

A new species of *Sisymbrium* (Brassicaceae) from Turkey: morphological and molecular evidence

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Received: 08.04.2014 • Accepted: 05.10.2014 • Published Online: 16.03.2015 • Printed: 10.04.2015

Abstract: *Sisymbrium malatyanum* Mutlu & Karakuş is identified as a new species from Malatya Province, East Anatolia, Turkey. Morphological and molecular (entire ITS region) studies proved that this species especially resembles *Sisymbrium brassiciforme*, *S. heteromallum*, *S. altissimum*, *S. damascenum*, and *S. austriacum* subsp. *contortum*. New species and related taxa are discussed in terms of morphological and molecular differences. Epicuticular wax on the fruit surface of the new species was determined. SEM images (hairs on leaf, stem, fruit, stylus, and epicuticular wax crystalloids) and field photographs (habitat, flowering and fruiting shape of habitus, and fruit) are presented.

Key words: Brassicaceae, epicuticular wax, ITS, new species, *Sisymbrium*

1. Introduction

The family Brassicaceae is the richest in the United States (616 species, 148 endemic) and the second richest in Turkey (606 species, 39 subspecies, 18 varieties, and 226 endemics) in terms of species number (Al-Shehbaz et al., 2007; Al-Shehbaz, 2010; Mutlu, 2012). Forty species belonging to the family Brassicaceae in Turkey were published as new species in the last decade (Mutlu, 2012), and this number has continued to increase.

The authors collected numerous species in Malatya Province between 2011 and 2013, and one of these species was published as a new species (Mutlu and Karakuş, 2012). One of the collected samples could not be designated according to the current literature (Bush, 1939; Ball, 1964; Hedge, 1965; Zohary, 1966; Rico, 1996; Al-Shehbaz, 2001, 2010; Khodashenas and Assadi, 2007) and it seems to represent a new-to-science species of the genus *Sisymbrium* L. (English: yellow rocket; German: Rauken; Turkish: bülbül otu) (Figure 1).

The genus is distributed mainly in the Old World, and only one species, *S. linifolium* Nutt., is native to North America (Al-Shehbaz, 2006). Morphological and extensive molecular studies identified that yellow flowers, strongly 2-lobed stigmas, often pinnately divided but never auriculate or amplexicaul cauline leaves, and simple or no trichomes (except *S. burchellii* DC.) are synapomorphies for the genus (Warwick et al., 2002; Warwick and Al-Shehbaz, 2003; Al-Shehbaz, 2004, 2006, 2012; Bailey et

al., 2007). These studies have also demonstrated that *Sisymbrium* consists of only about 40 species.

Sisymbrium is one of 98 genera within the family Brassicaceae (tribe *Sisymbrieae* DC.) in Turkey. The genus is represented by 10 species in Turkey (Hedge, 1965; Al-Shehbaz, et al., 2007; Mutlu, 2012). These species are *S. altissimum* L., *S. confertum* Stev., *S. elatum* K.Koch, *S. irio* L., *S. loeselii* L., *S. officinale* (L.) Scop., *S. orientate* L., *S. polyceratium* L., *S. runcinatum* Lag. ex DC., and *S. septulatum* DC.

Five species of the genus *Sisymbrium* in Turkey were examined in this study and one of them was identified as a new species according to morphological and molecular (including ITS1, 5.8S, and ITS2) data.

2. Materials and methods

2.1. Plant material

Plant materials for the new species were collected during a field survey conducted in 2013 in Malatya Province in Turkey. These specimens were kept in the INU herbarium. Three specimens (one was flowering and the other 2 were fruiting) of the new species and 4 different species (one specimen each of *S. altissimum*, *S. officinale*, and *S. septulatum* and 2 specimens of *S. orientale*) were examined.

2.2. DNA amplification and sequencing

DNA of the specimens was extracted from dried leaves of the herbarium samples.

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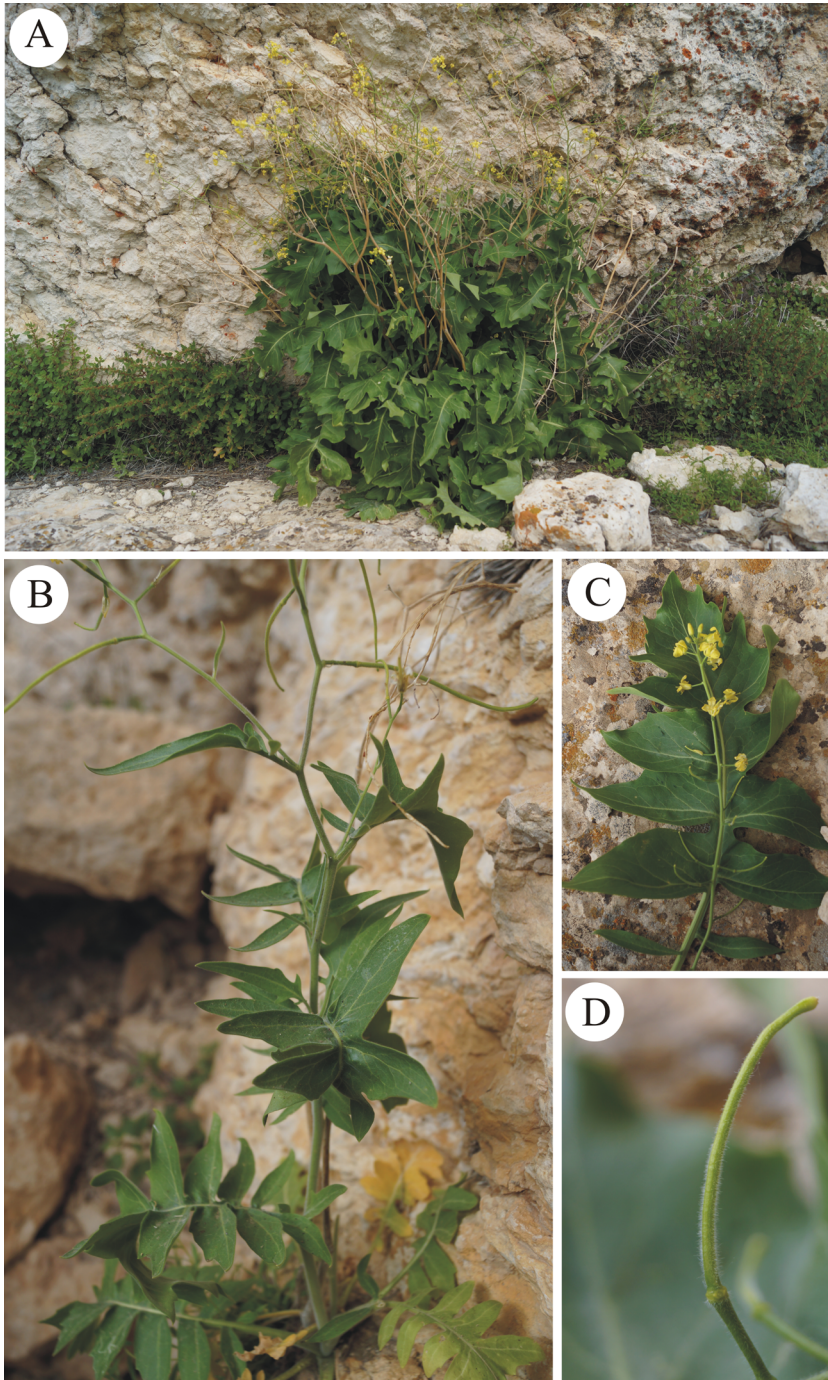


Figure 1. *Sisymbrium malatyanum* sp. nov. (photographed by B Mutlu): **A-** habit in flowering; **B-** habit in fruiting; **C-** basal leaf and flowers; **D-** fruit.

Total genomic DNA was extracted by the method of Warwick et al. (2002) using a modified CTAB extraction method. The entire ITS region was amplified, and primers ITS1-18S and ITS4 as described by O'Kane et al. (1996) and White et al. (1990) were used. The amplification reaction mixture (25 $\mu\text{L} \times 2$) for PCR was prepared as

follows: 10X PCR buffer 4 μL , dNTP mix (10 mM) 2 μL , MgCl_2 (25 mM) 4.0 μL , each primer (10 pmol/ μL) 1 μL , Taq polymerase (5 U/ μL) 0.5 μL , sample DNA (50 ng/ μL) 2.5 μL , and dH_2O 10 μL . Protocol for PCR was as follows: 5 min at 95 $^\circ\text{C}$, 32 cycles (94 $^\circ\text{C}$ for 30 s, 52 $^\circ\text{C}$ for 1 min, and 72 $^\circ\text{C}$ for 90 s), elongation at 72 $^\circ\text{C}$ for 7 min, and storage

at 4 °C until used. All the PCR examinations were carried out using a Thermo Px2 Thermal Cycler. Amplification products were cleaned using a PCR Clean-Up Gel Extraction Kit (Macherey-Nagel, Germany) in accordance with the manufacturer's instructions.

PCR products (25 µL) were mixed with 6X gel loading buffer (5 µL) and loaded onto agarose (1.4% w/v, with 4 µL EtBr/115 mL 1X TBE buffer) gel electrophoresis in 1X TBE buffer at 60 V for 120 min. DNA bands were visualized and photographed under UV light. A DNA molecular size marker (50-bp marker, BioLabs) was used.

The PCR products were sequenced using a Sanger automated DNA sequencer (Applied Biosystems) at İntek Research and Biotechnology Company (İntek Corp., İstanbul, Turkey). All DNA sequences in this study were deposited in GenBank, and Table 1 gives the accession numbers of these sequences.

2.3. Sequence alignment and phylogenetic analyses

DNA sequences of 24 species (2 outgroups) were obtained for comparison from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). Two species (*Capsella bursa-pastoris* (L.) Medik. and *Neotorularia torulosa* (Desf.) Hedge & J.Léonard) were selected as outgroups. Table 1 lists GenBank accession information for the sequences of compared and studied species.

The entire ITS region (ITS-1, 5.8S, and ITS2) sequences were automatically aligned by CLUSTALW (Thompson et al., 1994).

Maximum parsimony (MP) analyses were performed using PAUP 4.0b10 with heuristic search strategy according to the tree bisection and reconnection branch-swapping algorithm. The MULTREES option and ACCTRAN optimization were activated (Swofford, 2003). Gaps in the alignment were treated as missing data. All characters were treated as unordered and equally weighted. Limitations of computer memory required constraining the maximum number of trees to 1000 per replicate and limiting the maximum tree number to 40,000.

Bayesian inference (BI) analysis, which is an alternative phylogenetic method, was performed using MrBayes 3.2.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The Markov chain Monte Carlo method was used for 3 million generations. The settings were as follows: 2 independent runs, with nucmodel = 4 by 4, Nst = 6, rates = invgamma, samplefreq = 1000, 4 chains = 1 cold and 3 hot, and sump burnin = 450 samples.

Results of MP and BI analyses were drawn as trees with TREEVIEW (Page, 1996).

2.4. SEM studies

Before the SEM studies were carried out, samples were coated with gold/palladium. Electron micrographs were obtained with an EVO 40XVP (LEO Ltd., Cambridge, UK) scanning electron microscope at an accelerating voltage of 10 kV.

3. Results and discussion

Sisymbrium malatyanum is morphologically similar to *S. altissimum* L. (fruiting pedicel as thick as mature fruit, fruiting pedicel longer than 5 mm), *S. damascenum* Boiss. & Gaill. (hairy stem and fruit, fruiting pedicel longer than 5 mm), and *S. austriacum* Jacq. subsp. *contortum* (Cav.) Rouy & Foucaud (silique longer than 20 mm, lower leaves deeply lobed, petal longer than 3 mm, fruiting pedicel as thick as mature fruit, ovule 30–35 in each loculus). *Sisymbrium altissimum* is cosmopolitan species growing in North America, Asia, Europe, North Africa, and East Australia. However, while *S. austriacum* subsp. *contortum* grows only in Spain and Portugal, *S. damascenum* grows only in Syria and Jordan (Ball, 1964; Hedge, 1965; Zohary, 1966; Rico, 1996; Al-Shehbaz, 2001, 2010; Khodashenas and Assadi, 2007).

Figure 2 illustrates results of MP and BI analysis as a comparative tree. Bootstrap values (in the MP tree) and posterior probabilities (in the BI tree) are given above branches at $\geq 50\%$ for the supported clade. Tree length (L) was 404, consistency index (CI) was 0.65, and retention index (RI) was 0.86 for the parsimony tree. MP and BI analysis based on molecular data showed that new species is more closely related to *S. brassiciforme* and *S. heteromallum*. As seen from Figure 2, the new species is most close to *S. brassiciforme*. Schultz placed these 2 species in the section of *Grypolobus* O.E.Schulz and the species in this section are distributed in Central Asia. *Sisymbrium brassiciforme* is a more common species in West Asia compared to *S. heteromallum*. Furthermore, the distribution area of *S. brassiciforme* is the closest to Turkey (Khodashenas and Assadi, 2007). Distinguishing characters of the section are petiolate leaves, simple trichomes, ebracteate racemes, slender fruiting pedicel, yellow petal, entire or 2-lobed stigma, 3-veined fruit valve, uniseriate seed, and incumbent cotyledons. Some of these characters are shown in the new species (Figures 3A–3D).

SEM studies conducted on the epidermal surface of *Sisymbrium malatyanum* showed that the epicuticular surface has wax crystalloids, observed especially on the fruit surface (Figures 3E and 3F). These crystalloids are flat and connected to the epicuticular surface by their narrow side. These epicuticular wax crystalloids were named as “platelets” by Barthlott et al. (1998). Epicuticular waxes were observed on some Brassicaceae species, especially on *Arabidopsis thaliana* (L.) Heynh. (Broun et al. 2004) and *Brassica* spp. (Baker, 1974; Ni et al., 2014). This epicuticular wax on the epidermal surface was first identified in the species of the genus *Sisymbrium*.

As a result of inclusion of this new species in Brassicaceae, the number of species of the family in Turkey has reached 607.

Table 1. Accession numbers for ITS regions and locations of studied taxa in GenBank. []: Acronyms of herbarium according to Holmgren et al (1990).

	Species	Location, collectors, and herbarium	Accession no.
1	<i>Sisymbrium altissimum</i> L.	Canada (QC), Warwick BCN 2984 [DAO] Canada (SK), Jenkins 6115 [DAO] Canada (BC), Anonymous [DAO 614012] Turkey, B.Mutlu 485 [INU 1542-2014]	AF531559 AF531569 AF531571 KJ557141
2	<i>Sisymbrium austriacum</i> Jacq.	Czech Rep., Warwick BCN 3519-1B [DAO] France, Exc. de Retz No. 12-4796 [DAO] Spain, Leadlay & Petty 8 [MO]	AF531576 AF531577 AF531578
3	<i>Sisymbrium brassiciforme</i> C.A.Mey.	Russia, Rybaeb & Ruszhuka 228-4 [DAO] Kashmir, Webste & Nasir 6049 [MO]	AF531579 AF531580
4	<i>Sisymbrium burchellii</i> DC.	South Africa, Merxmuller & Giess 32557 [MO]	AF531581
5	<i>Sisymbrium capense</i> Thunb.	South Africa, Mdhlui & Lerata 29/11/1976 [DAO]	AF531582
6	<i>Sisymbrium erysimoides</i> Desf.	Algeria, Exc. de Retz No. 12-4795 [DAO] Kuwait, Bajiva 161-76 [MO]	AF531584 AF531585
7	<i>Sisymbrium heteromallum</i> C.A.Mey.	China, Al-Shehbaz, Tai & Yang 9341 [MO]	AF531586
8	<i>Sisymbrium irio</i> L.	Belgium, Warwick BCN 8159-1, [DAO] USA (AZ), Holmgren & Holmgren 6586 [DAO] Mexico (Baja): Wiggins & Thomas 370 [DAO]	AF531558 AF531567 AF531568
9	<i>Sisymbrium linifolium</i> Nutt.	USA (ID), Rollins et al. 83268 [MO]	AF531613
10	<i>Sisymbrium loeselii</i> L.	Canada (ON), Garton 12675 [DAO] Kazakistan, Al-Shehbaz 9241 [MO]	AF531573 AF531587
11	<i>Sisymbrium luteum</i> (Maxim.) O.E.Schulz	Russia, Solomon & Barkalov 19351 [MO]	AF531588
12	<i>Sisymbrium malatyanum</i> sp. nov.	Turkey, Ş.Karakuş 4187 [INU 12536-2014] Turkey, Ş.Karakuş 4187 [INU 12545-2014] Turkey, Ş.Karakuş 3919 [INU 12534-2014]	KJ557138 KJ557142 KJ557140
13	<i>Sisymbrium officinale</i> (L.) Scop.	Canada (QC), Warwick BCN 8231-4 [DAO] Canada (BC), Lindsay & Woodbury 1086 [DAO] Turkey, B.Mutlu 1904 [INU 1546-2014]	AF531557 AF531564 KJ557136
14	<i>Sisymbrium orientale</i> L.	Australia (NSW), Coveny & Dalby 12257 [DAO] USA (CA), Twisselmann 8099 [DAO] USA (CA), Pollard 26/03/1958 [DAO] Turkey, B.Mutlu 9538 [INU 1541-2014] Turkey, Ş.Karakuş 4264 [INU 1544-2014]	AF531590 AF531591 AF531592 KJ557139 KJ557143
15	<i>Sisymbrium polyceratium</i> L.	France, Gavelle 28/06/1961 [DAO]	AF531594
16	<i>Sisymbrium polymorphum</i> (Murray) Roth	Hungary, Bano 19/05/1946 [DAO] Kazakhstan, Al-Shehbaz et al. 9431 [MO]	AF531595 AF531596
17	<i>Sisymbrium septulatum</i> DC.	Turkmenistan, Yakovleva & Nikitin [DAO] Iran, Grant 15451 [MO] Turkey, B.Mutlu 9561 [INU 1540-2014]	AF531600 AF531601 KJ557137
18	<i>Sisymbrium strictissimum</i> L.	Sweden, Smith 27/07/1945 [DAO] Switzerland, Comte 3534 [MO]	AF531603 AF531653
19	<i>Sisymbrium volgense</i> M.Bieb. ex E.Fourn.	Moravia, Vicherek 05/06/1973 [DAO]	AF531608

Table 1. (Continued).

		Poland, Aniol-Kwiatkowska [DAO]	AF531609
		Russia, Sagalae s.n. 22 May 1993 [MO]	AF531610
20	<i>Neuontobotrys lanata</i> (Walp.) Al-Shehbaz Syn: <i>Sisymbrium lanatum</i> (Walp.) O.E.Schulz	Peru, Pennell 13323 [GH]	AF531651
		Peru, Hutchinson & Wright 7239 [F]	AF531652
21	<i>Polypsecadium solidagineum</i> (Triana & Planch.) Al-Shehbaz Syn: <i>Sisymbrium solidagineum</i> Triana & Planch.	Ecuador, Laegaard et al. 103012 [MO]	AF531602
22	<i>Erucastrum supinum</i> (L.) Al-Shehbaz & Warwick Syn: <i>Sisymbrium supinum</i> L.	France, Carl 1133 [DAO] Sweden, Schäffer Aug.1947 [MO]	AF531604 AF531605
23	<i>Capsella bursa-pastoris</i> (L.) Medik.	Canada (ON), Warwick BCN 3557 [DAO]	AF531561
24	<i>Neotorularia torulosa</i> (Desf.) Hedge & J. Léonard	Iran, Rechinger 40534 [W]	AF137571

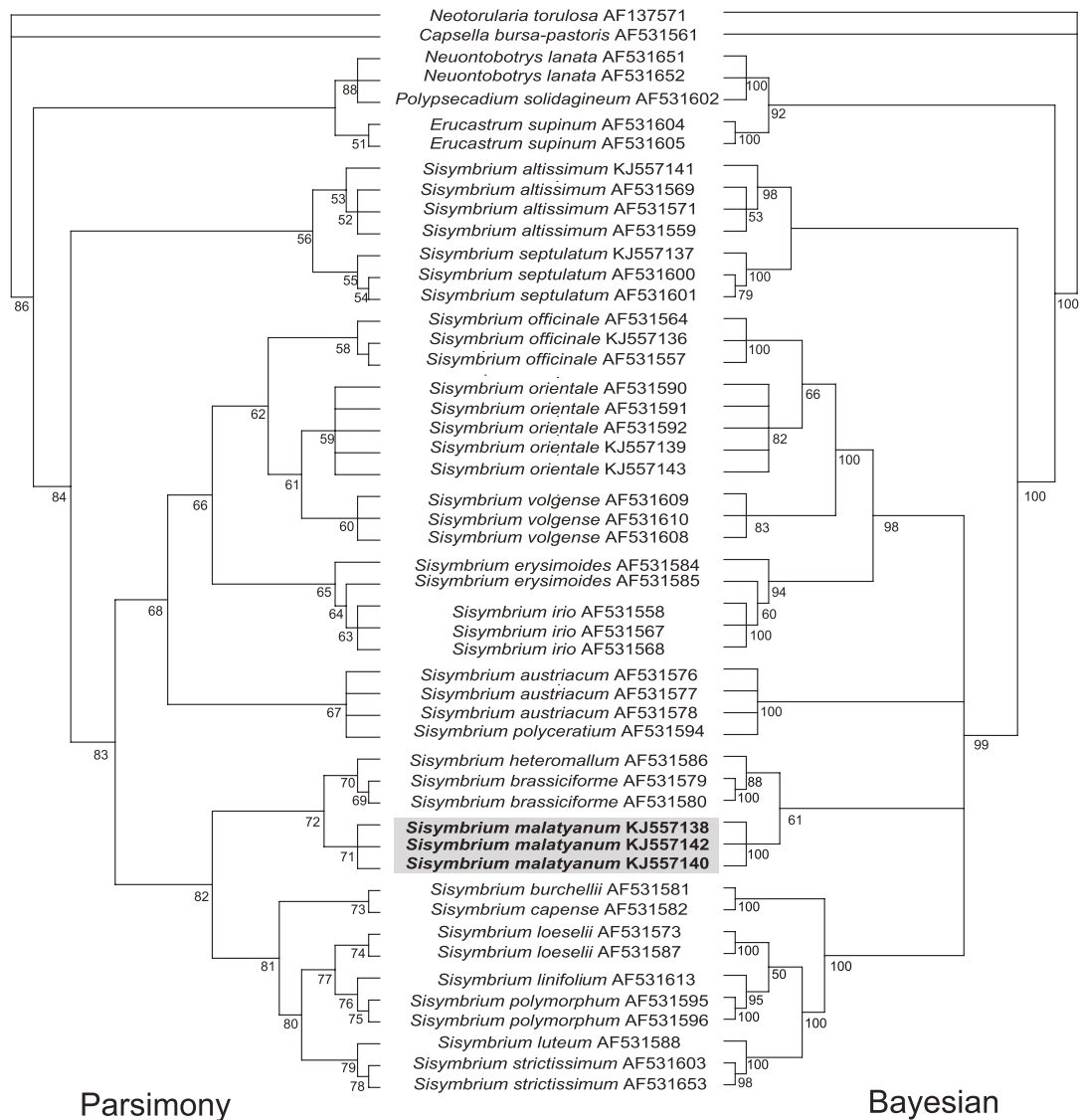


Figure 2. Most parsimonious and Bayesian inference trees (50% majority rule consensus trees) in ITS analysis for the species of *Sisymbrium*. Studied samples of the new species are indicated in bold.

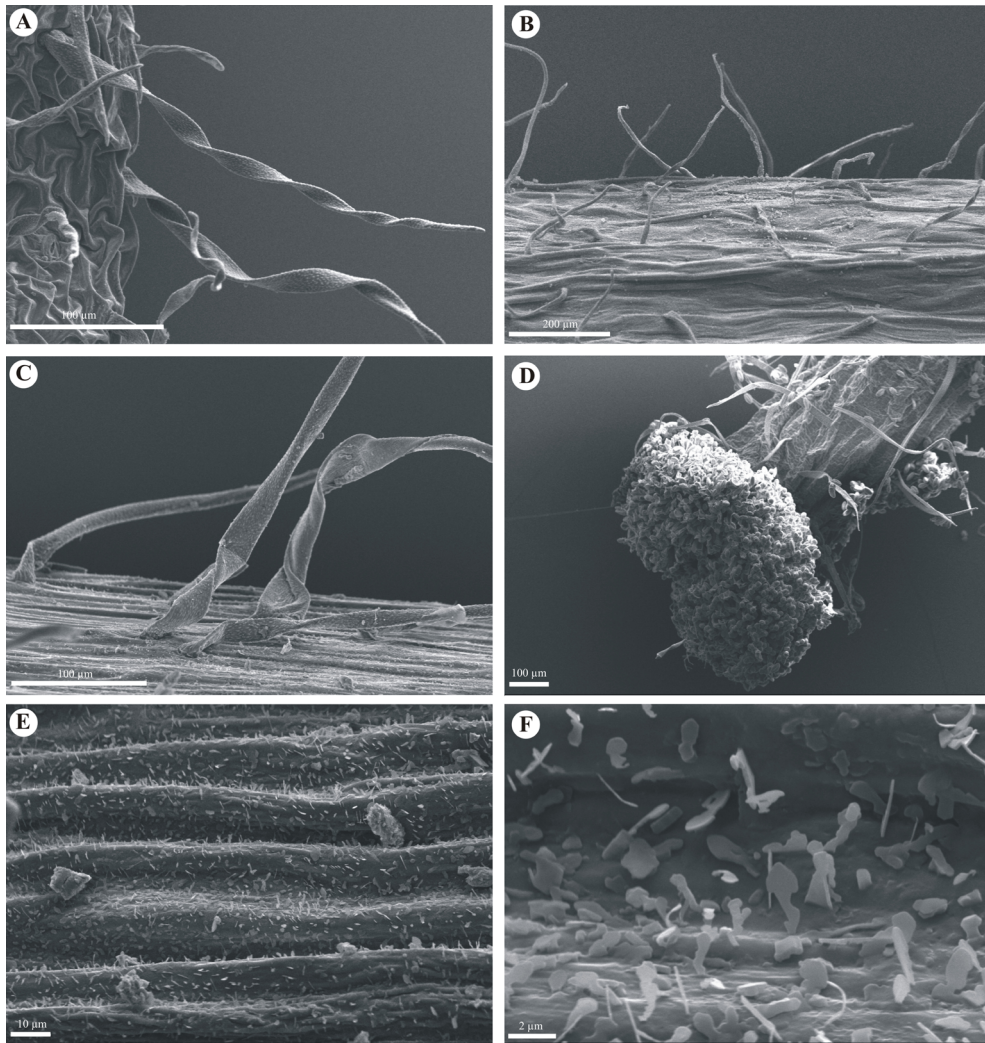


Figure 3. SEM photographs of hair, stigma, and epicuticular wax on *Sisymbrium malatyanum*: **A-** stem leaf hairs, **B-** stem hairs, **C-** fruit hairs, **D-** stigma and style hairs, **E-** epicuticular surface of fruit, **F-** wax crystalloids on epicuticular surface of fruit.

3.1. Type

Sisymbrium malatyanum Mutlu & Karakuş, **sp. nov.** (Figures 1 and 3). [Turkey] B7 Malatya: Akçadağ, Bayramuşağı village, İnikayası Hill, outside of cave, 1804 m, 09 v 2013, 38°20'158"N, 37°52'046"E, Ş.Karakuş 3919 (in flower) (holotype INU; isotypes INU, ANK, ISTE, HUB). 30 v 2013, Ş.Karakuş 4187 & B.Mutlu (in fruit) (paratype INU, ANK, ISTE, HUB).

3.2. Diagnosis

Sisymbrium malatyanum has morphological and molecular properties similar to *S. altissimum*, *S. damascenum*, *S. austriacum* subsp. *contortum*, *S. brassiciforme*, and *S. heteromallum*. The new species is mainly different from these related species by having bracteate raceme (only lower 2–3 flowers), thick pedicels (not narrower), densely

sericeous and pilose hairy (not glabrous), sepals length 6–7 mm (not 2–6 mm), and petal length 12–15 mm (not 3–12 mm). Detailed morphological differences among the related species are given in Table 2.

3.3. Description

Biennial herb (40–)50–70(–80) cm tall. Stem erect, branched below and above, hirsute with spreading hairs, trichomes 1.5–2 mm long at base. Rosette leaves not persisting. Leaves petiolate, the petioles 2–11 mm long, median leaves blade oblong in outline lyrate-pinnatisect 10–30 cm long, 2.5–11 cm wide, lateral lobes (3–)5–6 on each side of midvein, smaller than terminal, terminal lobe triangular ovate, acute, margins entire to coarsely lobed and toothed. Uppermost stem leaves smaller than lower leaves, sessile or shortly stalked. Inflorescence elongated

Table 2. Morphological differences among *Sisymbrium malatyanum*, *S. altissimum*, *S. damascenum*, *S. austriacum* subsp. *contortum*, *S. brassiciforme*, and *S. heteromallum*.

	<i>S. malatyanum</i>	<i>S. altissimum</i>	<i>S. damascenum</i>	<i>S. austriacum</i> subsp. <i>contortum</i>	<i>S. brassiciforme</i>	<i>S. heteromallum</i>
Habitus	Biennial	Annual	Annual or biennial	Annual or perennial	Annual	Annual
Fruit hairs	Pilose	Glabrous	Sparingly pilose	Glabrous or setose When young	Glabrous or pubescent	Glabrous
Seed number in fruit	60-70	90-120	?	60-78	90-150	(60-)90-160
Bract on lowermost flowers	+	-	-	-	-	-
Sepal length (mm)	6-7	4-6	2-4	2.5-3(-4)	4-6	3-4
Petal length (mm)	12-15	(5-)6-8(-10)	3-7	(4-)4.5-5(-6)	6-10(-12)	4-5
Fruit length (cm)	(5-)7-9	(4.5-)6-9(-12)	5-7	1.5-5	(5-)7-10(-12)	(5-)6-9.5(-11)
Fruiting pedicel length (mm)	7-15	(4-)6-10(-13)	6-10	4-6	4-8	5-15
Comparing fruiting pedicel thickness and mature fruit	Pedicels as thick as fruit	Pedicels as thick as fruit	Pedicels slightly narrower than fruit	Pedicels slightly narrower than fruit	Pedicels slightly narrower than fruit	Pedicels much narrower than fruit

and lax in fruit, only lowest 2 or 3 flowers bracteate. Flowers pedicellate, flowering pedicel 3–10 mm long. Sepal oblong, spreading, caducous, glabrous, narrowly scarious at margin, 6–7(–7.5) mm long, 2–2.5(–3) mm wide. Petal pale yellow, obovate, 12–15 mm long, 4–5 mm wide, rounded at apex, cuneate at base, claw slightly shorter than limb. Stamens slightly tetradynamous. Filament erect, 3.5–5 mm long, white, anthers oblong, sagittate at base, recurved at apex after dehiscence, 4–6 mm long, yellow. Style 0.5–1.5 mm long, stigma capitates emarginated, slightly 2-lobed. Ovules 60–70 per ovary. Fruiting pedicels divaricate or rarely ascending, stout, nearly as thick as fruit, 7–15 mm long. Fruit narrowly linear, terete, (5–)7–9 cm long and 0.7–1(–1.2) mm wide, pilose, valve 3-nerved. Ovules 30–35 in each loculus. Seeds pale brown.

3.4. Distribution and habitat

Sisymbrium malatyanum only grows at 1800–1850 m on the north side of limestone slopes of İnikayası Hill in Malatya Province. Recently several new species, including *Chaenorhinum semispeluncarum* H.Yıldırım, Kit Tan, S.Şenol & A.Pirhan (Yıldırım et al., 2010), *Rosa vanheurckiana* Crépin ex Boiss. var. *barbata* Tan (Tan et al., 2012), *Ornithogalum malatyanum* Mutlu (Mutlu and Karakuş, 2012), *Minuartia aksoyi* M.Koç & Hamzaoğlu (Koç et al., 2012), *Minuartia hamzaoglui* Koç & Aksoy (Koç and Aksoy, 2013), *Campanula alisan-kilincii* Yıldırım & Şenol (Yıldırım and Şenol, 2014), and *Bellevalia malatyaensis* Uzunh. & H.Duman (Uzunhisarcıklı et al., 2013), have been described from this province. The flora of the province is one of the richest in Turkey. There are 1914 taxa in the flora of the province, and 365 of them are endemic (unpublished data).

The new species grows at the type locality with *Lamium garganicum* L. subsp. *striatum* (Sm.) Hayek var. *striatum*, *Psephellus mucronifer* (DC.) Wagenitz, *Parietaria judaica* L., *Cruciata taurica* (Pall. ex Willd.) Ehrend., and *Arum elongatum* Steven. Flowering and fruiting periods of this species are early May and late June.

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3.5. Suggested conservational status

Because the known population is limited to an area of less than 10 km² (criterion of area of occupancy and number of locations is B2a) and total number of individuals is approximately 50 (criterion of very small or restricted population is D), this new species should be regarded as ‘Critically Endangered’ (CRB2a; D) according to the IUCN threat categories (IUCN Species Survival Commission, 2010).

3.6. Etymology

The epithet of the new species refers to its type locality in the province of Malatya, Turkey. The Turkish name of this species is stated in this article as “Akçadağ bülbülotu” according to Mutlu (2012) and Menemen et al. (2013).

3.7 Additional species examined

S. septulatum [Turkey]. B7 Malatya: İnönü University Campus, Science and Art Faculty, around car park, 970 m, 09 v 2005, *B.Mutlu* 9561, INU 1540-2014. – *S. altissimum* [Turkey]. B3 Isparta: Şarkikaraağaç, Kızıldağ National Park, between forest house-park entrance, openness of *Quercus* sp. forest, 1100–1250 m, 27 v 1994, *B.Mutlu* 485, INU 1542-2014. – *S. officinale* [Turkey]. A1 Tekirdağ: Around Vocational School, 450 m, 19 vi 1997, *B.Mutlu* 1904, INU 1546-2014. – *S. orientale* [Turkey]. B7 Malatya: İnderesi, XV. Regional Directorate, Ministry of Forestry and Water Affairs, Republic of Turkey, garden, 1100 m, 08 vi 2003, *Ş.Karakuş* 4264, INU 1544-2014; B7 Malatya: Gündüzbey, NW slopes of Beydağı, garden side, 1235 m, 08 v 2005, *B.Mutlu* 9538, INU 1541-2014.

Acknowledgments

This study was supported financially by the İnönü University Scientific Research Unit (BAP-Project No: 2013-49). Field studies were supported by the Republic of Turkey, Ministry of Forestry and Water Affairs, 15th Regional Directorate. The authors thank Assist Prof Dr Fatma Mutlu for assistance in molecular studies. Special thanks go to Dr Murat Özabacı (İnönü University Scientific and Technological Research Center) for SEM studies.

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