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RESEARCH ARTICLE

Pollen morphology and variability among Indian cultivars of *Chrysanthemum morifolium* and comparative analysis with genera of the Asteraceae family

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Abstract Chrysanthemums are important worldwide for their beauty and medicinal uses. This research analyzes the pollen morphology of 134 *Chrysanthemum morifolium* cultivars using scanning electron microscopy (SEM). Key pollen characteristics such as shape, spine length, ornamentation, and aperture type were observed in detail, highlighting the effectiveness of SEM in species and cultivar identification based on pollen features. Multivariate analyses, including hierarchical clustering and principal component analysis, categorized the cultivars according to their pollen traits. Notable findings among *Chrysanthemum morifolium* cultivars included Punjab Gold with larger pollen size, IAH Red with an equatorial diameter of 25.14 µm, and Kundan distinguished by a spine length of 13.42 µm. These differences suggest environmental factors may influence the observed variation. Comparison with other Asteraceae members, using data from the PalDat database, underscored the distinctiveness of chrysanthemum pollen morphology, validating its use as a classification tool. Pollen morphology study is vital for understanding plant biology, including reproduction, biodiversity, ecological interactions, and environmental adaptation. The findings have practical applications in agriculture and horticulture, enhancing knowledge of plant taxonomy and classification.

Keywords Pollen morphology · Palynology · Scanning electron microscope · PCA · Asteraceae · Cluster analysis

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Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat), a member of the Asteraceae family with a high ornamental value is one of the ten most popular traditional flowers in India and one of the four most popular cut flowers in the world; therefore, it is a commercially important species that occupies a large share of the global market of cut-flower production (Higuchi 2018); reportedly, the second largest floriculture crop in the world after roses (Spaargaren and Geest 2018). The term 'C*hrysanthemum*' comes from the Greek 'krus anthemon', which means gold flower, and was initially used in China (Eisa et al. 2022). The genus *Chrysanthemum*, which holds significant ecological and economic value, comprises approximately 40 species along with numerous hybrids and cultivars (Hao et al. 2022). The history of the chrysanthemum-group, a branch of the eco-geographical gradients and diverse macrohabitats in East Asia. Despite ongoing debates about its circumscription and monophyly, the group is characterized by features such as solitary flower heads or corymbose synflorescences, radiate or disciform capitula, and echinate Anthemis-type pollen grains (Shen et al. 2021). There exists a wide variety of chrysanthemum cultivars globally, which are dicotyledonous herbaceous annual or perennial plants, showcasing significant diversity in both morphology and popularity. These flowers are praised worldwide not only for their unique aesthetic characteristics, vibrant colors, and complex structures but also receive substantial recognition for their therapeutic and medicinal properties. (Hadizadeh et al. 2022).

Pollen grain is a microscopic structure of flower containing the microgametophyte of flowering plants, which produces the male gametes. The pollen unit serves as a fundamental entity essential for plant reproduction, representing an array of morphological traits essential for systematic characterization (Bedinger 1992). At the forefront of pollen morphology lies its polarity, a feature defined by the spatial arrangement of the microspore within the grain. There are two forms of polarity: isopolar and heteropolar, where isopolar pollen grains often exhibit symmetrical characteristics across the equatorial plane, whereas heteropolar grains lack such symmetry. Integral to pollen morphology is its diverse organization of shapes, ranging from simple spheroids to complex geometries like cups, boats, or prisms. These shapes combined with the presence and arrangement of colpi, which are the surface furrows facilitating pollination; contribute considerably to overall structural identity of the grain (Weber 1998). Additionally, the exine, the outer layer of the pollen wall, shows distinct sculpturing patterns (McCormick 1993), expanding analytical features for taxonomic classification. Further factors enhancing morphological complexity of the pollen grain are surface ornamentations adorning colpi, offering unique identifiers for taxonomic classification (Halbritter et al. 2018). Pollen size, quantified in micrometers, varies among species, often represented as a range representing its length and width. Significantly, the P/E ratio which is a metric indicative of the ratio of the polar axis length to the equatorial diameter, serves as an important descriptor, interpreting overall shape and morphological profile of the pollen grain. Pollen characteristics have been extensively studied, including its origin, morphology, and physiology. Bahadur et al. (2022) studied pollen morphology of some selected tribes of the Asteraceae of Hainan Island South China using light and scanning electron microscopy, revealed significant taxonomic understandings at the tribe and genus levels. The utility of pollen features, such as spine length and aperture traits, were featured in explaining species boundaries and refining taxonomic classifications within the family. Additionally, the potential of pollen features as an additional tool for regrouping taxa within Asteraceae was underlined, leveraging both light and scanning electron microscopic techniques. Recent progress in understanding Asteraceae family has been reported, drawing on joint efforts by specialists in palaeobotany, cytogenetics, comparative genomics, and phylogenomics, emphasizing the noteworthy phenotypic diversity and global distribution of the family, which positioned it as a model for addressing a broad range of eco-evolutionary questions (Palazzesi, et al. 2022). Its role was also studied in DNA identification, flower pollination, germination, and fertilization. Their adaptation to distinct pollination strategies resulted in observable anatomical variations, as detailed by Halbritter et al. (2018). The current investigations into pollen have played a crucial role in enhancing the knowledge of plant classification, taxonomy and biodiversity. Modern taxonomies are seen to be relied on palynology for precise differentiation and classification of closely related taxa and thereby palynology is regarded as one of the most effective tools for achieving identifications and defining the boundaries of plant groups; discriminating and describing intraspecific as well as interspecific diversity for the species (Khan et al. 2012). While the causes of pollen morphology variation are largely unknown, its evolutionary implications depend on environmental and genetic factors. Pollen diversity aids survival in varying conditions, influenced by size, apertures, wall ornamentation, and thickness (Ejsmond et al. 2011; Sahli et al. 2023). According to Matamoro-Vidal et al., (2016), aperturate pollens with thin exine walls and crotonoid ornamentation are more flexible and may retain their integrity better under intense desiccation stress. Plants in hotter environments tend to produce larger pollens with a lower surface-to-volume ratio, which could be used to reduce the rate of water loss. Different findings suggested that the relationships between ornamentation type and pollination system are taxonomic (Ferguson 1985; Sannier et al. 2009; van der Ham et al. 2010).

Pollen morphology, including wall morphology, polarity, symmetry, shape, and size, is crucial in paleoecology, paleontology, and archaeology (Stephen 2014). Palynology investigates nature, distribution, and preservation of pollen, crucial for disciplines like paleoecology and forensics (Rahmawati et al. 2019). In recent years, scanning electron microscopy (SEM) has emerged as a powerful technique for evaluating the morphology of pollen grains across different species (Parakhia 2017). Pollen morphology research has advanced understanding of evolution and systematics, aiding in identifying past plant assemblages and environmental changes (Moore et al. 1991). However, using pollen morphology for taxonomy is challenging and should be combined with other characteristics. Traditional plant biodiversity classification methods involve observable traits, while modern molecular tools like DNA barcoding and SNPs offer more efficient categorization means (Arif et al. 2010). Over the past two decades, several investigations have considered variations in pollen characteristics among different cultivars (Messora et al. 2017). Yet, there is an uncertainty regarding the evolutionary processes that have led to the diverse shapes of pollen found in flowering plants. Key knowledge gaps exist concerning the relationship between pollen morphology and factors such as genetics, environment, pollination ecology, and the evolution of pollinators (Kriebel et al. 2017; Mander et al. 2021). Despite investigations showing that genome size does not predict pollen size well, little research has been done on how genome size or events like whole-genome duplication relate to the evolution of pollen morphology. This study builds upon a similar concept that pollen grain morphology can serve as a meaningful criterion for distinguishing cultivars and exploring biodiversity in flowering plants (Jardine, 2022).

The study of pollen morphology provides the information on genetic identity and parentage of genotypes which is important for the exploration of germplasms aimed at maximizing the use of genetic diversity (Adedeji and Akinniyi 2015). Although there has been a broad research on the morphology and pollen characteristics of Asteraceae, the daisy family over the years, there remains a lack of specific information regarding the palynology of numerous subfamily members. The current study is aimed to address this gap by conducting thorough palynological examinations of 134 chrysanthemum cultivars using scanning electron microscopy analysis unveiling subtleties in shape, size, ornamentation, and aperture type. The objectives involved a multifaceted investigation, aiming to study into chrysanthemum pollen morphology intricately while situating it within the broader context of Asteraceae family diversity and taxonomy. This research serves to observe and describe the pollen morphology variations and enhance the identification of chrysanthemum cultivars through their palynological attributes. The pollen morphology of species from various genera within the Asteraceae family was comparatively examined, utilizing the data present in the PalDat database. Moreover, the pollen morphological information sourced from PalDat for the different genera was compared with the morphological data obtained for 134 *Chrysanthemum* cultivars through SEM analysis. The comparison of these traits with data from the PalDat database, covering 14 genera within Asteraceae, aimed to illuminate distinctive features of chrysanthemum pollen morphology. Statistical analyses, including cluster and principal component analyses, were employed to categorize chrysanthemum cultivars based on their pollen characteristics, shedding light on intrageneric variation and potential evolutionary influences. While several genera within the Asteraceae family have undergone palynological research, as documented and deposited in PalDat database, a thorough examination of *Chrysanthemum* genus pollen remains absent. This gap in systematic research is yet to be addressed. Furthermore, the study aimed to underscore the taxonomic significance of pollen characters and the utility of SEM techniques in species and cultivar identification. Ultimately, by suggesting future research directions, the study aimed to contribute to a comprehensive understanding of chrysanthemum taxonomy and evolutionary history, advocating for a holistic approach to plant classification and biodiversity conservation.

Material and methods

Sample collection

In this study, a total of one hundred and thirty-four chrysanthemum (*C. morifolium*) cultivars, each possessing high ornamental value, had been selected for analysis. In addition to this, one sample of another species, *C. coronarium* was also studied for its pollen morphology. The selected Indian cultivars were collected and are maintained at ICAR-Directorate of Floricultural Research (DFR) in Pune. Flowers were collected from the field and transported to the lab in plastic pouches to avoid contamination. The anthers were separated and the pollens were precisely collected at the stage of anthesis using sterile forceps in clan, sterile 2 mL tube containing 100% glacial acetic acid to maintain sample integrity. Pollens were primarily confirmed by using the compound microscope and used for further study. Various chrysanthemum cultivars subjected to SEM examination and analysis can be found enlisted in Table 1, providing a reference for further investigation.

Sample preparation and SEM analysis

The pollens were acetolyzed as per protocol described by Erdtman (1966) for SEM sample preparation. Pollen grains in glacial acetic acid were centrifuged at 3000–4000 rpm for 3 min. The pellet was then suspended in 5 ml of acetolysis mixture (9:1 acetic anhydride: Conc. sulfuric acid) and heated at 100 °C for 20 min until brown. After cooling, the mixture was centrifuged and the pellet was washed three times with distilled water. Further the pellet was rinsed with 10% glycerin. For SEM, the acetolyzed pollen was suspended in 70% ethanol, centrifuged, then replaced with 80% ethanol followed by centrifugation again, and coated with gold (gold sputtering) to prevent shrinking. Qualitative attributes, such as the shape of pollen in both polar and equatorial views, pollen class, exine, ornamentation, pollen size, polarity and aperture type were examined using scanning electron microscope. On the other hand, quantitative characteristics, including polar length, equatorial diameter, exine thickness, spine length, and spine rows between colpi were measured. Pollen measurement data were calculated from at least 10 grains per sample. Measurements and analysis of SEM images carried out by using Fiji win 64 software. The shape of pollen was determined using methods described by Erdtman (1945), characterizing pollen shape based on the equatorial view, by calculating the P/E ratio, ratio of the polar axis to the equatorial diameter (P:E) was calculated with following equation:

$$\text{Form Index (FI)} = \frac{\text{P}}{\text{E}} \times 100$$

where, P- Polar axis, E- Equatorial diameter (Erdtman 1966). Shape classes and relations between polar axis (*P*) and equatorial axis (*E*) of grains in equatorial view when one of the apertures lies exactly at the center.

Statistical analysis

Two analyses were conducted using StatistiXL software, a data analysis package (Coutinho et al. 2021). Cluster analysis (CA) was performed using the average taxonomic distance, specifically the Euclidean distance matrix, and the UPGMA clustering method, as implemented in StatistiXL software in MS Excel. Here, we investigated the relations among chrysanthemum cultivars on the basis of similarity by employing Hierarchical Cluster Analysis (HCA) with the assistance of pollen morphology data. HCA was conducted to explore how these cultivars could be grouped based on the observed dissimilarities in their characteristics. Multivariate ordination analysis of the pollen characteristics from 134 cultivars were subjected to numerical analysis, including techniques such as principal component analysis (PCA) in addition to cluster analysis.

Database screening and data collection

The morphometric data included in PalDat database (https://www.paldat.org/; accessed on 12 September, 2023), was obtained for species from 14 various genera within the Asteraceae family, which had also been subjected to SEM analysis, summarized in Table 2. Data collected was compared for their pollen morphological features with chrysanthemum cultivars as considered in this study.

Table 1 Morphometric data of pollen grains in equatorial view of *Chrysanthemum morifolium* cultivars obtained on SEM analysis (n = 134) represented as arithmetic mean ± standard deviation; P/E ratio – polar equatorial diameter ratio

Chrysanthemum cultivars		Polar length	Equatorial diameter	P/E ratio	Form index	Exine thickness	Spine length	Spine rows between colpi	Shape in equatorial view
1.	Accession 1	28.80 ± 3.46	28.48 ± 3.99	1.01 ± 0.17	101.12 ± 13.15	3.11 ± 0.44	5.13 ± 0.87	4.0 ± 0.7	Prolate Spheroidal
2.	Accession 12	30.10 ± 3.61	30.99 ± 4.34	0.97 ± 0.16	97.13 ± 12.63	4.31 ± 0.60	5.28 ± 0.90	5.0 ± 0.9	Oblate Spheroidal
3.	Accession 13	31.04 ± 4.35	31.55 ± 3.47	0.98 ± 0.19	98.38 ± 6.89	2.28 ± 0.32	6.57 ± 0.79	4.0 ± 0.5	Oblate Spheroidal
4.	Accession 2	29.49 ± 8.88	29.27 ± 9.07	1.01 ± 0.01	100.75 ± 97.86	3.18 ± 0.14	5.83 ± 0.31	5.0 ± 0.3	Prolate Spheroidal
5.	Accession 3	31.11 ± 1.56	29.91 ± 3.29	1.04 ± 0.03	104.02 ± 8.32	3.32 ± 0.56	4.53 ± 0.59	4.0 ± 0.1	Prolate Spheroidal
6.	Akitha	30.86 ± 4.01	29.34 ± 3.81	1.05 ± 0.14	105.19 ± 13.67	2.26 ± 0.29	6.46 ± 0.84	4.0 ± 0.5	Prolate Spheroidal
7.	Anmol (PAU)	28.32 ± 4.25	28.48 ± 3.14	0.99 ± 0.11	99.43 ± 12.93	2.57 ± 0.50	5.01 ± 0.85	5.0 ± 0.9	Oblate Spheroidal
8.	Aparajita	31.54 ± 3.70	30.92 ± 4.11	1.02 ± 0.18	101.98 ± 13.67	3.53 ± 0.32	5.91 ± 1.10	4.0 ± 0.7	Prolate Spheroidal
9.	Autumn Joy	28.63 ± 6.76	29.93 ± 7.28	0.96 ± 0.01	95.68 ± 92.92	3.24 ± 0.06	5.51 ± 0.32	5.0 ± 0.3	Oblate Spheroidal
10.	Bidhan Agnishikha	28.52 ± 3.78	29.77 ± 4.33	0.96 ± 0.17	95.81 ± 13.26	3.73 ± 0.49	6.93 ± 1.00	5.0 ± 0.7	Oblate Spheroidal
11.	Bidhan Gold	29.46 ± 8.40	31.75 ± 9.45	0.93 ± 0.01	92.78 ± 88.89	2.50 ± 0.09	5.05 ± 0.35	6.0 ± 0.3	Oblate Spheroidal
12.	Bidhan Jayanti	30.38 ± 3.94	29.93 ± 4.33	1.02 ± 0.18	101.51 ± 13.80	3.92 ± 0.47	6.14 ± 0.89	6.0 ± 0.7	Prolate Spheroidal
13.	Bidhan Lalima	29.53 ± 3.54	29.86 ± 4.45	0.99 ± 0.16	98.91 ± 12.06	3.30 ± 0.35	4.83 ± 0.86	6.0 ± 1.1	Oblate Spheroidal
14.	Bidhan Madhuri	28.55 ± 3.65	29.06 ± 4.19	0.98 ± 0.17	98.27 ± 13.20	2.56 ± 0.55	5.63 ± 1.04	5.0 ± 1.1	Oblate Spheroidal
15.	Bidhan Mum	29.65 ± 3.54	28.66 ± 4.18	1.03 ± 0.17	103.45 ± 12.86	3.04 ± 0.46	6.00 ± 0.82	6.0 ± 1.1	Prolate Spheroidal
16.	Bidhan Neeta	31.05 ± 3.43	28.83 ± 4.07	1.08 ± 0.17	107.69 ± 12.78	3.40 ± 0.36	5.78 ± 0.96	5.0 ± 0.9	Prolate Spheroidal
17.	Bidhan Poonam	30.14 ± 3.56	28.94 ± 4.01	1.04 ± 0.18	104.16 ± 13.45	3.50 ± 0.43	6.16 ± 1.02	5.0 ± 1.1	Prolate Spheroidal
18.	Bidhan Purna	30.80 ± 3.73	29.78 ± 4.04	1.03 ± 0.18	103.44 ± 14.00	2.35 ± 0.48	5.70 ± 0.98	5.0 ± 0.9	Prolate Spheroidal
19.	Bidhan Rajat	30.45 ± 4.42	29.20 ± 3.49	1.04 ± 0.10	104.26 ± 12.06	2.86 ± 0.26	5.64 ± 0.86	5.0 ± 1.1	Prolate Spheroidal
20.	Bidhan Rupanjali	28.01 ± 3.70	27.65 ± 4.17	1.01 ± 0.18	101.29 ± 13.45	2.62 ± 0.33	5.51 ± 0.97	4.0 ± 0.9	Prolate Spheroidal
21.	Bidhan Sabita	30.33 ± 4.55	29.11 ± 3.21	1.04 ± 0.11	104.19 ± 13.54	3.50 ± 0.50	5.17 ± 0.88	4.0 ± 0.7	Prolate Spheroidal
22.	Bidhan Shova	30.38 ± 3.36	29.50 ± 3.87	1.03 ± 0.17	103.00 ± 13.17	2.54 ± 0.37	4.87 ± 0.94	4.0 ± 0.0	Prolate Spheroidal
23.	Bidhan Sweta	32.42 ± 4.42	31.59 ± 3.49	1.03 ± 0.10	102.64 ± 12.06	2.38 ± 0.50	5.14 ± 0.86	5.0 ± 1.1	Prolate Spheroidal
24.	Bidhan Swapna	28.95 ± 3.47	31.61 ± 4.43	0.92 ± 0.16	91.59 ± 11.91	3.40 ± 0.50	5.99 ± 1.02	5.0 ± 0.9	Oblate Spheroidal
25.	Bidhan Tarun	35.15 ± 5.27	32.34 ± 3.56	1.09 ± 0.12	108.69 ± 14.13	2.43 ± 0.50	6.21 ± 1.06	6.0 ± 1.1	Prolate Spheroidal
26.	Chandrika	30.68 ± 4.60	29.97 ± 3.30	1.02 ± 0.11	102.35 ± 13.31	2.42 ± 0.50	5.96 ± 1.01	5.0 ± 0.9	Prolate Spheroidal
27.	Charlie	30.28 ± 4.54	29.79 ± 3.28	1.02 ± 0.11	101.65 ± 13.22	2.58 ± 0.50	5.17 ± 0.88	5.0 ± 0.9	Prolate Spheroidal
28.	Cherabu	26.48 ± 3.97	25.99 ± 2.86	1.02 ± 0.11	101.86 ± 13.24	3.23 ± 0.50	5.00 ± 0.85	4.0 ± 0.7	Prolate Spheroidal
29.	Classic	28.27 ± 4.24	29.89 ± 3.29	0.95 ± 0.10	94.58 ± 12.30	3.59 ± 0.50	5.06 ± 0.86	4.0 ± 0.7	Oblate Spheroidal
30.	Coffee	29.70 ± 4.45	28.51 ± 3.14	1.04 ± 0.11	104.18 ± 13.54	3.59 ± 0.50	5.47 ± 0.93	5.0 ± 0.9	Prolate Spheroidal
31.	Coimbatore Semi-Double	30.11 ± 4.52	30.82 ± 3.39	0.98 ± 0.11	97.69 ± 12.70	3.48 ± 0.50	5.55 ± 0.94	4.0 ± 0.7	Oblate Spheroidal
32.	Corom Small	27.58 ± 2.09	28.50 ± 3.14	0.97 ± 0.09	97.00 ± 8.73	2.64 ± 2.38	6.88 ± 1.95	5.0 ± 1.1	Oblate Spheroidal
33.	Daity White	32.46 ± 4.87	30.83 ± 3.39	1.05 ± 0.12	105.29 ± 13.69	3.44 ± 0.50	5.28 ± 0.90	6.0 ± 1.1	Prolate Spheroidal

Table 1 (continued)

	Chrysanthemum cultivars	Polar length	Equatorial diameter	P/E ratio	Form index	Exine thickness	Spine length	Spine rows between colpi	Shape in equatorial view
34.	Dark Eyes	28.88 ± 3.47	27.56 ± 3.86	1.05 ± 0.18	104.78 ± 13.62	4.85 ± 0.68	5.41 ± 0.92	4.0 ± 0.7	Prolate Spheroidal
35.	Dark Red	28.53 ± 3.42	31.72 ± 4.44	0.90 ± 0.15	89.94 ± 11.69	2.91 ± 0.41	4.19 ± 0.71	6.0 ± 1.1	Oblate Spheroidal
36.	Devi	29.63 ± 3.56	30.47 ± 4.27	0.97 ± 0.16	97.24 ± 12.64	3.00 ± 0.42	5.12 ± 0.87	5.0 ± 0.9	Oblate Spheroidal
37.	DFR C-7	28.39 ± 3.41	29.35 ± 4.11	0.97 ± 0.16	96.74 ± 12.58	3.68 ± 0.51	5.19 ± 0.88	4.0 ± 0.7	Oblate Spheroidal
38.	DFR C-2 (DFR Megha)	31.05 ± 3.73	29.08 ± 4.07	1.07 ± 0.18	106.77 ± 13.88	3.33 ± 0.47	5.90 ± 1.00	5.0 ± 0.9	Prolate Spheroidal
39.	DFR C-1 (DFR Pallavi)	29.10 ± 3.49	29.93 ± 4.19	0.97 ± 0.16	97.21 ± 12.64	3.17 ± 0.44	5.37 ± 0.91	5.0 ± 0.9	Oblate Spheroidal
40.	DFR C-3 (DFR Swarna)	28.26 ± 3.39	28.78 ± 4.03	0.98 ± 0.17	98.16 ± 12.76	2.63 ± 0.37	5.09 ± 0.87	5.0 ± 0.9	Oblate Spheroidal
41.	DFR C-4 (DFR Swarna Bindu)	29.85 ± 3.58	28.30 ± 3.96	1.05 ± 0.18	105.49 ± 13.71	2.72 ± 0.38	5.39 ± 0.92	4.0 ± 0.7	Prolate Spheroidal
42.	DFR C-5	32.36 ± 3.88	32.80 ± 4.59	0.99 ± 0.17	98.69 ± 12.83	3.48 ± 0.49	4.92 ± 0.84	5.0 ± 0.9	Oblate Spheroidal
43.	DFR C-6	30.00 ± 4.20	28.95 ± 3.18	1.04 ± 0.20	103.62 ± 7.25	2.06 ± 0.29	5.48 ± 0.66	5.0 ± 0.7	Prolate Spheroidal
44.	Dignity	28.25 ± 4.24	28.55 ± 3.14	0.99 ± 0.11	98.95 ± 12.86	3.03 ± 0.00	5.10 ± 0.87	6.0 ± 1.1	Oblate Spheroidal
45.	Discovery	30.26 ± 4.54	28.17 ± 3.10	1.07 ± 0.12	107.40 ± 13.96	3.76 ± 0.00	8.23 ± 1.40	5.0 ± 0.9	Prolate Spheroidal
46.	Draguma	27.95 ± 4.19	28.49 ± 3.13	0.98 ± 0.11	98.14 ± 12.76	4.42 ± 0.57	5.43 ± 0.92	5.0 ± 0.9	Oblate Spheroidal
47.	Fire Ball	31.15 ± 4.67	32.04 ± 3.52	0.97 ± 0.11	97.22 ± 12.64	3.49 ± 0.00	5.57 ± 0.95	4.0 ± 0.72	Oblate Spheroidal
48.	Gaity	27.44 ± 4.12	28.02 ± 3.08	0.98 ± 0.11	97.92 ± 12.73	4.46 ± 0.00	5.46 ± 0.93	4.0 ± 0.65	Oblate Spheroidal
49.	Garden Beauty	28.38 ± 4.26	29.74 ± 3.27	0.96 ± 0.11	95.46 ± 12.41	3.97 ± 0.36	5.68 ± 0.97	6.0 ± 0.9	Oblate Spheroidal
50.	Gauri	32.43 ± 4.86	31.81 ± 3.50	1.02 ± 0.11	101.96 ± 13.26	3.542 ± 0.18	5.22 ± 0.89	5.0 ± 0.9	Prolate Spheroidal
51.	Gitanjali	29.32 ± 4.40	28.84 ± 3.17	1.02 ± 0.11	101.66 ± 13.22	2.65 ± 0.00	5.92 ± 1.01	5.0 ± 0.0	Prolate Spheroidal
52.	Gulmohar	29.70 ± 4.16	29.71 ± 3.27	1.00 ± 0.19	99.97 ± 7.00	4.69 ± 0.00	4.70 ± 0.56	5.0 ± 0.0	Spheroidal
53.	Gunjan	29.30 ± 4.10	29.42 ± 3.24	1.00 ± 0.19	99.61 ± 6.97	4.47 ± 0.00	5.30 ± 0.64	5.0 ± 0.9	Spheroidal
54.	Himani-3	29.52 ± 4.43	28.88 ± 3.18	1.02 ± 0.11	102.21 ± 13.29	4.285 ± 0.47	7.12 ± 1.21	5.0 ± 0.0	Prolate Spheroidal
55.	Honey Comb	30.68 ± 4.60	30.54 ± 3.36	1.00 ± 0.11	100.44 ± 13.06	3.18 ± 0.00	6.02 ± 1.02	5.0 ± 0.0	Spheroidal
56.	Houston	31.53 ± 4.73	30.94 ± 3.40	1.02 ± 0.11	101.90 ± 13.25	2.99 ± 0.39	5.89 ± 1.00	6.0 ± 1.0	Prolate Spheroidal
57.	HYDC-12	29.42 ± 4.41	30.14 ± 3.32	0.98 ± 0.11	97.60 ± 12.69	2.75 ± 0.25	4.94 ± 0.84	5.0 ± 1.0	Oblate Spheroidal
58.	HYDC-19	28.40 ± 4.26	28.51 ± 3.14	1.00 ± 0.11	99.62 ± 12.95	2.24 ± 0.00	6.07 ± 1.03	5.0 ± 0.9	Spheroidal
59.	HYDC-2	29.38 ± 4.41	28.38 ± 3.12	1.04 ± 0.11	103.53 ± 13.46	3.22 ± 0.00	6.70 ± 1.14	3.0 ± 0.0	Prolate Spheroidal
60.	HYDC-20	31.12 ± 4.36	29.68 ± 3.26	1.05 ± 0.20	104.86 ± 7.34	3.53 ± 0.49	5.32 ± 0.64	5.0 ± 0.9	Prolate Spheroidal
61.	HYDC-26	28.32 ± 4.25	29.55 ± 3.25	0.96 ± 0.11	95.83 ± 12.46	3.24 ± 0.50	5.28 ± 0.90	5.0 ± 0.9	Oblate Spheroidal
62.	HYDC-28	28.24 ± 4.24	27.33 ± 3.01	1.03 ± 0.11	103.33 ± 13.43	2.47 ± 0.50	5.66 ± 0.96	5.0 ± 0.65	Prolate Spheroidal
63.	HYDC-29	29.37 ± 4.41	29.87 ± 3.29	0.98 ± 0.11	98.32 ± 12.78	3.25 ± 0.50	6.12 ± 1.04	4.0 ± 0.0	Oblate Spheroidal
64.	HYDC-41	28.52 ± 4.28	29.45 ± 3.24	0.97 ± 0.11	96.85 ± 12.59	2.33 ± 0.50	5.37 ± 0.91	4.0 ± 0.72	Oblate Spheroidal

Table 1 (continued)

	Chrysanthemum cultivars	Polar length	Equatorial diameter	P/E ratio	Form index	Exine thickness	Spine length	Spine rows between colpi	Shape in equatorial view
65.	HYDC-42	28.23 ± 4.23	29.90 ± 3.29	0.94 ± 0.10	94.40 ± 12.27	2.66 ± 0.50	5.73 ± 0.97	5.0 ± 0.9	Oblate Spheroidal
66.	HYDC-55	28.67 ± 4.30	29.70 ± 3.27	0.96 ± 0.04	96.24 ± 3.92	2.85 ± 0.50	6.05 ± 0.91	5.0 ± 0.9	Oblate Spheroidal
67.	HYDC-56	30.29 ± 4.63	30.81 ± 3.28	0.98 ± 0.11	98.31 ± 13.45	4.41 ± 0.50	6.20 ± 1.01	4.0 ± 0.9	Oblate Spheroidal
68.	HYDC-77	27.45 ± 3.84	30.21 ± 3.32	0.91 ± 0.17	90.87 ± 6.36	3.49 ± 0.49	6.08 ± 0.73	4.0 ± 0.52	Oblate Spheroidal
69.	HYDC-9	29.20 ± 4.38	30.16 ± 3.32	0.97 ± 0.11	96.79 ± 12.58	3.20 ± 0.50	6.75 ± 1.15	6.0 ± 0	Oblate Spheroidal
70.	HYDC-Local Yellow	28.71 ± 4.31	30.03 ± 3.30	0.96 ± 0.11	95.62 ± 12.43	2.91 ± 0.50	6.25 ± 1.06	5.0 ± 0.9	Oblate Spheroidal
71.	IAH Red	24.86 ± 3.73	25.14 ± 2.77	0.99 ± 0.11	98.85 ± 12.85	2.46 ± 0.50	5.21 ± 0.89	5.0 ± 0.9	Oblate Spheroidal
72.	Jaya	30.89 ± 4.63	29.85 ± 3.28	1.03 ± 0.11	103.46 ± 13.45	2.99 ± 0.50	5.93 ± 1.01	6.0 ± 1.08	Prolate Spheroidal
73.	Karnal pink	28.60 ± 4.29	28.60 ± 3.15	1.00 ± 0.11	100.00 ± 13.00	2.98 ± 0.50	5.51 ± 0.94	5.0 ± 0.9	Spheroidal
74.	Kiev Orange	30.50 ± 4.27	31.67 ± 3.48	0.96 ± 0.18	96.30 ± 6.74	3.54 ± 0.50	5.32 ± 0.64	5.0 ± 1	Oblate Spheroidal
75.	Kiev Yellow	30.01 ± 4.20	30.07 ± 3.31	1.00 ± 0.19	99.80 ± 6.99	4.87 ± 0.50	5.65 ± 0.68	6.0 ± 0.78	Spheroidal
76.	Kunchit	28.29 ± 3.96	29.86 ± 3.28	0.95 ± 0.18	94.73 ± 6.63	3.62 ± 0.51	5.51 ± 0.66	5.0 ± 0.65	Oblate Spheroidal
77.	Kundan	27.94 ± 4.19	28.65 ± 3.15	0.98 ± 0.11	97.52 ± 12.68	2.98 ± 0.50	13.42 ± 2.28	6.0 ± 1.08	Oblate Spheroidal
78.	Liliput	29.84 ± 4.48	27.14 ± 2.99	1.10 ± 0.12	109.96 ± 14.30	2.84 ± 0.50	5.49 ± 0.93	5.0 ± 0.65	Prolate Spheroidal
79.	Magenta	30.14 ± 4.52	29.32 ± 3.23	1.03 ± 0.11	102.77 ± 13.36	3.07 ± 0.50	5.73 ± 0.98	4.0 ± 0.52	Prolate Spheroidal
80.	Maghi White	28.18 ± 3.95	28.59 ± 3.14	0.99 ± 0.19	98.57 ± 6.90	3.13 ± 0.44	5.81 ± 0.70	5.0 ± 0.65	Oblate Spheroidal
81.	Mahatma Gandhi	29.00 ± 3.19	29.54 ± 3.25	0.98 ± 0.11	98.14 ± 14.72	3.19 ± 0.50	5.72 ± 0.86	4.0 ± 0.52	Oblate Spheroidal
82.	Mauve Sarah	30.75 ± 4.61	29.15 ± 3.21	1.05 ± 0.12	105.49 ± 13.71	3.96 ± 0.50	4.98 ± 0.85	6.0 ± 0.78	Prolate Spheroidal
83.	Mini Jessie	32.39 ± 4.86	30.74 ± 3.39	1.05 ± 0.12	105.34 ± 13.69	3.76 ± 0.50	5.07 ± 0.86	4.0 ± 0.52	Prolate Spheroidal
84.	Mother Teresa	31.13 ± 4.67	30.28 ± 3.33	1.03 ± 0.11	102.79 ± 13.36	2.27 ± 0.50	6.02 ± 1.02	4.0 ± 0.52	Prolate Spheroidal
85.	Mountaineer	29.76 ± 4.46	28.74 ± 3.16	1.04 ± 0.11	103.54 ± 13.46	3.76 ± 0.50	6.05 ± 1.03	5.0 ± 0.9	Prolate Spheroidal
86.	Navy pride	29.34 ± 1.47	30.15 ± 3.32	0.97 ± 0.03	97.00 ± 2.91	3.26 ± 0.50	4.91 ± 0.64	4.0 ± 0.52	Oblate Spheroidal
87.	NBRI little orange	33.09 ± 4.63	32.71 ± 3.60	1.01 ± 0.19	101.16 ± 7.08	2.36 ± 0.33	6.21 ± 0.75	5.0 ± 0	Prolate Spheroidal
88.	NBRI little pink	30.09 ± 4.21	30.42 ± 3.35	0.99 ± 0.19	98.92 ± 6.92	2.84 ± 0.40	6.64 ± 0.80	4.0 ± 0.72	Oblate Spheroidal
89.	NBRI yellow delight	31.22 ± 4.68	29.74 ± 3.27	1.05 ± 0.12	104.98 ± 13.65	3.72 ± 0.50	6.62 ± 1.13	4.0 ± 0.72	Prolate Spheroidal
90.	OPCH double red	28.66 ± 4.01	29.16 ± 3.21	0.98 ± 0.19	98.29 ± 6.88	3.22 ± 0.45	5.04 ± 0.60	4.0 ± 0.72	Oblate Spheroidal
91.	OPCH 12-2-1213	31.03 ± 4.34	30.36 ± 3.34	1.02 ± 0.19	102.20 ± 7.15	2.57 ± 0.36	5.49 ± 0.66	4.0 ± 0.52	Prolate Spheroidal
92.	OPCH 12-7-1112	30.57 ± 4.28	30.16 ± 3.32	1.01 ± 0.19	101.37 ± 7.10	2.80 ± 0.39	6.10 ± 0.73	5.0 ± 0.9	Prolate Spheroidal
93.	OPCH 13-2	30.07 ± 4.21	30.51 ± 3.36	0.99 ± 0.19	98.55 ± 6.90	3.60 ± 0.50	6.99 ± 0.84	5.0 ± 0.9	Oblate Spheroidal
94.	OPCH 27-1	29.58 ± 4.14	29.72 ± 3.27	1.00 ± 0.19	99.54 ± 6.97	2.27 ± 0.32	4.90 ± 0.59	5.0 ± 0.65	Oblate Spheroidal
95.	OPCH double yellow	31.00 ± 4.34	30.14 ± 3.32	1.03 ± 0.20	102.84 ± 7.20	2.52 ± 0.35	4.82 ± 0.58	5.0 ± 0.9	Prolate Spheroidal
96.	Punjab Shyamli	27.22 ± 3.81	29.99 ± 3.30	0.91 ± 0.17	90.76 ± 6.35	3.46 ± 0.48	5.77 ± 0.69	4.0 ± 0.72	Oblate Spheroidal

Table 1 (continued)

	Chrysanthemum cultivars	Polar length	Equatorial diameter	P/E ratio	Form index	Exine thickness	Spine length	Spine rows between colpi	Shape in equatorial view
97.	PAU-107	30.02 ± 4.50	28.62 ± 3.15	1.05 ± 0.12	104.89 ± 13.64	4.09 ± 0.50	5.01 ± 0.85	5.0 ± 0	Prolate Spheroidal
98.	PAU-147	31.37 ± 4.39	28.76 ± 3.16	1.09 ± 0.21	109.06 ± 7.63	3.59 ± 0.50	5.76 ± 0.69	6.0 ± 1.08	Prolate Spheroidal
99.	PAU-35	29.95 ± 4.49	29.48 ± 5.01	1.02 ± 0.15	101.60 ± 15.24	2.56 ± 0.50	5.46 ± 0.82	5.0 ± 0.9	Prolate Spheroidal
100.	PAU-55	32.07 ± 4.81	29.32 ± 3.23	1.09 ± 0.12	109.39 ± 14.22	2.40 ± 0.50	5.84 ± 0.99	5.0 ± 0.65	Prolate Spheroidal
101.	PAU-58	29.24 ± 4.39	29.52 ± 3.25	0.99 ± 0.11	99.04 ± 12.88	2.78 ± 0.50	6.51 ± 1.11	6.0 ± 1.08	Oblate Spheroidal
102.	PAU-66-2	30.04 ± 4.51	30.36 ± 3.34	0.99 ± 0.11	98.95 ± 12.86	3.72 ± 0.50	5.46 ± 0.93	5.0 ± 0	Oblate Spheroidal
103.	PAU-D-11	30.13 ± 4.22	30.96 ± 3.41	0.97 ± 0.18	97.30 ± 6.81	2.33 ± 0.33	8.71 ± 1.05	5.0 ± 0.9	Oblate Spheroidal
104.	Pink Cloud	30.13 ± 4.52	29.45 ± 3.24	1.02 ± 0.11	102.31 ± 13.30	2.08 ± 0.00	5.38 ± 0.91	4.0 ± 0.72	Prolate Spheroidal
105.	Preet Shringar	30.34 ± 4.55	29.43 ± 3.24	1.03 ± 0.11	103.10 ± 13.40	3.80 ± 0.00	4.69 ± 0.80	4.0 ± 0.64	Prolate Spheroidal
106.	Punjab Gold	43.60 ± 6.10	44.08 ± 4.85	0.99 ± 0.19	98.91 ± 6.92	3.27 ± 0.46	5.81 ± 0.70	5.0 ± 0.9	Oblate Spheroidal
107.	Purple Quill	30.66 ± 4.60	31.41 ± 3.46	0.98 ± 0.11	97.62 ± 12.69	3.48 ± 0.00	6.59 ± 1.12	6.0 ± 1.08	Oblate Spheroidal
108.	Pusa Aditya	29.21 ± 4.38	27.49 ± 3.03	1.06 ± 0.12	106.25 ± 13.81	3.48 ± 0.00	5.26 ± 0.89	5.0 ± 0.9	Prolate Spheroidal
109.	Pusa Centenary	28.49 ± 1.42	28.84 ± 3.17	0.99 ± 0.03	99.00 ± 2.97	3.48 ± 0.00	5.48 ± 0.71	6.0 ± 1.08	Oblate Spheroidal
110.	Pusa Chitraksha	26.90 ± 4.03	27.62 ± 3.04	0.97 ± 0.11	97.40 ± 12.66	3.48 ± 0.00	4.91 ± 0.83	5.0 ± 0.9	Oblate Spheroidal
111.	Pusa Guldasta	27.28 ± 4.09	28.95 ± 3.19	0.94 ± 0.10	94.23 ± 12.25	3.48 ± 0.00	5.51 ± 0.94	5.0 ± 0.9	Oblate Spheroidal
112.	Pusa Kesari	30.97 ± 4.34	28.07 ± 3.09	1.10 ± 0.21	110.33 ± 7.72	2.20 ± 0.00	5.14 ± 0.62	5.0 ± 0	Prolate Spheroidal
113.	Pusa Sweta	27.28 ± 4.09	27.29 ± 3.00	1.00 ± 0.11	99.96 ± 12.99	2.73 ± 0.00	4.93 ± 0.84	5.0 ± 0.9	Spheroidal
114.	Pusa Sona	29.21 ± 4.38	30.80 ± 4.63	0.95 ± 0.10	94.81 ± 7.58	2.73 ± 0.00	4.42 ± 0.49	5.0 ± 0	Oblate Spheroidal
115.	Ravi	30.74 ± 4.61	29.90 ± 3.29	1.03 ± 0.11	102.83 ± 13.37	2.05 ± 0.00	5.88 ± 1.00	5.0 ± 0	Prolate Spheroidal
116.	Ravikiran	29.49 ± 4.42	28.91 ± 3.18	1.02 ± 0.11	102.03 ± 13.26	3.66 ± 0.00	5.92 ± 1.01	4.0 ± 0	Prolate Spheroidal
117.	Red Gold	29.80 ± 3.28	30.03 ± 5.11	0.99 ± 0.12	99.22 ± 14.88	4.98 ± 0.50	6.40 ± 0.51	4.0 ± 0	Oblate Spheroidal
118.	River City	29.35 ± 4.40	30.37 ± 3.34	0.97 ± 0.11	96.64 ± 12.56	3.30 ± 0.50	6.12 ± 1.04	5.0 ± 0	Oblate Spheroidal
119.	Royal purple	28.23 ± 4.23	27.89 ± 3.07	1.01 ± 0.11	101.22 ± 13.16	2.62 ± 0.50	5.18 ± 0.88	5.0 ± 0	Prolate Spheroidal
120.	Sadwin yellow	30.00 ± 4.50	30.84 ± 3.39	0.97 ± 0.11	97.28 ± 12.65	4.76 ± 0.50	6.01 ± 1.02	4.0 ± 0	Oblate Spheroidal
121.	Salmon	30.50 ± 4.57	30.63 ± 3.37	1.00 ± 0.11	99.57 ± 12.94	3.40 ± 0.50	7.03 ± 1.19	5.0 ± 0	Oblate Spheroidal
122.	Seema	32.08 ± 4.81	31.90 ± 3.51	1.01 ± 0.11	100.55 ± 13.07	2.64 ± 0.50	5.69 ± 0.97	6.0 ± 0	Prolate Spheroidal
123.	Sensation	30.82 ± 4.62	30.41 ± 3.35	1.01 ± 0.11	101.35 ± 13.18	2.41 ± 0.50	5.65 ± 0.96	5.0 ± 0.9	Prolate Spheroidal
124.	Silk Brocade	31.08 ± 4.66	30.21 ± 3.93	1.00 ± 0.15	100.08 ± 8.23	2.75 ± 0.50	5.27 ± 0.79	5.0 ± 0.0	Spheroidal
125.	Texas Gold	31.19 ± 4.68	29.29 ± 3.22	1.07 ± 0.12	106.47 ± 13.84	3.05 ± 0.50	6.40 ± 1.09	5.0 ± 0.9	Prolate Spheroidal
126.	Thai Chen Queen	31.24 ± 1.56	29.57 ± 3.29	1.06 ± 0.03	106.00 ± 3.12	2.66 ± 0.56	5.58 ± 0.84	5.0 ± 0.0	Prolate Spheroidal
127.	TQP—06	28.84 ± 3.75	30.21 ± 3.93	0.96 ± 0.00	95.45 ± 0.00	4.04 ± 0.50	5.44 ± 0.82	5.0 ± 0.0	Oblate Spheroidal
128.	Vanity Pink	27.20 ± 4.08	30.07 ± 3.31	0.90 ± 0.10	90.47 ± 11.76	3.55 ± 0.50	5.66 ± 0.96	4.0 ± 0.0	Oblate Spheroidal
129.	Veena	29.66 ± 4.45	30.74 ± 3.38	0.96 ± 0.11	96.49 ± 12.55	2.73 ± 0.50	5.03 ± 0.86	4.0 ± 0.0	Oblate Spheroidal
130.	Vijay	31.52 ± 4.73	31.38 ± 3.45	1.00 ± 0.11	100.47 ± 13.06	4.00 ± 0.50	6.51 ± 1.11	5.0 ± 0.0	Spheroidal

Table 1 (continued)

	Chrysanthemum cultivars	Polar length	Equatorial diameter	P/E ratio	Form index	Exine thickness	Spine length	Spine rows between colpi	Shape in equatorial view
131.	Vijay Kiran	30.88 ± 3.40	30.93 ± 5.26	1.00 ± 0.12	99.84 ± 14.98	4.93 ± 0.50	5.89 ± 0.47	5.0 ± 0.0	Spheroidal
132.	HYDC pink	29.99 ± 3.30	29.82 ± 5.07	1.01 ± 0.12	100.57 ± 15.09	3.06 ± 0.50	6.46 ± 0.52	4.0 ± 0.0	Prolate Spheroidal
133.	Winter Queen	30.64 ± 3.37	31.91 ± 5.42	0.96 ± 0.12	96.01 ± 14.40	3.06 ± 0.50	5.51 ± 0.44	5.0 ± 0.0	Oblate Spheroidal
134.	Yellow Delight	30.33 ± 3.34	31.59 ± 5.37	0.96 ± 0.12	96.00 ± 14.40	4.03 ± 0.40	5.04 ± 0.40	6.0 ± 0.0	Oblate Spheroidal

Multivariate analysis of *Asteraceae* family members

The pollen morphological data obtained from the PalDat database for various Asteraceae members were compared to the data obtained through SEM in the present study. The methodology for multivariate analysis was based on study by Marinho et al. (2018) where principal component analysis (PCA) was conducted on the pollen data to validate if species could be classified. The variance was extracted using the variance/covariance (centered) matrix and coordinates in a distance-based scatter plot. The results were plotted in a two-dimensional graph of the first two principal components. Hierarchical cluster analysis (HCA) was also performed to verify that pollen traits could differentiate and thereby group plants of Asteraceae family members. The HCA dendrogram as created using Ward's method on the squared Euclidean distance measure and linkage method. Statistical analysis was carried out using StatistiXL in MS Excel.

Results

Scanning *electron* microscopic research

In the study of the morphology of chrysanthemum pollen grains, several key features were examined. Data are presented in Table 1, and microscopic (SEM) views of pollen grains revealing several systematic characteristics from different cultivars are represented in Fig. 1. The pollen units exhibited tetrahedral tetrad polarity, showcasing radial symmetry with shapes varying from oblate, prolate-spheroidal, and spheroidal in equatorial view to circular lobate in polar view (Fig. 2). The pollen grains were tricolporate with apertures that were either lacunate or non-lacunate, and the apertural membrane was echinate. A distinctive characteristic of the pollen grains was the presence of spines, predominantly pyramidal-shaped, commonly conical with a broad base and a blunt to sharp apical portion. The number of spine rows between colpi ranged from 3 to 6 in *Chrysanthemum* species, providing a useful character for species differentiation within the genus and highlighting the taxonomic potential of these features.

Exine sculpturing exhibited diverse patterns, including caveate and echinate, with varying spine lengths. The exine surface in some *Chrysanthemum*

Table 2 Description of the pollen morphology of members of Asteraceae family as obtained from PalDat Database

Organism	Pollen shape	Polar length	Equatorial diameter	P/E ratio	Form index	Aperture-number	Aperture type
Artemisia vulgaris	Spheroidal	16–20	16–20	1.14	113.89	3	Colporus
Helenium autumnale	Spheroidal	–	–	–	–	3	Colporus
Inula britannica	Spheroidal	16–20	16–20	1.00	100.00	3	Colporus
Solidago virgaurea	Spheroidal	16–20	21–25	0.89	89.13	3	Colporus
Calendula officinalis	Spheroidal	31–35	31–35	1.00	100.00	3	Colporus
Leucanthemum vulgare	Spheroidal	16–20	21–25	1.00	100.00	3	Colporus
Bellis perennis	Spheroidal	16–20	16–20	1.00	100.00	3	Colporus
Leucanthemopsis alpina	Spheroidal	16–20	16–20	1.00	100.00	3	Colporus
Doronicum columnae	Spheroidal	21–25	21–25	1.00	100.00	3	Colporus
Tanacetum parthenium	Spheroidal	21–25	21–25	1.00	100.00	3	Colporus
Achillea clavennae	Spheroidal	21–25	16–20	1.12	112.20	3	Colporus
Anthemis tinctoria	Spheroidal	16–20	21–25	1.00	100.00	3	Colporus
Bidens pilosa	Spheroidal	–	21–25	1.00	100.00	3	Colporus
Senecio abrotanifolius	Spheroidal	21–25	26–30	1.00	100.00	3	Colporus
Chrysanthemum coronarium	Spheroidal	20.08–27.15	20.2–28.4	1.00	100.00	4	Colporus
Chrysanthemum morifolium—Ravi	Spheroidal	26.13–35.35	26.6–33.2	1.03	103.00	5	Colporus
Chrysanthemum morifolium—Punjab Gold	Spheroidal	37.5–49.7	39.2–48.9	0.99	99.00	6	Colporus
Chrysanthemum morifolium—DFR C1	Spheroidal	25.61–32.59	25.7–34.1	0.97	97.00	7	Colporus
Chrysanthemum morifolium—Dark Red	Spheroidal	25.11– 31.95	27.3–36.2	0.90	90.00	8	Colporus

species appeared granulated, and the intine was thinner than the exine. These species had somewhat aggregated and granulated columella. Exine thickness varied between 2.1 µm and 5 µm among different chrysanthemum cultivars, with Red Gold having the thickest exine and Ravi, Pink Cloud, and DFR-C-6 sharing the thinnest.

The examination of 134 chrysanthemum cultivars revealed a significant range of variation in polar length, spanning from 24.86 to 43.6 µm. The maximum pollen size of 43.60 µm was observed in the Punjab Gold cultivar in polar view, while the minimum size of 24.86 µm was recorded in the IAH Red cultivar. Similarly, in equatorial view, the maximum pollen size of 44.08 µm was observed in Punjab Gold, and the minimum size of 25.99 µm was noted in the Cherabu cultivar. The polar-to-equatorial diameter (P/E) ratio varied among the different cultivars, indicating differences in pollen shape, with ratios ranging from 0.9 to 1.1. All examined chrysanthemum pollen grains exhibited tetrahedral tetrad polarity.

Heteromorphy was also observed among the chrysanthemum cultivars under examination. The photosensitive cultivars, including PAU-55, Bidhan Gold, Bidhan Sweta, Bidhan Sabita, Bidhan Rajat, Bidhan Neeta, and Bidhan Agnishikha, had an average spine length of 5 µm and form indices ranging from 92 to 109. Conversely, the photo-insensitive cultivars, such as Bidhan Tarun, Mother Teresa, Vanity Pink, Autumn Joy, ACC-2, Aparajita, IAH Red, and Royal Purple, featured spine lengths ranging from 5 to 6 µm and exhibited spheroidal shapes that were prolate to oblate in equatorial view. Overall, spine length among the cultivars varied from 4.19 to 13.42 µm. The obtained morphometric data were further analysed for detailed insights.

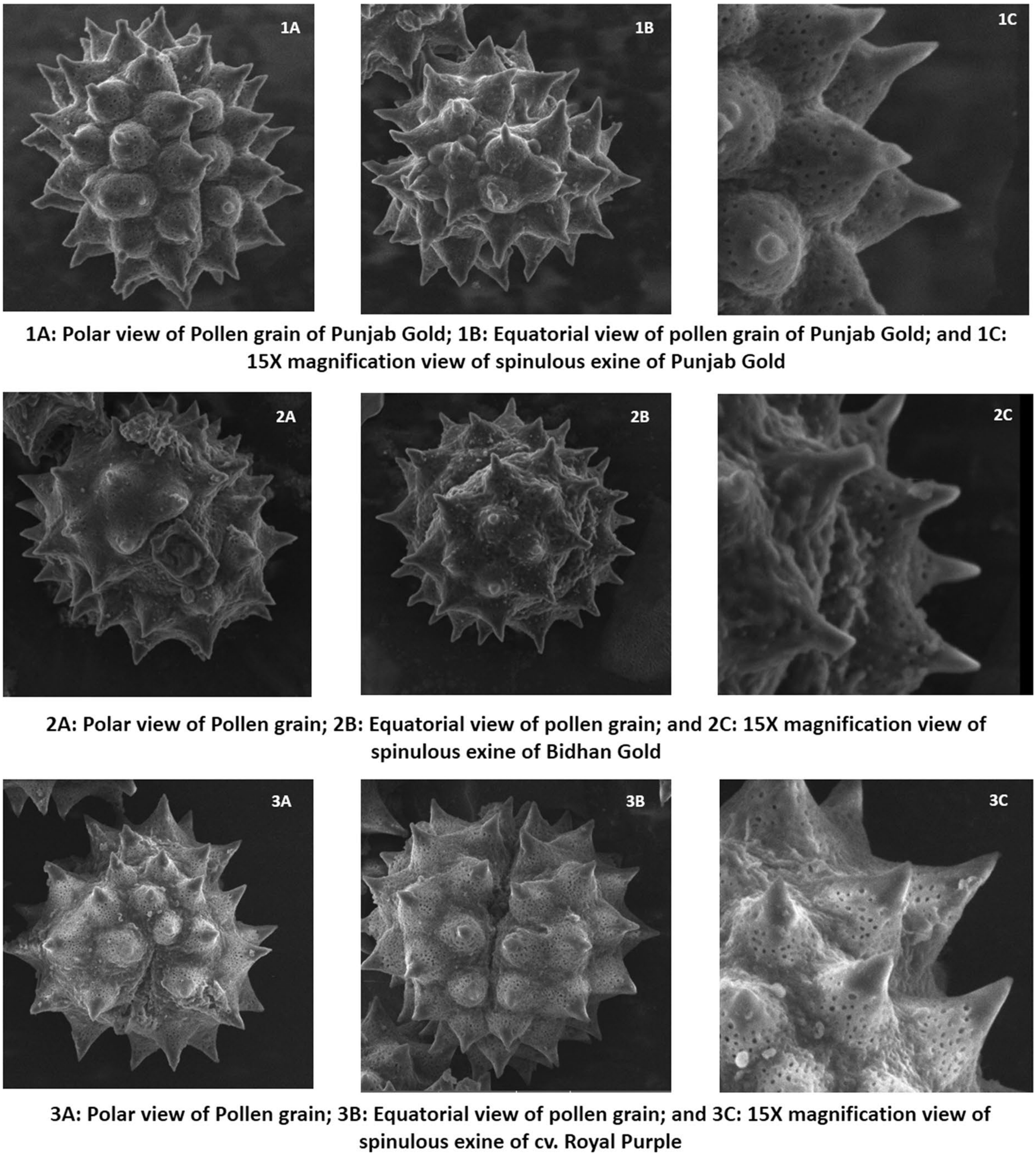

Fig. 1 Scanning electron microscopic-polar view, equatorial view and 15 × magnification view for spinulous exines of pollen grains of various chrysanthemum cultivars

Statistical analysis

For multivariate analysis, hierarchical cluster analysis was performed using StatistiXL, with Euclidean distance as the similarity measure and Ward's linkage as the algorithm. The resulting dendrogram showed that the cultivars were initially divided into two distinct groups, A and B, based on their overall

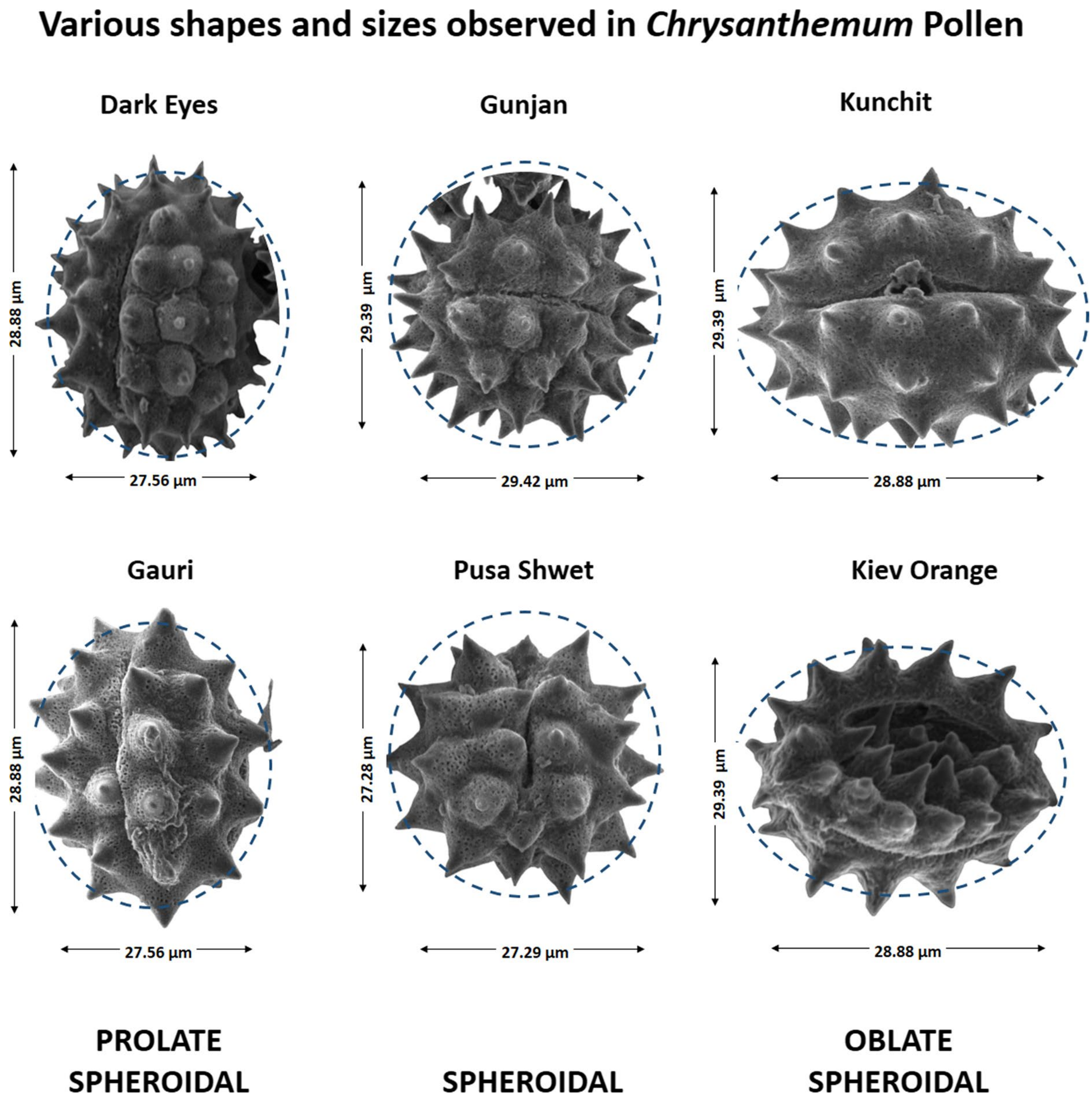

Fig. 2 Morphological differences observed in pollen grain shape and sizes

similarity in characteristics, with a squared Euclidean distance of 3381.234. Each group was further categorized into three clusters (Fig. 3). The chrysanthemum cultivars were primarily partitioned based on the number of spine rows between colpi. Group A exhibited 3–4 spine rows, while group B had 5–6 spine rows between colpi. Cultivars such as Kundan, Bidhan Gold, Vanity Pink, Bidhan Tarun, Liliput, Pusa Century, and PAU-55 formed distinct smaller outgroups at comparatively greater distances. The larger clusters were in close proximity to each other and included the majority of members, with 44 and 45 individual cultivars, respectively.

The differentiation or variation observed in chrysanthemum cultivars could be correlated with geographic and environmental factors (Hao et. al., 2022). PCA was employed to ascertain if the morphological pollen data facilitated the grouping of cultivars. The

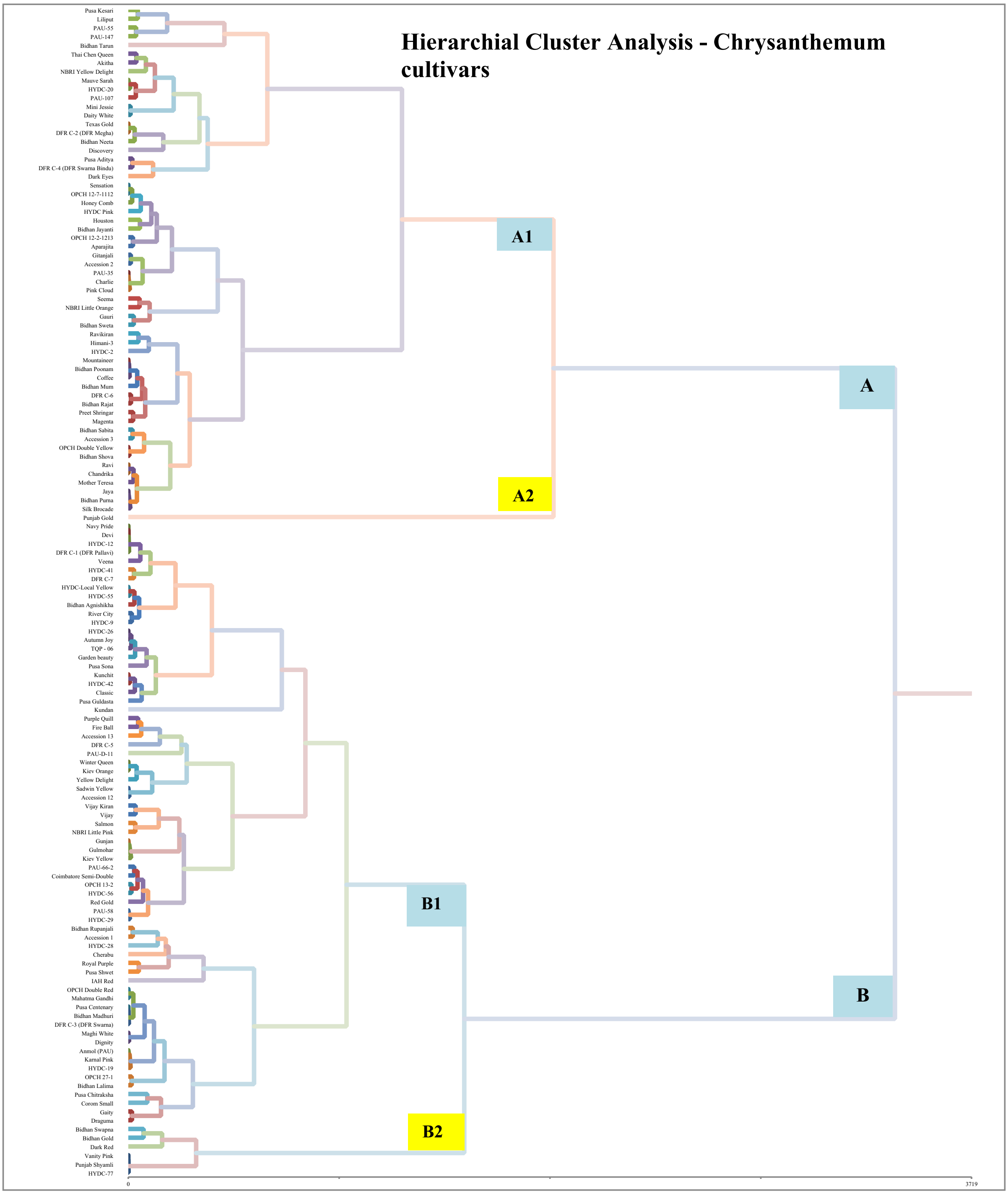

Fig. 3 Dendrogram obtained on Hierarchical Cluster Analysis as the multivariate analysis for 134 chrysanthemum cultivars

first two principal components (PCs) with eigenvalues exceeding one were depicted. These findings were visualized in a two-dimensional plot of the initial and secondary principal components. The

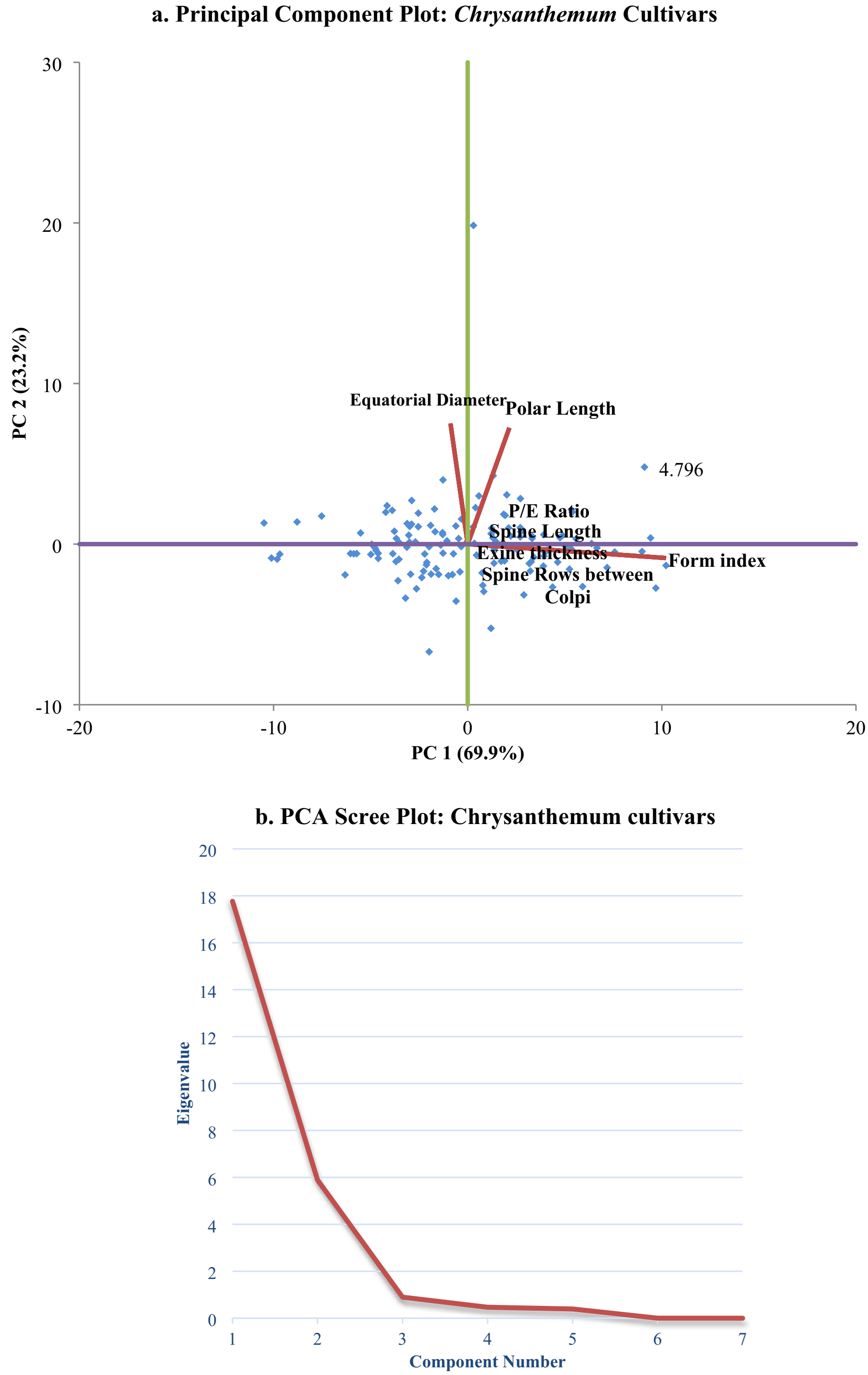

Fig. 4 **a** Scatter plot as a result of Principal Component Analysis (PCA) of chrysanthemum pollen morphological data as multivariate ordination analysis; **b** Scree plot obtained as a result of Principal Component Analysis (PCA) of *Chrysanthemum* cultivars' pollen morphological data as multivariate ordination analysis

eigenvalues, representing the highest character variation, were graphed on a two-dimensional scatter plot using the first two principal component axes (PCA1 and PCA2). Figure 4 shows the same for the pollen morphological data of chrysanthemum cultivars obtained using scanning electron microscopy. Particularly, the first two principal component axes are of significance, collectively explaining 93.084%

of the variance, with the first accounting for 69.911% and the second for 23.174%. According to the biplot, the morphological characters pollen length, form index, and equatorial diameter were the most significant variables.

Database screening and data collection

PalDat provided pollen morphological data for 14 genera in Asteraceae family including *Artemisia vulgaris, Helenium autumnale, Inula britannica, Solidago virgaurea, Calendula officinalis, Leucanthemum vulgare, Bellis perennis, Leucanthemopsis alpine, Doronicum columna, Tanacetum parthenium, Achillea clavennae, Anthemis tinctoria, Bidens pilosa,* and *Senecio abrotanifolius.* Table 2 provides detailed information about the shapes and sizes of pollen from various plant genera in the Asteraceae family. Most species in this family have spherical pollen grains with colporate or tricolporate apertures, showing a high level of similarity in these features. The size of the pollen grains varies, with some species having small grains (10–25 µm) and others having medium-sized grains (26–50 µm). Despite these differences in size, the overall pollen characteristics remain consistent across most species in the Asteraceae family, including *Chrysanthemum*, which have larger pollen grains but share the same general traits.

Multivariate analysis of *Asteraceae* family members

Multivariate analysis methods like PCA and HCA were utilized to study data structure, identify similarities, and detect outliers. Principle component analysis, represented as a visualization approach, while HCA served as an agglomerative algorithm, both widely employed for their efficiency in data analysis (Granato et al. 2018) were used here for the morphometric data obtained through SEM and from PalDat Database. When PCA was carried out for Asteraceae family members for their morphological characters, it was observed that the first principal component (PC1), representing 72.51% of the variance, seems to signify overall size, with larger grains on the right and smaller on the left and second principal component (PC2) with 26.627% variance captured shape differences, with elongated grains at the top and rounder at the bottom (Fig. 5). The vector rows indicated the direction and magnitude of the original morphological variables, according to which the longer arrows like polar length and equatorial diameter correlate positively with PC1, while form index correlate negatively with PC2. It revealed that size and elongation/roundness were the major factors those contributed to the variance. The spread of points indicates diversity, with some cultivars tightly clustered (less diverse) and others like *Solidago virgaurea* more diffuse (more diverse). PC1 played a crucial role, differentiating cultivars by traits like polar length, equatorial diameter, and P/E ratios. Some taxa, including *Artemisia vulgaris* and *Achillea clavennae*, clustered together due to similar pollen size and rounded shape, while *Chrysanthemum morifolium* cultivars showed separation based on elongation and protrusion. PC2, though less dominant, contributed to distinctions in aperture numbers. HCA revealed similarity relationships amongst the Asteraceae members, based on pollen morphological data. Overall, the hierarchy showed morphological relationships from trait similarities and differences among Asteraceae family members- ranging from predominant separation of certain genera to sub-clusters distinguishing even close cultivars (Fig. 6). The dendrogram contained two primary clusters, further subdivided into about five main sub-groupings. Within the sub-clusters, genera like *Bellis perennis, Inula britannica, Leucanthemum alpina* were clustered closely, indicating similarity in floral pollen traits. On the other hand, tighter sub-groupings were emerged of the chrysanthemum cultivars of our interest where *C. morifolium*-Dark Red showing similar traits with *Solidago virgaurea* were clustered together with a squared Euclidean distance of 141.276; *C. morifolium*–Ravi was clustered with *Calendula officinalis* independently with a squared Euclidean distance of 23.719; *C. morifolium*-DFR-C1 exhibiting similar traits with *Senecio abrotanifolius* were placed together in another cluster with a squared Euclidean distance of 13.936 while *C. morifolium*-Punjab Gold cultivar was seen as an out group individually showing characteristics comparatively distinguishing. *Helenium autumnale, Leucanthemum vulgare, Doronicum columnae, Tanacetum parthenium, Anthemis tinctoria, Bidens pilosa,* and *Chrysanthemum coronarium* were observed to cluster together because of their similar P/E ratios displaying *C. coronarium* distinguished from *C. morifolium* in its morphological traits yet similar to the other Asteraceae family

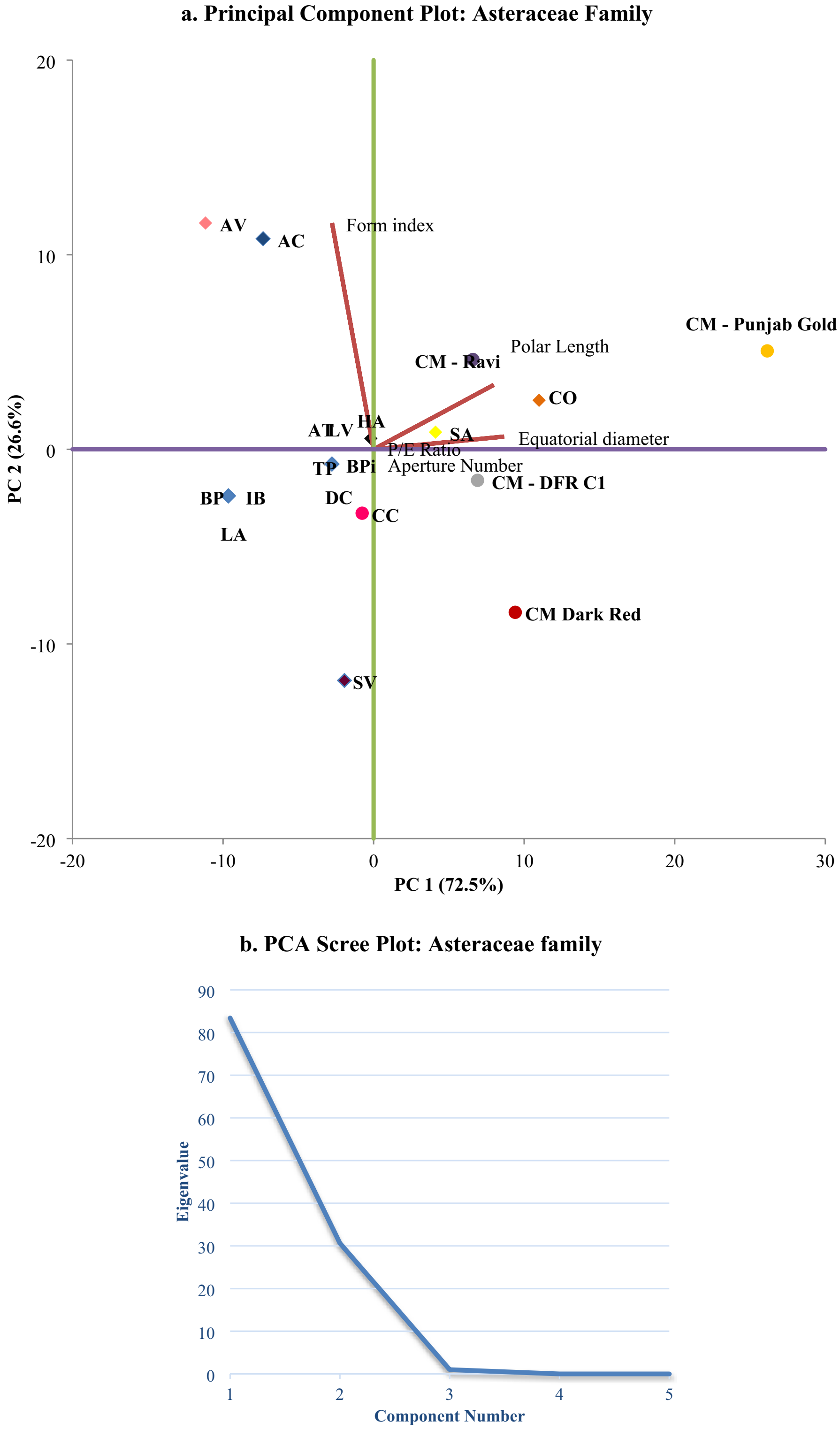

Fig. 5 **a** Scatter plot obtained on Principal Component Analysis for pollen morphological data obtained from PalDat database in comparison with representative chrysanthemum cultivars; **b** Scree Plot obtained on Principal Component Analysis for pollen morphological data obtained from PalDat database in comparison with representative chrysanthemum cultivars

members in the group. The chrysanthemum variants exhibited the greatest similarity, branching off last on the vertical axis. Their late divergence reflects finer-scale morphological variations among the cultivated types. Besides, genera *Artemisia vulgaris* and *Achillea clavennae* sharing a common characteristic of having a higher P/E ratio and, accordingly, a higher form index showed a squared Euclidean distance of 15.369, clustering together and diverging from the rest of the groups earlier. This suggests a more distinct morphology compared to others. Thus, when compared to other Asteraceae family members, morphological variables like P/E ratio, equatorial diameter provide continuous quantitative differences.

As a result, the study of pollen morphological characters in chrysanthemum specifically size, shape, type, spine, ornamentation, and echini features proved to be informative and contributed to a better understanding of their intergeneric relationships. Additionally, the importance of SEM techniques for identifying examined species using palynomorphological traits was further clarified. The foundational information provided by pollen morphological data revealed that the cultivars could be studied for their biodiversity.

Discussion

The study conducted an in-depth analysis of pollen morphology in a diverse collection of chrysanthemum cultivars, alongside comparative assessments with other Asteraceae taxa. Through systematic scanning electron microscopic (SEM), researchers scrutinized various characteristics such as size, shape, ornamentation, and aperture type of pollen grains. Among the remarkable cultivars noted in the study, Punjab Gold was distinguished for its large pollen size, with a maximum polar length of 43.60 µm and an equatorial diameter of 44.08 µm, distinguishing it among the examined cultivars. This cultivar's significant pollen size can further be appreciated for adding to its aesthetic appeal and underscoring its potential for breeding programs aimed at enhancing desirable traits. Wodehouse (1935a, b) reported pollen diameter range from 24.2 to 34.2 µm in *Chrysanthemum* species which gives support to the present findings. Bidhan Gold, with its average spine length of 5 µm and a form index ranging from 92 to 109, was recognized for being distinct in ornamental traits, offering understandings in genetic diversity and selection criteria for breeding purposes. Also, Bidhan Sweta, was observed to exhibit distinct spine lengths and shapes, contributing to its visual appeal and differentiation, thereby serving as a valuable genetic resource for further research and cultivation. Bidhan Tarun, with its unique spheroidal and prolate pollen shapes, was considered captivating for studying pollen morphology and its implications for plant evolution and adaptation. Vanity Pink, known for its aesthetic appeal, was found to showcase distinctive pollen characteristics, including spine lengths ranging from 5 to 6 µm, and tricolporate apertures, emphasizing its potential as a genetic source for breeding programs aimed at developing novel cultivars with enhanced ornamental traits. The overall spine length among the chrysanthemum cultivars ranged from 4.19 to 13.42 µm, aligning with Wodehouse's (1935a; b) study, which reported spine lengths of 2.3 to 8.1 µm in *Chrysanthemum* species. The morphometric data obtained were further analyzed, revealing exine thickness ranging from 2.1 to 5 µm across different cultivars. These findings are consistent with Huang's (1972) study, which observed exine thickness of 2.0–2.5 µm in four *Chrysanthemum* species from Taiwan, and with Meo and Khan's (2006) study, which reported exine thickness ranging from 4.1 to 7.9 µm in chrysanthemum cultivars from Pakistan. The cultivars examined in this study, among others, are noted for enhancing the diversity of chrysanthemum cultivars. The pollen grains exhibited tricolporate features with apertures that were either lacunate or non-lacunate, and the apertural membrane displayed echinate characteristics. These findings are consistent with those reported for medicinal plant species in the Asteraceae (Compositae) family, as documented by Khan et al. (2012) in their study of the flora of Kaghan Valley. Within *Chrysanthemum* species, the number of spine rows between colpi varied from 3 to 6, providing valuable traits for distinguishing species within the genus and highlighting their taxonomic significance. This variation aligns with the study by Meo and Khan (2006), which examined the pollen morphology of 7 *Chrysanthemum* species (Compositae–Anthemideae) from Pakistan. The observed variation in spine row numbers demonstrates a correlation with taxonomic classification, enhancing the potential for species segregation within the genus. Moreover, the detailed

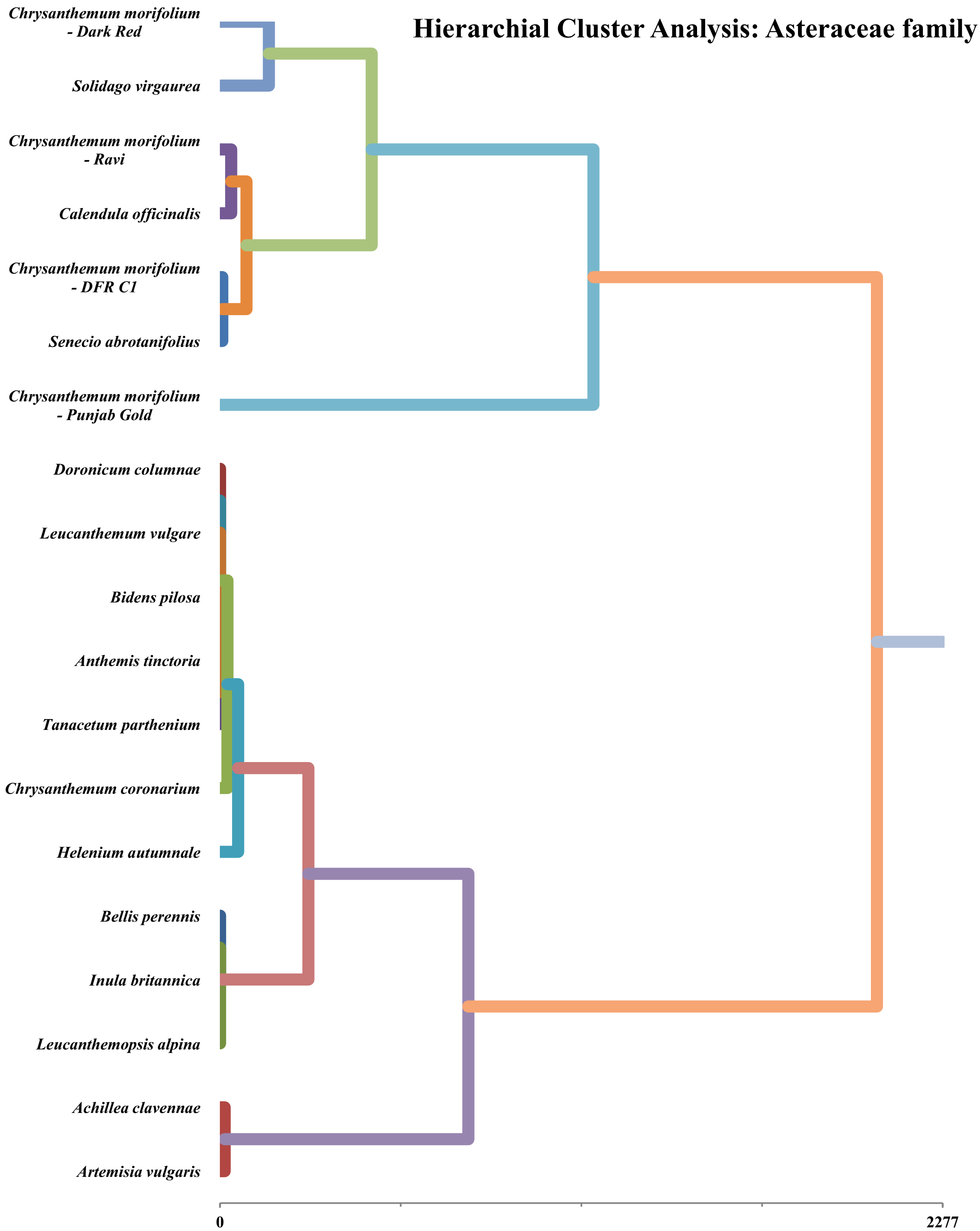

Fig. 6 Dendrogram representing Cluster Analysis of selected Asteraceae members for morphological characters of pollen grains

examination of pollen morphology in these cultivars could be acknowledged for contributing to their classification and identification in taxonomy, aiding researchers in precisely categorizing *Chrysanthemum* species based on their unique pollen characteristics. The differentiation or variation observed in chrysanthemum cultivars could be correlated with geographic and environmental factors (Hao et. al., 2022). For further analysis of the pollen morphological data, multivariate statistical techniques like principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used. PCA helped reduce the dimensionality of the data and identify the key sources of variation, with the first two principal components capturing over 93% of the variance in *C. morifolium* cultivars, driven by traits like pollen length, form index, and equatorial diameter. For Asteraceae genera, PCA revealed overall size and shape as the major axes of variation. HCA was then used to explore relationships and groupings based on morphological similarities. It divided *C. morifolium* cultivars into clusters based on spine row numbers, while for Asteraceae genera, the dendrogram showed distinct sub clusters of closely related genera sharing pollen traits. Especially, *C. morifolium* cultivars formed tighter sub clusters, indicating finer-scale variations among them. These multivariate analyses facilitated visualizing pollen diversity, identifying key contributing traits, and understanding the taxonomic implications within and across the studied groups. By examining the variation in pollen traits among cultivars, researchers could infer evolutionary relationships, genetic divergence, and adaptation to different environments. Thus, when compared to other Asteraceae genera, shared characteristics like spheroidal pollen grains with colporate or tricolporate apertures were observed, indicating common evolutionary origins. Additionally, hierarchical cluster analysis and comparative analysis with other members of the Asteraceae family revealed distinct groupings based on morphological traits, allowing for broader identifications into cultivar relationships, evolutionary patterns, and phylogenetic relationships within the family. Further, new chrysanthemum cultivars with enhanced ornamental or agronomic properties can be developed by using breeding strategies informed by the identification of various pollen traits and their link with desired phenotypic attributes. Ultimately, results revealed significant diversity in pollen size, shape, ornamentation, and aperture type between cultivars, which contributes to their differentiation, clustering of genera within the family, and genetic diversity evaluation.

The study on morphological, cytological, and physiological features of ripe pollen by Pacini and Franchi, (2020) disclosed correlations among the traits (shape, size, and dispersal units), the female counterpart, and environmental factors. Despite their shared reproductive function, diverse morphological and physiological characteristics were observed during dispersal. Analyzing factors such as pollen grain types, shape, size, and dispersal units contributed to a slight understanding of complex interactions influencing plant reproduction. The importance of studying the diversity in plant shapes and forms was elaborated by Jardine et al. (2022); it was suggested that understanding morphological differences among plants is crucial for biodiversity research, complementing traditional diversity metrics and the differences were distributed across space and time, zooming in on specific plant parts like pollen and flower will be more useful. A further thorough understanding of the evolutionary processes influencing pollen diversity may be possible with additional research into the genetic basis and molecular mechanisms behind the observed variations in pollen traits. The adaptive significance of pollen features and their role in plant adaption and dispersion patterns may be understood by studying potential relationships between pollen morphology and environmental factors or geographic distribution. The knowledge of phylogenetic relationships and the evolution within the Asteraceae family may be improved by widening the study by including a wider range of Asteraceae genera and species.

Conclusion

An analysis of pollen morphology in 134 chrysanthemum flower types was conducted using scanning electron microscopy (SEM). The detailed examination of pollen characteristics, such as shape, spine length, ornamentation, and aperture type, underscores SEM's importance in species identification. Among *C. morifolium* cultivars, Punjab Gold, IAH Red, and Kundan were notably distinct due to their unique pollen traits. The formation of different clusters suggests that environmental factors contribute to the observed biodiversity. Comparative analysis of Asteraceae members from the PalDat database highlights pollen morphology as a reliable classification tool. Understanding pollen morphology is crucial for insights into plant reproduction, biodiversity, ecological interactions, and environmental adaptation, with practical applications in agriculture and horticulture. For a comprehensive taxonomy of the *Chrysanthemum* genus, future research should include morphological, karyological, and molecular analyses.

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Declarations

Conflict of interest All the authors listed have agreed with the submission and declared no conflict of interest.

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