

Comparative morphological, fruit anatomical and micromorphological studies of two *Trinia* species

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ABSTRACT

Background and Aims: The aim of the present study was to review and compare the morphological, micromorphological, and anatomical characteristics between *Trinia scabra* Boiss. & Noe and *T. glauca* (L.) Dumort.

Methods: The fruit micromorphologies were examined using scanning electron microscopy. For anatomical studies, the Paraffin method was used. Transverse sections were cut using a rotary microtome and then were stained with safranin.

Results: Expanded descriptions, phenologies, geographic distributions, and photos of the *T. scabra* and *T. glauca* are provided and the differences between the two species are discussed.

The morphological, micromorphological, and anatomical characteristics of *T. glauca* and *T. scabra* are presented. Fruit anatomy is the best discriminating characteristic between the two species, which is entirely different in each. There is one val- lecular vitta and there are two commissural vittae in *T. scabra*, but one to three vallecular vittae and one to two commissural vittae in *T. glauca*. The vittae are relatively large in *T. scabra* but small in *T. glauca*. There are no sclerenchyma cells toward the end of the ribs in *T. scabra* although they are found toward the end of the ribs in *T. glauca*. Also, the presence of *T. leiogona* C.A.Mey in Turkey has been reported by Grossgeim in *Flora Kavkaza* and confirmed our study.

Keywords: *Trinia*, morphology, Apiaceae, Turkey

INTRODUCTION

The flowering plant family Apiaceae (Umbelliferae) comprises approximately 450 genera and 3700 species (Pimenov & Leonov, 1993). It is widely distributed in the temperature zones of both northern and southern hemispheres and exhibits great diversity in Central Asia (She et al., 2005). The family consists of 105 genera and around 493 species in Turkey.

Trinia Hoffm. is distributed throughout Europe and southwest Asia and comprises approximately 10 species. The genus was re- vised by Hedge & Lamond (1972) for *Flora of Turkey and the East Aegean Islands*, in which *T. glauca* (L.) Dumort and *T. scabra* Boiss. & Noë, were accepted as two species. Despite that *Trinia* is a small genus, there is considerable confusion about its taxonomy and nomenclature at inter- and intra-generic levels (Wolff, 1910; Uribe-Echebarría, 1990). The high variability in some of the most important characteristics that define and characterize the species are factors for intrageneric confusion. It is classically accepted that *Trinia* species contain unisexual flowers (dioecious or rarely monoecious plants) (Cannon, 1968), although this characteristic is still being studied, because hermaphrodite flowers have occasionally been observed, which could lead researchers to consider that some species in this genus are polygamous (Ferrer-Gallego et al., 2013; Gómez-Navarro et al., 2015). In addition, the height, branching system, number and length of rays, and length of leaf lobes vary considerably between male and female plants of the same species (Hedge & Lamond, 1972).

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Trinia was established by Hoffm. in 1814 as a genus that is distinct from *Rumia*. *Trinia* and *Rumia* Hoffm. were repeatedly subjected to revisions and many researchers transferred species from one genus to the other or reunited (Fedoronchuk, 1978). In *Flora Altaica* (Ledebour, Meyer, & Bunge, 1829), *R. seseloides*, described by Hoffmann, was transferred into *Trinia*; therefore, *Trinia* and *Rumia* were combined as one genus. In *Prodromus Systematis Naturalis* (De Candolle, 1830), *Trinia* and *Rumia* were classified in tribe Ammineae as separate genera. Later in *Flora Rossica* (1844), Ledebour not only separated these genera, but placed them to different tribes. *Trinia* was placed in tribe Ammineae and *Rumia* was placed tribe Seselineae. Boissier (1872), following De Candolle (1830), evaluated two genera in tribe Ammineae. The results of the study by Ajani et al. (2008) showed that *T. hispida* Hoffm. was very closely related to members of tribe Selineae and Downie et al. (2010) confirmed these positions and considered *Rumia* to be a separate genus.

In the current study, we contribute to the knowledge about *T. scabra* and *T. glauca*, and the presence of *T. leiogona* in Turkey is confirmed. In this paper, the macro- and micromorphological properties of the fruits were investigated. A detailed anatomical description of the cross sections of the mature fruits is provided. An expanded description of the species is also provided.

MATERIALS AND METHODS

Samples of *Trinia scabra* and *T. glauca* were collected from different localities in Turkey (Appendix 1). Plant samples were identified according to Shishkin (1951), Canon (1968), Hedge and Lamond (1972), Rechinger (1987), Güner et al. (2012). The abbreviations of the authors of plant names were checked from Brummitt and Powell (1992).

The fruits were directly mounted on the prepared stubs and coated with gold for SEM studies. Photographs were taken with a Zeiss LS-10 after coating with a Polaron SC7620 sputter coater.

Mature fruits were kept in 70% ethanol. Each mericarp was rehydrated and placed in formalin-acetic acid- alcohol (1:1:8) for a minimum of 24 h. Rehydrated materials were embedded into paraffin blocks following the traditional paraffin section method. A transverse section about 5-10 μm thick was cut using a Thermo microtome and stained with a safranin solution. Micrographs were taken using a Nikon light microscope.

RESULTS AND DISCUSSION

Trinia scabra Boiss. & Noe

Erect biennial with much branched, herbs. Root fusiform, 6-15 mm in diam., covered with densely fibrous collar at the base. Stem 15-35 cm, puberulous in the lower part and sparsely puberulous in the upper part, ribbed, partly glaucous. Basal leaves 2-pinnate, ovate-lanceolate, 4-20 x 2-6 cm, petiole with broadly membranous sheathing base, terminal segments linear-lanceolate, 2-12 mm long, acute to acuminate at apex, margins and veins usually scabrid. Median and upper cauline leaves gradually smaller towards flowering part; terminal segments mostly linear, up to 25 mm long. Umbel compound,

rarely simple, 3-8 rayed, 0.3-4.5 (-5) cm long, very unequal, ribbed, 0.5-0.8 mm thick, usually glabrous. Bracts 0-3, unequal, 0.9-7.5 x c. 0.5 mm, simple, rarely divided into linear lobes. Bracteoles 1-3, \pm linear, unequal, up to 6 mm. Umbellula (4-7)-15 flowered, 5-10 mm in diam, pedicels 0-6 (-10) mm, unequal on female plants, \pm equal on the male. Sepal obsolete, petals white, incurved at apex, c. 0.6 mm long, ovate. Fruit broadly oblong-ovoid, laterally compressed. Mericarp oblong-elliptic, 2.8-3.2 x 1.2- 1.8 mm, scabrid. Stylopodium depressed. Stylus short, recurved, flat (Figure 1 and 2).

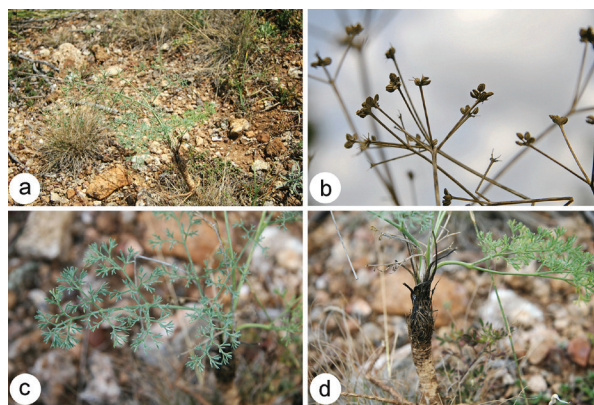


Figure 1. *Trinia scabra* and a- habitus, b- fruit, c- basal leaves, d- root.



Figure 2. *Trinia scabra*. a- b- fruit, c- stem, d- petiolule, e- leaf, f- ultimate segment.

Phenology: Flowering time from June to July.

Distribution: This taxon is known only from Anatolia. It is endemic to Turkey.

Phytogeographic region: Irano-Turanian

***Trinia glauca* (L.) Dumort**

Perennial with much-branched, ± glaucous. Root fusiform, 8-15 mm in diam., covered with densely fibrous collar at the base. Stem 15-30(-50) cm, angled, glabrous, ribbed. Lower leaves 2-3-pinnate, petiole with sheathing base, terminal segments linear, acute, glabrous or rarely margins and veins scabrid. Median and upper cauline leaves gradually smaller towards the flowering part; terminal segments mostly linear, 3-20(-35) mm. Umbel (3-)4-8(10) rayed, (5-) 8-30 mm long, subequal, ribbed, glabrous. Bracts 0-1, entire or trifid. Bracteoles absent or few, ± linear, up to 1-2 mm. Umbellula 3-8, peduncles 1-7 mm, unequal on female plants. Sepal obsolete, petals white, incurved at apex. Fruit elliptic to oblong, glabrous, 2-3(-3.3) x (1-)1.5-2 mm., slightly laterally compressed. Stylopodium short conical, undulate at margin. Stylus short, recurved, 0.5-0.8 mm long (Figure 3 and 4).



Figure 3. *Trinia glauca*. **a-** habitus, **b-** fruit, **c-** basal leaves, **d-** root.



Figure 4. *Trinia glauca*. **a-** umbellule, **b-** fruit, **c-** bracteole, **d-** stem, **e-** leaf, **f-** ultimate segment.

Phenology: Flowering time from June to July.

Distribution: West & South Europe, northwards of S. England.

Micromorphological results

The fruit of *Trinia scabra* is broadly oblong. It is composed of two equal mericarps which easily split open at maturity. Mericarps are oblong-elliptic and 2.8-3.2 x 1.2-1.8 mm, somewhat laterally compressed. Each mericarp has five strongly projecting primary ribs, all ribs nearly equidistant each other, with four deep valleculae. Stylus short, recurved, flat and 0.8-1.2 mm long. The ornamentation of the fruit surface is striate. The pericarp surface is covered with hairs (Figure 5).

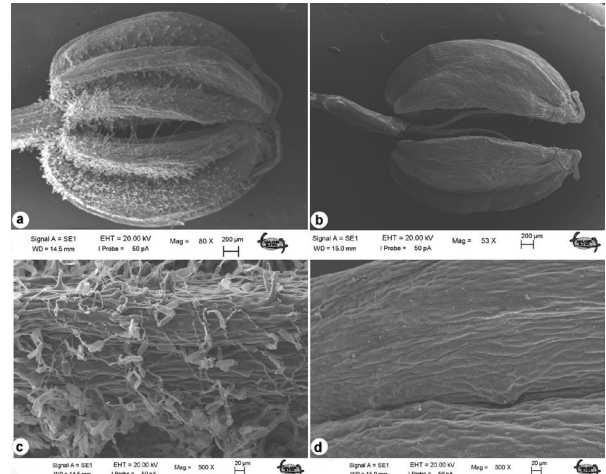


Figure 5. SEM photos of fruits of *Trinia scabra* **a-** general shape of fruit, **c-** surface details, *T. glauca* **b-** general shape of fruit, **d-** surface details.

The fruit of *Trinia glauca* is elliptic to oblong, glabrous, 2-3 x (1-)1.5-2 mm. It is composed of two equal mericarps that easily split open at maturity. The mericarps are ovoid to oblong, and somewhat laterally compressed. Each mericarp has five strongly projecting primary ribs, with the three dorsal ribs wider and closer to each other than the others, and the two marginal ribs somewhat narrower with four deep valleculae. The stylopodium is short conical, and undulate at the margin. The stylus is short (0.5-0.8 mm long), and recurved. The pericarp surface contains elongated rectangular-like or irregularly shaped pentagonal cells. Fine striations can be seen on the cell surfaces (Figure 5).

Anatomical results

The mericarps of *T. scabra* are nearly semi-circular in the transverse section. They have five strongly projecting ribs. Secondary ribs are absent. The ribs are 0.4-0.6 mm long in depth. The width of furrows are 0.15-0.30 mm, they slightly narrow at the base and top of the fruit. The exocarp is a single layer that consists of rectangular-like cells (10-30 x 5-12 µm) with a thickened outer wall covered with a thick, scaly, or nearly smooth cuticle layer. The exocarp layer is interrupted at the base of the marginal ribs from the commissural side. The mericarp consists of multi-rowed parenchymatous and sclerenchymatous cells. The rib ducts within the ribs are surrounded by mericarp cells and a vascular bundle. They are round, 120-250 µm in diameter, and occupy nearly the entire rib cavity. The vittae are relatively large (150-200 x 60-100 µm), lined with thin-walled epithelial cells, and some what destroyed in the mature fruit. There is one vallecular vitta and there are two commissural vit-

tae. There are two vascular bundles on both sides of the base of the rib duct. The endocarp is 20–30 μm thick and composed of two to three layers of thin-walled cells that are heavily elongated in a longitudinal direction. The seed coat adheres to the inner part of the pericarp. The endosperm on the transverse section is oval and slightly impressed into the commissural side. Endosperm cells are large and filled with dense granular contents (Figure 6).

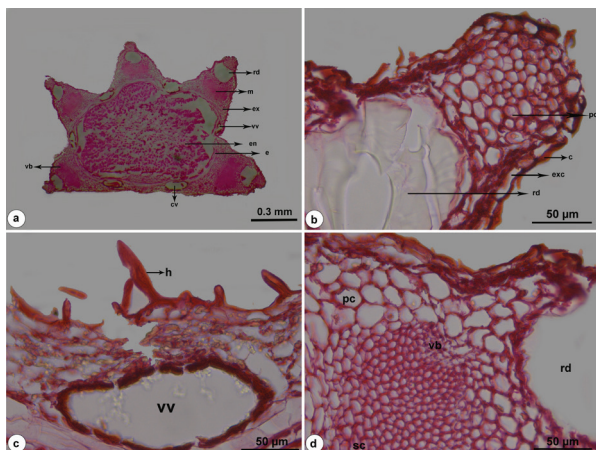


Figure 6. Fruit anatomy of *Trinia scabra* **a-** mericarp; **b-** dorsal rib area; **c-** vallecular view; **d-** the view of the vascular bundle and rib duct. (rd-rib duct, m-mesocarp, e-endocarp, ex-exocarp, cv-commissural vitta, vv-vallecular vitta, vb-vascular bundles, en-endosperm, pc-parenchymatous cells, sc-sclerenchyma cells, exc-exocarpcell, c-cuticle, h-hair, s-seed coat, enc-endosperm cell with glandular content).

In the transverse section, mericarps of *T. glauca* are nearly semi-circular. They have five distinctly angled and strongly projecting ribs. Secondary ribs are very rare. Ribs are 0.1- 0.2 mm long in depth. The furrows are wide, 0.18-0.40 mm, they narrow at the base and top of the fruit. The exocarp is a single layer. It consists of rectangular-like cells (5-20x5-12 μm) with a strongly thickened outer wall covered with a thick, weakly scaly or almost smooth cuticle layer. At the base of the marginal ribs from the commissural side, the exocarp layer is interrupted. The mericarp consists of multi-rowed parenchymatous and sclerenchymatous cells. Rib ducts are located into the ribs and surrounded by mericarp cells and vascular bundle. They are large, elliptic or round (150-320 x 70-260 μm) and occupy almost the entire cavity of the rib. Mesocarp tissue under the rib ducts is formed 4-7 layer sclerenchyma cells, which at the base of the ribs gradually turns into oblong cells of a larger diameter with thickened walls. Also, there are several layer sclerenchyma cells towards the end of ribs. Vittae are small, lined with thin-walled epithelial cells, often destroyed in a mature fruit. The number of the vallecular vittae is 1-3 and the number of the commissural vittae is 1-2. There are two vascular bundles which are collateral type located on both sides of the base of the rib duct. Endocarp is 30-45 μm in thickness. It is composed of 2-3 layers of thin-walled cells, strongly elongated in the longitudinal direction. The seed coat is closely adherent to the inner part of the pericarp and is represented by an external epidermal layer well preserved in the mature fruit, the cells of

which are thin-walled, strongly elongated in the longitudinal direction. Between this layer and the endosperm there is a thin layer of lysed cells. Endosperm on the transverse section has an oval shape, slightly impressed in the commissural side. Endosperm cells are large, densely filled with granular contents (Figure 7).

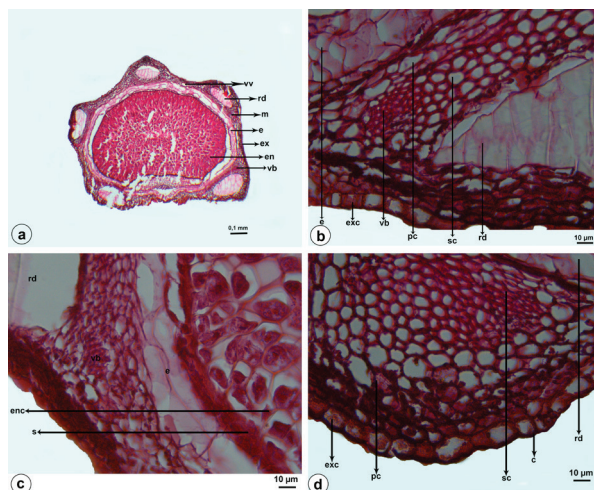


Figure 7. Fruit anatomy of *Trinia glauca* **a-** mericarp; **b-** pericarp view; **c-** marginal view of mericarp; **d-** dorsal rib area.

DISCUSSION

Trinia comprises approximately 10 species that are distributed throughout Europe and southwest Asia. In *Flora of the U.S.S.R.*, eight *Trinia* species were divided into two sections—*Leptopus* and *Pachypus*—based on pedicel thickness (Shishkin, 1951).

The genus was revised by Hedge & Lamond (1972) for *Flora of Turkey and the East Aegean Islands*, in which *T. glauca* (L.) Dumort and *T. scabra* Boiss. & Noë, were accepted as two species. In *Flora Kavkaza* (1967), Grossheim noted that *T. leiogona* was collected from two different localities (A8 Erzurum and A9 Kars) in Turkey. The presence of the species in Turkey is confirmed here.

Fruit and inflorescence characteristics are considered to be the most important features for identifying *Trinia* species. The presence or absence of bracteoles, glabrous or scabrous fruit, rib shape, and the pedicel thickness in the fruit are important diagnostic features and are used to determine species limits.

Fedoronchuk (1978) divided the genus *Trinia* into 2 subgenera (subgenus *Trinia* and subgenus *Triniella*) based on primary and secondary ribs, leaf petiole anatomy, micromorphology of pollen grains, chromosome numbers. Subgenus *Triniella* included two species,—*T. daleshampii* (Ten.) Janchen and *T. guicciardii* (Boiss. & Heldr.) Drude. Subgenus *Trinia* was divided into three sections—*Pachypus*, *Trinia*, and *Levigatus*.

Trinia glauca is distributed from Europe to western Asia. The northwestern and western parts of Turkey and Crimea form the species' eastern distribution boundary. *Trinia glauca* is known only from the Karabük, Bursa, and Kırklareli regions of Turkey.

According to Hedge & Lamond (1972), *T. glauca* is distinguished from *T. scabra* by its one to three bracteoles and scabrid leaf veins and margins; however, it appears that these characteristics might not be suitable for distinguishing these species. The presence of bracteoles in *T. glauca* was mentioned by Jury (2003) and Aizpuru et al. (1999) has mentioned that its leaf margin is scabrid. Fruit anatomy is the best discriminating characteristic between the two species, which is entirely different in each. There is one vallecular vitta and there are two commissural vittae in *T. scabra*, but one to three vallecular vittae and one to two commissural vittae in *T. glauca*. The vittae are relatively large in *T. scabra* but small in *T. glauca*. There are no sclerenchyma cells toward the end of the ribs in *T. scabra* although they are found toward the end of the ribs in *T. glauca*.

Trinia scabra was described by Boissier & Noë (1856), who considered *T. scabra* and *T. leiogona* to be two separate species. (Boissier, 1856). Since its description (Boissier, 1856), a detailed

study on it had never been conducted. Moreover, the description in the protologue is short and contains no information about fruit; therefore, species delimitations are very difficult or even impossible. In *Flora Orientalis*, the fruit of *T. scabra* was described as "fructus junioris asperuli tandem glabrio vati jugis crassiselevatis obtusis, valleculis late univittatis". The stem was described "pruinoso-scabra" in both the protologue and *Flora Orientalis*. Hedge & Lamond (1972) provided an expanded description of *T. scabra* and noted that the fruit of the species is glabrous or scabrid, and the stem is puberulous or glabrous. In this description, *T. leiogona* and *T. scabra* were evaluated as the same species, therefore, this description is artificial.

Trinia leiogona has a limited geographic distribution that comprises Iran, Armenia, Azerbaijan, south Russia, and northeast Turkey. The west shore of the Caspian Sea is the northern distribution boundary and south Russia and northeast Turkey comprise the western distribution boundary of this species.

Table 1. Comparison of the morphological features of the *Trinia glauca*, *T. hispida*, *T. leiogona* and *T. scabra*

| Species Characters | <i>Trinia hispida</i> | <i>T. leiogona</i> | <i>T. scabra</i> | <i>T. glauca</i> |
|-----------------------------|---|--|--|---|
| Stem | much-branched, up to 35 cm | single, 10-50 cm high | much branched, 15-35 cm | much branched, 15-25(-50) cm. |
| Stem hairy | glabrous to hispid with short stiff hairs hairy or sub-glabrous | very short scabrous-hairy or glabrous | densely puberulous in the lower part and sparsely puberulous in the upper part | glabrous |
| Lower leaves | 10-20 cm long, bi- or tripinnate | 8-12 x 4-6 cm, ovato-oblonga, tripinnate | 4-20 x 2-6 cm, ovate-lanceolate, 2-pinnate | 2- to 3-pinnate |
| Umbel | up to 10 rayed (male plants) and up to 9 markedly unequally (female plants) | unequal, 5-7 rayed | very unequal, 3-11 rayed | subequal, 4-8(10) rayed |
| Bracts and bracteole | bracteole absent | bracts and bracteole 0-1 | bracts 0-3, bracteoles 1-3 | bracts 0-1, trifid or simple, bracteole 0-5 |
| Umbellula | 9-20 | 7-11, 2-19 mm long | (4) 7-18 | 3-8, (5-) 8-30 mm long |
| Fruit size | 3-4 x 2.5-3 mm | 3,5-4x 2-2.5 mm | 2.8-3.2 x 1.2- 1.8 mm | 2-3,3 x (1-) 1.5-2 mm |
| Fruit shape | broadly ovoid | ovoid, ovoid-oblong | broadly oblong | elliptic to oblong |
| Fruit hairs | nearly always covered with short stiff hairs | glabrous | scabrid | glabrous |
| Peduncle | unequal, (1-2)- 4 mm long | very unequal, 1-19 mm long, thickened in fruit | unequal (female plants), ± equal (male plants), 0-10 (-15) mm, | unequal on female plants, ± equal (male plants), 1-7 mm |
| Fruit surface ornamentation | - | - | striate | contains elongated rectangular-like and irregularly shaped pentagonal cells. Fine striations can be seen on the cell surfaces |
| Stylus length | - | - | 0.8-1.2 mm long | 0.5-0.8 mm long |
| Secondary ribs | - | - | absent | very rarely |
| Vallecular vitta | - | - | large, one vallecular vitta | small often destroyed in a mature fruit 1-3 vallecular vittae |
| Commissural vittae | - | - | two commissural vittae | 1-2 commissural vittae |

According to Fedoronchuk (1979) and Shner, Pimenov, & Kljuykov (in Kamari, Blanche, & Garbari 2004) *T. scabra* and *T. leiogona* are synonyms, the latter name having priority in species ranking; however, Hedge & Lamond (1972) noted that the nomenclature of *T. scabra* should be investigated together with that of the Caucasian *T. leiogona*, which differs from *T. scabra* by its absence of bracteoles and indumentum. The presence of scabrid hairs on the fruit of *T. scabra* suggests that the species is more closely related to *T. hispida* than to *T. leiogona*. According to the regional flora, the presence of bracteoles in *T. scabra* separate the species from *T. hispida* and *T. leiogona*. According to the description in *Flora of U.S.S.R.*, both the bracts and bracteoles in *T. hispida* are absent, however, according to the description in *European Flora*, only the bracteoles are absent. Bracteoles are also absent in *T. leiogona* according to descriptions in both publications. When we examined images of *T. leiogona* from the Moscow Herbarium (MW0698699) and the specimens collected from Turkey, we noted that some specimens of *T. leiogona* had both bracts and bracteoles. The fruits of *T. scabra*, *T. leiogona*, and *T. hispida* also have a similar anatomical structure, making them unsuitable characteristics for separating this complex. Determination of intraspecific variations of *T. hispida* and *T. leiogona* is a critical consideration for defining a synonym for these species. A comparison of the morphological features of *T. glauca*, *T. hispida*, *T. leiogona*, and *T. scabra* is presented in Table 1.

The morphological, micromorphological, and anatomical results from this study clearly show that enough differences exist between *T. glauca* and *T. scabra* and support their treatment as distinct species. Our findings indicate that *T. scabra* differs from *T. leiogona*, therefore, this should be accepted at species ranking. Nonetheless, further studies, including compilations of morphological and micromorphological data, as well as new sequences of taxa related to *Trinia*, such as *T. hispida* and *T. leiogona*, will shed new light on refining the taxonomic relationships among all these species of concern.

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Conflict of Interest: The authors have no conflict of interest to declare.

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APPENDIX 1. Examined species

Trinia glauca

A4 Karabük: Keltepe, 1900 m, 11.09.1997, steppe, N.A. 3058 & A. Kaya (GAZI); Karaağaç, Keltepe, 1855 m., 21.07.2018, stony steppe, 41°05'20"N, 32°27'40"E, M.Çelik 533.

Spain: Valladolid: Tiedra, 08.06.1981 (MA 310705); Valladolid: Encinas de Esgueva monte de encinas, 12.06.1981, F. Alonso 478JF (MA 424183); Valladolid: Canillas de Esgueva, 22.06.1984 (MA 311202); Valensia S de Enguera, XJ81, 900 m, matorrales calciolas, 12.06.1984, G. Mateo, R. Figuerola sn. (MA 426567); Leon: Puente Orugo, 30TTN5060, en grietas de roca caliza, 01.07.1983, E. Bayon, S. Castroviejo 8594, P. Galan & G. Nieto (MA 426305).

Trinia scabra

B3 Eskişehir: İnönü, from İnönü to Kütahya, 8. km, 990 m, 02.07.2015, rocky slopes and open *Quercus*, 39°47'45"N, 30°12'56"E, M.Çelik 379; ibid. 22.07.2018, M.Çelik 534 & Ö.Çetin.

Trinia leiogona

B5 Nevşehir: Zelve, Akdağ, 1280-1300 m, 19.06.1989, *Thymus* steppe, M.Vural 5233 & et al.

A9 Kars: Arpaçay, from Doğruyol to Çanaksu, c. 1 km, 2008 m., 04.08.2018, rocky steppe, 41°03'25.81"K- 43°18'59.74"D, M.Çelik 535.

A9 Kars: Arpaçay, Karakale, c. 2 km north of the Karakale, 2048 m., 04.08.2018, rocky steppe, 40°53'13.94"K- 43°27'13.44"D, M.Çelik 536.