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A new *Achillea* (Asteraceae) species from southwestern Turkey

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Abstract: *Achillea alimeana* Semiz & Uysal sp. nov. of sect. *Santolinoidea* DC. (Asteraceae), was described, diagnosed and illustrated as a new species. It was collected from Akdağ Mountains in Denizli Province. In this paper, the diagnostic characters of *A. alimeana* and its similar species were discussed in the morphological, palynological and molecular findings. As regards the new species, some notes on the ecology, conservation status, and distribution were submitted. A diagnostic key was given for related taxa.

Key words: Compositae, ITS, rDNA, palynology, *Santolinoidea*, SEM, Anatolia

1. Introduction

The Asteraceae (Compositae) is one of the largest flowering plant families in the world (Hattori and Nakajima, 2008). The transformation of systematics, particularly molecular analysis, has occurred on a large scale as a result of the family's recent milestone. Currently, the family includes approximately 24,000–30,000 species in more than 1600 genera (Funk and Robinson, 2005; Kadereit and Jeffrey, 2007; Kubitzki, 2007) throughout the world.

The tribe *Anthemideae* Cass. has 111 genera and around 1800 species, according to the most current generic conspectus in Compositae (Oberprieler et al., 2007a). *Achillea* L. is a member of the *Anthemideae* tribe and is represented by about 115 taxa in the world (Aytaç et al., 2016). According to the last check list of the Flora of Turkey, *Achillea* has 6 sections and 60 taxa, so that 55 % (33) of them are endemic to Turkey (Arabacı, 2012; Aytaç et al., 2016). Many sections of the tribe have more than 10 capitula on their inflorescence excepting sect. *Arthrolepis* Boiss. and sect. *Santolinoidea* DC. (Huber-Morath, 1975, 1986). Additionally, *Achillea* species in sect. *Santolinoidea* are characterized by typical leaf shapes, with primary leaflets appearing transverse to the rhachis, and segments being positioned imbricate, phyllaries persistent, not articulate at base (Huber-Morath, 1975; Valant-Vetschera and Kästner, 1998; 2000). Mainly, the genus is distributed in inner, eastern, and southeastern Anatolia, but *Achillea cretica* L., *Achillea nobilis* L. subsp. *densissima* (O. Schwarz ex Bässler) Hub.-Mor., and *Achillea grandifolia* Friv. are distribution in the southwestern parts of Anatolia (Aytaç et al., 2016).

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According to the first detailed light microscopic studies of *Anthemideae* pollen (Wodehouse, 1926, 1935), it is described that the pollen is tricolporate, echinate having a coarse-granular two layered exine. Afterwards, many studies have carried out about the tribe palynology (Stix, 1960; Çigurjaeva and Tereškova, 1983; Benedí i González, 1987; Federončuk and Savitskii, 1988; De Leonardis et al., 1991; Oberprieler et al., 2009). These studies revealed that the presence of *Anthemis* L. type pollen grains, which are rather uniformly sphaeroidal, trizonocolporate and spiny with tenuimarginate, sharply pointed colpi and alonate. The genus *Achillea* is represented by *Anthemis*-type pollens (Stix, 1960).

Phylogenetical studies including *Achillea* proposed a new subtribal classification within Compositae-*Anthemideae* (Oberprieler et al., 2007b; Himmelreich et al., 2008) and it was broadly adopted. The subtribes are divided into four groups based on their biogeographical patterns in the evolutionary reconstructions: (1) Southern Hemisphere Grade, (2) Asian-Southern Africa Grade, (3) Eurasian Grade, and (4) Mediterranean Clade (Oberprieler et al., 2009). As a result, *Achillea* was evaluated as a member of *Matricariinae* into the third grade.

During the field studies concerning floristic botany in southwestern Anatolia, some interesting specimens belonging to *Achillea* were collected from the Akdağ Mountain (C2, Çameli district of Denizli). All samples were compared with other similar *Achillea* taxa and their specimens which are deposited in various herbaria in Turkey (KNYA) and abroad such as BM and MNHN.

Additionally, the relevant literature was searched (Huber-Morath, 1975, 1986; Richardson, 1976; Valant-Vetschera, 1996, 2000; Duman, 2000; Arabacı and Yıldız 2006a, 2006b, Oberprieler et al., 2009). After the detailed comparisons based on morphology, palynology and molecular studies, it has been decided that our samples belonged to hitherto undescribed species of sect. *Santolinoidea* and the new species was related to *Achillea aleppica* DC. subsp. *aleppica* and *Achillea tenuifolia* L.

2. Materials and methods

2.1. Plant materials

Specimens were collected from the Akdağ Mountain in Çameli district (around Kargın Plateau), Denizli Province in the southwestern Turkey by the first author. The specimens were identified using the current literature (Boissier, 1875; Huber-Morath, 1975, 1986; Duman, 2000; Arabacı, 2006, 2012) and compared with herbaria materials stored in KNYA, BM and MNHN. Plant names were checked using the IPNI database (2012), the study of Brummit and Powell (1992) was used to check the author names of plants and pollen morphology terminology was employed in accordance with Punt et al. (2007).

2.2. Morphological and palynological studies

The morphological data were obtained from micro- and macromorphological examinations of light binocular and scanning electron microscopy (SEM) techniques. These microscopes were used for the detailed description of the new species. SEM was used to analyze the pollen and seed morphologies at Pamukkale University. For the SEM investigation, the pollen was treated with 70% ethanol and then dried before being mounted on stubs with gold-palladium. Samples were coated with Quorum 150 RS Au-Pd, and SEM photomicrographs were taken with a Zeiss Supra 40 VP Electron Microscope at Pamukkale University. For the seed SEM analyses, dehydration of at

least 10 seeds of the species was conducted by utilizing an alcohol series, which comprised concentrations of 70%, 80%, 96%, and 100%, for the purpose of cleaning. The achenes were then coated using gold-palladium, followed by an examination that was conducted at magnifications of 137, 486 and 554× using an SEM high-vacuum microscope (Zeiss Supra 40 VP) to conduct an observation of the surface structures. The terminology that was used for the achene characteristics was that which was given by Stearn (1983) as well as that of Koul et al. (2000).

2.3. Molecular studies

Molecular studies had been carried out based on the DNA sequence analysis, ITS gene region of ribosomal DNA. The DNA extractions on the new species and the closest taxon were performed in our laboratory (MPSL-KNYA). *Achillea alimeana* and morphologically related taxa were identified. Next, the new taxon and *A. aleppica* subsp. *aleppica* (A.Duran 6573) were isolated (Doyle and Doyle, 1987; Soltis et al., 1991; Cullings, 1992) and sequenced (for ITS protocol; Uysal et al., 2016). Sequences of other related species were downloaded from the gene bank (Table 1). Sequences of all taxa were aligned in Bioedit (version 7.0.5.3; Hall 1999). Parsimony analyzes with the PAUP program and Bayesian analyzes with MrBayes were performed from the data set obtained from ITS data. For parsimony analysis; heuristic searches were performed by utilizing PAUP software (version 4.0b10; Swofford, 2002), in addition to tree bisection and reconnection branch swapping, and character states were categorized as unweighted and unordered. For the bootstrap (BS) analyses (Felsenstein, 1985), 1000 heuristic search replicates were utilized and set at the default settings. Both the retention index (RI) and consistency index (CI) were given to the strict consensus tree, with the exclusion of the uninformative characters. For Bayesian analysis, the analyses were conducted by utilizing random starting

Table 1. Voucher specimens for the taxa in the ITS study.

Taxa	GenBank	Author, year
<i>Achillea alimeana</i>	OL378196	Current study
<i>A. aleppica</i> subsp. <i>aleppica</i>	OL378197	Current study
<i>A. pseudoaleppica</i>	DQ267612	Ehrendorfer and Guo 2006
<i>A. vermicularis</i>	DQ267613	Ehrendorfer and Guo 2006
<i>A. teretifolia</i>	AY603255	Guo et al., 2004
<i>A. alpina</i>	KX670802	Jung et al., 2016
<i>A. tenuifolia</i>	KM589804-KM589830	Tomasello et al., 2014
<i>A. millefolium</i>	MZ191016	Huixia and Kejian 2021
<i>A. cretica</i>	AY603252	Guo et al., 2004
<i>Tanacetum vulgare</i>	AY603264	Guo et al., 2004

trees, which were then run for 1×10^5 generations, for 2 independent 4 Metropolis-coupled chain runs. For every 1000 generations that were run, only 1 was recorded. The run output was examined for convergence, by considering the standard deviation of the split frequencies that were near 0.001. The first 1000 samples (20%) were considered to be burned-in and were removed after they were visually examined with regards to the likelihood score plots. In PAUP, a model search was performed according to the Akaike information criterion (AIC) with the automatic model selection command and the best fit evolution model was TPM3+I (ITS). Bayesian phylogenetic analyzes were performed with the MrBayes, under the TPM3+ I model. The stationary of the runs, as well as the convergence between the runs, were examined by utilizing Tracer software (version 1.7.0; Rambaut et al., 2018).

3. Results

Achillea alimeana Semiz & Uysal **sp. nov.** (Figures 1–3; Table 2).

Type:—TURKEY. Denizli: Çameli, Akdağ Mountain, Kargın Plateau, on limestone, 1700–1900 m, 19 June 2020, G.Semiz GSE 2146 (holotype: PAUB!; isotype: KNYA!).

Diagnosis: The peduncle of the new species is distinct and longer than its relatives and it rises to 2 cm (not without peduncle, but further than 4 or 7 mm). The corymb width in *A. alimeana* is less compared to the other two close taxa and the furthest they can broaden to 2 cm (not as being

7 cm in *A. tenuifolia* and up 5 cm in *A. aleppica* subsp. *aleppica*). The ligules of *A. alimeana* are more number (5–6 many) and yellow (not 3–5 or 2–4, not golden yellow or ivory white). Additionally, the disc flowers of the new species are more than *A. aleppica* subsp. *aleppica* [not 6–15(–20)]. The fruits are similar in *A. alimeana* and *A. aleppica* in which are oblong or oblong-narrowly obovoid unlike *A. tenuifolia* with ovate-oblong one.

Description: Perennial herb with woody rootstocks. Stems erect ascending, numerous, 40–60 cm long, with short sterile shoots, branched from the base, with stria and adpressed to subadpressed floccose hairy. Leaves homomorphic, with arachnoid or floccose hairy, linear to filiform, median cauline ones 8–18(–20) \times 0.8–1.2 mm, pinnatisect, segments ovate or semiobicular, 0.5–0.8 \times 0.2–0.5 mm, 1–3-denticulate, apiculate. Peduncles 2–22 mm. Capitula (2–)4–8(–10) per stem, (4.5–)5–6 \times (3–)3.5–4 mm, broadly rounded at base, corymbs extend to 2 cm. Involucre ovoid to hemispherical and angular, (3.8–)4–5 \times (3.4–)4–5(–6) mm, arachnoid hairy. Phyllaries in 3–4 series, outer ones 10–12 mm, ovate-oblong, median ones oblong-orbicular, inner ones lanceolate, all phyllaries scarious-margined, outers ones pannose on the middle vein. Receptacle paleaceous; palea 1.5–2 \times (1–)0.2–0.3 mm, linear-lanceolate, membranaceous. Ligules 5–6, yellow, 3–4.2 mm long, with three lobes; tube 2.3–2.5 mm long, lobes 1.2–1.7 \times 1.2–1.5(–1.8) mm, almost orbicular in outline. Anthers cream, slightly exserted from the tube.

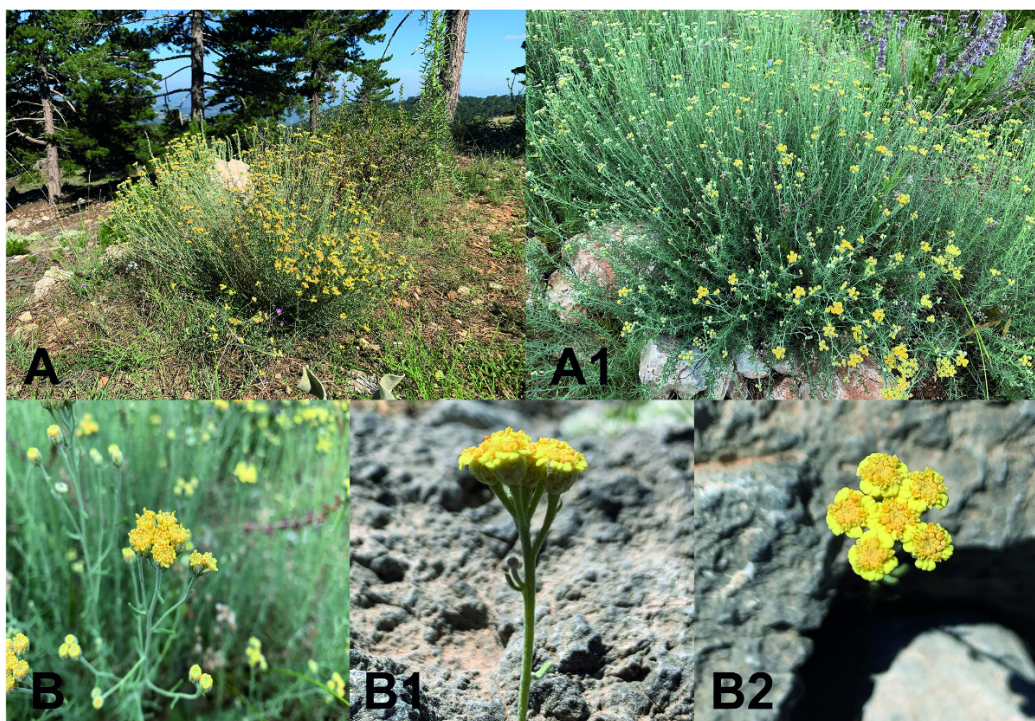


Figure 1. *Achillea alimeana* A-A1: Plant habit B: Inflorescens B1-B2: Corymbs.

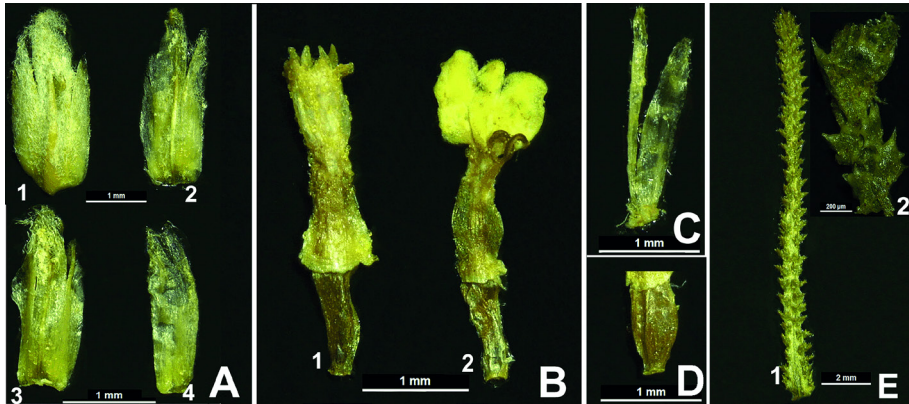


Figure 2. *Achillea alimeana* A- outer (1, 2) and inner phyllaries (3, 4), B- tubular (1) and ray (2) flowers, C- palea, D- achene, E- stem leaves.



Figure 3. Scanning electron micrographs of achene in *Achillea alimeana*.

Disc flowers creamish yellow, 20–30, 3.5–4 mm, slightly exserted from involucre; style orange, 1–2 mm long, stigma two-partite as long as style. Cypselae oblong to narrowly obovoid, 2–3 mm long, compressed dorsally; pappus absent.

Phenology:—Flowering June–July and fruiting July–September.

Group B [This key has been modified from *Flora of Turkey* (Volume 5, pp. 226–227)]

1. Ligules 3–8

Table 2. The morphological comparison of *Achillea alimeana* and related taxa.

Character	<i>A. alimeana</i>	<i>A. aleppica</i> subsp. <i>aleppica</i> (Huber-Morath, 1975; Arabacı, 2006)	<i>A. tenuifolia</i> (Arabacı, 2006)
Plant	Erect-ascending, 40–60 cm	Ascending, 5–60 cm	Ascending 25–90 cm
Indumentum	Floccose hairs	Adpressed pilose	White tomentose
Stem	Branched, deeply furrowed	Simple, longitudinally striped	Branched, deeply furrowed
Leaves	Linear to filiform, median cauline ones 8–18 (–20) × 0.8–1.2 mm, pinnatisect, densely imbricate, with ovate or semiorbicular, 1–3-denticulate and apiculate segments	Linear-filiform, median cauline (5–) 10–40 (–50) × 0.8–1.5 cm, pinnatisect, segments densely, at last ± loosely, imbricate, 0.5 mm, 3-lobed, lobes broadly ovate to orbicular, denticulate	Linear, median cauline (1–)2–4 × (0.1–)0.2–0.6(–1) cm, pinnatisect, segments simple, 3-dentate or 3-lobed, cuspidate, loosely arranged, not imbricate, linear-lanceolate, rarely narrowly cuneate, margins entire
Peduncle	Up to 20 mm	Up to 4 mm	Up to 7 mm
Capitula	(2–) 4–8 (–10) per stem, corymbs 2 cm broad	(4–) 6–120 per stem, corymbs 1–4.5 cm broad	(10–) 20–70 (–100) per stem, corymbs 2–5(–7) cm broad
Involucrum	Ovoid to hemispherical and, angular, (3.8–) 4–5 × (3.4–) 4–5 (–6) mm	Oblong cylindrical, angular, 3.5–5.5 × 1.5–2.5 mm	Ovoid to hemispherical, 3–4.5 × 2.5–4.5 mm
Ligules	5–6, yellow	2–4, ivory white	3–5, pale or golden yellow
Disc flowers	creamish yellow, 20–30, 3.5–4 × 0.3–0.4 mm	yellow, 6–15 (–20), 2.5–3 × 0.5 mm	yellow, 20–35, 3 × 0.5 mm
Palea	Linear-lanceolate, 1.5–2 × (1–) 0.2–0.3 mm	Lanceolate, 3.5 × 1 mm	Lanceolate, 3.5–4 × 1 mm
Cypselae	Oblong, 2–3 × 0.3–0.6 mm	Ovate-oblong, 1.4–2.5 × 0.4–1 mm	Oblong-oblong, 1.5–2 × 0.5–0.8 mm

5. Involucre oblong-cylindrical, about 2× as long as broad

6. Lowest phyllaries triangular-ovate to lanceolate, adpressed *aleppica*

6. Lowest phyllaries subulate, linear or linear-lanceolate, spreading *pseudoaleppica*

5. Involucre oblong ovoid to hemispherical, not more than 1.5× as long as broad

7. Stem terete, longitudinally striate or obtuse-angled, not furrowed; ligules white or yellow

7. Stem deeply furrowed; ligules yellow

8. Involucre hemispherical or somewhat depressed, 5–6 × (5–)6–8 mm

8. Involucre ovoid to hemispherical, 3–4.5 × 2.5–4(–5) mm

9. Leaves with loosely arranged, not imbricate, linear lanceolate, simple or tripartite, cuspidate segments, margins entire

10. Peduncle absent or sometimes up to 7 mm; fruit ovate-oblong *tenuifolia*

10. Peduncle up to 20 mm in length, fruit oblong to narrowly obovoid *alimeana*

9. Leaves with dense or loosely imbricate, tripartite, oblong to lanceolate segments, margins denticulate

..... *lycaonica*

1. Ligules 3–8 others

Pollen structure:—Pollen grains of *A. alimeana* are oblate spheroidal, tricolporate. P = (19.35)22.01 ± 1.29(24.57), E = (20.09)23.09 ± 1.48(25.05). Ornamentation is echinate to microperforate (Figure 4), P/E is oblate spheroidal (Erdtmann, 1957).

Achene structure:—Achene oblong-lanceolate, 1.76 ± 0.16 × 0.62 ± 0.08 mm, ribbed, glabrous, slime cells over the entire surface (Figure 2D).

Phylogeny:—The new taxa is phylogenetically taken place together with the taxa of *Santolinoidea* section (Figure 5). In phylogram, the most close of the new taxa is seen *A. aleppica* subsp. *aleppica* as well as other relatives comprising *Achillea vermicularis* Trin., *Achillea pseudoaleppica* Hub.-Mor., *Achillea teretifolia* Willd., *A. cretica*, respectively (PP:0.84/BS:78).

Etymology:—Ms. Alime Çetiner was a nature lover and made great motivational contributions to her husband Rasim Çetiner during the discovery of the species. Hereby,

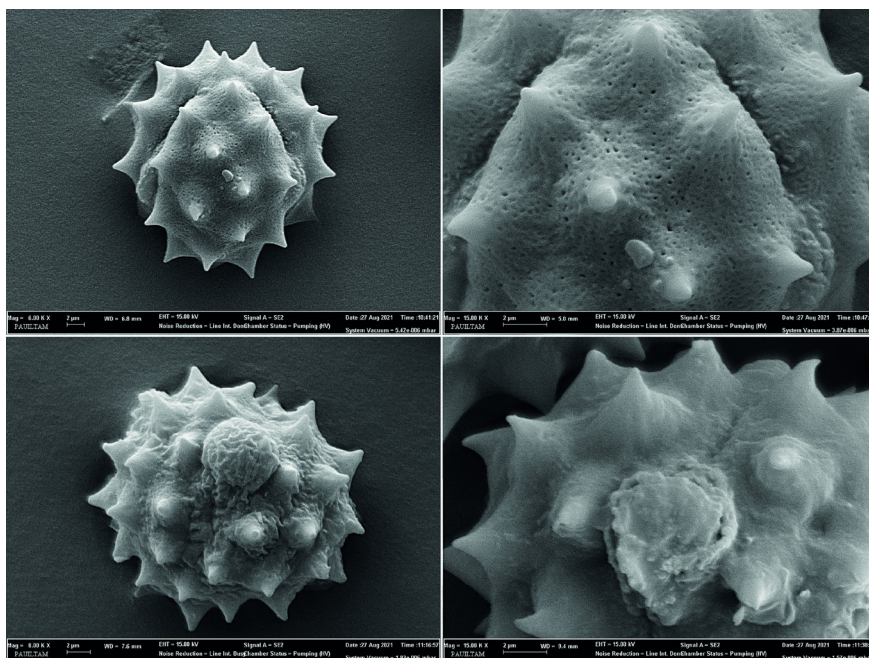


Figure 4. Scanning electron micrographs of pollen grains of *Achillea alimeana*.

A. alimeana was named in honor of the deceased wife of Rasim Çetiner who is the forest engineer and also is known as a “citizen scientist”.

Proposed Turkish name: The Turkish name of this species was given as “Hanım perçemi” according to the guidelines of Menemen et al. 2016.

Ecology:—*Achillea alimeana* grows on limestone under *Pinus nigra* J.F.Arnold subsp. *pallasiana* (Lamb.) Holmboe forest at altitudes of 1700–1900 m, together with *Scutellaria brevibracteata* Stapf subsp. *brevibracteata*, *Echinophora tournefortii* Jaub. & Spach, *Echinops emiliae* P.H.Davis, *Nepeta nuda* L. subsp. *lydiae* P.H.Davis and *Salvia chrysophylla* Stapf.

Conservational status: The specimens were collected at Denizli, near places of the summits of Akdağ Mountains (Çameli; Denizli Province). The distribution of *A. alimeana* can be defined as very local and is restricted to a single location (IUCN Criteria B1a). The current populations of the species are pure, with an occupied area of less than 10 km², and field observations suggest that the total number of individuals of this endemic species in a single locality does not exceed 250 (Criteria B2a). Based on our expertise, we believe that the species could be classified as critically endangered (CR), but we believe that further data are required to accurately quantify or estimate its threat category.

4. Discussion

It has been reported that the region of Central Anatolia is the main differentiation center of the *Santolinoidea* section for

Turkish Flora (Huber-Morath, 1975). The locality (Denizli Province) of the new species situates a transition gateway between Aegean, Central Anatolia and Mediterranean Regions. Therefore, it can be thought that this transition point would be the most extreme southernwest position of the main differentiating center for *Santolinoidea*. Interestingly, another species *Rindera cetineri* Yıldırım and *Linum punctatum* Presl subsp. *pyncnophyllum* (Boiss. & Heldr.) Gustavsson have also been discovered recently from the same locality with *A. alimeana* (Yıldırım, 2019; Semiz and Yılmaz, 2020). In point of bedrock, the species of *Achillea* may show different behaviors or choices, but they usually prefer stony rocky places, forest clearings and steppes. The members of *Santolinoidea* section spread in volcanic, limestone, conglomerate and limestone rocks. Especially, *A. alimeana* displays a distribution in the limestone cliffs of *Pinus nigra* forest clearings. In *Achillea* species, whether the stem structure is simple or branched, the number of capitula is important in the determination of the species. It is a rare feature in the genus that the stem shows continuous branching starts from the base. As well as being large and flamboyant, *A. alimeana* and *A. tenuifolia* are also the species that attract attention at first glance with their dense stem branching in their natural life. In terms of capitula number, the genus displays a broad variation, so that few species have one capitulum while others have 1–10 and sometimes more than 10 (Aytaç et al., 2016). *A. alimeana* has up to ten capitula in each stem. *Achillea alimeana* is related to *A. tenuifolia* and *A. aleppica* subsp. *aleppica*, but it has some important differences

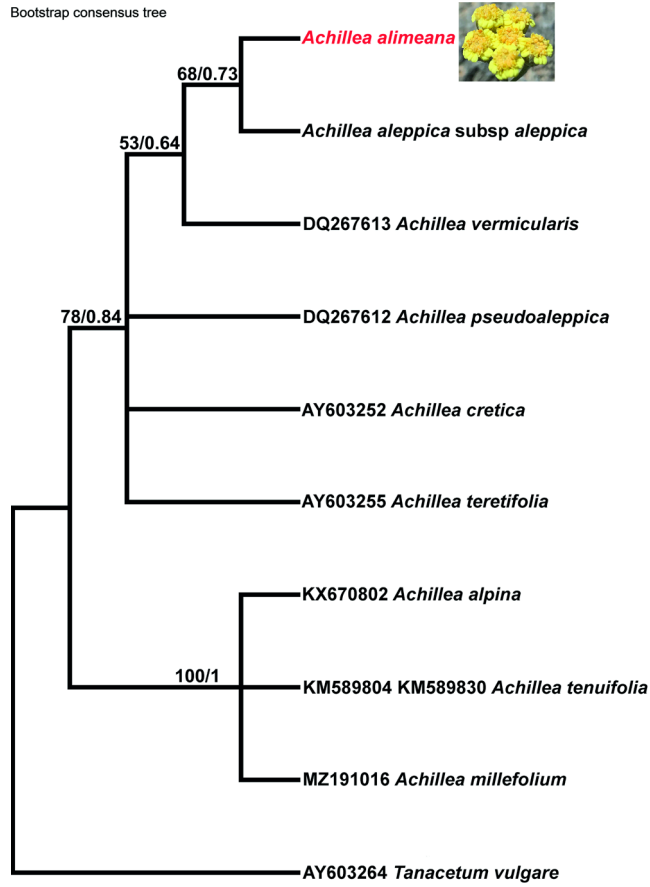


Figure 5. Majority rule consensus tree obtained from Parsimony and Bayesian analysis of the ITS data for *Achillea alimeana* and related taxa (tree length: 619, CI: 0.857, RI: 0.737, HI: 0.143). Values in the branches are shown as maximum parsimony bootstrap/ Bayesian posterior probabilities.

from similar species in terms of characters such as stem structure and branching shape, leaf type, peduncle length, capitula number, corymbs width, ligules numbers and fruits. *A. aleppica* was represented with two subspecies in Check List of the Flora of Turkey (Arabacı, 2012). The new species seems to be more close to *A. aleppica* subsp. *aleppica* comparing to the other subtaxon. For this reason, taxonomic and morphological relationships between the new species and this taxon were emphasized in here. The new taxon is also similar to *A. tenuifolia* in point of stem and branching and both of them have long and include deeply furrowed and many-branched stems; the ascendant and long lateral stems continue from the base to upwards repeatedly unlike *A. aleppica* subsp. *aleppica* which has usually simple stem. In point of the indumentum, *A. alimeana* is clearly different by having floccose hairs (not adpressed pilose or glabrescent). The new taxon and its relatives share a similar leaf shape but also there is a certain

difference among them; the leaves are pinnatipartite or pinnatisect in all taxa and their segments are divided into three lobes. While the leaf segments in *A. alimeana* are neither loose nor tight, they are further tight in *A. aleppica* subsp. *aleppica*. As a common feature, the segments are ovate-orbicular and denticulate for both of them. Comparison to these taxa, the leaf segments are too loose, linear-lanceolate and entire in *A. tenuifolia*. Besides these, the new taxon has many specific morphologic characters which are discussed as a diagnosis in the text and the determinative features have been submitted comparatively in Table 2.

In recent years, palynology and molecular findings of flowering plants have been very supportive and effective in the given decision of the new species (Aytaç et al., 2016; Uysal et al., 2016; Özbek, 2021; Özbek et al., 2021; Yin et al., 2021; Ertuğrul et al., 2021). Pollen structure for two species of *Santolinoidea* section, recently published for Flora of

Turkey, has been reported as oblate-spheroidal (Aytaç et al., 2016). It is also known that most of the species have pollen grains with the same characteristics (Akyalçın et al., 2011; Akyalçın et al., 2014). The pollens of *A. alimeana* are exactly suitable with the given literature; they are oblate-spheroidal. The pollen ornamentation of the new species is echinate in light microscopy and echinate-microporate and microporate in SEM. Therefore, we point out that many taxa of *Achillea* are fairly stable in the shapes of pollen grains and it is important to indicate that these features do not supply a serious conclusion in the description of the new species here. DNA sequence analyzes and the created phylogenetic trees have helped us to understand the phylogenetic relationships of the new species with the relatives within the relevant section. The new species is clearly related to *A. aleppica* subsp. *aleppica*. It is located in a more distant position with *A. tenuifolia*, the other species of the same section with which it is morphologically similar, and therefore shows comparatively a weaker phylogenetic relationship. Although morphologically *A. alimeana* and *A. tenuifolia* are similar in terms of capitula shape and branching characteristics, this morphologic similarity does not support at the same level in terms of phylogenetic relationships. As a result, *A. alimeana* is a new species for science and it has been described by a broad concept based on morphology, palynology and molecular data in this paper.

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