pollen grains were found in *R. purpurea* (Zingiberaceae; Zingiberales), monoaperturate in *A. stracheyi* (Amaryllidaceae; Asparagales), monocolpate in *T. govanianum* (Melanthiaceae; Liliales), and monosulcate in *C. arundinaceum* (Liliaceae; Liliales), whereas inaperturate pollen were recorded in *A. propinquum* (Arecaeae; Alismatales). In the studied eudicots species, tricolporate pollen grains were found in *A. heterophyllum, A. violaceum, B. lycium, M. aculeata, R. webbiana, Q. oblongata, G. wallichianum, B. ciliata, B. stracheyi, P. acinosa, R. companulatum, A. millefolium, J. dolomiaea, S. obvallata, L. fischerii var. euodon, A. glauca, P. kurroa,* tricolpate in *A. pubiflora, A. indica, R. australe, R. webbianum, O. digyna,* whereas triporate pollen grains were found in *M. longifolia* (Table 3). In *S. hexandrum,* monoporate pollens were observed. In *S. hians* (Lamiaceae; Lamiales) and *G. kurroo* (Gentianaceae; Gentianales), hexacolpate pollens were observed, whereas pentacolpate pollen grains were found in *A. benthamii.*

Exine ornamentation

The exine surface portrayed tremendous variation in its ornamentation in both monocot and eudicot groups. In monocot, the exine was echinate in *R. purpurea* (Zingiberales) and *A. propinquum* (Alismatales), striate-rugulate, perforate in *A. stracheyi* (Asparagales), gemmate in *T. govanianum* (Liliales), and microreticulate in *C. arundinaceum* (Liliales).

In the dicot group, the studied species of the order Ranunculales had microechinate, echinate, perforate, granulate, psilate (Table 3). The exine surface was striate in *R. webbiana* (Rosales), verrucate, scabrate, granulate in *Q. Oblongata* (Fagales; Fagaceae), clavate in *G. wallichianum* (Geraniales; Geraniaceae), striate, perforate, rugulate with echini on colpus membrane in *A. indica* (Sapindales; Sapindaceae). The exine surface in the species of the order Saxifragales was rugulate, perforate with granulate colpus membrane (*B. ciliata*), and rugulate with smooth to granulate colpus membrane (*B. ciliata*). In the studied species of Caryophyllales, the exine ornamentation was microechinate and perforate whereas *R. companulatum* (Ericales; Ericaceae) had verrucate exine ornamentation. Exine was echinate, perforate in the studied species of order Asterales with some infrageneric variations (Table 3). The exine was rugulate in *A. glauca* (Apiales; Apiaceae), verrucate, perforate, with a conspicuous annulus in *M. longifolia* (Dipsacales; Caprifoliaceae). In the studies species of order Lamiales, exine ornamentation was bireticulate, reticulate in *S. hians,* and psilate in *P. kurroa.* Likewise, the exine surface was reticulate with perforations in *G. kurroo* (Gentianales: Gentianaceae), whereas psilate exine surface with microechinii was reported on the colpus membrane in *A. benthamii* (Boraginales; Boraginaceae).

Discussion

The present investigation gave an account of the pollen micromorphology of 23 angiosperm families encompassing 32 medicinally important RET plants of Western Himalaya. In this study, different qualitative and quantitative characters have been used to describe the pollen grain. The obtained results showed significant variations in pollen morphology at the interspecific level for their shape, size, aperture type, and exine ornamentation. The differences in these parameters can prove helpful in species delimitation in these taxa, thus showing potential taxonomic value. The present study results are in line with other previous studies (Singh and Dathan, 1980; Tomsovic, 1997) that the architecture of the pollen grains plays an important role in taxonomic descriptions, implications, and taxonomy of angiosperms that reveal inter-relationship among plant taxa.

Pollen grains arrangement is the key factor in the evolutionary line. The pollen grains were released as monad in all the studied monocot species, which corroborated with the previous study (Luo *et al*., 2015) and suggested that most studied taxa belong to primitive angiosperm on the evolutionary scale. Monads are simplest, while polyads are considered most advanced in the line of evolution (Panicker, 2004).

The pollen morphology of *R. purpurea* (Zingiberaceae) is reported for the first time. However, the reported pollen characters of this species such as prolate spheroidal, large size, inaperturate, and had long-sized echini on the exine surface have been studied previously for other species (Mangaly and Nayar, 1990; Chen and Xia, 2011; Halbritter and Buchner, 2016). The pollen morphological characters of *A. stracheyi* (Amaryllidaceae) are described for the first time. The obtained results are in line with the previous studies on family Amaryllidaceae (Meerow and Dehgan, 1988; Oybak Donmez and Islk, 2008; Baser *et al*., 2019). The monocolpate pollen grains reported for *T. govanianum* (Melanthiaceae) (Figures 3, 3b) in the present investigations have also been reported earlier (Nair and Sharma, 1965; Takahashi, 1983). In contrast, inaperturate pollen was found in other species of genus *Trillium* (Furness *et al*., 2015) thus could be used as distinctive character for species identification.

The pollen grain of *A. propinquum* (Araceae) was small, inaperturate, prolate spheroidal with echinate exine. The variation shown by the pollen size and exine ornamentation in the present study substantiates the report of (Wortley *et al*., 2015). The pollen characters however, showed some differences with the previous study and were medium sized, aperturate, perforate to fossulate in *Epipremnum pinnatum*, pantoporate in *Alisma orientale*, size gigantic, and absence of exine in *Zostera marina* of family Araceae (Luo *et al*., 2015). This indicates the importance of these pollen characters in delimiting the genus *Arisaema* from the remaining genera. The functions of exine architecture in angiosperms are mainly protection (against adverse atmospheric conditions such as desiccation and UV radiation), storage of physiologically active substances, pollen grain clustering, and harmomegathy (Muller, 1979).

In *B. lycium* and *S. hexandrum* (Berberidaceae), pollen grains were prolate spheroidal, granulated, psilate, and perforate (Figure 4, 6c and 7c). The pollen grains were monoporate in *S. hexandrum* and tricolpate in *B. lycium*. Nowicke and Skvarla, (1981) found furrow-like aperture and gemmae of variable sizes on exine surface of *Sinopodophyllum hexandrum*. Similarly, Perveen and Qaiser, (2010) found that pollens of *B. lycium* had a spheroidal shape and sub-psilate exine ornamentation. The pollen's shape, size, and exine ornamentation completely distinguished *A. heterophyllum* from *A. violaceum* (Ranunculaceae) in the present study. The pollen characters of *A. violaceum,* such as tricolpate, prolate, microechinate, and perforate, are completely different from those of *A*. *fragrans* (Ahmad *et al*., 2018), suggesting the importance of these pollen characters in the taxonomy of Ranunculaceae. In *A. pubiflora* (Ranunculaceae) pollen grains were medium sized with tricolpate aperture and microechinate perforate ornamentation. Although Perveen and Qaiser, (2006) studied same taxa and found the small pollen grains with spinulose exine ornamentation. The subprolate, tricolpate echinate perforate pollen in *M. aculeata* (Papaveraceae) described here have also been found in different genera of this family (Pérez-Gutiérrez *et al*., 2015; Keshavarzi *et al*., 2011). Small, monad, tricolporate, prolate, striate pollen recorded in *R. webbiana* (Rosaceae) in the current investigation resembles the results of the earlier studies on the family Rosaceae (Singh *et al*., 2020; Hebda and Chinnappa, 1994). Colpate, tuberculate, verrucate, 3-zonocolporate, rugulate pollen were previously reported in the genus *Quercus* (Hayrapetyan and Bruch, 2020). However, scabrate and granulate pollen exine were found in present study and therefore can prove helpful in distinguishing *Q. oblongata* from other species of this genus.

The pollen characters reported by Shehata, (2008) for *Geranium wallichianum* (very large, tricolporate, oblate spheroidal and clavate) were similar to those reported in the present study. Furthermore, similar characters have been observed for the genus *geranium* (Geraniaceae) (Başer *et al*., 2016).

The pollen description for *Aesculus indica* (Sapindaceae) is reported for the first time and is in agreement with the previous study on genus *Aesculus* (Pozhidaev, 1995). In additions to striate and perforate exine ornamentation reported here rugulate, reticulate, granular and smooth pattern have also been reported in genus *Acer* of family Sapindaceae (Siahkolaee *et al*., 2017). The two studied species of Saxifragaceae, i.e., in *Bergenia ciliata* and *B. stracheyi,* can be easily distinguished based on pollen shape and exine ornamentation, which was spheroidal and perforate in the former and prolate spheroidal and without perforation in the latter. However, in a previous study of Perveen and Qaiser, (2009), exine was reticulate-rugulate and punctate rugulate in *Bergenia ciliata* and *B. stracheyi* respectively, whereas the pollen was sub prolate and prolate spheroidal in *B. ciliata* and *B. stracheyi,* respectively.

The results obtained for the pollen grains of Polygonaceae species showed similarities and dissimilarities with the previous reports. Hong *et al*. (1998) studied 30 taxa of *Polygonella* and *Polygonum* and observed pollens prolate to spheroidal. Yang *et al*. (2001) analyzed 40 species of *Rheum* of family Polygonaceae in which *R. australe* and *R. webbianum* had sub-spheroidal pollen. Our observations are different from Yang *et al*. (2001), who found the pollen grains of *R. australe* to be sub-spheroidal and medium, while the pollen of *R. webbianum* to be sub-spheroidal and medium. Pollen shape could be an attribute of evolution and used to know pollination mode (Lemmens *et al*., 2003; Olga *et al*., 2013). Pollen grains of *P. acinosa* were prolate, tricolporate, microechinate, perforate, granulate, and psilate, whereas subprolate pollen with reticulate exine ornamentation was observed previously for this species (Nowicke, 1968). The importance of aperture type in delimiting the genus *Phytolacca* from *Rivinia* has been demonstrated previously (Simpson and Skvarla, 1981). In *Rhododendron companulatum* (Ericaceae) medium-sized pollen with a rugulate exine pattern was observed, which partially corroborates with the previous study of (Sarwar and Takahashi, 2013), in which the species other than *R. companulatum* possessed small-sized pollen. Another study reported the pollen grains to be of oblate and sub oblate with gemmae type exine ornamentation in other species of *Rhododendron* (Namgay and Sridith, 2021). This character may prove useful in distinguishing this species from other species of the genus.

In the studied species *Achillea millefolium*, *Jurinea dolomiaea*, *Saussurea obvallata*, *Ligularia fischerii* var. *euodon* (Miq.) of Asteraceae family, pollen grain was prolate-spheroidal, oblate-spheroidal with echinate perforation on exine. Only the differences were found in the sizes, frequency, and spinules' density among the studied species. Our result is thus in line with the findings of (Ceter *et al*., 2013). Akyalcin *et al*. (2011) studied six species of genus *Achillea* from Turkey and found that pollen grains were generally tricolporate with oblate-spheroidal, prolate spheroidal, subprolate with echinate microperforate, and echinate-rugulate microperforate exine ornamentation. In contrast to the present study, Khan *et al*. (2012) found spheroidal and prolate with tricolporate aperture in *Achillea millefolium* with exine thickness 2.5 μm, which was more than we recorded; however, the pollen grains were of the almost identical size. The pollen characters of *Jurinea dolomiaea* were similar to that reported previously (Bordbar and Mirtadzadin, 2015). The pollen description for the *S. obvallata* and *L. fischerii* reported for the first time.

In *Angelica glauca* (Apiaceae) the pollen grains were small, tricolporate, prolate, and had rugulate exine ornamentation. No pollen data is available in the literature to be compared with our results. Therefore, these pollen characters can act as delimiting characters for this species. However, sub-rhombus, oval, sub-rectangle, and equator constricted pollen were reported in genus *Angelica* except species *A. glauca* from China (Chen *et al*., 2007). Triporate pollen having large funnel-shaped protrusions at the equator described in the present study for *M. longifolia* (Caprifoliaceae) was similar to the findings of (Blackmore and Cannon, 1983). Similarly, the pollen characters such as oblate–spheroidal or prolate–spheroidal hexacolpate having reticulate; perforate ornamentation for *Salvia hians* (Lamiaceae) were agreement with those reported for other species of genus *Salvia* (Kahramanet *et al*., 2010; Ozler *et al*., 2011). This indicates that the studied pollen characters do not have significant role in species identification.

The pollen grains in *Picrorhiza kurroa*, (Plantaginaceae) were small, tricolporate, sub prolate, psilate exine ornamentation and are reported for the first time. Mohsenzadeh *et al*., (2020) however, reported medium-sized, prolate-spheroidal to prolate, pantoporate pollen in other species of genus *Plantago*. Therefore, these reported pollen characters may prove helpful in delimitation of this species from others of the same genus. The medium, hexacolpate, reticulate and perforate pollen grains in *Gentiana kurroo,* (Gentianaceae) were reported for the first time in this study. In contrast, 3-colporate, prolate, and medium pollen have been reported in the only studied species of *Gentiana* i.e., *bambuseti* (Hsieh *et al*., 2007). This indicated that the studied pollen characters of *G. kurroa* are useful in species delimitation in the genus *Gentiana*. In addition to this, variation in several pollen morphological characters has been observed in family Gentianaceae (Chassoti and Von Hagen, 2008).

The pollen characters of *A. benthamii* (Boraginaceae) in the present study concurs with that reported in previous studies on several genera of Boraginaceae (Khatamsaz, 2001), therefore, suggesting that these characters are not useful in species discrimination.

Conclusions

In the present investigation, the pollen morphology analysis of 32 important RET plants of western Himalaya was conducted. Pollen description of *Roscoea purpurea, Allium stracheyi*, *Chlorophytum arundinaceum, Arisaema propinquum*, *Aquilegia pubiflora*, *Meconopsis aculeata*, *Quercus oblongata*, *Aesculus indica*, *Oxyria digyana*, *Rhododendron companulatum*, *Jurinea dolomiaea Saussurea obvallata*, *Ligularia fischerii*, *Angelica glauca*, *Salvia hians*, *Picrorhiza kurroa*, *Gentiana kurroo*, and *Arnebia benthamii* at species level have been reported for the first time. The studied pollen characters portrayed a wide range of variation and showed taxonomic significance at various levels. The present information can provide valuable information to plant taxonomist for further studies on these medicinal plants.

Authors' Contributions

Conceptualization: SG and BK; Data curation: SG; Formal analysis; BK and KS; Funding acquisition: SG; Investigation: BK; Methodology: BK; Project administration: SG; Resources: SG; Software: BK and SKD; Supervision: SG; Validation: SG; Visualization: SG; Writing - original draft: BK and KS; Writing - review and editing: SG, KS, PK and ZB. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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