

## PALYNOLOGICAL INVESTIGATION OF THE TRIBE HELIANTHEAE (ASTERACEAE) IN PAKISTAN AND ITS TAXONOMIC IMPLICATIONS

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Received 2023.07.03; accepted for publication 2023.12.03

Perveen, A. & Rauf, T. 2023.12.30: Palynological investigation of the tribe Heliantheae (Asteraceae) in Pakistan and its taxonomic implications. -*Iran. J. Bot.* 29(2): 132-146. Tehran.

Pollen morphology of eight genera and 11 species belonging to the tribe Heliantheae -Asteraceae viz., *Blainvillea* Cass., *Eclipta* L., *Helianthus* L., *Parthenium* L., *Sphagneticola* O. Hoffm., *Verbesina* Cav., *Xanthium* L. and *Zinnia* L. have been examined under the Light Microscope (LM) and Scanning Electron Microscope (SEM). Pollen grains are generally radially symmetrical, isopolar, mostly prolate-spheroidal to prolate rarely oblate-spheroidal or spheroidal. Tectum is usually echinate except in *Xanthium* and *Parthenium* which have micro echinate (spinulose) tectum. However, significant variation in qualitative and quantitative pollen morphological characters has been observed in pollen shape, polar length and diameter of equatorial axis (13-25  $\mu\text{m}$ ), exine thickness 1.31-3  $\mu\text{m}$  and exine ornamentation micro echinate-echinate, spine length >1 to 4  $\mu\text{m}$ , and tectum in between spines such as, punctate, to sub-psilate, rarely rugulate or granulate. Based on the exine ornamentation and cluster analysis two distinct pollen types are recognized viz., Pollen type-I *Helianthus* type (based on echinate), and pollen type-II *Xanthium* type (Micro echinate). The results of this study have shown that palynological characters are significantly helpful at specific levels.

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**Keywords:** Asteraceae; Pollen morphology; Pakistan; SEM; Tribe Heliantheae

تحقیقات گرده‌شناسی روی قبیله **(Asteraceae) Heliantheae** در پاکستان و کاربرد آن در رده‌بندی

انجم پروین: استاد، مرکز حفاظت گیاهان، دانشگاه کراچی، کراچی، پاکستان

طابه رؤف: دانشجوی دکتری، مرکز حفاظت گیاهان، دانشگاه کراچی، کراچی، پاکستان

مورفولوژی دانه گرده در ۸ جنس و یازده گونه از قبیله Heliantheae. خانواده آفتابگردان از پاکستان، شامل جنس‌های *Blainvillea* Cass., *Eclipta* L., *Helianthus* L., *Parthenium* L., *Sphagneticola* O. Hoffm., *Verbesina* Cav., *Xanthium* L. و *Zinnia* L. با استفاده از میکروسکوپ نوری و الکترونی، بررسی گردید. دانه‌های گرده معمولاً متقارن، جور قطب و عموماً prolate-spheroidal تا prolate و به‌ندرت oblate-spheroidal و یا spheroidal هستند. پوسته معمولاً خاردار است، بجز در جنس‌های *Xanthium* و *Parthenium* دارای خارهای کوتاه (تیغ دار) هستند. در هر حال تفاوت‌های کیفی و کمی زیادی در مشخصات مورفولوژی دانه‌های گرده مشاهده گردید. از جمله شکل دانه گرده، طول محور قطبی، عرض محور استوایی (۱۳-۲۵ میکرون)، اندازه پوسته ۳-۱/۳۱ میکرون و تزیینات سطح دانه گرده از خاردار تا با خارهای ریز، طول خار کوتاه‌تر از یک تا چهار میکرون و پوسته در فواصل بین خارها منقوط تا تقریباً صاف و یا چروکیده و یا دانه‌دار. آنالیز خوشه‌ای بر اساس تزیینات سطح دانه گرده، دو نوع دانه گرده را معرفی می‌نماید؛ دانه‌های گرده نوع ۱ و یا نوع *Helianthus* (با تزیینات خاردار) و نوع ۲ یا نوع

Xanthium (با تریبنات با خارهای کوتاه یا تیغ دار). نتایج این مطالعه نشان می‌دهد که خصوصیات دانه گرده برای شناسایی آرایه‌ها در سطح گونه مفید هستند.

## INTRODUCTION

Tribe Heliantheae was established by Cassini (1819) which included sunflowers with primarily radiate capitula, paleaceous receptacles, opposite leaves, and yellow corollas. The circumscription of tribe Heliantheae had been a matter of controversy among different workers and it was accepted variously based on the broader (s. lat.) or narrow concept (s. str.), (Cassini 1829; Bentham & Hooker 1873; Robinson 1981; Karis 1993; Bremer 1996) until the phylogenetic studies based on molecular data were conducted by Baldwin, & al. 2002; Panero & Funk 2002; Panero 2007; Baldwin 2009). These molecular studies clearly proved that Heliantheae could no longer be recognized in its traditional broader sense –rather it has to be recognized in its strict sense as a monophyletic taxon (s. str.) along with 13 other tribes such as Tageteae, Coreopsideae, Millereae, which were previously recognized under the broad canopy of Heliantheae (s.lat.). This assemblage of 13 tribes is now commonly known as the Heliantheae alliance (Panero 2005; Baldwin 2009).

Heliantheae (s. str.) is a large tribe of the family Asteraceae including about 300 genera, and more than 3300 species, distributed mostly in the new world's subtropical, tropical, and warm-temperate regions (Barkley 2006). In Pakistan, the family Asteraceae is represented by 188 genera and ca. 770 species, distributed in 19 tribes including Heliantheae (Qaiser & Abid, 2021). Tribe Heliantheae is represented by 12 genera and 17 species from Pakistan; of which five genera are cultivated (Perveen & Qaiser 2021).

This tribe is economically important for one of the most important oil-yielding plants –*Helianthus annuus*, horticulturally important plants like *Rudbeckia*, *Sphagneticola*, *Verbesina*, and *Zinnia*, food as *Helianthus tuberosus* and herbal drugs such as *Xanthium strumarium* (Fouche & al., 2008), *Eclipta prostrata*, and *Zinnia elegans* (Mandak 1976; Smoljaninova & Vassilczenko 1999).

Pollen morphology of the tribe Heliantheae has been examined by several workers such as Wodehouse (1928 & 1935); Erdtman (1952); Skvarla & Larson (1965); & Salamah & al. (2019). We studied the unexplored pollen ultrastructure of the tribe Heliantheae taxa in Pakistan using scanning electron microscopy methods and cluster analysis.

Coutinho & al. (2016) studied pollen morphology and exine ultrastructure of *Eclipta prostrata* under transmission electron microscopy and scanning

electron microscopy. A few species are reported on the pollen morphology of the tribe Heliantheae from Pakistan, such as Perveen (1999) who studied the pollen of *Blavillea acmella* and *Eclipta prostrata*. Zafar & al. (2007) examined the pollen of *Eclipta prostrata*, *Parthenium hysterophorus*, *Sphagneticola trilobata*, and *Zinnia elegans* under the light microscope only. Pollen morphology of a few species of the tribe Heliantheae by SEM and light microscope was also studied by Mazari & al. 2012; Usma & al. 2022 and Bahadur & al. 2022. The palynological studies of the tribe Heliantheae with their taxonomic implications have not yet been extensively studied in Pakistan.

The current study was undertaken with the following objectives, (1) to investigate and evaluate the pollen morphology of available species of tribe Heliantheae from Pakistan, (2) to identify palynological features for the taxonomic delimitation of genera and species using both light and scanning electron microscopic techniques and (3) also to compare the relationship of genera and species within the tribe Heliantheae based on pollen data through cluster analysis.

## MATERIALS AND METHODS

Polleniferous material of eleven species of Heliantheae belonging to *Blainvillea* Cass., *Eclipta* L., *Helianthus* L., *Parthenium* L., *Sphagneticola* O. Hoffm., *Verbesina* Cav., *Xanthium* L. and *Zinnia* was collected from the field and herbarium specimens (KUH). Voucher specimens were deposited in the Karachi University S.I. Ali Herbarium (KUH) Centre for Plant Conservation, University of Karachi (Table 1).

Pollen slides were prepared according to the standard technique of Erdtman (1960). Pollen was examined under a light microscope Nikon-type-102 (E100, 1.08), and 20-30 pollen grains for each species were measured. The following parameters were recorded: polar length (P), equatorial diameter (E), exine thickness, colpus length, spine length, exine ornamentation, apocolpium, and mesocolpium (Table 2). For scanning electron microscopy, one part of the sample was suspended in a drop of water and directly transferred with a fine pipette to a metallic stub covered by double-sided scotch tape. Dried stubs were coated with gold in a vacuum chamber Joel (JFC-1500), the coating was restricted to 150 Å for 10 minutes and pollen was examined by Joel (JSM-

6380A) Scanning Electron Microscope at Centralized Science Laboratory (CSL), University of Karachi.

A Hierarchical cluster analysis of 11 species of the tribe Heliantheae was carried out. The numerical analysis was performed using the Euclidean distance and Ward's method for a group linkage method with the computer program IBM SPSS Statistics 20, for the finding of the similarities of the species of the tribe. A total of 18 quantitative and qualitative pollen

characters were coded as 1 and 0 to show the presence or absence of the character respectively while average values were used for the quantitative characters. These characters and their state are presented in Tables 3 and 4.

The terminology used in the present paper was according to Erdtman (1952), Walker & Doyle (1975), Faegri & Iverson (1989), and Punt & al., (2007).

Table 1. Voucher specimens of the studied taxa.

S. No.	Name of Taxa	Collection Data
1	<i>Blainvillea acmella</i> (L.) Philip.	Pakistan, Hamdard University, 2020-10-07, Taba Rauf, 95726 KUH
2	<i>Eclipta prostrata</i> L.	Pakistan, Karachi University, Dept. Zoology, 2019-07-11, Taba Rauf & Rabia Akhlaq, 95838 KUH.
3	<i>Helianthus annuus</i> L.	Pakistan, Karachi University campus Pakistan Institute of Pharmaceutical Sciences, 2019-07-11, Taba Rauf & Rabia Akhlaq, 95832 KUH.
4	<i>Helianthus tuberosus</i> L.	Pakistan, Neelum Valley Azad Kashmir, 2018-09-15, Shabir Ijaz, 95297 KUH
5	<i>Parthenium hysterophorus</i> L.	Pakistan, Nasir jump, Korangi, Karachi, 2019-12-10, Shabir Ijaz, 95597 KUH
6	<i>Sphagneticola calendulacea</i> (L.) Pruski.	Pakistan, Near water canal Istaqlalabad, Sargodha, 2021-03-20, Anjum Perveen, 97648 KUH
7	<i>Sphagneticola trilobata</i> (L.) Pruski.	Pakistan, Karachi University beside BRC, near Dept. of Botany, 2020-12-29, Taba Rauf, 95687 KUH
8	<i>Verbesina encelioides</i> (Cav.) Benth. & Hook. f. ex A. Gray	Pakistan, Karachi University, Botanical Garden, 2019-07-16, Taba Rauf & Rabia Akhlaq, 95619 KUH
9	<i>Xanthium spinosum</i> L.	Pakistan, Zhob City, 2019-10-24, Kamran Ishaq Bahadikhet, 97730 KUH
10	<i>Xanthium strumarium</i> L.	Pakistan, Thatta, 2008-08-12, Yasir, 93411 KUH
11	<i>Zinnia elegans</i> Jacq.	Pakistan, Karachi University, Administration office, 2019-07-11, Taba Rauf & Rabia Akhlaq, 95833 KUH

## RESULTS

### General pollen characters and cluster analysis of the tribe Heliantheae

Pollen grains are usually radially symmetrical and isopolar, small to medium in size, tricolporate, usually circular, round, spheroidal to prolate-spheroidal or oblate-spheroidal rarely sub-prolate. Polar length and diameter of equatorial axis 13.5-24.9  $\mu\text{m}$  (Fig. 6), P/E ratio 0.94-1.18  $\mu\text{m}$ , colpi length 10.5-19.24  $\mu\text{m}$ , mesocolpium 10.5-18.91  $\mu\text{m}$ , apocolpium 6.57-12.27  $\mu\text{m}$ . Exine 1.69-2.63  $\mu\text{m}$  thick (excluding spines), sexine is usually thicker than nexine while in some cases, sexine is as thick as nexine. Tectum is echinate or spinulate (microechinate) with a microperforated pattern, finely or coarsely punctate, perforate to subsilate, irregularly rugulate, and rarely granulate in between spines. Spines are long or short, 1.31-3.68  $\mu\text{m}$ , sharp, pointed or blunt at tips, base broad, swollen or indistinct, and perforated rarely imperforated spine base in almost all taxa (Table 2, Figs. 1-5).

The hierarchical clustering divided all the studied species into two pollen types viz., Pollen type-I,

*Helianthus* type (cluster-I), and Pollen type-II, *Xanthium* type (cluster-II). Furthermore, cluster-I (Pollen type-I) was further divided into 2 subtypes, sub-cluster I and II. These pollen types are explained in detail below:

The pollen type-I (*Helianthus* type) is characterized by echinate exine, whereas the pollen type II (*Xanthium* type) is characterized by micro echinate exine. Cluster (type-I) consisted of 9 species and was further divided into 2 sub-clusters based on tectum ornamentation in between spines. Sub-cluster I was segregated due to the rugulate tectum and represented by two species of *Sphagneticola*. However, both species of *Sphagneticola* are separated due to the difference in pollen shape. While sub-cluster II accommodated those species that had sub-psilate to punctate tectum ornamentation in between spines. Sub-cluster II was further divided into 2 subtypes i.e. II-A and II-B based on the diameter of the polar and equatorial axis.

Sub-cluster II-A is characterized by more than 20  $\mu\text{m}$  diameter of the polar and equatorial axis,

comprised of 3 genera viz. *Verbesina*, *Zinnia* and *Helianthus*. *Verbesina* and *Zinnia* are closely linked by a short branch due to the spine length of up to 3  $\mu\text{m}$ . Both genera are delimited based on pollen shape and spine base perforation. In *Verbesina encelioides*, pollen is spheroidal with an imperforated spine base whereas, pollen is prolate-spheroidal with a perforated spine base in *Zinnia elegans* (Fig. 2a-h, 3e-f) while the genus *Helianthus* is segregated from above-mentioned genera by more than 3  $\mu\text{m}$  long spine. Furthermore, both species of *Helianthus* are separated based on pollen shape, tectum ornamentation in between spines, and spine shape. *Helianthus annuus* pollen is prolate spheroidal, spines monomorphic, and sub-psilate tectum in between spines. Whereas, in *Helianthus tuberosus* pollen are sub-prolate, dimorphic spines, and tectum punctate in between spines. (Fig1, e-h)

Sub-cluster II-B is characterized by less than 20  $\mu\text{m}$  diameter of polar and equatorial axis and comprised of 3 genera (Fig. 8). *Blainvillea* and *Eclipta* are closely linked due to sharing the similar feature of spine length (more than 1  $\mu\text{m}$ ) and tectum ornamentation (punctate) in between spine, whereas *Parthenium hysterophorus* is separated due to shorter spine length (<1  $\mu\text{m}$ ) and sub-psilate tectum in between spines (Fig. 2a-b).

Pollen type II (Cluster II) consisted of two species of *Xanthium* viz., *X. spinosum*, and *X. strumarium*. Pollen prolate-spheroidal, micro echinate ornamentation (Spines <1  $\mu\text{m}$ ) and granulate to scabrate tectum in between spines. Both the species can be delimited based on <2  $\mu\text{m}$  exine thickness. In *X. spinosum*, exine is less than 2  $\mu\text{m}$  and *X. strumarium* has more than 2  $\mu\text{m}$  thick exine (Fig. 3a-d).

**Pollen type-1:** *Helianthus annuus* (Figs. 1-2, 3e, f). The pollen in this type is tricolporate, echinate; P/E ratio is 0.94-1.18. The pollen outline is circular in the equatorial view and triangular in the polar view; pollen shape is prolate-spheroidal to oblate-spheroidal and rarely sub-prolate; sexine is as thick as nexine, rarely thicker than nexine. The tectum is sub-psilate to punctate, and rarely rugulate in between echinae.

The Polar axis (P) is (13.72-) 21.22 (-24.99)  $\mu\text{m}$ , equatorial diameter (E) is (13.53-) 20.33 (-24.26)  $\mu\text{m}$ , Colpus (10.52-) is 16.10 (-19.24)  $\mu\text{m}$  in length. Mesocolpium is (10.52-) 16.11 (-18.91). Apocolpium is (6.57-) 10.17 (-12.27). Exine (1.69-) 2.21 (-2.63)  $\mu\text{m}$  thick. Echinae (<1) 2.74 (3.68).

**Species included:** *Blainvillea acmella*, *Eclipta prostrata*, *Helianthus annuus*, *Helianthus tuberosus*, *Parthenium hysterophorus*, *Sphagneticola calendulacea*, *Sphagneticola trilobata*, *Verbesina encelioides*, *Zinnia elegans*.

Within this pollen type, two subtypes can be identified:

**Subtype-I:** Tectum rugulate in between spines, monomorphic echinae with indistinct swollen and perforated spine base.

**Species included:** *Sphagneticola calendulacea*, *Sphagneticola trilobata*

**Subtype-II:** Tectum sub-psilate to punctate in between spines, mostly monomorphic rarely dimorphic with distinct or indistinct swollen and perforated rarely imperforated spine base.

**Species included:** *Blainvillea acmella*, *Eclipta prostrata*, *Helianthus annuus*, *Helianthus tuberosus*, *Parthenium hysterophorus*, *Verbesina encelioides*, *Zinnia elegans*.

**Pollen Type-II:** *Xanthium strumarium*-type (Figs. 3a-d). The pollen in this type is also tricolporate, micro echinate (spinules); P/E ratio is 1.02-1.05. The pollen outline is circular in the equatorial view and slightly triangular in the polar view; the pollen shape is prolate-spheroidal; sexine is as thick as or thicker than nexine. The tectum is granulate to scabrate in between echinae.

The Polar axis (P) is (20.48-) 21.79 (-23.11)  $\mu\text{m}$ , equatorial diameter (E) is (19.36-) 20.91 (-22.46)  $\mu\text{m}$ , colpus length is (10.52-) 12.66 (-14.80)  $\mu\text{m}$  in length. Mesocolpium is (15.29-) 16.59 (-17.89)  $\mu\text{m}$ . Apocolpium is (10.52-) 10.91 (-11.31). Exine is (1.69-) 2.06 (-2.44)  $\mu\text{m}$  thick. Echinae (<1).

**Species included:** This pollen type consisted of two species i.e., *Xanthium spinosum*, and *X. strumarium*.

Table 2. Qualitative and quantitative morphological characteristics of pollen of the tribe Heliantheae.

Species	Polar Axis (P) ( $\mu\text{m}$ )	Equatorial Axis (E) ( $\mu\text{m}$ )	P/E Ratio	Shape	Spine Length ( $\mu\text{m}$ )	Colpi length ( $\mu\text{m}$ )	Mesocolpium ( $\mu\text{m}$ )	Apocolpium ( $\mu\text{m}$ )	Exine thickness ( $\mu\text{m}$ )	Tectum
<i>Blainvillea acmella</i> (L.) Philip.	(21.05-) 21.87 (-23.68)	(15.78-) 19.73 (-21.05)	1.10	Prolate-spheroidal	(2.63-) 2.63 (-2.63)	(15.78-) 17.76 (-18.42)	(14.47-) 15.52 (-15.78)	(9.21-) 10.26 (-10.52)	(1.31-) 1.78 (-1.97)	Echinate Punctate
<i>Eclipta prostrata</i> L.	(15.78-) 17.76 (-21.05)	(15.78-) 16.66 (-18.42)	1.06	Prolate-Spheroidal	(1.31-) 1.31 (-1.31)	(10.52-) 11.84 (-13.15)	(13.15-) 13.68 (-15.78)	(7.89-) 8.28 (-9.21)	(1.31-) 2.16 (-2.63)	Echinate Punctate
<i>Helianthus annuus</i> L.	(23.68-) 24.99 (-27.63)	(23.68-) 24.26 (-27.63)	1.03	Prolate-Spheroidal	(2.63-) 3.43 (-3.94)	(17.10-) 17.95 (-18.42)	(15.78-) 17.59 (-18.42)	(11.84-) 12.27 (-13.15)	(2.63-) 2.63 (-2.63)	Echinate Subpsilate
<i>Helianthus tuberosus</i> L.	(23.68-) 24.99 (-26.31)	(18.42-) 21.05 (-23.68)	1.18	Sub-Prolate	(3.28-) 3.68 (-3.94)	(15.78-) 17.62 (-19.73)	(15.78-) 16.31 (-17.10)	(10.52-) 11.84 (-13.15)	(1.97-) 2.34 (-2.63)	Echinate punctate
<i>Parthenium hysterophorus</i> L.	(13.15-) 13.72 (-15.13)	(13.15-) 13.53 (-14.47)	1.01	Prolate-Spheroidal	< 1 $\mu\text{m}$	(10.52-) 10.52 (-10.52)	(10.52-) 10.52 (-10.52)	(6.57-) 6.57 (-6.57)	(1.31-) 1.69 (-1.97)	Micro echinate, Subpsilate
<i>Sphagneticola calendulacea</i> (L.) Pruski.	(18.42-) 19.35 (-21.05)	(19.73-) 20.48 (-21.05)	0.94	Oblate-spheroidal	(2.63-) 3.18 (-3.94)	(15.78-) 16.90 (-18.41)	(13.15-) 16.15 (-18.41)	(9.20-) 8.79 (-10.52)	(1.31-) 2.34 (-2.63)	Echinate Rugulate
<i>Sphagneticola trilobata</i> (L.) Pruski.	(21.05-) 22.85 (-26.31)	(21.05-) 22.69 (-24.99)	1.00	Spheroidal	(2.63-) 3.12 (-3.28)	(18.42-) 19.24 (-21.05)	(18.42-) 18.91 (-19.73)	(10.52-) 11.34 (-12.49)	(1.97-) 2.30 (-2.63)	Echinate Rugulate
<i>Verbesina encelioides</i> (Cav.) Benth. & Hook.f. ex A.Gray	(23.02-) 24.15 (-25.65)	(23.68-) 24.05 (-24.99)	1.00	Spheroidal	(1.31-) 1.87 (-2.63)	(15.78-) 17.63 (-18.42)	(15.78-) 18.81 (-21.05)	(10.52-) 11.70 (-12.49)	(1.97-) 2.44 (-2.63)	Echinate Subpsilate
<i>Xanthium spinosum</i> L.	(18.42-) 20.48 (-21.05)	(15.78-) 19.36 (-21.05)	1.05	Prolate-Spheroidal	<1 $\mu\text{m}$	(13.15-) 14.80 (-15.78)	(14.47-) 15.29 (-15.78)	(10.52-) 10.52 (-10.52)	(1.31-) 1.69 (-1.97)	Micro echinate, granulate to scabrate
<i>Xanthium strumarium</i> L.	(21.05-) 23.11 (-26.31)	(21.05-) 22.46 (-26.31)	1.02	Prolate-Spheroidal	<1 $\mu\text{m}$	(10.52-) 10.52 (-15.78)	(15.78-) 17.89 (-21.05)	(10.52-) 11.31 (-13.15)	(1.97-) 2.44 (-2.63)	Micro echinate, granulate to scabrate
<i>Zinnia elegans</i> Jacq.	(21.05-) 21.38 (-23.68)	(18.42-) 20.55 (-23.68)	1.02	Prolate-Spheroidal	(2.63-) 2.74 (-3.28)	(14.47-) 15.46 (-15.78)	(15.78-) 17.54 (-18.42)	(10.52-) 10.52 (-10.52)	(1.31-) 2.25 (-2.63)	Echinate Subpsilate

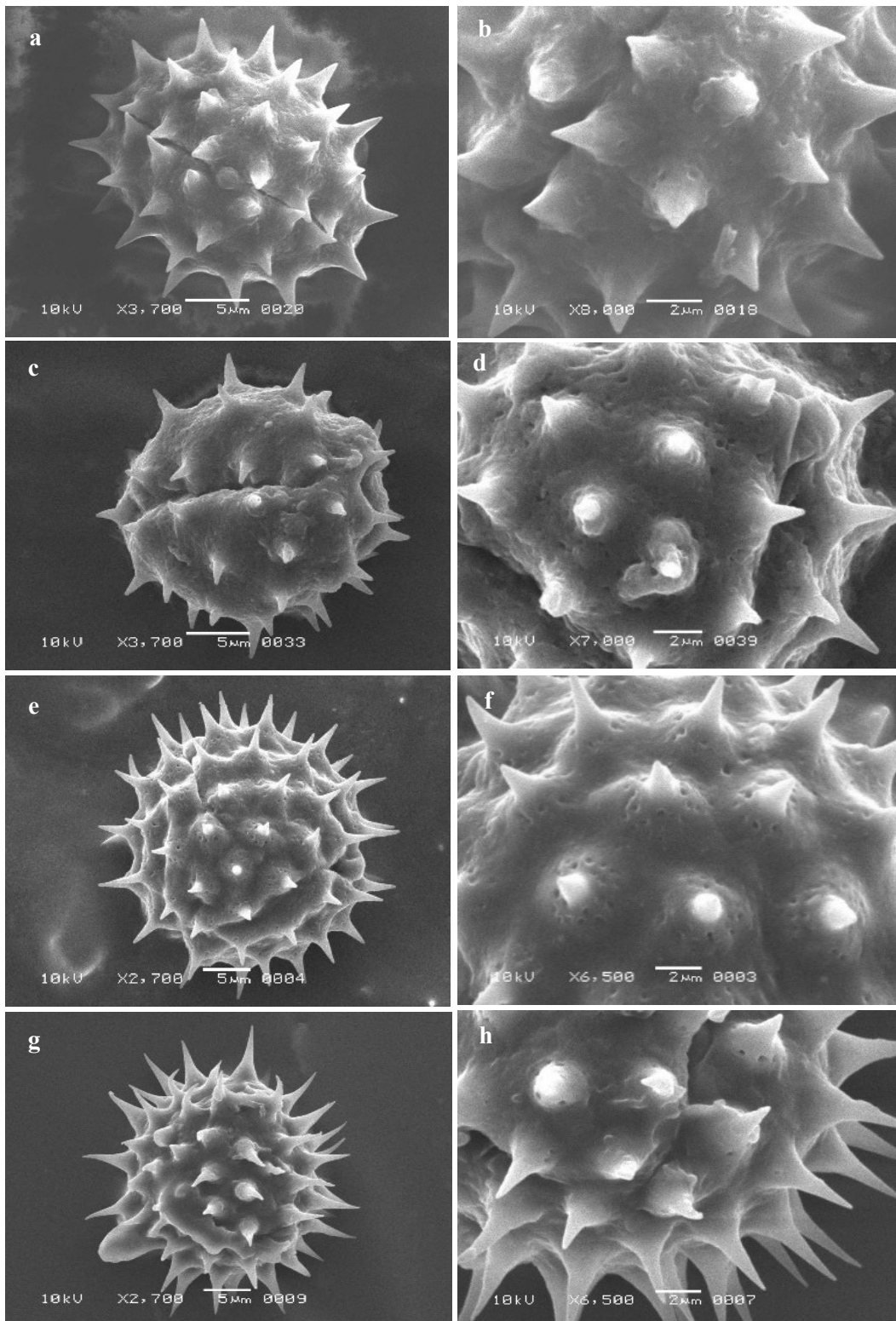


Fig. 1. Scanning electron micrographs (SEM) of the pollen of studied species. a-b, *Blainvillea acmella*; c-d, *Eclipta prostrata*; e-f, *Helianthus annuus*; g-h, *Helianthus tuberosus*. Scale bars: (a, c, e, g = 5μm), (b, d, e, f = 2μm).

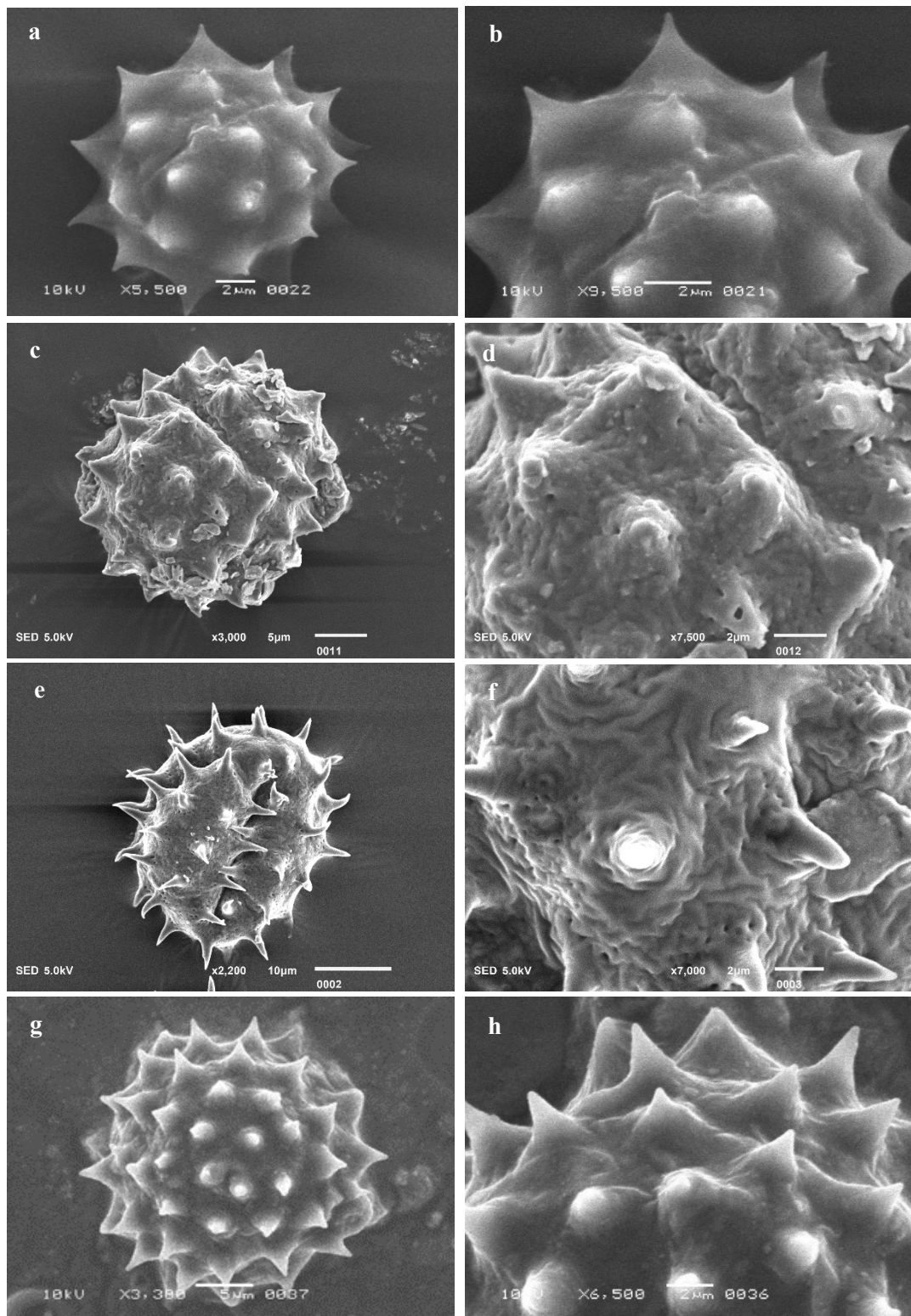


Fig. 2. Scanning electron micrographs (SEM) of the pollen of studied species. a-b, *Parthenium hysterophorus*; c-d, *Sphagneticola calendulacea*; e-f, *Sphagneticola trilobata*; g-h, *Verbesina encelioides*. Scale bars: (a, c, e, g= 5µm), (b, d, e, f= 2µm).

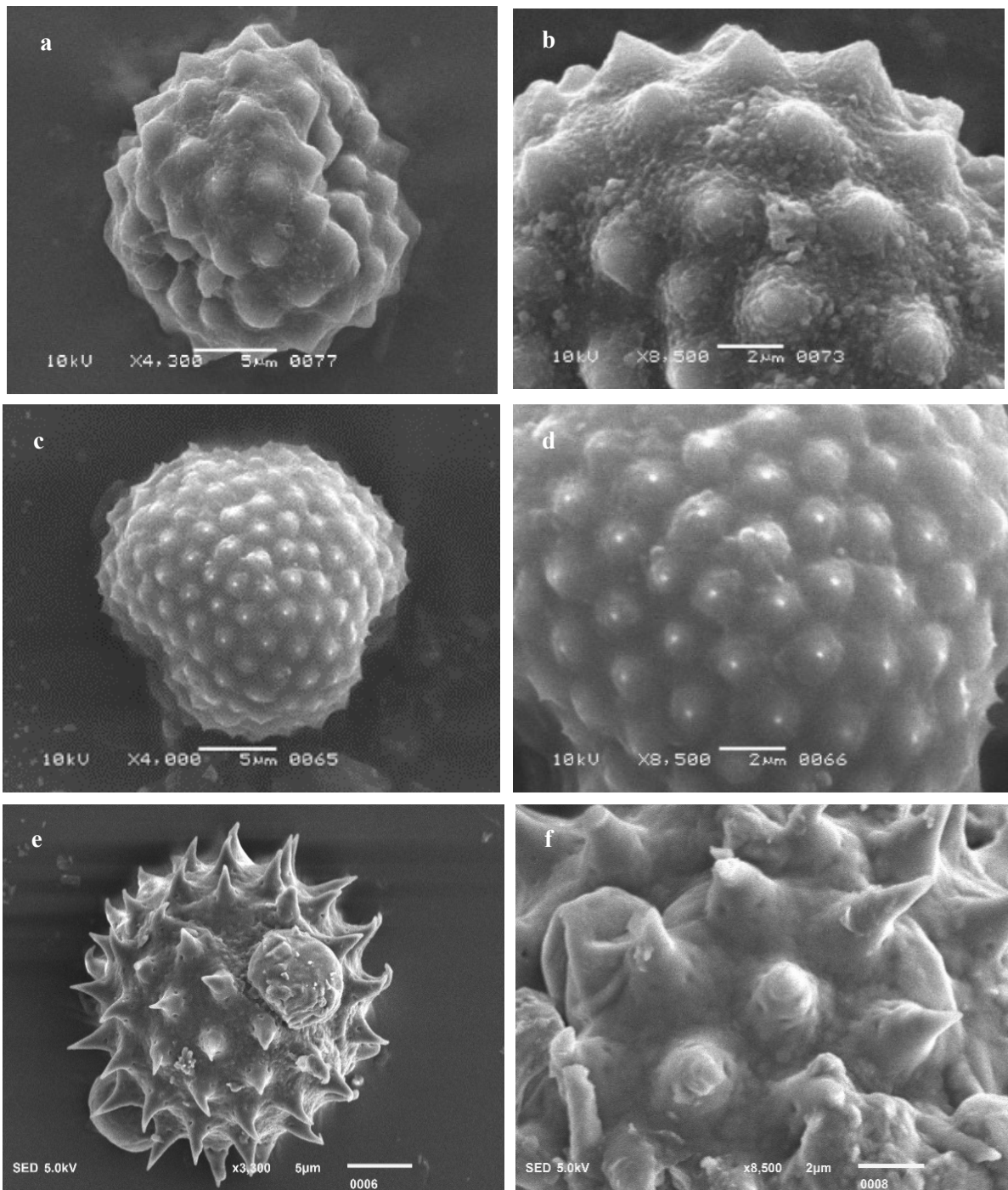


Fig. 3. Scanning electron micrographs (SEM) of the pollen of studied species. a-b, *Xanthium spinosum*; c-d, *Xanthium strumarium*; e-f, *Zinnia elegans*. Scale bars: (a, c, e, g= 5µm), (b, d, e, f= 2µm).



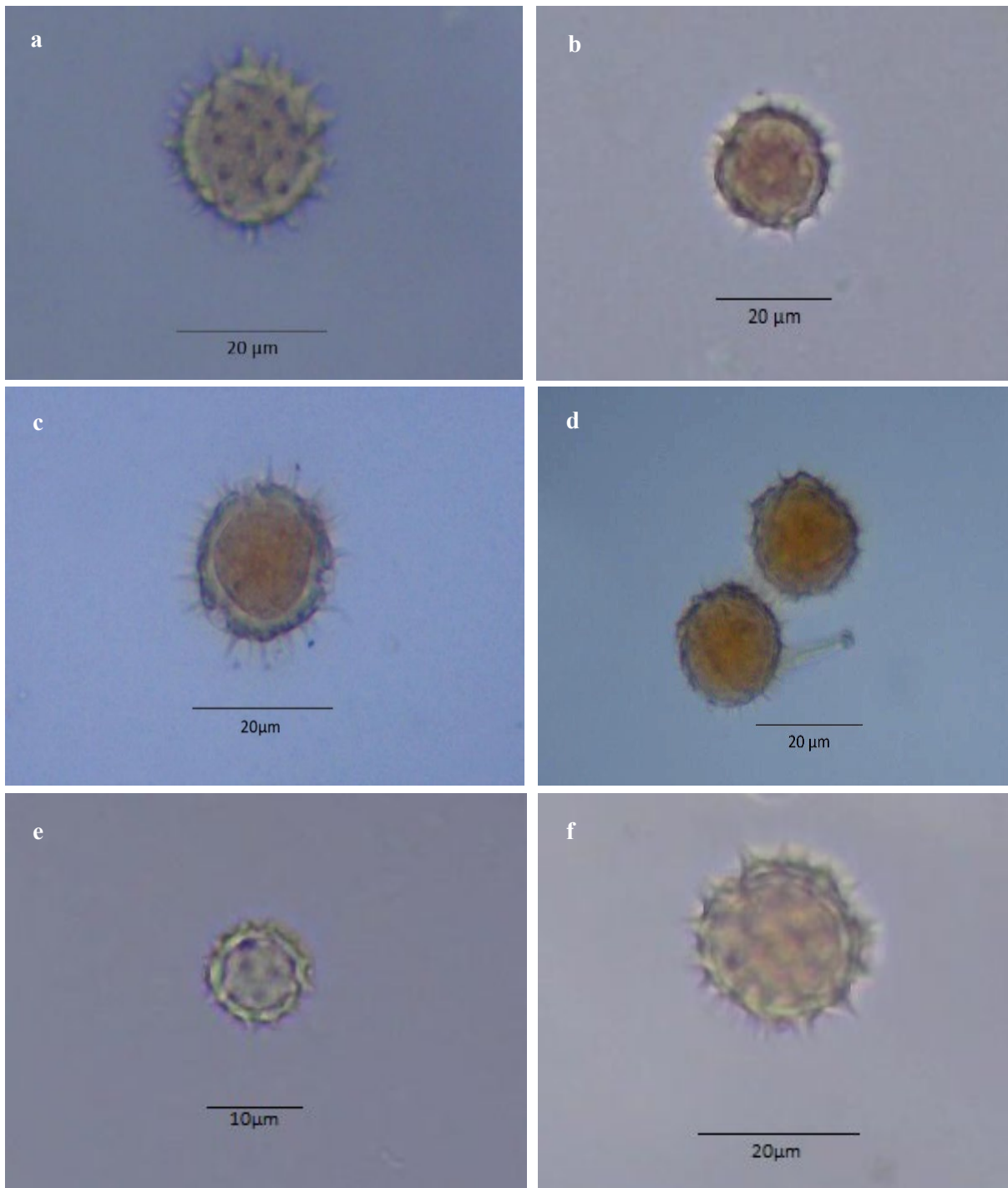


Fig. 4. Light micrographs (LM) of pollen of studied species. a, *Blainvillea acmella*; b, *Eclipta prostrata*; c, *Helianthus annuus*; d, *Helianthus tuberosus*; e, *Parthenium hysterophorus*; f, *Sphagneticola calendulacea*. Scale bars: (a-d, f= 20 $\mu$ m), (e = 10 $\mu$ m).

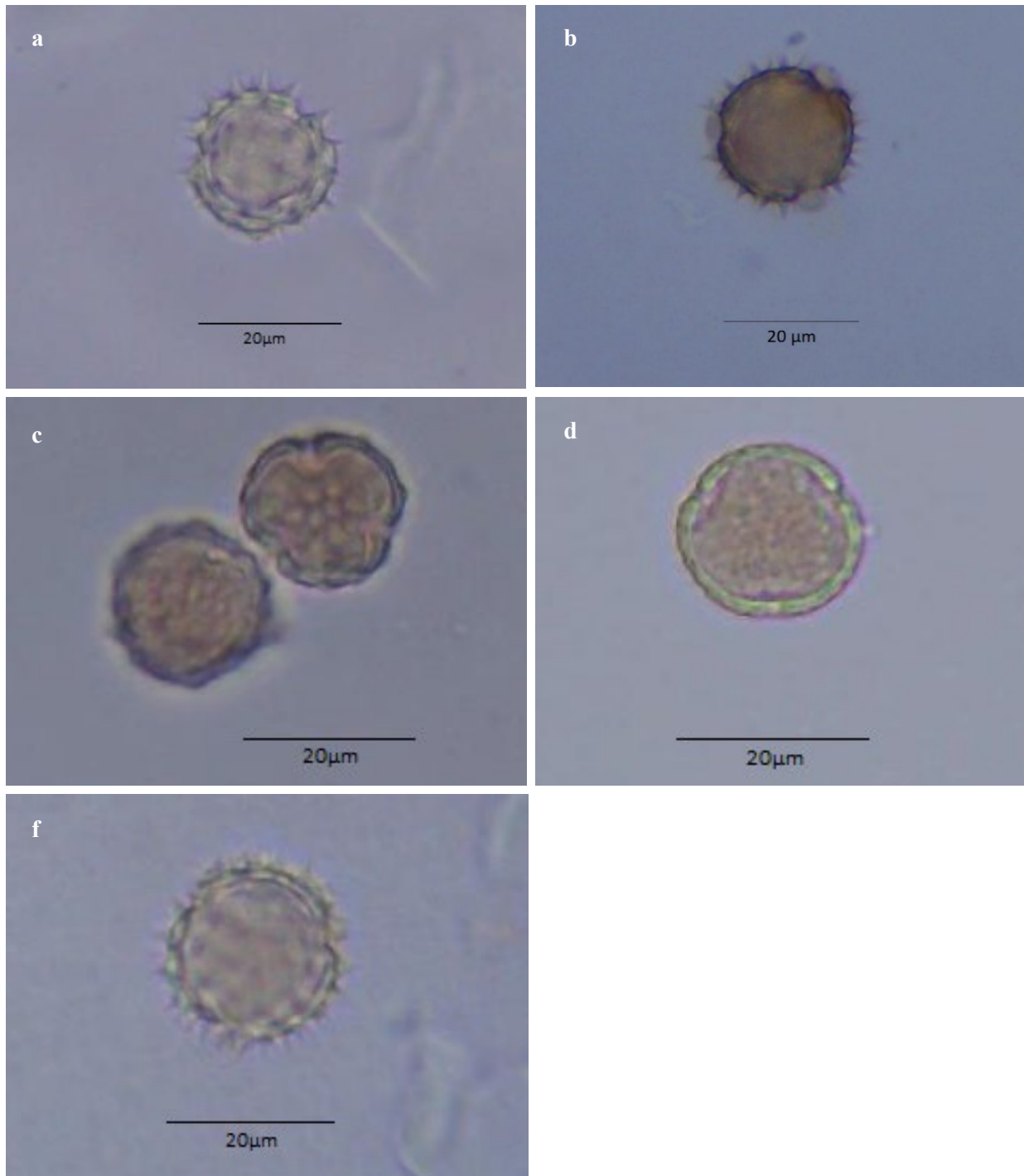


Fig. 5. Light micrographs (LM) of pollen of studied species. a, *Sphagneticola trilobata*; b, *Verbesina encelioides*; c, *Xanthium spinosum*; d, *Xanthium strumarium*; e, *Zinnia elegans*. Scale bars: (a-e= 20µm).

Table 3. List of characters scored for hierarchical cluster analysis for the taxa of the Tribe Heliantheae.

Name of Taxa	1	2	3	4	5	6	7	8	9	10	11
<i>Blainvillea acmella</i>	2	1	1	2	1	3	1	2	1	2	1
<i>Eclipta prostrata</i>	1	1	1	2	2	2	1	2	2	2	1
<i>Helianthus annuus</i>	2	2	1	1	2	4	1	1	1	2	1
<i>Helianthus tuberosus</i>	2	2	1	2	2	4	2	1	1	3	2
<i>Parthenium hysterophorus</i>	1	1	2	1	1	1	1	1	2	3	1
<i>Sphagneticola calendulacea</i>	1	2	1	3	2	4	1	2	1	2	1
<i>Sphagneticola trilobata</i>	2	2	1	3	2	4	1	2	1	2	1
<i>Verbesina encelioides</i>	2	2	1	1	2	2	1	1	2	2	2
<i>Xanthium spinosum</i>	2	1	2	4	1	1	1	1	2	2	1
<i>Xanthium strumarium</i>	2	2	2	4	2	1	1	1	2	1	1
<i>Zinnia elegans</i>	2	2	1	1	2	3	1	1	1	3	2

Table 4. The data matrix of the taxa representing the tribe Heliantheae scored for 11 characters presented in Table 3.

Characters descriptions	
1.	Max. length of Polar axis ( $\mu\text{m}$ ): Less than 20 (1), 20-30 (2)
2.	Max. length of Equatorial axis ( $\mu\text{m}$ ): Less than 20 (1), 20-30 (2)
3.	Exine ornamentation: Echinate (1), Micro echinate (2)
4.	Tectum ornamentation: Sub-psilate (1), Punctate (2), Rugulate (3), Granulate to scabrate (4)
5.	Max. Exine Thickness ( $\mu\text{m}$ ): 1-2 (1), 2-3 (2)
6.	Max. length of echinate ( $\mu\text{m}$ ): Less than 1 (1), 1.1-2.0 (2), 2.1-3.0 (3) 3.1-4.0 (4)
7.	Echinae morphism: Monomorphic echinae (1), Dimorphic echinae (2)
8.	Base of Echinae: Distinct swollen base (1), Indistinct swollen base (2)
9.	Perforation base: Perforated Echinae base (1), Imperforated echinae base (2)
10.	Apex of echinae: Blunt (1), Acute (2), Acuminate (3)
11.	Echinae tip: Straight (1), Curved (2)

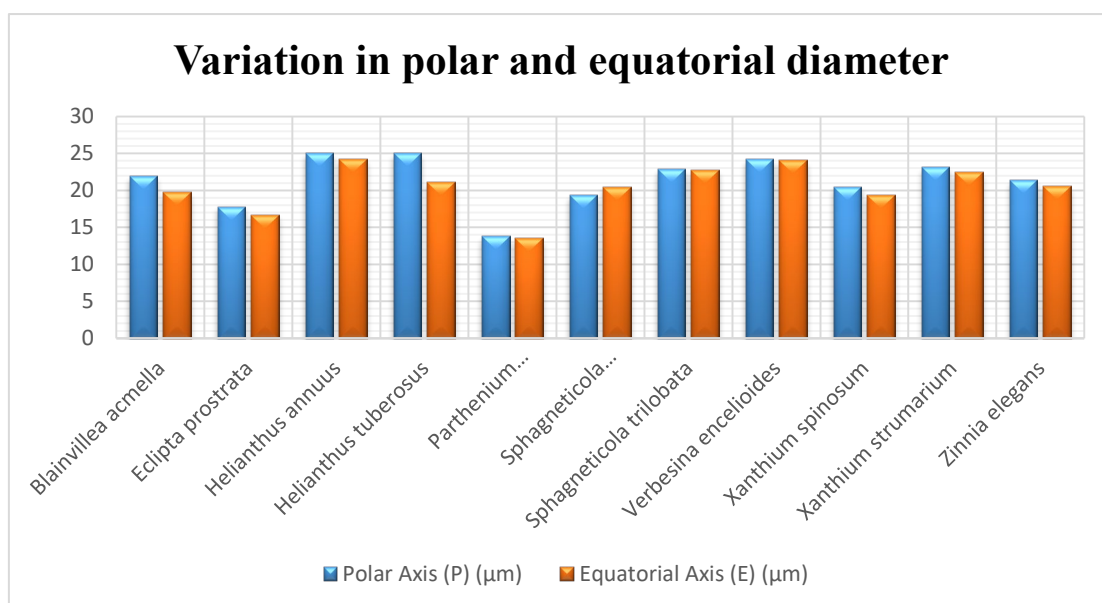


Fig. 6. The variations represented in the polar and equatorial diameter within the tribe Heliantheae.

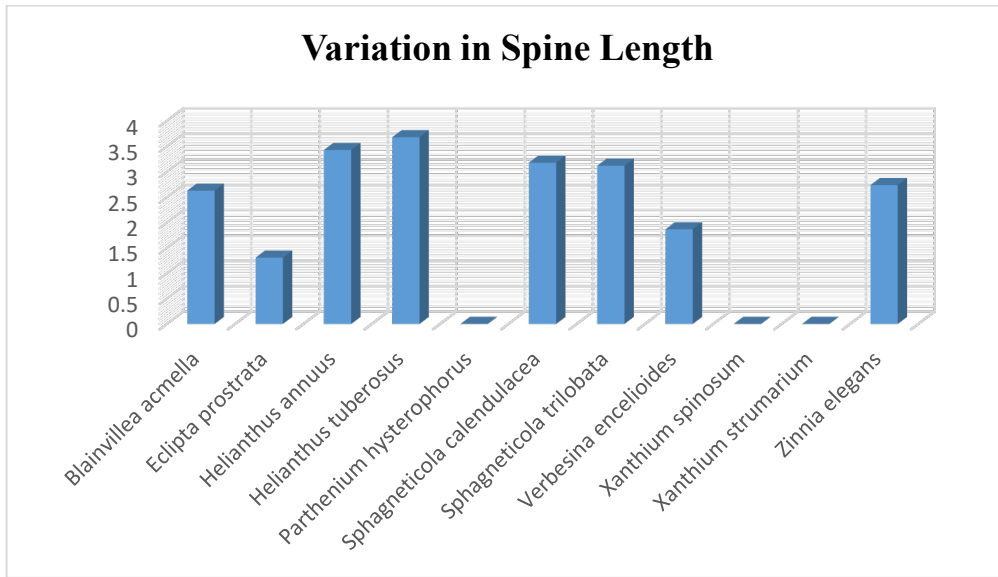


Fig. 7. The variations found in spine length within the tribe Heliantheae.

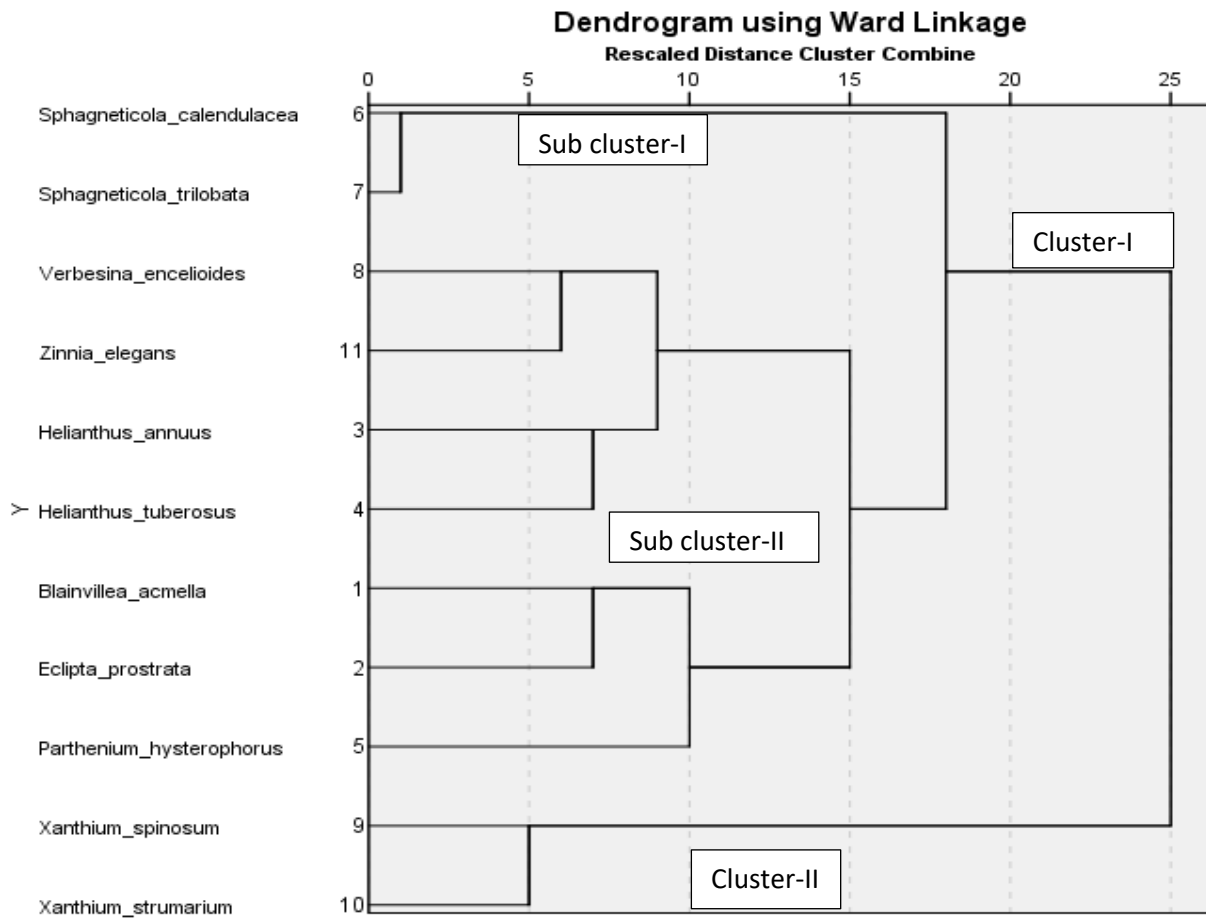


Fig. 8. Dendrogram obtained by Ward's cluster analysis, showing the relationship of the studied taxa of tribe Heliantheae based on pollen evidence.

## DISCUSSION

In the present study, pollen of 11 species from 8 genera of tribe Heliantheae from Pakistan were examined by light and scanning electron microscope (SEM). The palynological study revealed that these species had a significant variation in pollen characteristics such as shape, size, exine ornamentation pattern, spine length, and type. Similar pollen morphological characters were also reported by earlier researchers (Wodehouse 1928; Skvarla & Larson 1965; Skvarla & Turner 1966; Perveen 1999; Zafar & al. 2007; Blackmore & al. 2009; Mazari & al. 2012; Stanski & al. 2013; Coutinho & al. 2016; Salamah & al. 2019; Coutinho & al. 2021; Bahadur & al. 2021; Usma & al. 2022; Bahadur & al. 2022). However, Usma & al. (2022) reported tetracolporate pollen with echinolophate tectum in *Parthenium hysterophorus*. Whereas, our results did not support Usma's findings. According to our studies, pollen grains of *Parthenium hysterophorus* were tricolporate with micro echinate tectum, this was in agreement with the findings of Blackmore & al. (2009) and Stanski & al. (2013). However, due to cluster analysis and other pollen morphological characteristics of *Parthenium hysterophorus* (such as pollen size, and diameter of polar and equatorial axis), it is placed in Pollen type-I, which also supports the species placement in the tribe Heliantheae.

In the present study, the most variable feature was exine ornamentation. Wodehouse (1928) advocated the placement of *Xanthium* under Ambrosiaceae due to its spinules and drew the relationship of the Ambrosiaceae to Heliantheae because of the length of spines in this genus. It was first considered spineless and was placed under the tribe Ambrosiaceae. Later, it was treated under the tribe Heliantheae because it showed advanced pollen features of Compositae. In our studies, it was found that this genus had very small spines (<1 µm). Subsequently, these taxa are the most diversified species from the rest of the taxa of the tribe Heliantheae, indicating that these are the most distinct and advanced species of this tribe by having micro-echinate exine surface. Thus, this palynological study also supports the placement of this genus under the tribe Heliantheae.

Moreover, a considerable variation found in echinate and micro echinate types are notable diagnostic features to differentiate the taxa from each other within these types such as the presence or absence of perforation on the spine base, likewise, some taxa are also characterized by swollen or indistinct spine base. Such diversified pollen morphological characteristics made the palynological

study a significant taxonomic tool. In addition, the tectum in between spines was also one of the most diverse characters (viz. Punctate, sub-psilate rarely rugulate or granulate) and also delimit the taxa at generic (*Sphagneticola* and *Xanthium*) and specific levels (*Helianthus annuus* and *Helianthus tuberosus*). This character of tectum in between the spines was also studied by other workers (Skvarla & Larson 1965; Perveen 1999; Blackmore 2009; Coutinho & al. 2016; 2021; Bahadur & al. 2020 & 2022).

In conclusion, the pollen grains of the tribe Heliantheae taxa from Pakistan were different in terms of pollen type, size, and ornamentation. Based on the ornamentation of tectum two pollen types were recognized which corresponded with the dendrogram obtained from the cluster analysis. The present study also provided pollen micro-morphological characters useful for the genera and species delimitation and confirmed the eurypalynous nature of the family by showing significant pollen variations.

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