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CARPOLOGICAL INVESTIGATIONS ON SOME *CIRSIUM* (ASTERACEAE, CARDUOIDEAE) TAXA FROM NE ANATOLIA

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In the present study, morphological and anatomical structures of cypsela – 12 *Cirsium* Miller (Carduoideae, Asteraceae) taxa belonging to two sections (sect. *Cirsium* and sect. *Cephalonoplos*) were investigated in detail with using stereomicroscope and light microscope. The taxa were evaluated comparatively in the aspect of carpological variations and their anatomies were presented in here for the first time. Morphological features including size, shape and colour of cypselae were examined. From anatomical observations, anatomical structures of pericarp, as well as the structure of testa were described. Cypselae colours differ from light brown to stramineous, sometimes with blackish striations. Their shapes change from oblong to oblanceolate, rarely obovate. The largest cypselae are present in *C. echinus* (1.59±0.03 mm × 4.68±0.07 mm) and the smallest ones are found in *C. subinerme* (1.20±0.02 mm × 2.97±0.05 mm). The pericarp is characterised by almost parenchymatous cells, while the testa is composed of lignified sclerenchymatous cell lines and crushed cells group. Secretory structure in testa bundle was evaluated. Results obtained from this study were compared with the present data in literature. Overall, morphological and anatomical characteristics of cypselae provide useful taxonomic markers in their classifications of the studied taxa of *Cirsium* but not distinctive for their sectional levels.

Key words: Asteraceae, carpology, *Cirsium*, cypsela, NE Anatolia

INTRODUCTION

Several different studies show that cypsela (achene) morphology is one of the constant characters and less affected by environmental factors (Sears 1922). Cypsela is determined as a fruit of Asteraceae and differed from cypsela by an additional layer over the pericarp (Barroso *et al.* 1999, Frangiote-Pallone and de Souza 2014, Marzinek *et al.* 2008, Spjut 1994). Cypsela anatomy have been widely used in elucidating taxonomic relationships in Asteraceae (Abid and Ali 2010, Abid and Qaiser 2009, Barthlott 1981, 1984, Blanca and Guardia 1997, Bruhl and Quinn 1990, Cron *et al.* 1993, Garg and Sharma 2007, Geng *et al.* 1994, Inceer *et al.* 2012, 2018, Lavialle 1912, Pandey and Kumari 2007, Singh and Pandey 1984, Stebbins 1953, Zhu *et al.* 2006), and the tribes Cynareae (Kadereit and Jeffrey 2007) and Cardueae (Dittrich 1977, 1985, Häffner 2000, Ozcan and Akinci 2019, Zarembo and Boyko 2008).

Cirsium Miller is one of the largest genera in the subfamily Carduoideae (Asteraceae) and comprises almost 250–300 taxa (Smith 1977, Stevens 2001, Zomlefer 1994). This spiny genus has a Holarctic distribution extending from the northern Mediterranean area over Turkey to Caucasus (Häffner 2000). Some species are highly invasive weeds in field or cultivated areas. It has several diversity centres and Turkey is one of the gene centres. According to recent taxonomic studies, *Cirsium* is represented by 80 taxa, dividing into three sections (*Cephalonoplos* (Neck.) DC., *Cirsium*, *Epitrachys* DC.) and growing in very diverse altitudes from sea level to 3,000 m in Turkey (Davis and Parris 1975, Yıldız 2012, Yıldız *et al.* 2013, Duman *et al.* 2017).

Cirsium is closely related to the genus *Centaurea* L. and has complex taxonomy (Charadze 1998). Some *Cirsium* species also have morphological affinities with *Carduus* L., *Cnicus* L., and *Centaurea*. Some taxa display large morphological variations or have a high degree of overlapping morphological characters. Primary morphological characteristics leading to the complexity of the genus *Cirsium* are the structure of the florets and cypsela (achene), leaf shape and spiny habits (Davis and Parris 1975).

The species belonging to the genus *Cirsium* are used as weeds for animal breeding or cultivated as decorative plants (Charadze 1998). Several scientific examinations were carried out for this genus in the areas of vegetative anatomy (Ozcan *et al.* 2015), cytotaxonomy (Ozcan *et al.* 2008, 2011, Yüksel *et al.* 2013), chemotaxonomy (Boğa *et al*. 2014, Ozcan *et al.* 2016), and micromorphology (Köstekci and Arabacı 2011, Ozcan 2016, 2017) to obtain additional data for the phylogeny of complicated *Cirsium* taxa. However, cypselar anatomy in this genus has not received as much attention as it should have received.

In this context, the present investigation deals with detailed studies of cypsela morphologies and anatomies of all *Cirsium* taxa (12 taxa, 21 populations) distributed in NE Anatolia belonging to the two different sections. Special emphasis has been given to the compositions of pericarp and testa. The aim of this study is to obtain comparative information for the taxonomy of these taxa. This study presents for the first time a detailed account of cypsela anatomy of 12 *Cirsium* taxa from NE Anatolia. Data obtained from this study were evaluated to their potential value and in relation to the previous work in the genus *Cirsium.*

MATERIAL AND METHODS

Plant collections

A list of taxa (12 taxa, 21 populations) along with two sections examined was collected from natural populations of NE Anatolia in Turkey, and their names and voucher information are given in Table 1. Species identification

and nomenclature follow Davis and Parris (1975) and Yıldız (2012) and IPNI (International Plant Name Index). Herbarium materials were deposited in Artvin Coruh University Herbarium (ARTH).

Morphological studies

For each taxon, cypselae obtained from at least three different individuals were examined by using a stereomicroscope to ensure size, shape, colour and maturity. Fifteen to twenty cypselae were used for morphological analyses. They were photographed by using a stereomicroscope (Leica M60 with digital camera attachment DFC 295). Totally 21 populations were used for morphological calculations and measurements (Table 1).

Anatomical examinations

Mature cypselae were obtained from different plant capitula, and softened by boiling water for 3–4 days. Selected populations were used for anatomical preparations and several characters were evaluated (Tables 1–2). Cross sections made of middle of the fruits were prepared using cryostat. Sections were cut to a thickness of 15–20 μm. All sections were stained in haematoxylin for about 15 min (Algan 1981). Glycerol solution was added on slides to obtain semi-permanent slides. Observations were made using Olympus BX53 Research microscope with digital camera attachment DP73 and the images of well stained sections captured digitally.

Five cross sections from at least three different samples were measured to assess the consistency of anatomical characters and to calculate the means and standard error among different cross sections using LM.

Statistical analyses

Data analysis was carried out on SPSS (version 19) software. Based on the data, Duncan's multiple-range test, one-way analysis of variance, was used in 10 selected quantitative values to identify the statistical importance of distinctions among the data. Differences between means were considered significant at $P < 0.05$ (Table 2).

RESULTS

Variations in cypsela morphological and anatomical structures were characterised and showed in Figures 1–4 as well as Tables 2–3.

Table 1
Collection data of studied *Cirsium* taxa.* = selected population for anatomical observations

Taxon	Locality	Voucher
Section *Cephalonoplos*		
C. arvense (L.) Scop. (Syn: *C. arvense* (L.) Scop. subsp. *arvense*)	*Artvin: Şavşat-Ardahan, Kocabey high plateau, roadsides, 2,346 m, 30.09.2011	M. Ozcan 493
(Syn: *C. arvense* subsp. *vestitum* (Wimm. et Grab.) Petr.)	Bayburt: Kop Mountain, roadsides, 2,415 m, 28.08.2013	M. Ozcan 656
	*Bayburt: Erikdibi village, near cultivated area, 1,618 m, 05.08.2007	M. Ozcan 130
Section *Cirsium*		
C. echinus (M. Bieb.) Hand.-Mazz.	*Gümüşhane: Zigana Pass, roadsides, 1,750 m, 15.09.2011	M. Ozcan 473
	Trabzon; Araklı, Dagbaşı, near Pazarcık, 1,350 m, 31.07.2005	M. Ozcan 065
C. hypoleucum DC.	Giresun: Near Tamdere, under forest, roadsides, 1,100 m, 19.08.2009	M. Ozcan 307
	*Rize: Çamlıhemşin, Zilkale near Çat, 1,044–1,170 m, 29.08.2008	M. Ozcan 223
C. obvallatum (M. Bieb.) Fisch.	*Rize: Çamlıhemşin, Yukarı Kavrun High plateau, alpine meadows, 2,299 m, 05.08.2011	M. Ozcan 453
	Artvin: between Şavşat and Ardahan, roadsides, eroded blanks, 2,200–2,350 m, 31.08.2008	M. Ozcan 231
C. pseudopersonata subsp. *kusnezowianum* (Sommier et Levier) Petr.	Artvin: Borçka, Camili road, downward Macahel Pass, near streamsides, 1,631 m, 10.10.2008	M. Ozcan 279
C. pseudopersonata Boiss. et Balansa subsp. *pseudopersonata*	*Rize: Anzer road, near humid areas, 1,433 m, 19.08.2007	M. Ozcan 136
	Trabzon/Bayburt: Soğanlı Pass, humid areas, among grass, 2,291 m, 18.08.2008	M. Ozcan 462
	Artvin: Murgul Şavval hill, streamsides, under forest, 2,243 m, 20.09.2008	M. Ozcan 262
C. pubigerum var. *caniforme* Petr.	Rize: İkizdere, Cimil yolu, Termal near stream, under forest, humid areas, 896 m, 21.09.2013	M. Ozcan 664
C. pubigerum var. *glomeratum* (Freyn et Sint.) P. H. Davis et Parris	Rize: Çamlıhemşin, Ayder, Yukarı Kavrun High plateau, near cultivated area, 2,322 m, 15.08.2008	M. Ozcan 187

Table 1 (continued)

Taxon	Locality	Voucher
C. rhizocephalum C. A. Mey. subsp. *rhizocephalum*	Gümüşhane: near Köse, Gökçe village, among grass, 1,677 m, 24.08.2008	M. Ozcan 201
C. rhizocephalum ssp. *sinuatum* (Boiss.) P. H. Davis et Parris	Trabzon / Bayburt: near Limonsuyu, among alpine grass, 2,369 m, 22.09.2013	M. Ozcan 665
C. simplex subsp. *armenum* (DC.) Petr.	*Rize: İkizdere, Cimil High plateau, alpine meadows, 2,700–2,900 m, 16.09.2007	M. Ozcan 175
	Rize, Çamlıhemşin, Ayder, Yukarı Kavrun High plateau, streamsides, 2,280 m, 05.08.2011	M. Ozcan 452
C. subinerme Fisch. et C. A. Mey. (Syn: *C. elodes* M. Bieb.)	*Rize, İspir road, streamsides, 1,301 m,12.09.2008	M. Ozcan 250
	Bayburt; Kop Mountain, alpine meadows, 2,438 m, 18.08.2011	M. Ozcan 465

Cypsela morphology

Cypsela colours are light brown to stramineous, sometimes with black striations (Fig. 1). They are compressed slightly on both sides and mostly asymmetric or more or less symmetric (*Cirsium hypoleucum*, *C. arvense*) in outline, and has smooth surface and non-ribbed structure. Hilum is located at the base of cypsela. It has narrow base and acute apex. Carpopodium is undeveloped in all taxa. The mean lengths of cypselae range from 2.97 (*C. subinerme*) to 4.68 mm (*C. echinus*). Similarly, the mean width varies among 1.13 (*C. arvense*) and 1.68 mm (*C. pubigerum* var. *glomeratum*) (Table 2). The shapes of cypselae differ from oblong to oblanceolate, rarely obovate (two varieties of *C. pubigerum*) (Fig. 1). Colours vary from sordid-white to brown with blackish striations (Table 2). Pappus is plumose and caducous. The cypsela of *C. echinus* has the longest umbo in upper part as 0.62 μm, while *C. pseudopersonata* subsp. *kusnezowianum* has the smallest one as 0.20 μm (Table 2).

Cypsela anatomy

Cypsela shapes in cross section are elliptic or ovate in outline (Figs 2–4). Their diameters parallel to cotyledons differ in maximum in *Cirsium obvallatum* (1,856.53±46.58 μm) (Fig. 2D) as well as minimum in *C. arvense* (1,227.09±33.69 μm) (Fig. 2M). Similarly, vertical diameters are observed

Table 2

Size and morphology of cypselae in *Cirsium* taxa investigated. All measures are in mm. * = mean±standard error

Taxon	Cypsela shape	Length mean (min.–max.)	Width (mm) mean (min.–max.)	Ratio (l/w)	Umbo (mm) mean (min.–max.)	Colour
C. arvense	oblong	3.19±0.03 (2.92–3.54)	1.13±0.02 (0.94–1.46)	2.46–3.77	0.26±0.01 (0.15–0.38)	stramineous
C. echinus	oblong	4.68±0.07 (4.05–5.26)	1.59±0.03 (1.32–1.76)	2.41–3.47	0.62±0.02 (0.42–0.79)	light brown-stramineous
C. hypoleucum	oblong	3.64±0.06 (3.25–4.13)	1.29±0.02 (1.08–1.46)	2.25–3.53	0.30±0.02 (0.17–0.42)	stramineous-dark brown (blackish striate below)
C. obvallatum	oblong-oblanceolate	4.12±0.06 (3.26–4.74)	1.66±0.01 (1.5 –1.87)	2.0–2.87	0.42±0.01 (0.16–0.47)	sordid-white with black stripes
C. pseudopersonata subsp. *kusnezowianum*	oblong	3.91±0.14 (3.32–4.74)	1.55±0.02 (1.42–1.71)	2.11–3.16	0.20±0.01 (0.11–0.26)	brown-stramineous with reddish brown striations
C. pseudopersonata subsp. *pseudopersonata*	oblong	3.88±0.10 (3.04–4.68)	1.39±0.02 (1.10–1.83)	2.01–3.19	0.20±0.01 (0.13–0.37)	brown-stramineous with brown stripes
C. pubigerum var. *caniforme*	obovate-oblong	3.52±0.04 (3.29–3.68)	2.53±0.34 1.37–1.68)	2.07–2.40	0.26±0.02 (0.16–0.34)	stramineous
C. pubigerum var. *glomeratum*	obovate-oblong	3.99±0.303 (3.79–4.16)	1.68±0.02 (1.58–1.75)	2.31–2.53	0.44±0.03 (0.25–0.54)	stramineous-light brown
C. rhizocephalum subsp. *rhizocephalum*	oblong	4.05±0.06 (3.79–4.26)	1.59±0.204 (1.42–1.79)	2.12–2.72	0.29±0.02 (0.21–0.37)	sordid-white with black-ish stripes
C. rhizocephalum subsp. *sinuatum*	oblong	3.81±0.08 (3.50–4.17)	1.56±0.02 (1.46–1.63)	2.15–2.74	0.49±0.03 (0.31–0.67)	stramineous with brown striations
C. simplex subsp. *armenum*	oblong-oblanceolate	3.79±0.07 (3.44–4.05)	1.32±0.04 (1.16–1.53)	2.61–3.35	0.43±0.02 (0.32–0.53)	light brown with black stripes
C. subinerme	oblong-oblanceolate	2.97±0.05 (2.67–3.21)	1.20±0.02 (1.0–1.29)	2.07–2.46	0.28±0.01 (0.21–0.33)	stramineous

in maximum in *C. obvallatum* (1,347.18±42.69 μm) as well as minimum in *C. arvense* (895.79±14.77 μm) (Fig. 2M, D, Table 3).

Cypsela is composed of four different regions; pericarp, testa, endosperm and cotyledons. The mature pericarp is divided into two zones; exocarp and mesocarp, and shows variation in the structure (Figs 2–4).

Fig. 1. Cypselae of the investigated taxa. A = *C. arvense*; B = *C. echinus*; C = *C. hypoleucum*; D = *C. obvallatum*; E = *C. pseudopersonata* subsp. *kusnezowianum*; F = *C. pseudopersonata* subsp. *pseudopersonata*; G = *C. pubigerum* var. *caniforme*; H = *C. pubigerum* var. *glomeratum*; I = *C. rhizocephalum* subsp. *rhizocephalum*; J = *C. rhizocephalum* subsp. *sinuatum*; K = *C. simplex* subsp. *armenum*; L = *C. subinerme*

Table 3a
Cypsela anatomical characteristics of investigated *Cirsium* taxa. * = mean value±standard error

Taxon	Pericarp thickness (μm)	Testa		Vascular bundle	
		Lignified cells thickness (μm)	Crushed cell thickness (μm)	Length (μm)	Width (μm)
C. arvense	23.80±1.77^{bc}	35.53±1.48^{a-d}	13.09±0.48^{bc}	27.69±2.46^{a-d}	48.12±5.09^{b-f}
	23.29±0.29^{bc}	36.79±0.97^{a-e}	14.85±1.23^{bcd}	29.36±2.01^{b-e}	43.42±3.82^{a-d}
C. echinus	52.98±6.96^{l}	61.95±1.92^{kl}	13.40±0.62^{bcd}	43.83±4.12^{g}	46.95±4.29^{a-e}
C. hypoleucum	15.97±1.64^{a}	46.71±0.80^{fgh}	17.40±1.19^{cde}	29.49±1.33^{b-e}	49.14±2.92^{b-f}
C. obvallatum	34.37±1.48^{ghi}	59.65±2.08^{jk}	23.93±2.45^{fgh}	35.27±2.06^{ef}	62.05±4.39^{fgh}
C. pseudopersonata subsp. *kusnezowianum*	41.99±2.03^{j}	65.23±4.34^{kl}	21.33±1.28^{efg}	39.09±1.40^{fg}	59.83±1.99^{e-h}
C. pseudopersonata subsp. *pseudopersonata*	33.04±1.22^{f-i}	51.17±1.06^{hij}	17.97±1.04^{cde}	33.52±0.84^{def}	52.87±4.20^{d-g}
C. pubigerum var. *caniforme*	32.71±0.80^{e-i}	59.59±1.05^{jk}	18.47±0.65^{de}	42.82±1.85^{g}	58.78±8.05^{e-h}
C. pubigerum var. *glomeratum*	36.31±2.33^{hij}	61.51±0.88^{ijk}	33.74±4.33^{kl}	36.44±0.69^{ef}	43.28±1.85^{a-d}
C. rhizocephalum subsp. *rhizocephalum*	42.51±2.14^{bc}	44.48±2.28^{ab}	20.59±0.75^{bcd}	29.39±2.55^{ab}	44.16±2.46^{abc}
C. rhizocephalum subsp. *sinuatum*	33.40±1.59^{jk}	44.01±1.13^{d-h}	18.18±1.04^{efg}	32.86±0.69^{b-e}	36.98±2.28^{a-d}
C. simplex subsp. *armenum*	23.17±0.94^{ghi}	30.05±0.82^{d-h}	13.72±0.83^{cde}	25.72±1.99^{c-f}	38.81±1.78^{ab}
C. subinerme	22.94±0.95^{bc}	39.10±0.57^{b-f}	11.90±0.35^{ab}	25.15±1.41^{ab}	33.62±3.62^{a}

Epidermal cells forming the exocarp of the pericarp are uniseriate, thick walled rectangular to oval and the mesocarp consists of isodiametric parenchymatous cells. Differently from others, *Cirsium echinus* has slightly longitudinally elongated epidermal cells (Fig. 2H, I). The thickest pericarp is found in *C. rhizocephalum* subsp. *rhizocephalum* (42.51±2.14 μm) (Fig. 4A–C), while the thinnest one is observed in *C. hypoleucum* (15.97±1.64 μm) (Fig. 2J–L). Four carpel bundle traces underlaid by a layer of parenchymatous cells (in the pericarp) and two testa bundles placed in opposite sides and between lignified cells and crushed cell groups are present. The testa body consists of two differentiated cell types, other zone with a single row longitudinally elongated, lignified cells (notable thickening and swelling on their radial walls) and inner zone with crushed parenchymatous cell group (inner zone). Lignified cell layer covers most of the testa. The highest and the smallest values of this layer are detected in *C. pseudopersonata* subsp. *kusnezowianum* (65.23±4.34 μm) and *C. simplex* subsp. *armenum* (30.05±0.82 μm), respectively. In the testa bundles

Table 3b
Cypsela anatomical characteristics of investigated *Cirsium* taxa. * = mean value±standard error

Taxon	Endosperm thickness (μm)	Epidermal cell		Cypsela diameter	
		Length (μm)	Width (μm)	Parallel to cotyledons (μm)	Vertical to cotyledons (μm)
C. arvense	09.60±0.54^{e-j}	05.83±0.22^{b-f}	07.62±0.43^{b-i}	1263.95±41.29^{a}	992.78±51.43^{ab}
	09.76±0.48^{f-j}	04.74±0.38^{abc}	6.93±0.39^{a-e}	1227.09±33.69^{a}	895.79±14.77^{a}
C. echinus	07.35±0.25^{a-d}	16.33±0.92^{p}	11.42±0.27^{no}	1712.32±15.17^{de}	1203.29±14.23^{cde}
C. hypoleucum	09.42±0.53^{d-i}	07.43±0.52^{f-j}	08.78±0.32^{e-k}	1475.29±28.53^{bc}	1044.47±55.89 ^{abc}
C. obvallatum	13.05±1.84^{l}	10.14±0.61^{mn}	09.39±0.65^{i-m}	1856.53±46.58^{e}	1347.18±42.69^{ef}
C. pseudopersonata subsp. *kusnezowianum*	11.58±1.09^{jkl}	11.51±0.42^{no}	11.52±0.94^{no}	1817.74±14.94^{de}	1267.85±23.45^{def}
C. pseudopersonata subsp. *pseudopersonata*	06.94±0.21^{abc}	09.93±0.36^{lm}	10.75±0.33^{k-n}	1623.51±33.37^{cd}	1102.89±12.55^{bcd}
C. pubigerum var. *caniforme*	12.75±0.70^{l}	11.86±0.45^{o}	12.05±0.31^{op}	1795.42±33.54^{de}	1249.82±36.63^{def}
C. pubigerum var. *glomeratum*	05.80±0.31^{a}	09.62±0.35^{klm}	13.85±0.39^{p}	1626.41±81.00^{cd}	991.73±34.96^{ab}
C. rhizocephalum subsp. *rhizocephalum*	08.72±0.66^{ab}	08.16±0.68^{c-g}	09.44±0.52^{mno}	1797.70±23.60^{bc}	1136.16±21.40^{b}
C. rhizocephalum subsp. *sinuatum*	09.13±0.46^{b-g}	06.49±0.24^{h-k}	10.93±0.72^{i-m}	1664.37±59.19^{de}	1306.56±42.49^{bcd}
C. simplex subsp. *armenum*	06.59±0.24^{d-h}	06.20±0.16^{d-h}	11.31±0.41^{l-o}	1467.54±20.72^{cde}	910.87±14.59^{ef}
C. subinerme	07.68±0.27^{a-f}	07.10±0.63^{e-j}	08.13±0.38^{d-j}	1394.25±54.18^{ab}	982.72±48.90^{ab}

of *C. hypoleucum*, two subspecies of *C. pseudopersonata* and two varieties of *C. pubigerum*, wide gaps were observed, differently from the others (Figs 2J, L, 3A, D–F, G, H, 4A).

A subjacent layer of the testa is endosperm or only remnants composed of one layer of elongated and thin-walled parenchymatous cells. The two cotyledons occupy a large region of in cypsela interior and they are oriented in anterior-posteriorly (Figs 2–4).

DISCUSSION

The genus *Cirsium* is widespread in the northern hemisphere and also mountains of southern Europe and in the Caucasus from sea level to high altitudes in the mountains. In this study, 12 *Cirsium* taxa from the two sec-

tions and distributed in NE Anatolia were evaluated from morphological and anatomical aspects by stereomicroscope and light microscope. All anatomical data were detailed and compared for the first time.

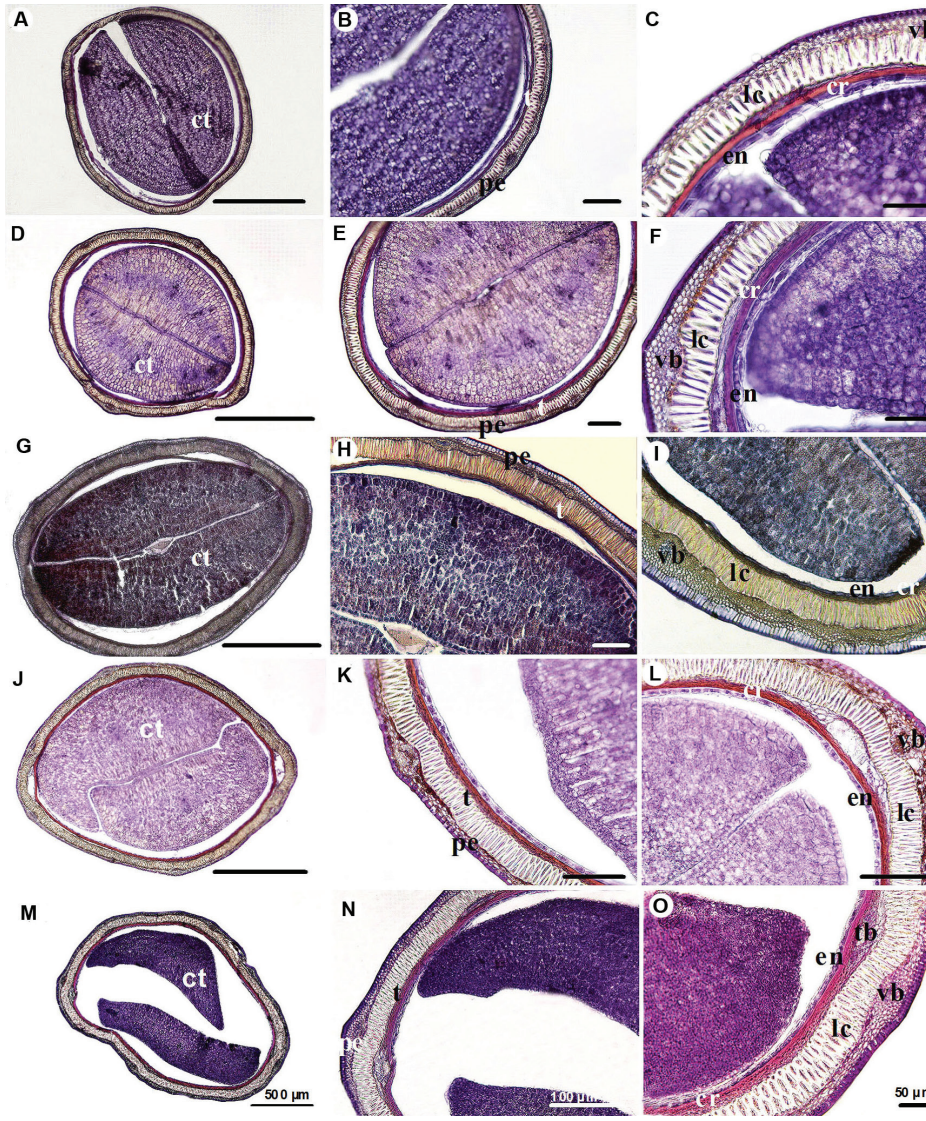

Fig. 2. Transverse sections of cypselae. A–F = *C. arvense*; G–I = *C. echinus*; J–L = *C. hypoleucum*; M–O = *C. obvallatum* (cr = crushed cell, ct = cotyledon, e = epidermis, en = endosperm, lc = lignified cell, pe = pericarp, t = testa, tb = testa bundle, vb = vascular bundle). Scale bars (left) = 500 µm, (middle) = 100 µm, (right) = 50 µm

Cypsela mean lengths of investigated taxa differ from 2.97 mm (*C. subinerme*) to 4.68 mm (*C. echinus*) (Table 2) in the two sections (sect. *Cephalonoplos* and sect. *Cirsium*). Like in the present investigated taxa, Köstekci and Arabacı (2011) previously reported the smallest cypsela for *C. pubigerum* var. *glomeratum*, while the biggest one was determined in *C. echinus*. The present results are in accordance with this previous report. On the other hand, Ozcan (2017) reported mean cypsela lengths in sect. *Epitrachys* have differed from 4.26 mm to 7.46 mm. In the other words, it can be concluded that cypselae of sect. *Cirsium* taxa have smaller than those of sect. *Epitrachys*.

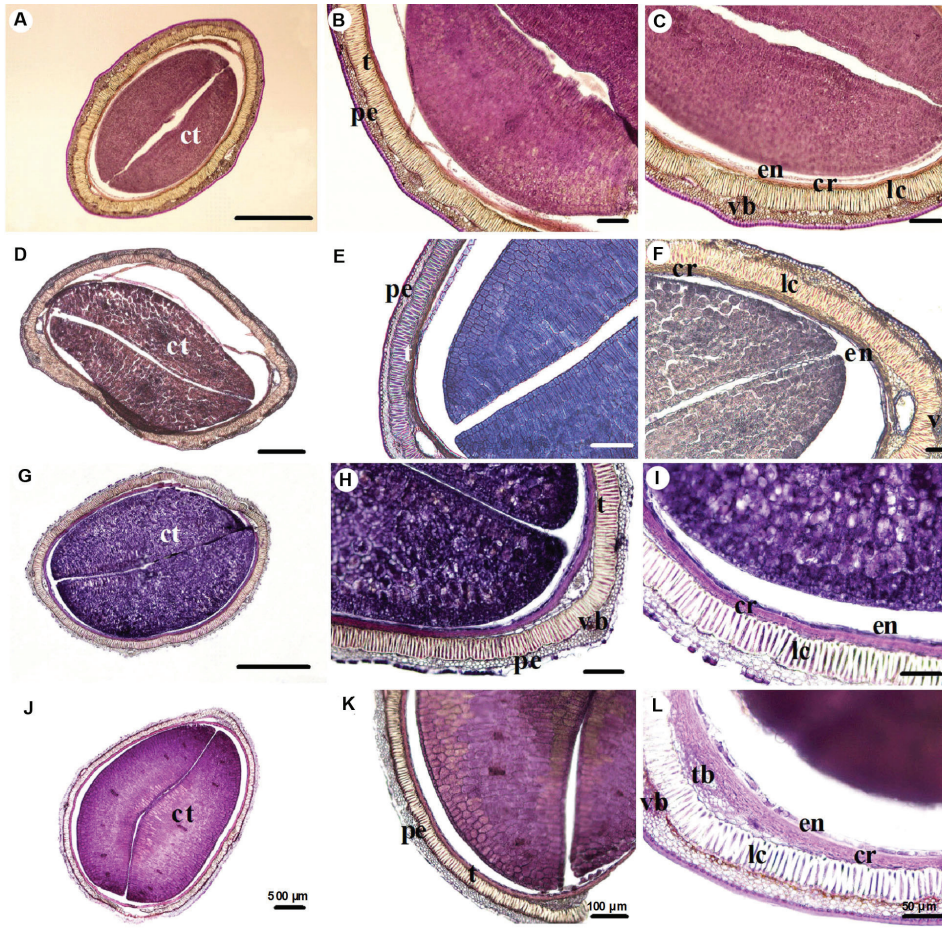

Fig. 3. Transverse sections of cypselae. A–C = *C. pseudopersonata* subsp. *kusnezowianum*; D–F = *C. pseudopersonata* subsp. *pseudopersonata*; G–I = *C. pubigerum* var. *caniforme*; J–L = *C. pubigerum* var. *glomeratum*. See Fig. 2 for abbreviations. Scale bars (left) = 500 µm, (middle) = 100 µm, (right) = 50 µm

The cypsela surfaces of all investigated taxa in this study are smooth (not ribbed), oblong to oblanceolate rarely obovate, with compressed dorsal and ventral margins (Fig. 1). However, both ribbed and non-ribbed cypselae were previously reported in the family Asteraceae by Abid and Qaiser (2009).

The taxa examined here have uniseriate and thick walled epidermis, which included no trichomes on their surfaces. Shabestari *et al.* (2013) described in some *Centaurea* taxa with in their seed (cypsela) types with or without hairs on the surfaces. Sparsely long hairs were also reported in the cypselae of *C. polyclada* by Uysal e*t al.* (2005). Andrés-Sánchez *et al.* (2015) referred to twin hairs in some species (e.g. *Filago* L.) in the family Asteraceae. However,

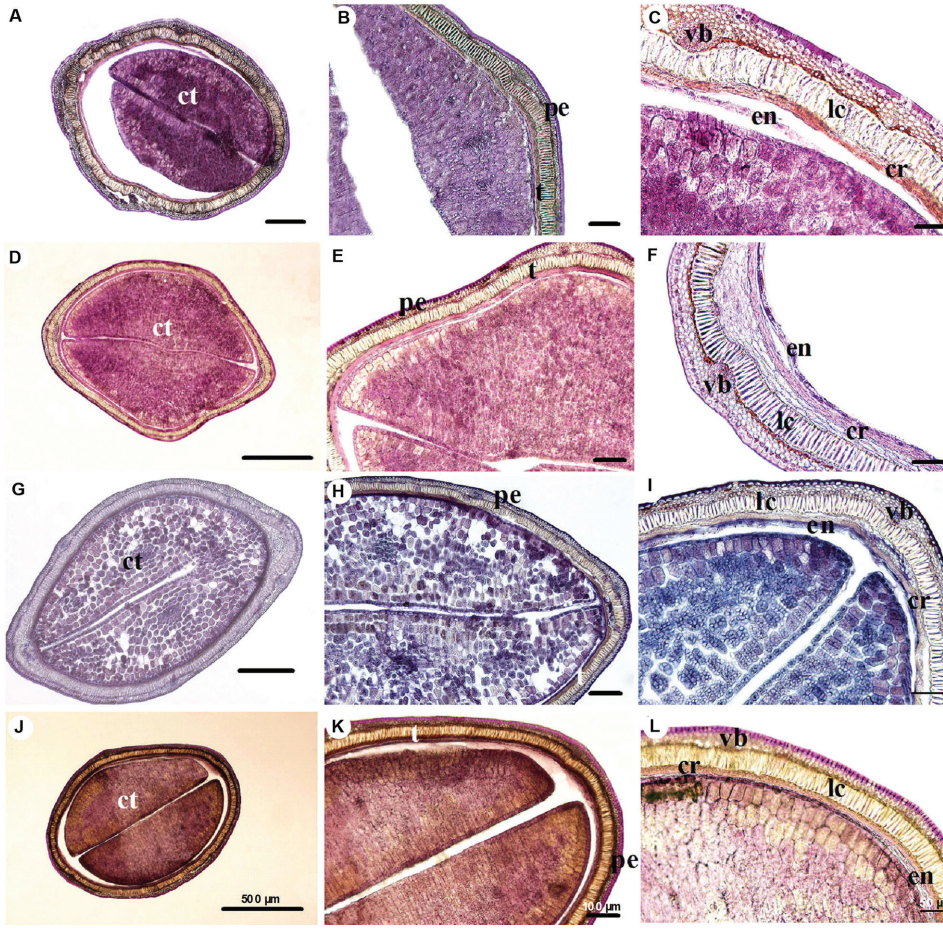

Fig. 4. Transverse sections of cypselae. A–C = *C. rhizocephalum* subsp. *rhizocephalum*; D–F = *C. rhizocephalum* subsp. *sinuatum*; G–I = *C. simplex* subsp. *armenum*; J–L = *C. subinerme.* See Fig. 2 for abbreviations. Scale bars (left) = 500 µm, (middle) = 100 µm, (right) = 50 µm

Dittrich (1977) reported that twin hairs are never present on the cypsela surface in the subtribe Carduinae and Centaureinae. No hair type was observed in *Cirsium* taxa investigated in the present study (Fig. 1).

The anatomical structure of the cypselae in the sect. *Epitrachys* seems to be rather similar in outline. In the cypsela anatomies of taxa, five distinct parts can be determined; exocarp, mesocarp, testa, one layer endosperm and two large cotyledons. The pericarp zones were determined as exocarp and mesocarp. The exocarp is composed of uniformly thickened epidermal cells, parenchymatous cells and collenchymatous/sclerenchymatous cells, which around vascular bundles cover the entire mesocarp in all taxa. Sclerified epidermal cells have been previously reported in *Carduus*, *Cirsium*, *Notobasis* Cass., *Picnomon* Adans., *Silybum* Adans. and *Trymnus* Cass. by Häffner (2000). Thin epidermal cells and parenchymatous mesocarp have been reported in *Rhaponticum* Vaill., while both parenchymatous and sclerenchymatous tissues were determined in the species of *Klasea* Cass. and *Serratula* L. by Zarembo and Boyko (2008). Mukherjee (2001) also mentioned sclerenchymatous braces and parenchymatous cell layers in the mesocarpic zone in the tribe Senecioneae.

The testa of all taxa investigated exhibits well-developed, elongated, with radially stretched lignified cell and crushed cell layers near the single row endosperm. 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 Pandey (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*. The thick testa is characterised in *Cirsium pseudopersonata* subsp. *kusnezowianum,* and a comparatively thin one is observed in *C. simplex* subsp. *armenum*. On the other hand comparatively thick pericarp is present in *C. echinus*, whereas *C. hypoleucum* has the thinnest one, among the investigated taxa (Table 3). *C. echinus* has also slightly elongated epidermal cells like palisade parenchyma, differently from others. Crushed cell zone shows little variations among the investigated taxa. Four main vascular traces are found in the taxa. Four to five, sometimes six to seven vascular traces have been reported in the taxa of the tribe Cardueae by Häffner (2000).

Differently from other investigated ones, between lignified cells and crushed cell groups, in opposite directions of five taxa, large hollows were observed. They are probably secretory structures developed by lysigenous origin. Similarly, three different secretory structures have been determined in roots or rhizome by Fritz and Saukel (2011) as endodermal resin ducts (*Cirsium vulgare*), secretory ducts (*C. arvense*) and secretory cavities (*Echinops sphaerocephalus*) in some species of the tribe Cardueae. In addition, phytomelanin-

coated cells were reported in roots of *Centaurea jacea* in that study. Detection of similar secretory structure, however, no phytomelanin-included cells were observed in the cypselae of investigated taxa in the present study.

A one-layer, elongated and thin-walled parenchymatous endosperm is observed in all taxa investigated. The thickest endosperm is determined in *C. obvallatum*, while the thinnest is shown in *C. pubigerum* var. *glomeratum* (Figs 2M–O, 3J–L, Table 3). Häffner (2000) reported that the appearance of endosperm does not differ significantly among the genera of the subtribe Carduinae. Grau and Hopf (1985) supposed that this structure probably provides an additional protection to the embryo.

Carpological features of some *Cirsium* taxa have been reported in two investigations till now. However, detailed investigations on morphological and anatomical data are still desirable. Three different reports were present in literature about cypsela of some *Cirsium* taxa; one is cypsela anatomy of *Cirsium eriophorum* (L.) Scop. (Häffner 2000), the second is carpology of *C. aggregatum* Ledeb. (Ozcan 2016) and the third is about micromorphology and anatomy of 17 *Cirsium* taxa (Ozcan 2017). In this study carpological characters were evaluated in detail. From anatomical points, all taxa investigated in the present study share similar patterns with *C. eriophorum* without sectional delimitations.

CONCLUSION

The data obtained from the present study demonstrate that the morphological and anatomical characters investigated are potentially informative. Especially, cypsela length, mesocarp and testa structure display diversity in *Cirsium* taxa. They are applicable for identification and can be used as a parameter to support species delimitation. Investigations of cypsela structures should therefore be included in future taxonomic and phylogenetic studies of the genus *Cirsium*, as well as in other genera within the tribe Cardueae.

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