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### *Thlaspi harungalipii* (Brassicaceae), Türkiye'den Yeni Bir Tür

Mehmet Cengiz KARAİSMAİLOĞLU<sup>1\*</sup>

#### **Öne Çıkanlar:**

- Türkiye'nin güneyinden yeni bir bitki türü keşfedilmiştir.
- Yeni türün cins içerisindeki filogenetik ilişkileri tartışılmıştır.
- Yeni türün IUCN kategorisi belirlenmiştir.

#### **ÖZET:**

*Thlaspi harungalipii* sp. nova (Brassicaceae) Türkiye'nin güneyinden betimlenmiş ve resimlenmiştir. Bu takson, çiçek ve meyve karakterlerinde açıkça farklılık gösterdiği *T. violascens* ile benzerdir. Bu çalışmada, yeni türün nükleer kodlu ribozomal iç transkripsiyonlu ayırıcı bölgesine dayalı olarak cins içindeki filogenetik ilişkileri ve tohum mikromorfolojik ve anatomik karakterleri belirlenmiştir. Ayrıca, yeni türün IUCN kategorisi tartışılmış ve dağılımı haritalanmıştır.

#### **Anahtar Kelimeler:**

- Brassicaceae
- Cruciferae
- ITS
- *Thlaspi*
- Taksonomi

### *Thlaspi harungalipii* (Brassicaceae), A New Species from Turkey

#### **Highlights:**

- A new plant species has been discovered from southern Turkey.
- The phylogenetic relationships of the new species within the genus are discussed.
- The IUCN category of the new species has been determined.

#### **ABSTRACT:**

*Thlaspi harungalipii* sp. nova (Brassicaceae) is described and illustrated from Southern of Turkey. This taxon is similar to *T. violascens*, from which it clearly differs in flower and fruit characters. In this study, the seed micromorphological and anatomical characters, and phylogenetic relations within the genus based on the nuclear-encoded ribosomal internal transcribed spacer region of the new species are determined. Furthermore, the IUCN category of the new species is discussed, and its distribution is mapped.

#### **Keywords:**

- Brassicaceae
- Cruciferae
- ITS
- *Thlaspi*
- Taxonomy

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## INTRODUCTION

The genus *Thlaspi* L. sensu lato (s. lato) is one of the biggest genera of the family Brassicaceae, and is represented by 75 taxa worldwide (Appel & Al-Shehbaz, 2003). Problems with the classification of *Thlaspi* s. lato continue to persist. The 12 genera separation of *Thlaspi* s. lato was suggested (Meyer, 1973; Meyer, 1979) (*Thlaspi* F.K. Mey. sensu stricto, *Callothlaspi* F.K. Mey., *Kotschyella* F.K. Mey., *Neurotropis* (DC.) F.K. Mey., *Microthlaspi* F.K. Mey., *Noccaea* Moench, *Thlaspiceras* F.K. Mey., *Syrenopsis* Jaub. & Spach, *Atropatenia* F.K. Mey., *Vania* F.K. Mey. *Noccidium* F.K. Mey., and *Masmenia* F.K. Mey.), which was mostly based on seed-coat anatomy. As a result, only 6 taxa were stated within the genus (sensu stricto).

Meyer's classification was not accepted by many researches because of limited taxonomical use (Greuter & Raus, 1983; Greuter et al., 1986; Al-Shehbaz, 1986; Appel & Al-Shehbaz, 2003). Some molecular phylogenetic research have shown that the generic restriction in *Thlaspi* previously (Meyer, 1973) was obviously unusual (Mummenhoff & Zunk, 1991; Mummenhoff & Koch, 1994; Mummenhoff et al., 1997a; Mummenhoff et al., 1997b; Koch et al., 1998; Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004; Al-Shehbaz et al., 2006; Koch et al., 2007). As the result of family-wide molecular phylogenetic studies (Khosravi et al., 2009; Warwick et al., 2010), it was reported that 10 genera were separated from *Thlaspi* s. lato (Meyer, 1973) and added to *Noccaea*. Subsequently, these genera were reduced as the synonymy of *Noccaea* (Al-Shehbaz, 2014). Notwithstanding numerous studies on the infrageneric and interspecific taxonomy of the genus, problems with classification have not yet been clarified.

In this controversial case, most of the performed molecular phylogenetic studies were based on herbarium materials, which usually consisted of incomplete samples or a single repeatless sample, and these investigations were sufficiently deprived of fieldwork. However, the main priority in systematics has exactly followed the developmental stages of plants and a perfect diagnosis. In addition, the research performed contained few samples from Turkey, which is a diversity center for the genus. Moreover, the presence of many endemic taxa is in doubt because of the unknown ripe fruit or flowering cases in Turkey. Hence, all assessments made on the genus must be renewed in line with detailed field work, especially those in Turkey (Karaismailoğlu, 2018).

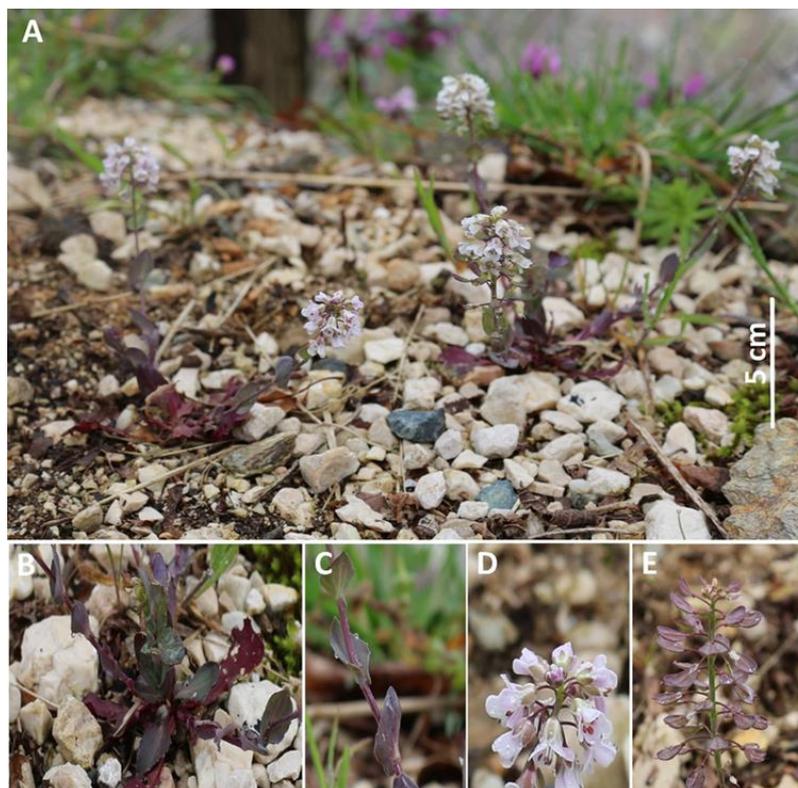
Al-Shehbaz (2014) performed a synopsis of the genus *Noccaea* having transferred many *Thlaspi* taxa to this genus. However, successive comprehensive studies on the genus in Turkey including morphological, anatomical, palynological, cytological and molecular data have showed that taxa in the sections *Nomisma*, *Thlaspi* and *Pterotropis* should be considered under the genus *Thlaspi* (Karaismailoğlu, 2018; Karaismailoğlu & Erol, 2018; Karaismailoğlu & Erol, 2019; Karaismailoğlu & Erol, 2020; Karaismailoğlu & Fidan, 2021; Karaismailoğlu et al., 2022).

In this paper, the specimens of *Thlaspi* taken from Dumanlı Mountain (Province of Osmaniye, southern Turkey) were analyzed by utilizing the extensive literature (Post, 1893; Hedge, 1965; Meyer, 1973; Meyer, 1979; Meyer, 1991; Meyer, 2001; Meyer, 2003; Meyer, 2006; Greuter & Raus, 1983; Greuter et al., 1986; Davis et al., 1988; Clapham & Akeroyd, 1993; Artelari, 2002; Al-Shehbaz, 2012; Al-Shehbaz, 2014; Özgüşi et al., 2018; Güzel et al., 2018). The investigation revealed that the specimens have unique features and are therefore suggested by the author as a new species, *T. harungalipii*.

## MATERIALS AND METHODS

The specimens were collected by the author in April 2015 and May, Jun, and July 2016-2019 during field trips to Dumanlı Mountain in the province of Osmaniye, in southern Turkey. The

specimens of the assumed new taxon were collected from 2 areas near each other and stored in the SUFAF herbarium and M.C. Karaismailoğlu collection. At first sight, due to resemblances in the overview, like the stems, basal and cauline leaves, and some flower characters, the specimens appeared to be similar to *Thlaspi violascens*. The collected specimens were compared with the key supplied by Hedge (1965) (Table 1). They were crosschecked with the related taxon (*T. violascens*) deposited in the ISTE, ISTF, ISTO, and ANK herbaria (Thiers, 2016). Images of the live material in nature were taken with a Canon EOS 650d digital camera (Figure 1).



**Figure 1.** *Thlaspi harungalipii*; A: Habitat, B: Basal leaves, C: Cauline leaves, D: Flowers, E: Siliculae and pedicels

Macromorphological examinations, such as fruit and seed features, were performed using an Olympus ZS51 stereomicroscope and Kameram Imaging Software (Figure 2).

For the micromorphological examinations, seeds obtained from the specimens were analyzed using a scanning electron microscope (JEOL Neoscope-5000) by mounting them to a table with silver adhesive, and covering them with gold (Karaismailoğlu, 2016) (Figure 3).

For the anatomical investigations, cross sections of the seeds were taken using a fully automatic microtome (Thermo Shonda Met Finesse). Next, they were passed through a variety of alcohol and xylene series, dyed with hematoxylin and Eosin-Y in a staining device (ASC 720 Medite), and covered with Entellan to observe the anatomical structures (Karaismailoğlu, 2015) (Figure 4). The anatomical characters were observed using an Olympus CX21FS1 microscope and Kameram Imaging Software.

DNA extractions, ITS region amplification and sequencing process and bioinformatic analysis of sequences of the new species have been performed according to Karaismailoğlu et al. (2022) (Sequence of the ITS region of *Thlaspi harungalipii*:  
 CTTGGTTCCAAACAGAACGACCCGAGAACGATTGATCATCACTCTCGGCGGGCCGGTTTC  
 TTATCCGATTCTGTGCCCGCCGATTCCGTGGTTTTGCGAGTGGTTCGATCAAGATTTTTAA  
 TCCTGATTGGGCTATGAGCTTAGCTTTCGGAAATTCACAAAACCACGGCACGATAAAGTG  
 TCAAGGAACATGCAACTAAACAGCCTGCTTCCGCCGCCCTGGAAACGGTGAGTGTGCGGG

ATGCTGTGCTGCGATCTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGC ATCGATGAAGAACGCAGCACAG). Total genomic DNA was acquired from leaves in accordance with the cetyltrimethylammonium bromide (CTAB) method advanced by Karaca et al. (2005). The amount of DNA was made utilizing a Thermo NanoDrop® Spectrophotometer. The ITS2 region sequences were gained from the genomic DNA was utilized as a template to intensify the ITS region with a MiniAmp Plus. Thermal Cycler device utilizing the primer pairs UniPlantF (5'-TGTGAATTGCARRATYCMG-3') and UniplantR (5'-CCCGHYTGAYYTGRGGTDC-3') (Moorhouse-Gann et al., 2018). PCR was organized in 25 µL volumes using the following reaction fundamentals: 3 µL template DNA, 11.25 µL water, 2.5 µL 10X buffer, 1 µL each of primers (50 ng µL<sup>-1</sup>), 4 µL MgCl<sub>2</sub> (2.5 mM), 1 µL dNTP mix (0.25 mM), 0.25 µL Taq DNA polymerase and 1 µL bovine serum albumin (BSA). Purification and sequencing were outsourced to Genoks. Also, the ITS sequences of 22 *Thlaspi* s.lato taxa belonging to the sections *Nomisma*, *Thlaspi* and *Pterotropis* were taken from Karaismailoğlu et al. (2022)'s results. Afterward, the first and last 30 bases were detached owing to poor quality by means of the BioEdit program (Hall, 2011) and these sequences were not included in the main analysis. The sequences were examined with the Ncbi-Blast algorithm to approve they belong to the examined material. After, it was utilized Mega X version 10.0.05 (Kumar et al., 2018) to perform phylogenetic analyses. The sequences were first loaded and then aligned with the out group, *Aethionema speciosum subsp. compactum*, which included using the base sequence, and Clustal W (Larkin et al., 2007). Bootstrap values for 1000 replicates were obtained in accordance with the maximum likelihood (ML) phylogenetic method (Figure 5).

The geographical locations of the new species were recorded by utilizing a GPS and the distribution in Turkey was mapped according to the grid system (Davis, 1965) (Figure 6). The conservation status of the new taxon was evaluated according to IUCN (2022).

## RESULTS AND DISCUSSION

### Taxonomic treatment of the new species

*Thlaspi harungalipii* KARAİSMAİLOĞLU, sp. nova.

### Type

Turkey; Osmaniye, Düziçi, Haruniye, Dumanlı Mountain, roadsides, stony slopes, 1200–1300 m, 19. iv. 2015, N 37°16.03, E 036°30.81, Karaismailoğlu 128a (holotype: SUFAF)

### Diagnosis

*Thlaspi harungalipii* clearly differs from *T. violascens* by its stem 8–20 cm tall (vs. 20–30 cm tall), sepals 1–2 mm in length (vs. 2–2.1 mm in length), petals 4–5 mm in length (vs. 3–4 mm in length), anthers reddish or violet, 0.3–0.5 mm in length (vs. black, 0.7–0.9 mm in length), pedicels spreading-descending, 4–6 mm in length (vs. spreading, 7–10 mm in length), siliculae obcordate, 6–7 mm in length (vs. obtriangular, 8–10 mm in length), apical sinus 0.5–1.5 mm in length, style exceeding sinus (vs. 1.5–2 mm in length, stilus not exceeding sinus), septums 0.5–1.5 mm in width (vs. 1.5–2 mm in width), seeds 2–4 in each loculus, ovate, 1.2–1.5 mm in length, surface clearly striped (vs. 4–5 in each loculus, elliptical, 1.5–1.7 mm in length, slightly striped).

### Description

Annual or biannual, with slender tap root, herb, single or several-stemmed, 8–20 cm, glabrous, glaucescent. Basal leaves rosette-forming, ovate-oblong, apex obtuse or acute, leaf margins dentate or cuneate, petiolate, 10–30 mm (length) × 5–12 mm (width). Caulin leaves ovate or lanceolate, amplexicaul, with 2 obtuse or acute auricles, apex acute, leaf margin entire or dentate, 8–28 mm × 5–10 mm. Inflorescence raceme or capitate-corymbose, elongating in fruit, raceme length 4–15 cm.

Sepals not saccate, ovate-oblong, apex rotund, broadly membranous-margined green or violet, 1–2 mm × 0.8–1.2 mm. Petals white, ovate or spatulate, apex rotund, 3–5 veins, with an indistinct claw and blade, 4–5.5 mm × 0.8–1.2 mm. Anthers reddish or blackish, elliptic, 0.3–0.5 mm. Filament narrow and linear, 1.2–2 mm. Stigma capitate. Ovary elliptic, 2–3 mm × 0.8–1 mm. Fruiting pedicels 4–6 mm, spreading-descending. Fruit a compressed silicula. Siliculae strongly glaucous, occasionally flushed purplish, narrowly obcordate, 6–7 mm × 3–5 mm. Fruit wings narrow, 0.5–1.5 in width, apex rotund or obtuse. Apical sinus narrow and shallow, 0.5–1.5 mm. Style exceeding sinus, 1–2.5 mm. Septum 5–7 mm × 0.5–1.5 mm. Seeds 2–4 in each loculus. Seeds ovate, brown, 1.2–1.5 mm × 0.8–1.1 mm, striped, not mucilaginous (Figure 2).



Figure 2. Fruit, seed number in loculus and seed of *Thlaspi harungalipii*

### Phenology

Flowering from March to April.

### Etymology

The name of this new species is given in honor of Harun Galip KARAİSMAİLOĞLU, who is the father of the author, retired worker, farmer, and amateur botanist.

### Seed micromorphology

Seed surface ornamentation of the new taxon is reticulate-areolate. Furthermore, seeds have a striped surface. While the anticlinal cell walls are sunken, the periclinal cell walls have a concave structure. Epidermal cells consist of polygonal cells (irregularly penta-hexagonal) (Figure 3). However, *T. violascens* is of the reticulate ornamentation, sunken anticlinal cell wall, concave periclinal cell wall and polygonal epidermal cells (Karaismailoğlu and Erol, 2018).

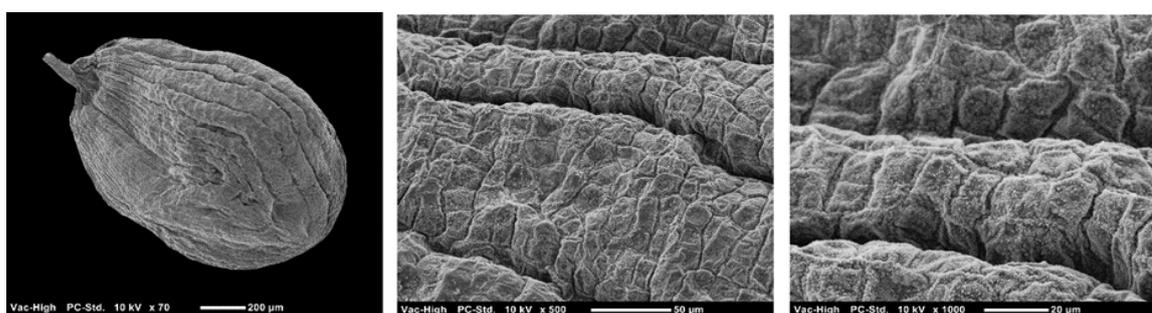
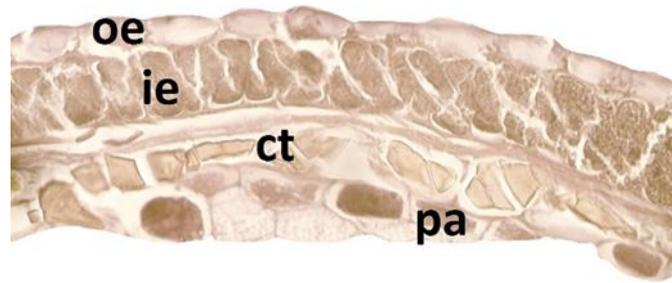


Figure 3. SEM microphotographs of the seed of *Thlaspi harungalipii*

### Seed testa anatomy

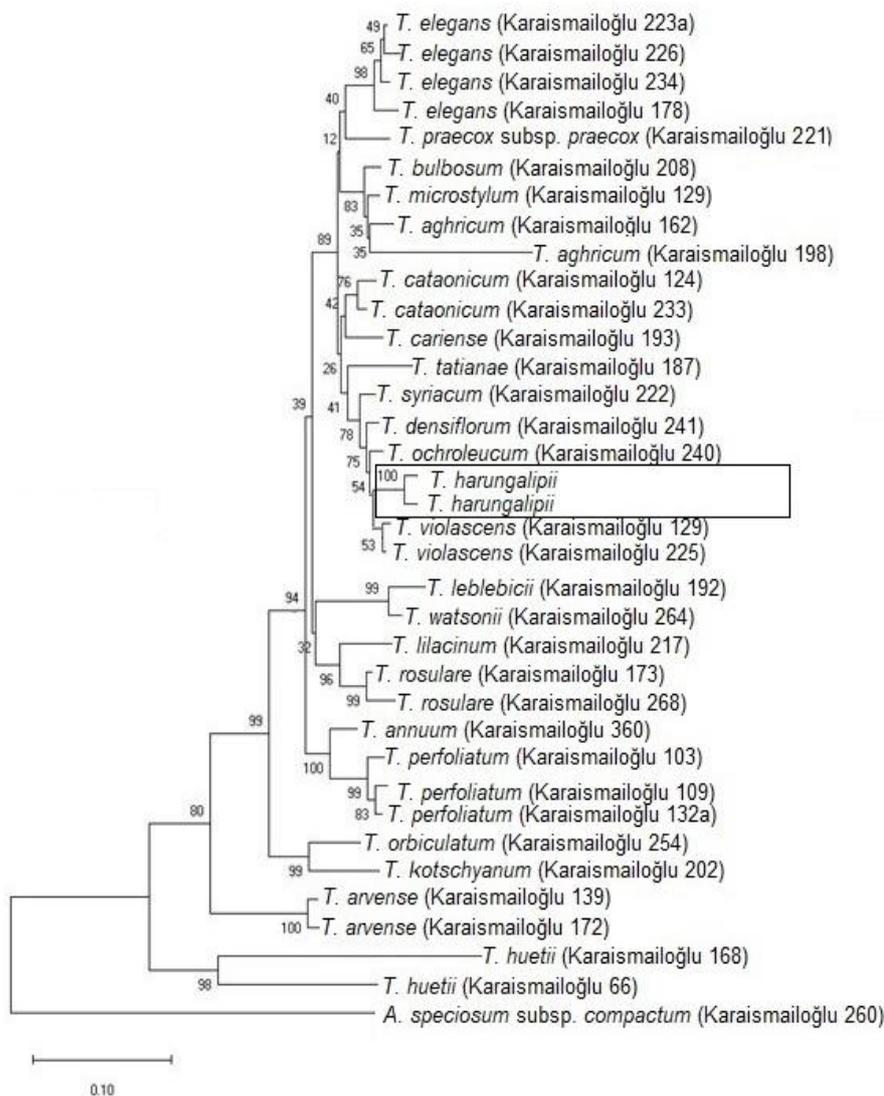
The seed coat has 2 layers, an outer testa consisting of the outer and inner epidermis layers, and an inner testa consisting of compressed tissue that contains no specialized structures like mucilage cells or protrusions (Figure 4). The outer epidermis consists of flat cells; however, cells in inner epidermis are oblong. The outer testa has a thickness of  $48.79 \pm 3.26 \mu\text{m}$ . Moreover, there is a parenchyma layer consisting of single flat cells in the innermost part.



**Figure 4.** Cross section of *Thlaspi harungalipii* seed coat (**oe**: outer epidermis, **ie**: inner epidermis, **ct**: compressed tissue, **p**: parenchyma, scale bar: 100 µm)

**Molecular phylogenetic outcomes**

The aligned ITS dataset involved 35 sequences belonging to 24 taxa and was 324 bp long, of which 95 were potentially parsimony informative. The phylogenetic backbone of the ITS tree corresponds to former phylogenetic study dealing with genus (Karaismailoğlu et al., 2022) (Figure 5).



**Figure 5.** Phylogenetic tree for representatives of the new taxon based on ITS region data. Numbers at nodes show the bootstrap values (The phylogenetic backbone of the ITS tree corresponds to former phylogenetic study dealing with genus (Karaismailoğlu et al., 2022)

All of the samples of *T. violascens* and *T. harungalipii* comprised of a monophyletic group and positioned a sister location to *T. ochroleucum*, *T. densiflorum* and *T. syriacum* concordantly with Karaismailoğlu et al. (2022) (Figure 5).

### Distribution

*Thlaspi harungalipii* shows a very limited distribution in 2 locations from Dumanlı Mountain in the province of Osmaniye (Figure 6). The species was taken from 2 localities on stony slopes and in meadows.

### Recommended IUCN category

The spread area of *Thlaspi harungalipii* is less than 2 km<sup>2</sup>. In the occurrence range, approximately 650 individuals were numbered. The grazing influences on the population were not monitored. In the direction of this information, the category of threat for *T. harungalipii* is recommended as 'Critically Endangered' (CR) [criteria B2b] (IUCN, 2022).

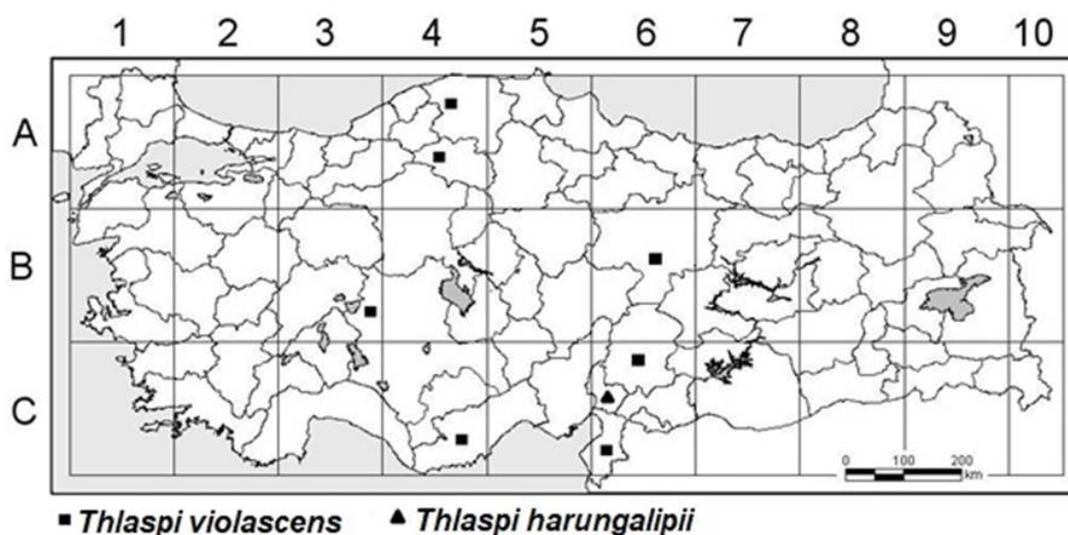


Figure 6. Dispersion map of *Thlaspi harungalipii* and closely related *T. violascens* in Turkey

The new species is placed in the genus *Thlaspi* because of its annual life form, with small herb stems, petiolate and denticulate basal leaves, auriculate cauline leaves, ascending or descending fruit pedicels, ovate or oblong sepals, white, ovate or spatulate petals, wingless filaments, obcordate silicula, style and apical sinus presence in fruit, capitate and unappendaged stigma, uniseriate, and wingless and non-mucilaginous seeds, as defined by Hedge (1965), Al-Shehbaz (2014) and Karaismailoğlu (2018).

In their studies, a similar seed testa anatomy was detected by Meyer (1979) and Aytac et al. (2006) in the genus *Thlaspi* s. lato. The outer integument consisted of 2 different epidermis layers, the outer and inner epidermis. Epidermis cells were flat, regular, simple, and of an unspecialized form in the outer layer, which most commonly originate in the basic group *N. cilicica* (Schott & Kotschy ex Boiss.) Al-Shehbaz and *N. sintenisii* F. K. Mey. (Meyer 1991).

*Thlaspi harungalipii* and *T. violascens* appear to be sister species in the maximum likelihood phylogenetic method of the ITS region (Figure 5). The ITS sequences of the samples taken from the two locations of the new taxon are exactly the same (BS value:100)

With the addition of *T. harungalipii*, the number of taxa appointed to the genus *Thlaspi* is increased to 76 species, 37 of which are distributed in Turkey. *Thlaspi harungalipii* is very different from the other species of the genus *Thlaspi*. The differences between the new species and the most similar taxon (*T. violascens*) are presented in Table 1.

**Table 1.** Diagnostic characters of *Thlaspi harungalipii* compared with the related *T. violascens*.

Characters	<i>Thlaspi harungalipii</i>	<i>Thlaspi violascens</i>
<b>Life form</b>	Annual or biannual	Annual or biannual
<b>Root</b>	Slender tap root	Swollen tap root
<b>Stem</b>	Single or several stemmed, 8-20 cm	Single or several stemmed, 20-30 cm
<b>Basal leaves</b>	Loose rosette forming	Dense rosette forming
<b>Cauline leaves</b>	Loose ovate or lanceolate	Dense ovate or lanceolate
<b>Sepals</b>	Green or violet, 1-2 mm in length	Violet, 2-2.1 mm in length
<b>Petals</b>	4-5.5 mm in length	3-4 mm in length
<b>Anters</b>	Reddish or violet, 0.3-0.5 mm in length	Black, 0.7-0.9 mm in length
<b>Pedicels</b>	Spreading-descending, 4-6 mm in length	Spreading, 7-10 mm in length
<b>Siliculae</b>	Obcordate, 6-7 mm in length, wings narrow, 0.5-1.5 mm in width, apex rotund or obtuse	Obtriangular, 8-10 mm in length, wings 1-2 mm in width, apex rotund
<b>Apical sinus</b>	Narrow and shallow, 0.5-1.5 mm in length, style exceeding sinus	Narrow, 1.5-2 mm in length, style not exceeding sinus
<b>Septums</b>	0.5-1.5 mm in width	1.5-2 mm in width
<b>Seeds</b>	(2-) 3 (-4) seeds in each loculus, ovate, 1.2-1.5 mm in length, striped	4 (-5) seeds in each loculus, elliptical, 1.5-1.7 mm in length, slightly striped

## CONCLUSION

This investigation including morphological, anatomical, and molecular data has revealed that the specimens have unique features and are therefore suggested by the author as a new species, *T. harungalipii*

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