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# A new Asperula L. (Rubiaceae) species from gypsum steppes of Çankırı province in Turkey

Bilal ŞAHİN<sup>1,\*</sup>, Mehmet SAĞIROĞLU<sup>2</sup>, Birol BAŞER<sup>3</sup>

<sup>1</sup>Cankırı Karatekin University, Yapraklı Vocational School, Cankırı, Turkey <sup>2</sup>Department of Biology, Faculty of Sciences and Arts, Sakarya University, Sakarya, Turkey <sup>3</sup>Department of Biology, Faculty of Sciences and Arts, Bitlis Eren University, Bitlis, Turkey

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Abstract: Asperula cankiriense B.Şahin & Sağıroğlu, a new species discovered in the central Anatolian region of Turkey (Çankırı), is described herein. A. cankiriense is similar to Asperula bornmuelleri Velen. However, A. cankiriense differs mainly in its habitus, stem, leaves, and inflorescence features. In this study, the diagnostic morphological features, distribution, habitat and ecology, etymology, conservation status, and taxonomic features of the new species are discussed. Additionally, pollen micromorphological characteristics of A. cankiriense and A. bornmuelleri are examined and compared using light microscopy (LM) and scanning electron microscopy (SEM) analyses. The pollen grains are subprolate, aperture type 6-7 or rarely 8 zonocolpate and heterosincolpate and their exine ornamentation is microechinate-perforate.

Key words: Anatolia, Asperula, gypsum, pollen, taxonomy, Çankırı

# 1. Introduction

Asperula is an important genus of the family Rubiaceae. This genus consists of 185 species and 232 taxa worldwide (Latrou, 1984; Thompson, 2009; Brullo et al., 2009; Minareci and Yıldız, 2010; Öztürk, 2013). Ehrendorfer and Schonbeck-Temesy revised the genus in Flora of Turkey and the East Aegean Islands (1982). In their study, the genetic diversification centre of the genus was determined as the Mediterranean phytogeographic region and south-western Asia (Ehrendorfer and Krandl, 1974); however, further studies were necessary to reinforce this claim (Senol and Yıldırım, 2010). Most of the wild and endemic plants of genus Asperula occur in Turkey (41 species) and Greece (38 species). Therefore, this region is considered the genetic diversification centre of the genus, presumably dating back to the Pleistocene and Holocene ice ages. In addition to these, Thompson (2009) reported that 21 natural species of the genus exist in Australia. Such data from Australia is important for diversification and occurrence of the genus (Öztürk, 2013).

Recently, Asperula samia Christodoulakis & Georgiadis, A. pseudochlorantha Ehrend. var. antalyensis (Ehrend.) Minareci & Yildiz, and A. anatolica M.Oztürk were added and defined for Flora of Turkey (Minareci et al, 2010; Öztürk, 2013). Asperula in Turkey comprises 52 taxa, in 6 sections, of which 27 are endemic (Güner et al., 2012;

Öztürk, 2013). The Turkish province of Çankırı contains 2 distinct phytogeographical regions. In the southern part of the province, the Irano-Turanian phytogeographical region is characterized by dry steppes, and northern part, the European-Siberian phytogeographical region, is characterized by forests. In the southern part of Çankırı, 5 to 15-million-year-old gypsum bedrock can be observed (Sevin and Oğuz, 2011). This bedrock holds a variety of gypsum steppe flora at altitudes of 500-900 m (Ekim et al., 2000; Şahin et al., 2015; Şahin and Şimşek, 2016). Alyssum nezaketiae Aytaç & H. Duman, Galium cankiriensis Yıldırımlı, Erysimum jacquemoudii Yıldırımlı, Genista vuralii Duran and Symirnium galaticum Czeczott were newly defined in Çankırı (Aytaç and Duman, 2000; Duran and Dural, 2003; Yıldırımlı, 2008; Yıldırımlı, 2010; Sağıroğlu et al., 2013).

During a field survey, specimens of Asperula were collected in Çankırı. These specimens appeared similar to A. bornmuelleri (Figures 1-3) at first sight. However, their dwarf caespitose habitus, short internodes, and inflorescence features differed. Following the collection of both A. bornmuelleri and this new specimen from the same area, a detailed study was performed and data was compared to herbarium samples of A. bornmuelleri. The specimens were also cross-checked with the account of Asperula in Flora Iranica, Flora Europaea, and other

<sup>\*</sup> Correspondence: felicyntoukand@yahoo.com





Figure 1. A specimen of Asperula bornmuelleri in Herb. JE (A), isotype in Herb. BR (B).

literature, and compared with herbarium specimens in the GAZI and ANK herbaria (Bornmüller, 1931; Czeczott, 1932; Ehrendorfer and Krendl, 1974; Ehrendorfer and Schonbeck-Temesy, 1982; Schonbeck-Temesy and Ehrendorfer, 2005; Özhatay and Kültür, 2006; Minareci, 2007; Özhatay et al., 2009; Minareci and Yıldız, 2010; Özhatay et al., 2011; Güner et al., 2012). Pollen morphology of the family has been examined by Erdtman (1952, 1971), Kuprianova and Alyoshina (1978), Nowicke and Skvarla (1979) Robbrecht (1982), Persson (1993), Puff et al. (1996), Block and Robbrecht (1998), Huysmans et al. (1994, 1999), Vinckier et al. (2000), Sotolongo (2002) Huysmans et al. (2003), Dessein et al. (2000, 2002, 2005), Minareci & Yıldız (2010), Minareci et al. (2010), Öztürk (2013) and Başer et al. (2020). Robbrecht (1982), stated that the pollen characteristics of genera such as Asperula are extremely important in systematic studies. Palynomorphological features of pollen grains of some genera have important taxonomic value (Block & Robbrecht, 1998; Dessein et al., 2000, 2002; Öztürk, 2013). Huysmans et al. (2003) examined the morphological structures of pollen of 29 species belonging to *Asperula*, *Crucianella* L., *Cruciata* Miller, *Galium* L., *Rubia* L. and *Sherardia* L. from Rubiaceae. They emphasized that the ornamentation of the exine is important in the separation of species rather than pollen size and shape. Perveen and Qaiser (2007) indicated that the Rubiaceae family is very suitable for pollen morphological studies. Başer et al. (2020) studied palynological features of 9 species belonging to *Asperula* L., *Galium* L. from Rubiaceae.

The aim of this study is to present a detailed description of *Asperula cankiriense* and to define similarities and differences of *A. cankiriense* and *A. bornmuelleri* based on morphology, ecology, and pollen morphological data in order to precisely determine their systematic identities (Figures 1–4, Tables 1, 2).

### 2. Materials and methods

The new species samples were collected at 3 different localities (as these 3 localities are in close geographical



Figure 2. Asperula cankiriense (A, B) A. bornmuelleri (C, D) habitus and habitat.

proximity to one another, they are illustrated as a single location in Figure 5). Each locality was visited a minimum of two times during the flowering and fruiting periods of the plant, and the population state of the species in their localities was determined. In the description below, each numerical value is the range of 10 measurements from different specimens. *A. bornmuelleri* samples were examined in the ANK and GAZI herbaria. Photos of the isotype samples were seen in the JE, W, PRC, WU, E, P, BR herbaria. The specimens of new species were compared morphologically with the specimens of *A. bornmuelleri* (Table 1).

For light microscopy (LM) analysis, pollen slides were prepared according to the Wodehouse's technique (Wodehouse, 1935). The material utilised in this research was either new or obtained from dried specimens. Measurements were performed on at least 30 pollen grains per specimen for each morphological character (Polar axis (P), equatorial diameter (E), Colpus width (Clt), Colpus length (Clg), mesocolpium area, and apocolpium diameter)



Figure 3. Asperula cankiriense (A, B) and A. bornmuelleri (C, D) flowers and fruits.

with LM using an oil immersion 100U objective lens. For scanning electron microscopy (SEM) analysis, pollen grains taken from the mature anthers were transferred onto stubs and then coated with gold, investigated and photographed with a Jeol JSM-6060 SEM and the number of microspines, microperforations' diameter and their number were performed 10 times from different areas (polar and equatorial regions) on the pollen surface in

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**Figure 4.** SEM microphotography of the pollen *A. cankiriense*; **a**- Equatorial view, **b**- Polar view, **c**- Exine surface in detail. *A. bornmuelleri*; **d**- Equatorial view, **e**- Polar view, **f**- Exine surface in detail.

each 5  $\mu$ m<sup>2</sup> (Figure 4). Terminology was adopted from Huysmans (2003), Punt et al. (2007) and Öztürk (2013).

#### 3. Results

**3.1.** *Asperula cankiriense* B.Şahin & Sağıroğlu **sp. nova** (Figures 2A,2B and 3A and 3B).

Section: Cynanchicae (DC.) Boiss.

**Type**: Turkey, A4 Çankırı: Süleymanlı village, Salt cave road, gypsum steppe, 600–700 m, 23.06.2016. B. Şahin 6526 & M. Sağıroğlu (holotype GAZI, isotypes: GAZI, HUB, ANK).

**Diagnoses:** Asperula cankiriense is closely related to *A. bornmuelleri*. It differs from *A. bornmuelleri* by its dwarf caespitose erect stem (not prostrate ascending), short internodes of 2–20 mm (not 30–50 mm) short inflorescence of 3–5 cm (not 8–16 cm), 20–35 flowered (not 50–80).

**3.2. Description**: Dwarf caespitose perennial. Stem numbers in young individuals (5–) 20–30, in adult individuals 40–70 (–100), 6–10 (–15) cm tall, erect upwards, mostly simple or rarely short branched (1–2 verticillasters), minutely setulose, woody at base. Internodes 2–10 mm long in lower parts and 10–20 mm long in upper parts, the lower and upper leaves nearly equal or longer than nodes. Leaves in whorls of 4, linear, linear-lanceolate, 4–10 (–15) ×0.5–1 mm, slightly reduced upwards, minutely setulose; margins revolute; apex acute-mucronate, 0.2–0.5 mm; leaves of the uppermost node sparsely setulose or glabrous.

Inflorescence spike-like, sessile to shortly pedunculate 3-5 cm long, verticillasters in interrupped spike-like thyrses, 2-6 cm long, verticillasters 3-6, each stem 20-35 flowered. Involucral bracts leaf-like, broadly ovate-lanceolate, 4-7  $\times$  1.5–2 mm, apex acuminate, hyaline margins minutely ciliate, gradually attenuating towards the base, expanded at base. Bracteoles broadly lanceolate, 2-2.5× 1-1.5 mm, apex acuminate-subulate, hyaline margins sparsely short ciliate, white margined. Pedicels sessile to 1 mm long, minutely sparsely setulose. Corolla white, infundibular, usually verruculose, 3.5-5 mm long with sparsely ciliate tube (1.5-2 mm) and 4 lobed limb, lobes  $2-3 \times 0.5-0.7$ mm linear, longer than tube, apex acute. Anthers 1-1.4 mm, nearly exserted from throat, brownish. Style 2-3 mm long, bifid with 2 stigmas globose, black, basally fused. Mericarps oblong-ellipsoid 2-2.5× 1.5-2 mm, papillose, brownish when mature.

**Phenology:** Flowering and fruiting times May to July. **3.3. Paratypes:** 

Çankırı: Center, Süleymanlı village, salt cave road, around 6th km, gypsum steppes, 600–700 m, 22.06.2016, B. Şahin 6484 (GAZI).

Çankırı: Center, Süleymanlı village, salt cave road, around 6th km, gypsum steppes, 600–700 m, 26.05.2018, B. Şahin 7131 (GAZI).

Çankırı: Center, Süleymanlı village, salt cave road, around 12th km, gypsum steppes 600–700 m, 26.05.2018, B. Şahin 7133 & M.Sağıroğlu (GAZI).

Characters	Asperula cankiriense	Asperula bornmuelleri	
Stem	Dwarf caespitose erect, 6–10 (–20) cm, woody at base	Prostrate ascending, (15–) 18–50 cm, semi-shrubs at base	
Lateral branch	Short, with 1–2 verticillasters	long, with 2–5 verticillasters	
Leaves	Linear-lanceolate, $4-10 \times 0.5-1$ mm	Linear, 6–15(–18) ×0.5–2 mm	
Internodes	10–20 mm long, shorter than leaves	30–50 mm long, Longer than leaves	
Inflorescens	Spike-like, 3–5 cm	Spike-like thyrses, 8–16 cm (partial inflorescens sparsely spiciform)	
Verticillasters numbers	3-6	5-9	
Flowers numbers	20-35	50-80	
Corolla	Usualy verruculose, 3.5–5 mm long	Sometimes verruculose, 4–6 mm long	
Anthers	1–1.4 mm long	07–1 mm long	
Style	2–3 mm long	to 2 mm long	

 Table 1. Diagnostic characters of A. cankiriense compared with morphologically similar A. bornmuelleri.

 Table 2. Pollen morphological characters for A. cankiriense and A. bornmuelleri.

Species		A. bornmuelleri M±SD	A. cankiriense M±SD
Polar axis (µm)		18.38 ± 1.42	20.10 ± 1.91
Equatorial diameter (µm)		15.27 ± 1.60	16.39 ± 2.86
P/E ratio and pollen shape		1.20 Subprolate	1.22 Subprolate
Exine thickness (µm)		$1.18 \pm 0.20$	$1.40 \pm 0.17$
Intine thickness (µm)		0.53 ± 0.10	0.69 ± 0.09
Colpus length (Clg) (µm)		14.10 ± 2.08	13.43 ± 1.58
Colpus width (Clt) (μm)		$1.28 \pm 0.57$	$1.31 \pm 0.48$
Mesocolpium (M) (µm)		$4.78 \pm 0.37$	$3.92 \pm 0.46$
Apocolpium (µm)		9.17 ± 1.83	$10.27 \pm 2.43$
Microperforation diameter (µm)		$0.23 \pm 0.08$	$0.18\pm0.07$
Number of colpus		6-7-(8)	6–7
At equatorial regions	Average the number of microspines in 5 $\mu$ m <sup>2</sup>	50 (30–70)	66 (50-82)
	Average the number of microperforation in 5 $\mu$ m <sup>2</sup>	15 (10–36)	12 (10–18)
At polar regions	Average the number of microspines in 5 $\mu$ m <sup>2</sup>	44 (32–52)	47 (38–51)
	Average the number of microperforation in 5 $\mu$ m <sup>2</sup>	11 (8–14)	26 (23-30)

**3.4. Etymology:** The specific epithet is derived from the name of the city, Çankırı, where the new species was first discovered.

**3.5. Vernacular name:** The Turkish name of this species is given as "Çankırı belumu", according to the guidelines of Menemen et al. (2013).

**3.6. Distribution and ecology:** Asperula cankiriense is endemic to central Anatolia and belongs to the Irano-Turanian floristic element. The specimens were collected from Çankırı province where the species appears to be restricted to a locality. A. cankiriense grows on gypsum stony slopes with Bromus tomentellus Boiss., Asperula



**Figure 5.** The distribution map of *Asperula cankiriense* (●) and *A. bornmuelleri* (●)

bornmuelleri Velen, Cruciata taurica (Pall. ex Willd.) Ehrend., Artemisia santonicum subsp. santonicum L., Achillea phrygia Boiss. & Balansa, Salvia absconditiflora (Montbret & Aucher ex Benth.) Greuter & Burdet, Genista albida Willd., Tanacetum germanicopolitanum (Bornm. & Heimerl) Grierson, Achillea aleppica DC., Gypsophila eriocalyx Boiss., Eryngium campestre L., Centaurea carduiformis DC., Paracaryum ancyritanum Boiss. (Figure 5).

**3.7.** Conservation status: Asperula cankiriense is an endemic species known in three separate and geographically proximal localities. According to the observations of this research and field studies, it has been determined that the area of occupancy of the new species is less than  $2 \text{ km}^2$ , its population size is estimated to be fewer than 500 mature individuals, and that the area of occupancy has experienced excessive soil erosion. Therefore, the conservation status of the new species should be evaluated as 'critically endangered' [CR: B1ab(iii) + B2ab(iii)] in accordance with IUCN criteria (2017).

### 3.8. Key to related Asperula species in Turkey

1-Corolla pale pink, 4–8 mm, with lobes  $\pm$  equalling tube to 3 × shorter; leaves  $10-20 \times 0.3-0.75$  mm, linear-subulate, always distinctly aristate with awns c.1 mm.....

# ....A. lilaciflora

1-Corolla white, 3.5-6 mm, with lobes as long as tube or longer; leaves 4-15 (-18) ×0.5-1 mm, linear, acute-mucronate to aristate, with awns 0.2–0.7 mm

**2**-Stem erect; inflorescence 3–5 cm long; internodes 2–20 mm long,

 **3.9.** Pollen morphology: Pollen morphologies were examined with LM and SEM. The pollen of *A. bornmuelleri* and *A. cankiriense* have monad, radial symmetry and isopolar.

Asperula bornmuelleri; The pollen grains are subprolate with polar axis 16.68-21.31 µm and equatorial axis of 13.58-19.27 µm. Aperture type is 6-7 or rarely 8 zonocolpate, rarely heterosyncolpate. The colpus is long (Clg:11.50-15.48 µm) and narrow (Clt: 1.05-2.45 µm); its margins distinct and ends acute. The ranges of the mesocolpium area and apocolpium diameter are 3.40-6.37 µm and 8.50-11.36 µm. The exine is tectate and its thickness ranges from 1.18 to1.30 µm. The intine has a thickness of 0.51-0.77 µm. Exine shows microechinateperforate ornamentation at polar and equatorial regions. At equatorial regions, the number of microspines in  $5 \,\mu\text{m}^2$ ranges between 30-70 and the microperforation number varies between 10-36, and diameter 0.20-0.32 µm. At polar regions, the number of microspines in 5  $\mu$ m<sup>2</sup> ranges between 23-52 and the microperforation number vary between 8-14 (Table 2, Figure 4).

Asperula cankiriense; The pollen grains are subprolate with polar axis 19.76–23.36  $\mu$ m and equatorial axis of 15.10–20.40  $\mu$ m. Aperture type is 6–7 zonocolpate, rarely heterosyncolpate. The colpus is long (Clg:11.50–18.50  $\mu$ m) and narrow (Clt: 1.20–2.35  $\mu$ m); its margins distinct and ends acute. The ranges of the mesocolpium area and apocolpium diameter are 2.91–5.49  $\mu$ m and 7.92–12.18  $\mu$ m. The exine is tectate and its thickness ranges from 1.20 to 1.60  $\mu$ m. The intine has a thickness of 0.77–1.02  $\mu$ m. Exine shows microechinate-perforate ornamentation at polar and equatorial regions. At equatorial regions, the number of microspines in 5  $\mu$ m<sup>2</sup> ranges between 50–82 and the microperforation number vary between 10–18 and diameter 0.18–0.37  $\mu$ m. At polar regions the number

of microspines in 5  $\mu$ m<sup>2</sup> ranges between 38-51 and the microperforation number vary between 23-30 (Table 2, Figure 4).

# 4. Discussion

A. cankiriense was collected from 3 localities, where it is typically found together with A. bornmuelleri. In the province of Çankırı, A. bornmuelleri is spread mainly on the gypsum steppes. A. cankiriense is superficially similar to A. bornmuelleri, although it differs typically by its habitus. Its small stems and internode, short lateral branches, dwarf caespitose habitus, and linear lanceolate leaves differentiate it from A. bornmuelleri (Table 1).

The synonyms of A. bornmuelleri (A. bornmuelleri subsp. cappadocica, A. bornmuelleri var. galatica, A. bornmuelleri var. paphlagonica) were also examined (Bornmüller, 1931; Czeczott, 1932; Ehrendorfer and Schonbeck-Temesy, 1982). A. cankiriense is always erect and A. bornmuelleri is prostrate and ascending, characteristics which have not yet been used to identify this species. As illustrated in Table 1, A. bornmuelleri differs in both morphological and generative character (Bornmüller, 1931; Czeczott, 1932; Ehrendorfer and Schonbeck-Temesy, 1982). Another synonymous species is A. refracta Czeczott; however, the author admits that his own species might be invalid by indicating that "Perhaps is identical with the A. bornmuelleri Velen. described by Bornmüller in 1931". Thus, A. refracta has been taxonomically synonymous since the beginning. The description of this species is the same as A. bornmuelleri (Czeczott, 1932).

In the south of the city of Çankırı, gypsum bedrocks of various ages are condensely present. These bedrocks are soft and tend to be highly exposed to the erosion process. The flow of the broken plate-like stacks, due to erosion, has formed a rugged geomorphological structure. Recently, there have been several reports claiming that endemic species exist in this special region. Hence, the floristic structure of this region needs to be examined in detail.

It has previously been determined that the apertures in the family Rubiaceae are mostly colpate or rarely colporate. Perveen and Qaiser (2007) reported that the number of colpus was generally 6–7, although sometimes 3 and 11. Minareci et al. (2010) and Öztürk (2013) determined the number of colpus as 6–7 (–8). In the present study, the number of colpus was 6–7 zonocolpate rarely heterosincolpate for *A. cankiriense* and 6–7 or rarely 8 zonocolpate, rarely heterosincolpate for *A. bornmuelleri*.

In a study related to pollen structures carried out in Turkey, Minareci et al. (2010) measured the maximum pollen size as 19  $\mu$ m and the mesocolpium as 6.4–6.6  $\mu$ m, whereas they were measured as 25  $\mu$ m and 4.8  $\mu$ m by Öztürk (2013), respectively. In the current study, the maximum the pollen size and mesocolpium were measured as 21.31  $\mu$ m and 6.37  $\mu$ m for *A. bornmuelleri* and 23.36  $\mu$ m and 5.49

um for A. cankiriense, respectively (Table 2). Polar axis and equatorial diameter of A. bornmuelleri and A. cankiriense are quite close to one another. Both show a similar ornamentation pattern: the tectum is microperforated and densely beset with tiny microspines, yet the size and density of the microperforation may vary between species. The average microperforation diameter is  $0.23 \ \mu m$  in A. bornmuelleri and 0.18 µm in A. cankiriense. According to these results, microperforation diameter is larger in A. bornmuelleri than in A. cankiriense. In contrast, the number of microperforations of A. bornmuelleri in 5 µm<sup>2</sup> is lower than in A. cankiriense on the mesocolpium and apocolpium area. Huysmans et al. (2003) determined that the number of microspines is greater than the number of microperforations on the same surface. The number of microspines and microperfoations indicates differences among the species. Also, the number of microspines of A. *cankiriense* in 5  $\mu$ m<sup>2</sup> is more frequent than *A. bornmuelleri* on the mesocolpium and apocolpium areas. The number of microspines and microperforations in 5  $\mu$ m<sup>2</sup> along with the diameter of microperforations and pollen size indicates differences and varies between A. bornmuelleri and A. cankiriense.

Thus, in this study, significant taxonomic differences between A. bornmuelleri and A. cankiriense in terms of pollen morphologic properties were clearly identified. These properties are pollen size, microspines, and microperforation numbers. In previously published studies, it has been found that these traits are the most useful characteristics in both the Rubiaceae family and the genus Asperula Dessein et al. (2005), Minareci and Yıldız (2010), Öztürk (2013), Başer et al. (2020). As a result of SEM analysis, the heterosyncolpate aperture type was observed in the pollen of both species examined in this study. When previous studies were reviewed (Huysmans et al., 2003, Dessein et al. (2005), Perveen and Qaiser (2007), Minareci and Yıldız (2010), Öztürk (2013), Başer et al. (2020), no information was found about heterosyncolpate type in the Rubiaceae family or genus Asperula. However, not enough is known about the presence of heterosyncolpate in the pollen of these species. Therefore, further studies are needed to determine whether this aperture in the genus Asperula has an adequate taxonomic character that can be used to describe pollen morphology.

The findings of this research clearly showed that the two species are different from each other. Thus, it can be concluded that these specimens represent a distinct species new to science. By adding *A. cankiriense*, as identified within this paper, the number of endemic species in Turkey rises to 22.

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# Additional specimens examined

# A. bornmuelleri:

Çankırı: Eldivan, around Seydiköy village, 1000-1200 m, gypsum steppes, 24.06.2016, B. Şahin 6519 (GAZI).

Çankırı: Center, around Hasakça village, 600-800 m, gypsum steppes, 23.06.2016B. Şahin 6507 (GAZI).

Çankırı: Center, around Bugay village, 600-800 m, gypsum steppes, 23.06.2016, B. Şahin 6506 & M. Sağıroğlu (GAZI).

Çankırı: Center, around Yanlar village, 600-800 m, gypsum steppes, 24.06.2016, B. Şahin 6510 (GAZI).

Çankırı: Center, around Onardüzü, 600-800 m, gypsum steppes, 24.06.2016, B. Şahin 6511 (GAZI).

Çankırı: Center, Kenbağ on the way to Kastamonu, 600-800 m, gypsum steppes, 23.06.2016, B. Şahin 6508 (GAZI).

Çankırı: Center, Ankara way, at the near of Ballıca MYO, 700-800 m, gypsum steppes, 26.05.2018, B. Şahin 7130 b (GAZI).

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