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A contribution to taxonomy of *Centaurea* including *Psephellus* (Asteraceae) based on anatomical and molecular data

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Abstract: Seven *Centaurea* L. s.l (Asteraceae) taxa endemic to Turkey were examined in terms of anatomical and molecular aspects to contribute to their taxonomic positions. Numerical analysis of the 13 anatomical traits showed that average row number of collenchyma and the arrangements of the vascular bundles in the stem, leaf type, and abaxial surface of leaf epidermal tissue were important to determine the investigated taxa. It was also found that the 7 investigated taxa were grouped into 2 distinct clusters based on anatomical traits and combined nrDNAITS/cpDNA data.

Key words: Anatomy, *Centaurea*, cpDNA, endemic, ITS, Turkey

1. Introduction

The genus *Centaurea* L. s.l. has about 800 species distributed mainly in the Mediterranean area and south-western Asia (Wagenitz & Hellwig, 1996). *Centaurea* has one of the highest rates of endemism in Turkey, with 112 endemics among 181 total species (Güner et al., 2000). The high endemism ratio shows that Turkey is one of the gene centres of this genus (Celik et al., 2008). Recently, the genus *Centaurea* has been divided into several distinct genera, namely *Centaurea*, *Rhaponticoides* Vaill., *Psephellus* Cass., and *Cyanus* Mill. (Wagenitz & Hellwig, 2000; Greuter, 2003a, 2003b; Susanna & Garcia-Jacas, 2006). *Centaurea* was divided into 34 sections by Wagenitz (1975) in the *Flora of Turkey*. In this classification, *C. sessilis* Willd. and *C. armena* Boiss. are treated under the sect. *Rhizocalthium* Tzvelev. *C. kilaea* Boiss., *C. helenioides* Boiss., *C. huber-morathii* Wagenitz, *C. hedgei* Wagenitz, and *C. appendicigera* C.Koch were treated under different sections. After that, the last 3 taxa, together with 32 species, were transferred to the genus *Psephellus* by Wagenitz and Hellwig (2000). More recently, a study based on combined genomic and cpDNA data was carried out by Garcia-Jacas et al. (2001) and supported the view of Wagenitz and Hellwig (2000). Furthermore, the palynological properties (Özler et al., 2009) and protein profiles (Uysal et al., 2010) of some Turkish *Centaurea* s.l. taxa supported the view of Wagenitz and Hellwig (2000). In the present study, we aimed to examine the 7 endemic taxa, which were not examined in the above cited studies, traditionally treated under the *Centaurea* s.l. with regard to anatomical and molecular data and contribute to their present systematic position.

2. Materials and methods

2.1. Plant materials

All plant materials used in the present study were collected from different regions of Turkey in 2005 and 2008. All specimens were dried according to standard herbarium techniques, identified according to *Flora of Turkey* (Wagenitz, 1975), and stored in the herbarium of Karadeniz Technical University's Department of Biology (KTUB). The detailed collection data of the examined specimens are given in Table 1.

2.2. Anatomical studies

The materials for anatomical studies were fixed in FAA for 24 h and then preserved in 70% alcohol in the field. Cross-sections of stems and leaves were taken with the help of a rotary frozen microtome at 15–20 µm thickness (Eo, 2012) and stained with hematoxylin and fast-green. Surface sections of leaves were cut by hand. Investigations were carried out under a light microscope (LM). Well-stained sections were photographed with an Olympus BH2-RFCA LM for permanent slides. A raw data matrix was created with observations of 13 characters related to stem and leaf anatomy (Table 2). These characters were assessed by numerical analysis. Two types of multivariate analyses (cluster analysis and principal components analysis) were performed using SYN-TAX PC 5.0 (Podani, 1993).

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Table 1. Locality information for the examined *Centaurea* taxa.

Taxa	Locality information
Centaurea kilaea Boiss. Syn.: *Acosta kilaea* (Boiss.) Holub	A2 İstanbul: Kilyos, 200 metres east of the Gümüşdere beach, 20 m, 31.08.2008, *Aydin* 010 (KTUB).
Centaurea sessilis Willd. Syn.: *Centaurea oltensis* Sosn.	B7 Erzincan: Ahmediye Pass, 1833 m, 39°51′204″N, 39°21′671″E, 09.07.2008, *Aydin* 003 (KTUB).
Centaurea armena Boiss. Syn.: *Centaurea taraxacifolia* Boiss. var. *armata* Freyn & Sint.	B7 Erzincan: Pöske Mountain, 2300 m, 09.07. 2008, *Aydin* 002 (KTUB).
Centaurea helenioides Boiss. Syn.: *Grossheimia helenioides* (Boiss.) Sosn. & Takht.	A8 Trabzon: Çaykara, between Demirkapı and Balıklı lake, 2000 m, 23.07.2008, *Aydin* 007 (KTUB).
Centaurea huber-morathii Wagenitz Syn.: *Psephellus huber-morathii* (Wagenitz) Wagenitz	B7 Erzincan: Ahmediye Pass, 2102 m, 39°53′506″N, 039°21′160″E, 09.07.2008, *Aydin* 006 (KTUB).
Centaurea hedgei Wagenitz Syn.: *Psephellus hedgei* (Wagenitz) Wagenitz	A9 Artvin: Şavşat, Yavuz Village, 1499 m, 41°13′506″N, 042°23′476″E, 10.07.2008, *Aydin* 011 (KTUB).
Centaurea appendicigera C.Koch Syn.: *Psephellus appendicigerus* (C.Koch) Wagenitz	A8 Rize: İkizdere, Ovit Mountain, 2850 m, 24.07.2005, *Coşkunçelebi* 553 (KTUB).

Table 2. List of characters used in numerical analysis.

Symbol characters	Unit/score
X_1 Average row number of collenchyma in the stem	Number
X_2 Sclerenchymatous cells in stem	Presence: 1; absence: 0
X_3 Chlorenchymatous tissue in the stem	Presence: 1; absence: 0
X_4 Arrangements of the bundles in the stem	One ring: 1; 2 rings: 0
X_5 Width of xylem / width of phloem in the stem	Rate μm/μm
X_6 Sclerenchymatous cells in the stem pith	Presence: 1; absence: 0
X_7 Arrangements of leaf bundles	One ring: 1; more than 1 ring: 0
X_8 Leaf type	Equifacial: 1; bifacial: 0
X_9 Layer of palisade parenchyma	Number
X_{10} Stomata index of the lower epidermis	Number
X_{11} Cambium in the leaf	Presence: 1; absence: 0
X_{12} Leaf type	Amphistomatic: 1; hypostomatic: 0
X_{13} Leaf surface of upper epidermis	Undulate: 1; straight: 0

2.3. Molecular studies

2.3.1. DNA isolation

Total genomic DNAs were extracted from either healthy leaves dried in silica gel or herbarium materials following the modified CTAB extraction procedure of Doyle and Doyle (1987). The gDNAs were resuspended in TE (Tris HCl-EDTA) and stored at 4 °C.

2.3.2. PCR amplification

Double-stranded DNA of ITS was amplified using universal ITS4 and ITS5 primers, which were designed by White et al. (1990). The *trn*L intron, *trn*T-*trn*L, and *trn*L-*trn*F intergenic spacers were amplified using the universal primers of Taberlet et al. (1991). The *trn*T-*trn*L intergenic spacer was amplified using a and b primers, c and d primers were used for amplification of *trn*L intron, and e and f primers were used for amplification of *trn*L-F intergenic spacer. The PCR amplifications were performed under the following conditions: for all regions (ITS, *trn*L intron, *trn*L-F intergenic spacer, *trn*T-L intergenic spacer), each amplification reaction (50 μL final volume) contained 2–6 ng/μL of template DNA, 10 mM of Taq polymerase reaction buffer, 200 mM of dNTP, 1 μM each of the primers, 1–2 units of Taq DNA polymerase, 2–6 ng (1 μL of 2-6 ng/μL) of total template DNA, and 14 μL of ddH2O.

2.3.3. Sequence and molecular data analysis

PCR product purification and DNA sequence analysis were performed by Macrogen Inc. (Seoul, Korea). The sequencing process was conducted with BigDyeTM terminator cycling protocols (Applied Biosystems Inc., Foster City, CA, USA). PCR products were purified using ethanol precipitation and run on an Automatic Sequencer (ABI 3730x1) by a contract laboratory. Sequencing of all examined regions was carried out using forward and reverse primers. The nucleotide sequences of all regions were combined and automatically aligned using BioEdit v.7.0 software (Hall, 1999). Maximum parsimony (MP) trees were built using the Molecular Evolutionary Genetics Analysis (MEGA 5) program (Tamura et al., 2011). All characters were unordered and equally weighted. The topology of the consensus tree was constructed and evaluated with 1000 bootstrap replications for the MP analysis (Felsenstein, 1985). Sequencing data of *Jurinea bererdioidea* (Franch.) Diels provided from GenBank was used as outgroup for molecular analysis.

3. Results and discussion

The current investigation was carried out in order to provide useful and additional taxonomical information for 7 *Centaurea* s.l. species endemic to Turkey. The current taxa were therefore examined in terms of both anatomical and molecular properties. Anatomical studies were performed on stem and leaf transverse sections and also leaf surface sections. The general stem structure is almost similar in all examined taxa, but a few differences were determined as shown in Figure 1. The epidermal tissue of the stem

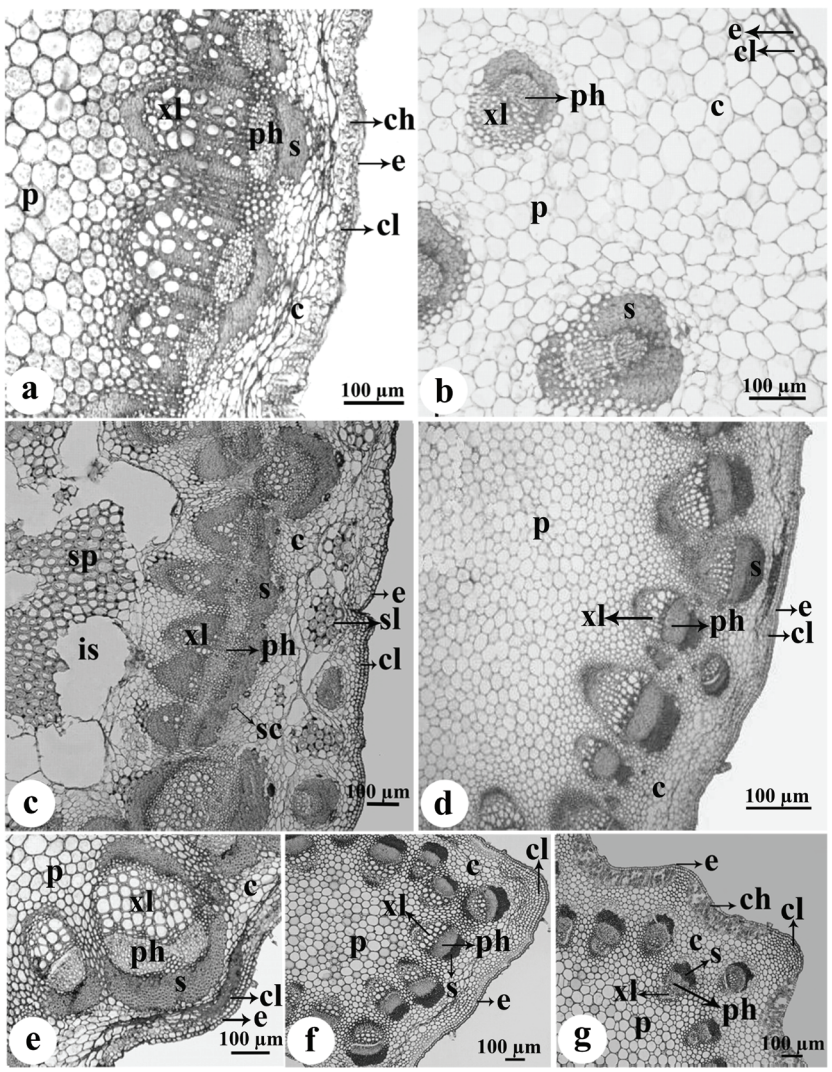

Figure 1. Stem cross-sections: a- *Centaurea kilaea*, b- *Centaurea sessilis*, c- *Centaurea armena*, d- *Centaurea helenioides*, e- *Centaurea huber-morathii*, f- *Centaurea hedgei*, g- *Centaurea appendicigera*. Abbreviations: c- cortex; ch- chlorenchyma; cl- collenchyma, e- epidermis, is- intercellular spaces, p- pith, ph- phloem, s- sclerenchymatic fibres, sc- secretory channel, sl- sclereid, sp- sclerenchymatic pith, xl- xylem.

consists of rectangular or orbicular cells in *C. kilaea* and *C. sessilis*; square or oval cells in *C. armena*, *C. helenioides*, and *C. huber-morathii*; and square or rectangular cells in *C. hedgei* and *C. appendicigera*. The surface of epidermal cells is covered by a thin cuticle layer in all examined taxa except for *C. kilaea*. It is well known that the position and the average row number of collenchyma tissue is important for comparative anatomical studies in plants (Metcalfe & Chalk, 1950; Özörgücü et al., 1991; Lersten & Curtis, 1997; Makbul et al., 2008). In the present study, it was observed that the collenchyma tissue was generally located very close to the epidermis with 1 row in *C. sessilis* and *C. armena* and 5–6 rows in the rest of examined taxa. Additionally, a chlorenchymatous tissue below the epidermis was observed in the stem cortex of *C. kilaea* and *C. appendicigera*. This kind of tissue was reported for the genus *Centaurea* in some previous studies carried out by Uysal et al. (2005), Celik et al. (2005, 2008) and Kaya et al. (2010). The stem cortex usually consists of parenchymatic oval cells with thin walls in all examined taxa, but it varies from 8 to 10 rows among the taxa (Figure 1). Yentür (2003) indicated that the arrangement of bundles provides valuable information in comparative anatomical studies. The vascular bundles in the stem are generally arranged as 2 rings, but they are arranged as 1 ring in *C. kilaea*. Celik et al. (2005, 2008) and Kaya et al. (2010) reported that vascular bundles are scattered in a circular manner as 1 ring in the stem of some *Centaurea* species. Clustered sclerenchymatic fibres are located in the upper and lower sides of the vascular bundles in all examined taxa (Figure 1). The distribution, presence, and absence of secretory channels are very important in the comparative anatomical studies (Makbul et al., 2011a). It was also indicated that the contents and distributions of secretory channels show variation among the Asteraceae members (Milan et al., 2006). In the present study, it was determined that there are several secretory channels in the stem cortex close to the vascular bundles of the examined taxa. Large intercellular spaces and clustered sclereids are clearly seen in the stem cortex of *C. armena*. Such sclereids were also reported in the root cortex of *Centaurea jacea* L. (Fritz & Saukel, 2010).

Leaf anatomical properties vary in many plants, and they are used as significant distinctive characters for plant taxonomy (Carlquist, 1961; De Villiers et al., 2010; Makbul et al., 2011b), such as in *Centaurea* (Uysal et al., 2005; Celik et al., 2005, 2008; Kaya et al., 2010). There was a typical arc-shaped vascular bundle in the central part of the midrib and it was capped by sclerenchymatic fibres at both sides in all examined taxa (Figure 2). However, the sclerenchymatic fibres were more prominent in *C. sessilis*, *C. armena*, and *C. helenioides*. Cambium tissue with several layers is prominent in the leaf midrib of *C. kilaea* and *C. hedgei*. There are also some distinct differences among the investigated taxa with respect to other foliar peculiarities, such as row number of the palisade tissue, shape of spongy cells, and distribution of intercellular cavities. *C. hedgei* has bifacial leaves with a mesophyll tissue that consists of 3 layers of palisade and 4–5 layers of isodiametric spongy parenchymatic cells. *C. sessilis*, *C. armena*, and *C. appendicigera* have equifacial leaves and mesophyll consists of 3 layers of palisade parenchymatic cells and a monolayer of isodiametric spongy tissue together with large intercellular spaces. *C. kilaea*, *C. helenioides*, and *C. huber-morathii* have equifacial leaves, but mesophyll consists of a palisade tissue with 2 layers of parenchymatic cells, and a monolayer of isodiametric spongy cells together with large intercellular spaces.

The adaxial surface of upper epidermal cells of the leaves are undulate in *C. sessilis*, *C. armena*, and *C. appendicigera*, but it is straight to wavy in *C. kilaea*, *C. helenioides*, *C. huber-morathii*, and *C. hedgei* (Figure 3) as previously reported by Kaya et al. (2010) for some *Centaurea* taxa. On the other hand, the leaves of the all examined taxa are amphistomatic, but only *C. helenioides* has hypostomatic leaves. Amphistomatic leaves were observed in *C. ptosimopappa* Hayek, *C. ptosimopappoides* Wagenitz, and *C. odyssei* Wagenitz by Celik et al. (2005, 2008), and in *C. solstitialis* subsp. *carneola* and *C. calcitrapa* subsp. *cilicica* by Kaya et al. (2010). In the present study, a distinct difference was found among the examined taxa in terms of stomata indexes. However, it is also well known that this anatomical character is a kind of trait influenced by environmental conditions (Özörgücü et al., 1991). Additionally, Kumar et al. (2012) reported that the frequency of the stomata varied among the *Morus* cultivars.

In order to explore the anatomical similarities among the examined taxa, all anatomical data were analysed with one of the most useful types of cluster analysis: the unweighted pair group method with arithmetic mean (UPGMA). The dendogram obtained from UPGMA is given in Figure 4. As seen in Figure 4, all examined taxa fall into 2 distinct clusters. The first cluster, labelled "a", consists of all the taxa traditionally treated under the genus *Centaurea*, but the second cluster, labelled "b", consists of the rest of examined taxa that were later transferred to the genus *Psephellus* by Wagenitz and Hellwig (2000). As it is understood from the above results, the anatomical properties support the separation of these taxa from the genus *Centaurea* as suggested by Wagenitz and Hellwig (2000). Similar findings were recorded by Uysal et al. (2010) for the genus *Centaurea* based on protein profiles using the SDS-PAGE method.

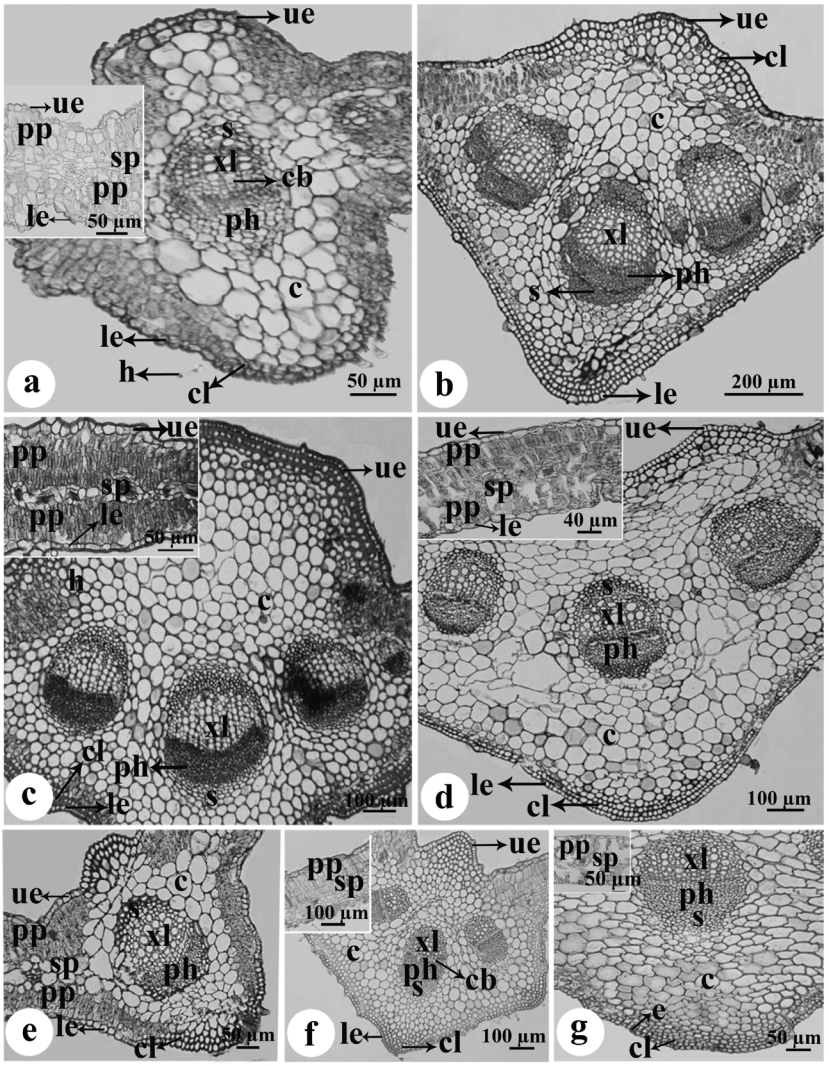

Figure 2. Leaf cross sections: a- *Centaurea kilaea*, b- *Centaurea sessilis*, c- *Centaurea armena*, d- *Centaurea helenioides*, e- *Centaurea huber-morathii*, f- *Centaurea hedgei*, g- *Centaurea appendicigera*. Abbreviations: c- cortex, cl- collenchyma, e- epidermis, h- hair, le- lower epidermis, ph- phloem, pp- palisade parenchyma, s- sclerenchymatic fibres, sp- spongy parenchyma, ue- upper epidermis, xl- xylem.

In the present study, a raw data matrix prepared according to the selected anatomical traits was also analysed by principal component analysis (PCA) to determine the characters that are important in explaining total variation among the examined species. Biplots resulting from PCA based on the first 2 components (Axis 1 and Axis 2) are given in Figure 5. Because of the high eigenvalues, only the first 5 components were taken into account. The first 3 components explained 73.01% of the total variance (Table 3). As a result of PCA analysis, it was found that the average row number of collenchyma tissue (X_1), arrangements of the bundles in the stem (X_4), leaf type (X_{12}), and shape of abaxial leaf surface (X_{13}) are important in explaining the variation among the examined taxa.

The lengths of the examined regions (ITS, *trn*L, *trn*L-F, *trn*T-L) are listed in Table 4. Molecular analysis of the regions separately revealed low resolution among the taxa, and so the ITS region and 3 cpDNA regions were combined and analysed to obtain more effective delimitations. As a result of this analysis, all examined taxa were clustered at 2 distinct clades with high bootstrap values (92%, 100%). *C. kilaea*, *C. sessilis*, *C. armena*, and *C. helenioides*, traditionally treated under the genus *Centaurea*, are located at Clade A. The rest of the taxa assessed under the genus *Psephellus* by Wagenitz and Hellwig (2000) are located at Clade B (Figure 6). This separation was also supported by pair-wise distance (PD) analysis (0.1%–3.3% in Clade A, 4.7%–17.4% in Clade B) (Table 5). Additionally, as seen

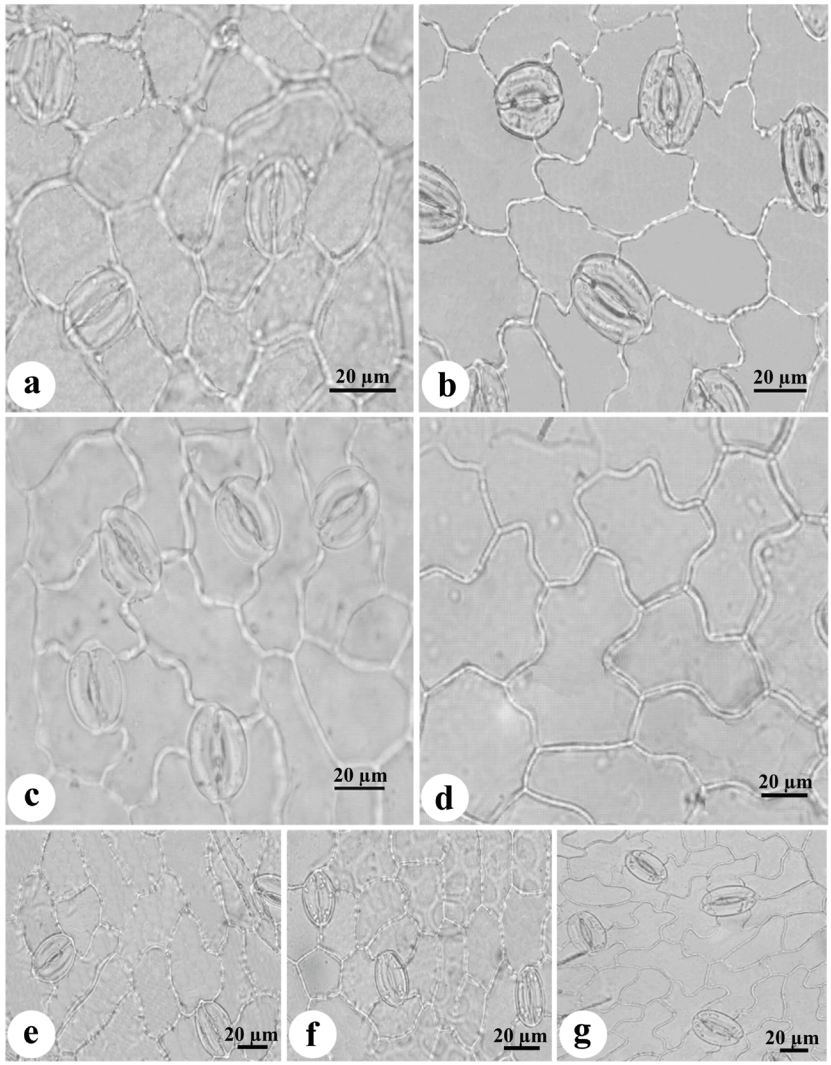

Figure 3. Superficial section of leaf: a- *Centaurea kilaea*, b- *C. sessilis*, c- *C. armena*, d- *C. helenioides*, e- *C. huber-morathii*, f- *C. hedgei*, g- *C. appendicigera*.

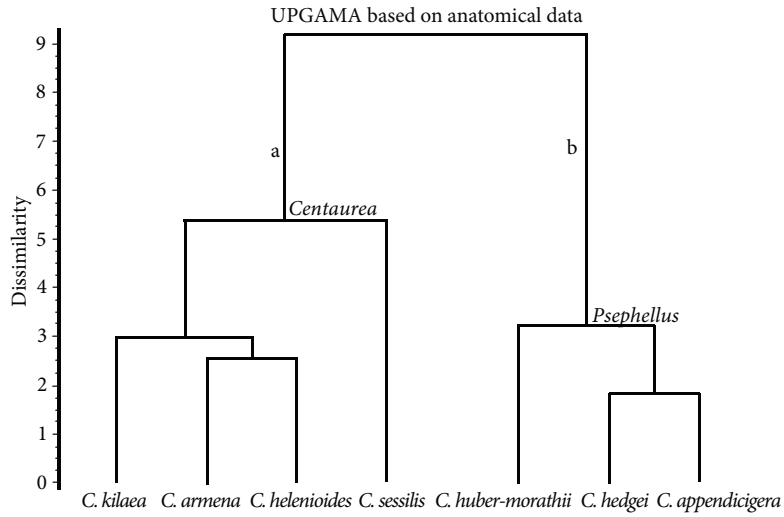

Figure 4. Cluster analysis – UPGMA.

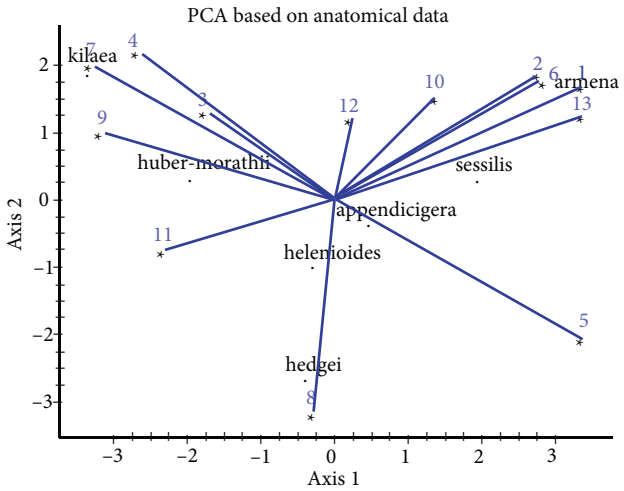

Figure 5. Principal component analysis of 7 taxa and 13 variables projected onto the first 2 axes. Variable numbers explained in Table 3.

Table 3. Percentage of variance and square roots of eigenvalues accounted for by first 3 components.

	PC-1	PC-2	PC-3
Square roots of eigenvalues	2.24	1.54	1.42
Percentages of variance explained	38.93	18.43	15.65
Cumulative variance explained	38.93	57.36	73.01

Table 4. Lengths (bp) of the examined regions and stomata index for taxa.

Taxa	ITS	*trn*T-L	*trn*L	*trn*L-F
Centaurea kilaea	633	567	506	407
Centaurea sessilis	630	563	506	407
Centaurea armena	630	561	505	407
Centaurea helenioides	630	584	504	407
Centaurea huber-morathii	638	564	506	407
Centaurea hedgei	633	525	505	408
Centaurea appendicigera	626	526	506	407

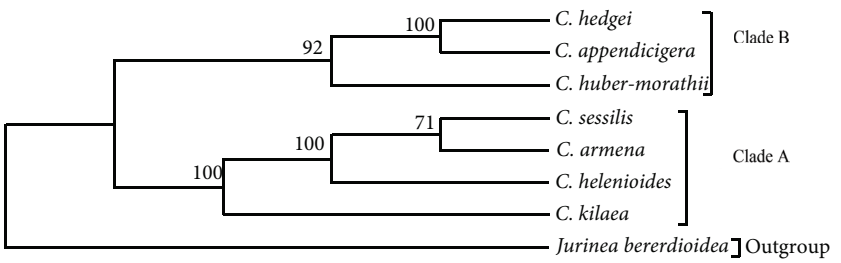

Figure 6. The dendrogram shows the genetic relationships based on the combined nuclear-chloroplast DNA sequences (ITS + cpDNA regions), evaluated by maximum parsimony.

Table 5. Pair-wise distance matrix of genetic divergence values of the 7 taxa obtained from MEGA.

		1	2	3	4	5	6	7
1	*Centaurea kilaea*							
2	*Centaurea sessilis*	0.023						
3	*Centaurea armena*	0.024	0.001					
4	*Centaurea helenioides*	0.023	0.003	0.002				
5	*Centaurea huber-morathii*	0.034	0.035	0.036	0.033			
6	*Centaurea hedgei*	0.068	0.068	0.069	0.067	0.047		
7	*Centaurea appendicigera*	0.195	0.195	0.195	0.191	0.174	0.164	

in Table 5, *C. armena* and *C. sessilis* are the closest species among the examined taxa, which is supported by MP. On the other hand, it is obviously seen in the PD analysis that *C. appendicigera* is the most distinct species among the examined taxa. We therefore compared the sequences of *C. appendicigera* with the NCBI database, and this comparison revealed that sequences of *C. appendicigera* was quite similar to *Psephellus* taxa. We concluded that the results of MP and PD analysis were congruent with the view of Wagenitz (1975).

In conclusion, it was found that there are some distinct anatomical differences among the examined endemic taxa that are traditionally assessed under the genus *Centaurea*, and these differences are congruent with molecular analysis. Furthermore, anatomical and molecular analysis supported the treatment of endemics *C. hedgei*, *C. appendicigera*, *and C. huber-morathii* under the genus *Psephellus*.

References

Carlquist S (1961). *Comparative Plant Anatomy*. New York: Holt, Rinehart & Winston.

Celik S, Uysal I & Menemen Y (2005). *Centaurea* species in Turkey (A): *Centaurea odyssei* Wagenitz (Asteraceae) in Kazdagi (Mt. Ida) National Park. *International Journal of Biodiversity Science and Management* 1: 113–120.

Celik S, Uysal I & Menemen Y (2008). Morphology, anatomy, ecology and palynology of two *Centaurea* species from Turkey. *Bangladesh Journal of Botany* 37: 67–74.

De Villiers BJ, Tilney PM & Van Wyk BE (2010). The taxonomic significance of leaf anatomical characters in *Cussonia* and related genera (Araliaceae). *Botanical Journal of the Linnean Society* 164: 246–263.

Doyle JJ & Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Eo JK (2012). A simple technique for cross-sectioning Gymnosperm needle leaves using microtome. *Turkish Journal of Botany* 36: 213–216.

Felsenstein J (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Fritz E & Saukel J (2010). Anatomy of subterranean organs of medicinally used Cardueae and related species and its value for discrimination. *Scientia Pharmaceutica* 79: 157–174.

Garcia-Jacas N, Susanna A, Garnatje T & Vilatersana R (2001). Generic delimitation and phylogeny of the subtribe Centaureinae (Asteraceae): a combined nuclear and chloroplast DNA analysis. *Annals of Botany* 87: 503–515.

Greuter W (2003a). The Euro+Med treatment of Cardueae (Compositae) – generic concepts and required new names. *Willdenowia* 33: 49–61.

Greuter W (2003b). The Euro+Med treatment Senecioneae and the minor Compositae tribes-generic concepts and required new names - with an addendum to Cardueae. *Willdenowia* 33: 245–250.

Güner A, Özhatay N, Ekim T & Baser KHC (eds.) (2000). *Flora of Turkey and the East Aegean Islands* (Suppl. 2), Vol. 11. Edinburgh: Edinburgh University Press.

Hall TA (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Kaya Z, Orcan N & Binzet R (2010). Morphological, anatomical and palynological study of *Centaurea calcitrapa* L. ssp. *cilicica* (Boiss. & Bal.) Wagenitz and *Centaurea solstitialis* L. ssp. *carneola* (Boiss.) Wagenitz endemic for Turkey. *Pakistan Journal of Botany* 42: 59–69.

Kumar V, Kodandaramaiah J & Rajan MV (2012). Leaf and anatomical traits in relation to physiological characteristics in mulberry (*Morus* sp.) cultivars. *Turkish Journal of Botany* 36: 683–689.

Lersten NR & Curtis JD (1997). Anatomy and distribution of foliar idioblasts in *Scrophularia* and *Verbascum* (Scrophulariaceae). *American Journal of Botany* 84: 1638–1645.

Makbul S, Coskuncelebi K & Beyazoğlu O (2011a). Notes on the stem anatomy of *Scorzonera* (Asteraceae) taxa from Northeast Turkey. *Phytologia Balcanica* 17: 113–121.

Makbul S, Coskuncelebi K, Türkmen Z & Beyazoglu O (2011b). Comparison of foliar anatomy of *Scorzonera* L. (Asteraceae) taxa from northeast Anatolia. *Pakistan Journal of Botany* 43: 135–155.

Makbul S, Türkmen Z, Coskunçelebi K & Beyazoglu O (2008). Anatomical and pollen characters in the genus *Epilobium* L. (Onagraceae) from Northeast Anatolia. *Acta Biologica Cracoviensia Series Botanica* 50: 57–67.

Metcalfe CR & Chalk L (1950). *Anatomy of the Dicotyledons*. Oxford, UK: Clarendon Press.

Milan P, Hayashi AH & Appezzato-da-Glória B (2006). Comparative leaf morphology and anatomy of three Asteraceae species. *Brazilian Archives of Biology and Technology* 49: 135–144.

Özler H, Kaya Z & Pehlivan S (2009). Pollen morphology of some *Centaurea* L., *Psephellus* Cass. and *Cyanus* Miller taxa. *Acta Biologica Cracoviensia Series Botanica* 51: 53–66.

Özörgücü B, Gemici Y & Türkan I (1991). *Karşılaştırmalı Bitki Anatomisi*. İzmir: Ege Üniversitesi Fen Fakültesi Yayını (in Turkish).

Podani J (1993). *Multivariate Data Analysis in Ecology and Systematic: A Methodological Guide to Syn-Tax 5.0 Package*. Netherlands: SPB Academic Publishing.

Susanna A & Garcia-Jacas N (2006). Tribe Cardueae. In: Kadereit JW & Jeffrey C (eds.) *The Families and Genera of Vascular Plants: Vol. 8: Flowering Plants: Eudicots Asterales*, pp. 123–146. Berlin, Heidelberg: Springer Verlag.

Taberlet P, Gielly L, Pautou G & Bouvet J (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S (2011). MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.

Uysal I, Celik S & Menemen Y (2005). Morphology, anatomy, ecology, pollen, and achene features of *Centaurea polyclada* DC. (Sect. *Acrolophus*) in Turkey. *Journal of Biological Sciences* 5: 176–180.

Uysal T, Arslan E, Tugay O & Ertuğrul K (2010). Determination of the relationship between some *Centaurea* species based on SDS-PAGE. *Turkish Journal of Biology* 34: 125–131.

Wagenitz G (1975). *Centaurea* L. In: Davis PH (ed.) *Flora of Turkey and the East Aegean Islands*, Vol. 5, pp. 465–585. Edinburgh: Edinburgh University Press.

Wagenitz G & Hellwig FH (1996). Evolution of characters and phylogeny of the Centaureinae. In: Hind DJN & Beentje HG (eds.) *Compositae: Biology and Utilization. Proceedings of the International Compositae Conference*, pp. 491–510. Kew: Royal Botanic Gardens.

Wagenitz G & Hellwig FH (2000) *Psephellus* Cass. (Compositae; Cardueae) revisited with a broadened concept. *Willdenowia* 30: 29–44.

White TJ, Bruns T, Lee S & Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: Innis M, Gelfand D, Sninsky J & White TJ (eds.) *PCR Protocols: A Guide to Methods and Application*, pp. 315–322. San Diego: Academic Press.

Yentür S (2003). *Bitki Anatomisi*. İstanbul: İstanbul Üniversitesi Fen Fakültesi Yayını (in Turkish).