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POLLEN MORPHOLOGY OF SECTION *CHEIROLEPIS* BOISS. OF THE GENUS *CENTAUREA* L. (ASTERACEAE) IN TURKEY

Burcu Yilmaz Çitak*, Hüseyin Dural, Tuna Uysal and Nur Münevver Pinar[1]

Department of Biology, Faculty of Science, Selcuk University, Konya.

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Abstract

In this paper, the palynomorphology of 17 taxa of section *Cheirolepis* in Turkey, were investigated by light (LM) and scanning electron microscope (SEM). Detailed descriptions of the pollen grains were given for each taxon and a well-resolved dendrogram was generated through numerical analysis of palynological diagnostic features. The pollen grains were found to be radially symmetric, isopolar, and generally 3-zonocolporate, with the exception of *C. derderiifolia*, *C. kotschyi* var. *floccosa*, and *C. saligna*, which were also 4-zonocolporate. The shape of the pollen grains were prolate-spheroidal, with the polar axes of 32.76–46.26 µm and equatorial axes of 31.86–45.82 µm. The sculpturing of the pollen grains was generally scabrate-perforate or rarely microechinate-perforate. The spines were conical with a changing base length. The length and the width of the spines varied between 0.48 and 2.28 µm and 0.4 and 3.39 µm, respectively. The number of perforations at the base of the spines ranged in two or more rows and they varied between 5 and 30. The number of spines was 16–70 in 10 µm^2. The results of this study showed that the polar axes, equatorial axes, aperture type, pollen shape, spine length, perforation number, and number of spines in 10 µm^2 are essential for distinguishing the studied taxa. The taxa were grouped by clustering analysis of selected pollen characters using the UPGMA method.

Introduction

One of the largest tribes of the family Asteraceae (2400 species) is the tribe Cardueae (thistles) with representatives in almost every continent (Barres *et al*., 2013). The main area of distribution of Cardueae is the Mediterranean region, with the centres of endemism in the eastern and western Mediterranean, the western Irano-Turanian region, and North Africa (Susanna and Garcia-Jacas, 2007). The Cardueae are traditionally subdivided into the four subtribes Echinopinae, Carlininae, Carduinae and Centaureinae (Bentham, 1873; Hoffmann, 1890-94) and this classification is still accepted. *Centaurea* L. genus is placed in Centaureinae subtribes. *Centaurea*is a relatively large genus in Flora of Turkey, comprised of ca. 250 species distributed across Eurasia, particularly in the Irano-Turanian and Mediterranean region (Anderberg and Elden, 2007). Recently, the number of taxa belonging to the this genus has reached to 201 taxa, of which 112 are endemic in Turkey (Uzunhisarcikli *et al*., 2007; Uysal *et al*., 2007; Uysal *et al.*, 2012; Uysal *et al*., 2017; Uysal and Hamzaoğlu, 2017; Armağan and Uysal, 2018; Sirin *et al*., 2019) and hence, the endemism rate is about 56%. The *Cheirolepis* section of *Centaurea* is represented by seven taxa in Flora of Turkey and the Checklist of The Flora of Turkey (Wagenitz, 1975; Uysal, 2012; Uysal *et al*., 2012). *Centaurea drabifolia* Sm. includes four subspecies and *Centaurea kotschyi* Boiss. & Heldr. includes four varieties. The others can be counted as *C. derderiifolia* Wagenitz, *C. deflexa* Wagenitz, *C. nivea* (Bornm.) Wagenitz, *C. sericea* Wagenitz

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*Corresponding author: burcuyilmaz@selcuk.edu.tr

[1] Department of Biology, Faculty of Science, Ankara University, Ankara

and *C. saligna* (C. Koch.) Wagenitz (Uysal *et al*., 2012). During the last decades, the endemic taxa *C. cankiriense* Duran & Dumanand *C. glabro-auriculata* Uysal & Demirelma have been described from the same section of *Centaurea*, and the number of species in *Cheirolepis* has now been increased to 15 (Uysal *et al*., 2007). In flora of Turkey, *C. ensiformis* P. H. Davis had been evaluated in '*incerta sedis*' and *C. isaurica* Huber-Morath had been written under the title of 'imperfectly known'. However, Ertugrul *et al.* (2004) has suggested the section of these two taxa as *Cheirolepis*, according to their morphological and molecular data. With the addition of the last two taxa to section *Cheirolepis*, the number of section has increased to 17, of which sixare known only by their type locality. All the selected and studied species are endemic in Turkey except *C. kotschyi* var. *persica* (Boiss.) Wagenitz.

There are several reports on chromosome count for the section *Cheirolepis* of *Centaurea* genus (Garcia-Jacas *et al*., 1997; Romaschenko *et al*., 2004; Uysal *et al*., 2009; Uysal *et al*., 2015). The basic chromosome numbers of section are x=9, x = 11 with 2n = 18 and 22. Karyological traits of the studied species except *C. ensiformis* and *C. glabro-auriculata* were reported by the above mentioned researchers. Ten of them were counted as 2n=18. *C. kotschyi* group were counted as 2n=4x=36 (tetraploid), *C. drabifolia* subsp. *floccosa* (Boiss.) Wagenitz & Greuter was counted as both 2n=4x=36 and 2n=6x=54. Chromosome number of *C. deflexa* was counted as 2n=6x=54 and those of both *C. saligna* and *C. sericea*as 2n=4x=36.

The family Asteraceae has remarkable pollen features that attract researchers due to its systematic importance, since the pioneer work of Wodehouse (1935) to recent studies of Wagenitz, 1955, Stix, 1960; Wagenitz, 1976; Pehlivan, 1995; Pınar and İnceoğlu, 1996; Ozler *et al*., 2009; Punt and Hoen, 2009; Erkara *et al*., 2012 and Pınar *et al*., 2016. Wagenitz (1955) distinguished eight pollen types based on their morphological structures in *Serratula*, *Crupina*, *Centaurium*, *Dealbata*, *Montana*, *Cyanus*, *Scabiosa*, and *Jacea* pollen types, where *Serratula* was recognized as more primitive and *Jacea* was more evolved. Stix (1960) examined 235 species of Asteraceae from all over the world and determined 45 types, mainly based on the properties of the sexine, and, in particular, of the echinae. Avestisjan (1964) identified five pollen types in *Centaurea* that are found in/reported from *Jacea, Centaurium, Scabiosa, Serratula,* and *Psephellus*' and six pollen types in *Jacea* that are reported from *Sulphurea, Tomentella, Diffusa, Alutaceae, Pergamacea,* and *Eremopappus*. Later on pollen studies have been increased with the availability of different magnification systems, for example Transmission Electron Microscopy (TEM), and new findings, for example establishment of three principal patterns of exine stratification *viz.*, Anthemoid, Helianthoid, and Senecioid, based on different combination of characters, particularly the presence or absence of a large cavea (space) within the pollen wall, and presence or absence of internal foramina (smaller spaces within individual structural elements). Wagenitz (1976) reported that the wealth of other forms of pollen may be explained by the combination of three main trends of evolution: first, is the reduction of the spines, next is the reduction and loss of the inner columellae (with formation of a cavity), and finally, is the formation of a pattern of ridges (lophate or fenestrate pollen). Turkish botanists who have studied the pollen morphology of *Centaurea* claimed that the pollen shape, exine structure, presence of single- or double-layer columellae, costa thickness, and sculpturing can be useful for the classification of taxa (Pehlivan, 1995; Ozler *et al*., 2009; Erkara *et al*., 2012). Punt and Hoen (2009) studied 187 species of Asteraceae16 of which belonged to the genus *Centaurea*, from northwestern Europe and proposed 37 types of pollen based on the apertures, apocolpium index, exine, cavea, costae, ornamentation, and outlines. They divided the species *Centaurea* into three pollen types: *C. scabiosa*, *C. nigra*, and *C. cyanus*. Recently, few studieshave been published on section *Cheirolepis* based on pollen morphology (Hayta *et al*., 2017; Baser *et al.*, 2019).

In light of these studies, we studied the pollen morphology of 17 taxa from section *Cheirolepis* of genus *Centaurea* from Turkey. With the exception of *C. saligna, C. kotschyi* var. *floccosa, C. drabifolia* subsp. *floccosa*, and *C. derderiifolia* the pollen features of the remaining species were examined for the first time in the present study. The aims of this report are to compare pollen characteristics of species with an efficient dendrogram and to clarify the usefulness of these features in terms of systematic implications.

Materials and Methods

Plant materials

The plant materials were collected from several localities during their flowering season between 2015 and 2016. Moreover, third author also provided us with his collections. All of the specimens used in this research were stored in the Selçuk University Herbarium (KNYA). The localities, geographical position, altitude, and the voucher number of the specimens are listed in Table 1.

Table 1. Locations and the number of collected samples of *Cheirolepis* (*endemic species).

Taxa	Location	Voucher number
Centaurea drabifolia* Sm. subsp. *drabifolia* **(C1)	**A2 Bursa:** Uludağ, the summit, above the rocks, 2100-2200 m., 20 August 2016	B. Çıtak 250
C. drabifolia* subsp. *austro-occidentalis* Wagenitz **(C2)	**C2 Denizli**: Honaz Mountain, The northeast of Babatepe, rocky places, 37º41′154″ N 29º17′345″ E, 2340-2500 m., 28 July 2016	B. Çıtak 218
C. drabifolia* subsp. *floccosa* (Boiss.) Wagenitz & Greuter **(C3)	**B5 Kayseri**: Pinarbaşı, Şirvan Mountain, roadside, 2210 m., 5 August 2015	B. Çıtak 177
	B2 Eskişehir: Between Kütahya-Afyon road, side of road, 39º14′094″ N, 30º07′046″ E, 1100 m., 30 June 2004	T. Uysal 583
	C3 Konya: Derebucak, Çamlık Town, Kızıldağ, 37º21′250″ N, 31º39′579″ E, 1350 m., 15 June 2016	H. Dural 3566-B.Çıtak
	B2 Eskişehir: 15 km from Bozhüyük to Kütahya, side of road, 39º33′400″ N, 30º03′842″ E, 905 m., 19 June 2003	T. Uysal 501
C. drabifolia* subsp. *cappadocica* (DC.) Wagenitz **(C4)	**B6 Sivas:** Gürün, between Gürün-Kangal road, Böğrüdelik gate, 38°57′098″ N, 37°16′887″ E, 1844 m., 14 July 2015	B. Çıtak 171
	C5 Niğde: Bolkar Mountain, Bulgar Mine, 37º27′507″ N, 34º40′138″ E, 1900 m., 28 July 2004	T. Uysal 854
	C5 Niğde: Ulukışla, Bolkar Mountain, East side of Lake Kara, west side of Koyunaşağı, rocky places, 2900 m., 01 September 2003	T. Uysal 855
	C3 Konya: Beyşehir, Doğanhisar-Hüyük road, 1800 m., 20 June 2016	H. Dural 3573-B.Çıtak
C. kotschyi* var. *kotschyi* (Boiss. & Heldr.) Hayek **(C5)	**C3 Konya:** Konya-Beyşehir road, 1 km to Altınapa, droughty stream bed, under the small trees, 37º 53′ 011″ K, 32º21′ 668″ E, 15 June 2015	H. Dural 3570 B. Çıtak
	C4 Karaman: Karadağ, 1600 m., 21.07.2015	K. Ertuğrul 5016 H. Dural
C. kotschyi var. *persica* (Boiss.) Wagenitz **(C6)**	**B5 Kayseri:** Pinarbaşı, Şirvan Mountain, around stone pit, 38°39.99′ N 36°22.97′ E, 1844 m.,14 July 2015	B. Çıtak 169
	C4 Konya: 8 km from Hadim to Korualan, side of roads,1850 m., steppe, 21 July 2015	K. Ertuğrul 5006 H. Dural
	C4 Konya: Hadim, around the Gevne Valley, 1650-1800 m.,	K. Ertuğrul 5007

	openings of forest, moving stones, 21 July 2015	H. Dural
	C4 Konya: Taşkent, 1 km to Beyreli Village, right side of road, 1762 m., 07 July 2004	T. Uysal 696
C. kotschyi* var. *decumbens* **(C7)	**C4 Konya**: Taşkent- Alanya, on the tableland, 9 km from Taşkent, before Belpinar, stony places, 1800 m., 12 July 2003	T. Uysal 508
	C4 Konya: Bozkır, Sorkun Plateau, before Dikilitaş Plateau, Sarnıç region, 1750 m.,15 July 2003	T. Uysal 510
	C4 Konya: Around Tosmur Plateau, 2000-2100 m., 21 July 2015	K. Ertuğrul 5009 H. Dural
C. kotschyi* var. *floccosa* **(C8)	**B5 Kayseri**: Pinarbaşı, Şirvan Mountain, roadside, 2210 m., 05 August 2015	B. Çıtak 176
C. derderiifolia* Wagenitz **(C9)	**B7 Elazığ**: Between Elazığ-Pertek, 16 km to Pertek, Saklaya Village, Yukarı stream region, in vineyard, 38°46′097″N, 39°12′350″E, 1002 m., 02 August 2004	T. Uysal 902
	B6 Kayseri: Gürün-Divriği road, 18 km to Divriği, small hills of side of road, 39º16′53″N, 037º59′27″E, 1500-1600 m., 27 July 2003.	B. Çıtak 172
C. deflexa* Wagenitz **(C10)	**C4 Antalya**: Gevne Valley, Küçüklü Village, 36°49′710″ N, 32°27′461″E, 1750-1800 m., 7 July 2004	T. Uysal 693
	C4 Konya: Hadim-Taşkent-Alanya road, 25 km to Taşkent, steppe,1750-1800 m., 21 July 2015	K. Ertuğrul 5011 H. Dural
	C4 Konya: Taşkent-Ermenek road, 5-6 km to Başyayla fork, loamy hills, 1800 m., 19 July 2012	K. Ertuğrul-4699- H. Dural
C. nivea* (Bornm.) Wagenitz **(C11)	**B3 Eskişehir**: 20 km from Mihaliçik to Alpu, the hill with jips, 39°49′592″ N, 31°16′890″ E, 940 m., 13 June 2016	B. Çıtak 182
C. sericea* Wagenitz **(C12)	**C3 Eskişehir**: Bozüyük-Kütahya road, openings of Pinus forest, 1140 m., 14 July 2016.	B. Çıtak 207-A. Ocak
C. cankiriense* A. Duran & H. Duman **(C13)	**A4 Çankırı**: Atkaracalar-Kalfat road, Ballı Plateau, 40º44′744″ N, 33º05′795″ E, 1500 m., 13 July 2016	B. Çıtak 201-B. Şahin
C. saligna* (Koch.) Wagenitz **(C14)	**C9 Hakkari**: Hakkari-Van road, Yüksekova fork, Bağışlar Village, side of road, droughty stream bed, 37°72′597″N, 44°045′108″ E, 1800 m., 10 July 2015	B. Çıtak 168
	B9 Erzurum: Karayazı- Göksu road, Göksu Valley, stream bed, 34°39′602″ N, 42°08′529″E, 2200 m., 30 July 2004	T. Uysal 891
	B9 Muş: 16 km to Solhan, 38°56′380″ N, 41°09′016″E, 1660 m., 31 July 2004	T. Uysal 897
	B9 Ağrı: Hamur, Buzhane Village, inside the fields, steppe, 1800 m., 26 July 2015	K. Ertuğrul 5117 H. Dural-T. Uysal
C. glabro-auriculata* Uysal & Demirelma **(C15)	**C3 Konya**: Derebucak, Kızıldağ, Tekneliyatak region, 37º20′128″ N, 31º29′465″ E, 1800-1900 m., 07 August 2016.	B. Çıtak 225-H. Dural-H. Demirelma
C. isaurica* Hub.-Mor. **(C16)	**C4 Konya**: Bozkır, Sorkun Village, Aşağı Sorkun Plateu, Top of stony places, 2000 m., 13 July 2003	T. Uysal 509
	C4 Konya: Hadim, Between Tosmur Valley and Gevne Valley,1900-1950 m., 27 June 2012	K. Ertuğrul 4638 H. Dural-T. Uysal
C. ensiformis* P. H. Davis **(C17)	**C2 Muğla**: Köyceğiz, Sandras Mountain, on serpentin places, 1700 m., 25 July 2016	B. Çıtak 193

Palynological analysis

Both Wodehouse (1935) and Erdtman (1960) techniques were used for the light microscopic investigations. However, the figures were given according to first technique (Figs 2-7). In the first technique, the pollen grains were obtained from mature anthers, stained with glycerin-jelly and safranin, and covered by a coverslip. In the second technique, the anthers were transferred to glass tubes and treated with potassium hydroxide for 20 min. Later, they were mixed in a water bath at 80°C and centrifuged at 4500 rpm. The supernatant was poured off. The acetolysis solution (9:1, acetic acid: sulfuric acid) was added to glass tubes that were carefully heated and centrifuged again. The acetolysis solution was carefully removed, and the pollen grains were washed and centrifuged again. A solution of 50% glycerine was added to the tubes, which was then poured out onto filter paper at room temperature. Next, the acetolysed pollen grains were collected from the tubes with a sterile needle and permanent slides were made with glycerine-jelly. All of the pollen slides were photographed using a Leica DM 1000 light microscope, which was attached to a Leica camera. The measurements were made based on 30 or more pollen grains per specimen.

For the scanning electron microscopy (SEM) studies, dried non-acetolysis pollen grains were directly transferred onto aluminum stubs and coated with gold. They were photographed with the SEM at the Advanced Technology Research and Application Centre at the Selçuk University in Konya, and the Turkish Petroleum International Company (TPAO). The pollen terminology was followed of the following literature *viz.*, Wagenitz (1955), Faegri and Iversen (1975), Punt *et al.* (2007), Punt and Hoen (2009) and Halbritter *et al*. (2018).

Numerical analysis

Determined total of twenty qualitative and quantitative pollen characters were recorded. Out of these, eight pollen characters were used to evaluate the taxonomic grouping and similarities among the taxa of *Cheirolepis*. These characters are listed in Table 2. The mean values of recorded qualitative and quantitative pollen characters were included in the data matrix (Table 2). The Simpson and Roe graphical test (Van der Pluym and Hideux, 1977) was used for the statistical calculations (Fig. 1). For the pollen characters of the 17 taxa, the coefficients of correlation were determined, and they were grouped using the cluster analysis method (UPGMA, dissimilarity, standardized variables). For the multivariate analysis, a primary matrix was created the using 17 taxa and 8 characters. The clustering analysis was based on Gower (1971) sgeneral coefficient similarity (Sneath and Sokal, 1973), which can be used directly with a mixture of character types (binary, qualitative, and quantitative characters). The UPGMA was selected because it is not only the most commonly used method, but it also appears to produce an accurate reflection similarity matrix, as measured by the co-phenetic correlation coefficient of Sokal and Rohlf (1962) and symmetrical hierarchical structure (McNeill, 1979), and has congruence with the classification derived by traditional methods (Ward, 1993). Untransformed, centred, and standardised data were used to create a covariance matrix. MVSP 3.22 software was used for all of the computations.

Results and Discussion

Pollen morphology

The palynological properties of the examined taxa of section *Cheirolepis* are given in Table 2, and their photographs are shown in Figs 2–7.

Size, symmetry and shape

The pollen grains of section *Cheirolepis* were monad, isopolar, and radially symmetrical. The pollen grains of taxa were prolate-spheroidal, with polar axes ranging from 33.77 to 46.26 µm and equatorial axes ranging from 33.77 to 45.82 µm. *C. cankiriense* were found to be smaller, while *C. ensiformis* and *C. kotschyi* var. *kotschyi* were larger. Their equatorial view was elliptic and compressed at the poles, while their polar view was circular, with intruding colpi or was slightly triangular with obtuse angles, and the colpi at the sides were amb inter-semiangular (Table 2, Figs 2-7).

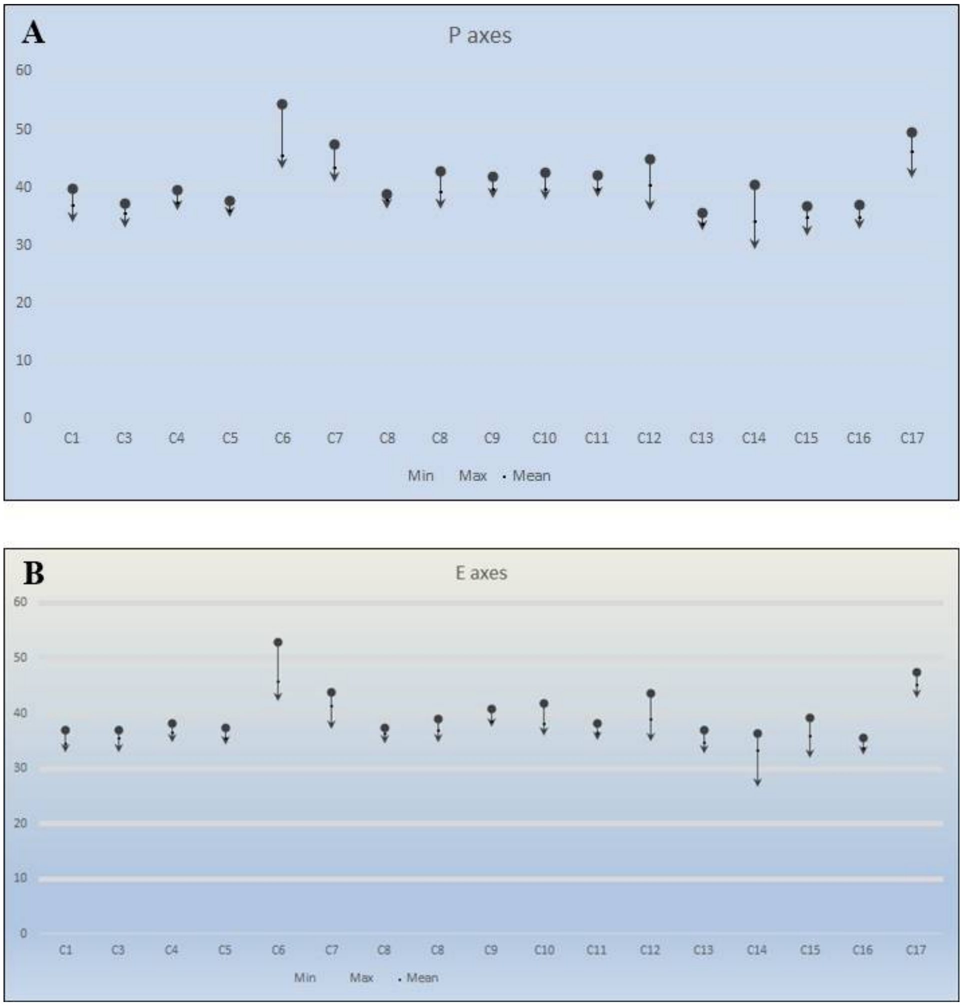

Fig. 1. Simpson and Roe test for the taxa of *Cheirolepis*. **A**. Polar axes (P); **B.** Equatorial axes (E).

Apertures

The pollen grains of *Cheirolepis* section were generally 3-zonocolporate or rarely tetracolporate or syncolporate. Some taxa had heteromorphic characteristics. For example, *C. derderiifolia* and *C. kotschyi* var. *floccosa* had 2% 4-zonocolporate pollen grains and 98% 3-zonocolporate pollen grains. The specimens of endemic species *C. saligna* collected from Hakkari and Ağrı provinces had 2% tetracolporate and 98% tricolporate pollens, whereas its specimens collected from

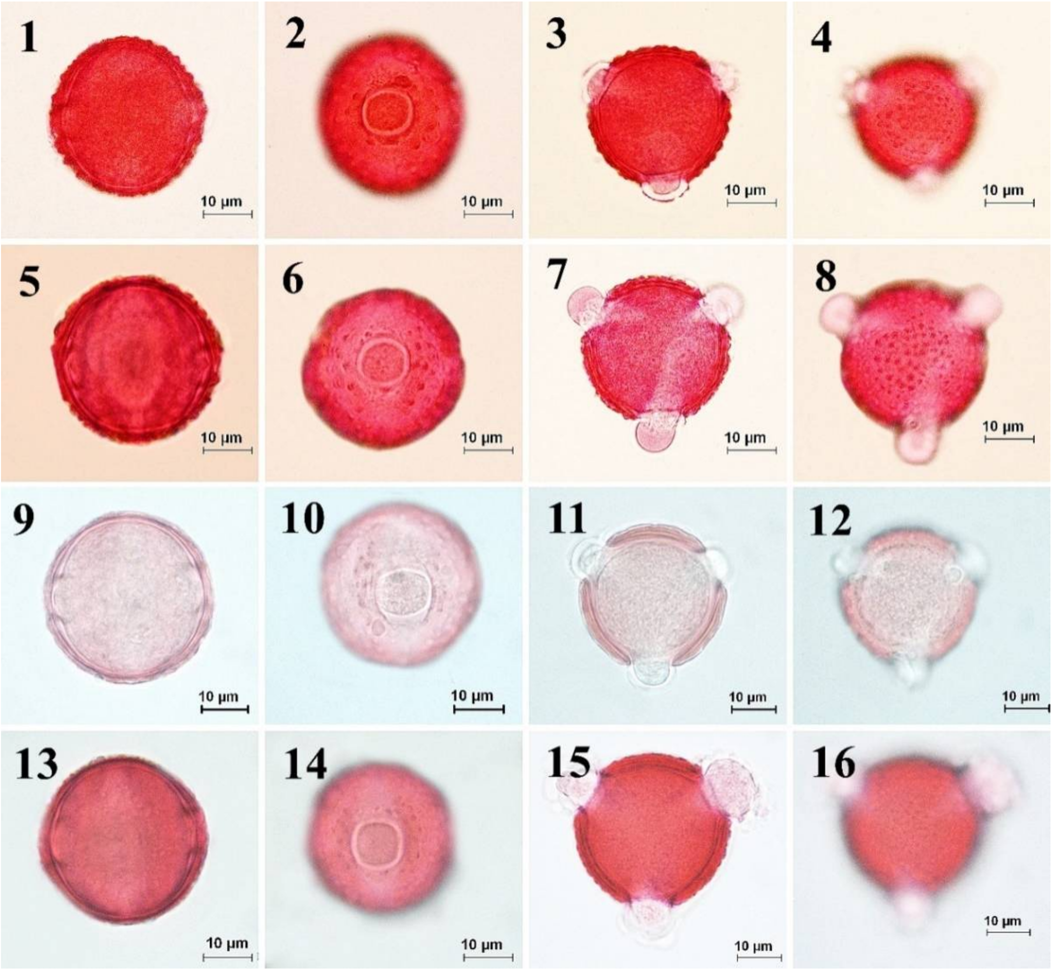

Fig. 2. Pollen morphology of the section *Cheirolepis* observed under light microscope (LM) according to Wodehouse method. 1-4. *C. drabifolia* subsp. *drabifolia*, 5-8. *C. drabifolia* subsp. *austro-occidentalis* 9-12. *C. drabifolia* subsp. *floccosa* 13-16. *C. drabifolia* subsp. *cappadocica*

Erzurum province had apertures that were 92% 3-zonocolporate, 5% syncolporate, and 3% 4-zonocolporate. The colpus was long (24.39–40.66 µm) and narrow (7.23–10.68 µm) and the ora was circular or lolongate in all of studied species. The highest values were observed in C. *kotschyi* var. *kotschyi*, *C. ensiformis*, *C. nivea*, *C. saligna*, and *C. drabifolia* subsp. *cappadocica* that have the smallest colpus. Margins were distinct, straight and ends were acute to obtuse in all examined taxa. The colpus membrane was granulate in all of the taxa (Table 2, Figs 2-7).

Exine and intine

The thickness of the exine and intine varied from 1.8 to 3.04 µm and 0.73 to 1.18 µm when the spines are excluded, respectively. Ectexine wasthicker than endexine without costae and cavea. A large cavea were present in *C. deflexa* and *C. sericea* (Fig. 4). The other taxa had a narrow and

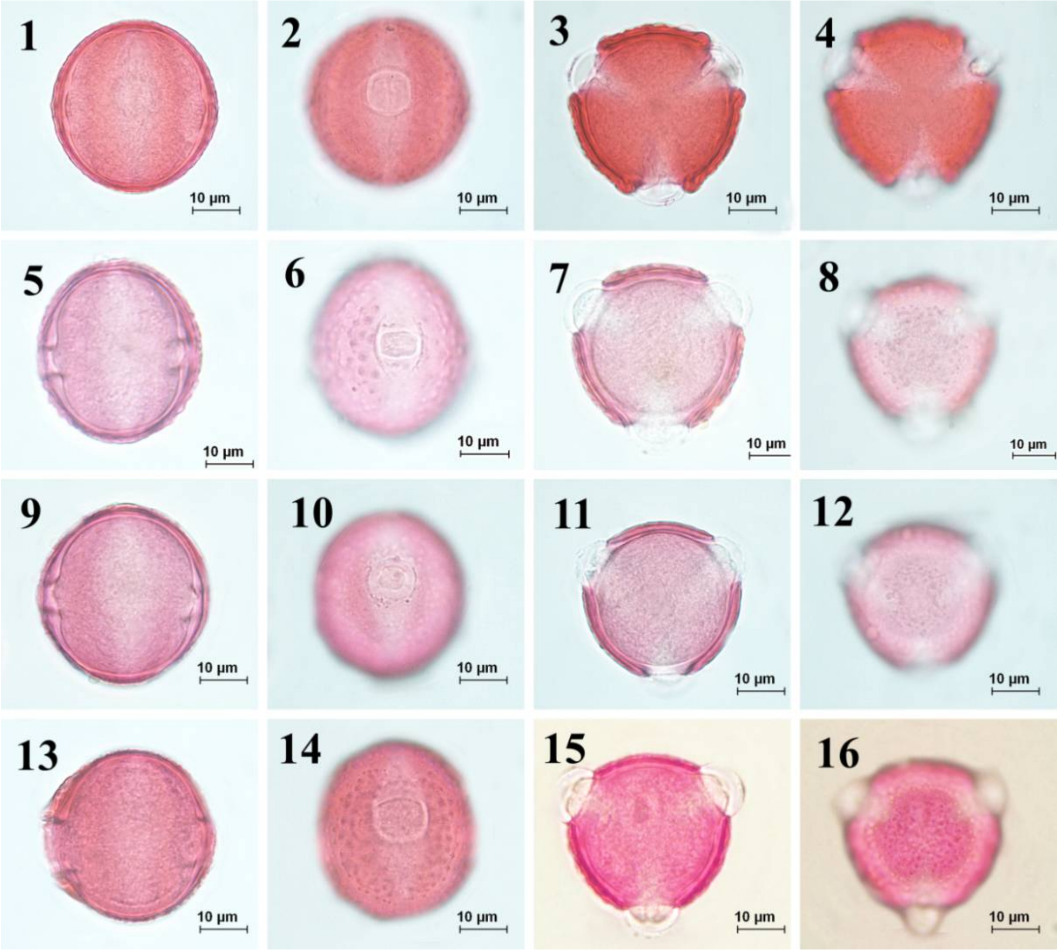

Fig. 3. Pollen morphology of the section *Cheirolepis* observed underlight microscope (LM) according to Wodehouse method. 1-4. *C. kotschyi* var. *kotschyi*, 5-8. *C. kotschyi* var. *persica*, 9-12. *C. kotschyi* var. *decumbens*, 13-16. *C. kotschyi* var. *floccosa.*

bad visible cavea (Figs 2-7). All of the taxa had a costae thickness ranging from 1.5 µm to 2.52 µm (Table 2). Intratectal columellae weremore distinct under spines than in inter-spinal region. The spines were commonly conical with a broad basis, the sides were straight or slightly convex and had a subacute tapered apical portion. Of the studied taxa, 13 had a scabrate-perforate ornamentation with spinule lengths of 0.48–0.9 µm, the remaining had a microechinate-perforate ornamentation with spinule lengths of 1.0–1.19 µm. The width of the spinules varied between 1.73 (*C. drabifolia* subsp. *floccosa*) and 4.45µm (C. *drabifolia* subsp. *austro-occidentalis*), while the base of the spinules in almost all of the studied species was irregular 1, 2, or 3 seriate with holes of equal size. The number of perforations was 5–10, 15–20, 20–25, 25–30, and 35–50. The distance between 2 spinules was 0.28–3.39 µm and there were 5–70 spinules in 10 μm^2 (Table 2, Figs 2-7).

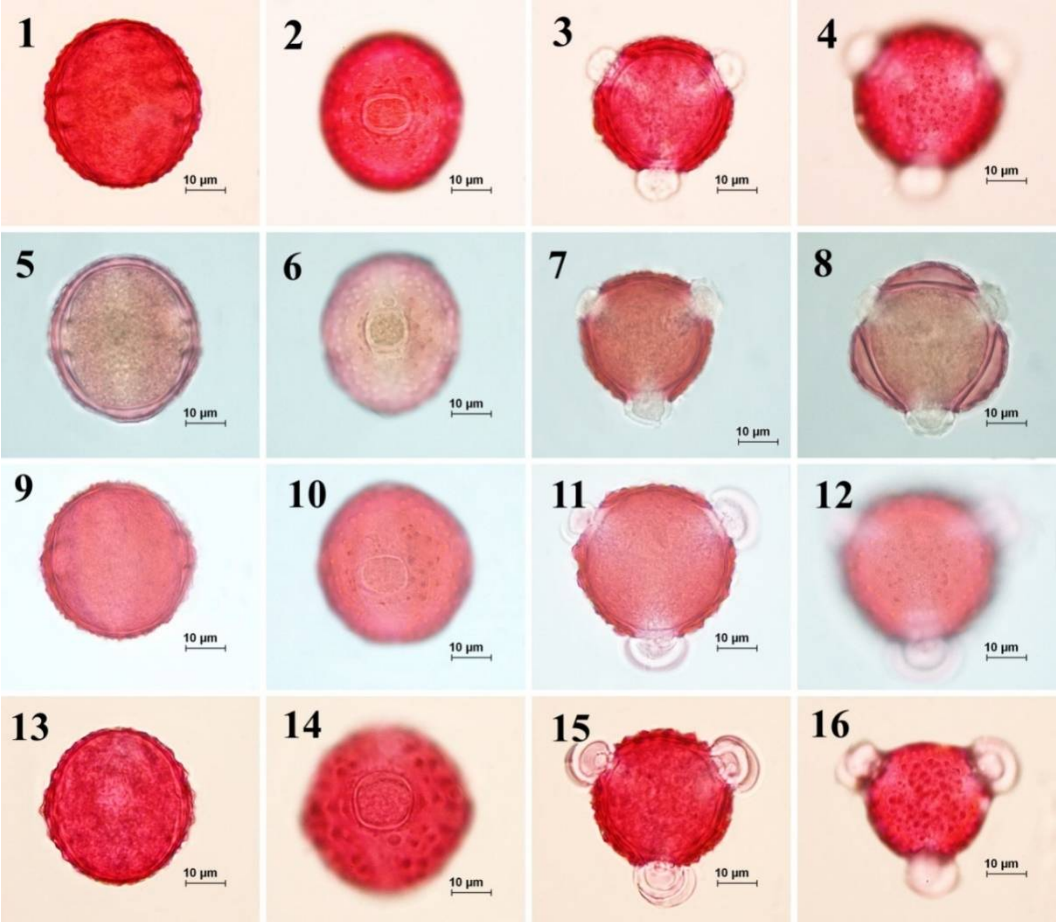

Fig. 4. Pollen morphology of the section *Cheirolepis* observed underlight microscope (LM) according to Wodehouse method. 1-4. *C. nivea*, 5-8. *C. sericea*, 9-12. *C. deflexa*, 13-16. *C. cankiriense.*

Numerical analysis of the pollen character states

The dendrogram obtained from the cluster analysis using the UPGMA was based on the eight palynological variables *viz.* polar axes/equatorial axes, the length of spines, number of spins, costae, cavea, apocolpium, amb, and perforation number at base of 17*Centaurea* taxa and is presented in Fig. 8. This dendrogram shows the similarities among the taxa being investigated. The dendrogram revealed two main groups with a 54% similarity; the first one contained *C. sericea* and *C. deflexa*, which are local and very distinct species with the similar appendage structure in section *Cheirolepis*, and the second one comprised the other 15 taxa. The second group, which consisted of two main clusters, recognized as Cluster A and Cluster B. Cluster A includes *C. ensiformis*, *C. cankiriense*, and *C. drabifolia* subsp. *drabifolia* with a similarity rate of 69%. Cluster B includes 12 taxa with three subgroups, namely B1, B2, and B3. Cluster B1 contained only *C. derderiifolia*, which is a huge and distinctive species without close relatives in section *Cheirolepis*. Cluster B2 included *C. kotschyi* and its varieties. Cluster B3 also included two subgroups, namely B3a and B3b. Cluster B3a also included two subclusters which contained *C.*

isaurica C. saligna, *C. nivea* and *C. glabro-auriculata* with a similarity rate of 83%. Cluster B3b included *C. drabifolia* subsp. *cappadocica*, *C. drabifolia* subsp. *floccosa* and, *C. drabifolia* subsp. *austro-occidentalis* subspecies, with a similarity rate of 83%.

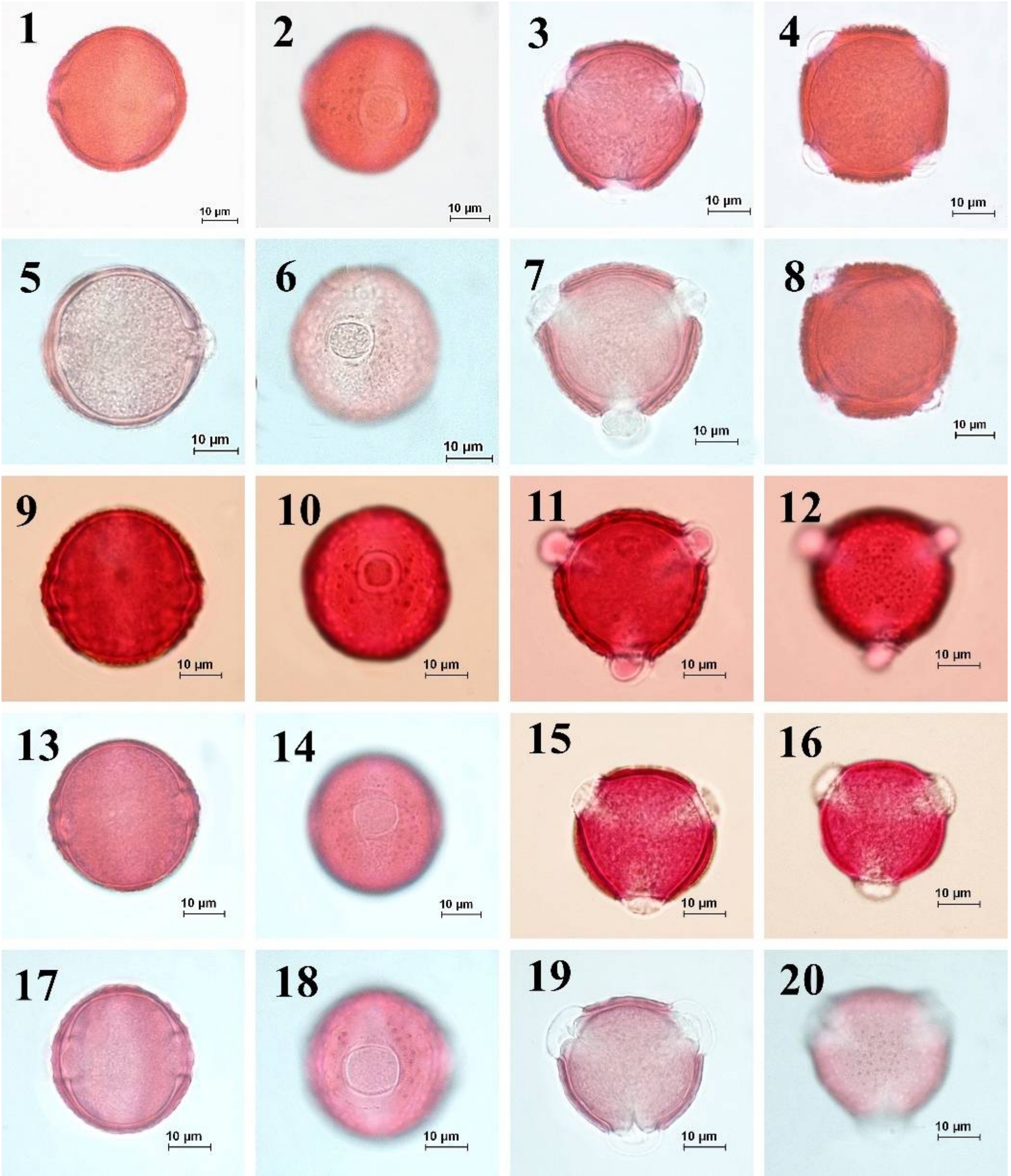

Fig. 5. Pollen morphology of the section *Cheirolepis* observed underlight microscope (LM) according to Wodehouse method. 1-4. *C. derderiifolia*, 5-8. *C. saligna*, 9-12. *C. glabro-auriculata*, 13-16. *C. isaurica* 17-20. *C. ensiformis*.

The pollen morphology of *Cheirolepis* section taxa show taxonomically significant characters. The main differences have been found at the species level.The pollen grains of the *Centaurea* taxa can be classified as *Centaurium*, *Dealbata*, *Montana*, *Cyanus*, *Scabiosa*, or *Jacea* types, according to Wagenitz (1955), *Centaurea* type according to Stix (1960) and *Centaurea nigra* L., *C. scabiosa* L., or *C. cyanus* L. types according to Punt and Hoen (2009). Pollen shape was prolate-spheroidal in *Cheirolepis*. The examined species pollen grains were determined as *Jacea* pollen type, which, despite a very diverse habit and morphology, was found to be monophyletic in a large group according to Wagenitz (1955) as indicated before Hayta *et al*. (2017). The fifteen species which were had narrow cavea placed into *Centaurea nigra* pollentype, and *C. sericea* and *C. deflexa* which had broad cavea placed into *Centaurea scabiosa* pollen type according to Punt and Hoen (2009, Figs 2-7).

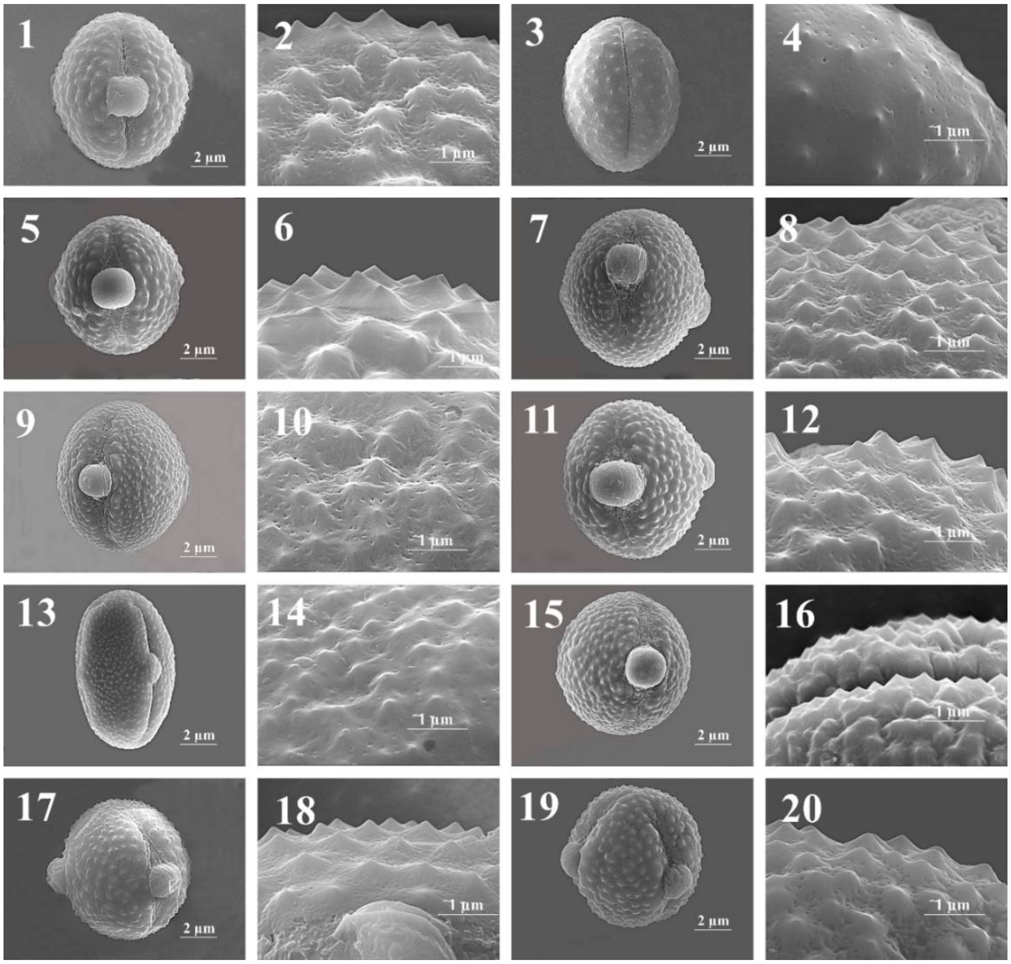

Fig. 6. Scanning electron microscope (SEM) images on the pollen grains of *Cheirolepis* section. 1-2. *Centaurea drabifolia* subsp. *drabifolia*, 3-4. *C. drabifolia* subsp. *austro-occidentalis*, 5-6: *C. drabifolia* subsp. *floccosa*, 7-8. *C. drabifolia* subsp. *cappadocica*, 9-10. *C. kotschyi* var. *kotschyi*, 11-12. *C. kotschyi* var. *persica*, 13-14. *C. kotschyi* var. *decumbens*, 15-16. *C. kotschyi* var. *floccosa*, 17-18. *C. nivea*, 19-20. *C. sericea.*

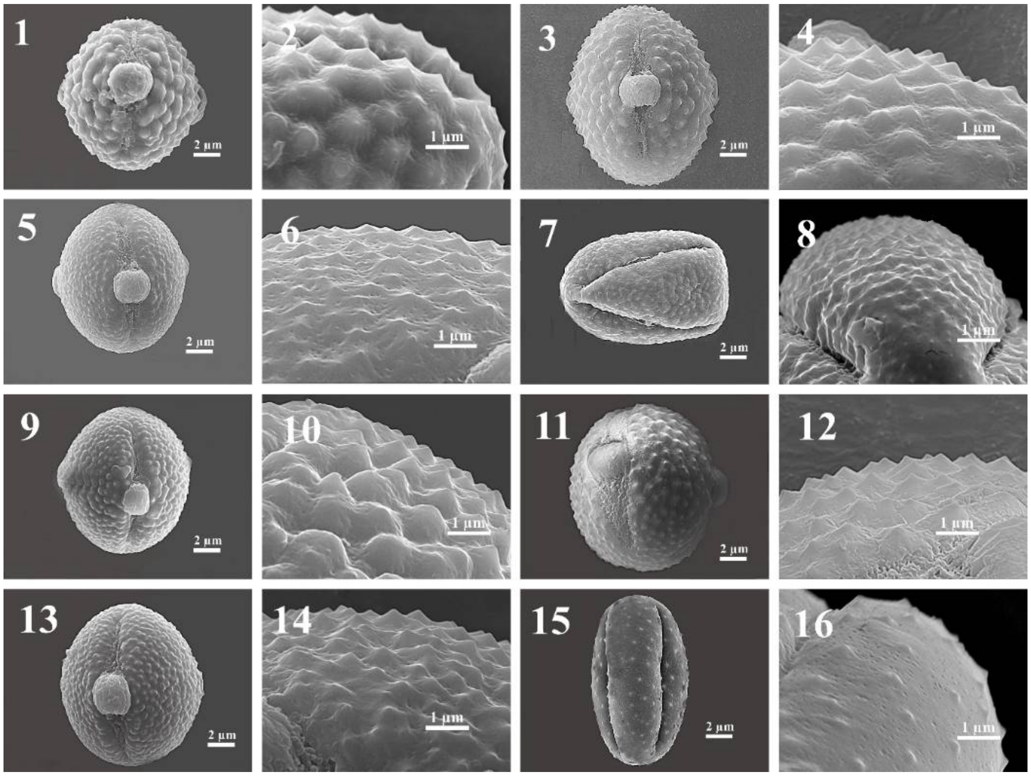

Fig. 7. Scanning electron microscope (SEM) images on the pollen grains of *Cheirolepis*, *Pteracantha*, *Pseudoseridia* and *Cheirolepis-Pseudoseridia* sections. 1-2. *Centaurea deflexa*, 3-4. *C. cankiriense*, 5-6. *C. derderiifolia*, 7-8. *C. saligna* (*T. Uysal*-891), 9-10. *C. saligna* (*B. Çıtak*-168), 11-12. *C. glabro-auriculata*, 13-14. *C. isaurica*, 15-16. *C. ensiformis*.

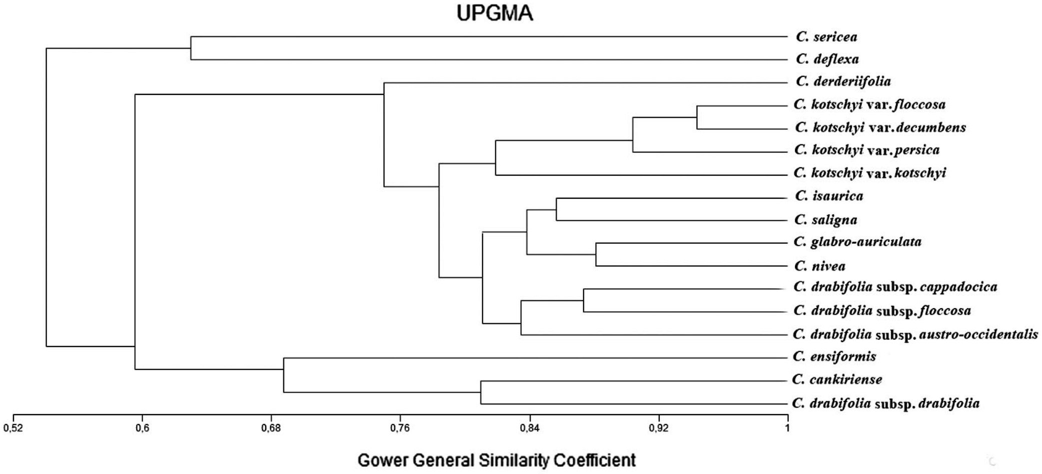

Fig. 8. Dendrogram based on pollen morphology of the examined taxa of *Cheirolepis* sectionindicating dissimilarity distance.

Table 2. Micromorphological characters of the pollens of section *Cheirolepis* in Turkey (values in µm).

Taxa	Chromosome number (2n)/ploidy level (x)*	Equatorial axes (E)		Polar axes (P)		P/E	Pollen shape	Exine	Intine	Costae	Cavea	Aperture type
		Min-Max	Mean ± SD	Min-Max	Mean ±SD							
C. drabifolia subsp. *drabifolia*	2n=18	32.92-37.06	34.58 ±1.04	34.03-39.84	36.87± 1.47	1.06	Prolate-spheroidal	2.16	0.73	2.24	0	%100 Tricolporate
C. drabifolia subsp. *austro-occidentalis*	2n=18	32.89-36.93	35.47 ±1.08	33.11-37.22	35.48± 1.46	1	Prolate-spheroidal	2.15	0.82	1.7	0	%100 Tricolporate
C. drabifolia subsp. *floccosa*	2n=54 2n=4x=36	34.7-38.24	36.58 ±1.11	35.97-39.44	37.36± 1.06	1.02	Prolate-spheroidal	2.2	0.73	2.09	0	%100 Tricolporate
C. drabifolia subsp. *cappadocica*	2n=18	34.33-37.31	35.46 ±1.61	34.94-37.66	35.99± 1.45	1.01	Prolate-spheroidal	3.04	0.73	2.28	0	%100 Tricolporate
C. kotschyi var. *kotschyi*	2n = 4x = 36	42.17-52.89	45.82 ±3.25	43.16-54.37	45.5±3.24	1.01	P-spheroidal	2.53	0.78	2.26	0	%100 Tricolporate
C. kotschyi var. *persica*	2n = 4x = 36	37.23-43.85	41.48 ±1.81	41.03-47.52	43.47± 1.98	1.04	Prolate-spheroidal	2.29	0.82	2.31	0	%100 Tricolporate
C. kotschyi var. *decumbens*	2n = 4x = 36	34.61-37.37	36.36 ±1.53	36.36-38.85	37.97± 1.39	1.06	Prolate-spheroidal	1.87	1.18	2.39	0	%100 Tricolporate
C. kotschyi var. *floccosa*	2n = 4x = 36	34.65-39.06	37.05 ±1.31	36.38-42.76	39.33± 1.71	1.04	Prolate-spheroidal	2.31	0.8	2.34	0	%98 Tricolporate %2 Tetracolporate
C. derderiifolia	2n=18	37.55-40.77	38.54 ±1.59	38.16-41.97	39.83± 1.61	1.03	Prolate-spheroidal	1.81	0.92	2.13	0	%98 Tricolporate %2 Tetracolporate
C. deflexa	2n = 6x = 54	35.98-41.75	38.17 ±1.25	37.91-42.62	39.75± 1.19	1.08	Prolate-spheroidal	1.99	0.86	2.52	1	%100 Tricolporate
C. nivea	2n=18	35.21-38.28	36.6 ±0.87	38.31-42.19	39.67± 1.36	1.08	Prolate-spheroidal	2.22	0.86	1.93	0	%100 Tricolporate
C. sericea	2n = 4x = 36	35.04-43.61	39.06 ±2.12	36.15-44.85	40.49± 2.01	1.12	Prolate-spheroidal	2.01	0.82	2.07	1	%100 Tricolporate
C. cankiriense	2n=18	32.68-37	34.77 ±2.7	32.47-35.6	33.77± 1.0	1.02	Prolate-spheroidal	2.26	0.73	1.50	0	%100 Tricolporate
C. saligna	2n = 4x = 36	26.77-36.33	33.39 ±2.74	29.24-40.57	34.3±2.65	1.02	Prolate-spheroidal	2.39	0.78	2.43	0	%98 Tricolporate %2 Tetracolporate
C. glabro-auriculata	?	31.89-39.27	35.95 ±1.69	31.59-36.65	34.95± 1.31	1.04	Prolate-spheroidal	2.02	0.76	1.82	0	%100 Tricolporate
C. isaurica	2n = 18	32.47-35.6	33.77 ±0.99	32.68-37	34.77± 1.1	1.02	Prolate-spheroidal	1.96	0.79	1.90	0	%100 Tricolporate
C. ensiformis	?	42.88-47.46	45.15 ±1.30	41.52-49.57	46.26± 2.37	1.02	Prolate-spheroidal	2.33	0.76	2.08	0	%100 Tricolporate

*Garcia-Jacas ve ark. (1997), Romaschenko ve ark. (2004), Uysal ve ark. (2009), Uysal ve ark. (2015), Uysal ve ark. (2016)

Table 2. (Continued)

Taxa	Colpus		Porus		Amb	Apo.*	Sculpturing	Spine				
	Clg	Clt	Plg	Plt				Length	Width at base	Distance between spines	Number of spines (10 μm^2)	Perforation number at base
C. drabifolia subsp. *drabifolia*	29.29	8.12	9.43	10.17	8.36	25.79	Microechinate-perforate	1.19	2.83	0.81	30-36	20-30
C. drabifolia subsp. *austro-occidentalis*	30.05	9.24	10.97	11.76	8.26	27.39	Scabrate-perforate	0.84	4.45	3.39	20-24	5-10
C. drabifolia subsp. *floccose*	27.76	9.52	10.12	12.02	10.53	25.69	Scabrate-perforate	0.7	1.73	0.28	15-20	15-20
C. drabifolia subsp. *cappadocica*	26.99	7.67	7.77	9.55	6.53	27.49	Scabrate-perforate	0.64	2.06	1.53	15-20	35-50
C. kotschyi var. *kotschyi*	40.66	10.68	12.57	14.09	8.22	36.19	Scabrate-perforate	0.65	2.06	1.80	25-30	26-30
C. kotschyi var. *persica*	33.1	8.88	12.06	13.51	7.89	29.83	Scabrate-perforate	0.55	1.58	0.47	25-30	25-30
C. kotschyi var. *decumbens*	27.33	8.56	10.39	11.14	7.99	29.94	Scabrate-perforate	0.48	1.48	0.45	30-40	15-20
C. kotschyi var. *floccosa*	27.76	9.52	10.12	12.02	8.30	29.18	Scabrate-perforate	0.58	2.09	0.45	30-40	15-20
C. derderiifolia	31.17	7.23	10.47	11.19	12.95	32.53	Scabrate-perforate	0.42	1.66	0.43	42-50	15-20
C. deflexa	30.7	7.96	9.36	10.67	10.1	29.42	Microechinate-perforate	1.10	2.42	0.66	16-20	25-30
C. nivea	35.1	10.43	9.50	13.0	8.50	24.88	Scabrate-perforate	0.76	1.94	0.43	36-48	20-25
C. sericea	31.55	10.13	10.53	12.75	11.86	30.52	Scabrate-perforate	0.69	3.09	0.79	30-40	15-20
C. cankiriense	28.41	8.62	10.03	10.77	9.11	24.37	Microechinate-perforate	1	2.58	0.56	34-39	25-30
C. saligna	24.39	7.36	8.02	8.01	8.56	27.15	Scabrate-perforate	0.9	2.26	0.36	26-40	15-20
C. glabro-auriculata	28.97	8.15	10.4	10.51	9.8	27.57	Scabrate-perforate	0.85	1.92	0.67	40-45	20-25
C. isaurica	28.49	8.02	9.65	10.28	8.63	27.46	Scabrate-perforate	0.57	1.57	0.40	65-70	5-10
C. ensiformis	37.47	10.49	12.29	13.41	14.31	34.5	Microechinate-perforate	1.15	3.78	2.51	18-20	5-10

*apo.:apocolpium

The chromosome number of the members of *Cheirolepis* section have been determined as 2n=18, 2n=36 or 2n=54 (Garcia-Jacas *et al.*, 1997; Romaschenko *et al.*, 2004; Uysal *et al.*, 2009; Uysal *et al.*, 2015). With the present study we confirm that the heteromorphy in apertural system of *Cheirolepis* and the presence of cavea reflects chromosomal variation in *C. saligna*, *C. deflexa* and *C. sericea* (Table 2, Fig. 4). However, the other polyploid taxa does not have such a state.

Chaturvedi *et al.* (1990)and Brochmann (1992) reported that the size of the pollen grains strongly correlates with the level of polyploidy, however, the data obtained herein does not show such a correlation. The mean values for the P and E in diploid and polyploidy species are very similar (Table 2, Figs 2-7). Similarly, a great variation of P and E in diploid, triploid and tetraploid species of *Tripleurospermum* Schultz, Carl (Karl) Hein. and *Matricaria* L. were not observed by Ceter *et al.* (2013).

The subapical perforations in the spinules or spine bases in the family Asteraceae are a well-specified taxonomic character for distinguishing the taxa (Mesfin *et al.*, 1995; Ceter *et al.*, 2013; Pinar *et al.*, 2016) . Moreover, according to Wagenitz (1976), the reduction of the spine is a progressive characteristic for Asteraceae. The number of spines varies from species to species in *Cheirolepis*. The smallest spines were found in *C. kotschyi* var. *decumbens*, while the biggest were found in *C. derderiifolia*. Although the number of perforations were different, the present paper has shown that the size of the perforations was similar in each taxa. In parallel, some researchers have claimed that the ornamentations between the spines are important pollen characters in Asteraceae (Mesfin *et al.*, 1995; Ceter *et al.*, 2013). However, we found only the perforate ornamentation between the spinesas useful pollen traits.

According to Kaya (1986), Özler *et al.* (2009) and Hayta *et al.* (2017) *C. drabifolia* subsp. *floccosa*, *C. kotschyi* var. *floccosa*, and *C. derderiifolia* have a reduction in their spine length which is evaluated as an advanced pollen character in *Centaurea*. The present study supports this observation.

Among the studied taxa, 14 of them had 3-zonocolporate aperture. However, *C. kotschyi* var. *floccosa* had 98% 3-zonocolporate and 2% 4-zonocolporate apertures. Kaya (1986) studied the pollen morphology of *C. derderiifolia* and *C. saligna* and claimed a tricolporate aperture, spheroidal, suboblate pollen shape, 38.61±2.22 µm equatorial axes, and 38.6±2.11 µm polar axes in *C. derderiifolia*, as well as a 40.45±1.88 µm equatorial axes and 41.06±1.83 µm polar axes in *C. saligna*. The examined species had a similar narrow colpus and distinct porus. In our study, the species *C. saligna* collected from the Hakkari (*B. Çıtak*-168) and Ağrı (*K. Ertuğrul*-5117-*H. Dural* & *T. Uysal*) provinces showed apertures that were 98% tricolporate and 2% tetracolporate (Fig. 7). The apertures of the specimens, collected from the type locality of *C. saligna* (Erzurum province, *T. Uysal*-891), were mostly tricolporate (92%), which was followed by syncolporate (5%) and tetracolporate (3%) ones (Fig. 7). Similarly, the apertures observed in *C. derderiifolia* were mostly tricolporate (98%) and a few tetracolporate (2%, Fig. 7). The other observations of Kaya (1986) overlap with our records.

The UPGMA dendrogram based on pollen morphological characteristics has been discriminated against the species of *Cheirolepis*. The positions and groupings of the species of *Cheirolepis* reflected as the clusters in the dendrogram were found to be agreeable with the classification made earlier on a large scale (Fig. 8). However, *C. drabifolia* subsp. *drabifolia* positioned in different subclade has raised the question of whether new taxonomic delimitation of these taxa are required or not. From the findings of the pollen analysis, we could affirm that there was a significant correlation between the previous taxonomical classifications.

Conclusions

The pollen morphology of 17 species of section *Cheirolepis* were observed bylight microscopy and SEM, and among the palynological characters, the pollen size and spine length were determined as the most useful for the systematics of genus *Centaurea*. The pollen size and spine characteristics has been proved to be the most useful characters of systematic value in section *Cheirolepis*. The *Jacea* pollen type determined for this section based on Wagenitz's pollen types is more evolved one. The cluster analysis revealed that the taxa could be divided into three main groups according to the pollen morphology. The polar axes, equatorial axes, spine length, apocolpium, amb, the presence of cavea, costae, and number of spines are the most important characters to explain the variations among the taxa studied. Different aperture types, and spine size and density among the taxa could be associated with their different dispersal mechanisms or germination requirements, which may have arisen as a result of an evolutionary adaptation independently of different habitat types or microclimatic areas, especially for the endemics. The systematic problems of the section of *Cheirolepis* can be solved more precisely by the combination ofanatomical, macro- and micromorphological and molecular data.

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