

POLLEN MORPHOLOGY OF ENDEMIC NE ANATOLIAN *CIRSIUM* TAXA (ASTERACEAE)

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Abstract

In this study, the detailed pollen morphological structures of three endemic *Cirsium* (Asteraceae) Miller taxa showing the natural distribution in NE Anatolia, Turkey: *C. trachylepis* Boiss., *C. sommieri* Petrak, and *C. pseudopersonata* Boiss. & Bal ssp. *pseudopersonata* were examined. The taxa were investigated for the first time by using light microscope and scanning electron microscope. Pollen grains are oblate spheroidal and suboblate with tricolporate aperture type. Sizes of the grains range from 45.22 to 46.55 μm on the equatorial axis mean and from 40.50 to 41.67 μm on the polar axis mean. Exine structure is tectate and mean thickness of exine varies from 1.22 to 1.92 μm . Pollen ornamentations are echinate-perforate-microreticulate or echinate-microreticulate with suprategal spines. According to the variance analysis, there are some similarities among taxa but most of values investigated have been found significantly different. The pollen morphologies observed in this study are in a harmony with their sectional classification of *Cirsium* based on morphological characteristics.

Key words: *Cirsium*, Pollen morphology, Endemic, SEM, Turkey.

Introduction

Cirsium Miller represented in the subfamily Carduoideae (Asteraceae) is composed of 250-300 taxa (Stevens, 2001). They grow in the northern hemisphere and have spiny habitus (Zomlefer, 1994). They spread in the Southern Europe Mountains and in the Caucasus (Werner, 1976; Meusel & Jäger, 1992, Garcia-Jacas *et al.*, 2001). While some species are used as decorative plants (Charadze, 1998), few species, especially *C. arvense*, are invasive in cultivated areas (Anser *et al.*, 2018). In Turkey, *Cirsium* is classified in three sections (*Epitrachys* DC, *Cirsium*, *Cephalonoplos* (Necker) DC.) and has 80 taxa, 68 of which are species level (Davis & Parris 1975; Güner *et al.*, 2000; Yıldız, 2012; Yıldız *et al.*, 2013; Duman *et al.*, 2017). Turkey is one of the gene centers and new taxa have been defined day by day for the Turkish flora. 30 endemic *Cirsium* taxa identified in Turkey and according to recent reports endemism ratio is 37.5% (Yıldız, 2012; Duman *et al.*, 2017). Three of these endemic taxa are spread in NE Anatolia.

Cirsium is closely related to the genus *Centaurea* L. and has complex taxonomy (Charadze, 1998). Species of *Cirsium* also stand for morphological affinities with *Carduus* L., *Cnicus* L., and *Centaurea* in conjunction with their morphological characteristics including the structure of the florets and achene, leaf shape and spiny habitus (Davis & Parris, 1975). While previous studies considered the species of *Cirsium* as constituents of the genus *Cnicus*, lately some American authors have considered uniting *Cirsium* and *Carduus*. The difference of *Carduus* from *Cirsium* is mainly based on a single characteristic which is often regarded as artificial (Bremer, 1994).

Several researches were carried out on morphology, anatomy and taxonomy of the genus (Ozcanet *et al.*, 2008, 2011; Arabacı & Dirmenci, 2011; Yıldız *et al.*, 2013; Ozcan *et al.*, 2015). However, few studies have been reported on the pollen morphology of *Cirsium* taxa (Erdtman, 1945; Yıldız *et al.*, 2011, Şafak & Özhatay, 2012).

Our study provides information about the pollen morphologies of endemic *Cirsium* taxa spread in Northeast Anatolia by using light and electron microscopy and presents comparative palynological characteristics of the taxa to contribute their taxonomy. Data obtained from this study and previous results of related genera in the tribe *Cardueae* have been discussed.

Materials and Methods

Plant samples were collected from natural habitats of NE Anatolia, Turkey during vegetation periods of the years between 2007 and 2013 (Table 1). Taxonomical descriptions of the plants were made according to Davis and Parris (1975). Pollen grains were taken from herbarium materials. Herbarium samples and pollen slides were deposited at the Artvin Coruh University Herbarium (ARTH).

Wodehouse (1935) method was used to prepare pollen slides from mature flowers. Microphotographs were taken with scanning electron microscope (SEM) and light microscope (LM). Measurements and microphotographs were taken with an Olympus BX 53 microscope (with DP73 digital camera attachment). Pollen grains were examined in detailed by using a Zeiss/Evo LS10 scanning electron microscope. Measurements for each species were made of 50 pollen grains. The equatorial axis (E, polar and equatorial view), polar axis (P), colpus width (clt) and length (clg), porus length (plg) and width (plt), length of one side of triangular polar area, length (dh) and width (dt) of spine, exine and intine thickness were measured and spines per 100 μm^2 were counted on the pollen grains (Table 2). In addition, P/E ratio and polar area index (PAI) were calculated. Pollen shape and exine ornamentation were determined. Pollen terminology used by Faegri & Iversen (1975), Aytuğ & Merev (2002) and Punt *et al.*, (2007), Hesse *et al.*, (2009) were followed.

In order to calculate the mean (M), standard deviation (S), and variation (V) of measurements, JMP 5.0 package software was used. The analysis of variance was applied in defining the differences among the pollen characteristics of the taxa.

Table 1. Collection data of *Cirsium* taxa investigated.

Section	Taxon	Locality	Voucher number
<i>Epitrichys</i>	<i>C. trachylepis</i>	Trabzon: Akçaabat, Hıdırnebi High Plateau, roadside, 1413 m, 39°25'33.5"E, 40°56'53.8"N	M. Ozcan 660
	<i>C. sommieri</i>	Gümüşhane, Vauk Mountain, roadside, 1800 m, 39°50'40.3"E, 40°22'0.04"N	M. Ozcan 652
<i>Cirsium</i>	<i>C. pseudopersonata</i> ssp. <i>pseudopersonata</i>	Rize: Çamlıhemşin, Ayder, Yukarı Kavrun High Plateau, 2195m, 41°07'55.4"E, 40°53'27.2"N	M. Ozcan 663

Table 2. The palynological measurements of *Cirsium* taxa.

Character	<i>C. trachylepis</i>	<i>C. sommieri</i>	<i>C. pseudopersonata</i> ssp. <i>pseudopersonata</i>
E (polar view)	49.24 ± 5.06 ^a	44.39 ± 2.48 ^c	45.92 ± 2.78 ^b
t	19.82 ± 1.32 ^b	18.53 ± 1.18 ^c	21.88 ± 1.86 ^a
E (equat. view)	46.55 ± 4.80	45.22 ± 3.73	45.92 ± 3.03
P	41.67 ± 4.77	40.50 ± 4.41	41.07 ± 3.05
P/E	0.95	0.89	0.88
PAI	0.41 ^b	0.42 ^b	0.48 ^a
clg	32.27 ± 2.64 ^a	30.25 ± 2.07 ^b	27.12 ± 2.34 ^c
clt	11.49 ± 1.74 ^a	9.30 ± 2.12 ^b	8.50 ± 1.41 ^c
plg	13.98 ± 1.68 ^a	12.22 ± 2.09 ^b	12.32 ± 1.43 ^b
plt	12.32 ± 1.26 ^a	10.66 ± 1.84 ^b	9.59 ± 1.18 ^c
dh	3.87 ± 0.45 ^b	4.14 ± 0.38 ^a	3.42 ± 0.36 ^c
dt	5.19 ± 0.73	5.06 ± 0.68	5.33 ± 0.53
Exine	1.22 ± 0.18 ^c	1.37 ± 0.18 ^b	1.92 ± 0.34 ^a
Intin	1.42 ± 0.26 ^b	1.19 ± 0.28 ^c	1.67 ± 0.33 ^a
Spine per 100 µm ²	2	2	2.25

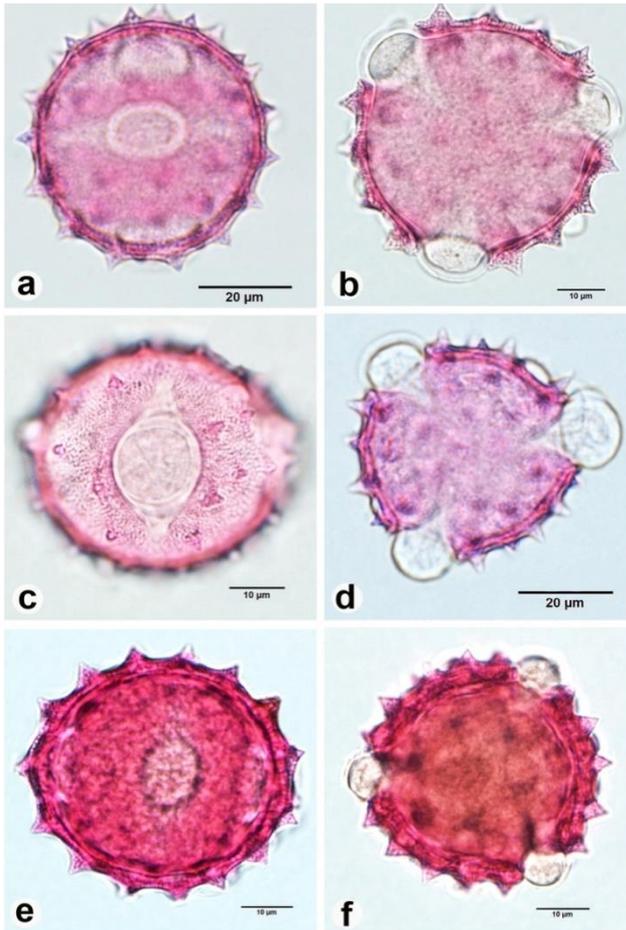


Fig. 1. Pollen micrographs (LM) of *Cirsium* taxa. a, b: *C. trachylepis*; c, d: *C. sommieri*; e, f: *C. pseudopersonata* ssp. *pseudopersonata*; a, c, e: equatorial view, b, d, f: polar view.

Results

The palynological characteristics of the taxa investigated in this study are illustrated in Table 2 and in Figures 1 and 2.

***Cirsium trachylepis*:** Pollen grains isopolar and tricolporate, 72% of pollen oblate spheroidal and 28% suboblate, amb circular (Fig. 1a, b and 2a-c). Polar axis is 41.67 (34.86-58.10) µm and equatorial axis is 46.55 (38.18-63.08) µm. Exine structure tectate, ornamentation echinate and interspinal area microreticulate with supratectal spines, reticules irregular. The exine is 1.22 (0.96-1.61) µm thick. Spines conic, blunt ended, 1-3 per 100 µm², 3.87 (3.11-4.86) µm in length, base diameter 5.19 (4.27-6.70) µm. The pollen has circular porus with distinct margin; Plg 13.98 (9.96- 18.26) µm, Plt 12.32 (9.96-14.94) µm. Colpus wide or slender at the equator, pointed, tapered or blunt towards the ends; Clg 32.27 (26.56-36.52) µm, Clt 11.49 (6.64-14.94) µm (Table 2).

***Cirsium sommieri*:** Pollen grains isopolar and tricolporate, 66% of pollen oblate-spheroidal and 34% suboblate, amb circular (Fig. 1c, d and 2d-f). Polar axis is 40.50 (33.2-54.78) µm and equatorial axis is 45.22 (39.84-58.1) µm. Exine structure tectate, ornamentation echinate and interspinal area microreticulate with supratectal spines, reticules irregular. The exine is 1.37 (1.02-1.74) µm thick. Spines conic, blunt ended, 1-3 per 100 µm², 4.14 (3.20-4.97) µm in length, 5.06 (3.80-6.51) µm in width. The pollen has circular porus with distinct margin; Plg 12.22 (8.30- 16.60) µm, Plt 10.66 (8.30-16.60) µm. Colpi wide or slender at the equator, pointed, tapered or blunt towards the ends; Clg 30.25 (23.24-34.86) µm, Clt 9.30 (6.64-14.94) µm (Table 2).

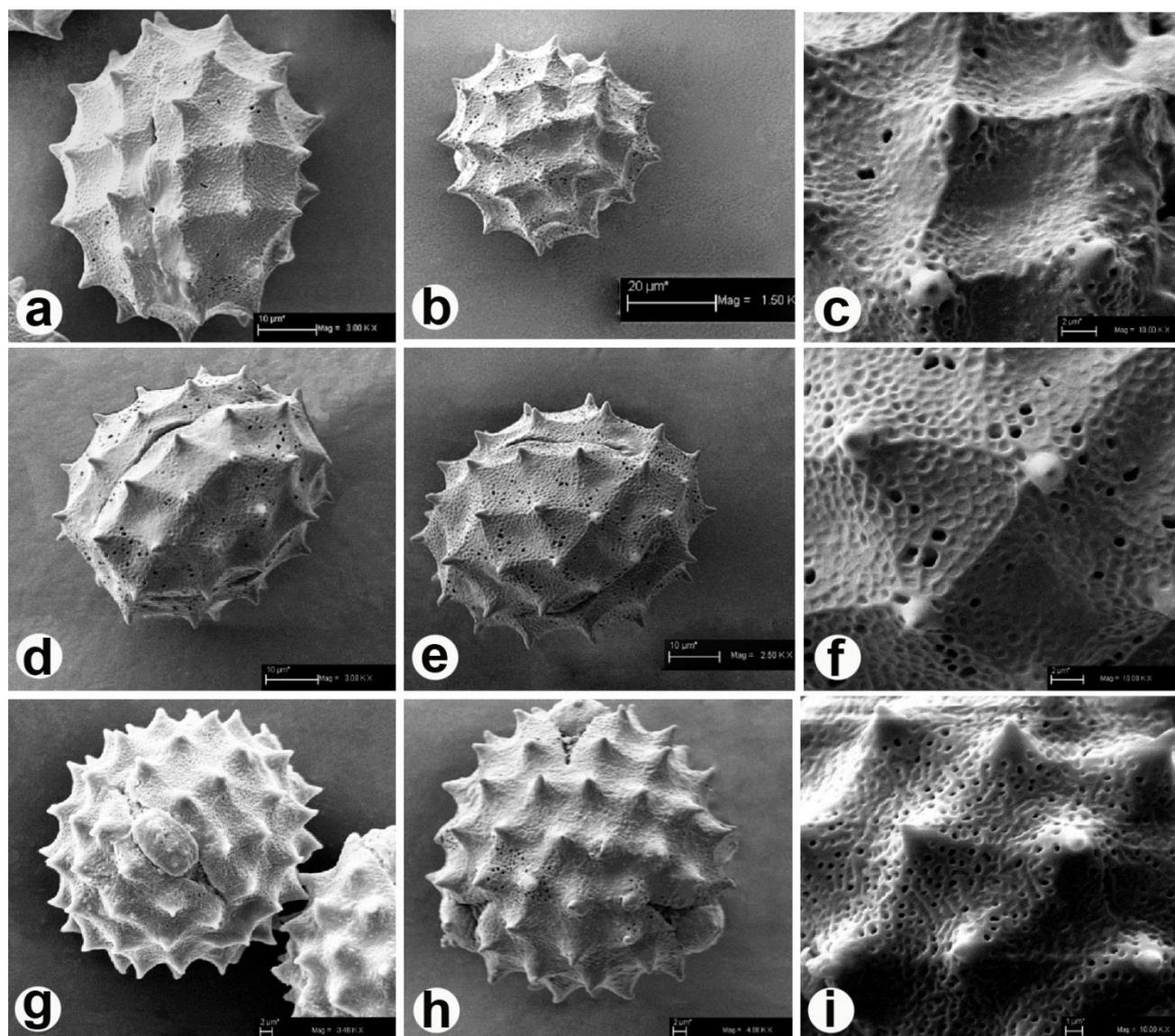


Fig. 2. Scanning electron micrographs (SEM) of the pollen grains. a, c: *C. trachylepis*; d, f: *C. sommieri*; g, i: *C. pseudopersonata* ssp. *pseudopersonata*; a, d, g: equatorial view, b, e, f: polar view, c, f, i: detail of spines and exine structure.

***Cirsium pseudopersonata* ssp. *pseudopersonata*:** Pollen grains isopolar and tricolporate, 72% of pollen oblate spheroidal and 28% suboblate, amb circular (Fig. 1e, f and 2g-i). Polar axis is 41.07 (34.86-48.14) μm and equatorial axis is 45.92 (36.52-49.80) μm . Exine structure tectate, ornamentation echinate and interspinal area perforate-microreticulate with suprategular spines, reticules regular. The exine is 1.92 (1.26-2.61) μm thick. Spines conic, pointed and curved, 2-3 per 100 μm^2 , 3.42(2.72-4.33) μm in length, base diameter 5.33 (4.29-6.53) μm . The pollen has circular porus with distinct margin; Plg 12.32 (9.96- 14.94) μm , Plt 9.59 (8.3-11.62) μm . Colpi wide or slender at the equator, pointed, tapered or blunt towards the ends; Clg 27.12 (24.90-36.52) μm , Clt 8.50 (6.64-11.62) μm (Table 2).

Discussion

The genus *Cirsium* has a widespread in the northern hemisphere and also on mountains of Southern Europe and in the Caucasus from sea level to high altitudes in the

mountains. In this study, endemic taxa from NE Anatolia were evaluated from palynological aspect. According to Davis and Parris (1975), two taxa of them are classified in *Epitrachys* section, while the other is indicated in *Cirsium* section (Table 1).

In this study, there are some similarities among the taxa but most of palynological values investigated are found significantly different (Table 2). *C. trachylepis* has the biggest pollen grains with 41.67 μm polar axis and 46.55 μm equatorial axis lengths, while *C. sommieri* has the smallest-sized pollen grains with 40.50 μm polar axis and 45.22 μm equatorial axis lengths. *C. sommieri* has the longest spine with 4.14 μm , while *C. pseudopersonata* subsp. *pseudopersonata* has the shortest with 3.42 μm (Table 2). However, the widest spines were measured in *C. pseudopersonata* subsp. *pseudopersonata*. It was emphasized that spine density and dimensions in Asteraceae are the most reliable characteristics for identification taxa in the flora of Turkey (Potoğlu Erkara, 2012). *C. trachylepis* and *C. sommieri*; two endemic species in section *Epitrachys* have very similar

morphological characteristics such as spiny upper leaf surface, nodding involucre. On the other hand, *C. pseudopersonata* subsp. *pseudopersonata* does not have these characteristics. PAI values of the two taxa (*C. trachylepis* and *C. sommieri*) are similar, while the other taxon has significantly the highest value. Namely, this value can be used for distinguishing the characteristics of these taxa in sectional levels. Our palynological results supported morphological identification of taxa. PAI value (Table 2) is also in agreement with sectional classification in the Flora of Turkey (Davis & Parris, 1975).

Many researches have been carried out on the pollen morphology of Asteraceae (Compositae) family. Akyalçın *et al.*, (2011) reported that the genus *Achillea* L. (the tribe Anthemideae) has heterogeneous pollen morphologies; oblate-spheroidal, prolate-spheroidal, subprolate and generally tricolporate, rarely tetracolporate or pentacolporate, echinate-microperforate and echinate-rugulate-microperforate. Pollen morphology of some *Artemisia* L. species was identified as tricolporate, isopolar, oblate-spheroidal, perspheroidal, subprolate and scabrate ornamentation (Hayat *et al.*, 2010). Çeter *et al.*, (2013) studied pollen morphology of closely relative two genera *Matricaria* L. and *Tripleurospermum* Sch. Bip. According to this study, *Tripleurospermum* pollens are determined as operculate and tricolporate, oblate-spheroidal, suboblate and prolate-spheroidal, and echinate granulate-perforate or reticulate-perforate, while the pollen grains of *Matricaria* is observed as operculate and usually tricolporate or rarely syncolporate, tricolporate and tetracolporate, oblate-spheroidal, and echinate rugulate-perforate.

In the tribe *Cardueae*, pollen morphology in some *Echinops* L. taxa trizonocolporate, spheroidal to prolate, microechinate/echinate-verrucate; in *Acantholepis orientalis* L. tricolporate, prolate, microechinate; in two *Xeranthemum* L. taxa trizonocolporate, prolate-spheroidal, verrucoid ornamentation; in *Chardinia orientalis* (L.) O. Kuntzetrizonocolporate, spheroidal, verrucoid ornamentation were reported, by Garnadje and Martin (2007), respectively.

In the genus *Centaurea* L., Pehlivan (1995) reported tricolporate, subprolate, tectate-perforate, microechinate-scabrate, triangular pollen grains in some endemic taxa. Özler *et al.*, (2009) investigated pollen morphology of some taxa and found that pollen grains are tricolporate, tetracolporate, isopolar, radially symmetrical, subprolate, spheroidal-subprolate, tectum perforate, scabrate and microechinate. Similarly, pollen morphologies 8 endemic taxa which have the characteristics of more or less spheroidal-subprolate, the amb triangular and tricolporate, tectate, microechinate-scabrate were reported by Potoğlu Erkara *et al.*, (2012).

Vilatersana *et al.*, (2005) find out that pollens were trizonocolporate, prolate-spheroidal to subprolate, echinate with spines contiguous by means of tectum elevation, with small perforations among spines in *Carthamus* L. complex. Bülbül *et al.*, (2013) reported pollen morphology of *Carthamustaxa* from Turkey as oblate-spheroid, spheroid, tricolporate rarely tetracolporate, echinate with micro perforations. The major difference of *Cirsium* from several different genera in Asteraceae is the echinateexine ornamentation with microreticulate and perforate-microreticulate.

In *Cirsium*, Yıldız *et al.*, (2011) described 3 types surface ornamentations: microreticulate with supratectalspinules (*C. rigidum* DC. and *C. sivasicum* Yıldız, Arabacı & Dirmenci), perforate-microreticulate with supratectal spines (*C. leuconeurum* Boiss. & Hausckn. and *C. peshmenianum* Yıldız, Arabacı & Dirmenci), rugulate with supratectal spines (*C. karduchorum* Petr.). It is found in our study that exine ornamentation is echinate, microreticulate with supratectal spines, reticules irregular in *C. trachylepis* and *C. sommieri*, and echinate perforate-microreticulate with supratectal spines, reticules regular in *C. pseudopersonata* subsp. *pseudopersonata*. The last taxon investigated in our study shows similar exine ornamentation with *C. leuconeurum* and *C. peshmenianum*.

Şafak & Özhatay (2012) reported sphaerooidal pollen grain in *Cirsium baytopae* Davis & Parris and *C. bulgaricum* DC. The fact that pollen grains of *C. karduchorum* is prolate-sphaerooidal (75%), the rest of four taxa are subprolate (55- 69%) were reported by Yıldız *et al.*, (2011). Our investigated taxa mainly different from the above mentioned reports in terms of pollen shape with oblate-spheroidal (66-72%).

According to our results, most of palynological characters (E, t, clg, clt, dh, plt, thickness of exine and intine) were found statistically important for three taxa; *C. trachylepis*, *C. sommieri*, *C. pseudopersonata* subsp. *Pseudopersonata* (Table 2). Şafak & Özhatay (2012) reported as we did in our study some distinctive differences between pollen sizes of their studied taxa.

The pollen morphologies observed in this study are coherent with their sectional classification of *Cirsium* based on morphological characteristics (Davis & Parris, 1975) and leaf anatomical patterns (Ozcan *et al.*, 2015). On the other hand, a few palynological studies have been reported in literature about *Cirsium* taxa so far. Further investigations should be continued to obtained wide interpretations about this genus.

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