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Morphology, anatomy, palynology and achene micromorphology of *Bellis* L. (Asteraceae) species from Turkey

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Abstract – In the present study, the morphological characters, root, stem and leaf anatomy, pollen and achene micromorphology of *Bellis* L. species (*Bellis annua* L., *B. perennis* L. and *B. sylvestris* Cirillo) distributed in Turkey have been investigated on light and scanning electron microscope. Palynological analysis showed that pollen characters were found as small to medium size, isopolar, radially symmetrical, oblate-spheroidal and prolate-spheroidal, tricolporate and echinate-perforate ornamentation in the three species. Achene characters were found dark brown to yellow in colour, often cylindrical, compressed, with thickened margin, obovate orobovoid shaped, pappus absent and the coat ornamentations are rectangular with short hairs on the surface. As a result of this study, leaf morphology and some pollen characteristics such as pollen size, shape, perforation and distance between spines were demonstrated to be different among the *Bellis* species.

Keywords: *Bellis*, common daisy, Compositae, taxonomy, SEM

Introduction

The genus *Bellis* L. (Asteraceae) has been included in the subtribe *Bellidinae* Willk. (tribe Astereae Cass.) along with 117 other genera representing more than 3000 annual or perennial taxa (Bremer 1994). It is native to western, central and northern Europe, Cyprus, western Syria and Azerbaijan but is commonly found as an invasive plant in North America (Webb 1976). In Turkey, according to the Flora of Turkey and the East Aegean Islands (Grierson 1975) and the last checklist of Turkish Flora (Güner et al. 2012), the genus is represented by three species: *Bellis annua* L., *B. perennis* L. and *B. sylvestris Cirillo.* It is known as „akbubeçlik, nineotu, çayır papatyası, koyungözü, koyun çiçeği and yoğurt otu" in Anatolia (Baytop 1994, Ekim 2012).

The genus *Bellis* (common daisy) is known as a traditional wound herb (Ai-Douri and Al-Essa 2010) and it was used for the treatment of bruises, broken bones, and wounds by Crusaders in the Middle Ages (Mitich 1997). It has also been used in traditional folk medicine for the treatment of sore throat (Uysal et al. 2010), headache, stomach ache, common cold, eczema, wound healing, diarrhea, peptic ulcer, gastritis, rheumatism, asthma, hemorrhoids and as a vulnerary, expectorant and laxative (Kavalcioğlu et al. 2010, Karakaş et al. 2012, Melikoğlu 2015).

Fiz et al. (2002) studied the phylogenetic relationships between *Bellis* and the closely related genera (*Bellidastrum* Scop, *Bellium* L. and *Rhynchospermum* Lindl.) and evolution of their morphological characters. Kavalcıoğlu et al. (2010) did a comparative RAPD analysis and pollen structure studies of *B. perennis* from Rize and Antalya cities of Turkey. Bozdağ et al. (2011) published data on the chromosome number and morphology of *Bellis* species of Turkey. The chromosome number was determined in *B. sylvestris*, *B. perennis* and *B. annua* as 2n = 36, 18 and 18, respectively. The previous studies reported that pollen and seed micromorphologies are efficient in the systematics of the Asteraceae family (Moore et al. 1991, Shabestari et al. 2013, Frangiote-Pallone and De Souza 2014). Akyalçın et al. (2011) studied the pollen morphology of six *Achillea* L. sect. *Achillea* (Asteraceae) species from Turkey. Their results show that pollen grains of *Achillea* species were oblate-spheroidal, prolate-spheroidal, subprolate and generally tricolporate, though at times tetracolporate or even pentacolporate. The structure of the exine is double tectate and mean exine thickness varied from 3 to 8.5 μm. Özler et al. (2009) investigated the pollen morphology of 29 taxa of the genera *Centaurea* L., *Psephellus* Cass. and *Cyanus* Miller from Turkey. They reported that

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pollen grains were tricolporate, rarely tetracolporate, isopolar, radially symmetrical, subprolate, spheroidal-subprolate, operculate, tectum perforate, with a microechinate and scabrate exine surface. Joujeh et al. (2019) conducted a palynological study on *Centaurea iberica* Trev. ex Spreng, *C. virgata* Lam., *C. verutum* L., *C. hyalolepis* Boiss., *C. solstitialis* L., *C. ammocyanus* Boiss. from Syria. They reported that pollen grains of the studied species were similar in shape, size, apertures characters, polarization and symmetry, while they differed in color, exine ornamentation pattern and density of the spines distributed on the exine surface and in quantitative measurements. However, studies about pollen and seed morphology of *Bellis* species from Turkey and near related countries are limited.

The aim of the present study is to give a detailed description of the anatomical features of root, stem and leaf, as well as of the achene and pollen morphology of *Bellis* species distributed in Turkey, and evaluate the systematic significance. To the best of our knowledge this is the first report on all of the morphological, anatomical and palynological characters of *Bellis* species growing in Turkey.

Materials and methods

The materials of this study includes 22 specimens belonging to 3 populations of *B. annua*, 102 specimens belonging to 15 populations of *B. perennis* and 32 specimens belonging to 4 populations of *B. sylvestris* that were collected from 22 different localities in Turkey during their flowering and fruiting time, between 2014 and 2016 (Tab. 1). The research area is given in Fig. 1 according to Davis's Grid system (Davis 1965-1985).

Specimens were dried according to standard herbarium techniques and are deposited in the Herbarium of Hatay Mustafa Kemal University, Hatay, Turkey (HMKU). The botanical identification was based on the Flora of Turkey and the East Aegean Islands (Grierson 1975). In addition, herbarium specimens from the Royal Botanic Garden (KEW), Royal Botanic Garden Edinburgh, ANK (Herbarium Turcicum), AEF (Ankara University Faculty of Pharmacy Herbaryum), GAZI (Gazi University Herbarium), HUB (Hacettepe University Herbarium), KNYA (Selçuk University Herbarium) and OMUB (Ondokuz Mayıs University Herbarium) herbaria were checked. Synonyms of taxa follow WFO (2019).

The plant samples were preserved in 70% ethanol for anatomical analysis. Transverse sections of root, stem and leaf samples were prepared. Slides were observed with a Zeiss Axiolab A1 light microscope. For anatomical description, the terminology of Metcalfe and Chalk (1957) was used. Pollen grains were obtained from mature anthers of dried flowers and measured under a light microscope from non-acetolyzed samples after preparation applying the Wodehouse (1935) method. The measurements of the polar axis (P), the equatorial diameter (E), the colpus length (clg), the ornamentation, the number of apertures (An), the aperture type, the spine length (spg), the spine width (spt), the exine (e) and the intine (i) thickness for 20 pollen grains were conducted under a Zeiss Axiolab A1 light microscope. In the course of scanning electron microscopy studies, mature achenes and pollen grains were mounted using double-sided tape on the stubs and coated with platinum-palladium in a Quorum Q150R EM sputter coater. They were examined

Tab. 1. Localities, habitats and sample codes of the studied *Bellis* L. species in Turkey. Abbreviations: NP – Number of population, NS – Number of specimens, FK – Faruk Karahan.

NP	NS	Taxa	Square, location and habitat	Altitude (m a.s.l.)	Co-ordinates	Collector number
1	5	*B. annua* L.	A4: Karabük: Ovacık, Boyalı village, grassland	623	41°07'01.5"N 32°49'25.6"E	FK 1210
2	6		B1: Manisa: Beydere village, wet grassland	210	38°40'53.7"N 27°14'54.6"E	FK 1218
3	11		C6: Hatay: Kırıkhan, Gölbaşı village, grassland	180	36°30'13.0"N 36°28'28.2"E	FK 1102
4	6	*B. perennis* L.	A1(E): Kırklareli: Armağan Dam, forest	448	41°54'14.9"N 27°25'40.2"E	FK 1124
5	7		A2: Kocaeli: Karamürsel, Senaiye village, forest	432	40°37'41.7"N 29°41'03.7"E	FK 1132
6	5		A3: Bolu: Mengen, Demirciler village, grassland	1450	40°57'23.0"N 32°05'42.2"E	FK 1138
7	6		A4: Kastamonu: Azdavay, Kart mountain, forest clearings	1227	41°45'02.9"N 33°17'58.9"E	FK 1204
8	7		A7: Giresun: Şebinkarahisar, Tamzara village, meadow	1332	40°19'42.7"N 38°26'07.6"E	FK 1103
9	5		B6: Sivas: Suşehri, Sökün village, wet slopes	1150	40°17'07.0"N 38°10'33.2"E	FK 1105
10	10		B6: Kayseri: Pınarbaşı, roadside	1520	38°43'13.9"N 36°23'10.4"E	FK 1104
11	5		B7: Elazığ: Altınkuşak village, wet grasslands	982	38°47'50.9"N 39°05'33.2"E	FK 1321
12	6		C3: Isparta: Yenişarbademli, wet grassland	1147	37°42'21.4"N 31°23'09.3"E	FK 1217
13	6		C4: Konya: Tatköy near,wet grasslands	1449	37°56'46.7"N 32°21'29.0"E	FK 1189
14	7		C5: Adana: Pozantı, Beypınarı, moist slopes	1532	38°05'53.6"N 36°14'07.9"E	FK 1139
15	8		C6: Hatay: Antakya Castle, wet grassland	440	36°13'08.8"N 36°10'48.0"E	FK 1106
16	8		C6: Hatay: Kırıkhan, Çataltepe village, grassland	128	36°31'03.8"N 36°25'43.0"E	FK 1108
17	6		C6:Osmaniye: Düziçi, Haruniye Castle, grassland	485	37°16'07.0"N 36°29'14.4"E	FK 1110
18	8		C7: Şanlıurfa: Birecik, Zeytinbahçe near, grassland	402	36°55'17.5"N 38°01'40.6"E	FK 1249
19	8	*B. sylvestris*	A2(E): Kırklareli: Vize, Kasatura, forest clearings	152	41°34'19.1"N 27°46'18.7"E	FK 1127
20	7	Cirillo	B1: Manisa: Beydere, forest clearings	232	38°40'53.7"N 27°14'54.1"E	FK 1219
21	8		C6: Hatay: Antakya castle,wet grassland	440	36°13'08.8"N 36°10'48.0"E	FK 1107
22	9		C6: Hatay: Hassa, Ardıçlı village, rocky slopes	320	36°43'54.6"N 36°31'10.9"E	FK 1109

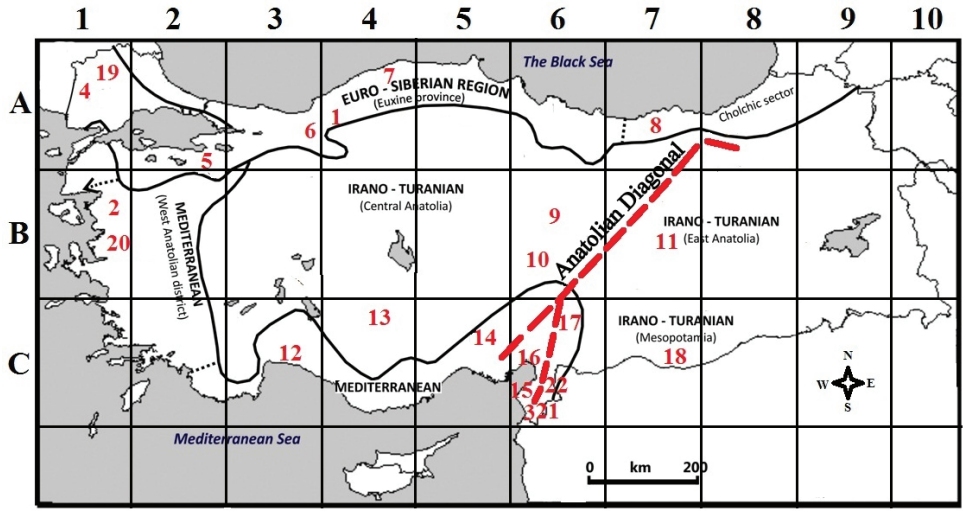

Fig. 1. Location of *Bellis* sampling sites from Turkey; (1–3: *Bellis annua*, 4–18: *B. perennis*, 19–22: *B. sylvestris*) modified from Avci (1996) and Davis's Grid system (after Davis 1965–1985). The thick red line shows the approximate midline position of the Anatolian diagonal.

with a Zeiss Supra 55VP FE-SEM at the Advanced Technology of Education, Research and Application Center of Mersin University. The pollen terminology follows Faegri and Iversen (1975) and Punt et al. (2007).

Results

In this study, we compared the morphology, anatomy, palynology and achene micromorphology of *Bellis* taxa growing in Turkey. Sampling sites are shown Fig. 1 (see also Tab. 1).

Morphological characters of the *Bellis* L. species

***Bellis annua* L., Sp. Pl 887 (1753).**

Type: Sicily, Spain and Montpellier (Hb. Linn. 1006/3) (Hassler 2019).

Syn: *Bellis annua* f. *annua*, *B. annua* f. *caerulea* Albo, *B. annua* f. *pubescens* Carano et Bamb., *B. annua* subsp. *annua* L., *B. annua* subsp. *majoricensis* (Gand.) Gand., *B. annua* subsp. *repens* (Lam.) Govaerts, *B. annua* subsp. *vandasii* (Velen. ex Velen.) D.A. Webb, *B. annua* var. *acutisquama* Pau ex Hunter, *B. annua* var. *annua*, *B. annua* var. *dentata* (DC.) Rouy, *B. annua* var. *vergens* (Pomel) Quézel et Santa, *B. annua* var. *vergens* Pomel, *B. dentata* (Viv.) DC., *B. majoricensis* Gand., *B. prostrata* Pomel, *B. radicans* Coss. et Durieu ex Batt. et Trab., *B. ramosa* Lam., *B. repens* Lam., *B. vandasii* Velen., *B. vandasii* Velen. ex Nyman, *B. vergens* (Pomel) Bég., *Bellium bellidioides* (L.) Desf., *B. bellioides* G.Don, *B. dentatum* (DC.) Viv., *Cineraria corymbosa* Moench, *Diplopappus annuus* (L.) Bluff et Fingerh., *D. dubius* Cass., *Erigeron annuus* Sessé et Moc., *E. bellidioides* Spenn., *E. diversifolius* Rich. ex Rchb.

Description: Annual herb. Stems erect or decumbent, 3-13 cm long, sparsely pubescent (Fig. 2A). Fibrous roots 1.5-7 cm long. Leaves mostly spatulate, sometimes lanceolate or ovate, 1-3.5 cm × 2-8 mm, acute or obtuse at apex, attenuate at base, entire or 2-3-toothed on each side near apex, ciliate near base, otherwise glabrous (Fig. 3A). Capitula on 2-10 cm long peduncles. Involucre cylindrical and 3-4 mm broad; phyllaries 8-16, sparsely or densely villose, green, lan-

Fig. 2. General morphology of *Bellis annua* (A, FK 1102/HMKU 13512), *B. perennis* (B, FK 1103/HMKU 13516) and *B. sylvestris* (C, FK 1127/HMKU 13520). Scale bars = 1 cm.

ceolate, 2.5-5 × 1-2 mm, apex acute or obtuse. Ray flowers 20-30; ligules 2-5 mm long, white to purple. Disc corollas 1.5-4 mm long and yellow (Fig. 2A). Achenes 1-1.25 long and 0.5 mm wide, finally pubescent, light brown to yellow in colour and without pappus.

Distribution: Mediterranean area. East Mediterranean element. In Turkey: A4 Karabük: Ovacık, Boyalı village, grassland, 623 m a.s.l., FK 1210; B1 Manisa: Beydere village, wet grassland, 210 m a.s.l., FK 1218; C2 Muğla: Fethiye, Kemer to Kestep, 50 m a.s.l. (above sea level), Davis 25471! (ANK); C3 Antalya: Antalya, 60 m alt., Balls 2081! (ANK), Isparta, çamurluk yayla, 2000 m a.s.l., 04.08.1949, Davis 16076 (ANK); C4 Antalya: Gazipaşa, Çobanlar köyü, 50-75 m a.s.l., 12.03.1981, H. Sümbül 1045 (ANK). C6 Hatay: Çorum Village (Kırıkhan), grassland, 12.06.2015, FK 1102 (HMKU 13512).

Habitat: Damp places at edges of fields and waste ground, 0-300 m a.s.l., flowering time: February-May.

***Bellis perennis* L., Sp. Pl. 886 (1753).**

Original elements: Herb. Linn. 1006.2 (LINN); herb. Clifford 418: Bellis 1, 2 sheets (BM); Herb. Burser XIV (2): 69 (UPS); Dodoens, Stirp. Hist. Pempt. 265, icon (1583). Type: Herb. Burser XIV(2): 69 (lectotype designated here: UPS).

Type: (Hb. Cliff. 418/1! Hb. Linn. 1006/1) (Calvo et al. 2012, Hassler 2019).

Syn: *Aster bellis* E.H.L.Krause, *Bellis alpina* Hegetschw., *B. armena* Boiss., *B. croatica* Gand., *B. hortensis* Mill., *B. hybrida* Ten., *B. integrifolia* DC., *B. margaritifolia* Huter, *B. minor* Garsault, *B. perennis* f. *discoidea* D.C.McClint., *B. perennis* f. *perennis*, *B. perennis* f. *plena* Sacc., *B. perennis* f. *pumila* (Arv.-Touv. et Dupuy) Rouy, *B. perennis* f. *rhodoglossa* Sacc., *B. perennis* f. *tubulosa* A.Kern., *B. perennis* subsp. *hybrida* (Ten.) Nyman, *B. perennis* subsp. *perennis*, *B. perennis* var. *caulescens* Rochebr., *B.* perennis var. *fagetorum* Lac., *B. perennis* var. *hybrida* (Ten.) Fiori, *B. perennis* var. *margaritifolia* (Huter) Fiori, *B. perennis* var. *microcephala* Boiss., *B. perennis* var. *perennis*, *B. perennis* var. *pusilla* N.Terracc., *B. perennis* var. *strobliana* Bég., *B. perennis* var. *subcaulescens* Martrin-Donos, *B. perennis* var. *tubulosa* F.J.Schultz, *B. pumila* Arv.-Touv. et Dupuy, *B. scaposa* Gilib., *B. validula* Gand., *Erigeron perennis* (L.) Sessé et Moc.

Description: Perennial, usually rosulate herb, 8-20 cm long, sparsely pubescent (Fig. 2B). Leaves mostly spatulate, sometimes obovate and lanceolate, 1-9 × 0.5-1.8 cm, obtuse or broadly acute at apex, rather abruptly attenuate into winged petiolar base, entire or with up to 5-6 shallow teeth on each side near apex, sparsely pubescent on both surfaces (Fig. 3C-D). Capitula on l.5-10 (-25) cm scapes. Involucre cylindrical and 0.5-1 cm broad; phyllaries 12-16, sparsely or densely villose, green, linear, ovate-lanceolate, 3-6 × 0.75-2.5 mm, obtuse or acute at apex. Ray flowers 30-50; ligules 5-10 mm long, white and often pink beneath. Disc corollas 1.5-2 mm long and yellow (Fig. 2B). Achenes rounded at apex, 1.3-1.75 long and 0.7-0.8 mm wide, light brown to yellow in colour and weakly pubescent.

Distribution: Europe, Cyprus, W. Syria, Soviet Azerbaijan. Euro-Siberian element. In Turkey: A1(E) Kırklareli: Armağan Dam, forest, 448 m a.s.l., FK 1124; A2 Istanbul: Heybeli Island, 30 m a.s.l., 24.03.1957, Davis 26237 (ANK); Kocaeli: Karamürsel, Senaiye village, forest, 432 m a.s.l., FK 1132; A3 Bolu: Seben, Demirciler plateau, grassland,

Fig. 3. Leaf morphology of *Bellis annua* (A, FK 1102/HMKU 13512), *B. sylvestris* (B, FK 1127/HMKU 13520), *B. perennis* (C, FK 1204/HMKU 13513; D, FK 1103/HMKU 13516; E, FK 1104/HMKU 13518). Scale bars = 1cm.

1450 m a.s.l., FK 1138; A4 Kastamonu: Kastamonu to Araç road, Tepelce Hill, 1300 m a.s.l., 24.04.1981, M. Demirörs, 230 (ANK); Azdavay, Kart mountain, forest clearings, 1227 m a.s.l., FK 1204; Tosya, Kösdağı, wet grassland, 1400 m a.s.l., 04.06.1976, M. Kılınç 6119 (ANK); Zonguldak: Kozlu, 26.04.1940, B. Kasaplıgil (ANK); Zonguldak, Cemetery area, 14.02.1945, B. Şazi 164 (ANK); Karabük: Düdüklük, *Pinus brutia* woodland, 810 m a.s.l., 11.05.1984, M. Demirörs, 1303 (ANK); A5 Yozgat: Çekerek, Hanözü, wet grassland, 1250 m a.s.l., 26.03.1980, R. İlarslan 727 (ANK); A7 Giresun: Tamzara village, wet meadow, FK 1103 (HMKU 13516); A8: Rize: Ardeşen, Fırtına Bridge-Bakoz, sandy places, woodland, 10-200 m a.s.l., 28.06.1980, A. Güner 2670 (ANK); B3: Eskişehir: Türkmen Mountain, Bayat Çayır, 1400 m a.s.l., 17.06.1976, T. Ekim 2078 (ANK); Konya: Sultan mountains, Doğanhisar kartaltepe, along streams, 1300 m a.s.l., 20.03.1979, H. Ocakverdi 359 (KNYA); B5: Yozgat: Yıldızeli, Akdağ mining site, highway park, 05.06.1980, T. Ekim 5025 (ANK); B6 Kayseri: Pınarbaşı, roadside, FK 1104 (HMKU 13518); Sivas: Sökün Village (Suşehri), wet slopes, FK 1105 (HMKU 13517); B7 Elazığ: Altınkuşak village, wet grasslands, 982 m a.s.l., FK 1321; B9 Bitlis: Por river, rocky steppe, 1300-1500 m a.s.l., 1988, N. Adıgüzel and T. Ekim 7524 (ANK); C3: Antalya: Akseki, Cevizli-Kuyucak, grassland area, 1050 m a.s.l, 04.04.2003, H. Demirelma 1893 (KNYA); Akseki, Cevizli-Beyşehir roadsides, grassland area, 1150 m a.s.l., 17.05.2003, H. Demirelma 2148 (KNYA); Antalya: İbradı, Üzümdere village, 750 m a.s.l., 05.04.2003, H. Demirelma 1906 (KNYA); Isparta: Yenişarbademli, wet grassland, 1147 m a.s.l., FK 1217; Konya: Derebucak, Pınarbaşı, Çukurören near, rocky places, 950 m a.s.l., 22.04.2002, H. Demirelma 1305 (KNYA); C4 Konya: Tatköy to Gevenli hill, wet meadows, 1450 m a.s.l., 24.04.2005, E. Yıldıztugay 719 (KNYA); Konya: Başarakabak Küngönü, wet meadow, 1350 m a.s.l. 19.05.2005, E. Yıldıztugay 840 (KNYA); Ermenek, Aktepe near, moist places, 1600 m a.s.l., 26.05.1978, M. Vural 495 (ANK); Konya: Altınapa dam, 1500 m a.s.l., 16.05.1989, A. Tatlı, B. Eyce, Memduh Serin 8909; Konya: Kazımkarabekir kozağaç, 350 m a.s.l., 18.05.1984, M. Serin 1891; Konya: Tatköy near, wet grasslands, 1449 m a.s.l., FK 1189; Mersin: Anamur, Kaldokan, 1000 m a.s.l, 13.04.1956, Davis 25898 (ANK); Anamur, Astım cave near, *Pinus* woodland, 420 m a.s.l., 07.04.2007, E. Yıldıztugay 1511 (KNYA); Mersin: Mut-Silifke road, 15.04.1971, Rıza Çetik 2015; C5 Adana: Pozantı, Beypınarı, moist slopes, 1532 m a.s.l., FK 1139; C6 Hatay: Kınzır Yaylası, Demiroluk *Cedrus* woodland, Amanos Mountain, 1700 m a.s.l., 20.06.1967, 7355 (ANK); Belen, Karlıktepe above Soğukoluk, Moist banks and ledges, 900 m a.s.l., 04.04.1957, Davis and Hedge 27063 (ANK); Antakya Castle (FK 1106) and Çataltepe Village (F. Karahan 1108), Osmaniye: Haruniye Castle, wet grassland, FK 1108 (HMKU 13515); C7 Şanlıurfa: Birecik, Zeytinbahçe near, wet grassland, 402 m a.s.l., FK 1249.

Habitat: Moist places, often in forests, 0-2000 m a.s.l., flowering time: March-August (throughout year *-fide* Boissier).

***Bellis sylvestris* Cirillo (1792, XII), Pl. Rar. Neap. 2:12 t. 4 (1792).**

Original elements: Cirillo, Pl. Rar. Neapol. 2: XII, icon (1792).

Type: Cirillo, Pl. Rar. Neapol. 2: XII, icon (1792), lectotype designated here. Epitype: Italy, Puglia, Foggia, San Giovanni Rotondo, basal part of Monte Calvo, 41°42′40″N, 15°45′38″E, 5 Apr 2011, J. Calvo and A. Quintanar (Calvo et al. 2012, Hassler 2019).

Syn: *Bellidastrum michelii* Planellas, *B. pappulosum* Bertol., *Bellis atlantica* Boiss. et Reut., *B. hirta* Host, *B. longifolia* Orph. ex Nyman, *B. pappulosa* Boiss., *B. perennis* subsp. *sylvestris* (Cirillo) Rouy, *B. sylvestris* subsp. *maroccana* Sennen, *B. sylvestris* subsp. *natalis-Jesus* Sennen et Mauricio, *B. sylvestris* subsp. *sylvestris*, *B. sylvestris* var. *atlantica* (Boiss. et Reut.) Batt., *B. sylvestris* var. *cyrenaica* Bég., *B. sylvestris* var. *major* Alleiz., *B. sylvestris* var. *pappulosa* Lange, *B. sylvestris* var. *rotundifolia* (Boiss. et Reut.) Batt., *B. sylvestris* var. *sylvestris*, *B. velutina* Pomel, *Bellium pappulosum* (Boiss. ex DC.) Kunze, *Brachyscome sylvestris* Klatt, *Doronicum bellidiastrum* Sm.

Description: Perennial rosulate herb, 25-30 cm long, stems erect or decumbent, densely pubescent (Fig. 2C). Leaves oblanceolate, spatulate and cuneate, 3-11 × 0.5-1.8 cm, ± distinctly 3-nerved, gradually attenuate at base, obtuse or broadly acute at apex, subentire or distantly denticulate, pubescent on both surfaces (Fig. 3E). Capitula on 7-30 cm long scapes. Involucre cylindrical and 1-1.5 cm broad; phyllaries 12-16, sparsely or densely villose, lanceolate, 6-11 × 2-3 mm, acute or obtuse. Ray flowers ca. 50; ligules white, often strongly tinged with pink, 0.75-1 cm long. Disc corollas 2.25-2.5 mm long and yellow (Fig. 2C). Achenes rather square and somewhat notched at apex, 1.6-2.25 long and 1-1.2 mm wide, quite strongly pubescent.

Distribution: Mediterranean area. East Mediterranean element. In Turkey: A2(E) Istanbul: Maslak (Şişli) wet grassland, 10.06.1940, B. Kasaplıgil (ANK); Kırklareli: Vize, Kasatura, forest clearings, 152 m a.s.l., FK 1127; A3 Zonguldak: Zonguldak, Nov. 1946, Dijkstra (ANK); B1 Manisa: Beydere, forest clearings, 232 m a.s.l., FK 1219; C4 Mersin: Anamur, Anamuryum ancient city, rocky places, 50 m a.s.l., 01.02.2008, E. Yıldıztugay 1764 (KNYA); C6 Hatay: Antakya castle (FK 1107) and Ardıçlı village (Hassa), rocky slopes, 12.11.2015, FK 1109 (HMKU 13520).

Habitat: Hillsides and gravelly soil, 0-800 m a.s.l., flowering time: September-January.

Anatomical characters

Root: A transverse section taken from the root samples was observed as follows. In cross section the root is circular in all studied taxa. Roots of *B. perennis* and *B. sylvestris* are perennial while root of *B. annua* is annual. The epidermis consists of 1-2 layered rectangular or oval cells in *B. perennis* and *B. annua*, while a 4-5 layered exodermis is located in *B. sylvestris.* Under the periderm, the parenchymatic cortex contains 10-12 layers of oval cells. The endodermis is 1-2

layered with rectangular or oval cells. In the root centre, the radial type vascular system include distinguishable phloem and xylem rays, while the cambium is not distinguishable in the three species of *Bellis* studied (Fig. 4A, D, G).

Stem: The transverse section of the stem, the epidermis consists of 1-2 layered rectangular or oval cells. There are glandular or eglandular hairs on the epidermis. Under the epidermis, the cortex contains 6-8 layers of differently sized and shaped parenchymatic cells. The sclerenchymatic cap above the phloem is 4-6 layered. The cambium layer can be distinguished in all species. The phloem cells have an area nearly equal to xylem and the vascular system is of the open collateral type. The pith is wide and consists of polygonal or oval parenchymatous cells although sometimes collapsed areas are observed in the center of stems (Fig. 4B, E, H).

Leaves: The anatomical studies on the leaf of the *Bellis* species shows that the upper and lower epidermises consist of uniseriate cells with different sizes and shapes. Under the epidermis, the mesophyll contains 10-12 layers of uniform cells in *B. perennis* and *B. annua* (unifacial), while the mesophyll is composed of 5-6 layers of, rectangular and oval palisade parenchyma cells and 4-5 layers of spongy parenchyma cells in *B. sylvestris* (bifacial). The vascular system includes 6-8 layers of oval xylem cells and 6-7 layers of oval phloem

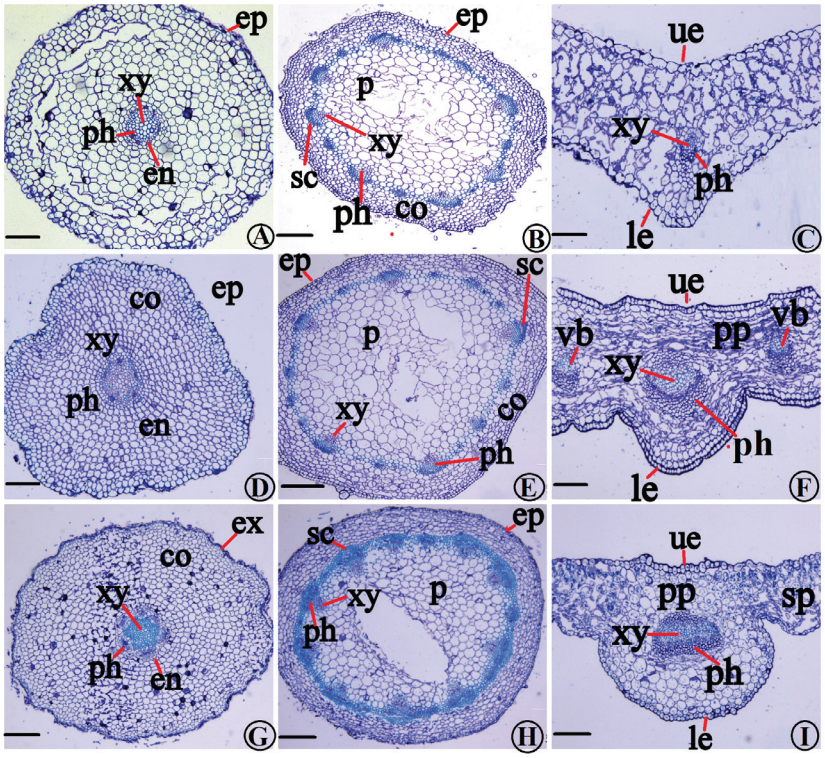

Fig. 4. Light microphotographs of the transverse sections of *Bellis* species: *B. annua* (A-C/FK 1102, HMKU 13512), *B. perennis* (D-F/FK 1104, HMKU 13518), *B. sylvestris* (G-I/ FK 1127, HMKU 13520), root (A, D, G), stem (B, E, H), leaf (C, F, I); co – cortex, en – endodermis ep – epidermis, le – lower epidermis, p – parenchyma, ph – phloem, pp – palisade parenchyma, sc – sclerenchyma, sp – spongy parenchyma, ue – upper epidermis, vb – vascular bundle, xy – xylem. Scale bars = 0.2 µm.

Tab. 2. The palynological measurements of *Bellis annua*, *B. perennis* and *B. sylvestris* taxa. Range and standard deviations are presented (n = 20).

Characters/Species	*B. annua*	*B. perennis*	*B. sylvestris*
Polar axis (µm)	24.84–33.75	15.44–26.02	18.77–23.89
	29.79±2.37	20.01±2.48	22.13±1.21
Equatorial axis (µm)	26.34–35.17	15.28–23.13	17.44–27.86
	30.17±2.16	19.17±1.58	22.17±2.65
Pollen shape	Oblate-spheroidal	Prolate-spheroidal	Oblate-spheroidal
Ornamentation	Echinate-perforiate	Echinate-perforiate	Echinate-perforiate
Perforation	Elliptic circular	Elliptic circular rectangular	Elliptic circular polygonal
Spine length (µm)	1.55–2.65	1.32–2.64	1.45–3.36
	2.10±0.40	2.15±0.28	2.49±0.37
Spine width (µm)	1.52–2.95	0.99–2.29	1.24–2.54
	2.08±0.33	1.51±0.29	1.73±0.35
Aperture type	Tricolporate	Tricolporate	Tricolporate
Distance between spine (µm)	2.78–5.71	2.67–5.56	3.75–5.63
	4.15±0.78	4.07±0.68	4.59±0.64
Number of spine (µm^2)	2–3	3–4	2–3

cells but they are larger in *B. perennis* and *B. sylvestris* than in *B. annua.* The xylem faces towards the upper surface, while the phloem faces the lower epidermis (Fig. 4C, F, I).

Pollen morphology

All of the morphological parameters investigated are given in Tab. 2. Under light microscope, the pollen grains are isopolar, radially symmetrical, oblate spheroidal and prolate spheroidal. Their apertures are tricolporate. The ornamentation is echinate-perforate in the three species (Fig. 5). The pollen size is small in *B. perennis* and *B. sylvestris*, but medium in *B. annua.* Type of perforation in the spine base is determined as elliptic-circular for *B. annua*, elliptic-circular-rectangular for *B. perennis* and elliptic-circular-polygonal for *B. sylvestris*. The polar axis (P) and equatorial axis (E) are the longest in *B. annua* (29.79 and 30.17 μm), and the shortest in *B. perennis* (20.01 and 19.17 μm). The spine lengths ranged from 2.10 μm (*B. annua*) to 2.49 μm (*B. sylvestris*) and the spine widths ranged from 1.51 μm (*B. perennis*) to 2.08 μm (*B. annua*). Under scanning electron microscope, the distance between spines ranged from 4.07 μm (*B. perennis*) to 4.59 μm (*B. sylvestris*). The number of spines counted per 10 μm^2 was the highest for *B. perennis*, and the lowest for *B. annua* and *B. sylvestris* (Fig. 5A-F).

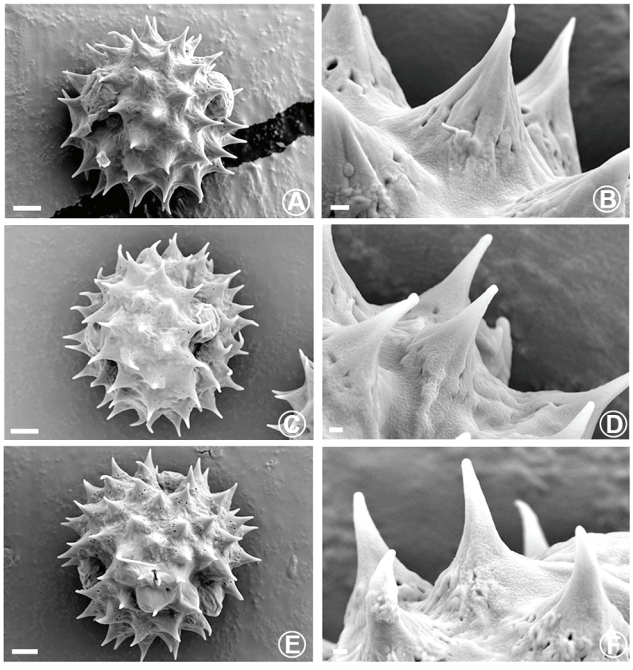

Fig. 5. Scanning electron micrographs (SEM) of pollen grains of *Bellis* species: *B. annua* (A-B/ FK 1102, HMKU 13512), *B. perennis* (C-D/ FK 1104, HMKU 13518), *B. sylvestris* (E-F/ FK 1127, HMKU 13520). Scale bars = 2 μm (A, C, E), and 0.2 μm (B, D, F).

Achene morphology

The achenes are simple, one-seeded, often cylindrical, compressed, with thickened margin, obovate or obovoid shaped, pappus absent. They are found in collective numbers attached to a common receptacle and dark brown to yellow in colour (Grierson 1975). The achene length ranged from 1.26 mm (*B. annua*) to 1.70 μm (*B. sylvestris*) and the achene width ranged from 0.54 mm (*B. perennis*) to 0.95 mm (*B. sylvestris*). The achene coat ornamentations are rectangular and short hairy on surface and the hilums are elliptic in the all investigated taxa (Fig. 6A-F).

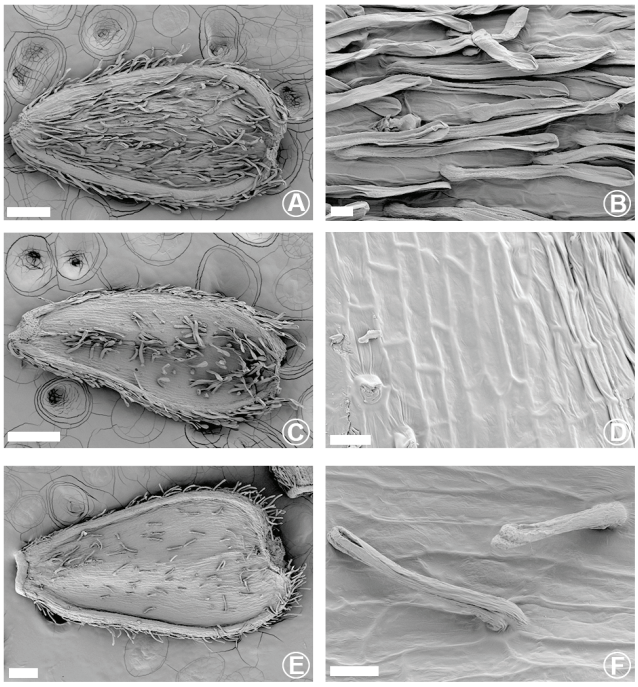

Fig. 6. Scanning electron micrographs (SEM) of achene grains of *Bellis* species: *B. annua* (A-B/ FK 1102, HMKU 13512), *B. perennis* (C-D/ FK 1104, HMKU 13518), *B. sylvestris* (E-F/ FK 1127, HMKU 13520). Scale bars = 200 μm (A, C, E), and 20 μm (B, D, F).

Discussion

The genus *Bellis* L. species includes annual or perennial herbs and it has a widespread distribution from Europe to Middle East. In the present paper, we investigated the morphological, anatomical, palynological, and micromorphological characters of *B. annua*, *B. perennis* and *B. sylvestris* in Turkey.

Avise (2000) reported that physical barriers such as mountain ranges are thought to be crucial for shaping the current population genetic and phylogeographic structures of many plant and animal species. Similarly, the Anatolian diagonal running from the southeastern to the northern part of Turkey is accepted as a major floristic barrier (see Fig. 1), and is thought to underlie much of the genetic diversity in this region (Mutun 2016). The phytogeographic origin of *B. sylvestris* is European-Siberian, so it spreads to the west of the Anatolian diagonal. Generally, Iran-Turanian elements are distributed in the south of the Anatolian diagonal. Our results demonstrate that the *Bellis* species studied are densely distributed to the west of the Anatolian diagonal and that these mountains can be considered a barrier especially for *B. sylvestris* in Turkey. However, thesespecies might be distributed from north to south due to the ecological conditions in the Mediterranean phytogeographic region in the southern part of the Anatolian diag-

onal (especially Hatay district) having been suitable after the last glaciation period. Results obtained from related literatures and field studies demonstrate that *Bellis* species studied are generally distributed in to the west of the Anatolian diagonal and these mountains can be considered a barrier especially for *B. sylvestris* (Euro-Siberian element) in Turkey (Fig. 1).

The results of our morphological analysis are in agreement with the description of all *Bellis* species in Flora of Turkey. However, there are no morphological characters such as achene colour, phyllary number, shape and size in the detailed description of the *Bellis* chapter in the Flora of Turkey. These characters were obtained from the present study. In addition, leaf shape and phyllary are important for the separation of taxa. Our studies showed that there is a large variation in leaf morphology of *Bellis* species especially *B. perennis* between both the interspecific and intraspecific levels. *B. annua* samples analyzed have 5 different leaf types, *B. sylvestris* samples have 6 different leaf types and *B. perennis* have 9 different leaf types (Fig. 3 A-E). Probably, this variation could be influenced by different ecological conditions. However, our comparative study determines several differences compared with the description in the Flora of Turkey: the stem is 3-13 cm (not up to 7 cm) long, leaves 1-3.5 cm × 2-8 mm (not 1-1.5 × 0.4-0.8 cm), capitula on 2-10 cm long (not 2-7 cm), phyllaries 2.5-5 × 1-2 mm (not 2.5-3 × 1-1.5 mm) and ligules 2-5 mm long (not 4-5 mm) in *B. annua*; leaves mostly spatulate, sometimes obovate and lanceolate (not only spatulate), leaves 1-1-9 × 0.5-1.8 cm (not 2-30 × 0.5-1.5 cm), ligules 5-10 mm long (not up to 7 mm) and achenes 1.3-1.75 × 0.7-0.8 mm (not 1.3-1.5 × 0.7-0.8 mm) in *B. perennis*; leaves mostly oblanceolate, spatulate and cuneate (not only oblanceolate) in *B. sylvestris*.

According to Metcalfe and Chalk (1957), Asteraceae species show various anatomical structures and some present ecological specialization due to the diversity of their habitats. Our observations of the root transverse section showed that *B. perennis* and *B. annua* have a 1-2 layered epidermis while a 4-5 layered exodermis is located in *B. sylvestris*. In root centre, the radial type vascular system includes distinguishable phloem and xylem rays and the cambium is not distinguishable in all taxa studied. The results of a stem transverse section showed that they have a distinct sclerenchymatic layer. A distinguishable endodermis was present between the cortex and the vascular tissue. Özörgücü et al. (1991) and Akçin and Akçin (2010) reported that endodermis is usually distinguishable in the stems of Asteraceae. Transversal sections of the leaves show that *B. perennis* and *B. annua* have unifacial leaves, but *B. sylvestris* has a bifacial type of leaf. Metcalfe and Chalk (1957) reported that generally bifacial mesophyll in the family Asteraceae and the anatomical diversity was commonly observed in the structure of leaves of Asteraceae. The vascular system includes xylem and phloem cells, which are larger in *B. perennis* and *B. sylvestris* than in *B. annua*. The xylem faces towards the upper surface, while the phloem faces the lower epidermis.

The results of the present study show that the palynological characters of *B. annua*, *B. perennis* and *B. sylvestris* are heterogeneous. Similarly, the many previous studies reported variations in pollen size, exine ornamentations, and spine length-width in the family Asteraceae (Türkmen et al. 2010, Akyalçin et al. 2011). The pollen grains are echinate-perforate, tricolporate, isopolar, radially symmetrical and oblate-spheroidal (*B. annua*) to prolate-spheroidal (*B. perennis* and *B. sylvestris*). The perforation of pollen surface ranged from elliptic to polygonal. According to Kremp (1965), *B. perennis* and *B. sylvestris* have small pollen sizes, while *B. annua* has a medium pollen size. Spine length is similar in the examined taxa, but the spine width and distance between spines varies among the taxa. The results obtained from palynological studies were generally similar to the results of Kavalcıoğlu et al. (2010). According to Ehrendorfer (1949) and Brochmann (1992) pollen grain size is frequently correlated with the ploidy level of the gamete. Sharbel et al. (2005) reported that some of the pollen-size variation resulted from differences in chromosome number. Furthermore, six species of the genus *Achillea* were examined by Akyalçın et al. (2011), who found some differences in pollen grains, size and exine ornamentation. Similarly, many previously studies reported some variation in the chromosome numbers and sizes of the genus *Bellis* even though they have commonly a diploid chromosome number (2n = 18) (Negodi 1936, Fernandes and Queiros 1971, Fiz et al. 2002). Bozdağ et al. (2011) determined variation in chromosome numbers and sizes of *B. annua* (2n = 18), *B. perennis* (2n = 18) and *B. sylvestris* (2n = 36). In this study, a large variation in palynological characters of *Bellis* species was determined and this variation may be caused by the polyploidy level or gamete divisions in microsporogenesis.

The shapes of achenes of the taxa were investigated morphologically and they were found to be one-seeded, compressed, obovate or obovoid shaped and without pappus (Fig. 6A-F). They are dark brown to yellow in colour. The achene coat ornamentations are rectangular and short hairy on surface. Therefore, no significant differences in seed morphology were observed among the taxa.

The present study provides useful information about the morphology, anatomy, palynology and achene morphology of *Bellis* species distributed in Turkey. This is the first comprehensive study on the root, stem and leaf anatomy, pollen and achene morphology of *Bellis* species growing in Turkey. We think that our investigations will contribute to systematics of this genus and further molecular characterization studies are required to solve the problems of relationships and taxonomy of this genus.

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References

Ai-Douri, N.A., Al-Essa, L.Y., 2010: A survey of plants used in Iraqi traditional medicine. Jordan Journal of Pharmaceutical Sciences 3, 100–108.

Akçin T.A., Akçin, A., 2010: Morphological and anatomical characteristics and taxonomical significance of achene micromorphology of *Achillea phrygia* and *A. gypsicola* (Asteraceae), endemic to Turkey. Nordic Journal of Botany 28, 65–73.

Akyalçin, H., Arabaci, T., Yildiz, B., 2011: Pollen morphology of six *Achillea* L. sect. *Achillea* (Asteraceae) species in Turkey. Turkish Journal of Botany 35, 183–201.

Avci, M., 1996. The floristic regions of Turkey and a geographical approach for Anatolian Diagonal. Review of the Department of Geography University of Istanbul 3, 59–91.

Avise, J.C., 2000: Phylogeography: The history and formation of species. Harvard University Press, Cambridge.

Baytop, T., 1994: A Dictionary of vernacular names of wild plants of Turkey. The Turkish Language Society, Ankara.

Bozdağ, B., Özdemir, C., Sepet, H., 2011: Karyotype analysis on the species of *Bellis* L. (Asteraceae) in Turkey. Caryologia 64, 251–255.

Bremer, K., 1994. *Bellis* L. In: Bremer, K., Anderberg, A.A (eds.), Asteraceae, cladistics and classification, 202–233. Timber Press, Oregon.

Brochmann, C., 1992: Pollen and seed morphology of Nordic *Draba* (Brassicaceae): Phylogenetic and ecological implications. Nordic Journal of Botany 12, 657–673.

Calvo, J., Quintanar, A., Aedo, C., 2012: Typification of four species names of *Bellis* (Compositae). Nordic Journal of Botany 30, 668–670.

Davis, P.H., 1965-1985: Flora of Turkey and The East Aegean Island, vol. 1-9. Edinburgh University Press, Edinburgh.

Ehrendorfer, F., 1949: Zur Phylogenie der Gattung *Galium*. I. Polyploidie und geographisch-ökologische Einheiten in der Gruppe des *Galium pumilum* Murray (Sekt. *Leptogalium* Lange sensu Rouy) im österreichischen Alpenarum. Österreichische Botanische Zeitschrift 96, 109–138.

Ekim, T., 2012: *Bellis*. Retrieved December 5, 2019 from http://www.bizimbitkiler.org.tr.

Faegri, K., Iversen, J., 1975: Textbook of pollen analysis. Alden Press, London.

Fernandes, A.,Queiros, M., 1971: Contribution a la connaissance cytotaxonomique des Spermatophyta du Portugal (ser. 2). Boletim da Sociedade Broteriana 45, 5–121.

Fiz, O., Valcárcel, V., Vargas, P., 2002: Phylogenetic position of Mediterranean Astereae and character evolution of daisies (*Bellis*, Asteraceae) inferred from nrDNA its sequences. Molecular Phylogenetics and Evolution 25, 157–171.

Frangiote-Pallone, S., De Souza, L.A., 2014: Pappus and cypsela ontogeny in Asteraceae: Structural considerations of the tribal category. Revista Mexicana de Biodiversidad 85, 62–77.

Grierson, A.J., 1975: *Bellis* L. In: Davis, P.H. (ed.), Flora of Turkey and the East Aegean Islands, vol. 5, 134–136. Edinburgh University Press, Edinburgh.

Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M.T. (eds.), 2012: Turkey plant list (Vascular plants). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul (in Turkish).

Hassler, M., 2019: World plants: Synonymic checklists of the vascular plants of the World. Retrieved December 5, 2019 from http://www.catalogueoflife.org/

Joujeh, R., Zaid, S., Mona, S., 2019: Pollen morphology of some selected species of the genus *Centaurea* L. (Asteraceae) from Syria. South African Journal of Botany, 125, 196–201.

Karakaş, F.P., Karakaş, A., Boran, Ç., Türker, A.U., Yalçin, F.N., Bilensoy, E., 2012: The evaluation of topical administration of *Bellis perennis* fraction on circular excision woundhealing in Wistar albino rats. Pharmaceutical Biology 50, 1031–1037.

Kavalcioğlu, N., Açik, L., Pinar, M., 2010: Comparative RAPD analysis and pollen structure studies of *Bellis perennis* L. Turkish Journal of Botany 34, 479–484.

Kremp, G.O.W., 1965: Morphologic encyclopedia of palynology: an international collection of definitions and illustrations of spores and polen. University of Arizona Press, Tucson.

Melikoğlu, G., 2015: The plants traditionally used to treatment of asthma in Turkey. Marmara Pharmaceutcal Journal 19, 1–11 (in Turkish).

Metcalfe, C.R., Chalk, L., 1957: Anatomy of the dicotyledons I. Clarendon Press, London.

Mitich, L.W., 1997: English daisy (*Bellis perennis* L.). Weed Technology 11, 626–628.

Moore, P.D., Webb, J.A., Collison, M.E., 1991: Pollen analysis. Blackwell Scientific Publications, Oxford.

Mutun, S., 2016: Review of oak gall wasps phylogeographic patterns in Turkey suggests a main role of the Anatolian diagonal. Türkiye Ormancılık Dergisi 17, 1–6.

Negodi, G., 1936: Cariologia delle specie italiane del genere *Bellis* e contributo all appressamento dello stato energetico dei genomi di specie omoploidi. Biological Genetics 12, 546–558.

Özler, H., Kaya, Z.,Pehlivan, S., 2009: Pollen morphology of some *Centaurea* L., *Psephellus* Cass. and *Cyanus* Miller taxa. Acta Biologica Cracoviensia Series Botanica 51, 53–66.

Özörgücü B., Gemici, Y., Türkan, I., 1991: Comparative plant anatomy. Ege Üniversitesi Fen Fakültesi Yayını, İzmir (In Turkish).

Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S., Le Thomas, A., 2007: Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology 143, 1–81.

Shabestari, E.S.B., Attar, F., Riahi, H., Sheidai, M., 2013: Pollen morphology of *Centaurea* L. (Asteraceae) in Iran. Acta Botanica Brasilica 27, 669–679.

Sharbel, T.F., Mitchell-Olds, T., Dobeš, C., Kantama, L., De Jong, H., 2005: Biogeographic distribution of polyploidy and B chromosomes in the apomictic *Boechera holboellii* complex. Cytogenetic Genome Research 109, 283–292.

Türkmen, Z., Makbul, S., Çoşkunçelebi, K., Beyazoğlu, O., 2010: Palynological observations on the genus *Scorzonera* L. (Asteraceae) from north-east Anatolia (Turkey). Turkish Journal of Botany 34, 495–512.

Uysal, I., Onar, S., Karabacak, E., Çelik, S., 2010: Ethnobotanical aspects of Kapıdağ Peninsula (Turkey). Biological Diversity and Conservation 3, 15–22.

Webb, D.A., 1976: *Bellis* L. and *Bellium* L. In: Tutin, T.G., Burges, N.A., Charter, A.O., Edmonson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H.,Walters, S.M.,Webb, D.A. (eds.), Flora Europaea 4, 111–112. Cambridge University Press, Cambridge.

WFO 2019: World Flora Online. *Bellis* L. Retrieved December 30, 2019 from http://www.worldfloraonline.org/.

Wodehouse, R.R., 1935: Pollen grains. McGraw-Hill, New York.