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ORIGINAL ARTICLE

Leaf anatomical notes on *Cirsium* Miller (Asteraceae, Carduoideae) from Turkey

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Abstract Here, we describe the leaf anatomical characters of 26 *Cirsium* taxa (33 accessions) native to the northeast region of Turkey, with the aim of evaluating their taxonomic significance within the taxa. Leaf anatomies of all taxa were characterized and compared using cluster analysis and multidimensional scaling analysis. The transverse sections of the leaves showed various numbers of vascular bundles and accessory bundles. In addition, significant differentiations were observed in the midrib and lamina thickness, the height and width of vascular bundle, and number of stomata, and epidermal cell wall patterns in the abaxial and adaxial surfaces among the taxa. These results demonstrate that the compared anatomical characters among taxa are partly in accordance with their sectional delimitation in the Flora of Turkey. However, our data suggest that some taxonomic re-arrangements might be necessary. Based on its anatomy, we propose that the *Cirsium* taxa can be treated as pioneers. Some ecological interpretations are also made.

Keywords Carduoideae · *Cirsium* · Leaf · Anatomy · Multivariate analyses · Turkey

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Introduction

Comparative leaf anatomy, including leaf epidermises and leaf transverse sections, can provide data on many taxonomic characters that have been proven to be of importance in species classification (Stace 1984; Lu et al. 2008). Such data have been widely used in taxonomic assignment (Ogunkunle and Oladele 2008; Gomes and Lombardi 2010; Jiang et al. 2010; Inceer and Ozcan 2011; Liu and Zhu 2011).

Cirsium is a large genus of the tribe *Carduoideae* (Stevens 2001) with complex taxonomy and nomenclature. The genus *Cirsium* comprises more than 250 taxa or variable number, depending on the study authors (Smith 1977; Zomlefer 1994). These perennial, biennial, or rarely annual, spiny species are distributed in Europe, North Africa, Siberia, Central Asia, West and East Africa, and Central America. Most of this species richness is concentrated in the mountains of southern Europe and Caucasia (Werner 1976; Meusel and Jäger 1992; Garcia-Jacas et al. 2002). According to recent studies, this genus is represented by 78 taxa at the level of species, subspecies, and variety in Turkey (Davis and Parris 1975; Davis et al. 1988; Guner et al. 2000; Daşkın et al. 2006; Yıldız 2012; Yıldız et al. 2013). Twenty-eight of these taxa are endemic to Turkey, resulting in an endemism ratio of 36 %. Endemic species are most frequently found in the section of *Epitrachys* DC (Davis and Parris 1975).

Cirsium is a typical example of a genus with a high affinity to form interspecific and intersubspecific hybrids. Under certain circumstances, the sterility barriers between closely related species of *Cirsium* break down, permitting hybridization and backcrossing. Hybrids also exhibit wide interfertility limits which translate into a large number of interspecific and intersubspecific hybrids. Several hybrids have been described from Caucasus and adjoining regions of Asia Minor (Davis and Parris 1975; Werner 1976; Charadze 1998; Bureš et al. 2004; Segarra-Moragues et al. 2007).

Several studies have been made to evaluate interspecific relationships within the genus *Cirsium* from Turkey using morphological and palynological features (Daşkın et al. 2006; Yıldız et al. 2009a, b; Arabaci and Dirmenci 2011; Yıldız et al. 2011, 2013), as well as karyological characters (Ozcan et al. 2008, 2011; Yüksel et al. 2013). However, there is as yet no report addressing the anatomy of the genus. Anatomical characters are well-established criteria and can offer significant assistance in plant taxonomy. Here, we aim to describe the leaf anatomy based on lamina midrib structure and stomatal characters of the taxa, to discuss these findings with respect to their potential systematic value. This work presents the first comprehensive evaluation of the systematic significance in the leaf anatomy of *Cirsium*.

Materials and methods

Plant collections

Examined samples (26 taxa, 33 accessions) were collected from natural populations in North-East Anatolia, Turkey (Fig. 1). The taxa were arranged in alphabetical order and their collection data are given in Table 1. Six taxa are indicated in this Table with recently reported new taxonomical names/status, differently from The Flora of Turkey. Specimens are deposited in Artvin Coruh University Herbarium (ARTH) and Karadeniz Technical University (KTUB).

Anatomical preparations

Fresh cauline leaves were fixed in the field with formalin–acetic acid–alcohol (FAA), or removed from herbarium material. Cross sections were prepared from the median part of the laminas. The leaves were sectioned by hand using commercial razor blades. The sections were stained with Hematoxylin solution for about 15 min (Algan 1981). Semi-permanent slides were mounted in glycerin. Well-stained sections were examined under a light microscopy and photographed using an Olympus BX53 research microscope with digital camera attachment DP73.

Several cross sections were obtained from three to five plants and, to assess the consistency of anatomical characters and to calculate the means and standard error among different cross sections, five of these sections were measured for each plant population. Ten paradermal slides (five from upper and five from lower surfaces) were prepared for each taxon and 50 stomatal lengths were measured on each slide. Epidermal and leaf structural features were described according to the terminology of Metcalfe and Chalk (1979) and the stomatal index was calculated according to the method described by Meidner and Mansfield (1968).

Statistical analyses

Multivariate analyses were performed to evaluate the anatomical characters using SPSS version 19.0. Anatomical characters (1 qualitative and 23 quantitative with mean value) of the taxa and their groupings were determined using the clustering analysis method (UPGMA, dissimilarity, standardized variable), as well as ordination based on multidimensional scaling analysis (MDS). In addition, discriminant analysis was performed to check the significance of the results obtained from cluster analysis. MDS based on the Euclidean distance model of stimulus configuration of measures was carried out to check taxonomic differentiation of the taxa investigated at the level of leaf anatomical characteristics.

Results

All investigated taxa had bifacial (dorsiventral structure) mesophyll; two or three layers, rarely one layer (*C. hypoleucum*) of compactly arranged palisade parenchyma, and three to eight layers of spongy parenchyma arranged with small spaces (Figs. 2, 3, 4). Stomata were found both on the adaxial and abaxial surfaces of all investigated taxa, except for *C. pseudopersonata* subsp. *pseudopersonata*, *C. pseudopersonata* subsp. *kusnezowianum* and *C. hypoleucum*. The stomata and other epidermal features were consistent within the same taxa and represented reliable characters for taxonomic purposes. The main leaf anatomical features observed through light microscopy are summarized in Tables 2 and 3, showing significant differentiations between taxa. More specific interpretations and illustrations of the anatomical features are described below.

Anticlinal cell wall patterns

The adaxial epidermal cells of the *Cirsium* taxa, as seen under light microscopy, are usually polygonal or irregular in form, with the anticlinal cell walls usually straight (Figs. 5, 6, 7). Twenty-one taxa appear to have straight anticlinal cell walls in adaxial surfaces, however, *C. pseudopersonata* subsp. *pseudopersonata*, *C. pseudopersonata* subsp. *kusnezowianum*, *C. hypoleucum*, *C. obvallatum*, and *C. simplex* subsp. *armenum* have sinuous cell walls. Whereas, the anticlinal cell walls of most taxa were sinuous on the abaxial surface, except for *C. pubigerum*

Table 1 Collection data of studied *Cirsium* taxa

Section		Taxon	Locality	Coordinates	Voucher
Cephalonoplos	(1)	*C. arvense* (L.) Scop. (Syn: *C. arvense* (L.) Scop. subsp. *arvense*; *C. arvense* subsp. *vestitum* (Wimm. & Grab.) Petr.)	Artvin: Ardanuç, Güleç village, near Geçitli, 1636–1736 m	42°13′15.2″E, 41°02′26.8″N	M. Ozcan 162
			Artvin: Şavşat, Sahara National Park road, Karagöl, 1500 m	42°25′40.1″E, 41°14′13.0″N	M. Ozcan 117
			Bayburt: Erikdibi village, near cultivated area, 1618 m	40°15′36.9″E, 40°26′11.1″N	M. Ozcan 130
Cirsium	(2)	*C. alatum* (S.G.Gmel) Bobrov (Syn: *C. subinerme* Fisch. & C.A.Mey., *C. elodes* M.Bieb.)	Gümüşhane: Gezge village, 1804 m	40°03′26.3″E, 40°33′39.2″N	M. Ozcan 171
	(3)	*C. echinus* (M.Bieb.) Hand.-Mazz.	Gümüşhane: Zigana Pass, roadsides, 1750 m	39°24′19.7″E, 40°02′27.2″N	M. Ozcan 058
	(4)	*C. hypoleucum* DC.	Rize: İkizdere, Anzer, under forest, 1468 m	40°32′80.1″E, 40°39′38.1″N	M. Ozcan 561
			Rize: Çamlıhemşin, Zilkale near Çat, 1044–1170 m	39°46′51.0″E, 40°59′35.1″N	M. Ozcan 223
	(5)	*C. obvallatum* (M.Bieb.) Fisch.	Rize: Çamlıhemşin, Yukarı Kavrun High plateau, alpine meadows, 2299 m	41°07′55.0″E, 40°52′40.2″N	M. Ozcan 453
	(6)	*C. pseudopersonata* subsp. *kusnezowianum* (Sommier & Levier) Petr.	Artvin: Borçka, Camili road, downward Maçahel Pass, near streamsides, 1631 m	41°50′30.7″E, 41°25′38.1″N	M. Ozcan 278
	(7)	*C. pseudopersonata* Boiss. & Balansa subsp. *pseudopersonata*	Rize: Anzer road, near humid areas, 1433 m	40°32′21.8″E, 40°39′25.3″N	M. Ozcan 136
			Rize: Çamlıhemşin, Verneçik road, 1392 m	39°46′51.0″E, 40°59′35.1″N	M. Ozcan 225
	(8)	*C. pubigerum* var. *caniforme* Petr.	Artvin: Ardanuç Tepedüzü village, humid areas, 1200 m	42°04′34.0″E, 41°05′14.0″N	M. Ozcan 160
	(9)	*C. pubigerum* var. *glomeratum* (Freyn & Sint.) P.H.Davis & Parris	Rize: Ayder, Yukarı Kavrun High plateau, near cultivated area, 2322 m	41°07′26.7″E, 40°52′35.5″N	M. Ozcan 187
	(10)	*C. rhizocephalum* C.A.Mey. subsp. *rhizocephalum*	Gümüşhane: near Köse, Gökçe village, among grass, 1677 m	39°42′28.4″E, 40°13′31.3″N	M. Ozcan 481
	(11)	*C. rhizocephalum* subsp. *sinuatum* (Boiss.) P.H.Davis & Parris	Trabzon: Çaykara, Soğanlı Dağı, High plateau, 2716 m	40°23′39.5″E, 40°32′35.4″N	M. Ozcan 254
			Trabzon/Bayburt: near Limonsuyu, 2369 m	40°05′04.2″E, 40°32′53.8″N	M. Ozcan 168
	(12)	*C. simplex* subsp. *armenum* (DC.) Petr.	Rize: Çamlıhemşin Yukarı Kavrun High plateau, 2280 m	41°07′47.2″E, 40°52′20.4″N	M. Ozcan 452
Epitrachys	(13)	*C. adjaricum* Sommier & Levier	Artvin: Şavşat, Sahara Milli Park road, roadsides, Kocabey village, 1654 m	42°24′49.4″E, 41°14′05.9″N	M. Ozcan 495
	(14)	*C. aggregatum* Ledeb.	Trabzon: Bayburt–Trabzon boundary, Soğanlı Pass, Çaykara road, alpine meadow, 2100–2210 m	40°17′25.1″E, 40°32′59.1″N	M. Ozcan 459
	(15)	*C. bulgaricum* DC.	Trabzon: Akçaabat, Hıdırnebi, Balıklı High plateau, 1516 m	39°24′48.7″E, 40°56′34.2″N	M. Ozcan 596
	(16)	*C. caucasicum* (Adams) Petr.	Artvin: Şavşat; Between Şavşat and Ardahan, roadsides, 2398 m	42°22′49.9″E, 41°13′06.8″N	M. Ozcan 490
	(17)	*C. cephalotes* Boiss.	Bayburt: Kop mountain roadsides, 2417 m	40°02′11.5″E, 40°30′42.1″N	M. Ozcan 466

Table 1 continued

Section	Taxon	Locality	Coordinates	Voucher
	(18) *C. kosmelii* (Adams) Fisch. ex Hohen. (Syn: *C. munitum* Fisch.)	Artvin: Between Şavşat and Ardahan, roadsides, 2261–2300 m	42°27′49.9″E, 41°13′06.8″N	M. Ozcan 488
		Rize: Ayder, Yukarı Kavrun High plateau, near cultivated area, 2283 m	41°07′56.7″E, 40°52′49.3″N	M. Ozcan 456
	(19) *C. leucocephalum* (Willd.) Spreng. subsp. *leucocephalum* (Syn: *C. lappaceum* subsp. *anatolicum* Petr.	Gümüşhane: Köse, Salyazı road, 1682 m	39°43′02.5″E, 40°13′26.6″N	M. Ozcan 480
	(20) *C. leucocephalum* subsp. *penicillatum* (K.Koch) Greuter (Syn: *C. lappaceum* (M.Bieb.) Fisch. subsp. *lappaceum*)	Bayburt: Kop Dağı, alpine glass, 2417 m	40°30′42.1″E, 40°02′11.5″N	M. Ozcan 467
	(21) *C. macrobotrys* (K.Koch) Boiss.	Gümüşhane: Sarıçiçek road, 2006 m	39°50′45.0″E, 40°23′0.8″N,	M. Ozcan 156
	(22) *C. osseticum* (Adams) Petr. subsp. *osseticum*	Rize: Çamlıhemşin, From Zilkale to Çat, roadsides, among rocks, 1132 m	39°46′51.0″E, 40°59′35.1″N	M. Ozcan 224
	(23) *C. rigidum* DC.	Artvin: Ardanuç, eroded areas, 542 m	42°03′45.3″E, 41°07′35.2″N	M. Ozcan 427
	(24) *C. sommieri* Petr.	Gümüşhane: Sarıçiçek road, roadsides, 2021 m	39°50′45.8″E, 40°23′24.4″N	M. Ozcan 155
	(25) *C. trachylepis* Boiss.	Trabzon: Çaykara, Uzungöl, near Taşkıran village, roadsides, 500 m	39°26′03.1″E, 40°39′55,6″N	M. Ozcan 167
	(26) *C. vulgare* (Savi) Ten.	Trabzon: KTÜ campus, roadsides, 160 m	40°15′18.5″E, 40°39′55.6″N	M. Ozcan 178
		Rize: İkizdere, Cimil, Ortaköy road, 1913 m	40°45′42.8″E, 40°45′22.2″N	M. Ozcan 268

var. *glomeratum*, *C. rhizocephalum* subsp. *sinuatum*, *C. kosmelii*, and *C. rigidum* (Figs. 5, 6, 7).

Trichomes and spinules/setae in epidermal surface

Uniseriate and multicellular trichomes (thin or thick) are present on the epidermal surfaces of all examined taxa, while adaxial surfaces of the taxa are more or less glabrous in the *Cirsium* section, especially in *C. echinus*. The trichomes consist of 1–2 small or 2–7 large basal cells and a long whip-like terminal cell. Numbers of basal cells are consistent among the taxa. In addition, *C. pseudopersonata* subsp. *pseudopersonata*, *C. pseudopersonata* subsp. *kusnezowianum, C. hypoleucum* (Fig. 2g–h) and *C. pubigerum* var. *caniforme* have both thin (1–2 small basal cells) and thick (2–7 large basal cells) trichomes. Trichome densities differ in abaxial parts. Their densities are more distinctive in the lower surfaces of eight taxa (*C. trachylepis*, *C. osseticum* subsp. *osseticum*, *C. caucasicum*, *C. macrobotrys*, *C. leucocephalum* subsp. *leucocephalum*, *C. echinus*, *C. hypoleucum* and *C. pseudopersonata* subsp. *pseudopersonata*) (Figs. 5, 6, 7).

All taxa present in the *Epitrachys* section have spinules/setae that vary in length and density (per mm^2) in the adaxial parts, but these are absent in the *Cirsium* section. *C. leucocephalum* subsp. *penicillatum* and *C. osseticum* subsp. *osseticum* had the highest density of spinules/setae in the adaxial part (Figs. 4b, h, 7a, g).

Stomatal apparatus

All investigated taxa are amphistomatic, except for three taxa (Fig. 5i, j, m–p). Stomatal density distinctly differs among taxa, and also between the adaxial and abaxial surfaces within a taxon (Table 3). The density is clearly significant in abaxial parts of all taxa than in the adaxial ones.

The stomata in investigated *Cirsium* taxa were determined as anomocytic–anizocytic type and appear at the same level with the epidermis.

The average stomatal frequencies ranged from 20 (*C. obvallatum*) to 195 (*C. leucocephalum* subsp. *penicillatum*) per mm^2 in adaxial parts and 56 (*C. pubigerum* var. *caniforme*) to 445 (*C. tracylepis*) per mm^2 in abaxial parts of the investigated taxa (Table 3). Stomatal indices ranged from 5.68 (*C. obvallatum*) to 18.04 (*C. rhizocephalum* subsp. *sinuatum*) in adaxial parts, and 11.16 (*C. leucocephalum* subsp. *penicillatum*) to 22.89 (*C. sommieri*) in abaxial parts.

Stomatal size varied considerably within the *Cirsium* taxa. The largest stomata were identified on the adaxial surfaces of *C. obvallatum*, *C. pubigerum* var. *glomeratum, C. rhizocephalum* subsp. *rhizocephalum*, and *C. rhizocephalum* subsp. *sinuatum*, while the smallest were

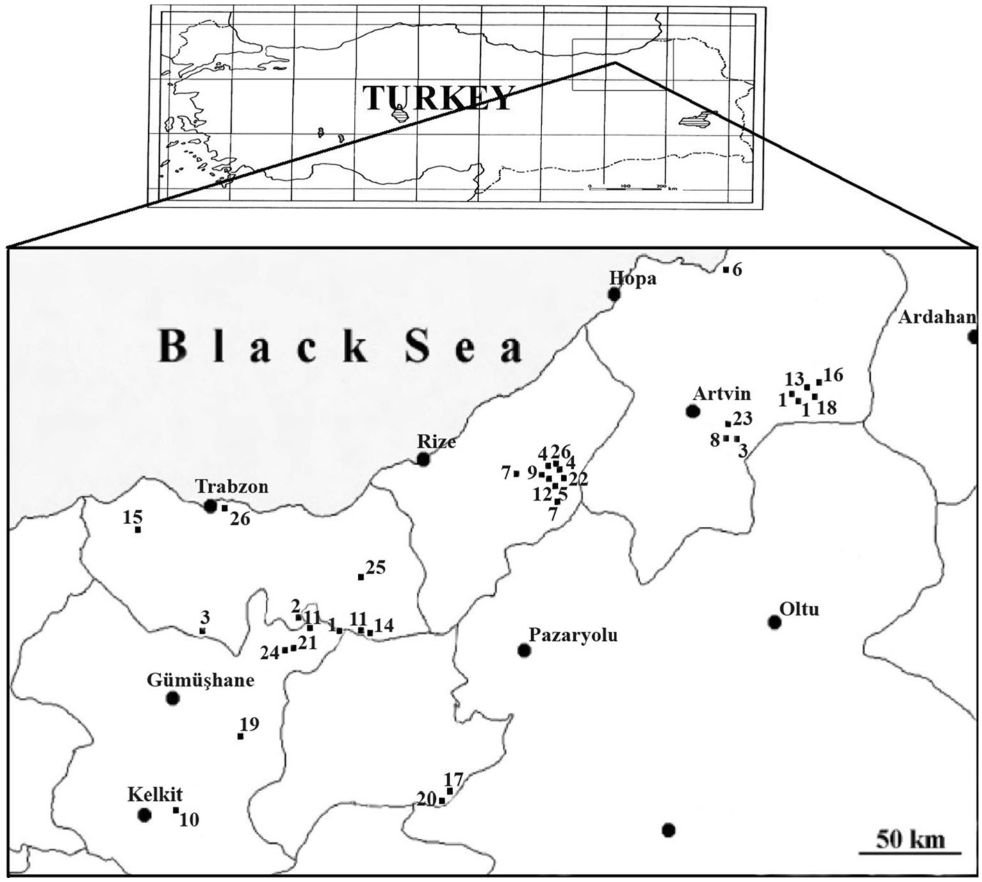

Fig. 1 Distribution map of *Cirsium* taxa investigated. Each *number* represents a taxon (Table 1)

observed on the abaxial surfaces of *C. hypoleucum* and *C. sommieri* (Table 3; Figs. 5j, k, t, 6a, c, 7l).

Midrib

Midrib thickness varies from 1120.8 (*C. pseudopersonata* subsp. *pseudopersonata*) to 3996 μm (*C. caucasicum*). Midrib thickness was much greater in the taxa of the *Epitrachys* section than in the *Cirsium* section (Table 3). The adaxial surface of the midrib is convex in most species, but this may vary in form, being flattish to concave in *C. aggregatum*, while the abaxial midrib is angular or convex.

Collateral vascular bundles are arranged in a single row and surrounded by parenchymatous sheath cells. One prominent and large vascular bundle ranging from 286.4 to 1116 μm (height) is present in the midrib area. Different numbers of vascular and accessory bundles are also seen in this region (Table 2). The number of bundles ranges from 1 (*C. arvense*) to 12 (*C. osseticum* subsp. *osseticum*, *C. kosmelii* and *C. leucocephalum* subsp. *penicillatum*). These bundles are more numerous in those taxa of the *Epitrachys* section than the taxa *Cirsium* and *Cephalonoplos* sections. Large secretory material containing parenchymatic cells (Fig. 3s) of various dimensions and numbers are located close to these bundles. The collenchyma is up to three or seven layers thick beneath the lower and upper epidermis of the lamina midrib, respectively (Figs. 2, 3, 4).

Leaf blade

All of the leaves in the sections were bifacial (dorsiventral mesophyll) type and are composed of a one-layered epidermis, palisade parenchyma, spongy parenchyma, and vascular tissue (Figs. 2, 3, 4). Leaf lamina thickness ranges between 116.5 (*C. hypoleucum*) and 461.6 (*C. caucasicum*) μm. The cuticle is much thicker in the adaxial epidermis than in the abaxial epidermis; ranging between 2.2 and 8.8 μm in adaxial parts and 1.5–5.4 μm in abaxial parts (Table 3). In transverse sections, adaxial epidermal cells in most taxa are bigger than abaxial cells, adaxial and abaxial epidermal cells in most taxa having greater width than height. The shapes of these cells are mostly regular, ranging from square to rectangular.

The mesophyll consisted of several layers of spongy parenchyma and two or three layers, rarely one layer (*C. hypoleucum*), of palisade parenchyma. The number of

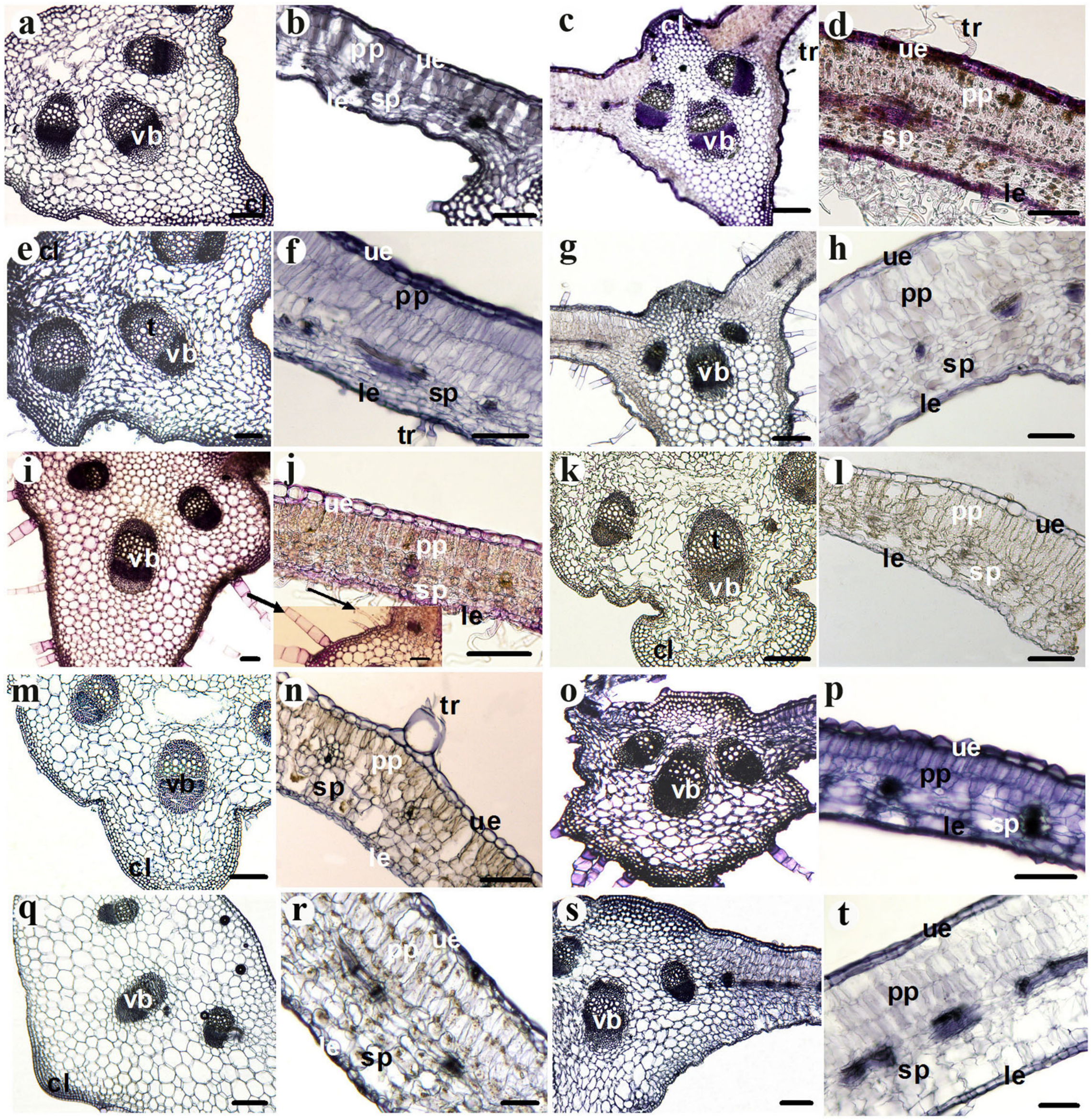

Fig. 2 Cross section of leaf in *Cirsium* taxa. **a, b** *C. arvense*, **c, d** syn: *C. arvense* subsp. *vestitum* (*C. arvense*), **e, f** *C. echinus*, **g, h** *C. alatum*, **i, j** *C. hypoleucum*, **k, l** *C. obvallatum*, **m, n** *C. pseudopersonata* subsp. *kusnezowianum*, **o, p** *C. pseudopersonata* subsp. *pseudopersonata*, **q, r** *C. pubigerum* var. *caniforme*, **s, t** *C. pubigerum* var. *glomeratum*. *le* lower epidermis, *cl* collenchyma, *vb* vascular bundle, *pp* palisade parenchyma, *se* setae/spinule, *sp* spongy parenchyma, *ue* upper epidermis, *t* trachea, *tr* trichome. *Arrows thick/thin* trichomes. *Scale bars* (midrib, on the *left*) 200 μm; (lamina, on the *right*) 100 μm

palisade cell layers varies among different species but is consistent within the same taxa, occurring in either one layer or two to three layers. The first palisade layer is distinctly longer than subsequent layers in *C. sommieri* (Fig. 4l). The layer closest to the spongy mesophyll is usually poorly differentiated and shorter than the next outermost layer. Palisade parenchyma occupies more or less equal area with spongy parenchyma in the mesophyll. The spongy parenchyma for the taxa studied is more or less densely arranged with small intercellular spaces and arranged in 4–8 layers.

Multivariate analyses

For the cluster analysis (UPGMA, Unweighted pair group method with arithmetic mean), 24 leaf anatomical characteristics of 26 taxa (33 accessions) were analyzed and their interspecific relationships were observed (Tables 2, 3; Fig. 8). The phenogram showed that the three major clusters were separated from all other species in a distinct position. The delimitation of these groups is mainly based on the occurrence of spinules/setae in adaxial parts, height and width of vascular bundle in the leaf midrib, thickness

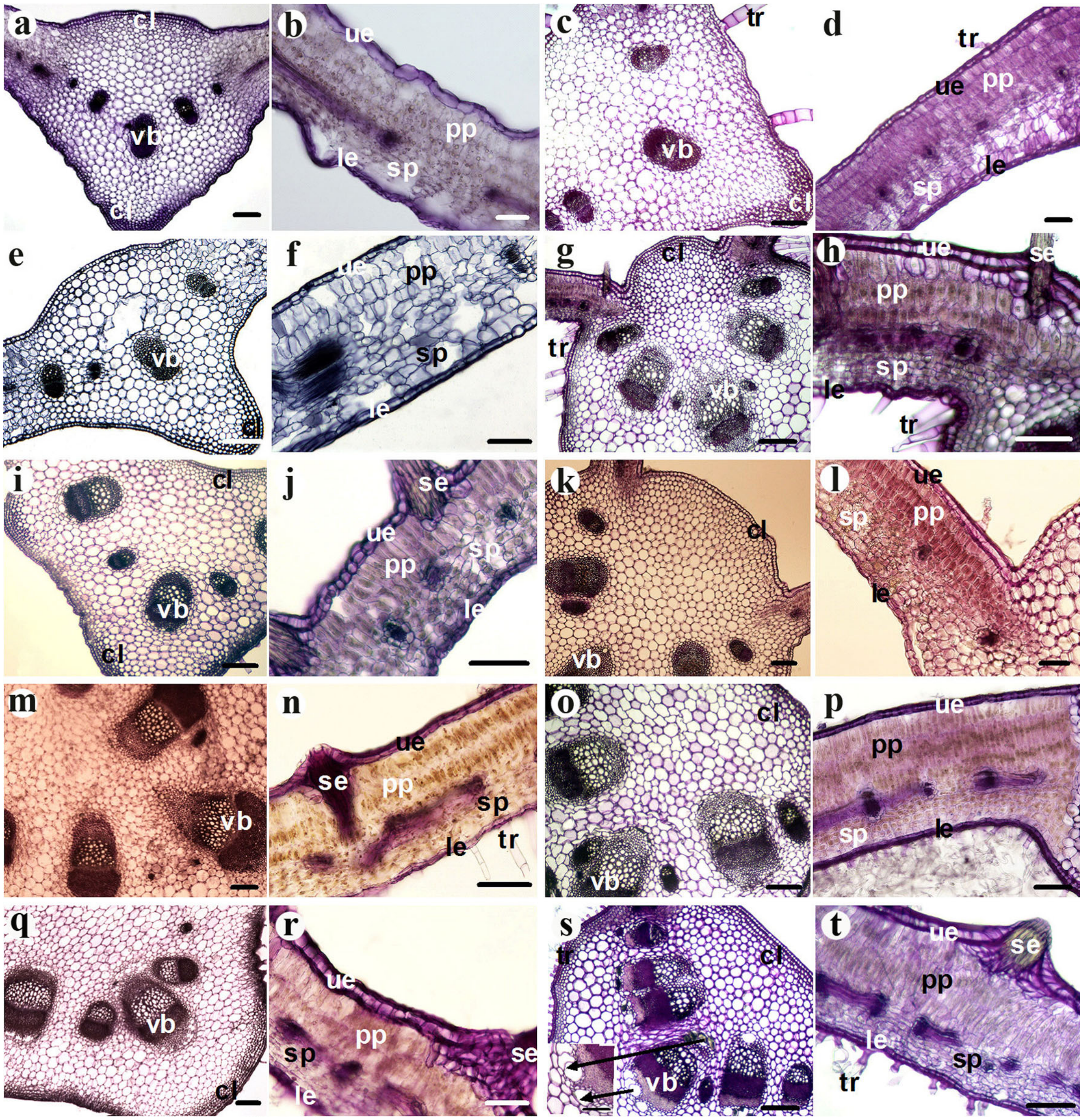

Fig. 3 Cross section of leaf in *Cirsium* taxa. **a, b** *C. rhizocephalum* subsp. *rhizocephalum*, **c, d** *C. rhizocephalum* subsp. *sinuatum*, **e, f** *C. simplex* subsp. *armenum*, **g, h** *C. adjaricum*, **i, j** *C. aggregatum*, **k, l** *C. bulgaricum*, **m, n** *C. caucasicum*, **o, p** *C. cephalotes*, **q, r** *C. kosmelii*, **s, t** *C. leucocephalum* subsp. *leucocephalum*. *Arrows* large parenchyma cells containing secretory material. *Scale bars* (midrib, on the *left*) 200 µm; (lamina, on the *right*) 100 µm

of phloem in this bundle, and mesophyll thickness in midrib ($P < 0.05$). In the dendrogram, 3 taxa, two subspecies of *C. pseudopersonata*, *pseudopersonata* and *kusnezowianum*, and *C. hypoleucum* were clearly delimited together in a separate group, but were separated from each other as three taxa at a dissimilarity level of about 2.0. The remaining (23 taxa) were divided into two major clusters at a distance coefficient of about 19.0.

The first cluster (14 taxa) was divided into three sub-clusters, the first of which included three sub-clusters: (1) *C. caucasicum*; (2) *C. leucocephalum* subsp. *leucocephalum*; and (3) and 12 other taxa. The former species, *C. caucasicum*, was split off from the others at a distance coefficient of about 15.0. *C. leucocephalum* subsp. *leucocephalum* resembles 12 taxa (*C. rigidum*, *C. vulgare*, *C. sommieri*, *C. aggregatum*, *C. macrobotrys*, *C. leucocephalum* subsp. *penicillatum*, *C. trachylepis*, *C. osseticum* subsp. *osseticum*, *C. adjaricum*, *C. cephalotes*, *C. kosmelii* and *C. bulgaricum*) at a distance coefficient of about 10.0. The third sub-cluster comprised 12 taxa with dissimilarity levels of about 7.0.

The second cluster (9 taxa) was divided into two sub-clusters, the first of which included two groups; one subgroup was comprised of *C. obvallatum* and the second

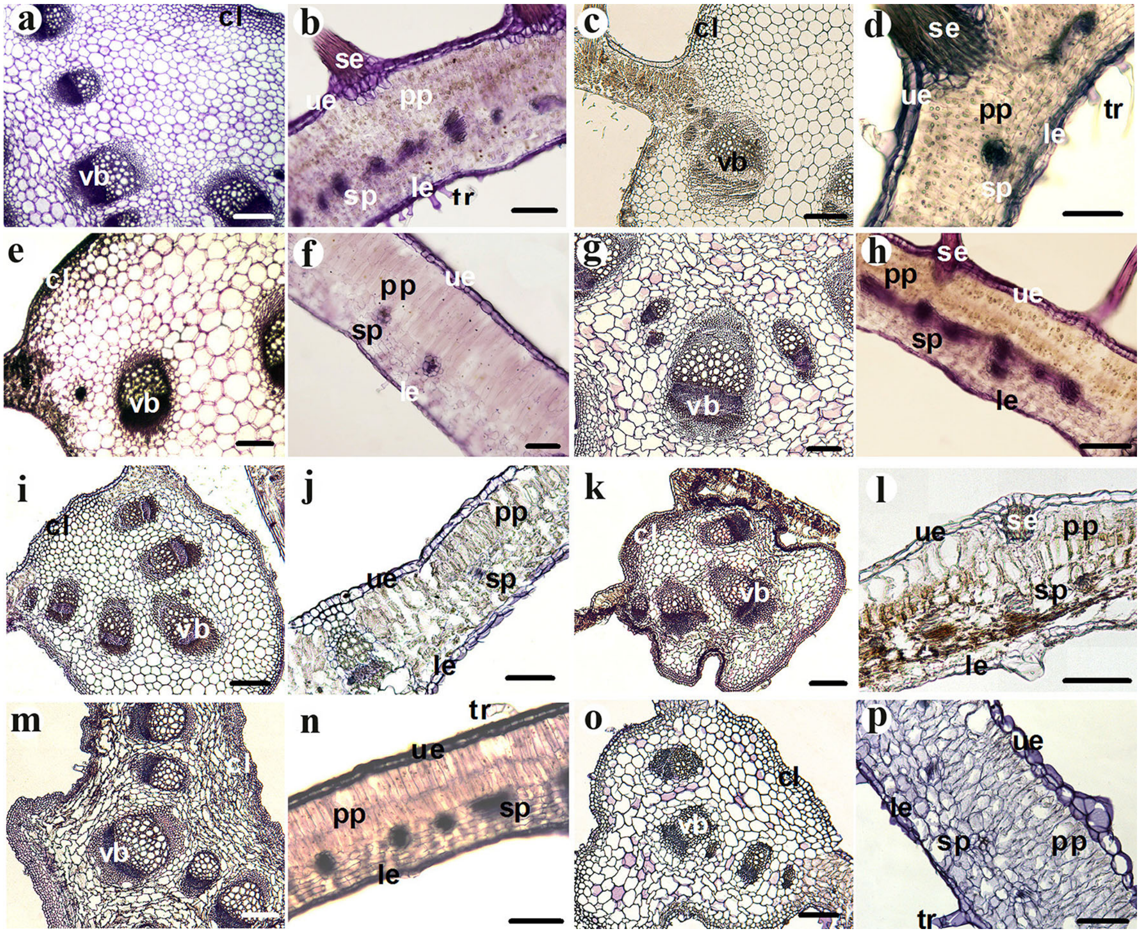

Fig. 4 Cross section of leaf in *Cirsium* taxa. **a, b** *C. leucocephalum* subsp. *penicillatum*, **c, d** *C. macrobotrys*, **e, f** syn: *C. munitum* (*C. kosmelii*), **g, h** *C. osseticum* subsp. *osseticum*, **i, j** *C. rigidum*, **k, l** *C. sommieri*, **m, n** *C. trachylepis*, **o, p** *C. vulgare*. *Scale bars* (midrib, on the *left*) 200 µm; (lamina, on the *right*) 100 µm

subgroup included *C. alatum*, *C. arvense*, and *C. echinus*. The former species (*C. obvallatum*) was separated from the latter three taxa at a distance coefficient of about 4.0. The second sub-cluster was divided into three groups at a dissimilarity level of about 4.0: the first group included the two varieties of *C. pubigerum*, *glomeratum* and *caniforme* and *C. simplex* subsp. *armenum*; the second group was comprised of *C. rhizocephalum* subsp. *rhizocephalum*; and the third group contained *C. rhizocephalum* subsp. *sinuatum*, which were clustered at a low distance coefficient of about 4.0. The clustering of the two varieties of *C. rhizocephalum* at a low distance coefficient of about 4.0, together with two varieties of *C. pubigerum*, with two varieties at a distance coefficient of about 3.0, reflects an underlying resemblance between them.

Multidimensional scaling analysis (MDS) of selected anatomical characters of the investigated taxa was applied to investigate the existence of clearly distinct groups of species (Fig. 9). The groups that were separated along the first two corresponding axes corresponded to the sections of genus *Cirsium*, illustrated as in the UPGMA analysis. Three distinct groups could be separated, according to the first two axes. The taxa coded as pse, kus, and hyp were separated as one group; 9 taxa coded as obv, ala, ech, arv, can, sim, rhi, sin, glo were delimited as a second group, and the remaining fourteen taxa were combined to form the third group (Fig. 9). Anatomical characters (1 qualitative and 23 quantitative) were rather homogeneous, being represented by states exclusive for these groups. The separation of the groups was mainly based on the occurrence of spinules/setae in adaxial parts, thickness of xylem in the bundle of leaf midrib, and number of stomata per mm^2 in the adaxial and abaxial surfaces.

Table 2 Leaf anatomical characteristics of *Cirsium* taxa investigated

Taxon	Length × width of ue (µm)	Midrib mesophyll thickness (µm)	Xylem height (µm)	Phloem height (µm)	Palisade thickness (µm)	Spongy thickness (µm)	Lamina thickness (µm)	Ct. vb		Trachea diameter (µm)	Length × width of le (µm)
								Height (µm)	Width (µm)		
C. arvense	17.60 × 34.47	2015.2 ± 48.9	294.7 ± 2.8	239.2 ± 5.4	71.40 ± 5.5	63.60 ± 9.2	288.7 ± 13.2	537.9 ± 8.3	332.8 ± 9.6	38.64 ± 2.1	12.68 × 21.2
	29.53 × 40.17	2027.1 ± 204.0	337.4 ± 20.0	268.8 ± 23.0	92.54 ± 1.1	133.5 ± 6.2	233.5 ± 3.9	532.0 ± 58.0	321.2 ± 37.0	36.81 ± 2.2	12.81 × 0.53
	25.26 × 33.75	1167.8 ± 46.2	180.5 ± 5.8	157.9 ± 7.1	162.9 ± 5.0	131.6 ± 9.6	148.0 ± 9.13	341.9 ± 9.6	265.1 ± 6.9	25.93 ± 0.8	14.81 × 20.7
C. alatum	22.17 × 35.40	1408.0 ± 31.7	215.2 ± 5.7	180.3 ± 1.6	146.0 ± 10.0	125.0 ± 5.8	286.8 ± 21.3	399.4 ± 9.5	268.0 ± 7.4	28.57 ± 1.2	16.20 × 27.3
C. echinus	22.02 × 32.00	1610.0 ± 40.2	284.8 ± 5.3	153.8 ± 12.0	122.7 ± 12.0	71.40 ± 7.9	190.0 ± 11.1	452.0 ± 3.1	282.4 ± 2.9	33.03 ± 0.9	11.63 × 18.3
C. hypoleucum	19.46 × 30.52	2154.6 ± 83.8	311.5 ± 13.0	232.8 ± 13.0	50.09 ± 1.5	76.12 ± 7.1	126.8 ± 8.16	545.8 ± 5.3	319.8 ± 11	33.78 ± 0.5	10.49 × 14.4
	22.45 × 34.72	2371.5 ± 21.5	337.3 ± 11.0	255.7 ± 3.2	33.48 ± 1.2	90.89 ± 8.5	116.5 ± 6.33	594.2 ± 12.0	309.2 ± 9.9	33.64 ± 1.3	12.97 × 15.8
C. obvallatum	26.08 × 43.25	1473.6 ± 123	331.2 ± 9.3	174.4 ± 11.0	94.93 ± 9.8	106.2 ± 14.0	213.0 ± 20.4	518.4 ± 18	344.0 ± 29.0	34.38 ± 2.9	19.33 × 27.6
C. pseudopersonata subsp. *kusnezowianum*	26.02 × 40.22	1650.0 ± 130	252.2 ± 7.5	229.8 ± 5.7	60.40 ± 6.2	133.2 ± 7.7	194.8 ± 13.0	481.6 ± 11	329.1 ± 6.1	40.17 ± 1.5	17.30 × 27.3
C. pseudopersonata subsp. *pseudopersonata*	18.33 × 35.67	1120.8 ± 116	222.4 ± 9.2	183.6 ± 10.0	68.70 ± 3.6	88.40 ± 5.8	152.8 ± 5.55	406.0 ± 18	278.0 ± 11.0	30.50 ± 1.3	15.89 × 20.6
	29.53 × 40.17	2027.1 ± 286	337.4 ± 20.0	268.8 ± 23.0	92.54 ± 1.1	109.9 ± 2.9	233.5 ± 3.96	532.0 ± 58.0	321.2 ± 37.0	36.81 ± 2.2	12.81 × 19.3
C. pubigerum var. *caniforme*	33.98 × 55.35	1734.6 ± 35.8	248.4 ± 3.7	176.4 ± 1.8	176.8 ± 12.0	266.4 ± 14	416.0 ± 10.2	423.6 ± 5.6	272.0 ± 8.1	27.30 ± 0.9	34.80 × 45.3
C. pubigerum var. *glomeratum*	24.80 × 38.33	1798.0 ± 16.9	274.8 ± 4.6	230.4 ± 11.0	196.0 ± 13.0	249.2 ± 8.1	427.2 ± 16.9	505.6 ± 11	304.0 ± 5.5	35.55 ± 1.2	23.80 × 37.3
C. rhizocephalum subsp. *rhizocephalum*	37.32 × 46.36	1953.9 ± 79.2	221.5 ± 10	182.9 ± 1.0	224.5 ± 15.6	191.69 ± 12.0	421.9 ± 21.0	402.7 ± 10	239.6 ± 6.5	22.67 ± 0.9	25.75 × 40.64
C. rhizocephalum subsp. *sinuatum*	25.08 × 35.08	1716.0 ± 96.3	184.0 ± 8.0	162.0 ± 5.6	192.8 ± 8.78	207.6 ± 7.9	387.2 ± 11.0	339.2 ± 9.6	231.2 ± 15	18.80 ± 1.3	23.43 × 34.93
C. simplex subsp. *armenum*	28.73 × 36.87	1336.0 ± 23.1	156.0 ± 1.7	129.8 ± 2.8	139.2 ± 6.14	240.0 ± 16.2	378.0 ± 9.6	286.4 ± 1.6	182.4 ± 1.6	18.55 ± 0.3	26.00 × 37.40
C. adjaricum	25.20 × 32.90	2136.0 ± 39.6	371.2 ± 22.0	316.6 ± 12.0	184.7 ± 20.1	113.7 ± 14.9	283.2 ± 24.0	696.0 ± 34.0	389.2 ± 20.0	36.85 ± 1.1	11.93 × 14.70
C. aggregatum	18.20 × 26.22	1466.0 ± 19.4	248.4 ± 7.6	199.6 ± 15.0	85.70 ± 8.23	95.00 ± 10.9	174.6 ± 12.0	432.8 ± 16.0	305.6 ± 10.0	29.95 ± 1.2	14.17 × 19.30
C. bulgaricum	25.18 × 37.36	3365.5 ± 24.2	419.6 ± 1.4	363.54 ± 7.0	155.9 ± 8.45	183.3 ± 18.4	356.1 ± 11.0	799.6 ± 8.4	394.4 ± 7.5	21.04 ± 0.2	19.91 × 27.80
C. caucasicum	33.33 × 27.73	3996.0 ± 231.	648.8 ± 46.0	472.0 ± 20.0	163.6 ± 21.5	96.40 ± 4.25	461.6 ± 46.0	1116.0 ± 49	676.8 ± 31.0	40.83 ± 1.3	19.90 × 15.80
C. cephalotes	31.71 × 39.50	2623.0 ± 23.4	375.2 ± 5.9	324.8 ± 5.8	217.8 ± 11.8	145.0 ± 6.90	251.0 ± 23.0	713.6 ± 4.8	376.0 ± 9.7	35.40 ± 0.7	12.33 × 15.30
C. kosmelii	19.40 × 26.20	3098.0 ± 79.2	516.0 ± 11.0	376.6 ± 13.0	137.3 ± 20.1	106.24 ± 15.0	239.4 ± 38.0	896.4 ± 30.0	457.6 ± 12.0	36.31 ± 0.5	17.10 × 18.67
	28.80 × 42.33	3140.0 ± 41.4	438.4 ± 57.0	306.4 ± 22.6	102.7 ± 20.0	116.2 ± 5.9	214.8 ± 22.0	739.2 ± 77.0	529.6 ± 32.0	45.80 ± 2.9	12.83 × 24.00
C. leucocephalum subsp. *leucocephalum*	24.60 × 32.80	1774.0 ± 41.8	283.2 ± 9.3	272.0 ± 2.5	169.2 ± 6.64	112.0 ± 13.9	275.2 ± 9.3	554.8 ± 8.5	339.2 ± 9.7	32.82 ± 0.7	11.27 × 32.80
C. leucocephalum subsp. *penicillatum*	19.80 × 25.00	1653.6 ± 26.9	290.0 ± 13.0	225.2 ± 7.8	67.20 ± 4.69	71.20 ± 8.00	122.4 ± 9.7	526.3 ± 16.0	357.1 ± 9.9	37.78 ± 1.1	12.30 × 15.97
C. macrobotrys	20.24 × 24.64	3136.0 ± 26.3	386.4 ± 7.10	301.2 ± 8.20	106.2 ± 7.2	90.30 ± 4.1	195.7 ± 9.3	688.8 ± 8.9	521.2 ± 9.5	32.90 ± 0.9	09.07 × 13.64

Table 2 continued

Taxon	Length × width of ue (µm)	Midrib mesophyll thickness (µm)	Xylem height (µm)	Phloem height (µm)	Palisade thickness (µm)	Spongy thickness (µm)	Lamina thickness (µm)	Ct. vb		Trachea diameter (µm)	Length × width of le (µm)
								Height (µm)	Width (µm)		
C. osseticum subsp. *osseticum*	21.72 × 31.40	2326.4 ± 40.9	592.6 ± 5.57	374.4 ± 2.99	82.60 ± 4.4	98.13 ± 10.0	233.8 ± 4.1	969.4 ± 6.7	585.6 ± 6.8	52.32 ± 1.3	12.27 × 14.97
C. rigidum	23.67 × 34.50	1650.4 ± 08.9	301.6 ± 4.30	208.4 ± 4.48	95.20 ± 2.2	102.9 ± 4.6	196.3 ± 9.7	509.6 ± 2.4	264.8 ± 6.9	36.10 ± 1.4	22.37 × 33.93
C. sommieri	15.39 × 30.47	1189.6 ± 40.2	278.2 ± 6.77	240.0 ± 7.14	110.0 ± 1.8	69.60 ± 2.8	177.6 ± 2.8	518.2 ± 9.8	306.3 ± 6.1	29.42 ± 0.5	13.47 × 23.90
C. trachylepis	21.50 × 33.15	1553.6 ± 35.9	457.6 ± 6.39	202.4 ± 4.30	115.9 ± 4.0	73.33 ± 1.5	188.0 ± 2.7	657.6 ± 4.1	417.6 ± 5.8	46.30 ± 0.6	10.53 × 17.13
C. vulgare	33.30 × 42.55	1468.8 ± 26.1	206.4 ± 2.99	201.6 ± 11.9	171.6 ± 3.5	114.5 ± 7.7	292.4 ± 8.3	400.0 ± 13.0	248.0 ± 9.8	30.27 ± 0.8	19.37 × 27.03
	24.66 × 39.64	1832.4 ± 120.0	333.9 ± 6.98	260.2 ± 12.9	150.1 ± 6.4	104.9 ± 1.6	242.2 ± 20.2	415.1 ± 24.0	596.5 ± 15.0	38.33 ± 6.9	18.60 × 28.54

mean ± standard error

ct.vb Central midrib vascular bundle, *le* lower epidermis, *ue* upper epidermis

Discussion

Cirsium is represented with 78 taxa including species, subspecies and varieties, and are part of three different sections in the Flora of Turkey (Davis and Parris 1975; Yıldız et al. 2013). In the present study, the importance of anatomical characters is evaluated and the relations among leaf anatomy and sectional delimitation are discussed. Here, we explain, for the first time (except for *C. arvense*), the leaf anatomical structures of all *Cirsium* taxa. The general leaf anatomy of *C. arvense* has been previously mentioned by Rancic et al. (2006).

All *Cirsium* taxa investigated in the present study show a dorsiventral mesophyll composed of between one to three layers of adaxial palisade parenchyma and four to eight layers of abaxial spongy parenchyma. Metcalfe and Chalk (1979) and Kadereit and Jeffrey (2007) previously mentioned that the dorsiventral mesophyll is commonly observed in the Compositae family, but the isobilateral type has also been reported. These types of leaf mesophyll are common in related genera, such as *Centaurea* L., *Psephellus* Cass., and *Silybum* Adans, being previously reported in seven *Centaurea* taxa by Ozcan et al. (2014) and in one *Psephellus* taxon by Özcan (2013). The dorsiventral type mesophyll has also been previously reported in *Silybum marianum* (Sidhu and Saini 2011) and *Cirsium arvense* (Rancic et al. 2006).

The occurrence of some accessory vascular bundles has been shown in the leaf anatomies (Figs. 2, 3, 4). The accessory bundles are more numerous in the *Epitrachys* section than in other sections. Accessory bundles have already been reported by many researchers (Inceer and Ozcan 2011; Ozcan et al. 2014) in related taxa and it is thought that they are present to meet the translocation requirements during unfavorable conditions (Sidhu and Saini 2011). Sidhu and Saini (2011) expressed that accessory vascular bundles must have been developed to overcome winters on reserve food material in *S. marianum*.

Big elongated parenchymatic cells of various numbers and dimensions are located close to the vascular bundles (Fig. 3t). These cells contain large volumes of secretory material. Similar secretory structures have been previously reported in related genera, such as *Centaurea* by Uysal et al. (2005), Celik et al. (2008), and Ozcan et al. (2014) and, in *Psephellus* by Özcan (2013). Metcalfe and Chalk (1979) and Kadereit and Jeffrey (2007) also mentioned this cell type in the family Asteraceae. Metcalfe and Chalk (1979) reported that secretory structures are present in the region of the stem endodermis, that they extend through the petiole to the lamina of the leaf in some cases, and that they

Table 3 Stomatal characteristics of *Cirsium* taxa investigated

Taxon	Upper epidermises						Lower epidermises				
	p (+) a (−)	Stomatal indexes	Stomatal length (μm)	Stomatal width (μm)	Stomatal number per mm^2	Cuticle thickness (μm)	Stomatal indexes	Stomatal length (μm)	Stomatal width (μm)	Stomatal number per mm^2	Cuticle thickness (μm)
C. arvense	−	8.45 ± 0.22	34.16 ± 1.09	26.80 ± 0.58	72.50 ± 3.18	2.90 ± 0.10	16.31 ± 0.88	27.64 ± 0.78	23.40 ± 0.46	73.75 ± 4.58	2.4 ± 0.19
	−	9.32 ± 0.92	33.72 ± 0.35	29.62 ± 0.29	105.0 ± 4.99	2.84 ± 0.16	12.64 ± 1.29	28.92 ± 0.59	26.38 ± 0.59	210.0 ± 26.9	2.41 ± 0.10
	−	9.76 ± 0.29	32.04 ± 0.43	27.60 ± 0.39	73.75 ± 7.22	4.90 ± 0.23	18.99 ± 0.22	23.07 ± 0.32	20,66 ± 0.19	87.50 ± 4.41	3.5 ± 0.41
C. alatum	−	8.81 ± 0.24	28.52 ± 0.65	23.36 ± 0.23	86.25 ± 5.37	3.10 ± 0.33	16.8 ± 0.29	23.36 ± 0.23	20.52 ± 0.31	332.5 ± 9.13	2.3 ± 0.20
C. echinus	−	12.56 ± 0.80	35.73 ± 0.76	29.28 ± 0.42	106.25 ± 8.37	3.70 ± 0.20	17.51 ± 1.86	22.64 ± 0.57	19.70 ± 0.63	275.0 ± 31.57	1.7 ± 0.20
C. hypoleucum	−	0	0	0	0	2.85 ± 0.15	11.47 ± 0.55	21.92 ± 0.40	19.85 ± 0.46	345.0 ± 19.96	1.5 ± 0.23
	−	0	0	0	0	3.27 ± 0.47	12.25 ± 1.36	22.68 ± 0.75	18.99 ± 0.15	260.0 ± 32.15	2.0 ± 0.22
C. obvallatum	−	5.68 ± 0.17	38.23 ± 0.70	28.37 ± 0.69	20.0 ± 2.33	2.45 ± 0.20	19.86 ± 0.17	32.47 ± 0.82	26.31 ± 0.69	136.25 ± 9.54	2.2 ± 0.20
C. pseudopersonata subsp. *kusnezowianum*	−	0	0	0	0	2.20 ± 0.12	20.20 ± 0.26	25.96 ± 0.84	20.93 ± 0.49	290.0 ± 5.07	1.9 ± 0.10
C. pseudopersonata subsp. *pseudopersonata*	−	0	0	0	0	2.50 ± 0.16	18.47 ± 0.40	27.99 ± 0.24	22.33 ± 0.46	200.0 ± 8.82	1.6 ± 0.24
	−	0	0	0	0	2.69 ± 0.17	21.93 ± 1.39	30.41 ± 0.52	21.87 ± 0.43	250.0 ± 26.17	1.7 ± 0.14
C. pubigerum var. *caniforme*	−	11.76 ± 0.17	36.59 ± 0.68	29.92 ± 0.53	45.0 ± 3.64	3.00 ± 0.27	12.55 ± 0.50	34.28 ± 1.20	29.14 ± 0.53	56.25 ± 6.54	2.2 ± 0.11
C. pubigerum var. *glomeratum*	−	12.33 ± 0.31	38.77 ± 1.11	31.19 ± 0.64	81.25 ± 5.92	2.80 ± 0.12	12.17 ± 0.24	35.56 ± 1.09	29.30 ± 0.46	97.50 ± 8.95	2.8 ± 0.12
C. rhizocephalum subsp. *rhizocephalum*	−	13.82 ± 0.93	38.57 ± 0.48	31.68 ± 0.44	88.75 ± 4.58	5.85 ± 0.21	16.40 ± 0.89	37.43 ± 0.35	32.00 ± 0.44	133.8 ± 10.55	4.5 ± 0.45
C. rhizocephalum subsp. *sinuatum*	−	18.04 ± 0.21	37.71 ± 1.02	32.10 ± 1.16	113.8 ± 7.99	3.90 ± 0.10	19.07 ± 0.80	36.86 ± 0.55	31.61 ± 0.65	146.3 ± 10.55	3.8 ± 0.20
	−	15.86 ± 0.83	39.89 ± 1.08	33.70 ± 1.38	110.0 ± 12.7	3.27 ± 0.26	13.57 ± 0.20	34.08 ± 0.48	29.19 ± 0.50	150.0 ± 13.67	2.39 ± 0.28
C. simplex subsp. *armenum*	−	9.03 ± 0.28	36.60 ± 1.01	30.36 ± 0.79	65.0 ± 2.50	3.37 ± 0.17	11.35 ± 0.49	36.28 ± 0.69	29.88 ± 0.34	81.25 ± 3.95	2.7 ± 0.12
C. adjaricum	+	09.04 ± 0.15	30.48 ± 0.57	25.46 ± 0.61	91.25 ± 6.72	4.30 ± 0.20	19.04 ± 0.62	20.46 ± 0.18	17.70 ± 0.36	685.0 ± 28.01	2.25 ± 0.16
C. aggregatum	+	06.93 ± 0.41	28.80 ± 0.71	24.56 ± 0.31	75.0 ± 5.22	3.60 ± 0.19	20.39 ± 0.43	26.04 ± 0.63	21.08 ± 0.59	367.5 ± 35.43	2.60 ± 0.24
C. bulgaricum	+	14.20 ± 0.44	31.83 ± 0.55	26.14 ± 0.34	130.0 ± 4.99	3.32 ± 0.19	19.46 ± 0.54	28.83 ± 0.72	24.78 ± 0.75	270.0 ± 32.9	2.89 ± 0.07
C. caucasicum	+	10.92 ± 0.37	25.64 ± 0.58	22.10 ± 0.32	103.8 ± 6.72	4.40 ± 0.40	13.75 ± 1.03	18.80 ± 0.65	17.14 ± 0.62	720.0 ± 78.68	2.20 ± 0.37
C. cephalotes	+	10.39 ± 0.51	31.32 ± 0.43	24.76 ± 0.17	130.0 ± 9.34	3.60 ± 0.19	14.28 ± 0.33	25.28 ± 0.32	21.56 ± 0.40	330.0 ± 21.47	2.60 ± 0.29
C. kosmelii	+	08.71 ± 0.50	30.82 ± 1.16	24.46 ± 0.87	88.75 ± 7.22	2.92 ± 0.05	14.45 ± 0.42	23.54 ± 0.38	21.20 ± 0.07	385.0 ± 18.68	2.10 ± 0.10
	+	06.59 ± 0.49	34.28 ± 0.54	25.44 ± 0.51	32.50 ± 5.37	3.10 ± 0.22	16.27 ± 1.09	27.90 ± 0.29	22.60 ± 0.34	315.0 ± 47.09	2.02 ± 0.13
C. leucocephalum subsp. *leucocephalum*	+	11.38 ± 0.78	27.86 ± 0.44	25.96 ± 0.49	180.0 ± 16.6	8.80 ± 0.80	12.87 ± 0.80	22.62 ± 0.12	20.46 ± 0.08	425.0 ± 37.85	5.40 ± 0.40

Table 3 continued

Taxon	p (+) a (−)	Upper epidermises: Stomatal indexes	Stomatal length (μm)	Stomatal width (μm)	Stomatal number per mm^2	Cuticle thickness (μm)	Lower epidermises: Stomatal indexes	Stomatal length (μm)	Stomatal width (μm)	Stomatal number per mm^2	Cuticle thickness (μm)
C. leucocephalum subsp. *penicillatum*	+	9.52 ± 0.60	25.02 ± 0.60	20.58 ± 0.46	195 ± 28.89	3.94 ± 0.06	11.16 ± 0.71	22.18 ± 0.39	19.20 ± 0.26	325.0 ± 31.57	3.21 ± 0.11
C. macrobotrys	+	14.77 ± 0.92	30.16 ± 0.84	26.46 ± 0.70	133.8 ± 9.38	3.50 ± 0.18	16.10 ± 0.79	23.46 ± 0.22	20.61 ± 0.28	250.0 ± 11.16	2.10 ± 0.10
C. osseticum subsp. *osseticum*	+	08.91 ± 0.31	30.08 ± 0.48	26.68 ± 0.48	81.25 ± 6.24	2.50 ± 0.22	20.32 ± 0.67	20.04 ± 0.36	18.16 ± 0.30	166.3 ± 13.75	1.80 ± 0.12
C. rigidum	+	14.02 ± 0.44	28.41 ± 0.58	22.96 ± 0.43	118.8 ± 6.54	3.25 ± 0.16	16.24 ± 0.53	25.99 ± 0.33	21.43 ± 0.21	173.8 ± 15.46	2.85 ± 0.19
C. sommieri	+	12.97 ± 0.30	30.33 ± 0.57	24.37 ± 0.35	130.0 ± 8.69	2.63 ± 0.22	22.89 ± 0.86	21.11 ± 0.34	18.18 ± 0.29	411.3 ± 11.57	3.38 ± 0.20
C. trachylepis	+	06.96 ± 0.34	29.94 ± 0.34	24.39 ± 0.46	61.25 ± 4.58	2.83 ± 0.12	17.42 ± 0.56	20.84 ± 0.17	17.88 ± 0.18	445.0 ± 26.28	1.50 ± 0.22
C. vulgare	+	13.57 ± 0.47	32.64 ± 0.56	25.92 ± 0.65	118.8 ± 6.54	3.90 ± 0.19	21.77 ± 0.40	26.64 ± 0.34	21.08 ± 0.19	97.50 ± 6.42	2.30 ± 0.12
	+	15.45 ± 0.69	25.65 ± 0.50	22.53 ± 0.49	130.0 ± 12.2	5.12 ± 0.42	17.68 ± 1.54	32.35 ± 0.35	29.52 ± 0.43	210.0 ± 12.7	3.32 ± 0.21

mean ± standard error

p spinule/setae present (+), *a* spinule/setae absent (−)

typically occur in the same tissues as the canals. In addition, Fritz and Saukel (2011) investigated these structures in the root and rhizomes of tribe *Cardueae*. 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. However, these cells are also present in the xylem part of the vascular bundles, as well as in the phloem parts.

Twenty-one of the investigated taxa have straight anticlinal cell walls, while five of the taxa do not (Figs. 5, 6, 7). These five taxa grow in shady banks and humid areas, while the 21 with straight anticlinal cell walls grow at high altitudes and dry habitats. Stace (1965) pointed out that epidermal cells with straight outlines are more common in xeromorphic plants than in mesomorphic plants, where they are typically undulate. Fahn (1990) also asserts that the epidermal cells of most leaves of shade-loving dicotyledons have sinuous anticlinal walls. Such sinuosity is probably due to the tensions that occur in the leaf and to cuticle hardening during cell differentiation (Alquini et al. 2003). Gomes and Lombardi (2010) stated that the cuticle of shade leaves hardens more slowly and its walls remain frail and plastic for longer periods, thus favoring the development of sinuosities. In some leaves, the epidermal walls harden more quickly, thus tending to be less undulate.

All investigated taxa are amphistomatic, except for three taxa (Fig. 5i, j, m–p). Stomatal density distinctly differs in their adaxial and abaxial surfaces (Table 3). The density is clearly significant in abaxial parts of all taxa. Arambarri et al. (2006) reported that most of the taxa with amphistomatic leaves have lower density of stomata in their adaxial face than the abaxial one. Our findings agree with this report. Stomata are usually anisocytic–anomocytic in the taxa. These types of stomata have been previously reported in the family Compositae by Metcalfe and Chalk (1979) and Kadereit and Jeffrey (2007).

Three of the 26 investigated taxa are polyploids (Ozcan et al. 2008, 2011). These polyploid taxa, namely *C. vulgare*, *C. pubigerum* var. *glomeratum* and *C. pubigerum* var. *caniforme*, have bigger stomata than the others. In this study, we observed that diploid taxa generally have smaller stomata than polyploids. However, some exceptions were also detected (Table 3); *C. simplex* subsp. *armenum*, *C. rhizocephalum* subsp. *rhizocephalum*, and *C. rhizocephalum* subsp. *sinuatum* (all diploid taxa) also have bigger stomata, such as in the polyploids. These taxa grow in dry conditions and are exposed to full sunshine. This could be an explanation for why they possess larger stomata similar to the polyploid taxa. Stace (1965) points out that polyploid plants have larger stomata than their diploid parents. In

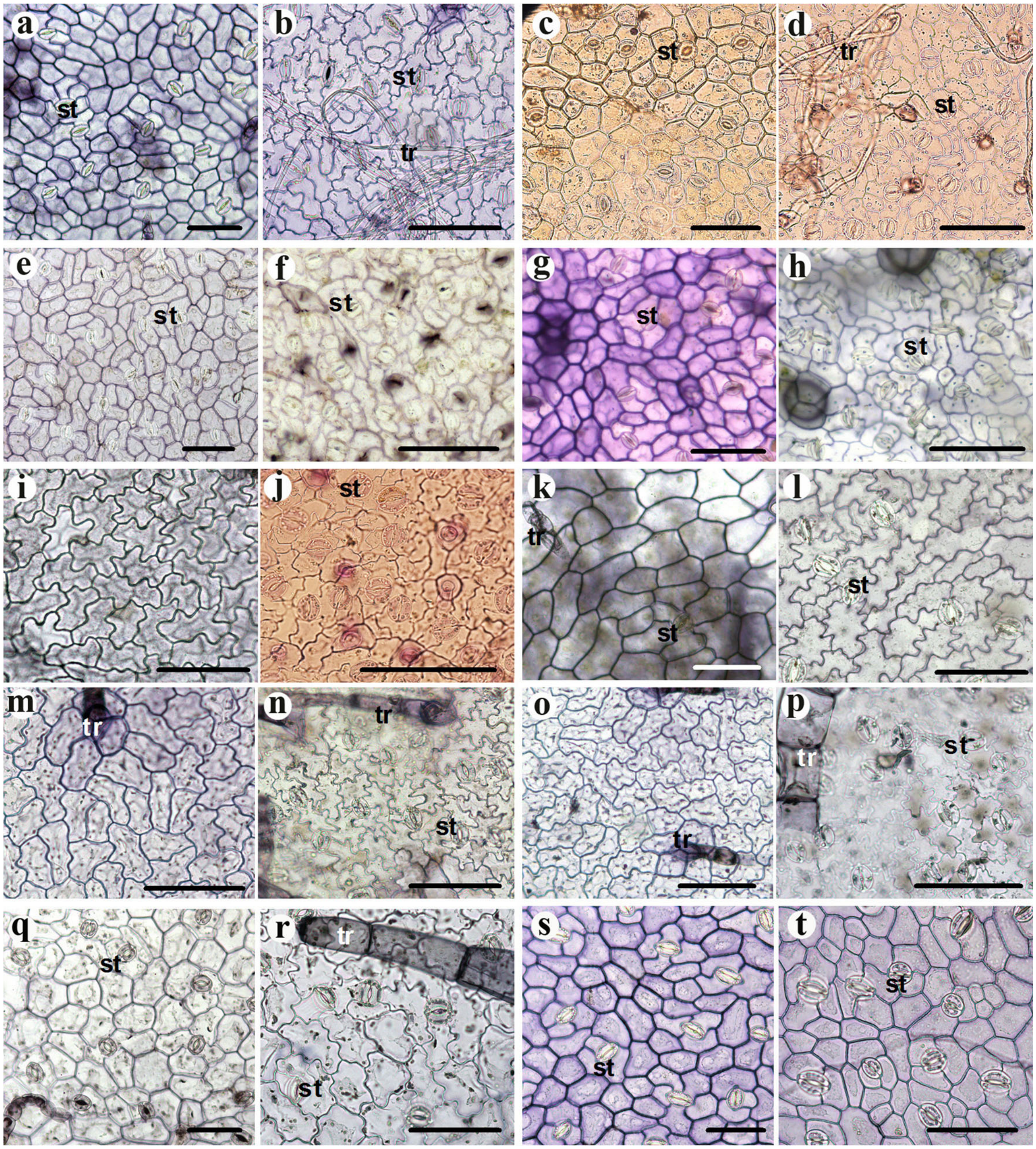

Fig. 5 Paradermal section of leaf in *Cirsium* taxa. Adaxial surface on the *left*, abaxial surface on the *right*. **a, b** *C. arvense*, **c, d** syn: *C. arvense* subsp. *vestitum* (*C. arvense*), **e, f** *C. echinus*, **g, h** *C. alatum*, **i, j** *C. hypoleucum*, **k, l** *C. obvallatum*, **m, n** *C. pseudopersonata* subsp. *kusnezowianum*, **o, p** *C. pseudopersonata* subsp. *pseudopersonata*, **q, r** *C. pubigerum* var. *caniforme*, **s, t** *C. pubigerum* var. *glomeratum*. *se* setea/spinule, *st* stoma, *t* trichome. *Scale bars* 100 μm

addition, he expressed that shade, atmospheric humidity, and soil moisture are conditions that coincide with smaller stomata, whereas full sun and dry conditions produce larger stomata.

The *Cirsium* taxa investigated grow at various extremes of altitude (from 10 to 3000 m) and in various ecological habitats. In our work, the investigated taxa growing at high altitudes (Table 1) and fully exposed to intense illumination have three layers of palisade parenchyma, whereas *C. hypoleucum* growing in low altitudes and in shadowed humid areas have a single layer of palisade parenchyma (Fig. 2j). It has been suggested that intense illumination and scarce water can enhance the development of palisade tissue, and consequently increase photosynthetic activity (Fahn, 1982); our data support this suggestion.

The phenograms showing the three major clusters (Figs. 8, 9) partly conform to Davis and Parris's

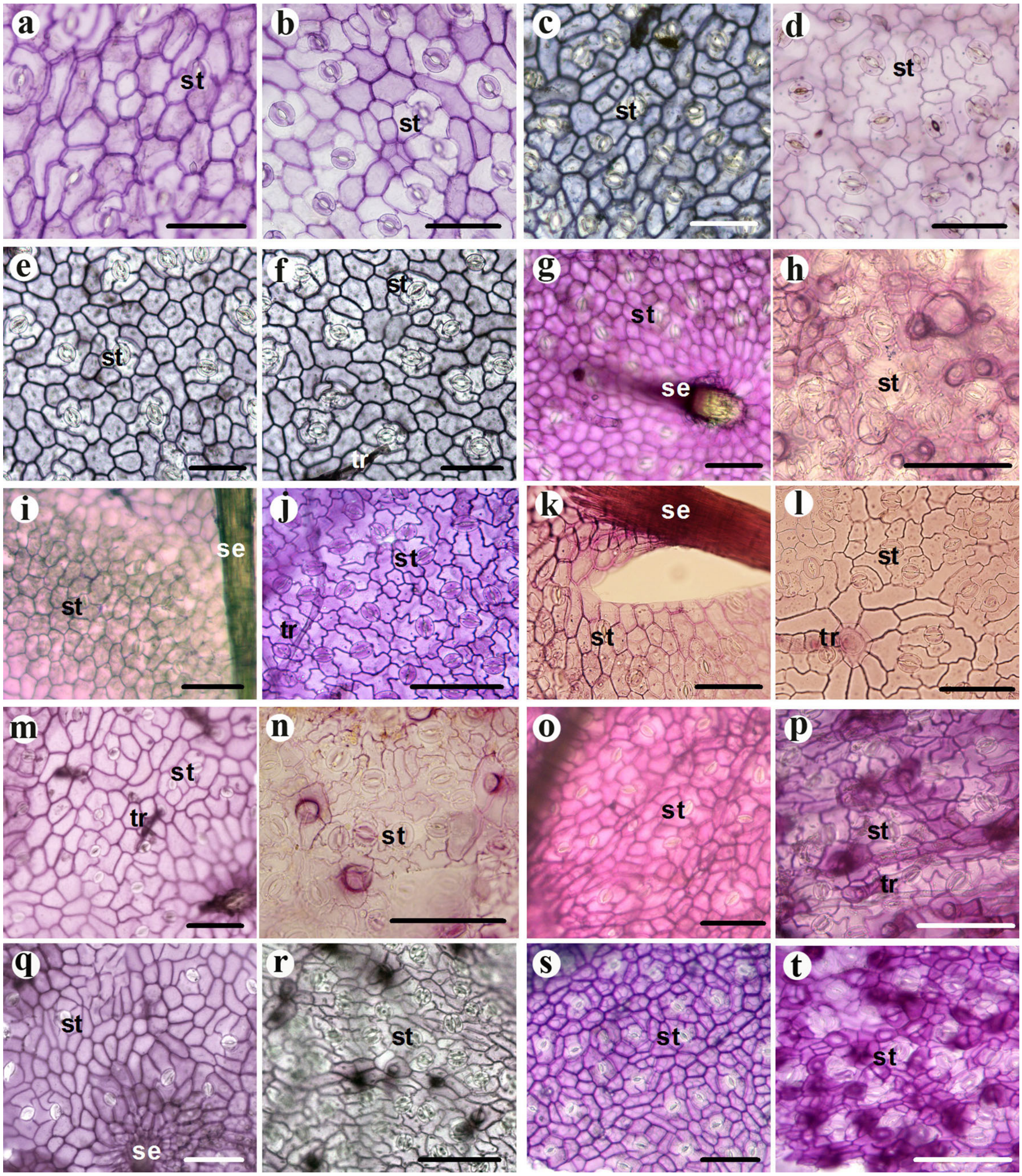

Fig. 6 Paradermal section of leaf in *Cirsium* taxa. **a, b** *C. rhizocephalum* subsp. *rhizocephalum*, **c, d** *C. rhizocephalum* subsp. *sinuatum*, **e, f** *C. simplex* subsp. *armenum*, **g, h** *C. adjaricum*, **i, j** *C. aggregatum*, **k, l** *C. bulgaricum*, **m, n** *C. caucasicum*, **o, p** *C. cephalotes*, **q, r** *C. kosmelii*, **s, t** *C. leucocephalum* subsp. *leucocephalum*. See Fig. 5 for abbreviations. *Scale bars* 100 µm

classification (1975), where 26 taxa were placed into three sections; *Epitrachys*, *Cirsium*, and *Cephalonoplos*. The *Cirsium* and *Cephalonoplos* sections are morphologically very similar. In Davis and Parris's classification, the delimitation of the *Cephalonoplos* section from *Cirsium* was based on its dioecious pattern. *C. arvense* represents the only species of the *Cephalonoplos* section with a dioecious pattern. However, leaf anatomical data are not consistent with Davis and Parris's classification (1975). In this study, we found that this species was located together in the *Cirsium* section. Therefore, a revision may be required in which this species are classified into the *Cirsium* section. The ten taxa assigned to the *Cirsium* section were located in the same cluster, except for three taxa (*C. pseudopersonata* subsp. *pseudopersonata, C. pseudopersonata* subsp. *kusnezowianum*, and *C. hypoleucum*). These three taxa

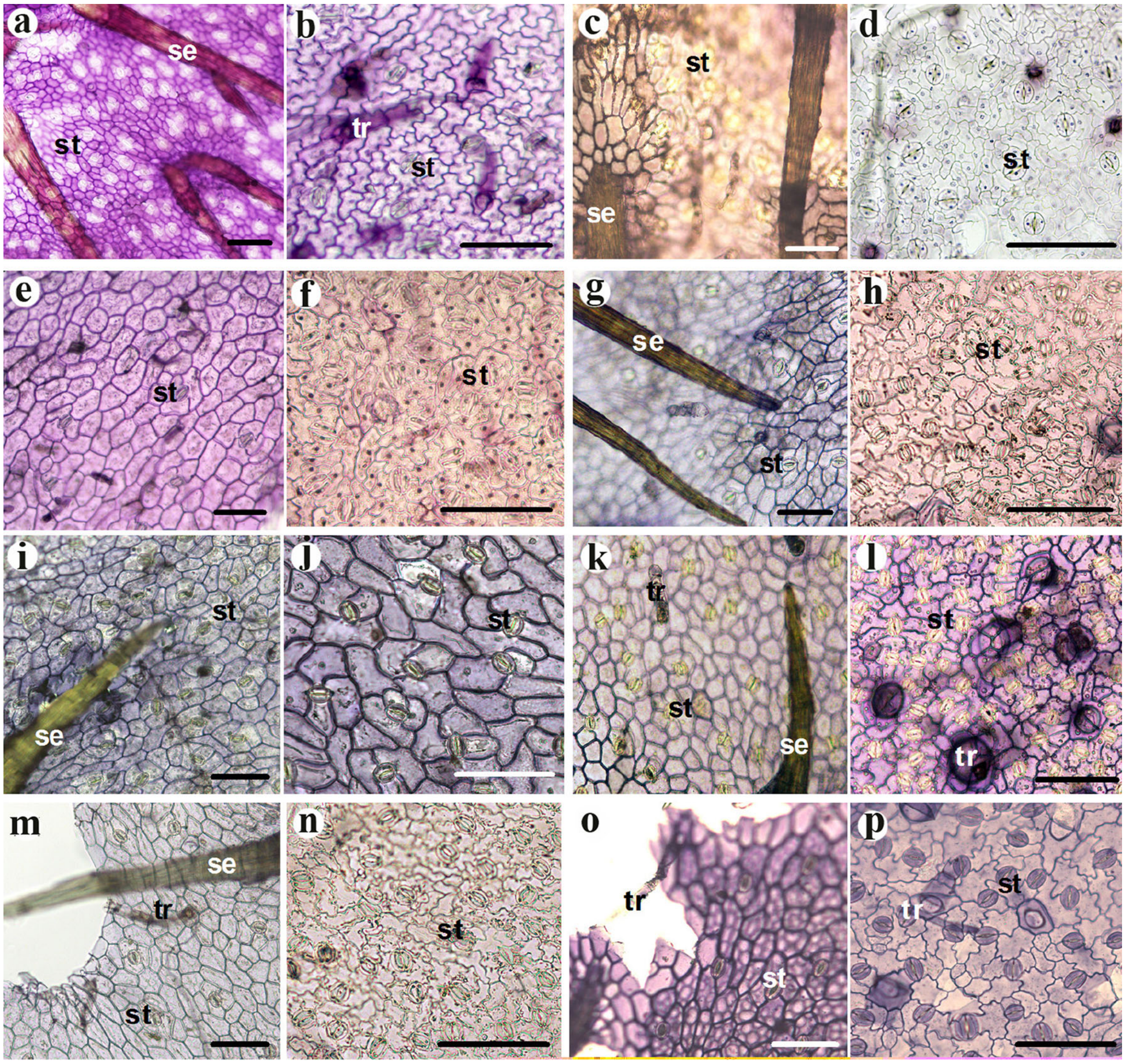

Fig. 7 Paradermal section of leaf in investigated *Cirsium* taxa. **a, b** *C. leucocephalum* subsp. *penicillatum*, **c, d** *C. macrobotrys*, **e, f** syn: *C. munitum* (*C. kosmelii*), **g, h** *C. osseticum*, **i, j** *C. rigidum*, **k, l** *C. sommieri*, **m, n** *C. trachylepis*, **o, p** *C. vulgare*. See Fig. 5 for abbreviations. *Scale bars* 100 μm

were delimited as a distinct group in both the UPGMA and MDS clusters. These taxa, with large lamina surfaces, narrow leaf lamina thickness, and hypostomatic leaf pattern sit apart from the other taxa within the *Cirsium* section. The taxa assigned to the *Cirsium* section, therefore, may be classified into a different section based on their qualitative and quantitative leaf characters. However, *C. leucocephalum* subsp. *leucocephalum* and *C. leucocephalum* subsp. *penicillatum*, that were assigned to the *Epitrachys* section, were separated from each other at a distance coefficient of about 10.0, as shown in Fig. 8. It has been indicated in the Flora of Turkey that this species is variable in habit and leaf shape. Therefore, new arrangements can be determined for these subspecies.

In conclusion, the compared anatomical characters among taxa are partly in accordance with their sectional delimitation presented in the Flora of Turkey. However, our results indicated that new arrangements may be necessary in the systematic positions of some taxa. Also, new sections may be determined as in the USRR Flora (11 sections) and Flora Iranica (5 sections). These anatomical characters are useful for separations based on morphology. However, these results should be considered preliminary and applicable to the populations from the analyzed region. Further studies will be required to verify these findings, and should include more species and wider ranges of populations from different geographical locations, as well as the analysis of seed anatomical characters, which should

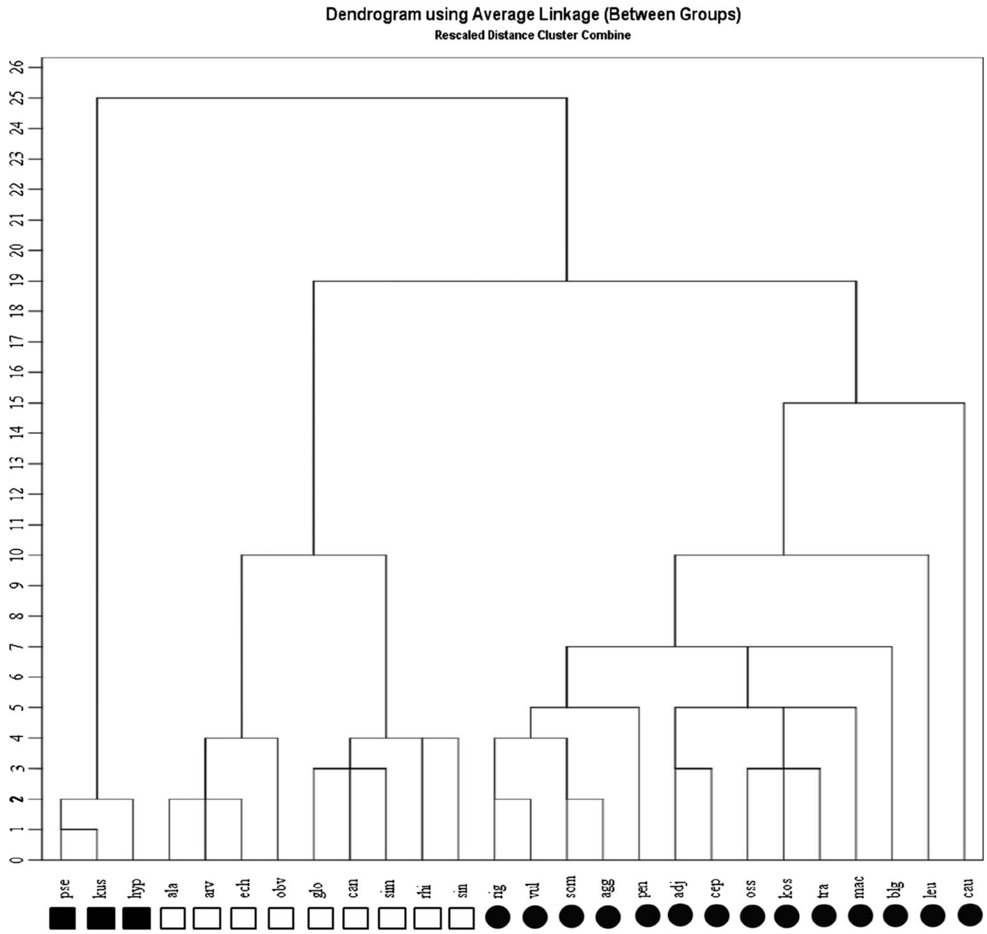

Fig. 8 UPGMA Cluster analysis using the leaf characteristics of investigated taxa. Each code represents a taxon and related group is indicated by each *different symbol*. Taxa codes; *adj C. adjaricum*, *agg C. aggregatum*, *ala C. alatum*, *arv C. arvense*, *blg C. bulgaricum*, *can C. pubigerum* var. *caniforme*, *cau C. caucasicum*, *cep C. cephalotes*, *ech C. echinus*, *glo C. pubigerum* var. *glomeratum*, *hyp C. hypoleucum*, *kos C. kosmelii*, *kus C. pseudopersonata* subsp. *kusnezowianum*, *leu C. leucocephalum* subsp. *leucocephalum*, *mac C. macrobotrys*, *obv C. obvallatum*, *oss C. osseticum* subsp. *osseticum*, *pen C. leucocephalum* subsp. *penicillatum*, *pse C. pseudopersonata* subsp. *pseudopersonata*, *rhi C. rhizocephalum* subsp. *rhizocephalum*, *rig C. rigidum*, *sin C. rhizocephalum* subsp. *sinuatum*, *som C. sommieri*, *tra C. trachylepis*, *vul C. vulgare*

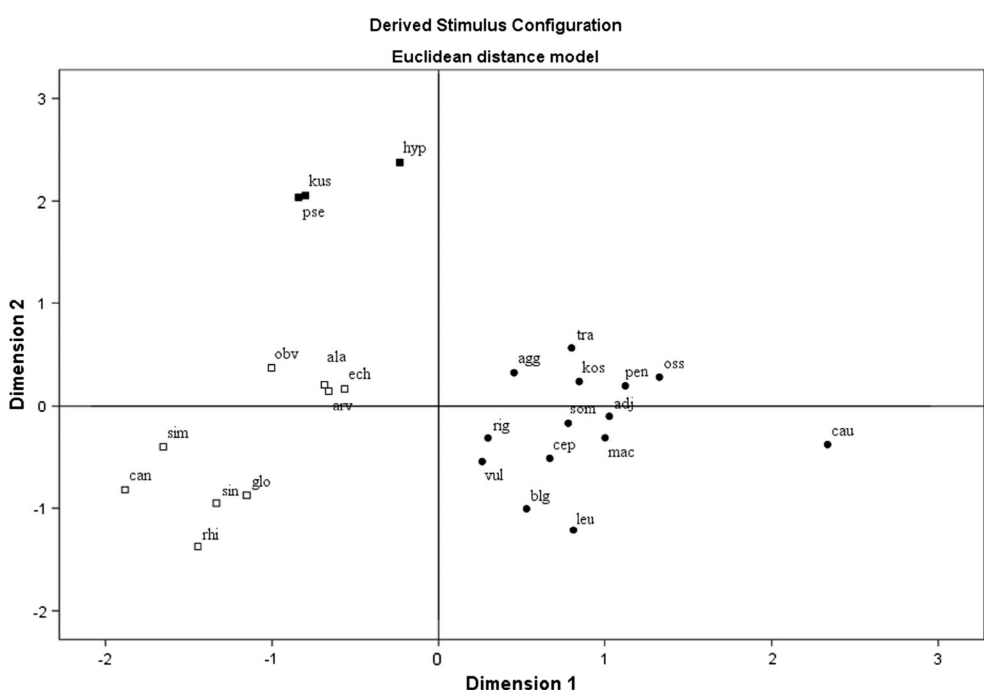

Fig. 9 Multidimensional scaling analysis (MDS) of investigated *Cirsium* taxa based on leaf anatomical data. Each code represents a taxon and related group is indicated by *different symbol*. See Fig. 8 for taxa codes

provide additional information and assist with distinct determination of this genus.

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