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Research Article

Pollen Morphology of 5 *Centaurea* L. and 3 *Psephellus* Cass. Taxa in Turkey

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Abstract: In this study, pollen morphology of 5 *Centaurea* (*C. antitauri*, *C. coronopifolia*, *C. iberica*, *C. patula*, and *C. pterocaula*) and 3 *Psephellus* (*P. hypoleucus*, *P. pulcherrimus*, and *P. simplicicaulis*) species distributed in Turkey have been examined based on the exine structure and sculpturing. Previous papers drew attention to great value of pollen morphology in the taxonomy of *Centaurea* and divided genus from *Centaurea* as *Rhaponticoides*, *Psephellus* and *Cyanus*. Pollen grain characters of 8 species were investigated by light microscope (LM). For morphological analysis, pollen grains were prepared according to Wodehouse (1935). Voucher specimens are deposited in the ISTE (Herbarium of Faculty of Pharmacy, Istanbul University). Types of pollen grains are tricolporate, isopolar and tectum complete of all specimens studied. The largest pollen in *Psephellus hypoleucus* whereas the smallest pollen in *Centaurea iberica*. The exine sculptures in the rest of the examined specimens are scabrate, microechinate or reticulate. The polar axis (P) ranges from 32.78±1.805 µm to 40.42±1.976 µm in studied *Centaurea* species and 36.76±0.8109 µm to 40.5±1.3092 µm in studied *Psephellus* species. The equatorial axis ranges from 30.77±1.43 µm to 39.20±0.632 µm in studied *Centaurea* species and 37.4878±2.1313 µm to 40.5789±1.7566 µm in studied *Psephellus* species. All of the morphological parameters investigated are given in detailed. Pollen shape of 8 species, which are we have been studied, is the same shape but their sizes are different. And there are some differences on the exine surface (sculpture). In the taxonomy pollen type gives an idea for these genera and it is also better to combine it with morphological, karyological and molecular data.

Türkiye'den 5 *Centaurea* L. ve 3 *Psephellus* Cass. Taksonunun Polen Morfolojileri

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Anahtar kelimeler
Centaurea,
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Polen morfolojisi.

Öz: Bu çalışmada Türkiye'de yetişen 5 *Centaurea* (*C. antitauri*, *C. coronopifolia*, *C. iberica*, *C. patula* ve *C. pterocaula*) ve 3 *Psephellus* (*P. hypoleucus*, *P. pulcherrimus* ve *P. simplicicaulis*) türünün polen morfolojileri ekzin skulptur ve strukturlerine göre incelenmiştir ve detaylı ölçümler verilmiştir. Daha önce yapılan çalışmalarda *Centaurea* ve ondan bölünen *Rhaponticoides*, *Psephellus* ve *Cyanus* cinslerinin taksonomisinde polen morfolojisinin önemine değinilmiştir. 8 türün polen morfolojisi ışık mikroskobuyla incelenmiştir. Morfolojik analiz için Wodehouse (1935) yöntemiyle polen materyalleri hazırlanmıştır. Çalışılan örnekler ISTE (İstanbul Üniversitesi Eczacılık Fakültesi Herbaryumu) herbaryumunda saklanmaktadır. Çalışılan tüm türlerin polenleri trikolporat, isopolar ve tektumdur. En büyük polen tanesi *Psephellus hypoleucus* türünde iken en küçük *Centaurea iberica* türüne aittir. Çalışılan tüm türlerde ekzin skulptur skabrat, mikroekinat veya

retikulattır. Polar aksis (P) aralığı çalışılan *Centaurea* türlerinde 32.78±1.805 µm ile 40.42±1.976 µm, *Psephellus* türlerinde 36.76±0.8109 µm ile 40.5±1.3092 µm'dir. Ekvatoryal aksis aralığı çalışılan *Centaurea* türlerinde 30.77±1.43 µm ile 39.20±0.632 µm, *Psephellus* türlerinde 37.4878±2.1313 µm ile 40.5789±1.7566 µm'dir. Araştırılan bütün morfolojik parametreler detaylı olarak verilmiştir. Sekiz türün polen şekilleri aynıdır ancak büyüklükleri farklıdır. Ayrıca ekzin yüzeyinde de farklılıklar vardır. Taksonomide polen tipleri bu cinsler için fikir vermektedir ve bu morfoloji, karyoloji ve moleküler çalışmalarla birlikte olduğunda daha iyi olmaktadır.

1. Introduction

Centaurea L. (Compositae) is one of the richest genus in the Turkish flora (Davis et al., 1988; Güner et al., 2000; Özhatay et al., 2013, 2015, 2017). Recently published several new species, and update the information it is divided into four genera, namely *Centaurea*, *Rhaponticoides*, *Psephellus* and *Cyanus* (Wagenitz and Hellwig, 2000; Greuter, 2003a, b). According to the latest studies and revision, genus *Psephellus* is represented by 37 taxa in Turkey (Ertuğrul and Uysal, 2013; Doğan et al., 2015; Özhatay et al., 2017). *Centaurea* is a large genus with 181 species and 112 of them are endemic in Turkey (Uysal, 2012).

According to Wagenitz (1955) the genus *Centaurea* is divided into eight groups on the basis of exine structure and sculpturing: *Centaurium*, *Crupina*, *Cyanus*, *Dealbata*, *Jacea*, *Montana*, *Serratula* and *Scabiosa*. Then five pollen types are defined by Avetisjan (1964) as *Centaurium*, *Jacea*, *Scabiosa*, *Serratula* and *Psephellus*. For phylogeny in the *Jacea* group pollen type is found one of the most reliable characteristics by Villodre and Garcia-Jacas (2000).

Most species of *Centaurea* are used as folk medicine in Turkey for asthma, chill, antipyretic, antidiaretic, appetitive and menstrual regulator (Baytop, 1984, Tuzlacı and Tolon, 2000, Koçyiğit and Özhatay, 2006). This genus is also a good source for honeybees as the honey plant (Kuś et al., 2014, Özler 2018). On the other hand some species are used in landscaping because of large and showy flowers of some members in the genus.

Pollen morphology of some Turkish *Centaurea* and *Psephellus* taxa have been studied (Pehlivan, 1995; Uysal et al., 2005; Çelik, 2007; Çelik et al., 2008; Özler et al., 2009; Erkara et al., 2012; Tasar et al., 2014; Bıyıklıoğlu et al., 2018). Previous papers drew attention to the great value of pollen morphology in the taxonomy of *Centaurea* and divided genus from *Centaurea*. In this study, 5 *Centaurea* and 3 *Psephellus* species distributed in Turkey have been examined for pollen grains characteristics.

2. Material and Methods

The pollen material was obtained from herbarium specimens kept in ISTE (Herbarium of Faculty of Pharmacy, Istanbul University). Examined specimens and their ISTE numbers are *Centaurea antitauri* Hayek (ISTE 37550), *C. coronopifolia* Lam. (ISTE 45502), *C. iberica* Trev. ex Sprengel (ISTE 18170), *C. patula* DC. (ISTE 25268), *C. pterocaula* Trautv. (ISTE 47403), *Psephellus hypoleucus* (DC.) Boiss. (ISTE 36989, 9028), *P. pulcherrimus* (Willd.) Wagenitz (ISTE 33479), *P. simplicicaulis* (Boiss. & A.Huet) Wagenitz (ISTE 19936)

Pollen grains for LM examination were prepared using the standard procedure of Wodehouse method (1935) and 8 pollen grain characters were investigated by a light microscope (BH-2 Olympus). Photos were taken with Spot Insight dijital camera at 100× magnification.

3. Results

The eight species member of *Centaurea* and *Psephellus* are studied. Distribution of the examined taxa is shown in the map and studied materials are marked with a star (Figure 1). The pollen photographs of the examined species are given in Figure 2. All parameters measured by using the Wodehouse method are given in Table 1.

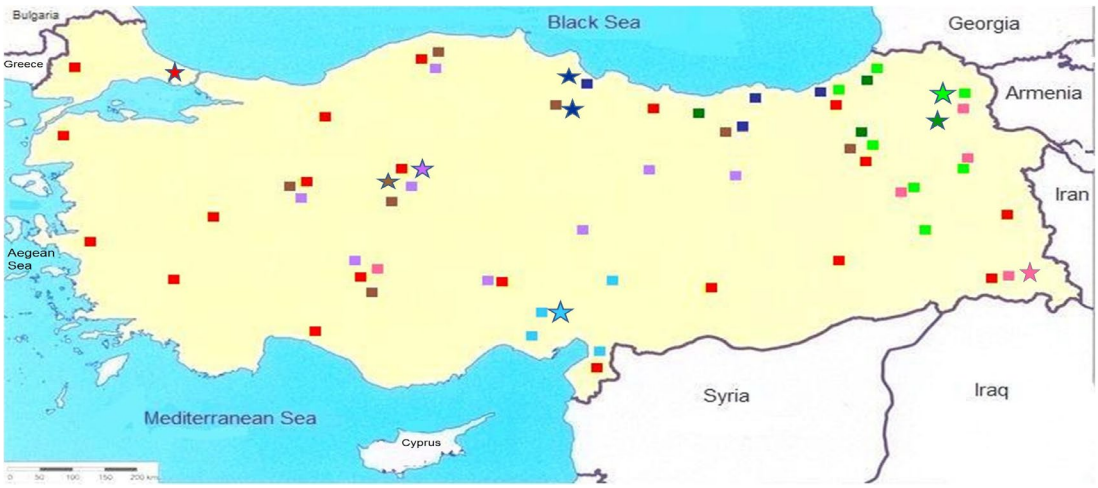

Figure 1. Distribution of studied species ■ *Centaurea antitauri* (ISTE 37550), ■ *C. coronopifolia* (ISTE 45502), ■ *C. iberica* (ISTE 18170), ■ *C. patula* (ISTE 25268), ■ *C. pterocaula* (ISTE 47403), ■ *Psephellus hypoleucus* (ISTE 36989, 9028), ■ *P. pulcherrimus* (ISTE 33479), ■ *P. simplicicaulis* (ISTE 19936).

Centaurea antitauri: The pollen grains are tricolporate and isopolar. The exine sculpture is scabrate- microechinate. The structure is tectate. Polar axis = 39.50±1.581 µm, equatorial axis = 39.20±0.632 µm; length of the colpus = 29.74±1.1191 µm, width of the colpus = 9.521±1.6712 µm.

***C. coronopifolia*:** The pollen grains are tricolporate and isopolar. The exine sculpture is reticulate-echinate. The structure is tectate. Polar axis = 34.59±1.1406 µm, equatorial axis = 33.89±1.4830 µm; length of the colpus = 22.20±0.1632 µm, width of the colpus = 10.4666±1.3232 µm.

***C. iberica*:** The pollen grains are tricolporate and isopolar. The exine sculpture is scabrate-microechinate. The structure is tectate. Polar axis = 32.78±1.805 µm, equatorial axis = 30.77±1.43µm; length of the colpus = 26.48±1.37 µm, width of the colpus = 8.9±3.13 µm.

***C. patula*:** The pollen grains are tricolporate and isopolar. The exine sculpture is scabrate. The structure is tectate. Polar axis = 37.58±1.640 µm, equatorial axis = 34.76±0.485 µm; length of the colpus = 29.896±2.143 µm, width of the colpus = 9.9±1.2475 µm.

***C. pterocaula*:** The pollen grains are tricolporate and isopolar. The exine sculpture is scabrate. The structure is tectate. Polar axis = 40.42±1.976 µm, equatorial axis = 36.97±0.474 µm; length of the colpus = 131.4615±0.990 µm, width of the colpus = 10.923±1.2783 µm.

***Psephellus hypoleucus*:** The pollen grains are tricolporate and isopolar. The exine sculpture is reticulate-scabrate. The structure is tectate. Polar axis = 40.5±1.3092 µm, equatorial axis = 40.5789±1.7566 µm; length of the colpus = 31.0416±2.0518 µm, width of the colpus = 11.625±2.7856 µm.

***P. pulcherrimus*:** The pollen grains are tricolporate and isopolar. The exine sculpture is scabrate. The structure is tectate. Polar axis= 36.76±0.8109 µm, equatorial axis= 37.4878±2.1313 µm; length of the colpus = 30.9714±0.1665 µm, width of the colpus = 11.9411±1.4539 µm.

***P. simplicicaulis*:** The pollen grains are tricolporate and isopolar. The exine sculpture is scabrate. The structure is tectate. Polar axis= 39.70±1.199 µm, equatorial axis= 38.56±0.706 µm; length of the colpus = 31.00±2.71 µm, width of the colpus = 12.076±1.902 µm.

Types of pollen grains are tricolporate, isopolar and tectum complete of all specimens studied. Pollen shape is sphaeroidea. The largest pollen in *Psephellus hypoleucus* (P=40.50, E= 40.5789) whereas the smallest pollen in *Centaurea iberica* (P=32.78, E=30.77). Exine was the thickest in *Psephellus hypoleucus* and the thinnest in *Centaurea coronopifolia.* The exine sculpture in *C. coronopifolia* is reticulate-echinate and in *Psephellus hypoleucus* is reticulate-scabrate. The exine sculpture in the rest of the examined specimens is scabrate and microechinate. The polar axis (P) ranges from 32.78±1.805 µm to 40.42±1.976 µm in *Centaurea* species and 36.76±0.8109 µm to 40.5±1.3092 µm in *Psephellus* species. The equatorial axis ranges from 30.77±1.43 µm to 39.20±0.632 µm in *Centaurea* species and 37.4878±2.1313 µm to 40.5789±1.7566 µm in *Psephellus* species.

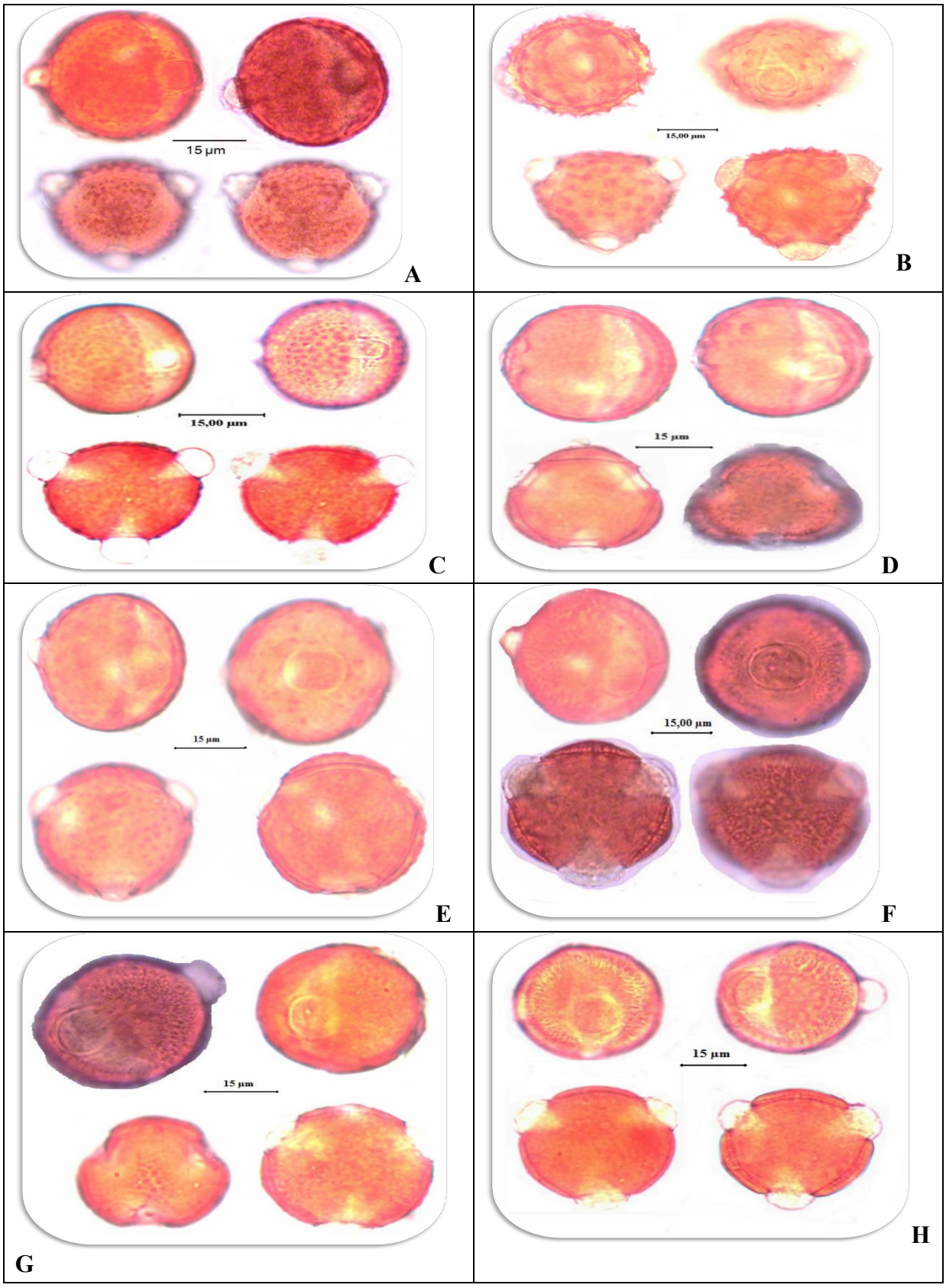

Figure 2. Light microscope (LM) microphotographs of *Centaurea* and *Psephellus* taxa.
A- *Centaurea antitauri* (ISTE 37550), B- *C. coronopifolia*(ISTE 45502), C- *C. iberica* (ISTE 18170), D- *C. patula* (ISTE 25268), E- *C. pterocaula* (ISTE 47403), F- *Psephellus hypoleucus* (ISTE 36989, 9028), G- *P. pulcherrimus* (ISTE 33479), H- *P. simplicicaulis* (ISTE 19936).

Table 1. The pollen characteristics of the examined taxa (Wodehouse).

TAXA		P (µm)	E (µm)	P/E	Ex (µm)	Int (µm)	clg (µm)	clt (µm)	plg (µm)	plt (µm)	plg/plt	t (µm)	Structure	Sculpture
Centaurea	M	39.50	39.20	1.0076(W)	2.555	1.00	29.74	9.521	11.6842	10.736	1.0883	18.00	Tectate	Scabrate -
antitauri	σ	±1.581	±0.632	Sphaeroidea	±1.832	±0	±1.1191	±1.6712	±1.0286	±0.908		±0.7550		microechinate
C.	M	34.59	33.89	1.0206 (W)	1.75	1.14	22.20	10.4666	11.7647	11.1764	1.0526	15.6470	Tectate	Reticulate-
coronopifolia	σ	±1.1406	±1.4830	Sphaeroidea	±0.75	±1.35	±0.1632	±1.3232	±0.2352	±0.2999		±2.0880		echinate
C. iberica	M	32.78	30.77	1.0653(W)	2.1	1.11	26.48	8.9	7.860	8.95	0.8782	9.105	Tectate	Scabrate -
	σ	±1.805	±1.43	Sphaeroidea	±0.160	±0.098	±1.37	±3.13	±0.364	±0.662		±0.571		microechinate
C. patula	M	37.58	34.76	1.0811(W)	2.50	1.00	29.896	9.9	9.6153	9.4230	1.0204	11.1379	Tectate	Scabrate
	σ	±1.640	±0.485	Sphaeroidea	±0.851	±0	±2.143	±1.2475	±1.6594	±1.5890		±2.2627		
C. pterocaula	M	40.42	36.97	1.0933(W)	2.4	1.125	31.4615	10.923	13.29	12.037	1.1040	11.9210	Tectate	Scabrate
	σ	±1.976	±0.474	Sphaeroidea	±1.389	±0.337	±0.990	±1.2783	±1.034	±1.1041		±1.5625		
Psephellus	M	40.5	40.5789	0.9980(W)	3.00	1.1167	31.0416	11.625	13.3793	11.769	1.1368	13.60	Tectate	Reticulate -
hypoleucus	σ	±1.3092	±1.7566	Sphaeroidea	±0	±1.761	±2.0518	±2.7856	±2.0071	±1.6245		±1.047		scabrate
P.	M	36.76	37.4878	0.9805(W)	2.2068	1.3448	30.9714	11.9411	12.8235	11.5714	1.1082	11.4848	Tectate	Scabrate
pulcherrimus	σ	±0.8109	±2.1313	Sphaeroidea	±1.095	±1.825	±0.1665	±1.4539	±1.3179	±1.4285		±1.7942		
P.	M	39.70	38.56	1.0295(W)	2.4	1.25	31.00	12.076	13.11	11.76	1.1147	14.15	Tectate	Scabrate
simplicicaulis	σ	±1.199	±0.706	Sphaeroidea	±1.105	±0.661	±2.71	±1.902	±1.9178	±0.637		±2.604		

P: Polar axis. *E:* Equatorial axis. *Ex:* Exine thickness. *Int:* Intine thickness. *clg:* Length of the colpus. *clt:* Width of the colpus. *plg:* Length of the porus. *plt:* Width of the porus. *t:* Apocolpium. *M:* Mean. σ: Standard deviations.

4. Discussion and Conclusion

For the first time pollen morphology of the genus *Centaurea* was studied in detail by Wagenitz (1955). Eight pollen types were recognized on the basis of exine structure and sculpturing. These are Centaurium, Crupina and Serratula as the most primitive; Cyanus, Dealbata and Montana as intermediate; Jacea and Scabiosa as the most advanced types. According to recent studies the genus *Centaurea* is divided into four genera, namely *Centaurea*, *Rhaponticoides*, *Psephellus* and *Cyanus* (Wagenitz and Hellwig, 2000; Greuter, 2003a, b). *Centaurea dealbata* Willd. is the type of the genus *Psephellus* Cass. Dealbata pollen type is a very conspicuous and it is shown that pollen type of the genus *Psephellus*. The Jacea type was divided into six subgroups based on morphological variations by Avetisjan (1964) and the Cyanus, Dealbata and Montana were also combined types into one large group, *Psephellus*.

Psephellus pecho (Albov) Wagenitz (syn. *Centaurea pecho* Albow.) and *P. hypoleucus* is the member of Sect. Psephellus, Özler et al. (2009) determined that exine sculpture in *P. pecho* as scabrate, spheroidal shape, in this study a similar result observed for *P. hypoleucus*, exine sculpture as reticulate-scabrate, and shape as spheroidal. Özler et al. (2009) determined that exine sculpture of *Psephellus appendicigerus* (K. Koch) Wagenitz (syn. *C.appendicigera* C. Koch), which is in the same section with *P. pulcherrimus*, is microechinate and differ from *P. pulcherrimus*, its scabrate.

Kaya et al. (2010) determined that the pollen shape of *C. calcitrapa* L. subsp. *cilicica* (Boiss. et. Bal.) Wagenitz and it is the same shape with as *C. iberica,* which is in the Sect. Calcitrapa, but pollen grains of *C. iberica* are bigger; the exine sculpture is scabrate in *C. calcitrapa* and scabrate-microechinate in *C. iberica.* According to Bıyıklıoğlu et al. 2018, the exine sculpture is microechinate in *C. iberica* and pollen shape is subprolate, P mean is 34.8; E mean is 30. Joujeh et al. (2019) studied pollen of some *Centaurea* species from Syria and one of them is *C. iberica*. According to their data the polar axis is 33.86 µm and the equatorial diameter is 31.86 µm in *C. iberica*. The pollen shape and the exine sculpture are prolate-spheroidal and scabrate in *C. iberica*. In our study the pollen shape and the exine sculpture are spheroidal and scabrate- microechinate. P mean and E means are 32.78±1.805 µm and 30.77±1.43 µm, respectively. There are some differences but P and E value of *C. iberica* are very close in these three studies.

Baser et al. (2019) shows that the polar axis and the equatorial axis are 53.63 µm and 48.06 µm respectively; shape is prolate-spheroidal; ornamentation is scabrate in *C. antitauri*. On the other hand, our data pointed out this value is 39.50 µm for the polar axis and 39.20 µm for the equatorial axis; the shape is spheroidal; ornamentation is scabrate -microechinate in *C. antitauri*.

For *C. pterocaula*, the polar axis and the equatorial axis are 31.58 µm and 27.14 µm in Baser et al. (2019); 40.42 µm and 36.97 µm in our study. Pollen shape and the exine sculpture of *C. pterocaula* show differences between Baser et al. (2019) and our study. The shape is subprolate; ornamentation is microechinate in Baser et al. (2019); spheroidal and scabrate in our study.

According to literature search, pollen morphology of *Psephellus simplicicaulis* and *Centaurea patula* is given for the first time in this study.

In conclusion, the pollen shape of 8 species, which are we have been studied, is the same shape but their sizes are different. And there are some differences on the exine surface (sculpture). In the taxonomy, pollen type gives an idea for these genera besides this it is better to combine it with morphological, karyological and molecular data.

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