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TAXONOMIC SIGNIFICANCE OF CYPSELAS MORPHOLOGY IN THE TRIBE COREOPSIDEAE (ASTERACEAE) FROM PAKISTAN AND KASHMIR

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The present studies dealt with the cypselas morphology of 11 species representing 4 genera of the tribe Coreopsideae (Asteraceae) from Pakistan and Kashmir. The following parameters of the cypselas viz., color, shape, surface, degree of pubescence, base, presence or absence of beak, awns, wings, and carpopodium have been examined by stereomicroscope and scanning electron microscope (SEM). The detailed cypselas morphological description of all the studied taxa along artificial keys of genera and species are given. SEM micrographs are provided to clearly show the diagnostic characters. The outcome results of cypselas morphological characters have been analyzed statistically by using SPSS 21, to search out the correlation between cypselas characters and gross morphological characters as well as to find the close affinities among the studied taxa of the tribe Coreopsideae from the study area. The present findings conclude that the cypsela characters can be used as diagnostic taxonomic characters for the delimitation of taxa.

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ارزش تاکزونومیکی صفات ریختی فندقه‌ها در قبیله Coreopsideae از خانواده Asteraceae از پاکستان و کشمیر

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مطالعات حاضر صفات ریختی فندقه در ۱۱ گونه از چهار جنس از قبیله Coreopsideae از خانواده Asteraceae را در پاکستان و کشمیر مورد بررسی قرار می‌دهد. صفات ریختی مختلف از جمله رنگ، شکل، سطح، درجه بلوغ، قاعده، وجود یا عدم وجود منقار، ریشک‌ها، بال‌ها و کارپوپودیوم توسط استریومیکروسکوپ و میکروسکوپ الکترونی روبشی (SEM) مورد بررسی قرار گرفته‌اند. شرح دقیق مورفولوژیکی cypselas از همه گونه‌های مورد مطالعه همراه با کلیدهای شناسایی جنس‌ها و گونه‌ها ارائه شده است. عکس‌های SEM برای نشان دادن واضح صفات تشخیصی ارائه شده است. نتایج حاصل از صفات مورفولوژیکی cypselas با استفاده از SPSS ۲۱ برای جستجوی همبستگی بین خصوصیات فندقه‌ها و خصوصیات ریخت‌شناسی ناخالص و همچنین برای یافتن قرابت‌های نزدیک در میان گونه‌های مورد مطالعه قبیله Coreopsideae از منطقه مورد مطالعه مورد تجزیه و تحلیل آماری قرار گرفته است. یافته‌های این مطالعه نشان می‌دهد که صفات فندقه را می‌توان به عنوان صفات تشخیصی در طبقه‌بندی و برای تعیین حدود گونه‌ها استفاده کرد.

INTRODUCTION

Coreopsideae is a well-known tribe of Asteraceae for its ornamental value. The tribe comprises of ca 25-30 genera and 500-550 species, present all over the world (Karis & Ryding 1994, Kaderert & Jaffrey 2007, Mort & al. 2008)**.** However, the main center of diversity is the New World particularly in Mexico, Central America, and North, and South America, and diversified by dispersal from pantropical regions of North America to dry regions of tropical and temperate regions of Asia and Africa (Crawford & al. 2009). In Pakistan, the tribe is represented by 5 genera and 12 species usually distributed in the northern areas (Chitral, Swat, Hazara, Hunza, Gilgit, Abbottabad, Azad Kashmir) and South Waziristan. A few species were also represented from various regions of Balochistan and Sindh (Qaiser & Perveen 2021).

The tribe Coreopsideae was initially described as a sub-group of Heliantheae by Cassini (1829), followed by Bentham & Hooker (1973), Hoffman (1890), Stuessy (1977), Turner & Powell (1977), and Robinson (1981). However, recent molecular studies changed the circumscription of taxa belonging to the Coreopsideae. Ryding & Bremer (1992) were the first who studied the molecular phylogeny of Coreopsideae. Later Funk & al. (2001) and Panero & Funk (2002) studied molecular phylogenetic studies of Heliantheae and its related tribes, and recognized that the Coreopsideae was an independent tribe. Mart & al. (2008) conducted phylogenetic studies of Coreopsideae inferred from nuclear and plastid DNA.

Coreopsideae not only showed diversification in their morphological characters but also exhibits a variety of cypselas morphological characters which are useful as a source of various taxonomic works. Morphologically, this tribe can be easily delimited by having dimorphic involucre phyllaries with prominent resin ducts; cypselas are radially compressed, with prominent reddish resin ducts in both ray and disc florets (Robinson 1981). Many species of this tribe are very useful for their horticulture as well as medicinal potential; for instance, *Coreopsis* and *Cosmos* are commonly used as ornamentals (Qasier & Perveen 2021). Leaf extract of *Bidens pilosa* shows activity against diarrhea (Mabberley 2008) and has a useful meditative value for cough (Burkill 1935). *Glossocardia bosvallia* is also a source of essential oils and the extract of the whole plant is significantly effective against *Staphylococcus*, *Streptococcus*, *Salmonella,* and *Klebsiella* (Ramakrishann & al. 2013, Darshani 2021).

Table 1. List of voucher specimens examined for cypsela morphology.

Taxa	Collection Data
Bidens bipinnata	*Mohindar Nath* 47017 (KUH); *Anjum Perveen* 2035 (KUH); *Anjum Perveen* 2085 (KUH); *Anjum Perveen* 2083 (KUH); *Anjum Perveen* s.n (KUH).
Bidens biternata	*M. Qaiser & Rizwan Y.Hashmi* 7923 (KUH); *Abdul Ghafoor & Tahir Ali* 3659 (KUH); *Kamal Athar Malik & S. Nazimuddin* 1880 (KUH); *Y. Nasir & Fazal Bhatti* 10148 (KUH); *S. Omer & M. Qaiser* 2274 (KUH); *M.Qaiser & Abdul Ghafoor* 4852 (KUH); *Abdul Ghafoor & Tahir Ali* 3659 (KUH); *M. Qaiser & Rizwan Yousuf Hashmi* 7912 (KUH); *M. Qaiser & Abdul Ghafoor* 4509 (KUH); *S. Omer & M. Qasim* 2233 (KUH).
Bidens cernua	*R. R. Stewart* 7499 (RAW); *R. R. Stewart & A. Rehman* 25436 (RAW); *Ziaullah* 1130 (KUH, RAW); *R.R. Stewart & I.D. Stewart* 6112 (RAW).
Bidens pilosa	*Hinna Fazal* 92830 (KUH); *Shabir Ijaz* 317 (KUH); *S. Omer & M. Qaiser* 2541 (KUH); *M. Qaiser* 255 (KUH); *Tahir Ali, M. Qaiser & M. Ajmal Khan* 69 (KUH).
Bidens tripartita	*M. Qaiser & Farooqi* 186 (KUH); *M. Qaiser & A. Ghafoor* 1702 (KUH); *A.Ghafoor & Tahir Ali* 3915 (KUH)
Coreopsis lanceolata	*Alma L.Moldenke & Harold N.Moldenke* 28493 (KUH); *Bushreen, Moin & Nadeem* 14 (KUH).
Coreopsis tinctoria	*Rabia Akhlaq & Taba Rauf* s.n (KUH); *Rabia Akhlaq & Taba Rauf* s.n (KUH).
Cosmos bipinnatus	*Mr.Abrar Hussain* 47562 (KUH); *Mr.Abrar Hussain* 47563 (KUH); *Zamarrud & Sultanul Abedin* 811 (KUH); *Zamarrud & Sultanul Abedin* 813 (KUH); *Zamarrud & Sultanul Abedin* 812 (KUH); *Zamarrud & Sultanul Abedin* 809 (KUH).
Cosmos sulfureus	*Zamarrud & Sultanul Abedin* 793 (KUH); *Zamarrud & Sultanul Abedin* 791 (KUH); *Zamarrud & Sultanul Abedin* 790 (KUH); *Zamarrud & Sultanul Abedin* 822 (KUH); *Zamarrud & Sultanul Abedin* 792 (KUH)
Glossocardia bidens	*Measurements extracted from the Flora of Pakistan,* no.224
Glossocardia bosvallia	*Rubina Akhter* 319 (RAW).

In Asteraceae, morphological characters of cypselas and pappus are found to be quite significant in taxonomic classification at the tribal level (Bremer 1994, Judd & al. 2002, Breistwieser & Ward 2005, Talukdar 2008, Frangiole-Pallone & Antonio de Souza 2014, Qaiser & Abid 2021). In addition, cypsela characters also contributed to the recognition of new genera, species, and combinations. Kallersjo (1985) studied 40 taxa of Anthemideae (Asteraceae) and recognized 25 new combinations based on cypselas characters. Bano & Qaiser (2009, 2010 & 2011) studied 33 species of Cichorieae and recognized new species based on cypselas morphology along with other morphological characters. Besides, comprehensive literatures are available on the morphological study of cypselas of various tribes. However, there is no comprehensive study of cypselas of Coreopsideae available, particularly from our region. Moreover, some previous reports are available on a few species of Coreopsideae which were previously placed under the tribe Heliantheae. For instance, Mukherjee & Jana (2014) studied cypsela characters of three species of *Bidens* and *Cosmos* and concluded that members of Coreopsideae showed great variation in morphological and anatomical features. Panero (2007) recognized that cypselas of Coreopsideae are usually isomorphic, varied in shape, presence, or absence of wings and awns.

Therefore, the present attempt has been made to determine whether cypselas morphological characters have taxonomic significance amongst the studied species by examining the detailed cypselas characters of Coreopsideae. Furthermore, to find the close affinities among taxa both at the generic and species level by cluster analysis.

MATERIALS AND METHODS

Mature cypselas of 11 species belonging to 4 genera namely *Bidens*, *Coreopsis*, *Cosmos,* and *Glossocardia* were mostly collected from herbarium materials. However, in a few cases, cypselae were also collected from fresh specimens. While there was insufficient availability of *Glossocardia* specimens, the data were extracted from the flora of Pakistan (Sultan 2021). The following parameters such as the main body, wings, beak, awns, and carpopodium were studied under a stereo microscope (Nikon Type 102,) and for detailed observations of cypselas, a scanning electron microscope (Joel JSM-6380A) was employed. For SEM, mature cypselas were directly mounted on a metallic stub with the help of double adhesive tape and coated with gold for a period of 6 minutes in a sputtering chamber and then the species were observed in SEM. Mostly 10 plants/ species and 10 cypselas/ plants were studied. It should be noted that the SEM micrographs for the two species of the genus *Glossocardia* could not be prepared, because of the unavailability of cypselas. The voucher specimens are kept in the herbarium of the University of Karachi (KUH). The details of examined materials are given in Table 1. The following characters were studied under a stereo and SEM microscope (Tables 2-5).

Cypselae: Shape, color, size, surface, ribs, appearance, number of ribs, angle, margins, apex, base, and hilum

Wings: Status, number, size, surface, and color

Beak: Status, number, size, surface, and color

Awn: Status, number, length, surface, shape, margins, and color

Carpopodium: The form, development, shape, symmetry, and diameter of carpopodium and foramen

The descriptive terminology used here is based on Strean (1992) and Roque & al. (2009).

Cluster analysis

The agglomerative cluster analysis was carried out by selecting the Euclidean distance as the resemblance function and Ward's method for a group linkage method (McCune & Grace 2002). This analysis is based on contrasting cypselas morphological characters to show the group structure in the examined taxa of the tribe Coreopsideae. The computations were performed using the computer program PC-ORD (version 6.0), (McCune & Grace 2002, Peck 2010). A total of 16 cypselas characters were selected (6 quantitative and 10 qualitative) to differentiate the studied taxa of the tribe Coreopsideae. The qualitative characters were recorded in the binary state i.e. terms of 1 and 2. In a few cases, multiple states were also used i.e. 1, 2, and 3. While in case of absence or presence, characters were coded as 0 and 1 respectively. The characters and character states used for performing hierarchical clustering are listed in Tables 6 and 7.

Table 2. Summarized representation of cypselas morphology in related genera of Coreopsideae.

Character	*Bidens*	*Coreopsis*	*Cosmos*	*Glossocardia*
Cypselas	Isomorphic to dimorphic	Isomorphic	Isomorphic	Isomorphic
Beak	Absent	Absent	Present	Absent
Wings	Absent	Present	Absent	Absent
Awns	Present	Present	present or Absent	Present
Ribs	4 to 5	only 1	3	1 to 3

Table 3. Cypselas characteristics of species of tribe Coreopsideae.

Cypselas										Beak				
Species	**Shape**	**Color**	**Length (mm)**	**Breadth (mm)**	**Surface**	**Number of ribs**	**Angle**	**Apex**	**Hilum**	**Status**	**Number**	**Length (mm)**	**Surface Texture**	**Colour**
Bidens bipinnata	Linear	Black	12-21	0.4-0.8	Glabrous	4	Quadrangular	Not truncate	Lateral	Absent	-	-	-	-
Bidens biternata	Linear	Black	18-22	1-1.5	Pubescent, Muricate	5	Quadrangular	Not truncate	Basal	Absent	-	-	-	-
Bidens cernua	Cuneate	Blackish brown	5-9	2-2.5	Pubescent, Muricate	4	Quadrangular	Truncate	Basal	Absent	-	-	-	-
Bidens pilosa	Linear	Apically brown, Black	14-20	0.5-1	Scabrid, Muricate	5	Quadrangular	Not truncate	Sub-basal	Absent	-	-	-	-
Bidens tripartita	Cuneate	Blackish brown	8-13	1.5-2	Glabrous, Muricate	4	Triangular	Truncate	Basal	Absent	-	-	-	-
Coreopsis lanceolata	oblanceolate	Black, brown granulated	2.5-3	1-1.5	Glabrous, Papillose, Muricate	1	Triangular	Not truncate	Lateral	Absent	-	-	-	-
Coreopsis tinctoria	oblong	Dark brown	2-2.5	1-1.5	Glabrous, Papillose, Muricate	-	Triangular	Not truncate	Basal	Absent	-	-	-	-
Cosmos bipinnata	Lanceolate	Dark brown, mottled	7-14	1.2-1.8	Glabrous	3	Triangular	Not truncate	Basal	Present	1	1-2	Glabrous	Black
Cosmos sulfureus	Linear	Black	16-24	0.8-1	Scabrid	3	Triangular	Not truncate	Lateral	Present	1	6-11	Antrorsely hispid	Brown
Glossocardia bidens	Lanceolate	Black	5-13	0.7-1.2	Glabrous	3	Quadrangular	Truncate	Basal	Absent	-	-	-	-
Glossocardia bosvallia	Linear	Brown	7-9	0.8-1.2	Glabrous	1	Quadrangular	Truncate	Basal	Absent	-	-	-	-

RESULTS

The detailed qualitative and quantitative cypselas morphological characters of studied taxa belonging to Coreopsideae are presented in summarized Tables 2 & 5 and shown in Figs. 1, 2, and 3. The general characters are given below:

Cypselas varied in shape as linear, oblanceolate to lanceolate, and oblong; color varied from black, blackish brown, brown-dark brown; measures, 1-24 mm long, 0.5-2.5 mm wide; glabrous, scabrid, papillose, muricate; margins entire, sometimes thickened or retrorsely barbed, truncate; ribs 1-5 in numbers, triangular - quadrangular, compressed; hilum basal or lateral, (rarely sub-basal in *Bidens pilosa*). Wings present (*Coreopsis*) or absent, 2 in number, 2-3 mm long, 0.2 - 0.5 mm wide, glabrous, brown. Beak mostly absent, except in *Cosmos*, 1-11 mm long, glabrous or antrorsely hispid, black or brown. Awns present or absent, 2-4 in number, 0.5-6 mm long, glabrous, barbed, retrorse (downward), sometimes scaly or slender, entire, pale yellow, sometimes light to dark brown. Carpopodium distinct, developed, with a complete ring, circular to elliptical or angular, symmetric or asymmetric, 249-800 µm in diameter, foramen distinct, 200-657 µm in diameter.

Analysis

The analysis showed that examined taxa were divided into two Clusters: Cluster I and Cluster II. Cluster I consisted of 7 species (63.63% of total species) distinguished by the presence of retrorsely barbed awns. While Cluster II accommodated 4 species (36.36% of total species) differed from Cluster I by the presence of glabrous awns or awns absent. Furthermore, Cluster I grouped into two subclusters with an optimal number of 3-4 species in each subgroup. Sub-Cluster IA accommodated 4 species of *Bidens* viz., *B. biternata, B. cernua, B. Pilosa,* and *B. bipinnata*, characterized by 4-5 ribbed with 2-4 (5) awns. Whereas Sub-Cluster IB accommodated 3 species *B. tripartita*, *Glossocardia bidens,* and *Glossocardia bosvallia*, characterized by usually 1-3 (4) ribbed with 2 awns.

Cluster II is further divided into subclusters IIA and IIB. Sub-Cluster IIA comprised two species of *Coreopsis*: *C. tinctoria* and *C. lanceolata* distinguished by winged and beakless cypselas. Awns are usually absent or if present, then awns are glabrous. Whereas Sub-Cluster IIB is characterized by wingless and beaked cypselas to which the genus *Cosmos* including *C. bipinnatus* and *C. sulfureus* belonged (Fig. 4).

Key to the genera

1. Cypselas beaked .... 1. Cosmos
- Cypselas not beaked 2
2. Cypselas winged, papillose, usually triangular ……..
.... 2. *Coreopsis*
- Cypselas are neither winged nor papillose, mostly quadrangular (rarely triangular) 3
3. Cypselas up to 22 mm long, up to 2.5 mm wide, 4-5 ribbed. Awns 2-4 (5) .... 3. *Bidens*
- Cypselas up to 13 mm long, less than 1.5 mm wide, 1-3 ribbed. Awns only 2 .... 4.*Glossocardia*

1. *Cosmos* Cav.

Two species including *C. bipinnatus* and *C. sulfureus* were examined. In these species cypselas are linear-lanceolate, dark brown-mottled or black, 7-24 mm long, 1-1.5 (-2) mm wide, glabrous or scabrid, 3-ribbed, triangular, pubescent at the base, brown, hilum basal or lateral. Wings absent. Beak only one, 1-11 mm long, glabrous or antrorsely hispid, black or brown. Awns are present or absent, if present then 2 in number, 0.5-1 mm long, glabrous, scaly, filiform, entire, pale yellow. Pappus absent. Carpopodium distinctly developed into a complete ring, circular or angular, symmetric or asymmetric, 393-709 µm in diameter, foramen 291-643 µm in diameter.

Key to the species of *Cosmos*

1. Cypselas oblanceolate, 7-14 mm long, glabrous, hilum basal. Beak 1-2 mm long, stout, glabrous. Awns absent. Carpopodium circular .... 1. *C. bipinnata*
- Cypselas linear, 16-24 mm long, scabrid, hilum lateral. Beak 6-11 mm long, slender, antrorsely hispid. Awns present. Carpopodium angular……….. 2. *C. sulfureus*

1. *Cosmos bipinnatus* Cav. (Fig. 3 E-H)

Cypselas lanceolate, dark brown-mottled, 7-14 mm long, 1.2-1.8 mm wide, glabrous, triangular, pubescent at the base, hilum basal, attenuate into 1-2 mm long, glabrous, black, stout beak. Awns absent. Carpopodium circular, symmetric, ca 393 µm in diameter, foramen ca. 291 µm in diameter.

2. *Cosmos sulfureus* Cav. (Fig. 3 I-L)

Cypselas linear - fusiform, black, 16-24 mm long, 0.8-1 mm wide, scabrid, ribs triangular, brown at the base, hilum lateral, attenuate into 6-11 mm long, antrorsely hispid, brown, slender beak. Awns present, 2 in number, 0.5-1 mm long, glabrous, scaly, filiform, entire, pale yellow, deciduous. Carpopodium angular, asymmetric, ca 709 µm in diameter, foramen ca 643 µm in diameter.

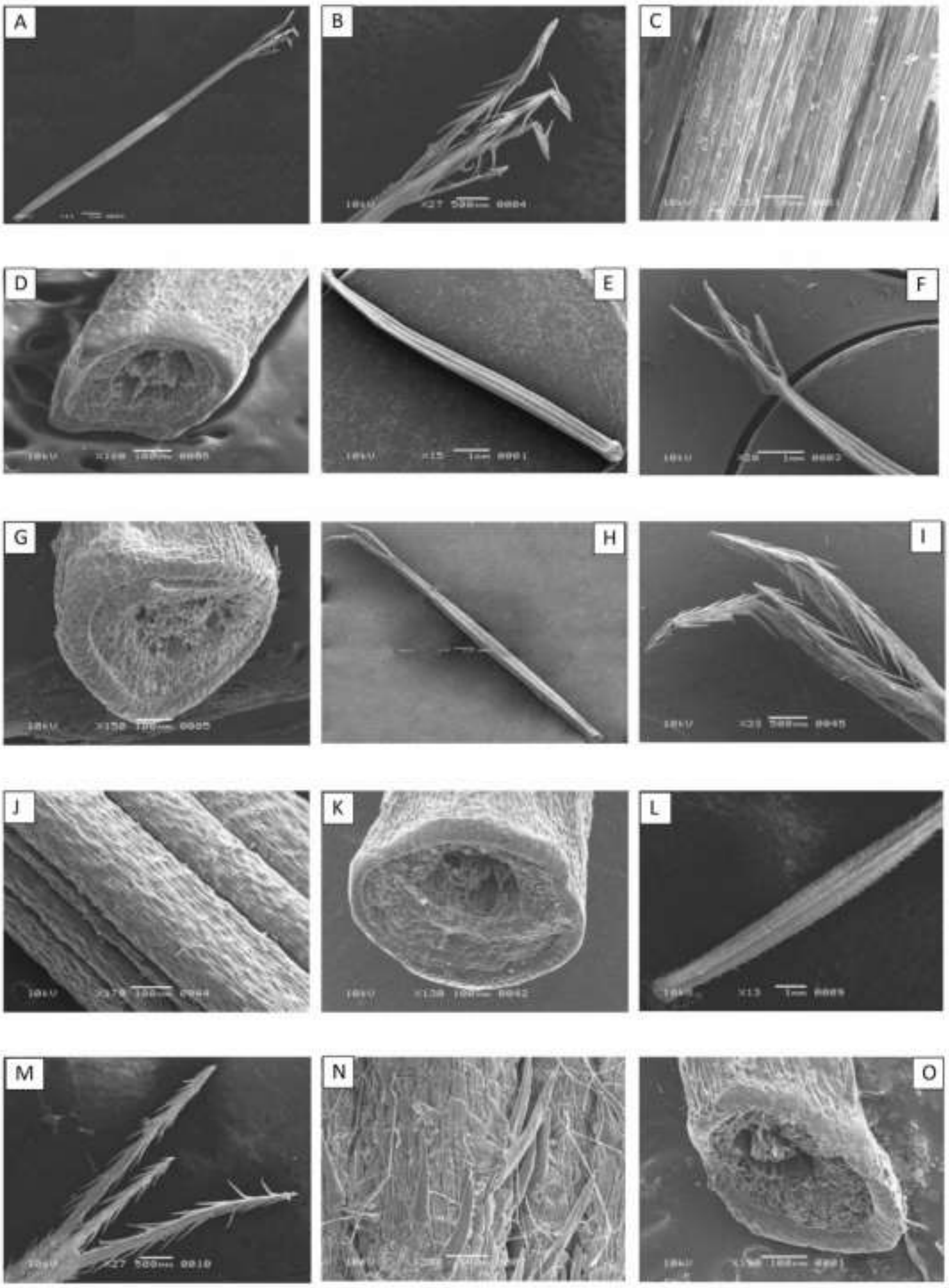

Fig. 1. Scanning Electron Micrographs (SEM) of the cypselas morphology. *Bidens bipinnata*: A, central cypsela; B, awns; C, surface; D, carpopodium. E, peripheral cypsela; F, awns; G, carpopodium. *Bidens biternata*: H, central cypsela; I, awns; J, surface; K, carpopodium; L, peripheral cypsela; M, awns; N, surface; O, carpopodium. (Scale bar: A, E, F, H, L = 1mm; B, I, M = 500µm; C, N = 50µm; D, G, J, K, O = 100µm).

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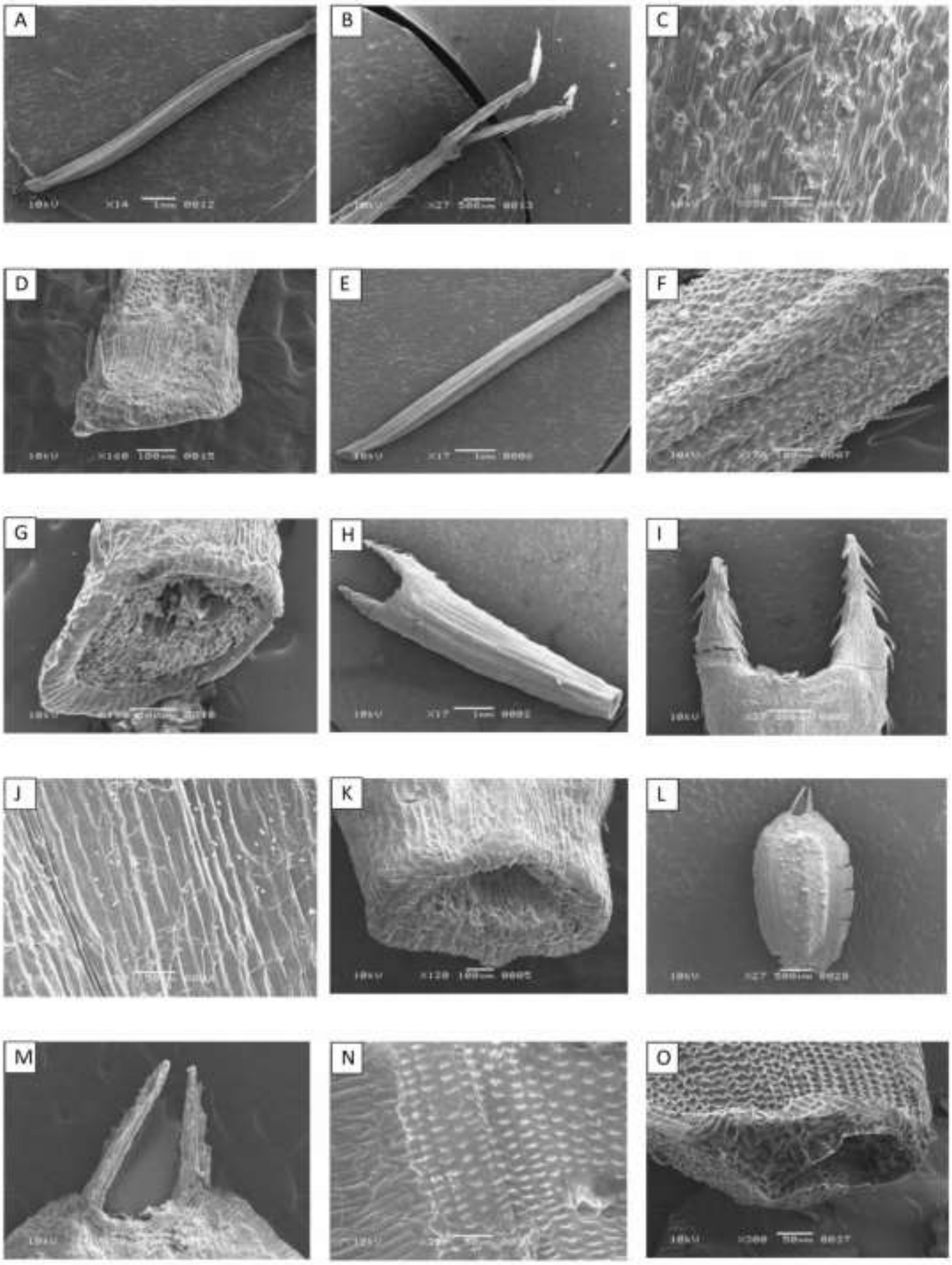

Fig. 2. Scanning Electron Micrographs (SEM) of cypselas. *Bidens pilosa*: A, central cypsela; B, awns; C, surface; D, carpopodium; E, peripheral cypsela; F, surface; G, carpopodium. *Bidens tripartita*: H, cypsela; I, awns; J, surface; K, carpopodium. *Coreopsis lanceolata*: L, cypselas; M, awns; N, surface; O, carpopodium. (Scale bar: A, E, H = 1µm; C, J, N, O = 50µm; D, F, G, I, K= 100µm; B, J, L = 500µm).

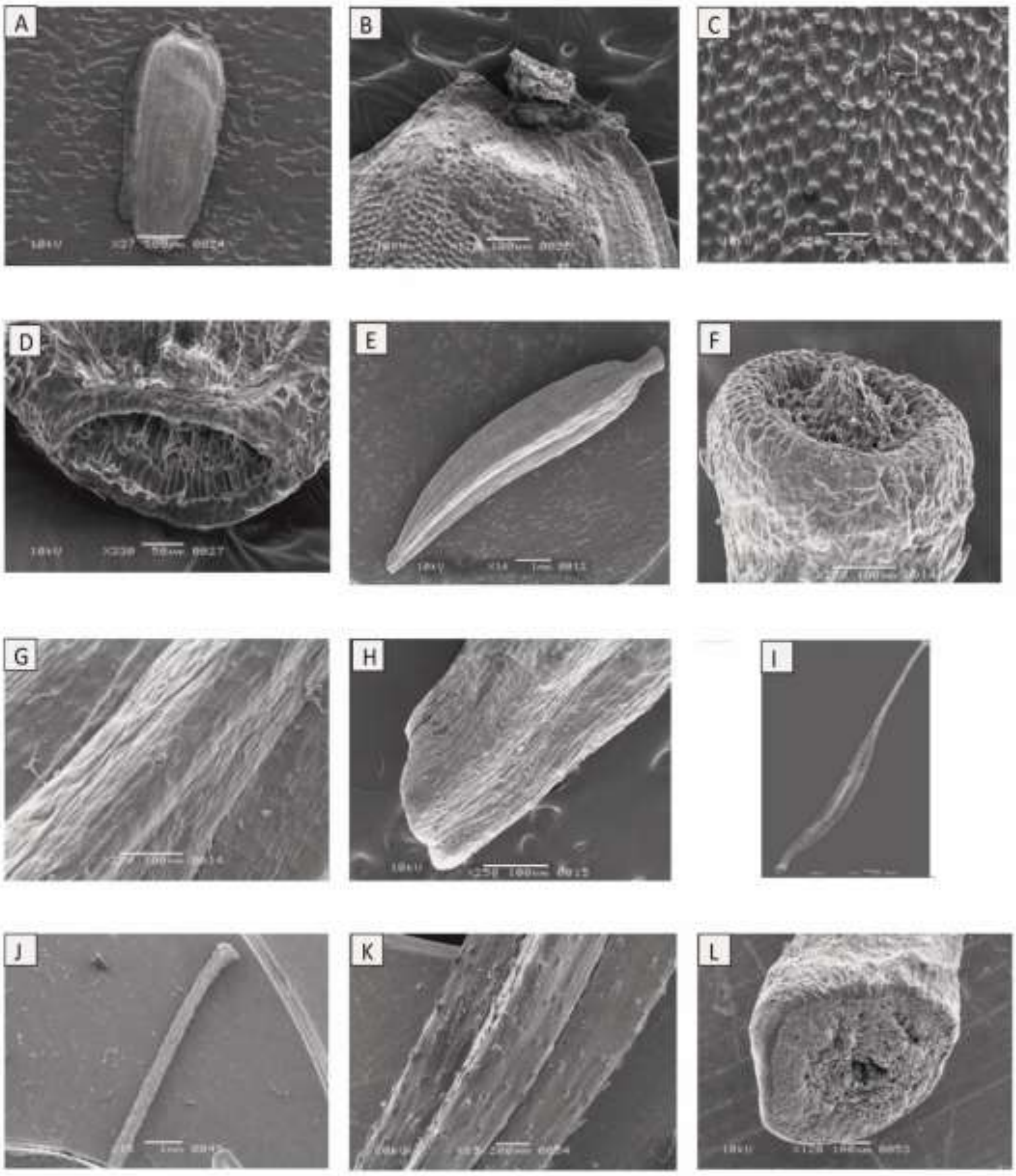

Fig. 3. Scanning Electron Micrographs (SEM) of cypselas. *Coreopsis tinctoria*: A, cypsela; B, awns; C, surface; D, carpopodium. *Cosmos bipinnatus*: E, cypsela; F, beak; G, surface; H, carpopodium. *Cosmos sulfureus*: I, cypsela; J, beak; K, surface; L, carpopodium. (Scale bar: A = 500µm; K= 200µm; B, F, G, H, L = 100µm; C, D = 50µm; E, I, J = 1µm).

2. *Coreopsis* L.

Two species including *C. lanceolata* and *C. tinctoria* were examined. Cypselas were oblanceolate - oblong, black or dark brown, 2-3 mm long, 1-1.5 mm wide, glabrous, papillose, muricate, ribs present or absent, if present then 1-median ribbed, triangular, compressed, curved inwards, hilum lateral or basal. Wings 2, 2-3 mm long, 0.2-0.5 mm wide, glabrous, brown. Beak absent. Awns 2, 0.5-1 mm long, glabrous, slender, entire, light brown, persistent or deciduous. Pappus absent. Carpopodium distinctly developed into a complete ring, without interruptions, elliptical, asymmetric, 249-260 µm in diameter, foramen 200-218 µm in diameter.

Key to the species of *Coreopsis*

1. Cypselas oblanceolate, black, with mid-dorsal rib, curved inwards, hilum lateral .... 1. *C. lanceolata*
- Cypselas oblong, dark brown, without rib, not curved inwards, hilum basal .... 2. *C. tinctoria*

1. *Coreopsis lanceolata* L. **(**Fig. 2 L-O)

Cypselas oblanceolate, black, brown tuberculate, 2.5-3 mm long, 1-1.5 mm wide (excluding wings), distinctly mid-dorsally 1-ribbed, curved inwards, hilum lateral. Wings 2.5-3 mm long, 0.2-0.5 mm wide. Awns persistent. Carpopodium elliptic, asymmetric, ca 249 µm in diameter, foramen ca 200 µm in diameter.

2. *Coreopsis tinctoria* Nutt**. (**Fig. 3 A-D)

Cypselas oblong, dark brown, 2-2.5 mm long, 1-1.5 mm wide (excluding wings), not ribbed, not curved inwards, hilum basal. Wings 2-2.5 mm long, 0.2-0.5 mm wide. Awns deciduous. Carpopodium elliptic, asymmetric, ca 260 µm in diameter, foramen ca 218 µm in diameter.

3. *Bidens* L.

Five species including *B. bipinnata, B. tripartita, B. cernua, B. pilosa, B. biternata* were examined. Cypselas were dimorphic (central and peripheral), linear to cuneate, black or blackish-brown, central cypselas 5-22 mm long, peripheral cypselas 8-14 mm long, 0.5-2.5 mm wide, scabrid or glabrous, muricate, ribs prominent, 4-5, unevenly distributed, triangular to quadrangular, margins thickened or retrorsely barbed, apex truncate, hilum basal or lateral, sub-basal in *Bidens pilosa*. Wings absent. Beak absent. Awns 2-4 (5), 2-6 mm long, barbed, retrorsely barbed, slender, entire, pale yellow or light brown to dark brown. Pappus absent. Carpopodium distinct, well developed into a complete ring, without interruptions, circular or angular, symmetric or asymmetric, 418-800 µm in diameter, foramen distinct, 313-657 µm in diameter.

Key to the species of *Bidens*

1. Cypselas homomorphic (only central), cuneate, truncate, more than 1.5 mm wide 2
- Cypselas dimorphic (central and peripheral), linear, not truncate, less than 1mm wide 3
2. Cypselas up to 9 mm long, quadrangular, 2-4 awned .... 1. *B. cernua*
- Cypselas up to 13 mm long, triangular, persistently 2 awned .... 2. *B. tripartita*
3. Cypselas glabrous, not muricate. Awn up to 6 mm long, pale yellow .... 3. *B. bipinnata*
- Cypselas scabrid or pubescent, muricate. Awns up to 5 mm long, light brown 4
4. Cypselas up to 20 mm long, 1 mm wide, brown at the apex. Awns 2-3. Carpopodium angular, asymmetric .... 4. *B. pilosa*
- Cypselas up to 22 mm long, 1.5 mm wide, and black at the apex. Awns 2-4. Carpopodium circular, symmetric .... 5. *B. biternata*

1. *Bidens cernua* L.

Cypselas only central, cuneate, blackish brown, 5-9 mm long, 2-2.5 mm wide, pubescent, muricate, 4-5 ribbed, quadrangular, margins thickened, truncate, hilum basal. Awns 2-4, 2-5 mm long, light brown.

2. *Bidens tripartita* L. (Fig. 2 H-K)

Cypselas only central, oblong to cuneate, blackish brown, 8-13 mm long, 1.5-2 mm wide, glabrous, margins muricate retrorsely barbed, truncate apically, triangular, 4-5 ribbed, hilum basal. Awns only 2, 2-4 mm long, barbed retrorse, brown. Carpopodium angular, asymmetric, ca 800 µm in diameter, foramen ca 572 µm in diameter.

3. *Bidens bipinnata* L. **(**Fig. 1 A-G)

Cypselas central and peripheral, black, 0.4-0.8 mm wide, 4 ribbed, hilum lateral. Awns 2-4, 3-6 mm long, pale yellow. Carpopodium distinctly developed into a complete ring, without interruptions, angular, asymmetric. Central cypselas linear - slender, 12-21 mm long, glabrous, quadrangular. Carpopodium ca 418 µm in diameter, foramen ca 313 µm in diameter.

Peripheral cypselas linear, black, 9-13 mm long, glabrous to slightly pubescent, ribbed 4, triangular. Carpopodium ca 615 µm in diameter, foramen ca 416 µm in diameter.

4. *Bidens pilosa* L. (Fig. 2 A-G)

Cypselas central and peripheral, linear, black with brown apex, 0.5-1 mm wide, scabrid, muricate, 5 ribbed, quadrangular. Awns 2-5 mm long, brown. Carpopodium distinctly developed into a complete ring, without interruption, angular, asymmetric. Central cypselas 14-20 mm long, hilum sub-basal. Awns 3. Carpopodium ca 505 µm in diameter, foramen ca 355 µm in diameter.

Peripheral cypselas 8-12 mm long, hilum basal. Awns 2-3.

5. Bidens biternata (Lour.) Merr. & Sherff (Fig. 1 H-O)

Cypselas central and peripheral, black, 1-1.5 mm wide, muricate, 5 ribbed, quadrangular, hilum lateral. Awns 2-5 mm long, light brown. Central cypselas linear - fusiform, 18-22 mm long, pubescent. Awns 2-4. Carpopodium circular, symmetric, ca 773 µm in diameter, and foramen ca 657 µm in diameter.

Peripheral cypselas linear, 10-14 mm long, scabrid. Awns 4. Carpopodium angular, asymmetric, ca 404 µm in diameter, foramen ca 318 µm in diameter.

4. *Glossocardia* Cass.

Two species including *G. bidens* and *G. bosvallia* Cypselas linear-oblong or lanceolate, black or brown, 5-13 mm long, 0.8-1.2 mm wide, glabrous to sparsely - densely pubescent or setose, 1 or 3- ribbed, quadrangular, obcompressed, truncate apically, hilum basal. Wings absent. Beak absent. Awns 2, 3-5 mm long, retrorsely barbed or sparsely setose, filiform or slender, entire, light to dark brown. Pappus absent.

Key to the species of *Glossocardia*

1. Cypselas linear-oblong, black, up to 13 mm long, margins usually smooth or slightly barbed, 1-3 ribbed. Awns retrorsely barbed, slender .... 1. *G. bidens*

- Cypselas lanceolate, brown, up to 9 mm long, densely setose (along the margins), 1 ribbed. Awns sparsely setose, filiform .... 2. *G. bosvallia*

1. *Glossocardia bidens* (Retz.) Veldkamp

Cypselas lanceolate, black, 5-8 (-13) mm long, 0.7-1.2 mm wide, glabrous, margins smooth or slightly barbed to densely pubescent, distinct, 1-3 ribbed, quadrangular, hilum basal. Awns 2 in number, up to 5 mm long, retrorsely barbed, slender, entire, light brown.

2. *Glossocardia bosvallia* (L. f.) DC.

Cypselas linear rarely oblong, brown, 7-9 mm long, up to 1 mm wide, densely setose along the margins, hair up to 2 mm long, 1-ribbed, quadrangular, hilum basal. Awns 2 in number, 3-5 mm long, sparsely setose, filiform, entire, dark brown.

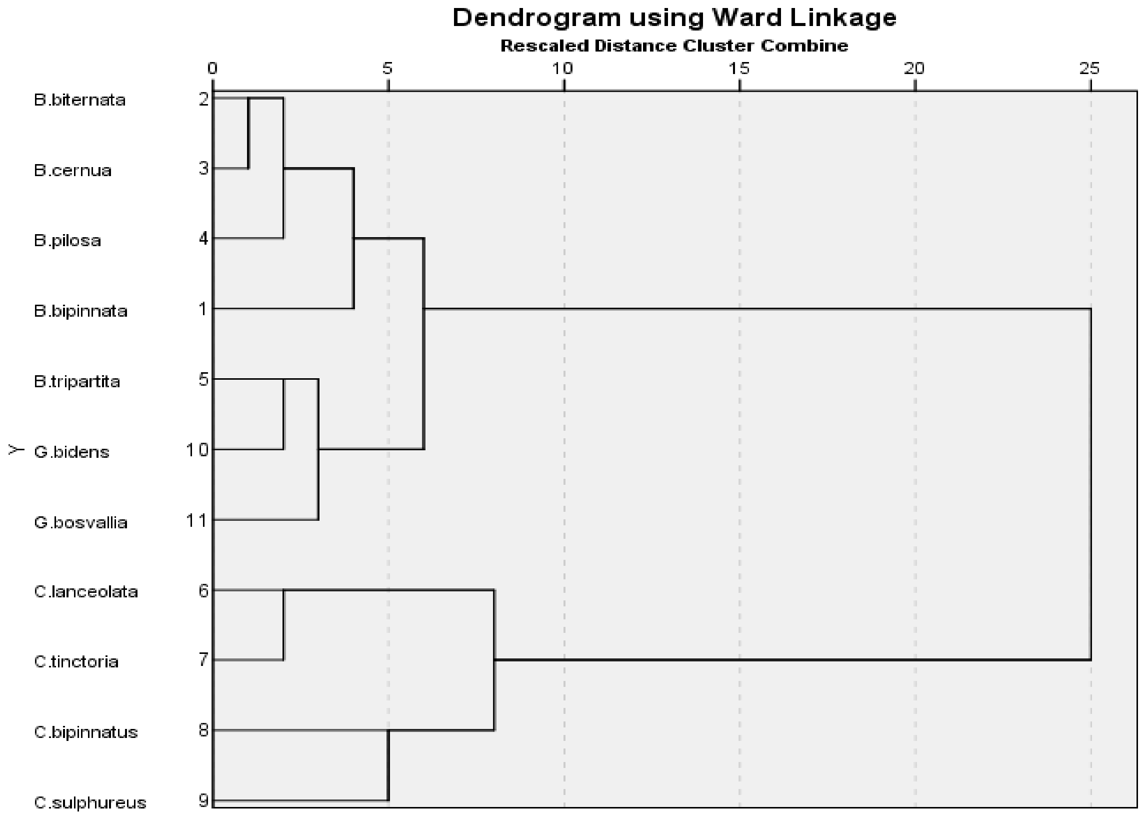

Fig. 4. Dendrogram showing the relationship of the species of tribe Coreopsideae.

Table 4. Cypselas Characteristics of tribe Coreopsideae (awns and wings of cypselas).

	Awns							Wings					
Name of Taxa	**Status**	**Number**	**Length (mm)**	**Surface Texture**	**Shape**	**Margins**	**Colour**	**Status**	**Number**	**Length (mm)**	**Breadth (mm)**	**Surface Texture**	**Colour**
Bidens bipinnata	Present	2-4	3-6	Retrorsely barbed	Slender	Entire	Pale yellow	Absent	-	-	-	-	-
Bidens biternata	Present	2-4	2-5	Retrorsely barbed	Slender	Entire	Light brown	Absent	-	-	-	-	-
Bidens cernua	Present	2-4	2-5	Retrorsely barbed	Slender	Entire	Light brown	Absent	-	-	-	-	-
Bidens pilosa	Present	2-3	2-4	Retrorsely barbed	Slender	Entire	Brown	Absent	-	-	-	-	-
Bidens tripartita	Present	2	2-4	Retrorsely barbed	Slender	Entire	Brown	Absent	-	-	-	-	-
Coreopsis lanceolata	Present	2	0.5-1	Glabrous	Slender	Entire	Light brown	Present	2	2.5-3	0.2-0.5	Glabrous	Brown
Coreopsis tinctoria	Present	2	0.5-1	Glabrous	Filiform	Entire	Light brown	Present	2	2-2.5	0.0-0.4	Glabrous	Brown
Cosmos bipinnata	Absent	-	-	-	-	-	-	Absent	-	-	-	-	-
Cosmos sulfureus	Present	2	0.5-1	Glabrous	Scaly filiform	Entire	Pale yellow	Absent	-	-	-	-	-
Glossocardia bidens	Present	2	4-5	Retrorsely barbed	Slender	Entire	Light brown	Absent	-	-	-	-	-
Glossocardia bosvallia	Present	2	3-5	Sparsely setose	Filiform	Entire	Dark brown	Absent	-	-	-	-	-

DISCUSSION

In the present work, cypselas morphology of 11 species from 4 genera belonging to the tribe Coreopsideae were studied for the first time from Pakistan and Kashmir. All the studied taxa were characterized by usually isomorphic sometimes dimorphic, black or brownish-black, sometimes corky or winged, with phytomelanin layer. Similar types of cypselas were observed by some previous authors such as Robinson (1981), Panero (2007), Crawford & al. (2009), and Jana & Mukherjee (2014). However, considerable variation has been found in a number of characters such as the presence or absence of beaks, wings, and awns, and the position of barbs. For instance, cypselas are usually isomorphic except for *Bidens* where the dimorphic cypselas also met. Robinson (1981) also reported a similar type of cypselas in Coreopsideae. The number of ribs is also found significant, such as cypselas 1-ribbed in *Coreopsis lanceolata* (Fig. 2) and *Glossocardia bosvallia* and more than 1-ribbed in *Bidens* and *Cosmos*, whereas absent in *C. tinctoria*. Moreover, quadrangular cypselas were observed in *Bidens* (except *B. tripartita*), (Fig. 2) and *Glossocardia*, while *Coreopsis* and *Cosmos* have triangular cypselas (Figs. 2 & 3). The narrowest cypselas were observed in *Cosmos sulfureus* (1-1.5 mm), (Fig. 3) whereas the widest cypselas were present in *Bidens cernua* (2-2.5 mm). Moreover, Basal is more common among the taxa, followed by lateral hilum whereas sub-basal hilum is only found in *Bidens pilosa* (Fig. 1). However, the length and shape of cypselas characters in the studied species overlapped and did not show significant variation.

The main dichotomy clearly is segregated into Cluster I and Cluster II, due to the presence or absence of retrorsely barbed awns (except *Glossocardia bosvallia*) and glabrous awns if present respectively.

Table 5. Cypselas characteristics of tribe Coreopsideae (Carpopodium).

Name of Taxa	Carpopodium	Development of Carpopodium	Shape	Symmetry	Diameter of Carpopodium (µm)	Foramen	Diameter of Foramen (µm)
Bidens bipinnata	Distinct	With a complete ring, without interruption	Angular	Asymmetric	418µm	Distinct	313µm
Bidens biternata	Distinct	With a complete ring, without interruption	Circular	Symmetric	773µm	Distinct	657µm
Bidens pilosa	Distinct	With a complete ring, without interruption	Angular	Asymmetric	505µm	Distinct	355µm
Bidens tripartita	Distinct	With a complete ring, without interruption	Angular	Asymmetric	800µm	Distinct	572µm
Coreopsis lanceolata	Distinct	With a complete ring, without interruption	Elliptical	Asymmetric	249µm	Distinct	200µm
Coreopsis tinctoria	Distinct	With a complete ring, without interruption	Elliptical	Asymmetric	260µm	Distinct	218µm
Cosmos bipinnata	Distinct	With a complete ring, without interruption	Circular	Symmetric	393µm	Distinct	291µm
Cosmos sulfureus	Distinct	With a complete ring, without interruption	Angular	Asymmetric	709µm	Distinct	643µm

The two genera *Bidens* and *Glossocardia* fell into Cluster I, however, due to some variation in the number of awns, the species of this group were further classified into sub-cluster IA and IB. Sub-cluster IA comprised four species of *Bidens*, that were further segregated on the basis of width (size), the number of ribs, apices, and position of hilum in their cypselas. Whereas sub-cluster IB comprised two species of *Glossocardia* and one species of *Bidens* (*B. tripartita*). Meanwhile, *B. tripartita* differentiated from *Glossocardia* by the width of cypselas (Table 3). Moreover, Cluster II was also segregated into sub-cluster IIA and IIB based on the presence of winged and beakless cypselas and wingless and beaked cypselas in *Coreopsis* and *Cosmos* respectively. The two species of each genus appeared as a sister on the same clade with short branches reflecting that they showed more similarity in their cypselas morphology. However, both species among the genus clearly differentiated on the basis of shape, color, size presence of ribs, the width of wings, length of the beak, and presence of awns (Table 3-5). Our results are in agreement with Qaiser & Perveen (2021).

Crawford & al. (2009) reported cypselas as linear-oblong, ellipsoid-oblanceolate, triangular to quadrangular with raised ribs, 2-5 pappus (retrorsely barbed awns) without wings in *Bidens* L. While the cypselas of *Glossocardia* Cass. reported as oblong to linear-lanceolate, ribbed with two short glabrous or aristate pappus (awns). These features have been found to be constant in our present findings. Mort & al. (2008) reported distinguished cypselas of *Coreopsis* L. as winged cypselas without awns or if awns were present then not retrorsely barbed, which was again matched to our finding (Tables 3 & 4, Figs. 2 & 3). Panero (2007) reported cypselas of *Cosmos* Cav. as fusiform to linear or lanceolate, usually black or dark brown, glabrous or hispid, curved outwards with age. This was also supportive in our present findings (Table 3) but our present investigation showed triangular cypselas whereas Panero observed quadrate cypselas (Fig. 3).

It is evident from the foregoing discussion and cluster analysis based on cypselas morphological characters clearly showed that all the taxa can be delimited both at the generic and species level. However, in a few cases, a lack of correlation was observed due to overlapping or integration in cypselas morphological characters.

Table 6. The character and their states for Cluster Analysis.

1	Length: Less than 10 mm (1), More than 10 mm (2)
2	Breadth: Up to 1 mm (1), More than 1 mm (2)
3	Surface: Glabrous or smooth (1), Pubescent or rough (2)
4	No. of Ribs: Absent or zero (0), 1-3 (1), 4-5 (2)
5	Angle: Triangular (1), Quadrangular (2)
6	Apex Truncate: Present (1), Absent (0)
7	Position of hilum: Basal (1), Sub-basal (2), Lateral (3)
8	Wings: Present (1), Absent (0)
9	Beak: Present (1), Absent (0)
10	Length of the beak (mm): 1 - 2.5 (1), More than 2.5 (up to 11) (2)
11	The surface of beak-Hispid: Present (1), Absent (0)
12	No. of Awns: Absent or zero (0), 2 (1), more than 2 (2)
13	Length of awns (mm): 0.5 - 1 (1), 2 - 6 (2)
14	The surface of awns: Glabrous (1), Sparsely setose (2), Retrorsely barbed (3)
15	The shape of awn: Filiform (1), Slender (2)
16	Color of awn: Pale yellow (1), Light brown (2), Brown (3), Dark brown (4)

Table 7. Data Matrix for cluster analysis for the tribe Coreopsideae.

Name of Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Bidens bipinnata	2	1	1	2	2	0	3	0	0	0	0	2	2	3	2	1
Bidens biternata	2	2	2	2	2	0	1	0	0	0	0	2	2	3	2	2
Bidens cernua	1	2	2	2	2	1	1	0	0	0	0	2	2	3	2	2
Bidens pilosa	2	1	2	2	2	0	2	0	0	0	0	2	2	3	2	3
Bidens tripartita	2	2	1	2	1	1	1	0	0	0	0	1	2	3	2	3
Coreopsis lanceolata	1	2	1	1	1	0	3	1	0	0	0	1	1	1	2	2
Coreopsis tinctoria	1	2	1	0	1	0	1	1	0	0	0	1	1	1	1	2
Cosmos bipinnata	2	2	1	1	1	0	1	0	1	1	0	0	0	0	0	0
Cosmos sulfureus	2	1	2	1	1	0	3	0	1	2	1	1	1	1	1	1
Glossocardia bidens	1	1	1	1	2	1	1	0	0	0	0	1	2	3	2	2
Glossocardia bosvallia	1	1	1	1	2	1	1	0	0	0	0	1	2	2	1	4

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