### **MELLIFERA**

### RESEARCH ARTICLE

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

Halime ATAR<sup>1\*</sup>. Barış BANİ<sup>1</sup>. Talip ÇETER<sup>1</sup>

<sup>1</sup> Kastamonu University, Arts and Sciences Faculty, Department of Biology, Kastamonu, Turkey

\*Corresponding author e-mail: hatar@kastamonu.edu.tr

Received: 05th December, 2018; accepted: 15th December, 2018; published: 28th December, 2018

#### ABSTRACT

Cyanus is a genus of family Asteraceae. 20 taxa belonging to Cyanus distributes in Turkey and 9 of them are endemics. In this study, pollen morphology and achene micro and macro morphology of 4 taxa belonging to Cyanus (C. depressus, C. triumfettii, C. pichleri subsp. pichleri, C. lanigerus) were investigated. The aim of the study was to determine taxonomic value of pollen and achene micro characteristic.

Pollen grains of Cyanus taxa observed as isopolar, radially symmetric, with tricolporate aperture and subprolate pollen shape. Polar axis range between 36-55.8  $\mu$ m while equatorial axis between 27.8-47  $\mu$ m. C. lanigerus pollen measured as biggest pollen wile C. depressus pollen is the smallest. Pollen surface ornamentation determined as scabrate.

The achene shape was observed as ovoid and oblong in studied taxa. SEM results showed that the achene surface had a striate-psilate sculpture and was depressed on both lateral surfaces, without rare hairs. The Achene length (4-5mm), width (1.6-2.4 mm) and pappus length (1-6.4 mm) ranges differed significantly. C. triumfettii achene determined as smallest (4.0 mm) in length with the smallest (1.1 mm) pappus while C. lanigerus were longest (5.0 mm) achene with smallest (1.0 mm) pappus.

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

Keywords: Asteraceae, Cyanus, Pollen, Achene, Light microscopy, Scanning Electron Microscopy

## Introduction

Cyanus is a member of the family Asteraceae. The family Asteraceae has a very large distribution around the world. In Turkey, there are approximately 1200 species, 450 of which are endemics, within 136 genera. Thus, it is the richest family of Turkey's flora in terms of both species and genus diversity. Leaves are usually alternate or opposite and rarely round, simple, or compound in the family Asteraceae. Flowers are grouped in capitulum, hermaphrodite, unisexual, or sterile. The capitulum is surrounded by one or more involucral bracts. The calyx may be

absent or modified into a papus. The ovary is inferior and has a single loculus and a single basal anatropous ovule. The fruit is usually an achene with often a papus or calyx residue at the apex [1-4].

There are about 27 species distributed around the world within the genus *Cyanus*. 20 species belonging to the genus *Cyanus* were identified in Turkey. 9 of these species are endemics to Turkey. The genus *Cyanus* contains both annual and perennial species. The stem of the plant is unbranched or slightly branched in perennial species,

while annual species are quite branched. Both surfaces of the leaves are hairy in species of the genus [5]. However glabrous species in the genus are rare. Flowers are violet and rose-pink/purplish or red. The pappus is usually short and the achene surface is rough due to stiff hairs. The pappus may be absent in some species [6-7].

Tasar, Kıran, and Doğan, (2017) examined the species *Cyanus depressus* (M. Bieb.) Soják in terms of karyology and palynology. The palynological studies showed that the pollen shape was subprolate, the aperture type was tricolporate, and the ornamentation was scabrate [8].

Kargün (2011) investigated the species belonging to the genera *Centaurea*, *Psephellus*, and *Cyanus* naturally growing in the Elazığ Region in terms of morphology and palynology and examined the pollen of *Cyanus depressus*, *C. pichleri* subsp. *pichleri*, and *C. triumfettii* using light microscopy [9]. Pınar (1989) and Pınar and İnceoğlu (1996) examined the pollen morphology of the A, B, and C groups of *Centaurea triumfettii* using light and electron microscopy [10.11].

Özler et al. (2009) was examined pollen morphology of 29 taxa, 24 of which are endemic to Turkey, by light and scanning microscopy. Pollen electron grains determined tricolporate, rarely as tetracolporate, isopolar, radially symmetrical, subprolate, spheroidalsubprolate, operculate, tectum perforate, scabrate and microechinate [12].

Atar (2006) examined pollen of *Centaurea kileae* and *C. cuneifolia* using LM and SEM [13]. Pınar (2007) investigate pollen of 5 sub-species of *Centaurea cariensis* with

light microscope. Researcher describe pollen of these taxa as isopolar, radially symmetric and tricolporate. Ornamentation determined as echinate or echinate-perforate [14].

Shabestari, Attar, Riahi and Sheidai (2013) examined pollen morphologies of 19 taxa of Centaurea using SEM and LM. Study results show that pollen grains are tricolporate, isopolar and radially symmetric. Pollen shape determined as oblate-spheroidal, prolate-spheroidal, subprolate. Ornamentation described as perforate and scabrate according LM studies. The exine ornamentation identified two types of pollen grains, designated type I (dense acute spinules) and type II (sparse spinules) according to SEM results [15].

Çeter et al. (2013) investigated pollen morphologies of 32 taxa belonging to the Matricaria and Tripleurosperumum using LM and SEM. Pollen of taxa described as radially isopolar, symmetric with tricolporate apertures. Pollen shape observed as suboblat and oblate-spheroidal. Exine sculpture determined as echinate and ornamentation of inter-spinal area as reticulate-perforate [16].

İnceer et al. (2012) investigated anatomical and morphological features of achene of 12 endemic Tripleurospermum Researcher observed that presence of corona and slime cell, achene colour and thickness, length, testa endosperm thickness, width of adaxial rib, thickness of lateral rib and achene surface ornamentation are valuable features for systematic of taxa [17].

Tarıkahya Hacıoğlu et al. (2012) investigated achene morphologies of 5 *Carthamus* taxa using SEM and stereomicroscopy. Researcher determine

the achene broadly obpyramidal, oblique, 4 angled, light brown to brown, upper part darker. Pappus, straw coloured to brown, inner pappus shorter than outer. Palea scabrous. Hilum obpyramidal or oval. ornamentation of achene surface striate or irregularly striate [18].

Bona (2014) examined achene of 7 *Centaurea* taxa and Bona (2015) examined achene morphologies of 23 taxa belonging to *Centaurea*, *Cyanus*, *Psephellus*, and *Rhaponticoides*. 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. 20].

The purpose of this study is to examine the pollen and achene properties of *C. depressus* (M.Bieb.) Soják, *C. triumfettii* (All.) Dostál ex Á.Löve & D.Löve, *C. pichleri* (Boiss.) Holub subsp. *pichleri*, and *C. lanigerus* (DC.) Holub using light and electron microscopy and determine the contributions of these properties to the systematic discrimination of taxa.

# **Materials and Methods**

The plant samples were collected from Adana and Kayseri provinces and prepared as herbarium material after identification (Table 1). The pollen samples prepared according to the Wodehouse (1935) method [21]. Pollen microphotograph taken using a light microscope (Leica DM3000) and the measurements were performed using the AlaMet S. 0.06 software. The achene from species samples the were photographed using a Leica S8APO stereomicroscope. For each micromorphological features measurements were performed form 20 microphotograph as described in literature [22, 23]. The pollen and achene samples were placed on aluminum stabs and coated with gold for the scanning electron microscopy (SEM) study. The pollen and achene microphotographs of the samples were taken in the Central Research Laboratory of Kastamonu University using a FEI Quanta FEG250 electron microscope. The pollens and achenes were analyzed in detail and identified using the photographs. Terminology was adopted from Faegri and Iversen (1975), Salgado-Labouriau (1982), and Punt and Hoen (2009) and shape classification follows that of Erdtman (1969) based on P/E ratio [24-27]

**Table 1.** Cyanus taxa and their location

Collector code	Taxa	Location
B.B. 3032	C. depressus	B6 Adana: Tufanbeyli 1560-1600 m
B.B. 2799	C. triumfettii	B6 Adana: Tufanbeyli, 1400-1500 m
B.B. 5091	C. pichleri supsp. pichleri	B6 Kayseri: Develi, 1700 m
B.B. 2915	C. lanigerus	B6 Adana: Tufanbeyli, 2250-2300 m

# **Results and Discussion**

The data obtained from detailed examination of pollens and achenes of 4 taxa belonging to the genus *Cyanus* 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 1-4.

The pollen studies of the taxa showed that the pollen of *C. depressus* were radially symmetrical and isopolar, the pollen shape was subprolate, the aperture type was tricolporate, and the ornamentation was psilate around the colpus and scabrate-perforate at other parts.

The pollen of *C. triumfettii* were radially symmetrical and isopolar, the pollen shape was subprolate, the aperture type was tricolporate and the ornamentation was scabrate-perforate. The pollen of *C.* 

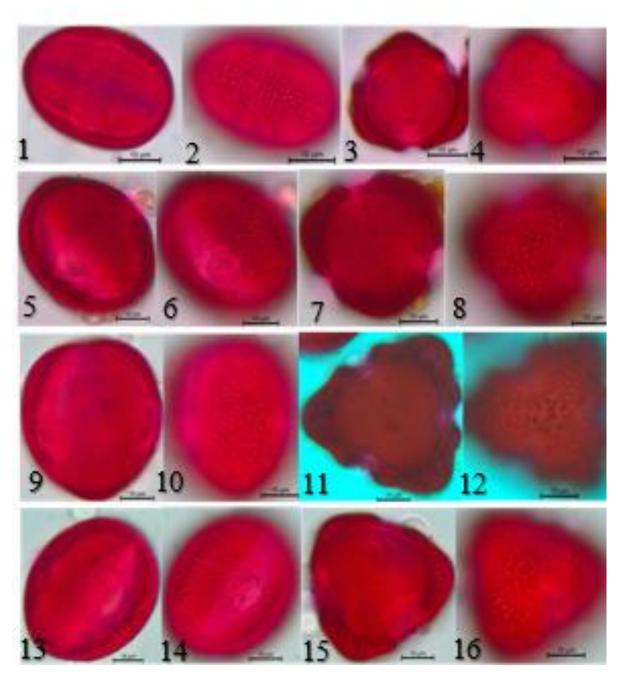
triumfettii were found to have similar properties in light microscopy examinations of Kargün [9] and studies performed by Pınar [11].

In this study, the pollen of *C. lanigerus* were radially symmetrical and isopolar, the pollen shape was subprolate, the aperture type was tricolporate, and the ornamentation was scabrate.

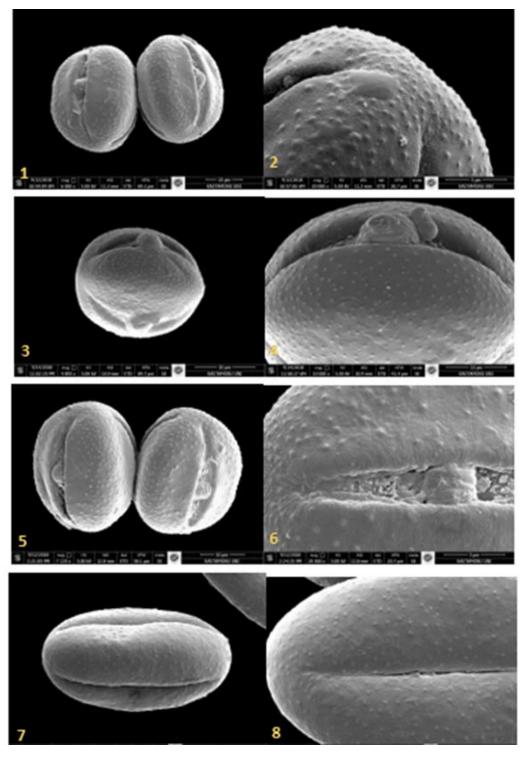
The pollen of *C. pichleri* subsp. *pichleri* were radially symmetrical and isopolar, the pollen shape was subprolate, the aperture type was tricolporate and the ornamentation was scabrate-psilate. Kargün (2011) found the same properties for the pollen of *C. pichleri* subsp. *pichleri* [9].

**Table 2.** Measurement and pollen features of studied *Cyanus* taxa (value in μm).

Taxa	Polar axis (P) (um)			Equatorial axis (E) (μm)			P/E	Polen Shape	Exine(µm)	İntine(µm)	Colpus				Aperture type	Ornamentation
	min	max	ort	min	max	ort					Clt	Clg	Plt	Plg		
C. depressus	29.8	39.5	36.2	25.5	29.5	27.8	1.3	subprolate		0.3 8	28.6	5.9	7.1	8.5	Trikolporat	Scabrate-perforate, Psilate at aperture surround
C.triumfettii	33.4	53.2	45.4	29	40.7	35.9	1.2	subprolate	4.1	0.5	34	7	7.6	8.1	Trikolporat	Scabrate-perforate
C. lanigerus	50.5	66.3	55.8	45	50.1	47	1.2	subprolate	4.8	0.5	44.3	10.1	12.5	12.7	Trikolporat	Scabrate
C.pichleri subsp. pichleri	42.4	53.4	49	36.6	43.2	40.7	1.2	subprolate	4.3	0.4	37	8.8	9.3	10	Trikolporat	scabrate-psilate



**Figure 1.** Light microscope microphotograph of *Cyanus* taxa, 1-4: *C.*, 5-8: *C. triumfettii*, 9-12: *C. lanigerus*, 13-16: *C. pichler*i subsp. *pichleri* (1,5,9,13: equatorial optic section, 2.6.10.14: ornamentation and aperture in equatorial view 3,7,11,15: polar optic section, 4,8,12,16: ornamentation in polar view)



**Figure 2.** Scanning electron microscope microphotographs of *Cyanus* taxa. 1-2: *C. depressus*, 3-4: *C. triumfettii*. 5-6: *C. lanigerus*, 7-8: *C. pichleri* subsp. *pichleri*.

According to the SEM results of the taxa, the achene surface had striate-psilate ornamentation. The achenes of the taxa had trichomes on both lateral surfaces. *Cyanus pichleri* subsp. *pichleri* had long and dense trichomes on the hilum and dense trichomes on the achene body. The achene length varied from 4 to 5 mm, the achene width varied from 1.6 to 2.4 mm, and the pappus length varied from 1 to 6.4 mm, and showed significant differences. *C. triumfettii* and *C. lanigerus* had the shortest inner pappus

length among the species with 1.1 mm and 1.0 mm, respectively. *C. triumfettii* had the shortest achene body length (4.0 mm). Where *C. lanigerus* had the longest achene body length (5.0 mm). The achene measurements showed that *C. depressus* had the widest hilum. The achene photographs and measurements of the species showed that the species had significant differences in terms of color and pappus length (Table 3) (Figure 3-4).

**Table 3.** Micro and macro morphological features of *Cyanus* achene (measurement in mm).

taxa	Achene length	Achene width	Achene Colour	Outer papus length	Inner papus length	Papus colour	Hillum
C. depressus	4.5±0.1	2.4±0.2	Dark straw	0.5±0.2	6.4±0.8	Dark straw	2.6±0.0
			pale straw				9
C.triumfettii	4±0.2	2±0.2	Brown	$0.4\pm0.1$	1±0.1	Dark straw	1.1±0.1
C. lanigerus	5±0.4	1.6±0.2	Brown	$0.4\pm0.1$	1±0.3	Pale straw	1.2±0.2
C. pichleri	4±0.8	2.2±0.2	Pale Brown	1.1±0.5	1±0.3	Pale straw	1.2±0.2
subsp. pichleri							

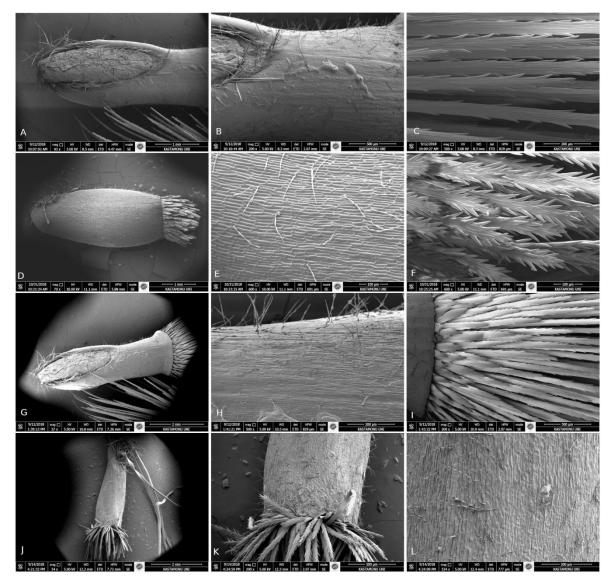
The pollen of the *Cyanus* species were found to be isopolar, tricolporate, and radially symmetrical. The pollen shape was subprolate, the polar axis varied from 36 to 55.8 µm and the equatorial axis varied from 27.8 to 47 µm. The pollen ornamentation was found to be scabrate, scabrate-perforate, and scabrate-psilate. *C. lanigerus* had the largest pollen size among the taxa, whereas *C. depressus* had the

smallest. The pollens of the species were observed to be similar in terms of morphological properties.

Microcharacters such as polar axis, equatorial axis, pollen shape, pollen surface ornamentation, intine and exine thickness, achene length, pappus length, and achene surface ornamentation were found to be significant properties for systematic differentiation between taxa.



**Figure 4.** Stereomicroscope photographs of *Cyanus* achene. A-B: *C. depressus*, C-D: *C.triumfettii*, E-F: *C. lanigerus*, G-H: *C.pichleri* subsp. *pichleri*.



**Figure 5.** SEM Photographs of *Cyanus* achene. A-C: *C. depressus*, D-F: *C. triumfettii*, G-I: *C. lanigerus*, J-L: *C. pichleri* subsp. *pichleri*.

# Türkiye'de Yayılış Gösteren Bazı Cyanus L. (Asteraceae) Taksonlarının Aken ve Polen Morfolojilerinin İncelenmesi

Öz: Cyanus, Asterace familyasına ait bir cins olup 9 taksonu endemik olmak üzere 20 taksonu Türkiye'de doğal yayılış göstermektedir. Bu çalışmada Cyanus cinsine ait 4 taksonun (C. depressus, C. triumfettii, C. pichleri subsp. pichleri, C. lanigerus) polen ve aken mikro ve makro

morfolojisi ışık mikroskobu ve taramalı elektron mikroskobu ile çalışılmıştır. Çalışmanın amacı taksonların polen ve aken morfolojik özelliklerini belirlemek ve taksonların sistematik ayırımına katkısını saptamaktır.

Çalışma sonucunda *Cyanus* taksonlarının polenleri izopolar, radyal simetrili ve trikolporat olarak saptanmıştır. Polen şekli subprolat olup polar eksen uzunluğu 36-55.8 µm arasında, ekvatoral eksen

uzunluğu 27.8-47 µm arasında saptanmıştır. Polen yüzey ornamentasyonu skabrat olarak belirlenmiştir. En büyük polenler *C. lanigerus* türünde saptanırken, en küçük polenler *C. depressus*'ta tespit edilmiştir.

Çalışılan taksonların akenleri pappuslu olup, oval veya oblong şekle sahiptir. Akenler iki yandan basık olup seyrek olarak tüylere rastlanmıştır. Stereo mikroskop fotoğraflarında aken yüseyi psilate görülürken, elektron mikroskop görüntülerinde sitriat-psilat olarak saptanmıstır. Aken uzunluğu 4-5 mm, eni 1.6-2.4 mm arasında ve pappus uzunluğu 1-6.4 mm arasında değişmektedir. En

küçük aken (4.0 mm) *C. triumfettii*'de saptanırken, en uzun aken (5.0 mm) *C. lanigerus* saptanmıştır.

Polar eksen, ekvatoral eksen, polen şekli, intin ve ekzin kalınlığı ile polen yüzey ornamentasyonu gibi polen mikromorfolojik özellikleri ile aken büyüklüğü, pappus boyu, aken yüzey ornamentasyonu gibi özelliklerin taksonların sistematik ayırımında kullanılabilecek ayırt edici karakterler olduğu saptanmıştır.

**Anahtar Kelimeler:** Asteraceae, *Cyanus*, Polen, Aken, Işık Mikroskobu, Taramalı Elektron Mikroskobu.

### REFERENCES

[1]GÜNER, A; ASLAN, S; EKİM, T; VURAL, M; BABAÇ, M T (2012) Türkiye Bitkileri Listesi (Damarlı Bitkiler). Flora Araştırmaları Derneği ve Nezahat Gökyiğit Botanik Bahçesi Yayını; İstanbul.

[2]WAGENITZ G (1986) *Centaurea* in South-west Asia; Patterns of distribution and diversity, Proceeding of the Royal Society of Edinburgh Vol: 89 Section: B, 11-21.

[3]GÜNER, A; ÖZHATAY, N; EKİM, T (2000) Flora of Turkey and the East Aegean Islands, Vol:11. Edinburg Press.

[4]DAVİS, P H (1975) Flora of Turkey And The East Aegean Islands, Vol:5. Edinburgh Univ. Pres; Edinburgh.

[5]WAGENİTZ, G; HELLWİG, G H (2000) The genus *Psephellus* Cass. (Compositae, Cardueae) revisited with a broadened concept. Willdenowia, 30(1): 29-44.

[6]GREUTER, W (2003) The Euro+Med treatment of Cardueae (Compositae) — generic concepts and required new names. Willdenowia, 33(1): 49-61.

[7]KAYA Z (1985) Endemik İki *Centaurea* Türü Üzerinde Taksonomik, Ekolojik ve Palinolojik Araştırmalar. Yüksek Lisans Tezi, Marmara Üniversitesi, Üniversitesi Sağlık Bilimleri Enstitüsü / Tıbbi Biyoloji Anabilim Dalı, İstanbul.

[8]TAŞAR, N; KIRAN, Y; DOĞAN, G (2017) *Cyanus depressus* (M. Bieb.) Soják Türünün Karyolojik ve Palinolojik Yönden İncelenmesi. Düzce Üniversitesi Bilim ve Teknoloji Dergisi, 5 (1): 299-305.

[9]KARGÜN K (2011) B7 Elâzığ Bölgesinde Yetişen *Centaurea*, *Psephellus* ve *Cyanus* Cinslerine Ait Türleri Üzerinde Morfolojik Ve Palinolojik Araştırmalar. Yüksek Lisans Tezi, Bartın Üniversitesi Fen Bilimleri Enstitüsü; Bartın.

[10]PINAR N M; İNCEOĞLU Ö (1996) A Comparative Study on The Pollen Morphology of *Centaurea triumfettii* All. groups A, B and C with Light and Electron Microscopy, Turkish Journal of Botany, (20): 395-398.

[11]PINAR N M (1989) Centaurea truumfettii All Grup, A, B ve C Polen Morfolojilerinin Işık ve Elektron Mikroskopunda Karşılaştırmalı İncelenmesi, Yüksek Lisans Tezi, Fen Bilimleri Enstitüsü, Ankara Üniversitesi, Ankara

[12]ÖZLER H; KAYA Z; PEHLİVAN S (2009) Pollen Morphology of Some *Centaurea* L., *Psephellus* Cass. and *Cyanus* Miller Taxa, Gazi Üniversitesi, Bartın Üniversitesi, Acta Biologica Cracoviensia Series Botanica 51 (2): 53–66.

[13]ATAR, M (2006) *Centaurea kilaea* Boiss. ve *Centaurea cuneifolia* Sm. Üzerinde Morfolojik ve Palinolojik Araştırmalar. Yüksek Lisans Tezi, Marmara Üniversitesi Fen Bilimleri Enstitüsü; İstanbul

[14]PINAR, A (2007) Türkiye İçin Endemik *Centaurea* cariensis Boiss. Alttürleri Üzerinde Morfolojik Ve Palinolojik Araştırmalar. Yüksek Mühendislik Tezi, Zonguldak Karaelmas Üniversitesi Fen Bilimleri Enstitüsü; Zonguldak

- [15]SHABESTARİ E; ATTAR F; RİAHİ H; SHEİDAİ M (2013) Pollen Morphology of *Centaurea* L. (Asteraceae) in Iran. Acta Botanica Brasilica, 27(4): 669-679.
- [16]INCEER, H; BAL, M; ÇETER, T; PINAR, N M (2012) Fruit structure of 12 Turkish endemic *Tripleurospermum* Sch. Bip. (Asteraceae) taxa and its taxonomic implications. Plant Systematics and Evolution, 298(4): 845-855.
- [17]ÇETER T; PINAR N M; İNCEER H; HAYIRLIOĞLU AYAZ S; YAPRAK A E (2013) The Comparative Pollen Morphology of Genera *Matricaria* L. and *Tripleurospermum* Sch. Bip. (Asteraceae) in Turkey, Plant Systematics and Evolution, 299(5): 959-977.
- [18]TARIKAHYA HACIOGLU B; ARSLAN Y; SUBASI İ; KATAR D; BÜLBÜL A S; ÇETER T (2012) Achene morphology of Turkish *Carthamus* species. Australian Journal of Crop Sciences, 6(8): 1260-1264.
- [19]BONA M (2015) Systematic implications of achene characteristics in genera *Centaurea* L., *Cyanus* Mill., *Psephellus* Cass. and *Rhaponticoides* Vaill. (Asteraceae). Bangladesh Journal of Plant Taxonomy, 22(2):125-136.
- [20]BONA M (2015) Achene characteristics of Turkish *Centaurea* (Asteraceae) and their systematic application. Bangladesh Journal of Botany, 43(2):163-168.

- [21]WODEHOUSE R P (1935) Pollen grains. Newyork, Mc Graw-Hill Press.
- [22] PINAR N M; DURAN A; ÇETER T; TUĞ G N (2009) Pollen and Seed Morphology of the Genus *Hesperis* L. (Brassicaceae) in Turkey. Turkish Journal Botany, 33(2): 83-96.
- [23]BANİ M; KARAKAYA M A; ÇETER T (2016) Fruit micromorphological characters of the genus *Grammosciadium* DC. (Apiaceae) in Turkey. Phytotaxa, 246 (3): 184-191.
- [24]FAEGRI K; IVERSEN J (1975) Textbook of pollen analysis, 4th edn. Wiley, New York.
- [25]SALGADO-LABOURIAU M L (1982) On cavities in spines of Compositae pollen. Grana 21:97–102.
- [26]PUNT W; HOEN P P (2009) The Northwest European Pollen Flora, 70: Asteraceae–Asteroideae. Rev Palaeobot and Palynol 157:22–183.
- [27]ERDTMAN G (1969) Handbook of palynology, morphology, taxonomy and ecology. Munksgaard, Copenhagen.