Helichrysum unicapitatum (Asteraceae), a new species from Turkey

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Helichrysum unicapitatum S.G. Şenol, Ö. Seçmen & B. Öztürk (Asteraceae) is described and illustrated from the SW province of Anatolia, Turkey. It grows on crystalline calcareous rocks between Evran Tepe and Karababa Tepe (1800–2300 m), situated within the Denizli Babadağ (Cadmus) Mountain Range. *Helichrysum compactum* seems to be its closest relative and their affinities are discussed based on morphological data and DNA sequences (nrDNA ITS and ETS).

Introduction

The tribe Gnaphalieae of Asteraceae comprises 185 genera and about 1240 species; it is cosmopolitan, but most diverse in the southern hemisphere (Bayer *et al.* 2007). *Helichrysum* is the largest genus of this tribe, with about 600 species, occurring in Europe, Asia, Africa and Madagascar (Bayer *et al.* 2007).

Since *Helichrysum* was revised by Davis and Kupicha (1975) and Davis *et al.* (1988), 18 species have been recognized in Turkey. Besides these works, one new subspecies and four new species have been described from the country: *H. plicatum* subsp. *isauricum* (Parolly 1995), *H. kitianum* (Yıldız & Kit Tan 1988), *H. sivasicum* (Yıldız & Kit Tan 1988), *H. orbicularifolium* (Sümbül *et. al.* 2003) and *H. yurterianum* (Gemici *et al.* 2008). However, according to Parolly and Eren (2006) *H. orbicularifolium* is a synonym of *H. chasmolycicum*. The author M. Galbany-Casals and her colleagues, who are currently working on a taxonomic revision of *Helichrysum* sect. *Helichrysum*, think that *H. kitianum* is a synonym of *H. graveolens*, *H. chionophilum* a synonym of *H. pallasii*, and *H. rubicundum* should be considered at the species level. After these amendments, the total number of *Helichrysum* species known from Turkey is 20.

Some specimens of *Helichrysum* originally identified as *H. chionophilum* were first collected by S. Oluk from Denizli (Babadağ), Turkey, in 1996 (EGE 34743). The authors of the present contribution examined that specimen, which lacks flowers and fruits. At first sight, the basal part looked similar to *H. compactum*, but the morphology of the capitula and especially the

brown colour of the involucral bracts did not match with that species.

In 2004 and 2005, we collected flowering and fruiting material from Denizli (Babadağ-Evran Tepe), and it became clear that the specimens were quite different from all known Turkish species due to the combination of solitary heterogamous capitula with brownish involucral bracts. A sample and several pictures of the species were sent to Dr. Arne Anderberg (Stockholm, Sweden). In accordance with his comments and the information he provided about the involucral bract characteristics, and our observations on the other of morphological traits, we describe these plants as a new species. This contribution raises the total number of *Helichrysum* species known from Turkey to 21.

The last author's molecular phylogenetic studies focused on the Mediterranean and Asiatic Helichrysum (Galbany-Casals et al. 2004, Galbany-Casals et al. 2009) made it possible to include ITS and ETS sequences of this odd Turkish species in phylogenetic analyses. Previous works already showed that the Mediterranean and Asiatic Helichrysum constitute a monophyletic group within the genus, derived from African species (Galbany-Casals et al. 2004, Galbany-Casals et al. 2009). Within this Mediterranean-Asiatic group, two groups are highly supported: one constituted by all members of sect. Stoechadina, and the other constituted by members of the traditional sect. Helichrysum and sect. Virginea. These two main clades also correlate with plant habit: sect. Stoechadina comprises shrubs or subshrubs without leaf rosettes, while sect. Helichrysum and sect. Virginea comprise plants with basal leaf rosettes (Galbany-Casals et al. 2009). In the present contribution we also determine the phylogenetic position of this new species and its evolutionary relationships with other Helichrysum species.

Material and methods

Morphological study

All morphological data presented and used in the description were directly observed by the authors. All the morphological characters traditionally used to identify *Helichrysum* species were studied and compared with those of *H. compactum* and other members of sect. *Helichrysum* and sect. *Virginea*. Features of gross morphology were examined under a stereoscopic microscope. Samples of pappus and pollen of the new species were examined gold-coated with a JEOL JSM-6060 scanning electron microscopy (SEM).

Phylogenetic study

We used the same dataset as Galbany-Casals *et al.* (2009), which included ITS and ETS sequences of 36 representatives of Mediterranean and Asiatic *Helichrysum*, five representatives of Macaronesia, and two African *Helichrysum*, these last two used as outgroup. ITS and ETS sequences of the new species were generated for this contribution (*see* Appendix).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted following the CTAB method of Doyle and Doyle (1987) as modified by Cullings (1992) from dried leaves of the type collection. ITS and ETS DNA regions were amplified, purified and directly sequenced as in Galbany-Casals *et al.* (2009).

Analyses

Nucleotide sequences were edited using Chromas 2.0 (Technelysium Pty. Ltd., Tewantin, Australia) and aligned visually by sequential pairwise comparison (Swofford & Olsen 1990).

Parsimony analyses involved heuristic searches conducted with PAUP* ver. 4.0b10 (Swofford 2002) using TBR branch swapping with character states specified as unordered and unweighted. The indels were were coded as missing data. All most-parsimonious trees (MPTs) were saved. To locate other potential islands of MPTs (Maddison 1991), we performed 1000 replications with random taxon addition, also with TBR branch swapping. Bootstrap analyses (BS; Felsenstein 1985) were performed with 100 replications and heuristic search with the default options. For the MP analyses, consistency index (CI), retention index (RI) and homoplasy index (HI) are given, excluding uninformative characters.

Bayesian inference (BI) estimation was calculated with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) using the GTR + Γ model (Yang 1996). Two simultaneous and independent analyses were performed, for each analysis four Markov Monte Carlo chains were run simultaneously starting from random trees. Each analysis was run for 2 000 000 generations sampling one out of every 200 generations, which resulted in 10 000 sample trees. A critical aspect of the Bayesian analysis is to ensure that the Markov chain has reached stationarity. With this aim, the first 1000 trees (burn-in) were excluded to avoid trees that might have been sampled prior to convergence of the Markov chains, before computing the majority-rule consensus tree. Posterior probability support (PP) was estimated to be significant for nodes with PP > 0.95.

Results

Description

Helichrysum unicapitatum S.G. Şenol, Ö. Seçmen & B. Öztürk, *sp. nova* (Figs. 1, 2 and 3)

Helichryso compacto Boiss. affinis sed scapis floriferorum brevissimis 0.4–2 cm altis (non 9–24 cm), foliis basalibus elliptico-orbicularis (non spathulatis), capitilis solitariis, rare 2–3 aggregatis (non in densum corymbum dispositis), bracteis involucralibus brunneis (non citrinis) differt.

TYPE: Turkey. C2 Denizli: Above Kızılcabölük district, Babadağ (Cadmus Mt.), Evran Mountain, 37°41′53.5′′N, 28°59′43.4′′E, 1920 m, on calcareous rocks, 13.VII.2004 *S. G. Senol & B. Öztürk* (holotype EGE 40730; isotypes BC, IZEF, S).

Caespitose, suffruticose plants, mat-forming, up to 70 cm in diameter, white-felted, eglandular. Flowering stems 0.4–2 cm, slender, erect, arising from short thick branching caudices. Basal leaves forming dense rosettes which arise directly from woody rootstock, $4-7 \times 2-5$ mm, elliptic-orbicular, concolor, densely tomentose, margin flat. Cauline leaves 5-8, more or less adpressed to stem, alternate, decreasing in size towards synflorescence; uppermost ones 4×1 mm, narrowly spathulate to linear, basal ones 6×2 mm, oblong to elliptic. Synflorescence terminal, one solitary capitulum, rarely 2-3 clustered. Capitula $4-5(-6) \times 5-6(-7)$ mm, broadly campanulate, heterogamous, disciform, with 35-65 yellow florets; pistillate florets 3-8, marginal; hermaphroditic florets 30-60, central. Involucral bracts regularly imbricate, more or less adpressed, arranged in 4 rows, brownish, papery, tomentose only in middle, stereome divided; outermost bracts $3-4 \times 1.2-1.5$ mm, ovate, obtuse; innermost bracts $4-5 \times 1-1.2$ mm, spathulate, obtuse. Corolla with tube glabrous, lobes 5, acute, outside covered by short stipitate-glandular hairs; corolla of pistillate florets with tube 3 mm long, lobes 0.3 mm long; corolla of hermaphroditic florets with tube 3.5 mm long, lobes 0.5 mm long. Style thickened at base, gradually narrowing upwards, in pistillate florets 2.5 mm long and in hermaphroditic florets 3-4 mm long. Stigma 0.8-1 mm long, with apices slightly thickened. Anthers ecalcarate, 2 mm long, apical appendage acute, basal appendages long-tailed, 0.2 mm long. Pollen grains 19–20 μ m in diameter, spheroidal, echinate (Fig. 2). Achenes ca. 0.7-1 mm long, cylindrical, light brown, with some scattered duplex hairs. Pappus bristles ca. 4 mm long, whitish, uniform, uniseriate, caducous, with ca. 20-25 barbellate hairs and short patent cilia at base; apical cells obtuse (Fig. 3). Flowering June-August; fruiting July-August.

ECOLOGY: Helichrysum unicapitatum grows on high mountains, at an altitude of 1800– 2300 m, on crystalline calcareous rocks, together with Helichrysum compactum, Astragalus angustifolius ssp. angustifolius var. angustifolius, A. angustifolius ssp. angustigolius var. violaceus, Linum aretioides, Pterocephalus pinardii, Galium aretioides, Minuartia pestalozzae and Dianthus leucophaeus var. leucophaeus.

DISTRIBUTION: *Helichrysum unicapitatum* is an endemic of the southwestern Anatolian province (Fig. 4). It occurs between the northeast of



Fig. 1. Helichrysum unicapitatum. — A and B: Habit. — C: Floret. — D: Achene. — E: Anther. — F: Basal leaves. — G: Lower cauline leaves. — H: Upper cauline leaves. — I: Involucral bracts (outer to inner). — Illustration by Beril Sümer Şenol.



Fig. 2. Pollen grain of *Helichrysum unicapitatum* (scale bar = $5 \ \mu$ m). SEM.



Fig. 3. Pappus bristles of Helichrysum unicapitatum (scale bar = 100 μm). SEM.



Fig. 4. Distribution map of *Helichrysum unicapitatum* (●) and *Helichrysum compactum* (□) in Turkey.

Denizli Babadağ mountain series Evran Tepe and Karababa Tepe.

Phylogenetic analyses

The number of parsimony informative characters was 151 (Table 1). Both parsimony and Bayesian inference analyses showed highly congruent topologies. Therefore, only the Bayesian consensus tree is shown (Fig. 5), with Bayesian posterior probabilities (PP) below branches and bootstrap values above branches.

Within the Mediterranean-Asiatic-Macaronesian clade, the four Madeiran endemic species constitute a monophyletic group (BS = 100%; PP = 1, clade E), a sister to the rest of the species (BS = 100%; PP = 1). Helichrysum gossypinum, from Lanzarote, is a sister to the whole Mediterranean-Asiatic group (BS = 88%; PP = 0.99, D). In the Mediterranean-Asiatic group, two main groups, sisters to each other, are highly supported: a clade that includes all the species of sect. Stoechadina (BS = 100%; PP = 1, clade C); and another clade that comprises all the representatives of the traditional sect. Helichrysum and sect. Virginea (BS = 100%; PP = 1, Clades A + B). *Helichrysum unicapitatum* constitutes a highly supported clade with H. compactum (BS = 86%; PP = 1), *H. noëanum* being sister to this clade (BS = no support; PP = 0.95). These three Turkish endemics are placed in an unresolved clade (clade B) which comprises several species from the central and eastern Mediterranean area and western Asia.

Discussion

Helichrysum unicapitatum bears a combination of morphological characters that makes it different from all other known Helichrysum species: its dwarf habit, with very short stems, and the solitary and heterogamous capitula with brownish involucral bracts. Regarding the habit, H. unicapitatum has basal leaf rosettes arising from woody stocks, as do also the species belonging to sect. Helichrysum and sect. Virginea (see Galbany-Casals et al. 2009), but the leaves of H. unicapitatum are orbicular and much smaller than in any other species. Solitary capitula are rarely found in Helichrysum, but they occur in H. doerfleri and H. sibthorpii, both Greek endemics but characterized by white involucral bracts and capitula noticeably larger than those of H. unicapitatum.

Helichrysum compactum is the most similar species in terms of the above mentioned vegeta-

Table 1. Results of numerical analyses. The consistency, retention and homoplasy indices are calculated excluding uninformative characters.

Number of taxa	44
Aligned length	1630
Parsimony analyses	
Parsimony informative characters	151
Number of MPTs	528
Number of steps	281
Consistency index (CI)	0.6655
Retention index (RI)	0.3345
Homoplasy index (HI)	0.8726
Bayesian inference	
Model of molecular evolution	GTR + Γ



Fig. 5. Consensus tree obtained from the Bayesian analysis of the combined ITS-ETS sequences dataset. ≥ 0.95 Bayesian posterior probabilities are shown below branches, > 65% bootstrap values from the parsimony analyses are shown above branches.



Fig. 6. *Helichrysum compactum* (**A**) and *H. unicapitatum* (**B**) in their natural habitat (Turkey, C2 Denizli, Babadağ-Evran Mountain, 1900 m). — Photograph by Serdar Şenol, 14 July 2005.

tive characters. The two species are sympatric over the Babadağ Mountains (Denizli, Turkey) (Fig. 4), but can be readily distinguished by the morphological features (*see* Table 2). *Helichrysum compactum* can be mostly found over the southern parts of the Babadağ Mountains (Denizli, Turkey) up to an altitude of 1900 m, while at higher altitudes it disappears and is replaced by *H. unicapitatum*, which occurs between 1800 and 2300 m. Between 1800 and 1900 m, *H. compactum* and *H. unicapitatum* can be found growing together (Fig. 6). According to Davis and Kupicha (1975), the type locality of *H. compactum* is "Turkey C2: Denizli] in rupibus calidis Cadmi pone urbem Denisleh, vi 1842, Boissier", also quoting other collections of this species from Antalya Elmalı Da., between 1200 and 1400 m. Based on our field observations, *H. compactum* occurs also at two other Turkish localities: C2, Burdur, Altınyayla, above Ballık (Maşda) village, Boncuk Mountains 1500–1600 m, on dolomite rocks (SG 3443), and B2, Denizli, Çivril-Işıklı village, 1000 m (EGE 25902). Therefore, the altitudinal range of *H. compactum* is 1000–1900 m, much broader than hitherto known.

Regarding the phylogenetic analyses, both Bayesian and MP consensus tree show *H. unicapitatum* closely related to *H. compactum* (Fig. 5), which supports our morphological con-

Characters	H. unicapitatum	H. compactum	
Flowering stems (cm)	0.4–2	9–24	
Lamina of basal leaves	elliptic-orbicular	spathulate	
Basal leaves (mm)	4–7×2–5	7–20 × 2–4.5(6.5)	
Lamina of cauline leaves	upper narrowly spathulate to linear, lower oblong to elliptic	linear	
Cauline leaf size (mm)	upper 4 \times 1; lower 6 \times 2	up to 10×2	
Capitula number per synflorescence	1(2–3)	6–35	
Capitula width (mm)	5-6(7)	3.5–5	
Sex distribution	heterogamous	homogamous	
Involucral bract colour	brown	lemon or straw yellow	

Table 2. Comparison of the diagnostic characteristics of Helichrysum unicapitatum and H. compactum.

clusions. Both species are placed in a nonsupported clade (B) mainly formed of species with a central or eastern Mediterranean distribution area, except H. plicatum, which is also distributed in western Asia, and H. noëanum, which is endemic to inner Turkey. Given that phylogenetic analyses do not show members of sect. Helichrysum and sect. Virginea in two independent clades (Fig. 5; see also Galbany-Casals et al. 2009), and that the infrageneric classification of this group is currently being revised (M. Galbany-Casals unpubl. data), H. unicapitatum is not formally assigned to any section, although by its morphological characters, i.e. the florets being as long as the involucral bracts, it would fit in sect. Helichrysum.

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Таха	Origin	ITS accession	ETS accession
H. amorginum Boiss. & Orph.	Greece, Amorgos, <i>Georgiadou 1494</i>	FJ211430,	FJ211562
H. arenarium (L.) Moench subsp. arenarium	Ukraine, Voroshilovgrad region, Deripova s.n. (LE)	FJ211488 FJ211425, FJ211483	FJ211553
H. arenarium (L.) Moench subsp. aucheri (Boiss.) P.H. Davis & Kunicha	Turkey, Erzurum, <i>Susanna 2384 & al.</i> (BCN 6126)	AY445197	FJ211570
H. armenium DC.	Turkey, Adiyaman, <i>Susanna 2346 & al.</i> (BCN 6127)	AY445195	FJ211577
H. artemisioides Boiss. & Hausskn.	Iran, Kerman, <i>Termeh & al. s.n.</i> (IRAN 35987,3)	FJ211438, FJ211496	FJ211574
H. chasmolycicum P.H. Davis	Turkey, Antalya, Mutlu 8591 (HUB)	FJ211432, FJ211490	FJ211565
H. compactum Boiss.	Turkey, Antalya, <i>Sorger 67-21-105</i> (W 1992-8330)	FJ211434, FJ211492	FJ211569
H. crassifolium (L.) D. Don	Spain, Balearic islands, Majorca, Galbany & Sáez s.n. (BCN 6117)	AY445200	FJ211541
H. devium J.Y. Johnson	Portugal, Madeira island, <i>Jardim s.n.</i> (MADJ)	FJ211441, FJ211499	FJ211579
H. doerfleri Rech. f.	Greece, Crete, Turland 776 (BM 98239)	FJ211431, FJ211489	FJ211564
H. gossypinum Sch. Bip.	Spain, Canary Islands, Lanzarote, Galbany & Arrabal s.n. (BCN 25226)	FJ211440, FJ211498	FJ211578
H. graveolens (M. Bieb.) Sweet	Turkey, Ankara, <i>Sorger 77-52-20</i> (W 1992-8328)	FJ211436, FJ211494	FJ211572
H. heldreichii Boiss.	Greece, Crete, <i>Garnatje 134 & Luque</i> (BCN 6123)	AY445204	FJ211552
H. italicum (Roth) G. Don subsp. italicum	Bosnia-Herzegovina, Herzegovina, Redžić & al. s.n. (BCN 20756)	FJ211422, FJ211480	FJ211548
H. italicum (Roth) G. Don subsp. microphyllum (Willd.) Nyman	Spain, Balearic islands, Majorca, Galbany & Sáez s.n. (BCN 6115)	AY445208	FJ211546
H. italicum (Roth) G. Don subsp. siculum (Jord. & Fourr.) Galbany, L. Sáez & Benedí	Italy, Sicily, Galbany s.n. (BCN 6116)	AY445205	FJ211547
H. lacteum Coss. & Durieu	Algeria, Batna, <i>Dubuis s.n.</i> (MA 370557)	AY445206	FJ211557
<i>H. leucocephalum</i> Boiss.	Iran, Prov. Kerman, <i>Assadi & al. 1785</i> (E 191281)	FJ211433, FJ211491	FJ211566
H. litoreum Guss.	Italy, Vesuvio, <i>Lluent & al. s.n.</i> (BCN 20755)	FJ211424, FJ211482	FJ211551
<i>H. maracandicum</i> Popov <i>ex</i> Kirp.	Kyrgyzstan, Tian-shan, <i>Vašák s.n.</i> (W 1992-16084)	FJ211427, FJ211485	FJ211558
H. melaleucum Rchb. ex Holl	Portugal, Madeira island, <i>Jardim s.n.</i> (MADJ)	FJ211443, FJ211501	FJ211581
H. milfordiae Killick	Lesotho, <i>ex</i> Roy. Bot. Gard. Kew (BCN 6101)	AY445209	FJ211537
H. monizii Lowe	Portugal, Madeira island, <i>Jardim s.n.</i> (MADJ)	FJ211444, FJ211502	FJ211582
<i>H. mussae</i> Nevski	Tadzhikistan, Zeravshchan mts., Filatov & al. 81 (LE)	FJ211426, FJ211484	FJ211555
<i>H. noeanum</i> Boiss.	Turkey, Sivas, <i>Aytaç 6710 & al.</i> (MA 590804)	AY445211	FJ211568
H. obconicum DC.	Portugal, Madeira island, <i>Jardim s.n.</i> (MADJ)	FJ211442, FJ211500	FJ211580
	(10211000	continued

Appendix. Origin of the material, herbaria where the vouchers are deposited, and GenBank accession numbers.

Таха	Origin	ITS	ETS
	C C C C C C C C C C C C C C C C C C C	accession	accession
H. oligocephalum DC.	Iran, Lorestan, Termeh & al. s.n.	FJ211439,	FJ211575
	(IRAN 35945,4)	FJ211497	
<i>H. orientale</i> (L.) Gaertn.	Greece, Crete, <i>ex</i> Roy. Bot. Gard. Kew (BCN 6098)	AY445212	FJ211567
H. pallasii (Spreng.) Ledeb.	Iran, Azerbaidjan, <i>Termeh s.n.</i> (IRAN 35925)	FJ211429, FJ211487	FJ211560
H. pamphylicum Davis & Kupicha	Turkey, Antalya, <i>Susanna 2268 & al.</i> (BCN 6128)	AY445213	FJ211545
H. pendulum (C. Presl) C. Presl	Spain, Balearic islands, Ibiza, <i>Sáez s.n.</i> (BCN 6118)	AY445220	FJ211539
H. plicatum DC.	Turkey, Sivas, <i>Susanna 2419 & al.</i> (BCN 6129)	AY445216	FJ211556
H. populifolium DC.	Republic of South Africa, <i>ex</i> Silverhill Seeds (BCN 8218)	AY445217	FJ211538
H. rubicundum (K. Koch) Bornm.	Iran, Azarbaidjan, <i>Termeh & al. s.n.</i>	FJ211437,	FJ211573
× ,	(IRAN 35924,4)	FJ211495	
H. sanguineum (L.) Kostel.	Palaestina, <i>P. Křivka 72</i> (W 2002-13225)	FJ211435, FJ211493	FJ211571
H. serotinum (DC.) Boiss. subsp.	Spain, Cádiz, c. del cap de Trafalgar, L.	FJ211423,	FJ211550
picardii (Boiss. & Reut.) Galbany,	Sáez 6666 & C. Benedí (L. Sáez herb. pers -BCB)	FJ211481	
H serotinum (DC.) Boiss subsp	Spain Tarragona <i>Blanco & al</i> sin	ΔΥ445222	F.1211549
serotinum	(BCN 6120)	AT 443222	
H. sibthorpii Rouy	Greece, <i>ex</i> Roy. Bot. Gard. Kew (BCN 6099)	AY445223	FJ211561
<i>H. stoechas</i> (L.) Moench (1)	Spain, Lleida, <i>Galbany s.n.</i> (BCN 6114)	AY445225	FJ211543
H. stoechas (L.) Moench (2)	Greece, Crete, Garnatje 138 & Luque (BCN 6122)	AY445226	FJ211544
H. subsimile Rech.f.	Afghanistan, Prov. Ghazni, K. H.	FJ211428,	FJ211559
	Rechinger 18591 (E 191265)	FJ211486	
<i>H. taenari</i> Rothm.	Greece, ex Bot. Gard.University of Copenhagen (BCN 6106)	AY445227	FJ211563
H. thianschanicum Regel	Ex Hortus Botanicus Táhor (BCN 10337)	AY445228	FJ211554
<i>H. unicapitatum</i> S.G. Şenol, Ö. Seçmen & B. Öztürk	Turkey, Denizli, Above Kızılcabölük district, Babadağ (Cadmus Mt.)–Evran Mountain, <i>S. G. Senol & B. Öztürk</i> (EGE 40730)	GQ913877	GQ913876