**MELLIFERA** 

#### RESEARCH ARTICLE

# Pollen and Achene Morphology of Some Centaurea L. Taxa (Asteraceae), Turkey

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#### ABSTRACT

*Centaurea* is one of the largest genus of the family Asteraceae. Turkey is one of the distribution areas of this genus. This genus represented with 194 taxa in Turkey, which 105 of them are endemics. In this study, pollen morphology and achene micro and macro morphology of 5 taxa belonging to *Centaurea* (*C. iberica, C. virgata, C. cheirolopha, C. urvelli* subsp. urvelli, C. solstitialis subsp. solstitialis) were investigated. The aim of the study was to determine taxonomic value of pollen and achene micro features.

Pollen grains of *Centaurea* taxa observed as isopolar, with tricolporate aperture and subprolate and prolate-spheroidal pollen shape. Polar axis ranges between  $30.4-49.9 \ \mu m$  while equatorial axis between  $25.7-41 \ \mu m$ .

The achene shape was observed as ovoid and oblong in studied taxa. SEM showed that the achene surface had a striate sculpture and was depressed on both lateral surfaces, without rare hairs.

The micro characters contain polar axis, equatorial axis, pollen shape, pollen surface ornamentation, intine and exine thickness, achene length, Pappus length, achene surface ornamentation was determined as important features for systematics of taxa.

Keywords: Centaurea, Pollen, Achene, Light microscopy (LM), Scanning Electron Microscopy (SEM)

#### Introduction

*Centaurea* L. is one of the most richest genera of the family Asteraceae. In flora of Turkey, it is third largest genus after *Astragalus* and *Verbascum* in terms of number of species. Turkey is one of the distribution centres of the genus *Centaurea*; 105 out of 194 species are endemic to Turkey [1, 2, 3].

The members of the genus are annual, biennial, or perennial plants and they are rarely evergreen large shrubs. Leaves are alternate. Leaf shapes vary from pinnatifid to pinnatipartite and sometimes they are decurrent at base. Occasionally rosette laves are present at base of the stem. The involucral bracts are multi-seriate and imbricate. Each of them has membranous or skinny appendage which are usually hard spiny or bristly at apex. The flowers are pink, blackish-purple, blue, yellow, or whitish [4, 5].

Palynological, karyological and molecular studies are very important unravelling the taxonomic status of the genus *Centaurea*. As a result of the studies performed in recent years, some taxa of the genus *Centaurea* were transferred to other genera such as *Psephellus* Cass., *Cyanus* Mill. and *Rhaponticoides* Vaill. [6, 7, 8].

Çeter et al. (2013) examined pollen morphologies of 4 taxa belonging to the genus *Matricaria* and 28 taxa belonging to the genus *Tripleurosperum* of the family Asteraceae using Light microscope (LM) and Scanning Electron Microscope (SEM). Pollen of taxa described as isopolar, radially symmetric with tricolporate apertures. Pollen shape observed as suboblate and oblate-spheroidal. Pollen surface ornamentation determined as echinate and ornamentation of inter-spinal area as reticulate-perforate [9].

Özbek et al. (2016) studied pollen morphologies of 22 taxa belonging to the genus *Cota* using LM and SEM. Pollen grains of *Cota* determined as radially symmetrical and isopolar. Their shape is oblate-spheroidal and aperture type are trizonocolporate. The outline is elliptic in equatorial view and triangular in polar view. Exine sculpturing is echinate. Interspinal region exhibits perforate and microreticulate-perforate ornamentations. [10].

Kaya (1985) examined pollen structures of two endemic species of the genus Centaurea, C. derderiifolia and C. saligna, using light microscopy [11]. Atar (2006) examined pollen of *Centaurea kileae* and *C*. cuneifolia using LM and SEM [12]. Pinar (2007) examined pollen of taxa belonging to 5 sub-species of Centaurea cariensis (Centaurea cariensis Boiss. subsp. cariensis. Centaurea cariensis Boiss. subsp. longipapposa Wagenitz, Centaurea cariensis Boiss. subsp. maculiceps (O. Schwarz) Wagenitz, Centaurea cariensis Boiss. subsp. microlepis (Boiss.) Wagenitz, cariensis Centaurea Boiss. subsp. niveotomentosa (Hub.-Mor.) Wagenitz) using LM. In general, author determined pollen of these taxa as isopolar, radially symmetric and tricolporate. Ornamentation determined as echinate or echinateperforate [13].

Kargün (2011) investigated pollen (LM) and general morphologies of 29 taxa of the genera *Centaurea*, *Psephellus*, and *Cyanus*, which include the taxa that we studied except for *Centaurea cheirolopha* [14]. Shabestari, Attar, Riahi, and Sheidai (2013) examined pollen morphologies of 19 taxa of some *Centaurea* species using SEM and LM [15].

Inceer et al. (2012) examined anatomical and morphological character of fruit structures (Achene) of 12 endemic species belonging to the genus Tripleurospermum (Asteraceae). They observed that presence of corona and slime cell, achene colour and length, testa thickness, endosperm thickness, width of adaxial rib, thickness of lateral rib and achene surface ornamentation are important characters for taxonomy of taxa [16].

Tarıkahya Hacıoğlu et al. (2012) examined Achene morphologies of 5 species belonging to the *Carthamus* using SEM and stereomicroscopy. Results of study indicate that achenes broadly obpyramidal, oblique, 4 angled, light brown to brown, upper part darker. Pappus, straw coloured to brown, inner pappus shorter than outer. Palea was observed as scabrous and hilum as obpyramidal or oval. Ornamentation of achene surface was observed as striate or irregularly striate. [17]

Bona (2015) examined achene morphologies of 23 species, including *C*. *cheirolopha* and *C*. *virgata* which are examined in our study as well, belonging to the taxa *Centaurea*, *Cyanus*, *Psephellus*, and *Rhaponticoides* [18]. Bona (2014) also studied *Centaurea iberica*, which is examined in this study as well, and 6 other taxa. Results determine the achene of taxa as greenish-brown when young, later black, generally with pappus except to *C*. *sivassica*. Achene surface pattern is smoot, glebulate-ruminate and rugose. [19].

Celik et al. (2005) examined the pollen and Achene morphology of *Centaurea consanguinea*. Pollen of taxa determined as isopolar, radially symmetric and tricolporate. Surface ornamentation of pollen was observed as echinate-perforate. [20].

# **Materials and Methods**

The plant samples were collected from Adana province between 2005-2008 (Table 1). The pollen samples to be examined under light microscope were prepared according to the Wodehouse (1935) method [21]. The pollen slides were examined and photographed using a LEICA DM3000 light microscope. The measurements were performed based on the photographs using the AlaMet S. 0.06 software. The achene samples were examined using a Leica S8APO stereomicroscope. The pollen and achene samples were placed on a doubleIn this study, pollen and achene micro- and macro-morphologies of 5 *Centaurea* taxa (*C. iberica, C. virgata, C. cheirolopha, C. urvillei* subsp. *urvillei, C. solstitialis* subsp. *solstitialis*) investigated to determine the contributions of micro-morphological characters to the systematic discrimination of taxa.

sided band attached to aluminium stubs and coated with gold for the SEM study. The stubs were examined in detail and photographed using a FEI Quanta FEG250 electron microscope. 10 samples were used for each of the polar and equatorial axis length, colpus and pore length and width, exine and intine thickness, pollen ornamentation, polar and equatorial view. The achene length and width, pappus length, achene and pappus structure were examined in detail on the achene samples.

Collector code	Таха	Location
B.B. 4625	C. iberica Trev. ex Sprengel	B6 Adana: Saimbeyli,
B.B. 2105	C. virgata Lam.	B6 Adana: Tufanbeyli
B.B. 3939	C. cheirolopha (Fenzl) Wagenitz	B6 Adana: Tufanbeyli
B.B. 3022	C. urvillei DC. subsp. urvillei	B6 Adana: Tufanbeyli
<b>B.B.</b> 4234	C. solstitialis L. subsp. solstitialis	B6 Adana: Tufanbeyli

Table 1. List of investigated taxa and locations.

### **Results**

The data obtained from detailed examination of pollen and achene of 5 taxa belonging to the genus *Centaurea* using light and electron microscopy can be seen

in Table 2 and Table 3. The pollen and achene photographs of the species are given in Figure 1-5.

The pollen studies showed that the pollen of the taxa were isopolar and radially symmetrical and the aperture structure was tricolporate. The surface examination of the showed microechinate pollen ornamentation for С. iberica. microechinate-perforateornamentation for C. virgata, C. cheirolopha, and *C*. solstitialis subsp solsititialis and scabrate, microechinate-perforate ornamentation for urvillei subsp. urvillei. С. Dense perforation was detected at the bottom of spinules of C. cheirolopha pollen. The perforation was less and spinules were quite dense in C. solstitialis subsp solsititialis. Spinules were more sparser and smaller in C. urvillei subsp. urvillei and C. virgata compared to others. The polar view was semitriangular for С. iberica, С. cheirolopha, C. virgata, and C. urvillei

subsp. *urvillei* and trilobate for *C. solsitalis*. The pollen shape was subprolate for C. iberica, C. urvillei subsp. urvillei and C. solstitialis subsp. solsititialis and prolatespheroidal for *C*. virgata and С. cheirolopha. The C. urvillei subsp. urvillei had the largest pollen size, while C. solstitialis subsp. solsititialis had the smallest pollen size according to the measurements. Under light microscope, the equatorial axis measurements of the pollen varied between 25.7-41µm, the polar axis measurements varied between 35.4-53 µm, the colpus length varied between 21.6-29.4 µm, the colpus width varied between 8.8-3.7 µm, the pore length varied between 10.4-7.3 µm, and the pore width varied between 12.3-8.6 µm.

Tuble 2. Medisarement of ponen readines of contained taxa.															
Taxa	Polar axis (P) (µm)			Ekvatoral axis (E) (µm)		P/E	len shape	zine(μm) tine(μm)		Colpus (µm)		Porus (µm)	Aperture Type	29 Ornamentation	
	min	max	mean	min	max	mean	-	Pol	EK	İn	Clt	Clg	Plt Plg		
Centaurea iberica	30.7	39.7	34.8	26	35	30	1.2	Subprolate	2	0.4	28.9	5.9	10.412.3	Tricolporate	Microechinate
C. urvillei subsp. urvillei	43.9	53	49.9	36.8	48.1	41	1.2	Subprolate	2.9	0.4	21.6	8.8	9.3 8.5	Tricolporate	Scabrate, Microechinate- perfolate
C. virgata	28.6	33.4	30.4	26.1	30.8	28	1.1	Prolate spheroidal	2.4	0.3	27.2	7.9	8.5 9.8	Tricolporate	Microechinate- perfolate
C. cheirolopha	28.1	37.2	33.9	24.1	35	31	1.1	Prolate spheroidal	2.3	0.4	29.4	6.7	9.4 9.4	Tricolporate	Microechinate- perfolate
C salstitialis subsn	26.6	35.4	31.8	22.1	283	25.7	12	Subprolate	22	03	26	37	73 86	Tricolnorate	Microechinate-

Table 2. Measurement of pollen features of Centaurea taxa.

The achene of the taxa had pappus and the pappus on the inner rings were longer compared to the pappus on the outer rings. The achene surface showed striate ornamentation in *C. urvillei* subsp. *urvillei* and *C. virgata*, psilate-striate ornamentation in *C. cheirolopha* and psilate ornamentation in *C. solstitialis* subsp. *solsititialis*. The pappus were oblate on both sides and a striate ornamentation was detected on the pappus in *C. urvelli* subsp.

solsititialis

*urvelli.* 3 rows of serrate echinules were observed on the pappus of *C. solstitialis* subsp. *solsititialis*, while these echinules were as two rows located on the sides of the pappus in other taxa.

perfolate

Sparse trichomes were observed on the achene of *C. iberica*, *C. virgata*, and *C. urvillei* subsp. *urvillei*, while the trichomes were denser in *C. cheirolopha*. There were no trichomes on the achene surface in *C.* 

solstitialis subsp. solsititialis. C. urvillei subsp. urvillei had the longest achene (5.5 mm) among the taxa examined in this study, while *C. solstitialis* subsp. solsititialis had the shortest achene 2.3 mm.

The hilum aperture varied from 0.8 mm to 0.5 mm and was located laterally close to the base.



**Figure 1.** Light microscope (LM) microphotographs of *Centaurea* taxa. A-D: *C. iberica* (A: polar optic section, B: ornamentation in polar view, C: equatorial optic section, D: ornamentation and apertur in equatorial view). E-H: *C. virgata.* I-L: *C. cheirolopha.* M-P: *C. urvillei* subsp. *urvillei.* R-U: *C. solstitialis* subsp. *solstitialis* 

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**Figure 2.** Scanning electron microscope (SEM) microphotographs of *Centaurea* taxa. A-B: *C. iberica*. C-D: *C. virgata*. E-F: *C. cheirolopha* 

#### Discussion

In agreement to the measurement results of our study, Kargün (2011) found a polar axis length of  $34.11 \pm 1.46 \,\mu\text{m}$  and an equatorial axis length of  $31.38 \pm 2.28 \,\mu\text{m}$  for *C*. *iberica* pollen. The polar axis length was measured as  $58.69 \pm 3.97 \,\mu\text{m}$  and the equatorial axis length was measured as  $52.02 \pm 3.15 \,\mu\text{m}$  for *C. urvillei* subsp.

*urvillei* pollen, while the pollen were smaller and the P/E ratio was higher in our study. For *C. virgata* group A pollen, Kargün (2011) found an equatorial axis length of  $26.66 \pm 2.69 \mu m$  and a polar axis length of  $27.95 \pm 2.21$  and the P/E value was given as 1.83. The polar axis length was measured as  $34.03 \pm 2.45\mu m$  and the equatorial axis length was measured as  $31.50 \pm 1.46 \mu m$  for *C. solstitialis* subsp *solsititialis*. The P/E ratio was found to be

higher in our study. Kargün (2011) did not perform a SEM study on the pollen [14].



**Figure 3.** Scanning electron microscope microphotographs of *Centaurea* taxa. A-B: C. urvillei subsp. urvillei. C-D: C. *solstitialis* subsp. *solstitialis* 

Bona (2015) found the achene length to be 3-4 mm, the achene width to be 1-2 mm, and the pappus length to be 2 mm for *C*. *virgata*. While these measurement results are consistent with our study, Bona (2015) did not find a difference between inner and outer pappus length. The author also examined *C*. *cheirolopha* achene and found results similar to those of our study [18]. Bona (2014) examined *C*. *iberica* achene in another study and measured the pappus length to be 0.5-1 mm. In our study, on the other hand, the pappus length was much higher, while other achene measurements were similar with those of the author [19]. Taşar et al. (2014) examined *C. virgata* achene similar to our study and obtained results similar to ours (Figure 4).

In the previous researches on achene structures, pappus structures have not been studied in detail [21]. However echinule shape arrangement and structure and also surface ornamentation of pappus were found to be significant character for systematic of taxa in our study.

Studies show that pollen, seed, achene and fruit micromorphological characteristics of taxa reveal valuable data in systematic discrimination of taxa [22-25].

# Conclusion

As a result of this study, the characteristics such as polar and equatorial axis, polar and equatorial view, pollen shape and ornamentation, intine and exine thickness, achene length and width, pappus length and structure and achene ornamentation and color were found to be significant in terms of taxonomic delimination of taxa.

Table 3. Micro and macro morphological features of Centaurea achene (Measurement in mm).
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Taxa	Achene length	Achene width	Achene colour	Outher Pappus length	Inner Pappus length	Pappus colour	Hilum
C. iberica	3.2±0	1.6±0.1	Black or dark brown	0.5±0.2	1.9±0.2	Bright white	0.6±0
C. urvelli subsp. urvelli	5.5±0.7	1.8±0.5	pale straw	0.5±0.1	9.6±1.6	Bright white	0.7
C. virgata	3.5±0.1	1.6±0	Pale brown	$0.7 \pm 0.4$	2±0.4	Bright white	0.5±0.1
C. cheirolopha	4.7±0.2	2.2±0	white- grey	0.6±0,2	1.1±0.3	White	0.8±0.1
C. solstitialis subsp. solsititialis	2.3±0.1	1.1±0	Dark brown to pale brown	1.7±1.1	4.1±0.3	Bright white	0.5±0.1



**Figure 4.** Stereomicroscope microphotograph of *Centaurea* achene. A: *C. iberica*. B: *C. virgata*. C: *C. cheirolopha*. D: C. *solstitialis* subsp. *solsititialis*. E-F: *C. urvillei* subsp. *urvillei* 

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Figure 5. SEM microphotograph of *Centaurea* achene. A-C: *C.iberica*. D-F: *C.virgata*. G-I: *C.cheirolopha*. J-L: *C. solstitialis subsp solsititialis*. K-M: *urvillei* subsp. *urvillei* 

# Türkiye'de Yayılış Gösteren BazıCentaureaL.Casteraceae)TaksonlarınınAkenMorfolojilerinin İncelenmesi

Öz: *Centaurea*, Asteraceae familyası içerisinde en büyük cinslerden birisidir. Türkiye bu cinsin ana yayılış alanlarından birisini teşkil etmektedir. Türkiyede 105 tanesi endemik olmak üzere Centaurea cinsine ait 194 takson vavilis göstermektedir. Bu çalışmada, Centaurea (C. iberica, C. virgata, C. cheirolopha, C. urvelli subsp. urvelli, C. solstitialis subsp. solstitialis) cinsine ait 5 taksonun polen ve aken morfolojisi 151k mikroskobu ve taramalı elektron mikroskobu ile ayrıntılı olarak incelenmiştir. Çalışmanın amacı polen ve aken mikro ve makro-morfolojik özelliklerinin taksonların sistematik ayırımındaki katkılarının ortaya konulmasıdır.

Çalışılan *Centaurea* taksonlarının polenleri izopolar kutuplu, trikolporat aperture yapısına sahip olup polen şekilleri subprolat ve prolat-sferoildal olarak saptanmıştır. Polar eksen uzunluğu 30.4-49.9 µm arasında saptanırken, ekvatoral eksen uzunluğu 25.7-41 µm arasında saptanmıştır.

Taksonların aken şekli oval veya oblong olarak saptanmıştır. SEM mikrofotoğraflarının analizi sonucunda akenlerin yüzeyinin striat ornamentasyon gösterdiği, trikomsuz veya seyrek trikomların olduğu ve lateral olarak iki yandan basık oldukları saptanmıştır.

Polar eksen, ekvatoral eksen, polen şekli, polen yüzey ornamentasyonu, intin ve ekzin kalınlığı, aken boyutu, pappus boyu, aken yüzey ornamentasyonu gibi mikro ve makro karakterler taksonların sistematik ayırımı açısından önemli ve ayırıcı karakterler olarak saptanmıştır.

AnahtarKelimeler:Asteraceae,Centaurea,Polen,Aken,Işıkmikroskobu,TaramalıElektronMikroskobu.

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