www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 11/2 (2018) 29-41

Research article/Araştırma makalesi

Anatomical and micromorphological investigations on some *Centaurea* (Asteraceae, Cardueae) taxa from NE Anatolia

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Abstract

This study evaluates the phylogenetic relationships of some *Centaurea* s.l. taxa native to Northeast Anatolia, Turkey. Stem, leaf and cypsela anatomical structures and cypsela micromorphologies were characterized and compared in detail by using variance analysis and cluster analysis (UPGMA). Macro and micromorphological characteristics of cypsela were investigated using a stereomicroscope and scanning electron microscope. Significant differences were observed in stem features, leaf midrib and lamina thickness, number of stomata, and epidermal cell wall patterns in the abaxial and adaxial surfaces among the taxa. Cypsela shapes were determined as ovoid or widely oblong. Cypselas can be distinguished into four types of surface ornamentations. The structure of the pericarp was described, as well as the testa, and the distribution of the sclerenchymatic zone of the ribs and secretory ducts (resin) in the mesocarp were evaluated.

Key words: anatomy, *Centaurea*, cypsela, micromorphology, SEM

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Kuzeydoğu Anadolu Bölgesi'ndeki bazı *Centaurea* (Asteraceae, Cardueae) taksonları üzerinde anatomik ve mikromorfolojik araştırmalar

Özet

Bu çalışma Kuzeydoğu Anadolu, Turkiye için doğal olan bazı *Centaurea* taksonlarının akrabalık ilişkilerini ele almaktadır. Gövde, yaprak ve sipsela anatomik yapıları ve mikromorfolojik özellikleri belirtilmekte ve varyas ile kümeleme analizi (UPGMA) uygulayarak detaylı olarak karşılaştırılmaktadır. Sipsela makro ve mikromorfolojik özellikleri stereomikroskop ve taramalı elektron mikroskobu kullanarak incelendi. Taksonlar arasında gövde özellikleri, yaprak orta damar ve lamina kalınlığı, stoma sayısı ve üst ile alt epidermis yüzey şekillerinde önemli farklılıklar gözlemlendi. Sipselalar şekillerine göre ovoid ve genişçe oblong olarak tanımlandı. Yüzey süslemelerine göre dört tip ayırt edildi. Testa yapısı ile perikarp yapısı tanımlandı ve damarlardaki sklerenkimatik kısımların ve mezokarpta salgı kanalarının dağılımı değerlendirildi.

Anahtar kelimeler: Anatomi, *Centaurea*, sipsela, mikromorfoloji, SEM

1. Introduction

Centaurea L. consists of approximately 600 species in the Mediterranean area and Southwest Asia (Wagenitz and Hellwig, 1996; Brummitt, 2004). In recent studies, combining morphological with molecular investigation, this genus has been divided into four genera; *Centaurea* L., *Rhaponticoides* Vaill., *Psephellus* Cass. and *Cyanus* Mill. (Wagenitz and Hellwig, 2000; Greuter, 2003a; 2003b). Susanna and Garcia-Jacas (2007), however, does not accept *Cyanus* as a separated genus. *Centaurea* and *Cyanus* are represented by ca. 190 species in Turkey, and most of *Centaurea* taxa are endemics (Davis et al., 1988; Duran and Duman, 2002; Kaya et al., 2010).

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 BioDiCon. 655-0217

Centaurea is one of the largest genera of the tribe *Cardueae* Cass. It has complex taxonomy characteristics. Through several studies were conducted on the genus (Romaschenko et al., 2004; Inceer et al., 2007; Kose and Yücel, 2007; Martin et al., 2009; Tekeli et al., 2010), some taxonomical problems are have not been resolved yet.

The stem, and particularly the leaves' anatomical characters provide important data for classification (Metcalfe and Chalk, 1979; Stace, 1984; Inceer and Ozcan, 2011; Eminagaoglu and Ozcan, 2014; Ozcan et al., 2015). Several studies have been carried out on the anatomy of *Centaurea* taxa (Uysal et al., 2005; Celik et al., 2008; Aydin et al., 2013; Ozcan et al., 2014), and important features in their anatomies have been reported. Fruit and seed surface ornamentations are also a useful tool for taxonomic considerations in several families (Sheikh Akbari and Azizian, 2006; Kaya and Dirmenci, 2008; Pinar et al., 2009; Bayrakdar et al., 2010; Shabestari et al., 2013). Cypsela (as a name of fruit in Asteraceae) external morphology and anatomy have been widely used for the classification of species in the Compositae family (Lavialle, 1912; Zhu et al., 2006; Lo Presti et al., 2010), and the tribes *Cynareae* (Kadereit and Jeffrey, 2007) and *Cardueae* (Dittrich, 1985; Häffner, 2000; Zarembo and Boyko, 2008; Abid and Qaiser, 2009; Abid and Ali, 2010; Ozcan, 2017).

The objectives of the present study were (i) to give detailed descriptions of stem and leaf anatomical characteristis in selected taxa of the genus *Centaurea* s.l, (ii) to investigate cypsela morphology and anatomy by using light and stereomicroscopes, and (iii) to discuss the taxonomic value of these characters using statistical analyses. The paper presents the first detailed account of the stem, leaf and cypsela anatomy, and cypsela micromorphology of four *Centaurea* s.l. taxa growing in Turkey. This is the first comprehensive study with several aspects, and taxonomical evaluations and comparisons with the literatures have been also discussed.

2. Materials and methods

2.1. Sampling

The plant samples (*Centaurea nigrifimbria*, one of them is indicated in here as *Cyanus nigrofimbrius*) examined were collected from natural populations. Taxonomical descriptions of the plants were made according to Wagenitz (1975) and Güner et al. (2012). Details of the voucher number are provided in Table 1. Voucher specimens were deposited in the Artvin Coruh University Herbarium (ARTH).

Table 1. Collection data of investigated taxa

Taxon	Locality	Voucher
Centaurea aggregata subsp. *albida* (K. Koch) Bornm.	Artvin, Salkımlı Village (Tolgum), eroded banks, 508 m, 18.viii. 2014	*M. Ozcan 699*
C. macrocephala Puschk. ex Willd.	Artvin, Ardanuç, Bilbilan high plateau, among grass, 1576 m, 30.vi. 2012	*M. Ozcan 546*
C. salicifolia M. Bieb. ex Willd.	Trabzon, Araklı Village, roadsides, 312 m, 17.viii.2013	*M. Ozcan 641*
Cyanus nigrofimbrius (K. Koch) Soják	Trabzon, Çaykara, near Soğanlı Mountain, 2236 m, 16.viii. 2014	*M. Ozcan 697*

2.2. Anatomical preparation

Anatomical observations were performed in the stem, leaf and cypsela of the taxa. Fresh cauline leaves and parts of the stems or removed parts from herbarium materials were used for preparations. Handmade cross-sections were prepared from the median part of the stem and leaf lamina using commercial razor blades. Peripheral sections of leaves were obtained by hand. Mature cypselas of at least three individual plants for each taxon were collected from the capitula, softened by boiling in water for 3-4 days and stored in glycerin. A cryostat was used for cross-sections of cypselas. Sections were cut to a thickness of 15- 20 μm. All sections were stained in hematoxylin for approximately 15 min (Algan, 1981). Semi-permanent slides were mounted in glycerin. Well-stained sections were examined under a light microscope and photographed using an Olympus BX 53 research microscope with a digital camera attachment DP 73.

Five cross-sections from at least three different individual plants from each taxon were measured to assess the consistency of anatomical characters and to calculate the means and standard error among different cross-sections. Ten peripheral slides were prepared, and 50 stomatal lengths were measured for each taxon. The stomatal index was calculated according to the method described by Meidner and Mansfield (1968). Fifty quantitative and qualitative characters were investigated (Tables 2-5).

2.3. Micromorphological examination

Micro and macromorphological features of the cypselas were studied using a stereomicroscope (Leica M60 with a digital camera attachment DFC 295) and a scanning electron microscope (Zeiss Evo LS10, ACU-Science

Research Center). Five specimens were selected from each taxon. The cypselas were first examined using a stereomicroscope to determine size, shape, color and maturity, and were then photographed. For scanning electron microscopy, mature cypselas were placed on stubs using double-sided adhesive tape, and coated with gold in a Cressington sputter coater 108 auto coating apparatus for 2-3 minutes. All cypselas were examined and photographed from the same region (from the middle part of the lateral region).

The terminology for cypselar characteristics proposed by Stearn (1985) was adopted to describe the fruit coat, size and shape, cell arrangements and primary sculpturing.

2.4. Cluster analysis

Data analysis was carried out on SPSS (version 19) software. Duncan's multiple-range test, a one-way analysis of variance technique, was used in 40 quantitative values to identify the statistical importance of distinctions among the data (Tables 2-5). Grouping of taxa was performed using the clustering analysis method (UPGMA)..

3. Results

In this study, stem, leaf and cypsela anatomical properties and cypsela micromorphology of four taxa were investigated in detail. A transverse section of stem, leaf blade, midrib and cypsela, and peripheral sections of leaf were investigated. The main anatomical characters, 42 being assessed among the taxa studied, are shown in Tables 2-5 and in Figures 1-6.

3.1. Stem

The taxa studied exhibited more or less similar morphological patterns. However important differences were also noted. *C. aggregata* subsp. *albida* and *C. salicifolia* have 7-8 corners, while *C. macrocephala* and *Cy. nigrofimbrius* exhibited two-three wings in addition to the corners. Wings were also previously reported in *Centaurea cheiranthifolia* var. *purpurascens* by Ozcan et al. (2014). All taxa investigated have collenchyma in the corners and chlorenchyma between the corners. The largest cortex and collenchyma thickness are seen in *C. macrocephala*, while the smallest ones are present in *C. aggregata* subsp. *albida* (Table 2). Collateral type vascular bundles appear in two layers in *C. macrocephala*, in contrast to the other taxa (Figure 1). They are arranged in one row in these three taxa (Figure 1D, Table 2). The number of vascular bundles in the stem is much higher (72-80) in *C. macrocephala*, but almost the same in the others. The largest sclerencymatic cap is present above the phloem in *C. macrocephala*, and the smallest occurs in *Cy. nigrofimbrius* (Figure 1, Table 2). Several secretory canals (ducts) were observed in the cortex opposite the bundles in all taxa near the bundles. These structures have been previously reported by Metcalfe and Chalk (1979) in some species of the family Asteraceae and by Celik et al. (2008), Kaya et al. (2010), Aydin et al. (2013), Özcan (2013) and Ozcan et al. (2014) in several *Centaurea* s.l. taxa. Parenchymatic pith cells are situated in the center of the stem. However, a large pith cavity is present at the center of the stem in *C. salicifolia* and *Cy. nigrofimbrius* (Figure 1G,J).

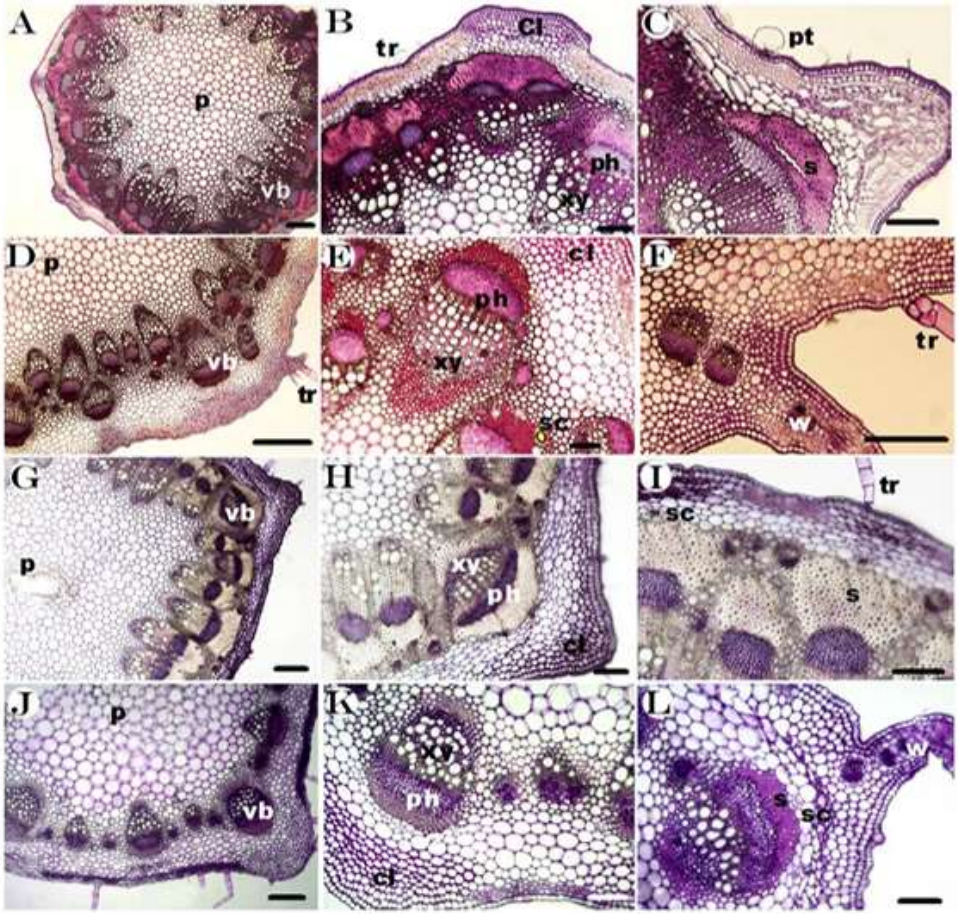

Figure 1. Stem anatomy of investigated taxa. A-C, *Centaurea aggregata* subsp. *albida*; D-F, *C. macrocephala*; G-I, *C. salicifolia*; J-L, *Cyanus nigrofimbrius*. cl = collenchymas, pp = palisade parenchyma, p = pith, ph = phloem, pt = peltat trichome, sc = secretory canal (duct), tr = simple trichome, vb = vascular bundle, w = wing, xy = xylem. Scale bars: A, D, G, J= 200 µm; B, C, E, F, K, L = 100 µm

Table 2. Stem features of investigated taxa. *mean± standard deviation

Character	*Centaurea aggregata* subsp. *albida*	*C. macrocephala*	*C. salicifolia*	*Cyanus nigrofimbrius*
Shape	angled	angled and winged	angled	angled and winged
Epidermis length (µm)	13.10±2.21^{a}	19.77±0.96^{b}	10.94±0.60^{a}	18.33±0.71^{b}
Epidermis width (µm)	19.63±3.76^{a}	20.77±0.72^{a}	14.92±0.93^{a}	20.67±035^{a}
Cortex thickness in the corner (µm)	179.1±3.10^{a}	389.69±17.52^{c}	221.3±22.59^{ab}	272.4±30.87^{b}
Number of cortex layer	8-9	15-20	10-13	8-14
Collenchyma thickness in the corner (µm)	114.19±5.53^{a}	264.07±12.56^{c}	133.59±10.72^{a}	193.2±18.61^{b}
Breadth of sclerenchymatic cap in phloem	121.70±6.21^{bc}	114.18±15.84^{b}	134.96±7.82^{c}	62.20±4.23^{a}
Number of vascular bundles	24-26 (1 row)	75-80 (2 rows)	22-35 (1 row)	15-23 (1 row)
Vascular bundle Length (µm)	606.2±29.63^{b}	717.20±32.78^{c}	552.99±14.79^{b}	355.2±18.26^{a}
Vascular bundle breadth (µm)	230.8±11.54^{ab}	318.82±20.48^{c}	190.65±7.40^{a}	262.9±17.13^{b}
Trachea (µm)	36.76±1.72^{a}	36.09±2.65^{a}	46.86±2.56^{b}	36.44±0.77^{a}
Pith cell (µm)	93.25±20.21^{ab}	120.1±5.18^{ab}	86.1±2.02^{a}	129.07±8.07^{c}

3.2. Leaf

The leaves are amphystomatic with eglandular and peltate glandular trichomes in adaxial and abaxial parts of the taxa, but *Cy. nigrofimbrius* does not have glandular trichomes (Figure 3). These trichome types have previously been reported in different *Centaurea* taxa by Ozcan et al. (2014). Stomatal numbers per mm^2 are lower in both of the

Character		*Centaurea aggregata* subsp. *albida*	*C. macrocephala*	*C. salicifolia*	*Cyanus nigrofimbrius*
Leaf type		isobilateral	isobilateral	dorsiventral	isobilateral
Upper epidermis	length(µm)	11.84±0.26^a	21.85±0.90^c	18.17±0.85^b	17.63±0.82^b
	width (µm)	16.33±0.88^a	25.77±1.26^c	26.53±1.46^c	20.93±0.97^b
Lower epidermis	length(µm)	11.81±0.41^a	19.12±0.63^c	12.82±0.56^a	15.50±0.53^b
	width (µm)	15.32±0.49^a	23.20^b±0.73	17.07±0.49^a	21.53±1.44^b
Midrib mesophyll size (µm)		652.98±31.10^a	1161.32±44.9^{bc}	988.63±104.5^b	1228.0±63.32^c
Midrib mesophyll breadth (µm)		410.54±12.16^a	1535.03±55.80^c	1108.59±66.24^b	1557.61±73.72^c
Lamina mesophyll breadth (µm)		223.42±13.41^c	179.42±5.86^b	127.93±3.95^a	215.6±2.70^c
Trachea diameter (µm)		20.34±1.41^a	27.58±1.46^b	22.63±0.94^a	27.25±0.98^b
Vascular bundle	Length (µm)	254.28±18.18^a	396.53±14.66^c	333.24±15.36^b	412.80±10.89^c
	breadth (µm)	167.29±13.41^a	249.07±6.59^c	249.5±9.49^c	214.40±11.95^b
	Number in midrib	1	4+1	3	3+1
Adaxial surface	Cuticle thickness (µm)	2.65±0.05^a	2.97±0.17^a	2.51±0.15^a	1.90±0.06^a
	Stomatal length (µm)	22.81±0.77^a	29.10±0.58^c	23.59±0.03^a	25.62±0.29^b
	Stomatal index	13.36±1.30^b	12.83±0.67^b	8.91±0.74^a	06.90±0.42^a
	Number of stomata (1 mm^2)	280.0±24.34^b	150.0±17.17^a	160.00±24.34^a	115.00±16.93^a
Abaxial surface	Cuticle thickness (µm)	2.47±0.13^b	2.57±0.16^b	2.08±0.09^a	1.80±0.12^a
	Stomatal length (µm)	21.29±0.19^a	30.41±0.65^c	23.36±0.59^b	23.90±0.16^b
	Stomatal index	15.44±0.68^{ab}	13.54±0.96^a	16.58±1.10^b	14.31±0.26^{ab}
	Number of stomata (1 mm^2)	400.0±31.43^b	180.00±19.96^a	380.0±37.18^b	220.0±24.20^a

Table 3. Leaf anatomical features of investigated taxa.*mean± standard deviation.

two leaf surfaces of *C. nigrofimbrius* than in the others. In addition, cypsela is ovoid for this species, in contrast to the others. These differences are in accordance with the classification of this species in the genus *Cyanus*. All the taxa studied have more or less sinuous-undulate anticlinal cell walls. However, less undulation was observed in *C. aggregata* subsp. *albida* and *C. salicifolia*, because of their growing habits in more or less dry conditions. This observation supports the idea that epidermal cells of most leaves of shade-loving dicotyledons have sinuous anticlinal walls (Fahn 1990). The leaf lamina is isobilateral in *C. aggregata* subsp. *albida, C. macrocephala* and *Cy. nigrofibrius*, whereas it is dorsiventral in *C. salicifolia*. Isobilateral and dorsiventral types of leaf lamina are commonly reported in the genus *Centaurea* and some related genera (Metcalfe and Chalk, 1979; Uysal et al., 2005; Celik et al., 2008; Aydin et al., 2013; Ozcan et al., 2014). One to three collateral vascular bundles are observed in the midrib of the leaf in investigated taxa. *C. aggregata* subsp. *albida* has only one large vascular bundle in the midrib, as in *C. pecho* as previously reported by Ozcan et al. (2014). These two taxa grow in very dry conditions, in contrast to the other taxa. Various numbers of secretory ducts (Figure 2) are observed near the bundles, as with the stems. It has been suggested that these ducts may act as a sieve tube in the transfer of organic material (Tetley, 1925; Williams, 1954), and this is probably correct for several *Centaurea* taxa, as in this study.

3.3. Cypsela

It is made up from pericarp, testa, endosperm and cotyledons. The mature pericarp is differentiated into two zones, i.e. exocarp and mesocarp, and exhibits variation in the structure (Figure 4). The exocarp is composed of relatively thickened and flat epidermal cells, and sclerenchymatous fiber cells cover the entire mesocarp in all taxa. Sclerified epidermal cells have been previously reported in *Carduus*, *Cirsium*, *Notobasis* Cass., *Picnomon* Adans., *Silybum* Adans.

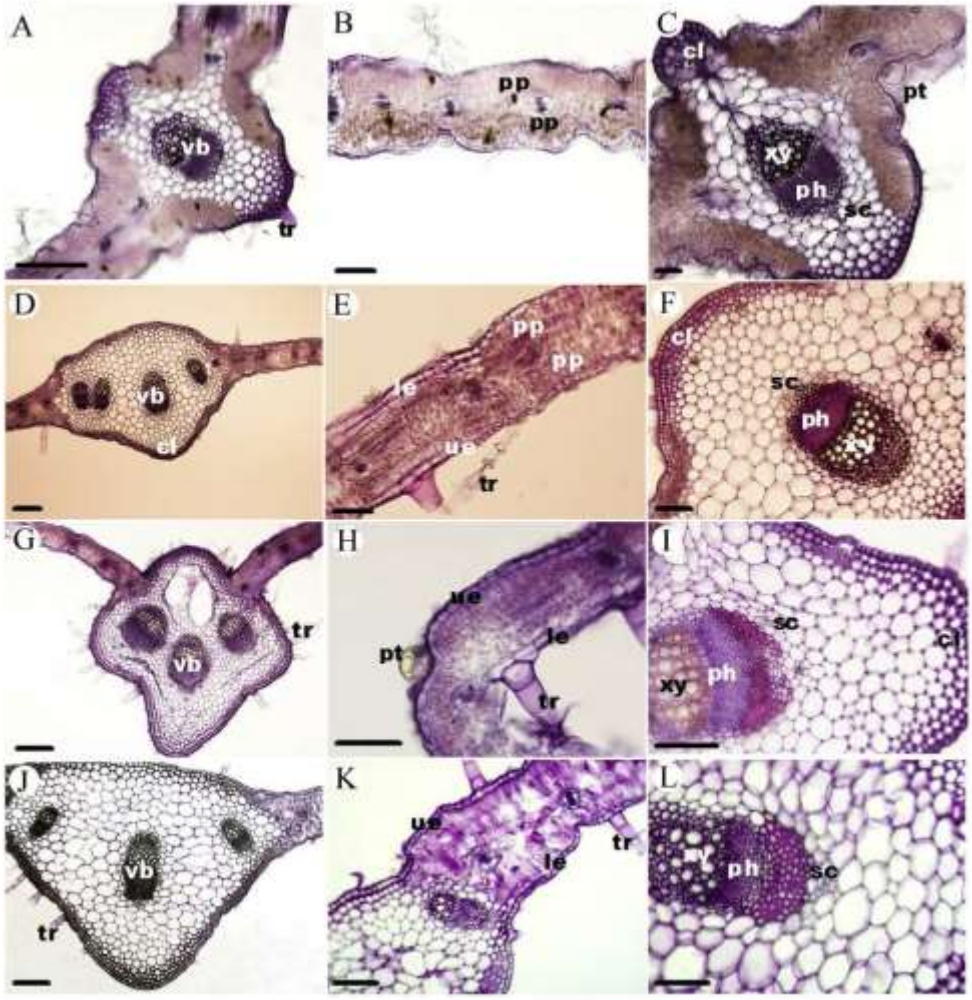

Figure 2. Leaf anatomy of investigated taxa. A-C, *C. aggregata* subsp. *albida*; D-F, *C. macrocephala*; G-I, *C. salicifolia*; J-L, *Cy. nigrofimbrius*. cl = collenchymas, le = lower epidermis, p = pith, ph = phloem, pp = palisade parenchyma, pt = peltat trichome, sc = secretory canal (duct), tr = simple trichome, ue = upper epidermis, vb = vascular bundle, xy = xilema. Scale bars: A, D, G, J = 200 µm; B, E = 100 µm; C, F, H, K = 50 µm

and *Trymnus* Cass. by Häffner (2000). In contrast, thin epidermal cells and parenchymatic mesocarp have also been reported in *Rhaponticum* Vaill. In the species of *Klasea* Cass. and *Serratula* L., the mesocarp is represented by both parenchymatous and sclerenchymatous tissue (Zarembo and Boyko, 2008). Mukherjee (2001) studied some taxa in the tribe Senecioneae and reported sclerenchymatic braces and parenchymatic cells layers in the mesocarpic zone of the pericarp. The testa is elongated, with radially stretched lignified cell and crushed cell layers near the single row endodermis. This type of pericarp and testa structure were previously reported by Häffner (2000) in some *Carduinae* and *Centaureinae* taxa, and by Zarembo and Boyko (2008) in some *Cardueae* genera, *Rhaponticum*, *Klasea*, *Serratula* and *Synurus* IIjin. Singh and Pandley (1984) reported an elongated and lignified testa structure in *Centaurea moschata* L. and in *Silybum marianum* (L.) Gaertn. A similar testa structure has been also determined in some species of *Klasea* and *Serratula*. *Cy. nigrofimbrius* is characterized by a very thick testa and a comparatively thin pericarp among the investigated taxa (Table 4).

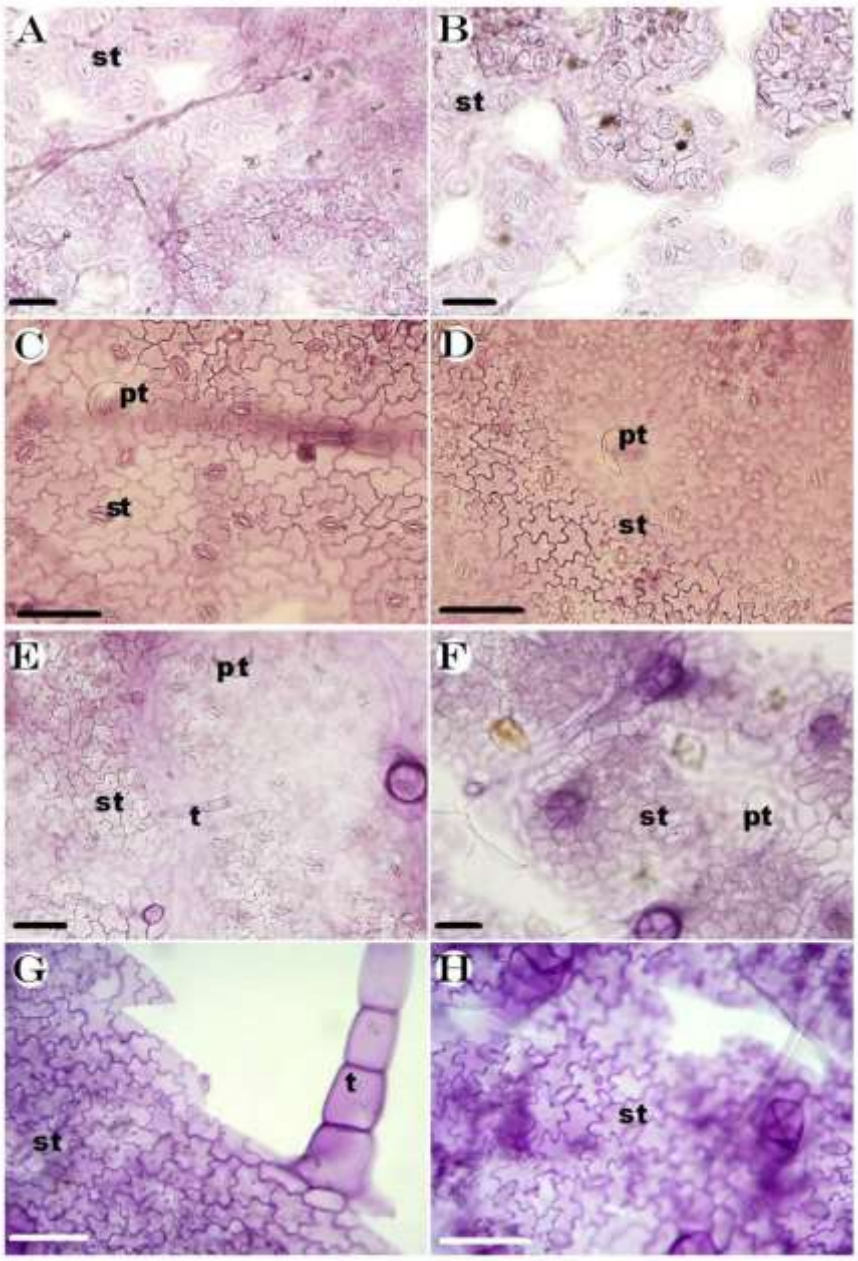

Figure 3. Stomata of investigated taxa. A-C, *C. aggregata* subsp. *albida*; D-F, *C. macrocephala*; G-I, *C. salicifolia*; J-L, *Cy. nigrofimbrius.* (A, C, E, G) adaxial surface. (E, D, F, H) abaxial surface. (pt) peltat trichome; (st) stomata; (t) trichome. Scale bars: 50 µm

Secretory ducts are clearly visible in *C. salicifolia* and *Cy. nigrofimbrius* near the bundle traces. Lavialle (1912) reported that secretory ducts are of great importance in the taxonomy of the *Cardueae*. These have been described in the species of *Carthamus* by Singh and Pandey (1984) and *Serratula*, some sections of *Centaurea* by Dittrich (1985), and some species of the tribe Senecioneae by Pandey and Singh (1982). A one-layer, elongated and thin-walled parenchymatous endosperm is observed in all taxa investigated. Häffner (2000) also reported that the appearance of endosperm does not differ significantly among genera of the subtribe *Carduinae*.

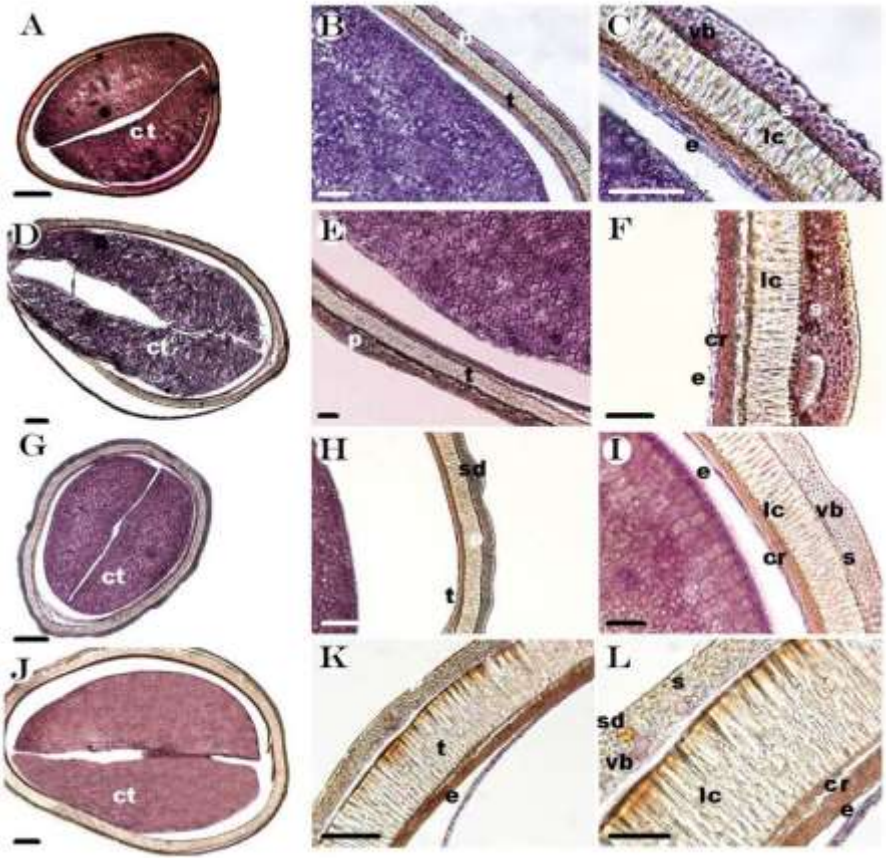

Figure 4. Cypsela anatomy of investigated taxa. A-C, *C. aggregata* subsp. *albida*; D-F, *C. macrocephala*; G-I, *C. salicifolia*; J-L, *Cy. nigrofimbrius*. cr = crushed cell, e = endosperm, lc = lignified palisade cell, s = sclerenchymatic cell, sd = secretory duct, vb = vascular bundle. Scale bars: A, D, G, J = 200 µm; B, E, H, K = 100 µm; C, F, I, L= 50 µm

Table 4. Cypsela anatomical characters of investigated taxa. *mean± standard deviation.

Character	*Centaurea aggregata* subsp. *albida*	*C. macrocephala*	*C. salicifolia*	*Cyanus nigrofimbrius*
Cuticle (µm)	1.65±0.18[b]	1.18±0.04[a]	1.49±0.07[a]	1.40±0.10[a]
Pericarp (µm)	27.02±1.56[a]	38.68±1.72[b]	39.44±1.34[b]	41.01±2.52[b]
Palisade sclerenchyma (µm)	35.96±1.41[a]	57.26±0.91[b]	54.74±0.64[b]	113.84±1.16[c]
Crushed cell layer (µm)	14.53±0.43[a]	22.83±1.12[b]	16.65±0.44[a]	25.71±0.95[c]
Vascular bundle length (µm)	14.87±0.39[a]	27.57±1.52[c]	14.42±0.46[a]	22.45±1.94[b]
Vascular bundle width (µm)	23.97±2.56[a]	64.80±1.29[d]	32.72±1.11[b]	48.08±3.12[c]
Endosperm (µm)	5.69±0.47[a]	6.54±0.30[a]	6.16±0.36[a]	5.44±0.21[a]
Cypsela length (µm) in transverse section	1297.8±43.29[a]	3257.9±101.73[c]	1459.9±11.29[a]	2799.35±71.5[b]
Cypsela width (µm) in transverse section	1002.2±26.81 [a]	1661.8±66.80[b]	1118.0±24.51[a]	2130.76±23.72[c]
Secretory cell in bundle	invisible	invisible	12.56±0.76	17.59±1.92

3.4. Micromorphology

Shape, size, color, and surface ornamentations of cypselas were observed and indicated in Table 5, as well as in Figures 4 and 5. Jana and Mukherjee (2012) reported that epidermal cell orientations are also important for characterization of taxa. In the present study, the cypselas of the investigated taxa are smooth (not ribbed), ovoid or widely oblong, with swollen or compressed dorsal and ventral margins (Figure 5). They also have a scabrous pappus

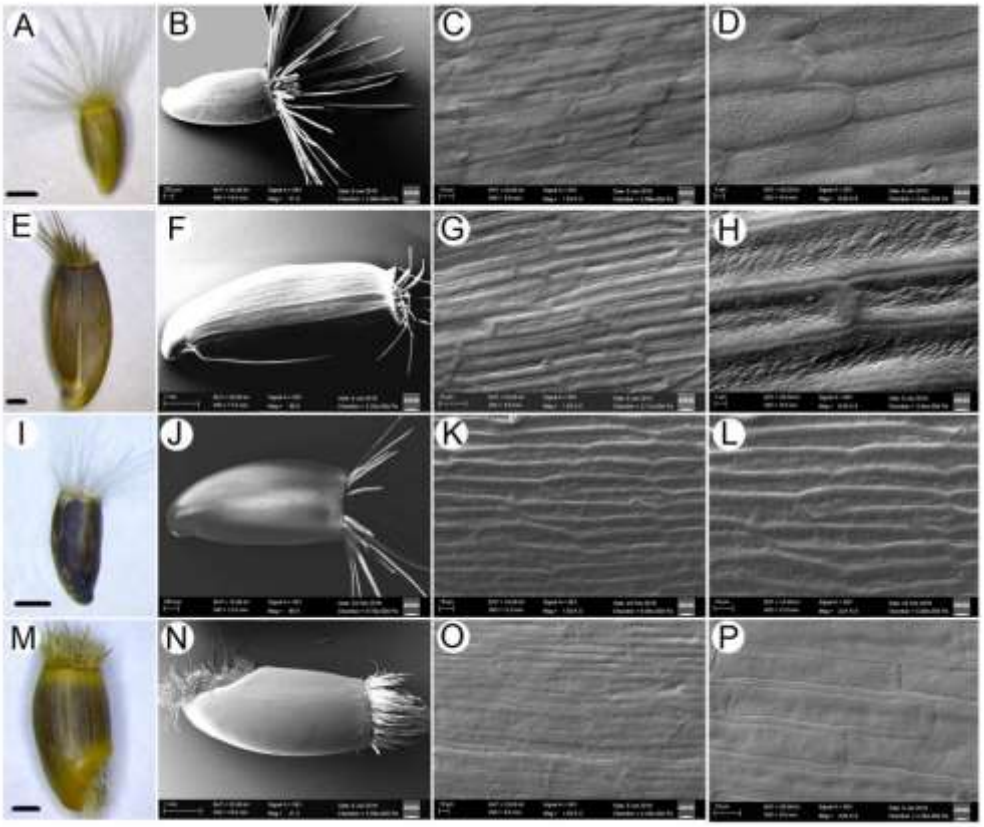

Figure 5. LM and SEM micrographies of Cypsela. A-D, *C. aggregata* subsp. *albida*; E-F, *C. macrocephala*; I-L, *C. salicifolia*; M-P, *Cy. nigrofimbrius.* Scale bars: A, E, H, L = 500 µm

with different sizes in above of cypselas (Table 5). However, both ribbed and non-ribbed cypselas were previously reported in the family Asteraceae by Abid and Qaiser (2009). Shabestari et al. (2013) previously examined various *Centaurea* taxa and described their seed (cypsela) types as a striate sculpture, strongly depressed on the both sides, with or without hairs on the surfaces.

Table 5. Cypsela micromorphology of investigated taxa. *mean± standard deviation.

Character	*Centaurea aggregata* subsp. *albida*	*C. macrocephala*	*C. salicifolia*	*Cyanus nigrofimbrius*
Length (cm)	2.40±0.05[a]	6.83±0.13[d]	2.72±0.08[b]	4.55±0.04[c]
Width (cm)	1.05±0.04[a]	2.82±0.10[c]	1.05±0.04[a]	2.42±0.05[b]
Shape (L/W)	widely oblong	widely oblong	widely oblong	ovoid
Color	brown	dark brown	light brown	brown-dark brown
Ornamentation	fine sulcate	scrobiculate-ribbed	Striate to scrobiculate-ribbed	scalariform
Anticlinal cell wall	straight	raised, straight	slightly raised, straight	straight
Periclinal cell wall	faintly rugulate	rugulate	coarse rugulate	smooth

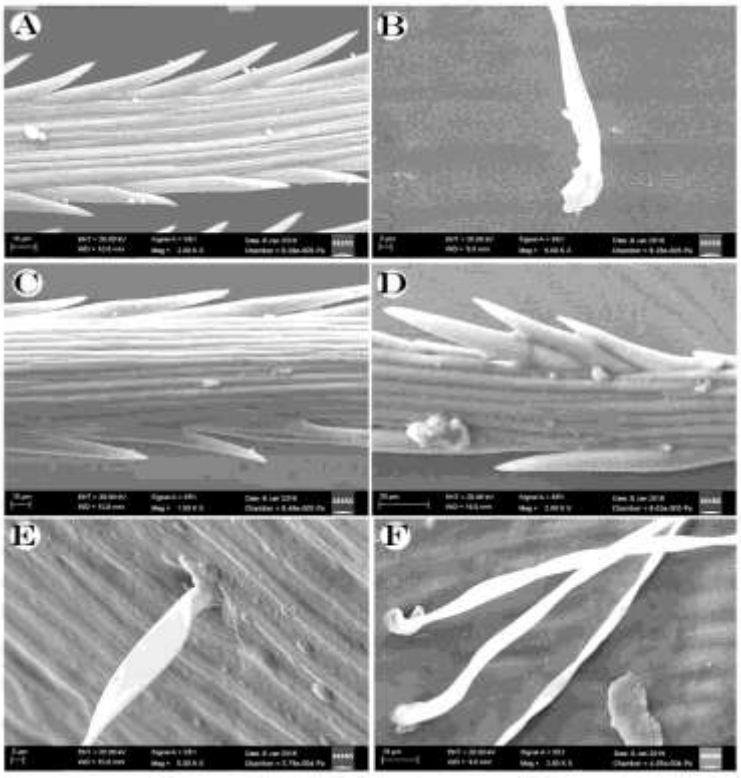

Figure 6. Pappus and trichomes. A-B, *C. aggregata* subsp. *albida*; C, *C. macrocephala*; D-F, *Cy. nigrofimbrius*

Cypsela colors vary from yellowish brown to brown or dark brown (sometimes blackish in the middle part). They are swollen in 3 taxa (*C. aggregata* subsp. *albida*, *C. salicifolia*, *Cy. nigrofimbrius*), and compressed slightly on both sides in *C. macrocephala*, and symmetric or asymmetric in outline. Cypselas have a narrow base, sublateral hilum, thin and acute apex. They are widely oblong in three *Centaurea* taxa, but ovoid in *Cy. nigrofimbrius* (Figure 5).

Anticlinal cell walls observed in the taxa are straight, forming rectangular cells uniformly organized into more or less distinct longitudinal columns, while the periclinal cell walls are smooth or with small irregular elevation (Figure 5, Table 5). The taxa can be divided into four types according to their surface ornamentations, fine sulcate (*C. aggregata* subsp. *albida*), scrobiculate-ribbed (*C. macrocephala*), striate (*C. salicifolia*) and scalariform (*Cy. nigrofimbrius*). In some fruits samples of *C. salicifolia* scrobiculate-ribbed was also observed. Striate structure and sparsely long hairs were also reported in the achene of *C. polyclada* by Uysal et al. (2005). Three different striation types have been described in Turkish *Carthamus* taxa by Tarıkahya-Hacıoğlu et al. (2012). Bona (2014, 2015) described 8 ornamentation types in some *Centaurea* and *Cyanus* taxa. In this mentioned study, surface ornamentation of *C. aggregata* subsp. *aggregata* was determined as glebulate-ruminate (Bona 2014) and smooth surface pattern was reported in four *Cyanus* species (Bona 2015). Candan et al. (2016) investigated some taxa present in the sect. *Acrolopus* and striate type ornamentation reported in *C. aggregata* subsp. *albida*. In the present study, the cypsela surfaces of 3 *Centaurea* taxa have more or less rugulate pattern, while *Cyanus nigrofimbrius* has smooth surface pattern and scalariform ornamentation. In one previous study, cypsela ornamentation of *Cy. nigrofimbrius* was reported by Şirin et al. (2017) as irregularly sulcate and not smooth surface, differently from this study. Striate ornamentation has been also reported by Shabestari et al. (2013) and Candan et al. (2016) in some *Centaurea* taxa.

In some species in the family Asteraceae, four ornamentations were determined as rectangular, rectangular with septae, hexagonal with septae or rugose by Kulkarni (2013). Based on the present results, the septum is not present in the surfaces of the *Centaurea* taxa investigated. Andrés-Sánchez et al. (2015) referred to twin hairs in some species (e.g. *Filago* L.) in the family Asteraceae. Dittrich (1985), however, reported that twin hairs are never present on the achene surface in the subtribe *Carduinae* and *Centaureinae*. Similarly, only sparsely long hairs were observed in the present study (Figure 6). Simple indumentum is sparsely found in cypselas from three taxa (*C. aggregata* subsp. *albida*, *C. salicifolia*, *Cy. nigrofimbrius*). The hilum densely covered with hair is only observed in *Cy. nigrofimbrius* (Figure 5M), similar to Şirin et al. (2017)'s report. Mean lengths of cypselas varied from 2.4 mm (*C. salicifolia*) to 6.83 mm (*C. macrocephala*), while their mean widths varied between 1.05 mm and 2.42 mm. The largest cypselas were characteristic of *C. macrocephala* while the smallest were in *C. aggregata* subsp. *albida* (Table 5). The pappus is scabrous, and one or two seriate in all taxa investigated. Mean length ranged from 0.3 mm to 5.2 mm in the taxa (Figure 6).

3.5. Multivariate Analyses

Analysis of variance analysis was performed on all quantitative (40) values (Tables 2-5). For the cluster analysis (UPGMA), 42 anatomical and micromorphological characteristics of stem, leaf and cypsela were analyzed (Figures 1-5; Tables 2-5). The phenogram (Figure 7) showed that clusters were separated from all species in a distinct position. The delimitation of these groups is mainly based on the occurrence of a winged structure in stem, height, width and number of vascular bundles and mesophyll thickness in the leaf midrib, thickness of pericarp in cypsela ($P<0.05$). In the dendrogram, four taxa were divided into two major clusters at a distance coefficient of approximately 25.0. Each cluster consisted of two different taxa. The first included *C. macrocephala* and *Cy. nigrofimbrius* and the second contained *C. aggregata* subsp. *albida* and *C. salicifolia*. The clustering of *C. macrocephala* and *Cy. nigrofimbrius* at a low distance coefficient of approximately 3.0, together with *C. aggregata* subsp. *albida* and *C. salicifolia* at a distance coefficient of approximately 1.0, reflects an underlying resemblance between them. In UPGMA, *C. aggregata* subsp. *albida* and *C. salicifolia* positioned together have also share similar morphological features such as small cluster capitula. On the other hand, *C. macrocephala* and *Cy. nigrofimbrius* found in second cluster have big single capitula and also winged type stems (Figure 7).

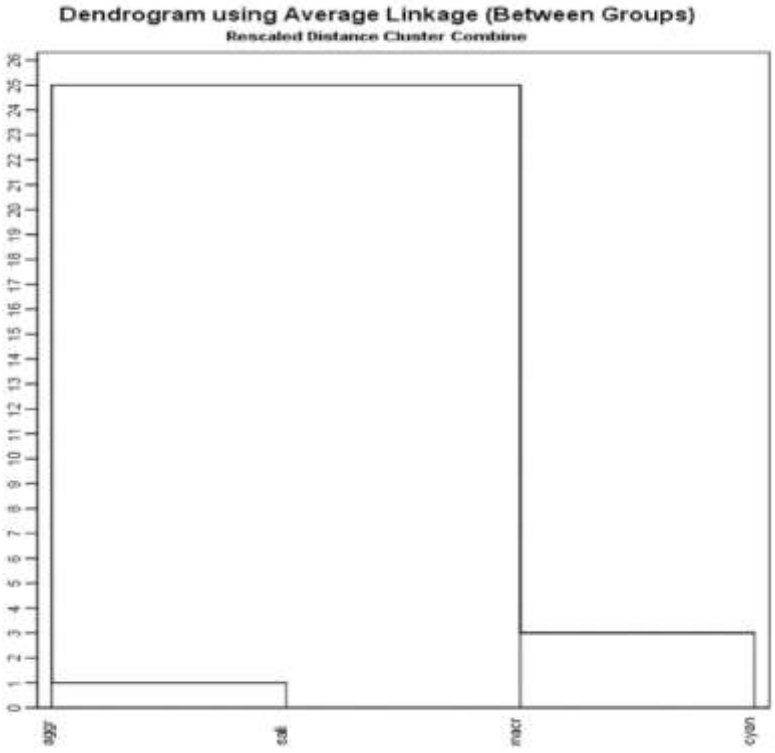

Figure **7.** UPGMA Cluster analysis using anatomical and micromorphological characters of investigated taxa. Each code represents a taxon. Taxa codes; aggr: *C. aggregata* subsp. *albida*, cyan: *Cy. nigrofimbrius*, macr: *C. macrocephala*, sali: *C. salicifolia*

Cluster analysis performed with 42 characters was useful in demonstrating the existence of clearly distinct groups of taxa, without generic delimitation. According to surface ornamentation, four different cypsela surface types were observed as fine sulcate, scrobiculate-ribbed, striate to scrobiculate-ribbed and scalariform (Table 5). Data obtained from the present study show that the anatomical and micromorphological characters investigated are mostly informative. They can be used as a parameter to support species identification. Investigation of vegetative parts and cypsela structures should therefore be included in future taxonomic and phylogenetic studies of the genus *Centaurea*..

Acknowledgements

The author is thankful to the Research Foundation of Artvin Coruh University for financial support (Project number: 2016.F10.01.08).

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(Received for publication 11 January 2017; The date of publication 15 August 2018)