

# PALYNOLOGICAL INVESTIGATIONS OF SOME SELECTED TAXA OF THE TRIBE COREOPSIDEAE (ASTERACEAE) FROM PAKISTAN AND KASHMIR AND THEIR TAXONOMIC IMPLICATIONS

TABA RAUF AND ANJUM PERVEEN

Centre for Plant Conservation, University of Karachi  
\*Corresponding author's email: [anjum\\_tahir@hotmail.com](mailto:anjum_tahir@hotmail.com)

## Abstract

Palynological characteristics of 9 species belonging to *Bidens* L., *Coreopsis* L., and *Cosmos* Cav., from Pakistan and Kashmir were examined using Light Microscope (LM) and Scanning Electron Microscope (SEM). Pollen grains were usually radially symmetrical, isopolar, Prolate-spheroidal to oblate-spheroidal, rarely sub-prolate in shape, generally tricolporate rarely tetracolporate (*Bidens pilosa*). Pollen showed a wide range of variation in size and shape. Exine ornamentation was echinate, but variation was found in the ornamentation in between spines such as punctate, rugulate and rarely granulate with microperforated tectum. Spines were long, sharp with pointed tips, perforated, mostly conical in shape with broad or narrow base. Based on the pollen size two pollen types viz., *Bidens pilosa* and *Coreopsis tinctoria* types are recognized.

**Key words:** Palynological characteristics, Pollen variation, Coreopsidae, *Bidens*, *Coreopsis*, *Cosmos*, Scanning microscope.

## Introduction

The tribe Coreopsidae –Asteraceae was first recognized by Lindley (1829), However, for long time this was never accepted as an independent tribe, and was treated as a subtribe of Heliantheae, Until Turner & Powell (1977) who resurrected it as a tribe. However, later workers Robinson (1981) and Bremer (1987) while studying the interrelationship of the tribe Asteraceae again accepted it as a subtribe Coreopsidinae of the tribe Heliantheae. The recent molecular studies of Heliantheae and related tribes conducted by Panero & Funk (2002) and Funk *et al.*, (2009) supported its independent status as a Coreopsidae.

Morphologically, the tribe Coreopsidae is characterized by its dimorphic phyllaries, outer ones differentiating from the inner ones in color and shape, outer phyllaries herbaceous and green, inner phyllaries membranous with orange-brown striations (resin ducts); Paleae with brownish-orange striations; and cypselas flat, obcompressed to quadrangular (Funk *et al.*, 2009).

The tribe Coreopsidae consists of 30 genera and approximately 550 species with a worldwide distribution. Most of the species are commonly found in South, North, and Central America, Mexico and few in Africa also. A number of species are introduced elsewhere as ornamental (Panero 2007; Daniel *et al.*, 2009). In Pakistan, the tribe is represented by 5 genera and 12 species, including 5 cultivated species (Qaiser & Perveen, 2021).

The tribe is commonly known for their ornamental plants which hold the beauty of gardens such as *Cosmos bipinnatus*, *C. sulphureus*, *Dahlia pinnata* and *D. hortensis* whereas, the species of the genus *Bidens* (*B. tripartita*, *B. pilosa*, and *B. cernua*) are being used in traditional medicines as anti-inflammatory, anti-malarial, anti-ulcer, anti-allergic, anti-cancer, anti-diabetic and antibacterial agents (Zahara, 2015). These plants are also used as a source of yellow dye for cloth.

Pollen morphology of some species of the genus *Bidens* have been studied by Ikuse (1956) and Huang (1972). Mesfin *et al.*, (1995) examined pollen of some North American species of the genus *Coreopsis*, Stanski *et*

*al.*, (2013) studied two species of the genus *Bidens* from Brazil. Meo (2005) described pollen of a few species from Pakistan of the tribe Coreopsidae based on light microscopic studies.

The objective of the present palynological study of the tribe Coreopsidae was to investigate and examine the taxa of the Tribe Coreopsidae and to identify novel pollen traits for taxonomic identification of the species by using light and scanning electron microscopy.

## Material and Methods

### Collection of plants and voucher specimens:

Polleniferous material was collected from the Herbarium specimens and of few species were obtained from fresh specimens from the field, the voucher specimens were deposited in Prof. Dr. S.I. Ali Botanical Herbarium (KUH), Centre for Plant Conservation, University of Karachi (Appendix-1).

**Light microscopy (LM):** Micro-morphological characters of the pollen of given taxa were examined by the method outlined by Erdtman (1943, 1952), 50% glycerin was added to polleniferous material prepared and centrifuged for 10-15 minutes, and glycerin was decanted. Pollen material was divided into two parts, one part was mounted on a slide for light microscopic work, Nikon-type-102 (E100, 1.08) was used. For each species 20-30 pollen grains were measured. Following measurements were taken: Polar length (P), equatorial diameter (E), exine thickness, colpus length, spine length, exine ornamentation, apocolpium, and mesocolpium.

**Scanning Electron Microscopy (SEM):** For scanning electron microscopy, the other part of the sample was suspended in a drop of water and directly transferred with a fine pipette to a metallic stub using double sided cello tape and coated with gold in a sputtering chamber, the coating was restricted to 15°A for 10 minutes. Pollen were examined and photographed by Joel (JSM-6380A) Scanning Electron Microscope.

Appendix-1. List of plant species and their collection sites along with voucher numbers.

S. No.	Name of taxa	Collection site	Collection date	Collector name	Voucher number
1.	<i>Bidens bipinnata</i> L.	Garda Babar Zhob City	03-08-2019	Kamran Ishaq Bahadikheth	KUH. No. 05
2.	<i>Bidens biternata</i> L.	On way to Gilgit	08-09-1981	Kamal Akhter Malik and S. Nazimuddin.	KUH. No. 188
3.	<i>Bidens cernua</i>	Kherai, Matta Swat	14-10-2020	Zahidullah	s. n. KUH
4.	<i>Bidens pilosa</i> L.	Between Chilas and Juglote	29-08-1988	S. Omer and M. Qaiser	KUH. No. 2274
5.	<i>Bidens tripartita</i> L.	Village Jarvanan near Bat Khela, Distt. Swat	18-06-1970	M. Qaiser and Abdul Ghafoor	KUH. No. 1702
6.	<i>Coreopsis lanceolata</i> L.	Karachi University campus	26-03-1987	Bushra, Moin, and Nadeem	KUH. No. 68641
7.	<i>Coreopsis tinctoria</i> Nutt.	Karachi University campus, Applied Physics	15-07-2019	Taba Rauf and Rabia Akhlaq	KUH No. 95835
8.	<i>Cosmos bipinnatus</i> Cav.	Karachi University, Dept. Botany	18-12-1968	Mr. Abrar Hussain	KUH. No. 47563.
9.	<i>Cosmos sulphureus</i> Cav.	Karachi University, campus Environmental Studies.	15-07-2019	Taba Rauf and Rabia Akhlaq	KUH. No. 95844

**Clusters analysis:** A Hierarchical cluster analysis of 9 taxa of the tribe Coreopsideae was performed by using the Euclidean distance and Ward's method for a group linkage method with the computer program IBM SPSS Statistics 20, for the comparing the species similarities of the tribe, based on contrasting characters of pollen morphology.

Total of 17 quantitative and qualitative pollen characters were chosen to differentiate the investigated species. The qualitative 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 represented in tables 2 and 3. The summary of qualitative and quantitative characteristics of pollen is given in Table 1 and LM and SEM micrographs are given in Fig. 1-3.

## Results and Observations

### General pollen characters of the studied taxa of tribe

**Coreopsideae:** Pollen grains radially symmetrical and isopolar, small to medium in size, mostly tricolporate, rarely tetracolporate, usually circular, round, prolate-spheroidal to oblate-spheroidal, rarely sub-prolate, 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 thickness varied from 1.31-5.26  $\mu\text{m}$ . Sexine thicker than nexine or sexine as thick as nexine, tectum echinate, sub-psilate to psilate rarely punctate, or granulate often rugulate in between spines. Spines long or short, sharp, pointed or blunt at tips, base broad or swollen and perforated in almost all taxa, some were conical in shape, spine length ranges from 1.31-5.26  $\mu\text{m}$ . In the present study on the basis pollen size two pollen types were recognized viz., *Coreopsis tinctoria*-type and *Bidens pilosa*-type.

### Key to pollen types

- 1 + Pollen small, diameter of polar axis 15-20  $\mu\text{m}$  and equatorial axis 15-20  $\mu\text{m}$  ..... *Coreopsis tinctoria*-type  
 - Pollen medium, diameter of polar axis 21-30  $\mu\text{m}$  and equatorial axis 21-30  $\mu\text{m}$  ..... *Bidens pilosa*-type

### Description of pollen types

**Pollen type-I: *Coreopsis tinctoria*** (Fig. 2A-F), 3(E-G)

**Pollen class:** Tricolporate. Zonoaperturate

**Shape:** Prolate-spheroidal rarely oblate spheroidal

**Apertures:** More or less circular.

**Ornamentation** Tectum Sub-psilate to sparsely rugulate in between the spines

**Measurements:** Polar axis P (15.78-) 18.41  $\pm$  1.07 (-21.05)  $\mu\text{m}$ , equatorial diameter E (13.15-) 17.10  $\pm$  1.9 (-21.05)  $\mu\text{m}$ , Colpus (10.52-) 13.15  $\pm$  1.09 (-15.78)  $\mu\text{m}$  in length. Mesocolpium 10.5-15.78  $\mu\text{m}$ . Exine 0.55-2.63  $\mu\text{m}$  thick, sexine thinner than nexine. 1.97-3.28.

**Species included:** *Coreopsis lanceolata* L., *Coreopsis tinctoria* Nutt., *Cosmos bipinnatus* Cav.

### Key to the species

1. + Pollen prolate-spheroidal, tectum sub-psilate in between spines ..... 2  
 - Pollen oblate-spheroidal, tectum sparsely regulate ..... *Coreopsis lanceolata*  
 2. + Max. polar length > than 20  $\mu\text{m}$ . Max. spine length less than 3  $\mu\text{m}$  ..... *Coreopsis tinctoria*  
 - Max. polar length < than 20  $\mu\text{m}$ . Max. spine length more than 3  $\mu\text{m}$  ..... *Cosmos bipinnatus*

**Pollen type-II: *Bidens Pilosa*** (Fig. 1(A-H), 2(G-H), 3(A-D, H)

**Pollen class:** Mostly tricolpate rarely tetracolporate

**Shape:** Prolate-spheroidal to oblate spheroidal rarely sub-prolate

**Apertures:** More or less circular.

**Ornamentation:** Tectum Psilate to granulate rarely punctate in between the spines

**Measurements:** Polar axis P (21.05-) 26.31  $\pm$  1.02 (-31.57)  $\mu\text{m}$ , equatorial diameter E (21.05-) 24.99  $\pm$  1.6

(-28.94)  $\mu\text{m}$ , Colpus (13.15-) 18.59  $\pm$  1.01 (-23.68)  $\mu\text{m}$  in length. Mesocolpium 10-22.36  $\mu\text{m}$ . Exine 1.31-5.0  $\mu\text{m}$  thick, sexine thinner than nexine. Spine's length 1.31-5.26.

**Species included:** *Bidens bipinnata* L., *Bidens biternata* L., *Bidens cernua* L., *Bidens pilosa* L., *Bidens tripartita* L., *Cosmos sulphureus* Cav.

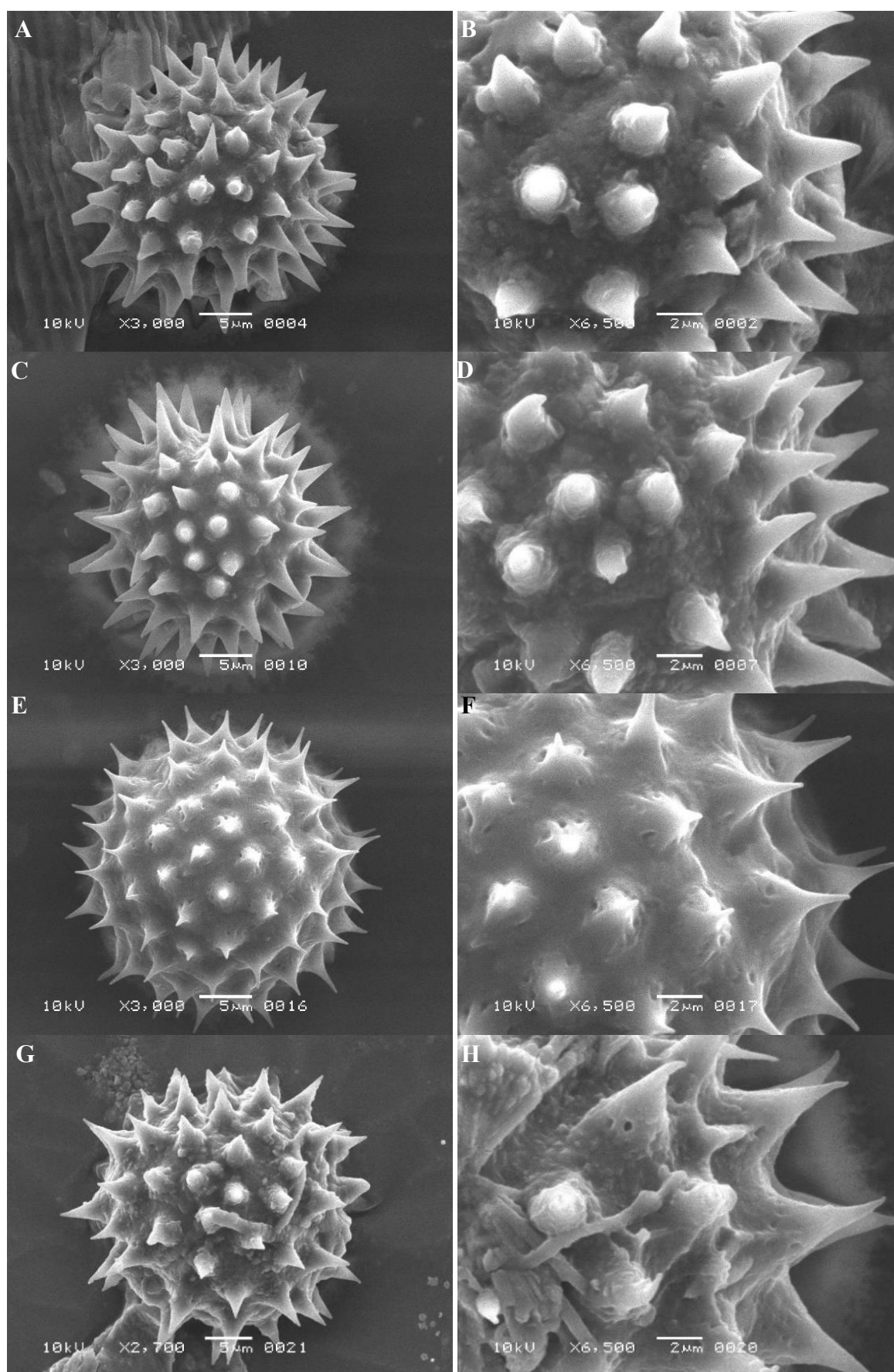


Fig. 1. Scanning electron micrographs of Tribe Coreopsidae pollens and their exine ornamentation. A-B *Bidens bipinnata*: A. Equatorial view of pollen; B. exine ornamentation. C-D *Bidens biternata*: C. Equatorial view of pollen; D. exine ornamentation. E-F *Bidens pilosa*: E. Polar view of pollen; F. exine ornamentation. G-H *Bidens tripartita*: G. Polar view of pollen; H. exine ornamentation. Scale bars: A,C,E,G = 5µm; B,D,F,H = 2 µm.

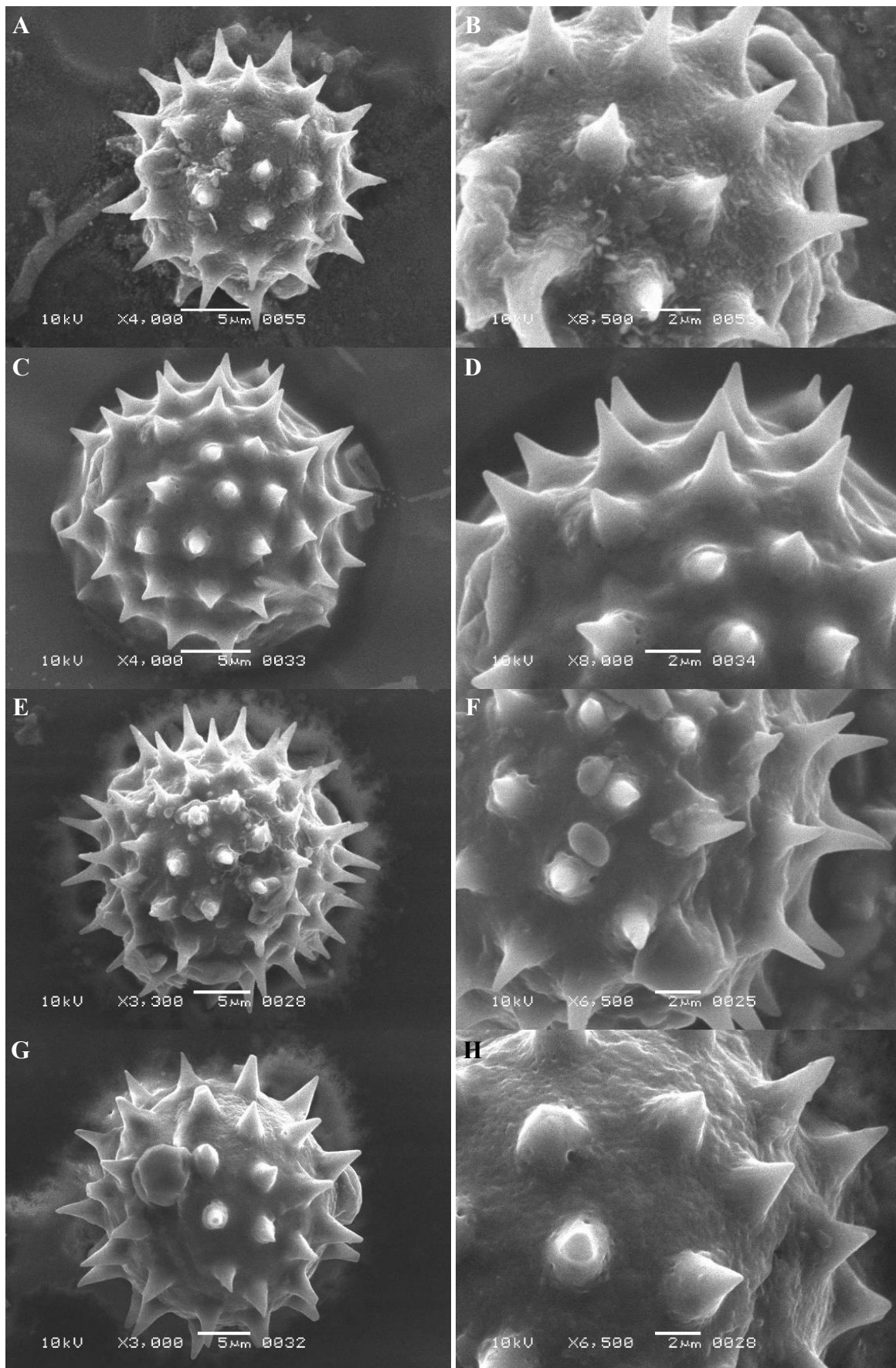


Fig. 2. Scanning electron micrographs of Tribe Coreopsidae pollens and their exine ornamentation. A-B *Coreopsis lanceolata*: A. Equatorial view of pollen; B. exine ornamentation. C-D *Coreopsis tinctoria*: C. Equatorial view of pollen; D. exine ornamentation. E-F *Cosmos bipinnatus*: E. Polar view of pollen; F. exine ornamentation. G-H *Cosmos sulphureus*: G. Polar view of pollen; H. exine ornamentation. Scale bars: A,C,E,G = 5  $\mu$ m; B,D,F,H = 2  $\mu$ m.

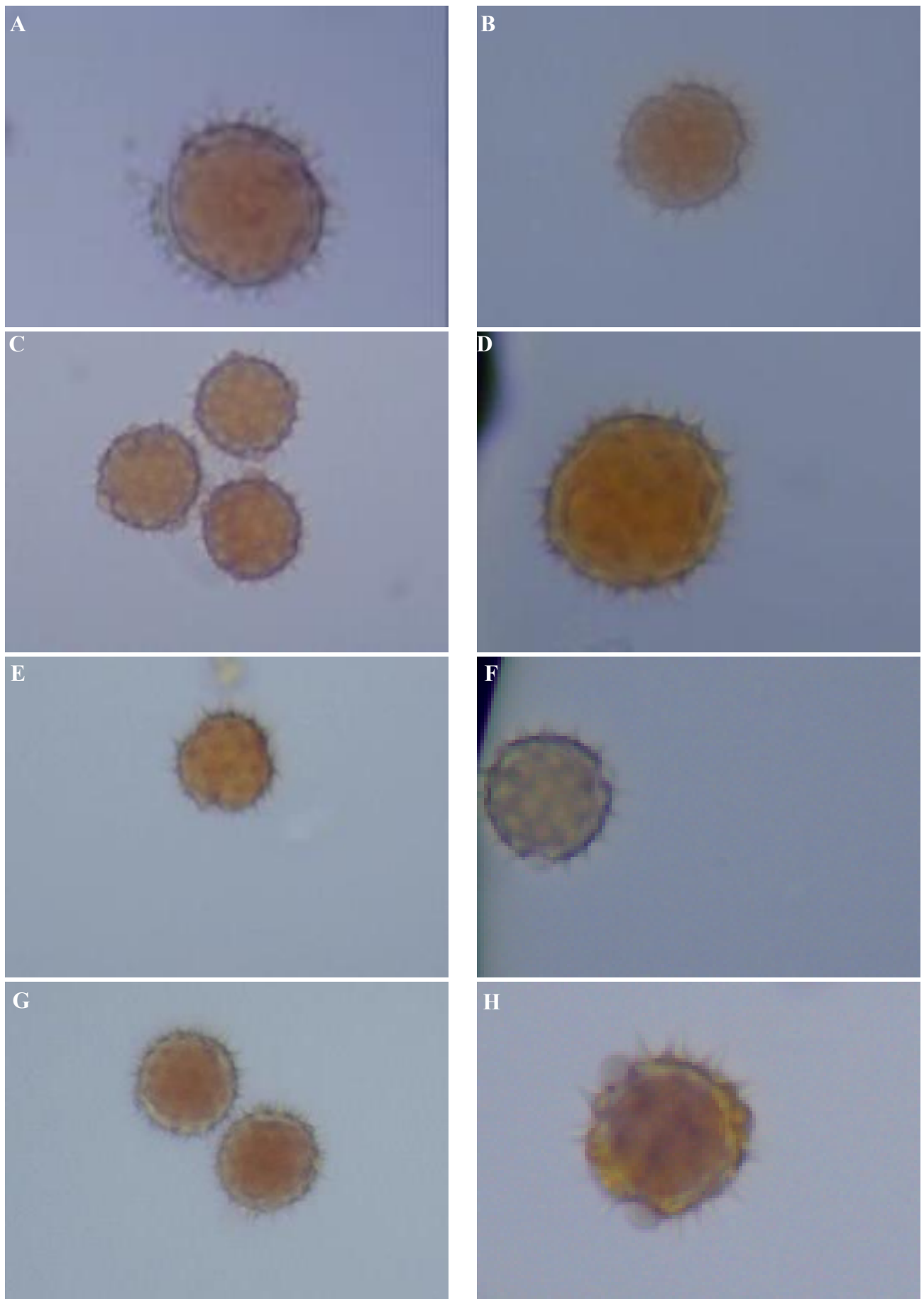


Fig. 3. Light Micrographs of Tribe Coreopsideae Pollen: A. *Bidens bipinnata*, B. *Bidens biternata*, C. *Bidens pilosa*, D. *Bidens tripartita*, E. *Coreopsis lanceolata*, F. *Coreopsis tinctoria*, G. *Cosmos bipinnatus*, H. *Cosmos sulphureus*.

## Key to the species

1. + Tectum punctate in between spines ..... *Cosmos sulphureus*  
 - Tectum Psilate to granulate in between spines ..... 2  
 2. + Tectum psilate in between spines ..... 3  
 - Tectum granulate in between spines ..... 5  
 3. + Pollen sub-prolate ..... *Bidens biternata*  
 - Pollen oblate-spheroidal ..... 4  
 4. + Pollen tricolporate ..... *Bidens cernua*  
 - Pollen tetracolporate ..... *Bidens pilosa*  
 5. + Max. polar length > than 30  $\mu\text{m}$  ..... *Bidens bipinnata*  
 - Max. polar length < than 30  $\mu\text{m}$  ..... *Bidens tripartita*

**Table 2. List of characters, scored for hierarchical cluster analysis for the taxa of the Tribe Coreopsidae.**

Character description	
1.	Length of Polar axis ( $\mu\text{m}$ )
2.	Length of Equatorial axis ( $\mu\text{m}$ )
3.	P/E Ratio
4.	Length of Spines ( $\mu\text{m}$ )
5.	Length of Colpi ( $\mu\text{m}$ )
6.	Length of Mesocolpium ( $\mu\text{m}$ )
7.	Length of Apocolpium ( $\mu\text{m}$ )
8.	Exine Thickness ( $\mu\text{m}$ )
Aperture type	
9.	Tricolporate: Absent (0), Present (1)
10.	Tetracolporate: Absent (0), Present (1)
Shape	
11.	Prolate-Spheroidal: Absent (0), Present (1)
12.	Oblate-Spheroidal: Absent (0), Present (1)
13.	Sub-Prolate: Absent (0), Present (1)
Exine pattern	
14.	Echinate, granulate: Absent (0), Present (1)
15.	Echinate, punctate: Absent (0), Present (1)
16.	Echinate, Psilate to Sub-psilate: Absent (0), Present (1)
17.	Echinate, irregular rugulate: Absent (0), Present (1)

## Discussion

In the present study pollen morphology of 9 species belonging to 3 genera of tribe Coreopsidae from Pakistan and Kashmir were investigated. Most of the pollen characters such as shape Prolate spheroidal, oblate spheroidal, rarely sub-prolate aperture (Tricolporate or Tetracolporate), exine sculpturing (echinate), as well as tectum in between spines (psilate to sub-psilate, punctate, rugulate rarely granulate) were found to be almost similar to the findings of earlier investigations (Mesfin *et al.*, 1995; Blackmore *et al.*, (2009); Stanski *et al.*, 2013).

Mesfin *et al.* (1995) also recorded the perforation at the base of almost all the species of *Bidens* and *Coreopsis*. Exine thickness ranged from 0.93- 2.631  $\mu\text{m}$ , the thickest exine was observed in *Bidens cernua* and the least thickness was found in *Coreopsis lanceolata*. Spines showed great variation and could be taken as a significant character as they were long, prominent, sometimes conical in shape with perforated, swollen, and broad bases (Fig. 4) Micro perforation was also observed in almost all the species. Our finding is in line with that of Blackmore *et al.*, (2009). During this study, the longest spines have been observed in *Bidens biternata* (5  $\mu\text{m}$ ) while the lowest was recorded in *Bidens pilosa* (1.86 $\mu\text{m}$ ).

Similar observation have also been recorded in the other tribes of the family Compositae by Jeffrey (2007) and Blackmore *et al.*, (2009). Mesfin *et al.*, (1995) also reported the irregular rugulate perforations in between spines in *Bidens* and *Coreopsis* species which was similar to our results.

A dendrogram arising from the hierarchical cluster analysis based on 17 pollen's morphological variables accommodated all the 9 species (Fig 5). All the species were grouped in two clusters viz., group I and group II showing the variation in morphological characters.

In the present study two pollen types were recognized on the basis of pollen morphological characters. Such as, Pollen type-I (*Coreopsis tinctoria*-type) had smaller pollen whereas the Pollen type-II (*Bidens pilosa*-type) was characterized by medium pollen grains with few exceptions more or less correlated with the cluster analysis.

Group-I (cluster-I/pollentype-I) comprised 3 of species belonging to 2 genera viz., *Coreopsis* and *Cosmos* characterized by the smaller pollen. This group contained small pollen with a diameter of polar area ranging from 15-20  $\mu\text{m}$  and a diameter of the equatorial axis ranging from 13-21  $\mu\text{m}$  (Fig.6). This pollen type showed considerable variation in pollen shape and exine ornamentation in between spines. The group /pollentype was further divided into two subtypes. Subtype-I (cluster-IA) having two genera each representing single species (*Coreopsis tinctoria* and *Cosmos bipinnatus*). whereas, in subtype - II (cluster-IB) single species of *Coreopsis* (*Coreopsis lanceolata*) was included. *Coreopsis lanceolata* has oblate-spheroidal shape pollen, tectum is irregular rugulate in between spines.

The group-II/cluster-II, characterized by medium sized pollen, comprised of 2 genera and 6 species. Group-II was further divided into two subgroups A & B. Subgroups A accommodated 4 species having larger pollen and long spine *Bidens* (*B. biternata*, *B. cernua*, and *B. tripartita*) and *Cosmos* (*C. sulphureus*). Subgroup B have two species of the genus *Bidens* (*B. bipinnata* and *B. Pilosa*).

*Bidens pilosa*-pollen type (group-II/cluster III) comprised of two genera and 6 species, including 5 species of the genus *Bidens* (*B. biternata*, *B. cernua*, and *B. tripartita*, *B. bipinnata* and *B. pilosa*) and *Cosmos sulphureus*. Group-II is further divided into two subtypes: subtype-1(cluster-IIA) and subtype-II(Cluster-IIB).

Table 1. General Pollen Morphology of Tribe Coreopsideae.

S. No.	Species name	Polar axis (P) (µm)	Equatorial axis (E) (µm)	P/E ratio	Shape	Spine length (µm)	Colpi length (µm)	Mesocolpium (µm)	Apocolpium (µm)	Exine thickness (µm)	Tectum
1.	<i>Bidens bipinnata</i>	(26.31-) 28.94 (-31.57)	(21.05-) 28.50 (-28.94)	1.01	Prolate-spheroidal	(2.63-) 3.069 (-3.94)	(21.05-) 22.80 (-23.68)	(18.42-) 20.17 (-22.36)	(13.15-) 14.25 (-15.78)	(1.97-) 2.25 (-2.63)	Echinata, granulate
2.	<i>Bidens biternata</i>	(26.31-) 28.28 (-30.26)	(21.05-) 23.68 (-22.36)	1.26	Sub-prolate	(4.60-) 5.09 (-5.26)	(13.42-) 14.86 (-15.78)	(15.78-) 16.44 (-17.10)	(10.52-) 11.84 (-13.15)	(1.97-) 2.25 (-2.63)	Echinata punctate
3.	<i>Bidens cernua</i>	(22.36-) 22.92 (-23.68)	(22.36-) 23.30 (-23.68)	0.98	Oblate-spheroidal	(2.63-) 3.35 (-3.94)	(15.78-) 18.41 (-21.05)	(15.78-) 16.22 (-17.10)	(11.18-) 11.62 (-11.84)	(2.63-) 3.65 (5.26)	Echinata Punctate
4.	<i>Bidens pilosa</i>	(23.68-) 29.82 (-31.57)	(28.94-) 28.94 (-28.94)	0.96	Oblate-spheroidal	(1.31-) 1.86 (-2.63)	(13.15-) 16.84 (-18.42)	(10.52-) 11.18 (-11.84)	(10.52-) 13.15 (-15.78)	(1.31-) 1.87 (-2.63)	Echinata Sub-psilate
5.	<i>Bidens tripartita</i>	(23.68-) 25.87 (-27.63)	(21.05-) 24.56 (-27.63)	1.05	Prolate-spheroidal	(2.63-) 2.96 (3.28-)	(15.78-) 17.10 (-18.42)	(13.15-) 15.13 (-15.78)	(10.52-) 12.49 (-13.15)	(1.31-) 1.78 (-2.63)	Echinata punctate
6.	<i>Coreopsis lanceolata</i>	(15.78-) 16.22 (-17.10)	(13.15-) 16.99 (-18.42)	0.95	Oblate-Spheroidal	(2.63-) 3.61 (-3.28)	(10.52-) 10.01 (-11.84)	(10.52-) 11.84 (-13.15)	(7.89-) 7.89 (-7.89)	(0.65-) 0.93 (-1.31)	Echinata irregular rugulate
7.	<i>Coreopsis tinctoria</i>	(18.42-) 18.60 (-19.73)	(15.78-) 16.72 (-18.42)	1.11	Prolate-Spheroidal	(1.97-) 2.30 (-2.63)	(13.15-) 14.47 (-15.78)	(13.15-) 13.48 (-14.47)	(9.21-) 9.86 (-10.52)	(1.31-) 1.59 (-2.63)	Echinata punctate
8.	<i>Cosmos bipinnatus</i>	(18.42-) 19.73 (-21.05)	(18.42-) 19.36 (-21.05)	1.01	Prolate-Spheroidal	(2.63-) 2.74 (-3.28)	(15.78-) 15.78 (-15.78)	(14.47-) 15.52 (-15.78)	(9.21-) 9.47 (-10.52)	(0.65-) 1.03 (-1.31)	Echinata punctate
9.	<i>Cosmos sulphureus</i>	(21.05-) 21.42 (-23.68)	(21.05-) 22.93 (-23.68)	0.93	Oblate-Spheroidal	(3.94-) 4.88 (-5.26)	(15.78-) 15.78 (-15.78)	(15.78-) 15.78 (-15.78)	(7.89-) 9.47 (-10.52)	(1.31-) 1.63 (-2.63)	Echinata punctate

Table 3. Data matrix of the taxa representing tribe Coreopsideae scored for 17 characters present in this table.

Specie name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
<i>B. bipinnata</i>	28.94	28.5	1.01	3.069	22.8	20.17	14.25	2.25	1	0	1	0	0	1	0	0	0
<i>B. biternata</i>	28.28	23.68	1.26	5.09	14.86	16.44	11.84	2.25	1	0	0	0	1	0	1	0	0
<i>B. cernua</i>	22.92	23.3	0.98	3.35	18.41	16.22	11.62	3.65	1	0	0	1	0	0	1	0	0
<i>B. pilosa</i>	29.82	28.94	0.96	1.86	16.84	11.18	13.15	1.87	0	1	0	1	0	0	0	1	0
<i>B. tripartita</i>	25.87	24.56	1.05	2.96	17.1	15.13	12.49	1.78	1	0	1	0	0	0	1	0	0
<i>C. lanceolata</i>	16.22	16.99	0.95	3.61	10.01	11.84	7.89	0.93	1	0	0	1	0	0	1	0	1
<i>C. tinctoria</i>	18.6	16.72	1.11	2.3	14.47	13.48	9.86	1.59	1	0	1	0	0	0	1	0	0
<i>C. bipinnatus</i>	19.73	19.36	1.01	2.74	15.78	15.52	9.47	1.03	1	0	1	0	0	0	1	0	0
<i>C. sulphureus</i>	21.42	22.93	0.93	4.88	15.78	15.78	9.47	1.63	1	0	0	1	0	0	1	0	0

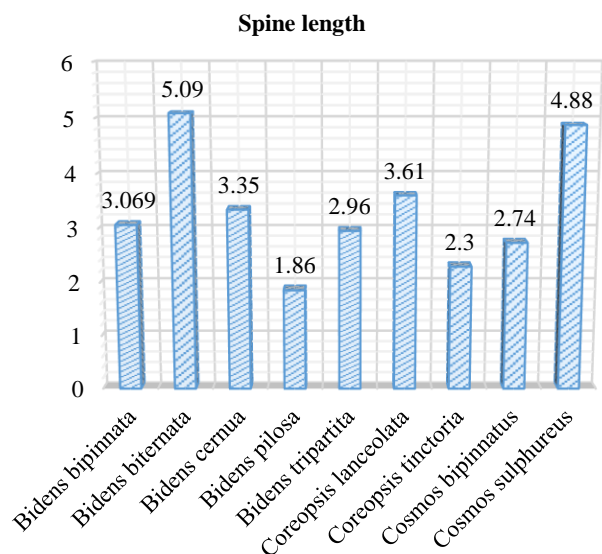


Fig. 4. The graph indicates the variations found in spine length within the Tribe Coreopsideae.

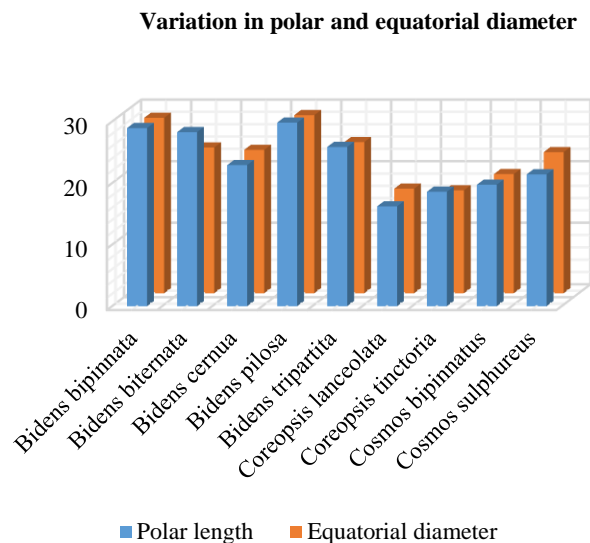


Fig. 5. The graph indicates the variations represented in the polar and equatorial diameter within the Tribe Coreopsideae.

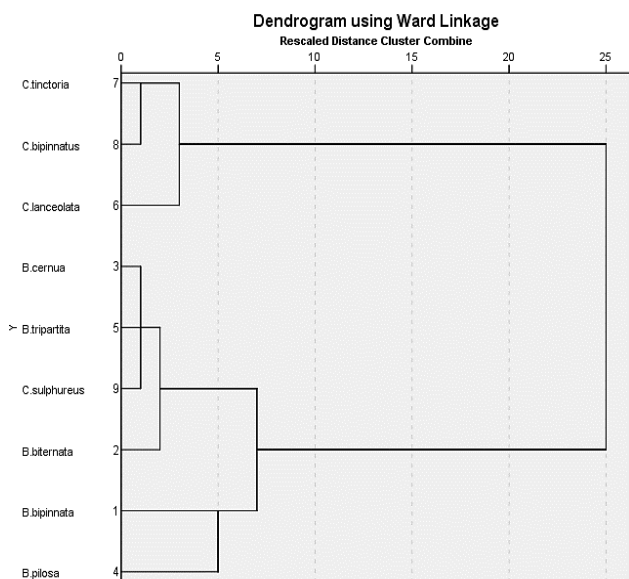


Fig. 6. Dendrogram obtained by Ward's cluster analysis, showing two groups of species belonging to Tribe Coreopsideae, separated on the basis of different pollen characteristics.

Subtype-1 (cluster-IIA) separated on exine ornamentation included *Bidens cernua*, *Bidens tripartita*, and *Cosmos sulphureus* that showed punctate ornamentation in between spines. These species could also be easily delimited based on exine thickness and shape, such as oblate-spheroidal found in both pollen types *Bidens cernua* and *Cosmos sulphureus* while prolate-spheroidal pollen were found in *Bidens tripartita*. In addition, longest spines were also observed. On the other hand, *Bidens biternata* is delimited on the basis of sub-prolate pollen, having the longest spines which are the most distinctive characteristics.

The subtype-II (cluster-II B)-II, comprised of 2 species *Bidens bipinnata* and *Bidens pilosa*, both the species are annual with opposite, 3-pinnatifid, ovate to lanceolate leaves, radiate capitula and angular cypsela. The exine thickness is almost similar in both the species. However,

they can easily be delimited on the basis of number of colpi. In *B. bipinnata* pollen are tricolporate, whereas in *B. pilosa*, tetracolporate pollen were observed. Moreover, in the former species the pollen were spheroidal with punctate ornamentation in between spines, whereas in latter species the pollen were oblate-spheroidal with sub-psilate ornamentation in between the spines.

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