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MEHTAP TEKŞEN

SEHER KARAMAN ERKUL

HAYRİ DUMAN

MEVLÜDE ALEV ATEŞ

MEHMET SAĞIROĞLU

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Tripleurospermum eskilensis (Asteraceae): a new halophytic species from Central Anatolia, Turkey

Mehtap TEKŞEN[1,*], Seher KARAMAN ERKUL[1], Hayri DUMAN[2], Mevlüde Alev ATEŞ[3], Mehmet SAĞIROĞLU[4]

[1]Department of Biology, Faculty of Science and Letters, Aksaray University, Aksaray, Turkey
[2]Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey
[3]Department of Agricultural Biotechnology, Faculty of Agriculture, Kırşehir Ahi Evran University, Kırşehir, Turkey
[4]Department of Biology, Faculty of Science and Letters, Sakarya University, Sakarya, Turkey

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Abstract: In the present study, a new species of the genus, *Tripleurospermum eskilensis* (Asteraceae), is described from Central Anatolia, Turkey. The new species has been collected from Eskil region in a restricted area (Aksaray Province). It grows in salty steppe, and is closely related to *T. decipiens* in terms of morphological characteristics. It has also been morphologically compared with discoid and disciform capitulated species in Turkey. This study includes its detailed description, diagnostic characters, original photographs, geographical distribution, habitat and ecology, conservation status, and identification key. The comparison of *Tripleurospermum eskilensis* with closely related species was conducted in terms of morphological characteristics, pollen, achene morphology and anatomy, stomatal length, and molecular phylogenetic analysis.

Key words: *Tripleurospermum*, morphological characters, phylogenetic analysis, Turkey

1. Introduction

Tripleurospermum Sch.Bip. belongs to the taxonomically complex tribe Anthemideae (Asteraceae), whose subtribal classification has not been fully resolved (Oberprieler et al., 2007). It spreads throughout Europe, North Africa, temperate Asia, and North America, and it is represented by about 40 species (Oberprieler et al., 2007; İnceer et al., 2012, 2018; Plants of the World Online (POWO), 2021; Özbek and Onaylı, 2020; İnceer, 2021).

The diversity center of *Tripleurospermum* is Turkey (İnceer et al., 2018; POWO, 2021). According to "Flora of Turkey and the East Aegean Islands", 24 species (26 taxa) are distributed in Turkey (Enayet Hossain, 1975). Subsequently, four new taxa have been described from Turkey, and two new records have been added, which increased the number of *Tripleurospermum* taxa in Turkey to 32 (İnceer and Beyazoğlu, 2004; Yıldırımlı, 2010; İnceer and Hayırlıoğlu-Ayaz, 2008, 2010, 2014; İnceer, 2012; Özbek and Onaylı, 2020). The Russian flora includes 17 species (Pobedimova, 2000), and flora of Iran includes six species (Podlech, 1986).

Tripleurospermum is often taxonomically confused due to its close morphological relationships with other related genera within Anthemidae such as *Anthemis* L. and *Matricaria* L. (Kay, 1976; İnceer et al., 2018). Many studies were made on *Tripleurospermum* in terms of pollen morphology (İnceoğlu and Karamustafa, 1977; Çeter et al., 2013), fruit morphology (Skilbeck et al., 2019; İnceer et al., 2012; Özbek and Onaylı, 2020), fruit (İnceer et al., 2012, İnceer and Özcan, 2021) and leaf anatomy (İnceer and Özcan, 2011, 2021), cytogenetic (İnceer and Hayırlıoğlu-Ayaz, 2010; İnceer and Beyazoğlu, 2004), and phylogenetic features (İnceer et al., 2018), and they were useful and reliable in solving some of the taxonomic problems of Anthemidae tribe.

Achene characterization is a very important criterion used in the distinction of *Tripleurospermum* species as well as those in Asteraceae family. Important taxonomic characteristics are achene size, shape, corona structure, number of ribs, thickness and width of lateral and adaxial ribs, and presence or absence of slime cells (Enayet Hossain, 1975; İnceer et al., 2012; Skilbeck et al., 2019; İnceer and Özcan, 2021). Enayet Hossain (1975), who conducted one of the few studies on the achene morphology of *Tripleurospermum* species, has provided a detailed morphology of the achene characteristics. İnceer et al. (2012) made multivariate analyses using the morphological and anatomical characteristics of the

* Correspondence: m_teksen@yahoo.com

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achene in 12 species, and Skilbeck et al. (2019) contributed to the morphological data of the achene on four species. In addition, İnceer and Özcan (2021) examined the achene anatomy of 15 taxa.

Similar morphological characteristics in species included in the same genus complicate the identification process. Therefore, DNA barcoding method has become an important tool with universal markers in the identification of species (Zhang and Jiang, 2019). Especially nuclear ribosomal DNA (nrDNA) internal transcribed spacer region (ITS) is recently used for such purposes. Entities new to science emerged based on the combination of both molecular and morphological data (Kress et al., 2005; Kress, 2017; Chen et al., 2010; China Plant BOL Working Group, 2011). Recently, İnceer et al. (2018) conducted a molecular study on *Tripleurospermum* by using ITS and ETS regions. Their results support species diversifications by using both phylogenetic relationships and genome size variations among the genus *Tripleurospermum.* This pesent study is the first and the unique molecular study carried out on the Turkish native species that belongs to this genus.

During the field studies in salty habitats around Salt Lake, an interesting *Tripleurospermum* species was found as it attracted attention with the succulent leaves in all the individuals of the population. The succulent leaf characteristic has continued in all individuals observed over the years since 2016. Although it is noteworthy that the individuals are especially similar to the species with discoid and disciform capitula (*T. decipiens* (Fisch. & C.A.Mey.) Bornm., *T. disciforme* (C.A.Mey.) Sch.Bip., *T. fissurale* (Sosn.) E.Hossain, *T. microcephalum* (Boiss.) Bornm., and *T. tempskyanum* (Freyn & Sint.) Hayek), the detailed examinations determined that *Tripleurospermum* species was different from those species with its leaf, flower, achene characteristics and that the individuals did not belong to any known *Tripleurospermum* taxa. Our purposes in the present study are 1) to analyse morphological, micromorhological, and anatomical characteristics of these individuals, 2) to compare these features with the related species, 3) to describe these plants as a new species, proposed as *T. eskilensis*, based on the morphological data and the results of phylogenetic analyses.

2. Material and methods

2.1. Plant materials

Particularly notable for their fleshy leaves, some *Tripleurospermum* samples were collected by the authors during a field trip near Eskil, Aksaray. These unknown specimens were checked from relevant literature: Kay (1976), Podlech (1986), Pobedimova (2000), Enayet Hossain (1975), Kral (1990), İnceer (2012), and Khayati et al. (2016). Morphological observations and measurements were based on living plants, dried specimens, and digital images from the herbaria E, GAZI, K, and LE (the acronyms follow Thiers, 2021). At least 20 measurements were taken for each morphological characteristic. Specific characteristics were photographed with a Leica S8 APO digital imaging system stereomicroscope (SM). The name of the species were checked within "International Plant Names Index (IPNI)" database (IPNI, 2021). A distribution map was prepared in Google Earth (2021) according to Güner et al. (2012). The threat category assessment of the new species follows IUCN criteria (IUCN, 2019).

Additionally, the examined herbarium specimens of *Tripleurospermum decipiens, T. disciforme*, *T. fissurale* subsp. *fissurale, T. microcephalum*, and *T. tempskyanum* are listed in Appendix.

2.2. Micromorphological studies

Pollen morphology was investigated with light microscopy (LM) and scanning electron microscopy (SEM). Pollen slides were prepared using Wodehouse (1935) technique for LM. LM studies were performed using a Leica DM 500 digital imaging system. For the preparation of SEM pictures, dried pollen grains which were isolated by Woodhouse method were transferred onto aluminium stubs using double-sided adhesive tape, and coated with gold particles in a sputter-coater (Çeter et al., 2013). Morphological observations were made in a Jeol JSM 6490LV SEM at Kastamonu University SEM Laboratory. The following characteristics of at least 30 pollen grains were measured and analysed with LM: pollen polarity, symmetry, shape, size, aperture characters, polar axis and equatorial diameter, exine and intine thickness, thickness under pores, colpus and pore length and width, and with SEM: ornamentation, spine size, perforation number at base, and ornamentation of interspinal area. Terminology was adopted from Faegri and Iversen (1992), Salgado-Labouriau (1982), Punt et al. (2007), Punt and Hoen (2009), Hesse et al. (2009), Çeter et al. (2013), and Halbritter et al. (2018). Pollen shape classification was adopted from the classification by Erdtman (1969) based on P/E ratio. For achene micromorphological analysis, achene shape and size (length, width, and thickness) characteristics of at least 20 dry mature achenes were measured with SM; ornamentation, corona length, lateral rib thickness, adaxial median rib thickness, ornamentation between rib area, abaxial median rib width; oil sac shape, size, and color, pericarp epidermal cells shape, anticlinal and periclinal cell walls; cuticle type, epicuticular wax structure were measured with SEM. The preparation of SEM images for the achenes follows the same method for the pollen grains. The achene micromorphological characteristics terminology was adopted from Enayet Hossain (1975), Barthlott (1981), İnceer and Hayırlıoğlu-Ayaz (2010), İnceer et al. (2012), and Skilbeck et al. (2019).

2.3. Anatomical studies

Transverse sections from the middle part of achene were cut by hand, stained and mounted in Sartur reagent for achene anatomical investigation (Çelebioğlu and Baytop, 1949). Five slides were prepared for each of the five individuals. The following anatomical characters were measured and photographed with a Leica DM 500 digital imaging system LM: testa, endosperm, adaxial rib, lateral rib, vascular bundle and palisade sclerenchyma, and thickness and width of the ribs in the pericarp. The achene anatomical characteristics terminology follows İnceer et al. (2012) and İnceer and Özcan (2021). Stomatal measurements were carried out on the abaxial and adaxial epidermis from fresh leaves with a Leica DM 500 digital imaging system LM (İnceer and Hayırlıoğlu-Ayaz, 2010; İnceer and Özcan, 2021). Ten leaf paradermal sections were prepared for stomatal lenght measurements, and five measurements were taken on each slide in random fields of view. Minimum, maximum, and standard deviation values were calculated for the measurements of all anatomical features. Line drawings were prepared to show the anatomical characteristics.

2.4. Molecular studies

To evaluate molecular data, leaf samples of *Tripleurospermum eskilensis* were obtained from its natural habitats. Total genomic DNA was extracted by using plant DNA extraction kit (MACHEREY-NAGEL) and the amplification of ITS regions (ITS1+5.8S+ITS2) was carried out by using the primer pairs of Hsiao et al. (1995). PCR amplifications were performed in a total volume of 20 µL containing 3.5 µL 5 × Hot FirePol Blend PCR Mix (Solis Biodyne) (15 mM $MgCl_2$), 0.5 µL each primer pair, 1 µL template DNA and 14.5 µL water. PCR reactions were performed with a Thermo cycler (MultiGENE, Cleaver Scientific Ltd) based on optimized cycling parameters as: 5 min at 94 °C for initial denaturation, followed by 30 cycles of 30 s at 94 °C for template denaturation, 30 s for annealing, 50 s at 72 °C for extension and 10 min at 72 °C for final extension. All products were checked on an agarose gel (2%) for getting perfect bands and the purification and sequencing of the samples were done by RefGEN Biotechnology Company (Ankara, Turkey). The obtained sequences were checked before analysis by Finch Tv software ver. 1.4.0-manufactured by Geopiza Research Team (Patterson et al., 2004-2006). MUSCLE (multiple sequence comparison by log expectation) tool (Edgar, 2004) of MEGA (Molecular Evolutionary Genetics Analysis) ver. 7.0.9 software (Kumar et al., 2016) was used for alignment of the sequences. The maximum likelihood (ML) method of GTR (general time reversible) model with gamma distribution with bootstrap test analysis was used for constructing the phylogenetic trees. Moreover, to evaluate clear genetic relationships among species, BEAST v 2.5.1 (Bayesian evolutionary analysis by sampling trees) package program was used with the same substitution model (GTR+G) for data partitions with a Yule tree prior and a randomly generated starting tree. Then, phylogenetic complex trees were summarized and combined with Tree Annotator v1.8.0 program with a posterior probability limit of 1 (Drummond et al., 2012). While constructing phylogenetic trees at MEGA and BEAST software, the sequences of *Anthemis chia* L., *Artemisia absinthium* L., *Artemisia santonicum* L., *Matricaria chamomilla* L. var. *chamomilla*, and *Matricaria aurea* (Loefl.) Sch.Bip. were studied to as a forementioned outgroups. Furthermore, to evaluate the genetic position of *T. eskilensis* among other species of *Tripleuspermum* genus, some different species from different sections of the genus were additionally obtained from NCBI data bank as outgroups (Hobbs and Baldwin, 2013, İnceer et al., 2018) (Table 1).

3. Results

3.1. Taxonomic treatment

Tripleurospermum eskilensis Tekşen & Karaman ***sp. nov.*** (Figures 1–3) (Table 2–4).

Type: Turkey. Aksaray: 3 km East of Eskil, 1000 m, salty steppe, 16 July 2020, *M. Tekşen* 3509 *& S. Karaman* (holo.: GAZI, iso.: ANK, AKSU, NGBB).

Paratypes: Turkey. Aksaray: Eskil, 3 km East of Eskil, 1000 m, salty steppe, 18 July 2016, *M. Tekşen* 2937 (AKSU); ibidem, 25 July 2021, *M. Tekşen* 4160, *S. Karaman & M. Sağıroğlu* (AKSU).

Diagnosis: *Tripleurospermum eskilensis* is closely related to *T. decipiens*, but it can easily be distinguished from it by 2–6 branched at base of the stem (vs. branched near apex), leaves fleshy, 9–13 mm long, leaflets linear to linear-lanceolate (vs. not fleshy, 60–90 mm long, leaflets filiform or lanceolate), capitula with 2–5 (vs. numerous capitula), adaxial median rib width 0.17–0.30 mm (vs. 0.07–0.13 mm) and adaxial median rib thickness of achene 0.14–0.29 mm (vs. 0.03–0.08 mm), corona length 0.13–0.25 mm (vs. 0.05–0.09 mm).

Description: Perennial, strongly tap rooted. Stem 10–25 cm tall, sulcate, decumbent or ascending, with 2–6 strongly branched at the base of the stem. Leaves 1-pinnatisect, fleshy, aristate at apex, glabrous or sometimes minutely pubescent. Basal leaves 9–13 × 6–10 mm, petiolate, petiole 5–25 mm long, sheathing at base, with 2–3 pairs spines at sheath margin and base, leaflets 3–10 × 0.5–1 mm, linear to linear-lanceolate. Cauline leaves 3–11 mm long, sessile, leaflets 2–10 × 0.5–1.5 mm, linear to linear-lanceolate, widened at base; upper cauline leaves strongly reduced, linear. Inflorescence solitary or a lax subcorymb with 2–5 capitulate, borne on long, unequal peduncles 15–60 mm long, elongated to 75 mm in fruit, bracteate, glabrous. Capitula homogamous, discoid, 3-9

Table 1. Accession numbers of the sequences of ITS regions of the species which were used to construct phylogenetic trees from NCBI data bank.

Taxa	NCBI accession numbers (ITS regions)	References
Tripleurospermum baytopianum	MG740678.1	İnceer et al., 2018
Tripleurospermum conoclinum	MG740711.1	
Tripleurospermum caucasium	MG740696.1	
Tripleurospermum tempskyanum	MG740704.1	
Tripleurospermum monticola	MG740701.1	
Tripleurospermum pichleri	MG740690.1	
Tripleurospermum parviflorum	MG740710.1	
Tripleurospermum repens	MG740691.1	
Tripleurospermum kotschyi	MG740679.1	
Tripleurospermum heterolepis	MG740684.1	
Tripleurospermum transcaucasicum	MG740693.1	
Tripleurospermum maritimum	MG740694.1	
Tripleurospermum melanolepis	MG740697.1	
Tripleurospermum callosum	MG740680.1	
Tripleurospermum sevanense	MG740681.1	
Tripleurospermum hygrophilum	MG740685.1	
Tripleurospermum elongatum	MG740687.1	
Tripleurospermum corymbosum	MG740706.1	
Tripleurospermum microcephalum	MG740689.1	
Tripleurospermum disciforme	MG740708.1	
Tripleurospermum decipiens	MG740682.1	
Tripleurospermum tenuifolium	MG740705.1	
Tripleurospermum fissurale subsp. *fissurale*	MG740703.1	
Tripleurospermum ziganaense	MG740700.1	
Tripleurospermum insularum	MG740707.1	
Tripleurospermum rosellum var. *album*	MG740692.1	
Tripleurospermum inodorum	MG740688.1	
Anthemis chia	MG740672.1	
Matricaria chamomilla var. *chamomilla*	MG740675.1	
Matricaria aurea	MG740674.1	
Artemisia absinthium	JX051763.1	Hobbs and Baldwin, 2013
Artemisia santonicum	JX051656.1	

mm in diameter, globose to hemispherical, 4–6 × 6–7 mm in fruiting head, bract on the peduncle 0.3–0.5 × 2–4 mm; involucre 2–5 × 2–3 mm, hemispherical, with ca. 20–30 involucral bracts arranged in 3 rows, imbricate; outer involucral bracts 1–2 × 0.5–1 mm, glabrous, lanceolate, obtuse, margins white membranous; inner involucral bracts 2–2.5 × 1–1.5 mm, glabrous, lanceolate, obtuse, margins white membranous. Receptacle 2.5–3.5 × 4.5–5 mm, ovoid-hemispherical, epaleate. Disc florets numerous, hermaphrodite, yellow with 5 lobes, lobes triangular, 0.25–0.5 mm long, tube 1–1.5 mm long, cylindric, not widening at base and not enveloping top of achene, corolla lobes with brownish-red gland at apex; anthers 5, 0.5–0.9 mm long, coherent along most of their length and forming a tube around style, appendages conical, acute at apex, slightly exerted from corolla tube; style 1–1.3 mm long,

Figure 1. Holotype of *Tripleurospermum eskilensis sp. nov.* (Collection no: M. Tekşen 3509) (Scale bar: 1 cm).

shorter than corolla tube; stigma 2-branched, branches 0.1–0.2 mm long, not exserted from anther tube. Achenes obpyramidal, 0.69–1.54 × 1.27–1.88 mm, abaxially rugose, tuberculate, with 2 brownish-red, oleiferous orbicular to oblong glands at apex, 0–1-ribbed white or brown, rib thin; adaxially rugose, tuberculate, 3 white ribbed, ribs thickened, with deep fissured; corona 0.13–0.25 mm long, white, membranous, 5-lobulate.

Flowering and fruiting time: June to August.

Etymology: The species epithet is derived from Eskil district in Aksaray Province, the locality where this species was collected for the first time.

Suggested Turkish name: The Turkish name of this species is suggested as "Eskil papatyası" according to the guidelines by Menemen et al. (2016).

Habitat and ecology: Currently found only at the type locality Tuz Lake in Eskil district, Aksaray Province, Turkey (Figure 4). *Tripleurospermum eskilensis* grows on

Figure 2. *Tripleurospermum eskilensis* (Collection no: M. Tekşen 3509). A. general habit (natural) B. capitulum C. fleshy stem leaves. Photographs taken by M. Tekşen at type locality.

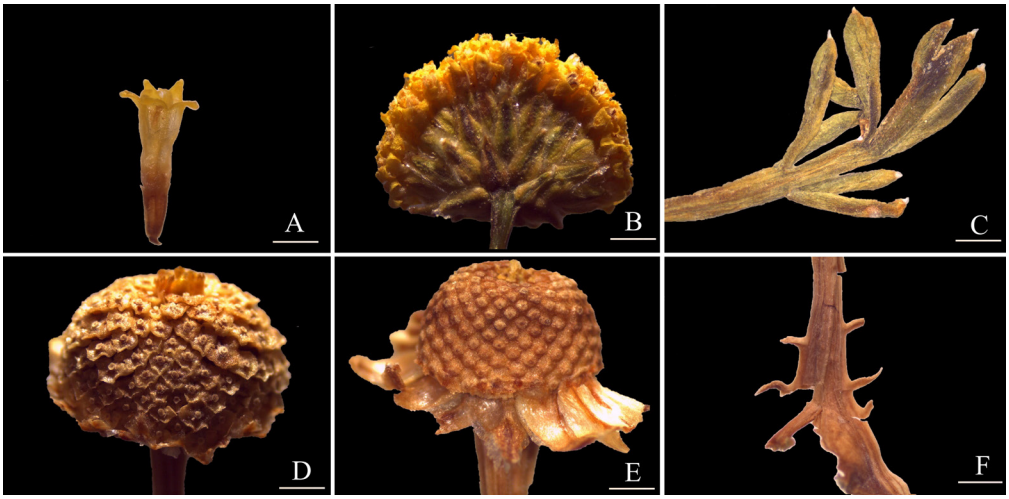

Figure 3. *Tripleurospermum eskilensis*. A. disc flower B. flowering capitulum and C. stem leaf D. fruiting capitulum E. receptacle F. basal leaf. Photographs taken by M. Tekşen (Scale bars: A: 1 mm; B–F: 2 mm).

Table 2. Comparison of pollen characteristics of *Tripleurospermum eskilensis* and *T. decipiens* (Çeter et al., 2013; specimens examined).

Pollen characteristics	***T. eskilensis***		***T. decipiens***
	Min-Max	Mean	Mean
P/E ratio and shape	0.93, oblate-spheroidal		0.80, subprolate
Polar axis (µm)	9.95–13.33	11.70	24.10
Equatorial axis (µm)	11.14–13.77	12.56	30.01
Colpus length (µm)	7.21–10.49	8.89	15.80
Colpus width (µm)	1.53–4.04	2.87	5.80
Pore length (µm)	3.61–4.92	4.12	7.00
Pore width (µm)	3.06–4.70	3.74	7.10
Exine thickness (µm)	0.98–2.51	1.73	3.00
Exine thickness under pore (µm)	0.33–0.87	0.60	Thinner
Intine thickness (µm)	0.22–0.44	0.33	0.30
Intine thickness under pores (µm)	0.66–1.97	1.18	thicker
Spine length (µm)	2.47–3.52	3.05	2.50
Spine width at base (µm)	3.18–4.88	3.81	4.10
Perforation number at base	10–60		15–40
Ornamentation of interspinal area	Reticulate-perforate		Granulate-perforate

salty steppes at an elevation of 950–1000 m. This habitat is associated with some endemic (*Allium scabriflorum* Boiss., *A. tchihatschewii* Boiss., *Asparagus lycaonicus* P.H. Davis, *Frankenia salsuginea* Adıgüzel & Aytaç, *Gladiolus halophilus* Boiss. & Heldr, *Limonium iconium* (Boiss. & Heldr.) Kuntze, *Onopordum davisii* Rech.f., *Puccinellia bulbosa* (Grosh.) Grosh. subsp. *caesarea* Kit Tan, *Salsola stenoptera* Wagenitz, *Salvia halophila* Hedge, *Verbascum pyroliforme* (Boiss. & Heldr.) Hub.-Mor, *Hypericum salsugineum* Robson & Hub.-Mor) and nonendemic taxa (*Frankenia hirsuta* L., *Juncus maritimus* Lam., *Halocnemum strobilaceum* (Pall.) M. Bieb., *Thymus sipyleus* Boiss. var. *sipyleus*, *Linum seljukorum* P.H. Davis, *Inula aucherana* DC., *Puccinellia convoluta* (Hornem.) P.Fourr.). An ecological comparison of *Tripleurospermum eskilensis* and *T. decipiens* is given in Table 4. It has also been compared ecologically with *T. disciforme, T. fissurale* subsp. *fissurale, T. microcephalum*, and *T. tempskyanum* (Table 4).

Table 3. Comparison of achene and stoma characteristics of *Tripleurospermum eskilensis* and *T. decipiens* (Enayet Hossain, 1975; İnceer and Özcan, 2021; specimens examined).

Achene characteristics	***T. eskilensis***		***T. decipiens***	
	Min-Max	Mean	Min-Max	Mean
Length (mm)	1.27–1.88	1.59	1.00–1.50	1.25
Width (mm)	0.69–1.54	1.01	0.30–0.70	0.53
Thickness (mm)	0.56–0.90	0.74	0.25–0.50	0.36
Corona length (mm)	0.13–0.25	0.21	0.05–0.09	0.07
Lateral rib thickness (mm)	0.15–0.24	0.19	0.07–0.12	0.08
Adaxial median rib width (mm)	0.17–0.30	0.23	0.07–0.13	0.10
Adaxial median rib thickness (mm)	0.14–0.29	0.18	0.03–0.08	0.06
Between rib area ornamentation	Rugose		Rugose	
Ornamentation	Reticulate-foveate		Rugose	
Abaxial median rib width (mm)	0.03–0.10	0.07	Absent	
Testa thickness (µm)	8.20–13.11	10.86		5.25
Endosperm thickness (µm)	9.11–11.47	10.79		8.75
Vascular bundle thickness in adaxial rib (µm)	59.01–74.40	66.67		48.40
Vascular bundle thickness in lateral rib (µm)	47.54–63.77	53.80		45.80
Palisade sclerenchyma thickness (µm)	21.31–44.26	31.91		18.25
Stoma length (µm)	23.82–27.73	26.19		28.07
Leaf epidermal cell walls	Undulate to sinuate		Undulate to sinuate	

Distribution and conservation status: *Tripleurospermum eskilensis* is known from a single locality in Eskil, Aksaray Province, Central Anatolia (Figure 4). The extent of occurrence (EOO) and area of occupancy (AOO) are less than 10 km^2 (criterion B1, B2). The only known population includes less than 2500 mature individuals and faces some immediate threats such as overgrazing, habitat loss because of agricultural activities, and reduced water availability due to human activities and climate change. As a result, a continuing decline in the area of occupancy, quality of habitat, and number of mature individuals of *Tripleurospermum eskilensis* is estimated for the near future. Thus, the species is assessed as "Critically Endangered": CR B1ab (iii, v)+ B2 ab (ii, iii, v), according to IUCN (2019).

Species identification key of closely related species:

1. Capitula disciform
2. Achene obpyramidal and corona absent .... ***T. disciforme***
2. Achene ± prismatic, linear and corona present .... ***T. decipiens***
1. Capitula discoid
3. Corolla lobes eglandular at tips; achene mucilaginous, adaxial fissure narrow, unvisible, corona 0.25–0.5 mm ....***T. fissurale*** subsp. ***fissurale***
3. Corolla lobes glandular at tips; achene not mucilaginous, adaxial fissure conspicously, corona up to 0.25 mm
4. Lower leaves sessile
5. Inflorescens densely corymbose .... ***T. microcephalum***
5. Inflorescens laxly corymbose ..... ***T. tempskyanum***
4. Lower leaves petiolate
6. Perennial; leaves fleshy; capitula 2-5 ..... ***T. eskilensis***
6. Annual or biennial; leaves not fleshy; capitula numerous ..... ***T. decipiens***

3.2. Micromorphological results

Pollen morphology: Pollen grains small, tricolporate, operculate, radially symmetrical, isopolar, oblate-spheroidal, (P/E ratio 0.93), equatorial outline elliptic, triangular in polar view, amb intersemiangular; polar axis (P) 9.95–13.33 µm (11.70 µm ± 0.88) and equatorial diameter (E) 11.14–13.77 µm (12.56 µm ± 0.76); colpus length 7.21–10.49 µm (8.89 µm ± 1.05), colpus width 1.53–4.04 µm (2.87 µm ± 0.76); pore length 3.61–4.92 µm (4.12 µm ± 0.35), pore width 3.06–4.70 µm (3.74 µm ± 0.40); exine 0.98–2.51 µm (1.73 µm ± 0.45) thick, exine under pore 0.33–0.87 µm (0.60 µm ± 0.15) thick, intine 0.22–0.44 µm (0.33 µm ± 0.08) in thickness, thicker under pores 0.66–1.97 µm (1.18 µm ± 0.35); exine ornamentation echinate, spine length 2.47–3.52 µm (3.05 µm ± 0.25), spine

Table 4. Diagnostic ecological and morphological features / comparison of *Tripleurospermum eskilensis, T. decipiens*, *T. disciforme, T. fissurale* subsp. *fissurale, T. microcephalum,* and *T. tempskyanum.* (Enayet Hossain, 1975; İnceer and Hayırlıoğlu-Ayaz, 2010; Güner et al., 2012; Özbek and Onaylı, 2020; İnceer, 2021; specimens examined).

Morphological characteristics	***T. eskilensis***	***T. decipiens***	***T. disciforme***	***T. fissurale* subsp. *fissurale***	***T. microcephalum***	***T. tempskyanum***
Habitat, altitute, and phenology (flowering/ fruiting time)	Salty steppe, 1000 m of elevation; July/July-August	Steppe, alpine steppe, meadows, rocky slopes, cultivated and fallow fields; 700–2350 m of elevation; April-July/ April- August	Meadow, riverbanks, dry river bed moist places, cultivated fields, and roadsides; 800–2286 m of elevation; June-September/June-December	Rocky slopes and crevices, near rivers; c. 400 m of elevation; May/May-June	Fallow fields, banks; 1400–1600 m of elevation; June-August/June-September	Meadows, damp places, open places, scrub, on granitic substrates; 1690-1900 m of elevation; June-July/July
Life cycle	Perennial	Annual or biennial	Biennial	Annual or biennial	Biennial or perennial	Perennial
Stem	10–25 cm	(10–)40–90 cm	(5–)40–70 cm	30–70 cm	30–60 cm	30–65 cm
Habit	decumbent or ascending; branched at base	usually erect or ascending, sometimes decumbent; branched near apex	erect; branched above	erect; branched at base	erect; laxly branched above	erect or ascending, corymbosely branched above
Leaves (mm)	1-pinnatisect, fleshy	1–2-pinnatisect, not fleshy	2–3-pinnatisect, not fleshy	3-pinnatisect, upper 1-2-pinnatisect, not fleshy	1–2-pinnatisect, not fleshy	2–3-pinnatisect, not fleshy
	Lower petiolate, middle and upper sessile	Lower petiolate, middle and upper sessile	Sessile	Sessile	Sessile	Sessile
	9–13 × 6–10	60–90 × 8–20	30–170 × 10–35	15–70 × 5–30	10–60 × 10–25	30–40 × 15–20
Leaf laciniae	Linear to linear-lanceolate, aristate	Filiform-setaceous or lanceolate-aristate	Filiform-setaceous	Small, triangular-acute or linear-acute, mucronate	Filiform-setaceous	Filiform-setaceous
Inflorescence	2–5, laxly corymbose	Numerous, laxly corymbose	3 to numerous, laxly corymbose	2-3	Numerous, densely corymbose	4–8(–20), laxly corymbose
Peduncle (cm)	1.5–7.5	5–15	3.5–10	8–25	0.5–2.5	2–10
Capitula	discoid	usually disciform (outer female with suppressed ligules), rarely discoid,	disciform, heterogamous	discoid	discoid	discoid
Receptacle	Ovoid to hemispherical	Ovoid	Ovoid-oblong	Ovoid	Ovoid-conical	Hemispherical
Corolla lobes	Glandular at tips	Usually glandular at tips, rarely eglandular	Red, resinous glandular at tips	Eglandular at tips	Glandular at tips	Glandular at tips
Achenes	Obpyramidal	Obpyramidal	± prismatic, linear	Oblong or obpyramidal	Obpyramidal	Oblong to obpyramidal
Achenes size (mm)	1.27–1.88 × 0.69–1.54	1.0–1.5 × 0.3–0.7	1.0–1.4 × 0.5	1.3–2.0 × 0.4–0.6	1.0–1.5 × 0.5–1.0	1.7–2 × 0.4–1.6
Slime cell	Absent, not mucilaginous	Absent, not mucilaginous	Present, mucilaginous	Present, mucilaginous	Absent, not mucilaginous	Absent, not mucilaginous
Achenes abaxial	Rugose, tuberculate	Rugose	± smooth, rarely tuberculate	Smooth or faintly tuberculate	Tuberculate-rugose	Rugose
Color of glands at apex	Brownish-red	Red	Red	Red	Red	Red
Abaxial rib	0–1, thin	0	0	0	0	0
Achenes adaxial	Rugose, tuberculate	Rugose	Obscurely tuberculate	Fissures narrow	Tuberculate-rugose	Rugose
Adaxial rib thickness	Thick	Thin	Very thin	Moderately thickened	Thick	Thick
Corona (mm)	Membranous, obviously 5-lobulate, 0.13–0.25	Marginiform, 0.05–0.09	Absent	Lobate, 0.25–0.8	Short, marginiform, ± lobed	Entire or lobed, marginiform, 0.2–0.4

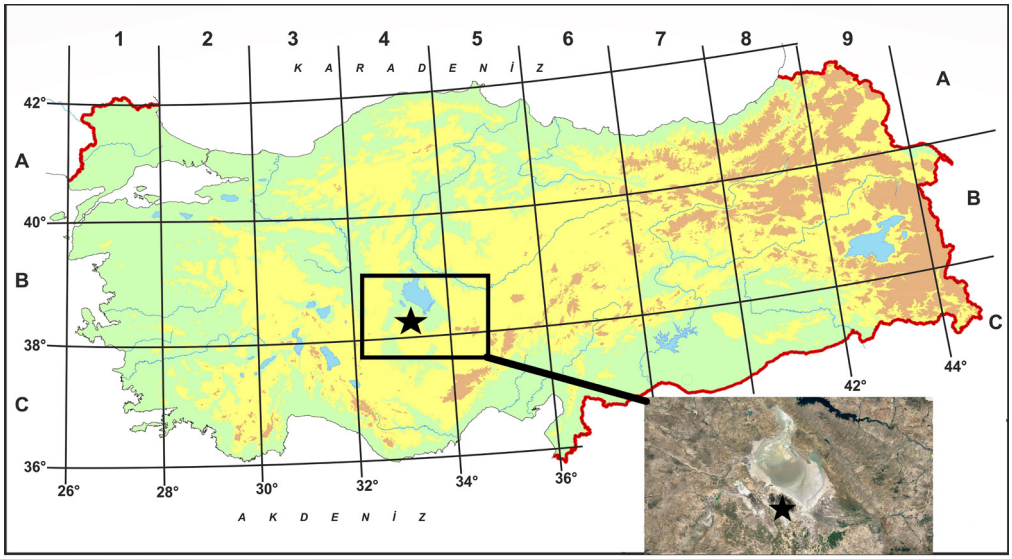

Figure 4. Distribution map of *Tripleurospermum eskilensis*.

width at base 3.18–4.88 µm (3.81 µm ± 0.42); perforation number at base 10-60; ornamentation of interspinal area reticulate-perforate (Figure 5) (Table 2).

Achene micromorphology: Achene shape oblong to obpyramidal, glabrous, achenes length 1.27–1.88 mm (1.59 mm ± 0.17), width 0.69–1.54 mm (1.01 mm ± 0.17) (maximum width), thickness 0.56–0.90 mm (0.74 mm ± 0.10) membranous, 5-lobulate, corona length 0.13–0.25 mm (0.21 mm ± 0.04), lateral rib thickness 0.15–0.24 mm (0.19 mm ± 0.03), adaxial median rib width 0.17–0.30 mm (0.23 mm ± 0.04), adaxial median rib thickness 0.14–0.29 mm (0.18 mm ± 0.04), between rib area ornamentation rugulate, deep fissured, abaxial median rib width 0.03–0.10 mm (0.07 mm ± 0.02), ornamentation reticulate-foveate, oil sac 2, orbicular to oblong, oil sac length 0.10–0.20 mm (0.15 mm ± 0.03), oil sac width 0.16–0.28 mm (0.21 mm ± 0.04), and brown to brownish-red (Figure 6, Table 3).

3.3. Anatomical results

Achene pericarp epidermal cells polygonal, oblong to linear or isodiametric, anticlinal cell walls irregularly thick, raised and straight, periclinal cell walls convex; cuticle type rugose, well developed or smooth, epicuticular wax smooth, slime cell absent, testa thickness 8.20–13.11 µm (10.86 µm ± 1.88), endosperm thickness 9.11–11.47 µm (10.79 µm ± 0.84), vascular bundle thickness in adaxial rib 59.01–74.40 µm (66.67 µm ± 2.03), vascular bundle thickness in lateral rib 47.54–63.77 µm (53.80 µm ± 3.32), palisade sclerenchyma thickness 21.31–44.26 µm (31.91 µm ± 2.17) (Figure 7a).

The stoma length is 23.82–27.73 µm (26.19 µm ± 1.11) and width is 7.03–12.10 µm (8.81 µm ± 1.12) on the abaxial epidermis. It is 23.70–27.50 µm (26.10 µm ± 1.10) long and 7.00–12.05 µm (8.79 µm ± 1.11) wide on the adaxial epidermis in the leaf sections taken superficially from *Tripleurospermum eskilensis* (Figure 7b) (Table 3).

3.4. Phylogenetic analysis

In the current study, ITS regions were sequenced, obtaining sequences of 666 bp in length (13 of them were variable sites). The mean genetic divergence is calculated as 0.007 between *Tripleurospermum eskilensis* and *Tripleurospermum* species from NCBI data bank, ant it means that they are genetically too close to each other. Firstly, in the current study, pairwise distance matrices were calculated by using *Tripleurospermum* species which were morphologically compared with *T. eskilensis* (*T. tempskyanum, T. decipiens, T. fissurale* subsp. *fissurale, T. disciforme,* and *T. microcephalum*) and it was found that *T. eskilensis* is genetically closer to *T. tempskyanum* (0.002) and *T. decipiens* (0.003) than the others. To compare the position of *Tripleurospermum eskilensis* in regard to available species from NCBI databank, a phylogenetic tree was constructed again with these species as outgroups (Figure 8). According to the phylogenetic tree, it could be said that all the analysed *Tripleurospermum* species are positioned under 3 different main clades in the same cluster except the outgroup. *Tripleurospermum microcephalum, T. inodorum, T. elongatum, T. transcaucasium,* and *T. corymbosum* have built clade 3, and *T. disciforme, T. hygrophilum, T. tenuifolium,* and *T. baytopianum* formed clade 2. The 3rd clade is composed of the rest of the studied samples. This 3rd clade is divided into 2 groups and *T. eskilensis* was positioned at the same group (Group 1) with *T. tempskyanum* and *T. fissurale* subsp. *fissurale*. Even though *T. decipiens* is positioned under a different branch at Figure 9, it is located at group 2 (Figure 8). Moreover, there is a *Tripleurospermum* cluster which

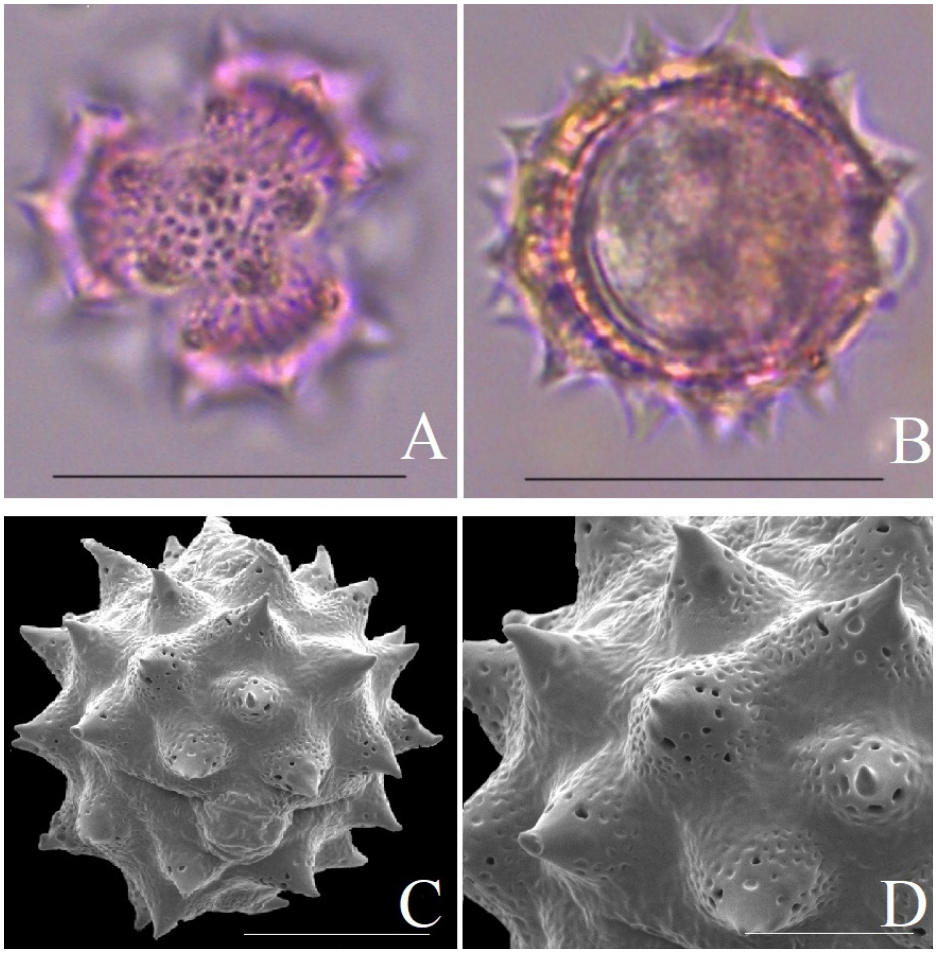

Figure 5. Pollen grain microphotographs of *Tripleurospermum eskilensis* (Collection no: M. Tekşen 3509) under LM (A, B) and SEM microphotographs (C, D) A. polar view, B. and C. equitorial view, D. ornamentation (Scale bars: A–B: 20 µm; C: 10 µm, D: 5 µm).

includs *T. disciforme, T. microcephalum,* and other species based on a phylogenetic tree which was constructed by using these species that were morphologically evaluated in the previous part of the study. *Tripleurospermum eskilensis* is positioned along with *T. tempskyanum* and separated from *T. fissurale* subsp. *fissurale* and *T. decipiens* which are in a different branch, and it forms a cluster with these four species as supposed. (Figure 9).

4. Results and discussion

In this study, *Tripleurospermum eskilensis* was compared with *T. decipiens* as the closest species, and the differences and similarities (in terms of morphology, habitat and altitude based on the data in literature) with other discoid and disciform capitulated species (*T. disciforme, T. fissurale* subsp. *fissurale, T. microcephalum,* and *T. tempskyanum*) are given in Table 4.

Tripleurospermum eskilensis differs from *T. disciforme* by having decumbent or ascending and 2–6 strong branches at base stem; 1-pinnatisect, fleshy leaves, petiolate lower leaves, sessile middle and upper leaves, 9–13 × 6–10 mm, linear to linear-lanceolate leaflets; 1.5–7.5 cm peduncle; homogamous discoid capitula; ovoid to hemispherical receptacle; 0.69–1.54 mm achenes width, obpyramidal to oblong achene shape, not mucilaginous, abaxial rugose, abaxial rib 0–1, thin, adaxial rib thick, membranous corona (in *T. disciforme* stem erect and branched above; leaves 2–3-pinnatisect, not fleshy, sessile, 30–170 × 08–35 mm, laciniae filiform-setaceous; peduncle 3.5–10 cm; capitula disciform, heterogamous; receptacle ovoid-oblong; achenes width 0.5 mm, shape ± prismatic, linear, mucilaginous, abaxial smooth, abaxial rib absent, adaxial rib very thin, corona absent) (Enayet Hossain, 1975).

The new species differs from *T. fissurale* subsp. *fissurale* by having perennial (vs. annual or biennial), 10–25 cm stem (vs. 30-70 cm), 1-pinnatisect, fleshy leaves, lower leaves petiolate (vs. lower leaves 3-pinnatisect, upper

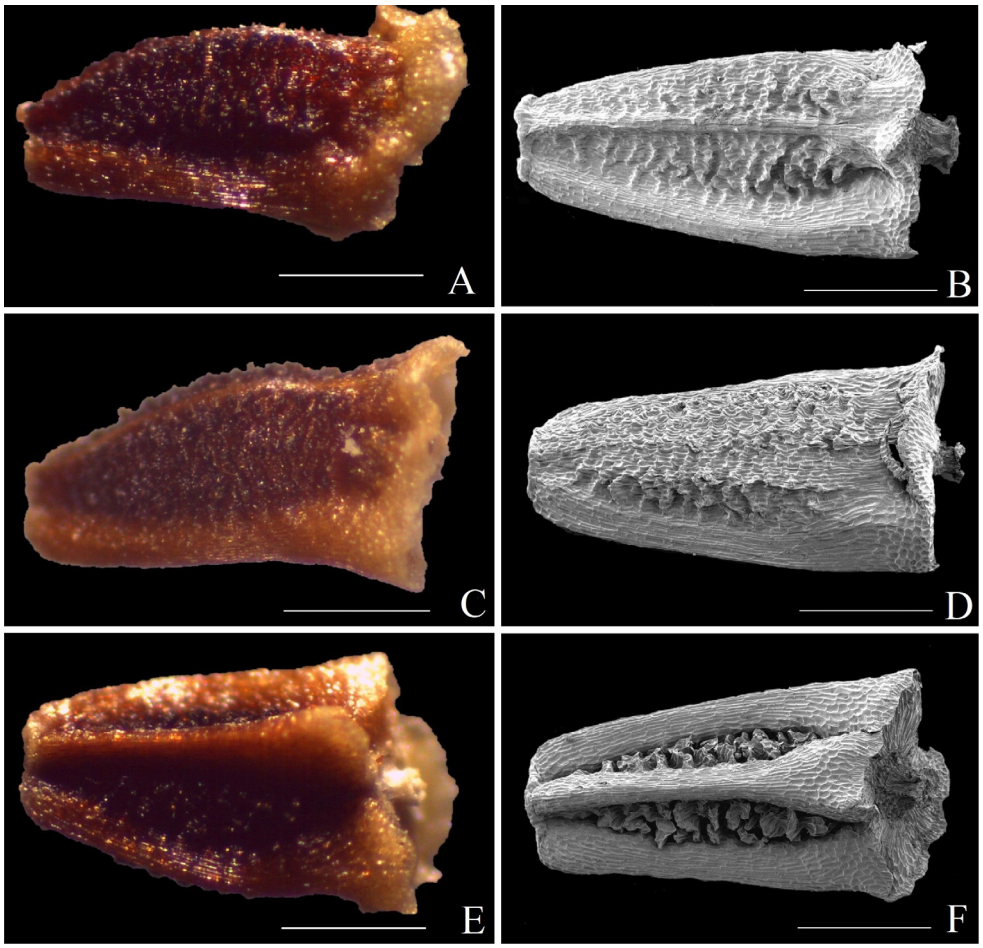

Figure 6. SM and SEM microphotographs of the achenes of *Tripleurospermum eskilensis* (Collection no: M. Tekşen 3509). A., B., C., and D. abaxial view by SM and SEM, E. and F. adaxial view by SM and SEM (Scale bars: A, C, E: 0.5 mm, B, D, F: 500 µm).

leaves 1–2 pinnatisect, not fleshy, sessile), peduncle 1.5–7.5 cm (vs. 8–25 cm), achenes not mucilaginous (vs. mucilaginous), achenes abaxial rugose, rib 0–1, thin (vs. smooth or faintly tuberculate, rib absent), adaxial rib very thick (vs. modaretely thickened), corona lobes 0.13–0.25 mm (vs. 0.25–0.8 mm) (Enayet Hossain, 1975; Özbek and Onaylı, 2020).

Tripleurospermum eskilensis differs from *T. microcephalum,* one of the other discoid capitulated species, by having stem branches at base and 10-25 cm long (vs. laxly branched above, 30-60 cm), 1-pinnatisect, fleshy leaves, lower leaves petiolate, leaflets linear to linear-lanceolate (vs. finely 1–2 pinnatisect, not fleshy, sessile, filiform-setaceous), inflorescence solitary or laxly corymbose with 2–5 capitula (vs. densely corymbose to ± umbel-like numerous capitulated), long, unequal peduncle, 1.5–7.5 cm (vs. short, ± equal, 0.5–2.5 cm), involucral bracts lanceolate (vs. ovate-lanceolate), corona lobate (vs. very short, marginiform) (Enayet Hossain, 1975).

The new species differs from *Tripleurospermum tempskyanum* by having 10–25 cm stem (vs. 30-65 cm), 1-pinnatisect, fleshy leaves, lower leaves petiolate, leaflets linear to linear-lanceolate (vs. finely 1–3 pinnatisect, not fleshy, sessile, filiform-setaceous), inflorescence with 2–5 capitulated (vs. 4–8(–20) capitulated), and corona membranous, obviously 5-lobulate, 0.13–0.25 (vs. entire or lobed, 0.2–0.4).

Pollen characteristics (Çeter et al., 2013), micromorphological and anatomical features of achene (Enayet Hossain, 1975; İnceer et al., 2012; Skilbeck et al., 2019; İnceer and Özcan, 2021), and stoma (İnceer and Hayırlıoğlu-Ayaz ,2010; İnceer et al., 2018; İnceer and Özcan, 2021) have been presented in detail and comparatively.

According to pollen analysis by Çeter et al. (2013) on 28 *Tripleurospermum* species, pollen size of genus *Tripleurospermum* in Turkey ranges from 15.6 to 32.2 µm in polar axis and 17.7 to 38.5 µm in equatorial axis.

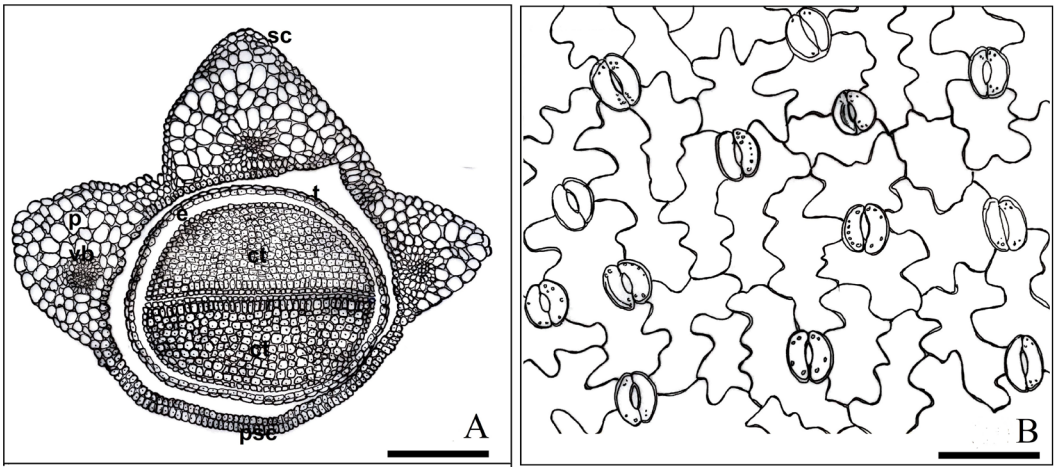

Figure 7. *Tripleurospermum eskilensis* (Collection no: M. Tekşen 3509). A. achene transverse section, B. stomata on abaxial leaf epidermal surface (ct: cotyledons; e: endosperm; p: parenchyma; psc: palisade sclerenchyma; sc: sclerenchyma; t: testa; vb: vascular bundle (Scale bars: A: 200 µm, B: 50 µm).

Pollen grains of *T. eskilensis* are smaller: 9.95–13.33 µm in polar axis and 11.14–13.77 µm in equatorial diameter. Pollen shape, ornamentation, spine, exine, and intine characteristics of *T. eskilensis* are in agreement with the data on *Tripleurospermum* presented by Çeter et al. (2013). The pollen morphologies of *T. eskilensis* and *T. decipiens* were compared based on our data and those by Çeter et al. (2013) and are given in Table 2. Pollen grains of *T. eskilensis* are similar to *T. decipiens* in its equatorial and polar outline, amb, exine ornamentation, intine thickness, and spine length and width. However, *T. eskilensis* differs from *T. decipiens* with some pollen characteristics, which are pollen size and shape, exine thickness, colpus width and length, and pore width and length (Table 2). Colpus and pore are short and narrow in *T. eskilensis*, and the exine is thinner than other *Tripleurospermum* species such as *T. decipiens* (Çeter et al., 2013).

The reason why the pollen grains of *Tripleurospermum eskilensis* are smaller among *Tripleurospermum* species may originate from their small genome size due to their preference for an extreme habitat such as salty steppe areas (Çeter et al., 2013; İnceer, 2011; İnceer et al., 2018). İnceer et al. (2018) stated that the diploid species they examined in their study were in the small genome category, and polyploids were in the intermediate genome category.

The comparison of achene characteristics between *Tripleurospermum eskilensis* and *T. decipiens* is given in Table 3. The achenes of *T. eskilensis* are different from *T. decipiens* by having larger achene measurements, corona length, width of adaxial median rib, thickness of lateral rib, adaxial median rib, testa, endosperm, vascular bundle in lateral rib, vascular bundle in adaxial rib, and palisade sclerenchyma (İnceer and Özcan, 2021). İnceer et al. (2012) and İnceer and Özcan (2021) stated that the thickness of adaxial rib and palisade sclerenchyma are supported the morphological separation of closely related species.

The examination of *Tripleurospermum eskilensis* achenes in SEM demonsrated that the absorption of water by gel helped germination in osmotically stressful and salty habitats and they did not have slime cells which are thought to be an adaptation for germination in limited water availability (İnceer, 2011; İnceer et al., 2012; Skilbeck et al., 2019; İnceer and Özcan, 2021) (Figures 6 and 7). The presence of slime cells in *Tripleurospermum* taxa is an important taxonomic characteristic, and slime cells have a relationship with the ploidy level and habitat. İnceer et al. (2012) studied 12 species of *Tripleurospermum* and stated that, despite the presence of slime cells in many rows and densities especially in diploid taxa, there are no slime cells in 7 polyploid species except for *T. monticola* (Boiss. & A. Huet) Bornm. and *T. repens* (Freyn & Sint.) Bornm.. Among the discoid and disciform capitulate species, the diploid *T. fissurale* and *T. disciforme* include slime cells on the achenes (İnceer et al., 2012; İnceer and Özcan, 2021). In addition, İnceer and Hayırlıoğlu-Ayaz (2010) and İnceer and Özcan (2021) stated in their studies that the slime cells seen in polyploid *T. monticola, T. repens,* and *T. tempskyanum* species growing in humid environments may not mature and function. Along with these three polyploid species without slime cells, there are also no slime cells in diploid species *T. decipiens* and *T. microcephalum* (Enayet Hossain, 1975; İnceer and Beyazoğlu, 2004; Skilbeck et al., 2019; İnceer and Özcan, 2021).

İnceer and Hayırlıoğlu-Ayaz (2010) stated that the stoma length in *Tripleurospermum* species are 24.87–29.69 µm (27.31 µm ± 1.99) in diploid individuals and 32.72–37.66 µm (35.00 µm ± 2.02) in tetraploid individuals. The abaxial/adaxial stoma length is 28.70/28.00 in *T. decipiens*,

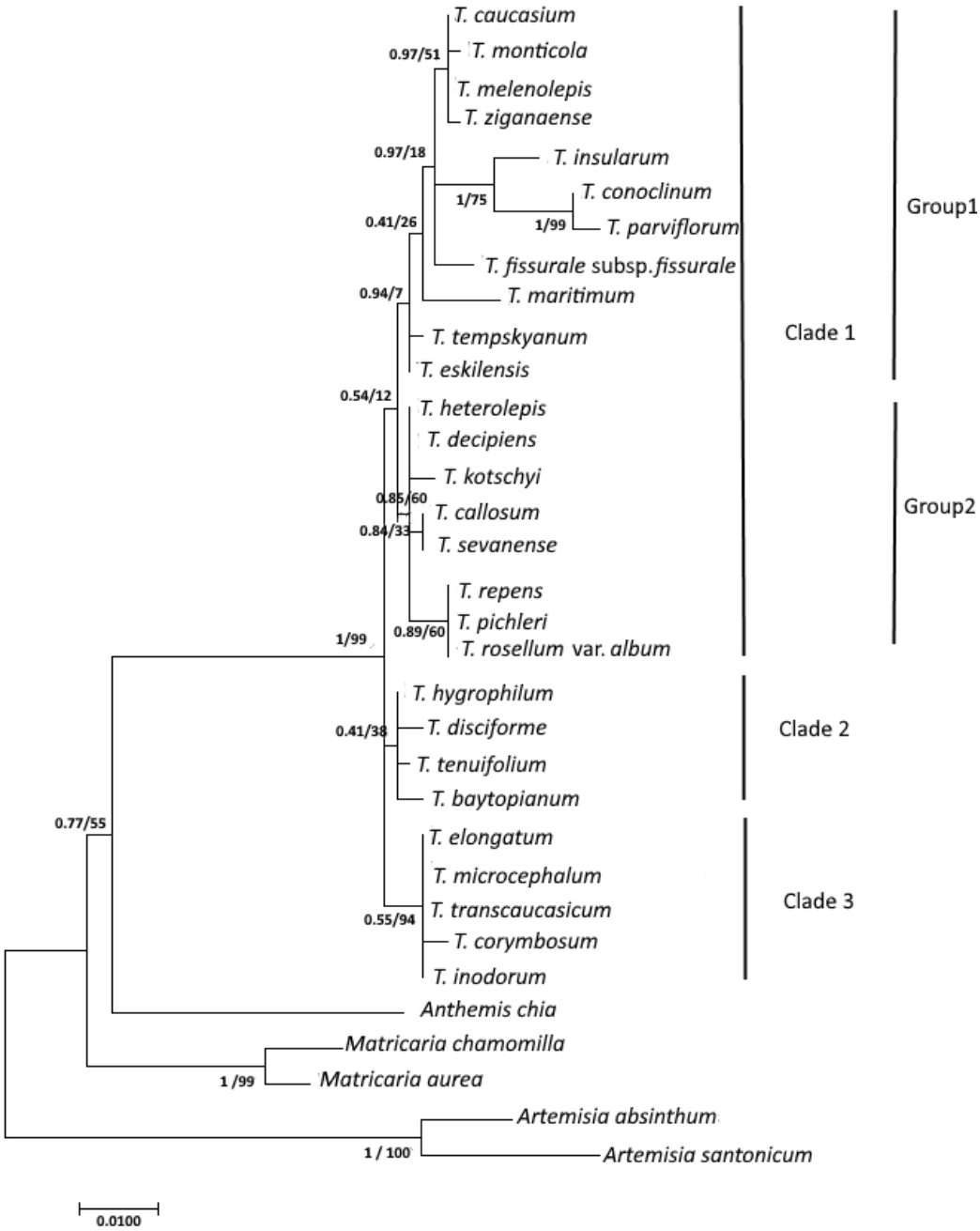

Figure 8. ML tree with GTR+G model of ITS gene region of *Tripleuspermum* species which were studied as aforementioned group. The results of ML analysis with subsequent optimization (bootstrap values with 1000 replicates) and posterior probabilities (PP) values of over 1 were given next to the nodes and separated with slashes.

25.20/25.00 µm in *T. microcephalum*, 27.08 µm in *T. fissurale* subsp. *fissurale,* 27.70/29.10 µm in *T. disciforme,* and 35.70/37.10 µm in *T. tempskyanum.* It is observed that stoma sizes of *T. eskilensis* (26.19/26.10 µm) are compatible with diploid species as well as other discoid and disciform capitulated species (İnceer and Hayırlıoğlu-Ayaz, 2010; İnceer and Özcan, 2021).

Comprehending the phylogenetic relationships within tribe Anthemideae has always been problematic; therefore, molecular evidence helped us distinguish a new species (İnceer et al., 2018). In the current study, we also carried out phylogenetic analysis based on the marker data (ITS). The phylogenetic tree indicates that the evidence from the morphology presents important

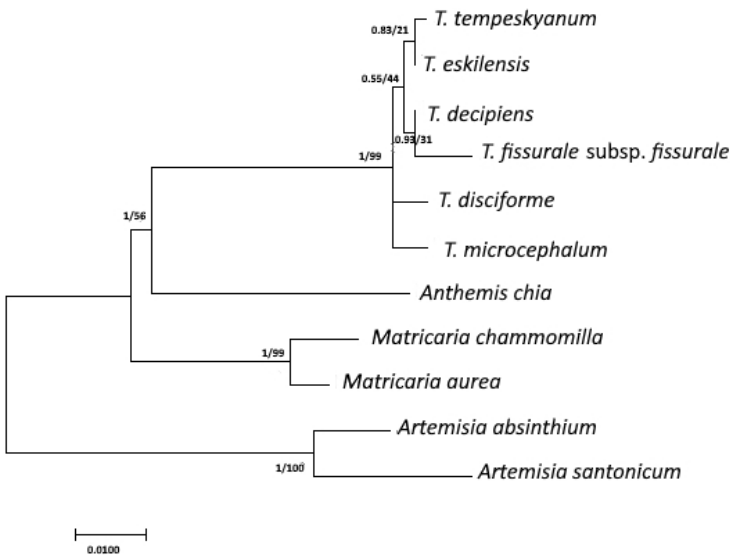

Figure 9. ML tree with GTR+G model of ITS gene region of morphologically compared species of *Tripleurospermum eskilensis*. The results of ML analysis with subsequent optimization (bootstrap values with 1000 replicates) and posterior probabilities (PP) values of over 1 were given next to the nodes and separated with slashes.

data towards a better understanding of character evolution of *Tripleurospermum*. The fact that *T. eskilensis* is in the same clade (Clade 1) with but in a group (Group 1) different from *T. decipiens* at the phylogenetic tree, supports the hypothesis that it is a new species. Their differences can be seen clearly both in Table 4 and in the diagnosis. *T. eskilensis* is closer to *T. tempskyanum* based on the phylogenetic tree. It differs from the new species especially in stem branching, nonsucculent, all sessile, longer and wider leaf sizes, achene characteristics with a longer and more prominent corona. These two species are allopatric, and *T. tempskyanum* grows in an especially narrow geographical area (South Marmara Section in Turkey) (Güner et al., 2012; İnceer, 2021). Morever, it is an expected result that *T. fissurale* subsp. *fissurale* is not close to *T.eskilensis* in the phylogenetic tree. In the same group, *T. fissurale* subsp. *fissurale* is located further away from *T. tempskyanum*, it differs from the new species with its plant height, leaf fragmentation, leaf size, and mucilage achene characteristics due to the presence of slime cells. It is also allopatric and grows in East Karadeniz Region in Turkey (Güner et al., 2012). In addition, *T. disciforme*, which is the only species having disciform capitula among the compared species, is located in Clade 2. It differs from *T. eskilensis* and other species with the characteristics of the presence of disciform, heterogamous capitula, achene with mucilage due to slime cells, and without corona. Furthermore, it is seen that *T. microcephalum*, which is distinguished by its leaf and flower morphological characters such as sessile, longer and wider leaf sizes, inflorescence densely corymbose and generally with short peduncul, is in Clade 3. The results of the phylogenetic analysis support that *T. eskilensis* is different from both *T. disciforme* and *T. microcephalum*.

All these clues indicate that although *T. eskilensis* which has many similar features regard to similar species, is a new species different from them. Therefore, the numbers of species and endemic species in Turkish *Tripleurospermum* have increased to 33 and 17, respectively, after this addition. The endemism ratio increased to 51.5% in Turkey.

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Appendix

Herbarium specimens examined of *T. decipiens, T. disciforme, T. fissurale* subsp. *fissurale, T. microcephalum,* and *T. tempskyanum*.

Tripleurospermum decipiens: TURKEY. Adana: Pozantı, 3 miles north on eastward slopes of Toros Dagi, 750–800 m, 16 July 1971, Aberdeen Univ. *Amanus Expedition* D3276 (E00580954!); Aksaray: Demirci town, Karabayır location, c. 1150 m, 16 June 1994, *F. Ertug* 56 (GAZI!); Afyon: Suhut, Kumalar Mountain, Ortapınar Village, field margin, 1100–1200 m, 19 May 1997, *E. Akcicek* 1677 (GAZI!); Ankara: Şereflikoçhisar Road, to Şereflikoçhisar, road margin, 18 June 1997, *N. Erdemoglu* and *A. K. Erdemoglu* s.n. (GAZI!); Antalya: Distr. Elmalı, Elmalı-Avlon Lake, fallow fields, rocky knolls, *S. Khan, G. T. Prance and D. Ratcliffe* 168 (E00580948!); Elmalı plain, 150 km from Fethiye, 20 km from Elmalı, 1050 m, road-sides step, 30 May 1962, *Dudley* 35207 (E00580950!); Denizli: 27 km sudlich Denizli zwischen dem Kazik beli und Sarabatkoy, 1200 m, 02 July 1973, *F. Holtz* 00.763 and *P. Hänel, T. Kesercioglu* (E00580961!); Kahramanmaraş: Engizek Mountain, around Kale Village, 1500-1600 m, 15 July 1987, field openings, *H. Duman* 3480 (GAZI!); Kırıkkale: Keskin, Bobrek Mountain, Müsellim Village, stony areas, 880 m, 28 June 1992, *Ü. Güler* 1801 (GAZI!); Koşubaba Town, region of vineyards, 1050 m, 29 April 1989, *A. A. Dönmez* 1351 (GAZI!); Konya: Beyşehir to Konya, 30 miles from Beyşehir, 1310 m, cult. fields, 15 June 1962, *Dudley* 35868 (E00580956!); Konya to Aksaray, 60 miles from Konya, 1000 m, dried up marsh, 17 June 1962, *Dudley* 35899 (E00580955!); Tunceli: zwischen Pertek und Tunceli bei Yeniköy, 1400 m, 21 July 1973, *F. Holtz* 00.764 and *P. Hänel, T. Kesercioglu* (E00584876!); Van: Tarihi İskele Cad., c. 4 km W of city centre, 1700 m, disturbed gravely ground, 25 July 1987, *R. M. Nesbitt* 1255 (GAZI!). Azerbaijan. Aderbeidshan: Ad aquaductus circa Seidchadzi in humidis ad urb, *J. N. Szovits* 473 (K000929416!, LE00018069!).

Tripleurospermum disciforme: Azerbaijan. Talysch: in pratis subalipinis humidis, 18-26 June 1830, *pr. Dryck & Swant*. (type E00531487 photo!). Turkey. Ağrı: Tutak, 1575 m, damp fields, 02 June 1966, *Davis* 43988 (E00584800!); Bitlis: Tatvan, 1750 m, roadsides and cornfield weed, 04 July 1954, *Davis* 23611 and *Polunin* (E00584823!); Hakkari: Yüksekova, 1950 m, 05 September 1967, *Duncan* and *Tait* 166 (E00584801!); İzmir: Boz Dağ, roadsieds, water meadows, 1021 m, 06 July 2008, *H. İnceer* 592 (E00544034!); Muğla: Muğla to Antalya, Girdev G., 1676 m, 04 August 1947, *Davis* 13761 (E00584821!); Van: Satak, 5 km N of Satak, river banks, 25 July 1954, *Davis* 23265 and *Polunin* (E00584790!).

Tripleurospermum fissurale* subsp. *fissurale: Turkey. Artvin: From İspir to Yusufeli, 10 km to Yusufeli, roadsides, 653 m, 31 May 2008, *H. İnceer* 533 (E00544032!); Yusufeli to İspir 2 km, rocky slopes, 605 m, 25 May 2019, *U. Özbek* 3119 (GAZI!); Yusufeli-İspir road, around Kozakura district, on the rock, 590–620 m, 17 May 2004, *H. Duman* 9416 (GAZI!); Kars: d. Olty prope p. Ukiam, 17 May 1912, *D. Sosnowsky* s.n. (type E00584829 photo!).

Tripleurospermum microcephalum: Turkey. Bingöl: In declivibus saxosis 3 km E of Solhan, 1500 m, 24 June 1974, *K. H. Rechinger* 49933 (E00584882!); Hakkari: 11 km from Semdinli to Yüksekova, 1600 m, edge of fields, 16 June 1966, *Davis* 44987 (E00584880!); Muş: prov. Musch ad radices australes Bimgoell montis ad Gumgum in districtu Warto; pagum Koweg, 15 August 1859, *T. Kotschy* 286 (K000929420!); 8 km N. of Muş, 1400 m, 9 July 1966, *Davis* 46114B (E00584887!); From Muş to Solhan, cultivated area, 1249 m, 10 July 2008, *H. İnceer* 596 (E00544031!).

Tripleurospermum tempskyanum: Greece. Iter thessalicum; Said-Pascha in Pindo tymphaeo, 9 July 1896, *P. Sintenis* 744 (B100093716!, E00385813!, K000929382!, M0030161!).