Pak. J. Bot., 46(4): 1287-1301, 2014.

COMPARATIVE ANATOMICAL AND ECOLOGICAL INVESTIGATIONS ON SOME C*ENTAUREA* (ASTERACEAE) TAXA FROM TURKEY AND THEIR TAXONOMIC SIGNIFICANCE

MELAHAT OZCAN[1]*, MEHMET C. UNVER[2] AND OZGUR EMINAGAOGLU[3]

[1]*Artvin Coruh University, Faculty of Science and Arts, Department of Biology, 08000 Artvin, Turkey*
[2]*Artvin Coruh University, Department of Science Education, Faculty of Education, 08000 Artvin, Turkey*
[3]*Artvin Coruh University, Department of Forest Engineering, Faculty of Forestry, 08000 Artvin, Turkey*
**Corresponding author e-mail: melahat.ozcan@artvin.edu.tr*

Abstract

The anatomical and ecological characteristics of 7 *Centaurea* L. s. l. taxa (*C. urvillei* DC. ssp. *stepposa* Wagenitz, *C. pseudoreflexa* Hayek, *C. simplicicaulis* Boiss. & Huet, *C. pecho* Albow, *C. hypoleuca* DC., *C. cheiranthifolia* Willd.var. *purpurascens* (DC.) Wagenitz, *C. woronowii* Bornm.), which three of them are endemic (*C. pseudoreflexa*, *C. pecho*, *C. woronowii*) to Turkey has been carried out. Cross sections of stem, leaf blade and midrib and peripheral sections of the leaves, and pH, total N, P, K and organic matter contents of soil samples have been investigated. Variance analysis and Tukey's Honest Significant Difference test were performed on ecological and anatomical data. Ecological characteristics obtained in this investigation were found mostly similar. On the other hand, anatomically important differences were determined in diversity of anticlinal cell walls, length and width of abaxial epidermis, number of bundles in the midrib and number of stomata per mm^2 both on the epidermis of the leaf, and the shape of transverse section of stem. According to Tukey's Honest Significant Difference test, especially length and width of abaxial epidermis, number of stomata both on the adaxial and abaxial epidermises have displayed three distinct groups. These results are in accordance with sectional delimitation in the Flora of Turkey and also support upgrading the sum of species of the genus *Centaurea*.

Introduction

Centaurea L. comprises approximately 600 species with the Mediterranean area and SW Asia (Wagenitz & Hellwig, 1996; Brummitt, 2004). The genus *Centaurea* is represented by 190 species in Turkey, excluding the species were placed in the genera *Psephellus* Cassini and *Rhaponticoides* Vaillant, according to recent studies (Uzunhisarcıklı *et al*., 2007; Uysal, 2008; Daskın & Yılmaz, 2009). This is the third largest genus in Turkey after *Astragalus* L. and *Verbascum* L., and most taxa of them are endemics (Davis *et al*., 1988; Duran & Duman, 2002; Kaya *et al*., 2010). Endemism ratio is 60% (Wagenitz, 1960, 1975; Guner *et al*., 2000). High endemism ratio shows that Turkey is one of the gene centers this genus (Wagenitz, 1986; Uzunhisarcıklı *et al*., 2007; Martin *et al*., 2009; Meriç *et al*., 2010).

Centaurea is a taxonomically difficult genus which needs further study. The problem of reasonable and natural divisions of this vast genus still remains to be resolved. The species delimitation is problematic in many sections and there are numerous intermediate species (Wagenitz, 1975). Its medicinal importance, as antidiabetic, antidiarrhoeal, antirheumatic, antiinflammatory, choleretic, digestive, stomachic, diuretic, menstrual, astringent, hypotensive, antipyretic, sitotoxic, antibacterial, has been widely emphasized by several workers (Orallo *et al*., 1998; Baytop, 1999; Arif *et al*., 2004; Guven *et al*., 2005).

Centaurea is the subject of active research (Hidalgo *et al*., 2008; Uysal *et al*., 2009) and new taxa in *Centaurea* are regularly described (Duran & Duman, 2002; Turkoglu *et al*., 2003; Aytac & Duman, 2005; Raimondo & Spadaro, 2008; Uysal, 2008; Dinc *et al*., 2009, Uysal & Kose, 2009). Some investigations have been carried out on the cytotaxonomy of *C. pecho*, *C. pseudoreflexa* and *C. hypoleuca* (Inceer *et al*., 2007), *C. simplicicaulis* (Poddubnaja-Arnoldi, 1931; Inceer *et al*., 2007) and *C. cheiranthifolia* var. *purpurascens* (Khaniki, 1995) and some other taxa (Romaschenko *et al*., 2004; Martin *et al*., 2009). In addition, chemical analysis of two taxa (Oksuz & Serin, 1997; Celik *et al*., 2005), ecological and biological properties of two endemic taxa (Kose & Yücel, 2007), antimicrobial activity of five endemic species (Guven *et al*., 2005), and fatty acid composition of six species (Tekeli *et al*., 2010) are given in literatures.

Many anatomical characters are useful in systematic, despite the influence of environmental factors, (Metcalfe & Chalk, 1950, 1979). Therefore the taxonomists have investigated anatomical characters that may support species identification. Such characterization aims to select anatomical data that may strengthen the taxonomy of the genera and provide features allowing better identification of the species. Stem, especially leaves provide many anatomical characters which are proven to be of importance in classification (Stace, 1984; Lu *et al*., 2008), and have been widely used in taxonomic treatments and systematic studies (Ogunkunle & Oladele, 2008; Araújo *et al*., 2010; Gomes & Lombardi, 2010; Jiang *et al*., 2010; Inceer & Ozcan, 2011; Liu & Zhu, 2011, Eminağaoğlu *et al*., 2012; Noman *et al*., 2014). Till now, anatomical characteristics of some *Centaurea* species were examined by Esau (1977) Metcalfe & Chalk (1950, 1979), Uysal (1991), Bhattacharya & Johri (1998), Kaya *et al*., (2000), Celik *et al*., (2005, 2008), Uysal *et al*., (2005), Altundağ & Gürdal (2009), Aydin *et al*. (2013) and Özcan (2013), and important features in their anatomies were reported.

This paper describes anatomical and ecological properties of the seven taxa, based on soil analyses, transverse sections of stem and leaf, and peripheral sections leaf and provides additional data which can contribute to the taxonomy of the genera *Centaurea*, *Psephellus* and *Cyanus*. Investigated characteristics are discussed with respect to their potential value and in relation to the previous work in *Centaurea*.

Materials and Methods

Plant collections: Plant taxa, including 3 endemics taxa to Turkey, were collected from natural habitats in NE Anatolia. The taxa are arranged phylogenetical order and their collections data are listed in Table 1. Specimens for morphological examinations were dried according to standard herbarium techniques and deposited in Artvin Coruh University Herbarium (ARTH).

Anatomical preparations: Anatomical observations were performed in the leaf and stem anatomy of the seven taxa. Fresh cauline leaves and parts of stem were fixed in the field with formaldehide, acetic acid–alcohol (FAA), or removed from herbarium material. The leaves and stems from herbarium specimens were softened by heating up in water. Then the plant parts were stored in 70% alcohol for anatomical studies. Handmade cross-sections were prepared from the median part of the stem. Transverse sections and peripheral sections of upper and lower epidermis of leaves were taken by hand using commercial razor blades and stained in Haematoxylin for about 15 min. To remove the excess stain, sections were washed in water several times (Algan, 1981). Semi-permanent slides were mounted in glycerin. Well stained sections were examined under a light microscopy and photographed using a Zeiss Primostar research microscobe with digital camera attachment Axiocam ERC 5S.

Five cross-sections from at least three different individual plants of each taxon were measured for each sample to assess the consistency of anatomical characters and to calculate the means and standard error among different cross-sections. Ten peripheral slides were prepared for each taxon and 50 stomatal lengths were measured on each slide. The length of stomata was measured under × 40 magnifications using a light microscope with an ocular micrometer using surface sections obtained from the adaxial and abaxial parts. The stomatal index was calculated according to the method described by Meidner & Mansfield (1968).

Ecological studies: During the flowering period the soil samples were collected from the same areas, where the plants were naturally growing. Soil samples were taken from 0 - 15 cm depths after the surface decomposed litter was removed. About 500 g of each sample were placed in polyethylene bags and brought to the laboratory. All samples were dried in air, passed through a 2 mm sieve and analyzed for different physico-chemical characteristics. Organic matter and pH were determined according to the method developed by Ozturk *et al*., (1997). The N contents were determined according to the Kjeldahl methods designed by Bremner (1965). Total potassium was determined using BWB flame photometer, following the method outlined by Pratt (1965). The total phosphorus was determined with spectrometer at a wavelength of 436 using Shimadzu UV 1800 spectrometer according to Anon., (1990).

Statistical analysis: Analysis of variance carried out to evaluate the anatomical characters and the data of soil analysis using Statistica version 7.0. Significant differences were determined using the Tukey's Honest Significant Difference (HSD) test (Zar, 1984). All of the test were performed at the significant level of $\alpha = 0.05$, with MICROSTA program.

Table 1. Collection data of investigated taxa in *Centaurea.*

Taxon	Locality	Coordinates	Voucher
C. urvillei DC. ssp. *stepposa* Wagenitz	Artvin, Seyitler Village, roadside, eroded banks, 540-568 m	41º11'59.2"N, 41º51'0.06"E	M. Ozcan 369
* *C. pseudoreflexa* Hayek	Gümüşhane, Keçi Kale road, eroded slopes, 1400 m	40º23'09.2"N, 39º41'50.4"E	M. Ozcan 410
C. simplicicaulis Boiss. & Huet	Artvin, roadside, rocky areas, 500 m	41º11'09.01"N, 41º49'08.52"E	O. Emin. 8748
	+ Artvin, Borçka, Aralık Village, Bayrak Tepe, rocky areas, 1367 m	41º22'46.16"N, 41º43'50.70"E	O. Emin. 7532
	+ Artvin, Şavşat, Veliköy, rocky areas, 1350 m	41º18'54.12"N, 42º26'14.44"E	O. Emin. 2773
**C. pecho* Albow	Artvin, Artvin-Yusufeli roadside, 266 m	41º10'59.53"N, 41º50'44.43"E	O. Emin. 8746
C. hypoleuca DC.	Gümüşhane, Gezge Village, alpine slopes, 1820 m	40º33'44.3"N, 40º03'26.8"E	M. Ozcan 420
C. cheiranthifolia DC. var. *purpurascens* (DC.) Wagenitz	Artvin, Şavşat, near Ardahan, Cenkelek high plateau, alpine meadow, 2439 m	41º11'58.66"N, 42º32'07.00"E	M. Ozcan 378
	+ Artvin, Borçka, Heba high plateau, alpine region, 2270 m	41º21'38.32"N, 41º52'07.83"E	O. Emin. 7892
	+ Artvin, Şavşat, Kirazli High Plateau, alpine meadow, 22350	41º14'20.61"N, 42º31'26.21"E	O. Emin. 2767
**C. woronowii* Bornm.	Artvin, Hatila Valley roadside, rocky areas, 627 m	41º11'39.02"N, 41º48'37.86"E	O. Emin. 8747

* : Endemic, + : The locality is used only for soil analysis

Results

Anatomy: A transverse section of stem, leaf blade, midrib and peripheral sections were investigated in this study (Figs. 1-7). The main anatomical characters, total of 33 assessed among the taxa studied are summarized in Tables 2 and 3. These tables compare detailed measurement of different cell and tissue types of stem and leaf of the studied taxa. The anatomical characters of all studied taxa were presented here for the first time.

Stem: The stems of all studied taxa show similar morphological patterns and they are more or less round or sometimes angled in transverse sections (Figs. 1, 2). It is almost round in *Centaurea simplicicaulis*, while is 5-6 angled in *C. pecho*, 6-7 angled in *C. urvillei* ssp. *stepposa* and *C. pseudoreflexa*, 6-8 angled in *C. cheiranthifolia* var. *purpurascens*, 5-8 angled in *C. woronowii* and 9-10 angled in *C. hypoleuca*. *C. cheiranthifolia* var. *purpurascens* has also leaf-like 2 wings (172-240 µm thick) which are located in opposite directions in addition to the angles (Fig. 2 c,d). The epidermis consists of single layered, flattened, roundish or ovate cells and surrounded by a more or less thick cuticle layer. It has covering multicellular hairs which are uniseriate with long whip-like terminal cell and glandular trichomes. Glandular trichomes with two stalked and four head cells are sparsely present in *C. cheiranthifolia* var. *purpurascens*, while they have short stalked or capitate trichomes and not much more in *C. woronowii* and *C. hypoleuca*. In the epidermal layers, stomata are visible in *C. urvillei* ssp. *stepposa*, *C. pseudoreflexa*, *C. pecho*, *C. hypoleuca* and winged parts of *C. cheiranthifolia* var. *purpurascens*. Multicellular hairs are abundant in *C. hypoleuca*, *C. cheiranthifolia* var. *purpurascens* and *C. woronowii*, while they are sparse in *C. simplicicaulis* and *C. pecho*. In addition to multicellular hairs, septate hairs are also present both *C. urvillei* ssp. *stepposa* and *C. pseudoreflexa* and *C. hypoleuca*.

The cortex of stem is different layered, with mostly isodiametric. Layer of cortex is the lowest number as 8-11 and 7-11 in *C. simplicicaulis* and *C. pecho*, while it is high number as 9-17 and 13-17 in *C. woronowii* and *C. hypoleuca*, respectively (Table 2). Underneath the epidermis, there is collenchyma with several layers in the corners. Number of these layers is seven to thirteen in *C. simplicicaulis*, five to eight in *C. pecho*, six to ten in *C. cheiranthifolia* var. *purpurascens*, five in *C. woronowii*, eigth to ten in *C. urvillei* ssp. *stepposa* and *C. pseudoreflexa* and eleven to fourteen in *C. hypoleuca*. Collenchyma and chlorenchyma take place of alternating segments below epidermis. Chlorenchyma cells are vertically elongated like palisade cells in *C. pecho* (Fig. 1 g,h). Beneath the collenchymas or chlorenchyma, a parenchyma was surrounded by vascular bundles. These bundles are collateral type and cambium is not distinguishable in all investigated taxa, except in *C. urvillei* ssp. *stepposa*. Number of vascular bundles in the stem is much more (35-42) in *C. cheiranthifolia* var. *purpurascens*, while is the lowest (12-17) in *C. pecho*. Phloem and xylem members are clear. Large sclerencymatic cap is present in above the phloem, except *C. cheiranthifolia* var. *purpurascens* (Fig. 2 c,d). This structure is not present above the phloem of this taxon. Several secretory canals (secretory ducts) are present in the cortex opposed to the bundles in all taxa (Figs. 1, 2). Parenchymatic pith cells situated centre of the stem are large, polygonal and rounded shape in five taxa (Figs. 1-2, Table 2). However, a large pith cavity is present at the center of the stem of *C. hypoleuca* and *C. cheiranthifolia* var. *purpurascens* (Fig. 2 a,c).

Leaf: Shape of leaf midrib ranges from flattened to convex on the ventral (adaxial) side, whereas it is convex on the dorsal (abaxial) side in all taxa.

There is a single-layered epidermis which is surrounded by thick cuticle on both the adaxial and abaxial surfaces of the leaf, except *Centaurea woronowii*. This taxon has thin cuticle (0.9-0.8 µm). In terms of size, upper epidermal cells are much larger than those of the abaxial ones in *C. simplicicaulis*, *C. pecho* and *C. hypoleuca* (Figs. 3 f,h, 4 b). Two or three central vascular bundles and two or six small vascular bundles can be seen in the midrib (Figs. 3, 4) and schizogen secretory canals are present near to these bundles. On the other hand, only one large central vascular bundle is seen in the midrib of *C. pecho* (Fig. 3 g). Collenchymatous cell layers are located below the both epidermises in the midrib region of all taxa. Midrib mesophyl thickness varies from 528.8 µm to 1235.4 µm (Table 3).

Lamina is bifacial (dorsiventral) in *C. simplicicaulis*, *C. pecho* and *C. hypoleuca*, and mesophyll composed compactly arranged two or three layers of palisade parenchyma which are cylindrical and a loosely arranged 4-8 layers of spongy parenchyma which are isodiametric and irregular shape (Figs. 3 f,h, 4 b, Table 3). On the other hand, it is equifacial (isobilateral) in other four taxa and mesophyll composed a loosely arranged 2-8 layer of spongy parenchyma and two or three layers of palisade parenchyma which are seen below the both epidermises (Figs. 3 b,d, 4 d,f).

In peripheral sections, adaxial (upper) epidermises are 4-6 angled, usually polygonal with straight anticlinal cell walls, while abaxial (lower) ones are irregular in shape and sinuous cell walls or more or less repand in five taxa. However, both epidermises are irregular shape and sinuous cell walls in other two taxa (Fig. 6 c-f). Multicellular eglandular trichomes are recognised easily on the upper and lower epidermis in the all studied taxa. Eglandular trichomes are more abundant in the abaxial surface in *C. simplicicaulis*, *C. pecho* and *C. hypoleuca*, while sparse in the adaxial one. They can be seen densely on both on the upper and lower side of the leaves of *C. cheiranthifolia* var. *purpurascens* and *C. woronowii*. On the other hand, glandular trichomes are clearly observed in upper epidermises of *C. simplicicaulis*, *C. hypoleuca* and *C. cheiranthifolia* var. *purpurascens* ((Fig. 4 a,b). They are short or medium stalked and big head cell. Moreover, septate hairs are present in *C. urvillei* ssp. *stepposa*, *C. pseudoreflexa* and *C. hypoleuca* (Fig. 3 a,c).

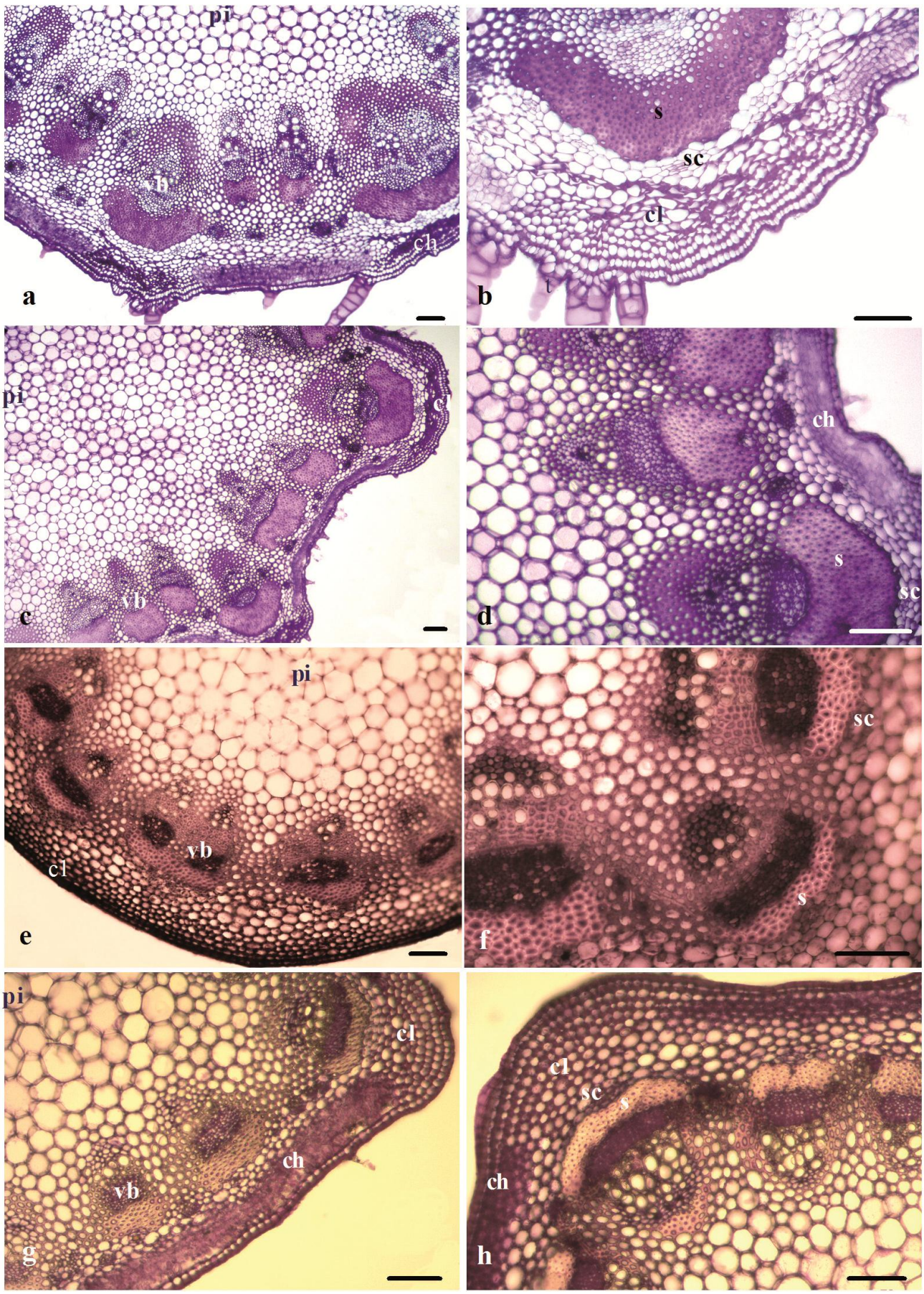

Fig. 1. Cross section of stem in sampled *Centaurea* taxa. (a, b); *C. urvillei* ssp. *stepposa*, (c, d); *C. pseudoreflexa*, (e, f); C. *simplicicaulis*, (g, h); *C. pecho*. ch: chlorenchyma, cl: collenchymas, pi: pith, s: sclerenchymatic cap, sc: secretory canal, wi: wing, vb: vascular bundle. Scale bars: 100 µm.

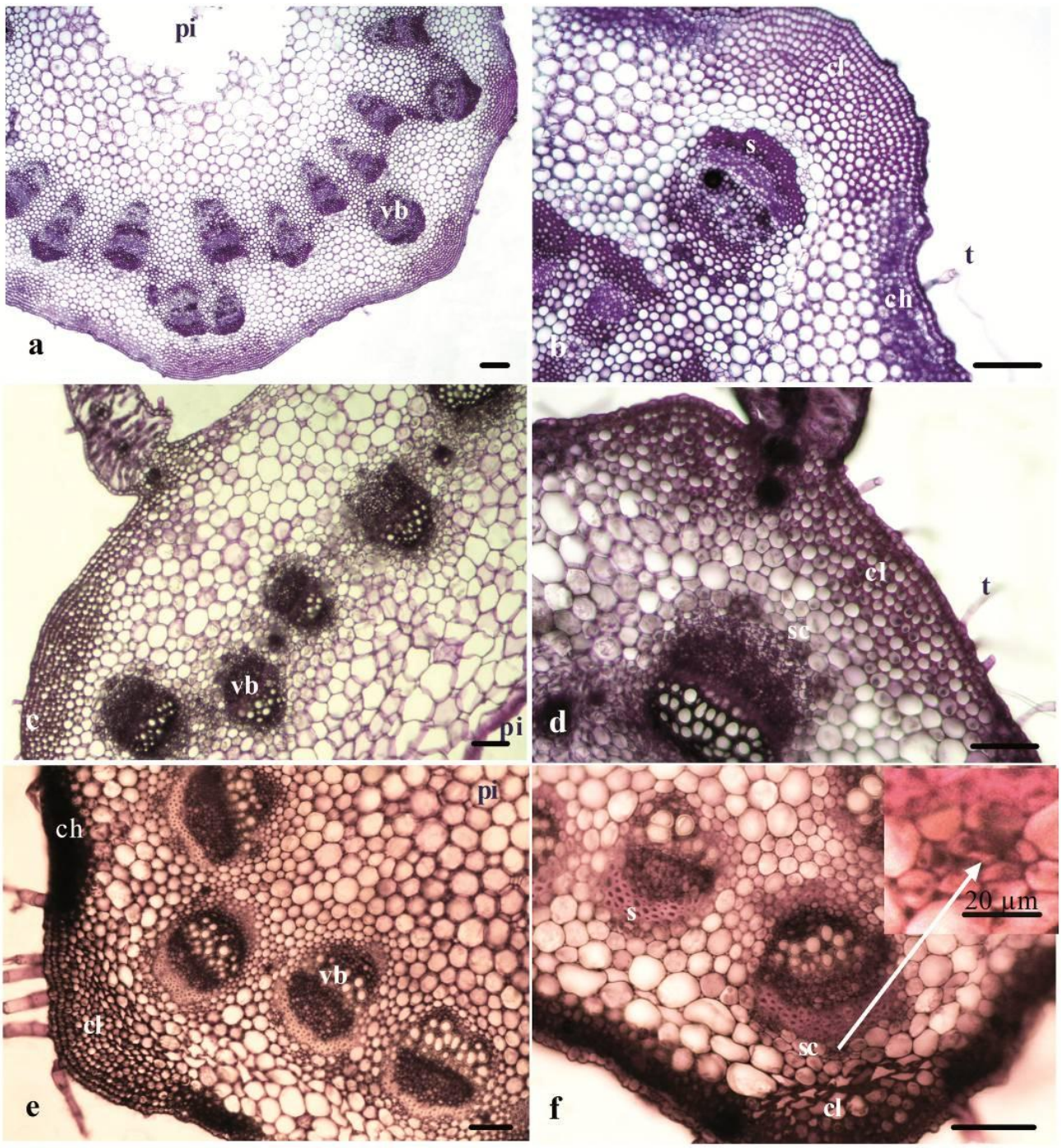

Fig. 2. Cross section of stem in sampled *Centaurea* taxa. (a, b); *C. hypoleuca*, (c, d); *C. cheiranthifolia* var. *purpurascens*, (e, f), *C. woronowii*. See Fig. 1. for abbreviations.

The leaf has anomocytic stomata cells with 3 or 5 neighboring cells. Stomata occur on the both surfaces (amphistomatic). Stomata cells are more abundant on the lower epidermis and slightly raised the epidermis level, whereas the pair of guard cells at the level with neighboring upper epidermal cells in *C. simplicicaulis*, *C. pecho* and *C. hypoleuca* (Figs. 3 f,h, 4b, Table 3). On the other hand, they are positioned on the both surfaces, at the same level with neighboring cells in *C. urvillei* ssp. *stepposa*, *C. pseudoreflexa*, *C. cheiranthifolia* var. *purpurascens* and *C. woronowii* (Figs. 3 b,d, 4 d,f, Table 3). In addition, stomata are in a cluster in *C. pseudoreflexa* and *C. urvillei* ssp. *stepposa* (Fig. 5 a,c,d).

Ecology: The result of the chemical analysis of the *Centaurea* taxa soils collected from study area is given in Table 4. According to variance analysis of pH, organic matter, total nitrogen and phosphorus all have meaningful group differences. *C. cheiranthifolia var. purpurascens*, *C. urvillei* ssp. *stepposa*, *C. hypoleuca*, *C. simplicicaulis*, *C. pecho*, *C. woronowii* and *C. pseudoreflexa* species grow in soil with pH values of 5.32, 6.80, 6.90, 7.26, 7.24, 7.18 and 7.20, respectively. The values of potassium content were 0.020% to 0.11% for all taxa *C. woronowii* has lowest content (Table 4). Average organic matter content in soil ranges from 1.58% to 6.55% (Table 4). The total nitrogen contents of *Centaurea* taxa studied vary from 0.08% to 0.42% (Table 4).

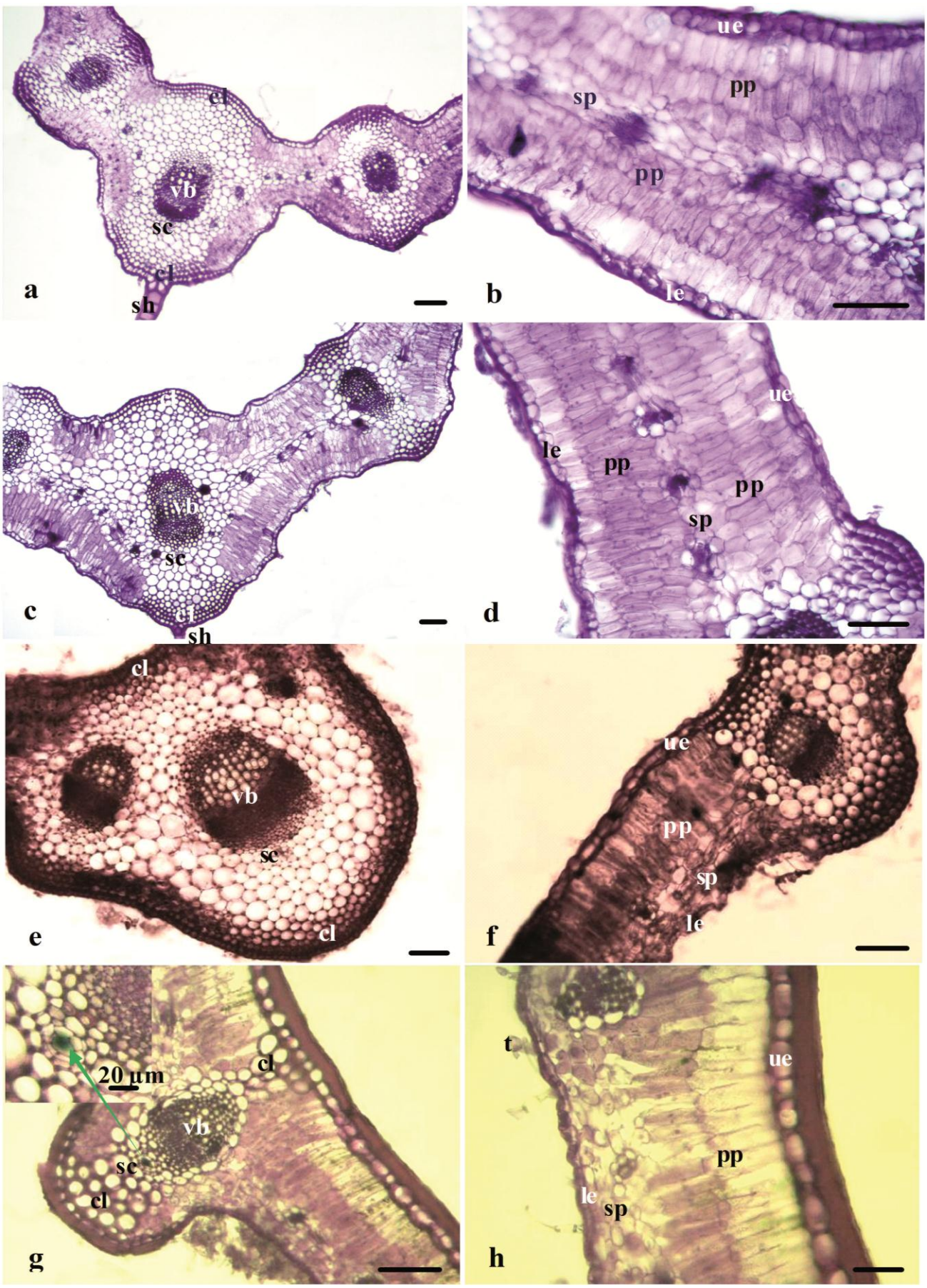

Fig. 3. Cross section of leaf in sampled *Centaurea* taxa. (a, b); *C. urvillei* ssp. *stepposa*, (c, d); *C. pseudoreflexa*, (e, f); *C. simplicicaulis*, (g, h); *C. pecho.* cl: collenchyma, le: lower epidermis, pp: palisade parenchyma, sc: secretory canal, sh: septate hair, sp: spongy parenchyma, ue: upper epidermis, vb: vascular bundle. Scale bars: 100 µm.

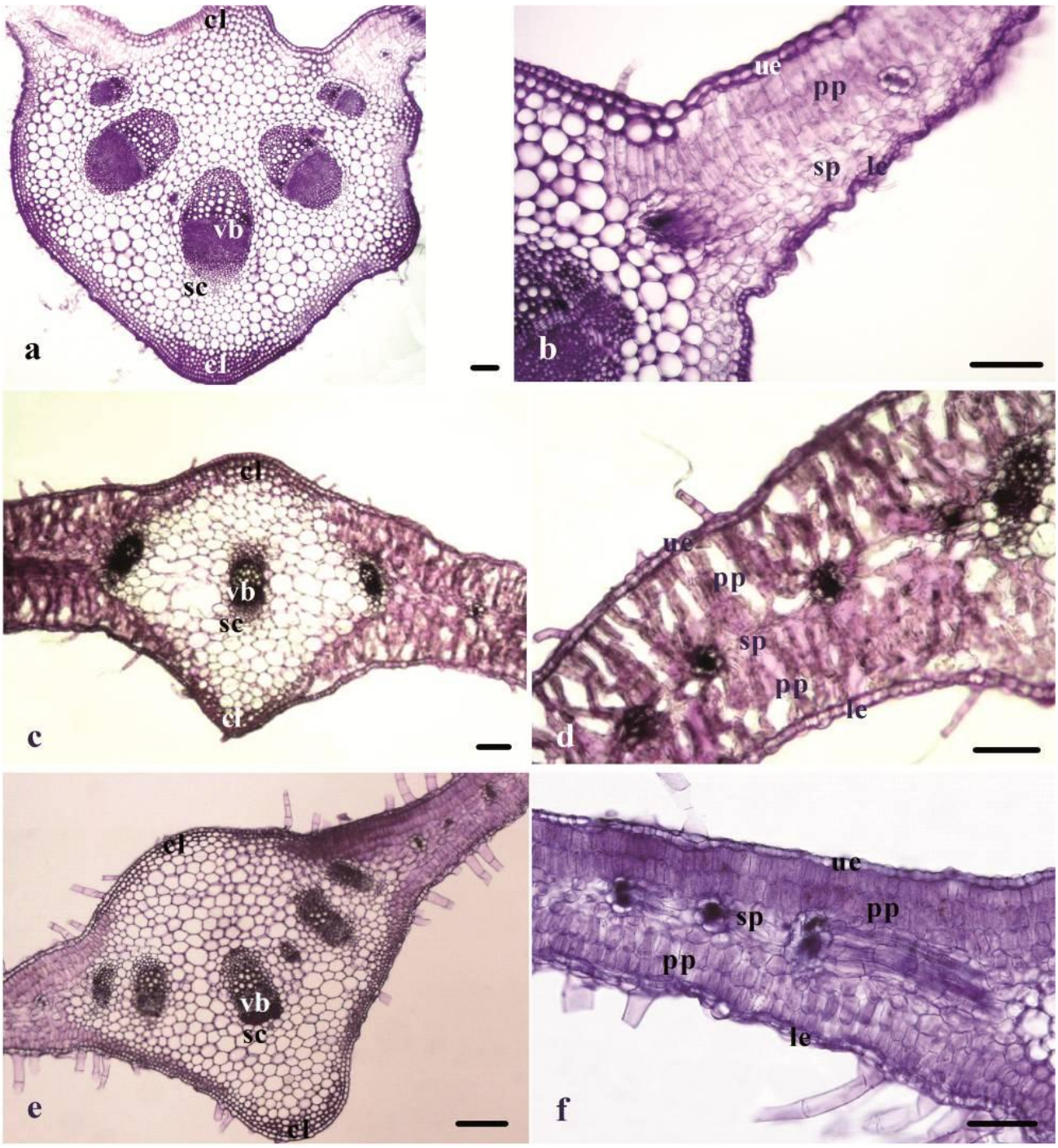

Fig. 4. Cross section of leaf in sampled *Centaurea* taxa. (a, b); *C. hypoleuca*, (c, d); *C. cheiranthifolia* var. *purpurascens*, (e, f); *C. woronowii*. See Fig. 3. for abbreviations.

Discussion

Several attempts have been carried out to subdivide the genus *Centaurea* (s.l.) which has 300 problematic species (Bremer, 1994; Wagenitz & Hellwig, 1996; Garcia-Jacas *et al*., 2006). In recent studies, four genera have been recognized viz., *Centaurea* L. (s.l), *Rhaponticum* Vaill., *Psephellus* Cass. and *Cyanus* Mill. (Wagenitz & Hellwig, 2000; Greuter, 2003a, 2003b). Some investigations were carried out on the genus *Psephellus* by Wagenitz & Hellwig (2000), Duran & Hamzaoglu (2005), and Duran *et al*., (2009) and Wagenitz & Hellwig (2000) expressed that the genus *Cyanus* resembles *Psephellus* some important characters, but some distinct differences were also reported between them. Wagenitz & Hellwig (2000) and Greuter (2003a, 2003b) remarked the taxa, *C. urvillei* ssp. *stepposa* and *C. pseudoreflexa* in the genus *Centaurea*, *C. simplicicaulis*, *C. pecho* and *C. hypoleuca* in the genus *Psephellus*, and *C. cheiranthifolia* var. *purpurascens* and *C. woronowii* in the genus *Cyanus*. On the other hand, Wagenitz (1975) placed these taxa in the three distinct sections of the genus *Centaurea*; *Acrocentron* (*C. urvillei* ssp. *stepposa*, *C. pseudoreflexa*), *Hyalinella* (*C. simplicicaulis*, *C. pecho*, *C. hypoleuca*) and *Cyanus* (*C. cheiranthifolia* var. *purpurascens*, *C. woronowii*) in Flora of Turkey.

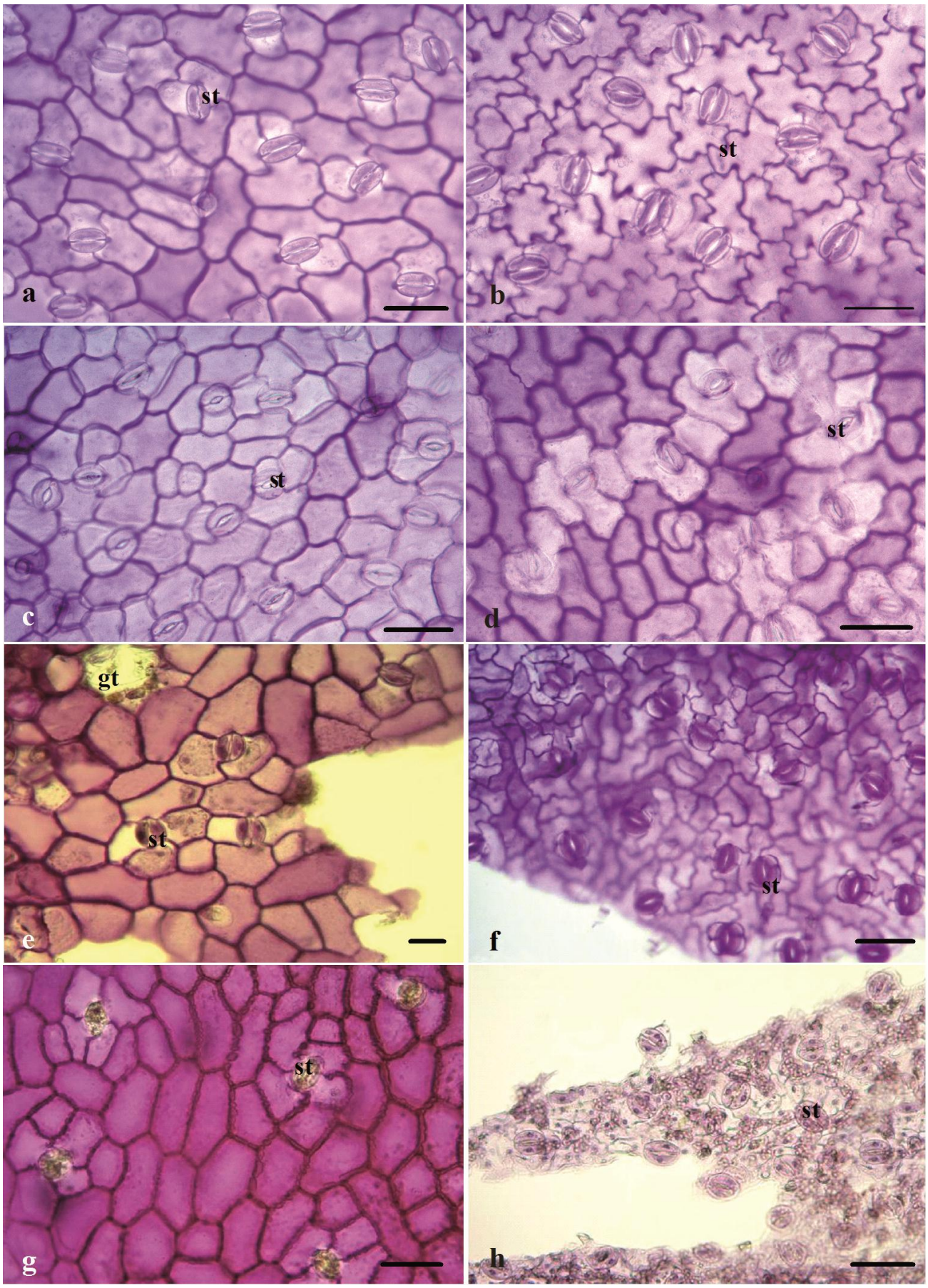

Fig. 5. Peripheral section of leaf in sampled *Centaurea* taxa. (a, b); *C. urvillei* ssp. *stepposa*, (c, d); *C. pseudoreflexa*, (e, f); *C. simplicicaulis*, (g, h); *C. pecho.* a, c, e, g: adaxial surface, b, d, f, h: abaxial surface, gt: glandular trichome, st: stomata, tr: simple trichome. Scale bars: 50 µm.

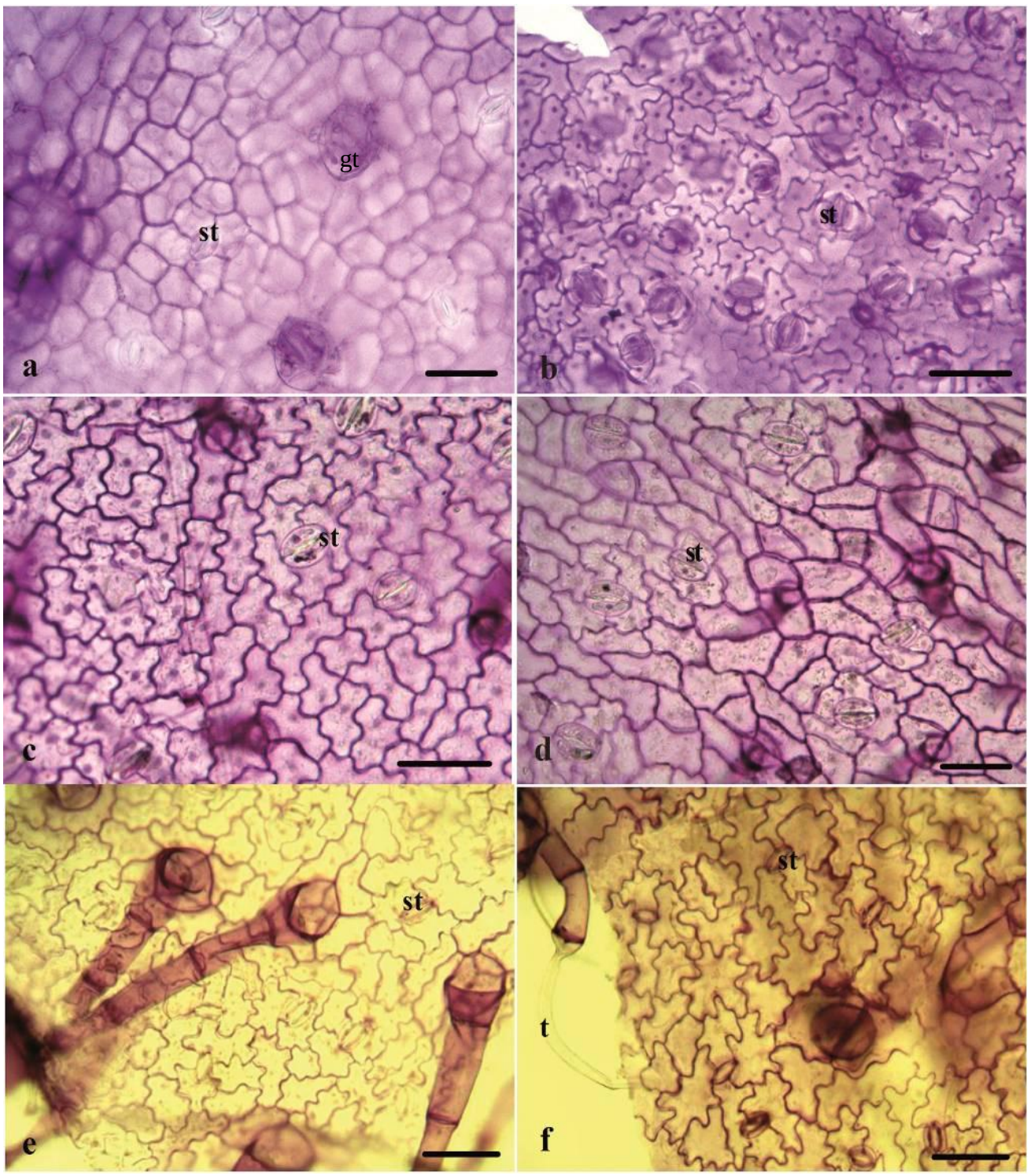

Fig. 6. Peripheral section of leaf in sampled *Centaurea* taxa. (a, b); *C. hypoleuca*, (c, d); *C. cheiranthifolia* var. *purpurascens*, (e, f), *C. woronowii*. See Fig. 5. for abbreviations.

Wagenitz (1975) explained morphological characters in the genus *Centaurea* and Metcalfe & Chalk (1979) gave information about the general anatomical characteristics of the family Asteraceae. In addition to some morphological and anatomical examinations, investigations were also made on palynology, phytogeographic distribution and ecology of some *Centaurea* s.l. species in Turkey (Pehlivan, 1994, 1996; Pinar & Inceoglu, 1996; Kaya *et al*., 2000; Reeves & Adiguzel, 2004; Celik *et al*., 2005; Uysal *et al*., 2005; Kose & Yücel, 2007; Özler *et al*., 2009; Potoğlu Erkaya *et al.*, 2012). However, no information on anatomical and ecological analyses of the investigated taxa was found in the literature.

In our study, stem transverse sections are almost similar in all the taxa, except for *Centaurea cheiranthifolia* var. *purpurascens* (Fig. 2 c,d, Table 2). This taxon has 2 wings and large pith cavity in its stem. All taxa investigated have collenchyma in the corners and chlorenchyma between the corners. The largest cortex and

collenchyma thickness in the corners are seen in *C. cheiranthifolia* var. *purpurascens*, while the smallest one are present in *C. simplicicaulis* (Table 2). Sclerenchymatic caps are present in the phloem parts of the bundles. On the other hand, sclerenchymatous cap is not present in the bundles of *C. cheiranthifolia* var. *purpurascens* (Fig. 2d, Table 2). In some *Centaurea* taxa and one *Psephellus* taxon studied, these kinds of tissues were previously reported by several authors (Metcalfe & Chalk, 1979; Uysal *et al*., 2005; Celik *et al*., 2005, 2008; Kaya *et al*., 2010; Aydin *et al*., 2013; Özcan, 2013). Our results are in agreement to these reports. Near the bundles secretory canals are observed in stem of all taxa. Kaya (1987), Celik *et al*. (2005), Kaya *et al*. (2010), Aydin *et al*. (2013) and Özcan (2013) have also mentioned this structure in several *Centaurea* s. l. taxa.

Anatomical features of leaves mostly differ in the taxa according to their sections or three genera (*Centaurea*, *Psephellus*, *Cyanus*). The leaves are amphystomatic with densely eglandular trichomes in abaxial parts and sparsely glandular hairs and sparsely or densely eglandular hairs in the adaxial parts of taxa. Glandular trichomes are present in adaxial parts of *C. simplicicaulis*, *C. hypoleuca* and *C. cheiranthifolia* var. *purpurascens*, while the others have only eglandular trichomes. The epidermis is surrounded by thick or thin cuticle. The amount of thickness is conspicuous in *C. pecho*. In terms of size, adaxial epidermal cells are also conspicuously larger than those of the abaxial ones in this species in the section *Hyalinella* (Table 3). The shape of epidermal cells and the pattern of anticlinal cell walls, which vary between taxa but are stable within a taxon and also within the same section, may represent good characters for taxonomy. In some taxa the anticlinal wall patterns are different on both surfaces; polygonal cells with straight to curved anticlinal cell walls on the adaxial epidermis and irregular cells with undulate (repand) anticlinal cell walls on the abaxial sides. This different pattern occurs in *C. urvillei* ssp. *stepposa*, *C. pseudoreflaxa*, *C. simplicicaulis C. pecho* and *C. hypoleuca* (Figs. 5, 6 a,b). Straigth to undulate epidermal shape have been also reported in several *Centaurea* or related taxa (Kaya *et al*., 2010; Aydin *et al*., 2013; Özcan, 2013). Amount of undulation is greater on the abaxial sides in these five taxa. According to Roth (1984), this is because the abaxial epidermis is exposed to conditions of greater shadow and humidity. The shapes of cells of *C. cheiranthifolia* var. *purpurascens* and *C. woronowii* on the adaxial epidermises with irregular and sinuous anticlinal cell walls are similar to abaxial ones (Fig. 6 c-f). Stace (1965) points out that epidermal cells with straight outlines are more common in xeromorphic plants than in mesomorphic plants, where they are typically undulate (repand). In addition, Fahn (1990) asserts that the epidermal cells of most leaves of shade-loving dicotyledons have sinuous anticlinal walls. It is also reported that such sinuosity is probably due to the tensions that occur in the leaf and to cuticle hardening during cell differentiation (Alquini *et al*., 2003). Our results are in agreement with these suggestions.

Table 2. Stem features of investigated taxa.

Character		*C. urvillei* ssp. *stepposa*	*C. pseudoreflexa*	*C. simplicicaulis*	*C. pecho*	*C. hypoleuca*	*C. cheiranthifolia* var. *purpurascens*	*C. woronowii*
Epidermis length (µm)		19.83 ± 1.07	17.90 ± 0.62	17.45 ± 0.17	15.93 ± 0.63	12.53 ± 0.34	13.57 ± 0.82	15.60 ± 0.40
Epidermis width (µm)		22.60 ± 0.51	25.40 ± 1.09	23.55 ± 1.12	20.40 ± 0.95	14.81 ± 0.55	18.00 ± 0.71	15.97 ± 0.66
Cortex thickness in the corner (µm)		190.2 ± 6.88	200.0 ± 28.2	156.2 ± 13.1	163.0 ± 15.1	167.5 ± 36.3	234.7 ± 9.14	230.0 ± 25.7
Number of cortex layer		9-14	10-13	8-11	7-11	13-17	9-15	9-17
Collenchyma thickness in the corner (µm)		149.9 ± 7.4	161.6 ± 22.8	78.20 ± 5.81	135.8 ± 15.7	168.4 ±4.76	170.3 ± 11.91	168.4 ± 18.8
Breadth of sclerenchymatic cap in phloem		149.5 ± 11.9	199.47 ± 9.59	60.67 ± 1.81	61.30 ± 3.63	78.23±3.72	absent	58.50 ± 4.55
Number of vascular bundles		21-25	27-29	24-26	12-17	31	34-45	20-23
Vascular bundle	Length (µm)	501.7 ± 55.8	543.9 ± 37.80	263.2 ± 18.9	293.7 ± 5.31	308.2 ± 10.4	273.6 ± 15.24	307.9 ± 4.8
	breadth (µm)	230.9 ± 10.4	196.3 ± 15.12	165.2 ± 6.55	178.1 ± 5.18	163.5 ± 6.08	211.60 ± 11.4	218.8 ± 7.9
Trachea (µm)		25.83 ± 1.64	25.83 ± 1.64	21.82 ± 1.17	27.05 ± 1.06	18.28 ± 0.31	24.78 ± 2.01	31.42 ± 0.53
Pith cell (µm)		80.0 ± 3.44	80.0 ± 3.44	89.9 ± 3.87	71.7 ± 2.32	77.3 ± 2.28	100.1 ± 3.91	76.0 ± 3.57

*mean ± : Standard error

Table 3. Leaf anatomical features of investigated taxa.

Character		*C. urvillei* ssp. *stepposa*	*C. pseudoreflexa*	*C. simplicicaulis*	*C. pecho*	*C. hypoleuca*	*C. cheiranthifolia* var. *purpurascens*	*C. woronowii*
Leaf type		equifacial	equifacial	bifacial	bifacial	bifacial	equifacial	equifacial
Upper epidermis	Length (µm)	26.13 ± 2.17	22.99 ± 0.97	29.17 ± 2.62	27.33 ± 1.24	21.73 ± 1.26	18.10 ± 0.93	17.56 ± 1.36
	Width (µm)	29.13 ± 1.84	31.73 ± 1.91	35.70 ± 2.09	39.67 ± 1.63	35.83 ± 2.90	23.98 ± 1.16	25.10 ± 3.61
Lower epidermis	Length (µm)	25.07 ± 1.76	23.37 ± 1.04	12.50 ± 0.60	08.35 ± 0.28	12.47 ± 0.28	17.10 ± 1.82	15.90 ± 0.58
	Width (µm)	31.10 ± 2.21	28.47 ± 1.62	18.20 ± 0.91	16.60 ± 1.08	17.28 ± 0.85	24.17 ± 1.75	22.10 ± 1.65
Midrib mesophyl breadth (µm)		811.2 ± 123.99	965.6 ± 17.29	909.20 ± 16.72	528.80 ± 19.24	1218.0 ± 138.59	1038.4 ± 40.42	1235.4 ± 52.33
Midrib adaxial shape		Flattish to convex	convex	flattish to concave	convex	convex	convex	convex
Lamina mesophyl breadth (µm)		333.60 ± 30.16	400.60 ± 16.89	217.57 ± 07.35	214.43 ± 07.02	224.33 ± 17.80	286.10 ± 18.25	243.96 ± 33.50
Trachea diameter (µm)		24.13 ± 2.94	23.07 ± 0.97	23.08 ± 0.76	17.12 ± 0.50	28.86 ± 02.29	22.55 ± 01.27	25.39 ± 0.79
Vascular bundle	Length (µm)	324.80 ± 26.95	352.0 ± 07.68	370.40 ± 06.87	215.80 ± 07.45	409.60 ± 32.18	336.00 ± 11.91	332.00 ± 4.37
	Breadth (µm)	251.20 ± 25.95	204.0 ± 18.21	300.00 ± 16.75	145.60 ± 09.31	267.20 ± 16.87	186.40 ± 17.39	198.40 ± 8.53
Number of the palisade cell line		3, 3	3, 3	2-3	2-3	2-3	2-3, 2-3	2-3, 2-3
Number of the spongy cell line		2	3	4-6	4-5	5-8	2-4	5-6
Adaxial surface	Cuticle thickness (µm)	5.0 ± 0.45	3.96 ± 0.04	03.95 ± 0.05	18.80 ± 1.85	3.0 ± 0.32	01.95 ± 0.05	0.90 ± 0.10
	Epidermal cell	polygonal shape, straight wall	polygonal shape, straight wall	polygonal shape, straight wall	polygonal shape, straight wall	irregular shape, straight wall	irregular shape, sinuous wall	irregular shape, sinuous wall
	Stomatal length (µm)	28.46 ± 0.84	29.20 ± 0.70	25.74 ± 0.15	26.14 ± 0.29	26.12 ± 0.66	29.90 ± 0.65	23.46 ± 0.13
	Stomatal index	18.45 ± 0.42	17.78 ± 0.80	07.83 ± 0.75	07.19 ± 0.72	06.31 ± 0.38	08.91 ± 01.21	10.71 ± 0.61
	Number of stomata (1 mm^2)	140.0 ± 10.18	122.00 ± 11.94	65.00 ± 9.98	50.00 ± 7.89	65.0 ± 8.95	130.0 ± 14.55	145.0 ± 09.34
Abaxial surface	Cuticle thickness (µm)	05.0 ± 0.45	03.40 ± 0.24	01.50 ± 0.22	02.10 ± 0.24	01.50 ± 0.22	01.96 ± 0.04	0.80 ± 0.12
	Epidermal cell	irregular shape, sinuous wall	irregular shape, repand wall	irregular shape, sinuous wall	irregular shape, sinuous wall	irregular shape, sinuous wall	irregular shape, sinuous or repand wall	irregular shape, sinuous wall
	Stomatal length (µm)	29.64 ± 0.37	29.16 ± 1.02	23.57 ± 0.56	24.08 ± 0.37	22.96 ± 0.30	33.06 ± 1.17	23.60 ± 0.26
	Stomatal index	20.63 ± 0.48	15.02 ± 0.78	14.49 ± 1.26	13.48 ± 0.27	15.05 ± 0.73	08.78 ± 1.07	11.68 ± 0.94
	Number of stomata (1 mm^2)	166.00 ± 10.37	150.0 ± 13.23	337.0 ± 23.68	380.0 ± 9.34	390.0 ± 45.06	110.0 ± 12.73	155.0 ± 19.96

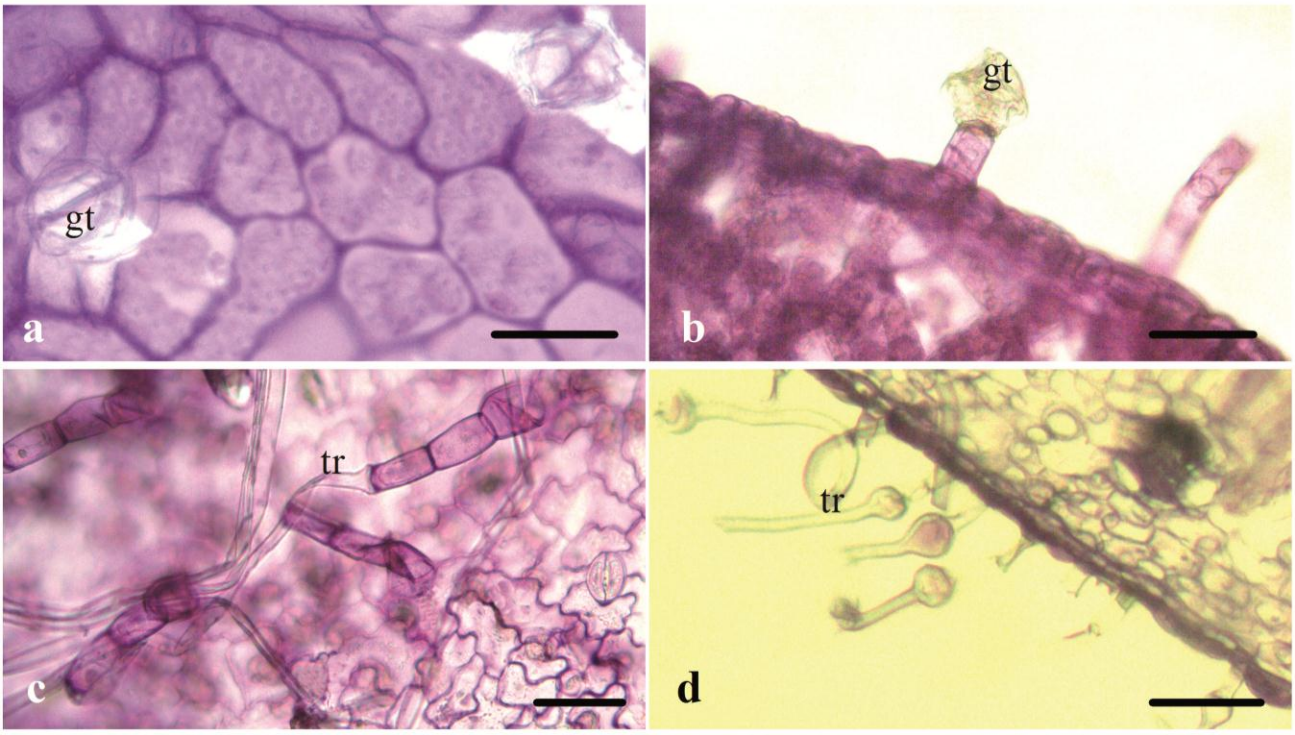

Fig. 7. Trichome type in sampled *Centaurea* taxa. (a, c); trichomes in peripheral section, (b, d); trichomes in cross section. gt: glandular trichome, tr: simple trichome. Scale bars: 50 µm.

Table 4. Soil analyses of investigated taxa in *Centaurea*.

Taxon	Organic matter (%)	Total N (%)	Ph	P_2O_5 (ppm)	K (%)
C. urvillei ssp. *stepposa*	1.58 ± 0.05	0.08 ± 0.03	6.80 ± 0.02	4.87 ± 0.05	0.023 ± 0.00
C. pseudoreflexa	2.12 ± 0.02	0.09 ± 0.03	7.21 ± 0.11	9.91 ± 0.52	0.040 ± 0.00
C. simplicicaulis	5.53 ± 0.07	0.12 ± 0.02	7.26 ± 0.01	20.99 ± 0.25	0.03 ± 0.00
C. pecho	2.08 ± 0.02	0.10 ± 0.00	7.24 ± 0.01	18.75 ± 1.15	0.11 ± 0.01
C. hypoleuca	4.20 ± 0.01	0.12 ± 0.02	6.90 ± 0.01	12.58 ± 1.02	0.080 ± 0.01
C. cheiranthifolia var. *purpurascens*	6.55 ± 0.08	0.42 ± 0.07	5.32 ± 0.07	29.64 ± 0.52	0.02 ± 0.00
C. woronowii	2.11 ± 0.03	0.16 ± 0.01	7.18 ± 0.01	9.38 ± 1.25	0.04 ± 0.00

The taxa have anomocytic or anomocytic-anisocytic stomata type. Stomata cells are more abundant on the lower epidermis, whereas it is a few in the upper parts of all taxa. Number of stomata per mm^2 is much dense in the lower parts of *Centaurea simplicicaulis*, *C. pecho* and *C. hypoleuca* than those of others four taxa. Three different sections or three different genera can be separated according to number of stomata in adaxial and abaxial parts of investigated taxa (Table 3). Average stomatal length for each species studied here varies from 33.06 to 23.46 and the average stomatal density ranges from 390.0 to 50.0 (Table 3). In addition, stomata are generally in the same level with epidermises, except for *Centaurea simplicicaulis*, *C. pecho* and *C. hypoleuca* (Table 3). In these species the stomata are positioned above abaxial epidermis level (Figs. 3 f,h, 4 b). This characteristic is also reported in *C. calcitrapa* ssp. *cilicica* and *C. solstitialis* ssp. *carneola* by Kaya *et al*., (2010). The guard-cells are oval-shaped and variable in size. Especially in the abaxial surfaces of *C. cheiranthifolia* var. *purpurascens*, guard cells are larger than adaxial ones (Table 3).

The adaxial surface of leaf midrib varies from concave, flattish to convex. The abaxial surface in all taxa is convex (Figs. 3, 4). The adaxial surface of midrib is flattish to concave in *Centaurea simplicicaulis*, while is convex in other six taxa. Leaf lamina is dorsiventral in *C. simplicicaulis*, *C. pecho* and *C. hypoleuca*, whereas it is isobilateral in the other four taxa. These three taxa grow in dry habitats, whereas the others which have isobilateral lamina grow in shadow or humid habitats. Isobilateral type of leaf lamina was reported in *Centaurea consanguinea* (Celik *et al*., 2005), in *C. polyclada* DC (Uysal *et al*., 2005), in *C. ptosimopappa* Hayek and *C. ptosimopappoides* Wagenitz (Celik *et al*., 2008), in *C. calcitrapa* L. ssp. *cilicica* (Boiss. & Bal.) Wagenitz and *C. solstitialis* L. ssp. *carneola* (Boiss.) Wagenitz (Kaya *et al*., 2010), and in the related taxon *Psephellus pulcherrimus* (Willd.) Wagenitz (Özcan, 2013). Dorsiventral and isobilateral type laminas are also common in the other family member of Asteraceae (Metcalfe & Chalk, 1979; Aydin *et al*., 2013).

There are three or five vascular bundles in three taxa studied, but in *Centaurea pecho* one large central vascular bundle is present in the midrib of leaf. Vascular bundles are collateral type and consist of xylem, phloem and sclerenchyma or collenchyma cells. Secretory canals are present near vascular bundles as in the stems (Fig. 1). According to Tetley (1925) and Williams (1954), since these ducts are so close to the phloem, they probably aid the sieve tube in the transfer of organic material. Kaya (1987), Uysal (1991), Uysal *et al*., (2005), Celik *et al*., (2008) and Özcan (2013) mentioned these secretory canals in some species of *Centaurea*. These structures have also frequently been reported in the family Asteraceae by Metcalfe & Chalk (1979).

Two basic types of trichomes can be distinguished in the investigated taxa: glandular and eglandular (Fig. 4). Leaf and stem surfaces are more or less densely covered by glandular and eglandular trichomes, forming an indumentum of variable texture and density. Among the examined taxa, *Centaurea simplicicaulis* and *C. pecho* are more or less glabrescent in the adaxial epidermises, while tomentose in the abaxial ones. All taxa studied have uniseriate and multicellular eglandular trichomes in abaxial surfaces. In addition, sepate trichomes are observed in *C. urvillei* ssp. *stepposa*, *C. pseudoreflexa* and *C. hypoleuca*. On the other hand, glandular thichomes are also observed in the stems of *C. cheiranthifolia* var. *purpurascens*, *C. simplicicaulis*, *C. hypoleuca* and *C. woronowii* and in the leaves of *C. simplicicaulis*, *C. hypoleuca* and *C. cheiranthifolia* var. *purpurascens* (Fig. 7). Our results about anatomy and also morphology obtained in this study are basically consistent with previous reports (Metcalfe & Chalk, 1979; Kaya, 1987; Uysal, 1991; Uysal *et al*., 2005; Celik *et al*., 2005; Kaya *et al*., 2010). On the other hand some important differences are also determined in the taxa. For example, in *C. urvillei* ssp. *stepposa* and *C. pseudoreflexa* in the section *Acrocentron*, stomata are in a cluster in the peripheral sections of leaves. In *C. simplicicaulis*, *C. pecho* and *C. hypoleuca* in the section *Psephellus*, have equifacial lamina and the stomata slightly raised the epidermis level. These differences are support sectional delimitation in the Flora of Turkey (Wagenitz, 1975) and the separation of the genus *Centaurea* as four genera (Wagenitz & Hellwig, 2000; Greuter, 2003a, 2003b).

According to soil analyses, *C. cheiranthifolia* var. *purpurascens* grows in habitats which contain plenty of organic matter, while others prefer medium amount of its. Six taxa, *C. urvillei* ssp. s*tepposa*, *C. pseudoreflexa*, *C. pecho*, *C. hypoleuca*, *C. woronowii* and *C. simplicicaulis* prefer soil which have poor nitrogen content and distribute in neutral or slight alkali medium, however *C. cheiranthifolia* var. *purpurascens* well grows in soil particules with medium amount of nitrogen and distributes in acidic soil. *C. cheiranthifolia* var. *purpurascens* well also grows in the soil containing rich phosphorus content, while *C. pseuporeflexa*, *C. pecho*, *C. hypoleuca* and *C. woronowii* prefer soil which has medium amount phosphorus content and *C. urvillei* ssp. *stepposa* prefers poor ones. On the other hand, all taxa investigated prefer soils contained rich potassium content (Table 4). Namely, ecological characteristics obtained in this investigation are mostly similar and do not support their separations.

To sum up, lamina type, and anticlinal epidermal cell wall, number of the vascular bundle structure, and the size, distribution and density of stomata in the leaves, and pith structure, the shape of transverse section, and wings in the stems are taxonomically significant characters in separating the taxa ($p<0.05$) (Tables 2, 3). Our anatomical findings are support to their sectional separations and also corroborate the dividing of the genus *Centaurea* as three different genera, and these results lead to understand of the phylogenetical positions of taxa. On the other hand, several taxa are present in these three sections and most of them have not been studied anatomically or ecologically till now. To make a detailed phylogenetic implication and better understand about their relationships among sections or the genera *Centaurea*, *Psephellus* and *Cyanus*, number of studied taxa should be increased.

Acknowledgements

This research was partly supported by the Research Fund of Artvin Coruh University, project No. 2011.F15.02.16. We express our thanks to the Research Fund of Artvin Coruh University for financial support and to the professors in Department of Biology, section Botany and section Zoology of Karadeniz Technical University for technical supports.

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(Received for publication 14 February 2013)