

Cypselæ micro-macromorphological characteristics
of selected taxa of *Centaurea* L. from Turkey
and their systematic importance

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Cypselas micro-macromorphological characteristics of selected taxa of *Centaurea* L. from Turkey and their systematic importance

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ABSTRACT

In this study, the cypselas micro-macromorphological characteristics of 15 *Centaurea* L. taxa, from the seven sections from Turkey were investigated using Light Microscopy and Scanning Electron Microscopy techniques. The studied taxa are *C. goksivriensis* M. Bona, *C. calcitrapa* subsp. *calcitrapa* L., *C. calcitrapa* subsp. *cilicica* (Boiss. & Bal.) Wagenitz, *C. glastifolia* L., *C. amanicola* Hub.-Mor., *C. cataonica* Boiss. & Hausskn. in Boiss., *C. haradjianii* Wagenitz, *C. haussknechtii* Boiss., *C. regia* Boiss. var. *regia*, *C. sclerolepis* Boiss., *C. spicata* Boiss., *C. solstitialis* L., *C. behen* L., *C. rigida* Banks & Sol., and *C. antitauri* Hayek. They belong to sections *Acrocentron* (Cassini) DC., *Calcitrapa* (Heister ex Fabr.) DC., *Chartolepis* (Cass.) DC., *Cynaroides* Boiss., *Mesocentron* (Cass.) DC., *Microlophus* (Cass.) DC., *Pseudophaeopappus* Wagenitz. Cypselas characteristics such as shape, size, colour, and surface pattern, hair structure, length, and colour of pappus are studied. Cypselas are 1.99 to 9.95 mm long and their width is between 1.01 and 3.73 mm. The pappus is 2.21 to 3.16 mm long. The pappus is generally straw-coloured, brown or whitish and barbellate but *C. goksivriensis* distinguished by its purple pappus and *C. glastifolia* by its plumose pappus. *C. calcitrapa* subsp. *calcitrapa* and *C. calcitrapa* subsp. *cilicica* do not have pappus. Scanning Electron Microscope analysis shows five different cypselas surface patterns for the studied species: reticulate, smooth, undulate, smooth-undulate, undulate-ruminate. A key to the taxa has been provided on the basis of these characteristics. The cypselas micro-macromorphological characteristics provide distinctive data for infrageneric classification for the studied species.

KEY WORDS
Centaurea,
Turkey,
achene,
cypselas,
pappus,
SEM.

RÉSUMÉ

Caractéristiques micro-macromorphologiques de la cypsèle de taxons sélectionnés de Centaurea L. de Turquie et leur importance systématique.

Dans cette étude, les caractéristiques micro-macromorphologiques de la cypsèle de 15 taxons de *Centaurea* L., provenant de sept sections de Turquie, ont été étudiées à l'aide de techniques de microscopie optique et de microscopie électronique à balayage. Les taxons étudiés sont *C. goksivriensis* M. Bona, *C. calcitrapa* subsp. *calcitrapa* L., *C. calcitrapa* subsp. *cilicica* (Boiss. & Bal.) Wagenitz, *C. glastifolia* L., *C. amanicola* Hub.-Mor., *C. cataonica* Boiss. & Hausskn. in Boiss., *C. haradjianii* Wagenitz, *C. haussknechtii* Boiss., *C. regia* Boiss. var. *regia*, *C. sclerolepis* Boiss., *C. spicata* Boiss., *C. solstitialis* L., *C. behen* L., *C. rigida* Banks & Sol. et *C. antitauri* Hayek. Ils appartiennent aux sections *Acrocentron* (Cassini) DC, *Calcitrapa* (Heister ex Fabr.) DC, *Chartolepis* (Cass.) DC, *Cynaroides* Boiss., *Mesocentron* (Cass.) DC, *Microlophus* (Cass.) DC et *Pseudophaeopappus* Wagenitz. Les caractéristiques des cypselles telles que la forme, la taille, la couleur et le motif de surface, la structure des poils, la longueur et la couleur du pappus sont étudiées. Les cypselles mesurent 1,99 à 9,95 mm de long et leur largeur est comprise entre 1,01 et 3,73 mm. Le pappus a une longueur de 2,21 à 3,16 mm. Le pappus est généralement de couleur paille, marron ou blanchâtre et barbelé mais *C. goksivriensis* se distingue par son pappus violet et *C. glastifolia* par son pappus plumeux. *Centaurea calcitrapa* subsp. *calcitrapa* et *C. calcitrapa* subsp. *cilicica* n'ont pas de pappus. L'analyse au microscope électronique à balayage montre cinq motifs de surface de cypsèle différents pour les espèces étudiées : réticulé, lisse, ondulé, lisse-ondulé, ondulé-ruminé. Une clé des taxons a été fournie sur la base de ces caractéristiques. Les caractéristiques micro-macromorphologiques de la cypsèle fournissent des données distinctives pour la classification infragénérique des espèces étudiées.

MOTS CLÉS

Centaurea,
Turquie,
akène,
cypsèle,
pappus,
SEM.

INTRODUCTION

Centaurea L. sensu lato (s.l.) is one of the largest genera in the Asteraceae with 400-700 species (Wagenitz 1975; Wagenitz & Hellwig 1996; Hellwig 2004; Garcia-Jacas *et al.* 2006; Susanna & Garcia-Jacas 2007; Negaresh *et al.* 2015; Özbek 2021). The systematics of the *Centaurea* has been progressed considerably based on morphological and molecular phylogenetic studies and the natural delineation of *Centaurea* was achieved on the basis of molecular data (Wagenitz & Hellwig 2000; Garcia-Jacas *et al.* 2001, 2002, 2006; Greuter 2003; Hellwig 2004; Susanna & Garcia-Jacas 2007; Negaresh *et al.* 2015; Herrando-Moraira *et al.* 2019). The genus distributes mainly in the Mediterranean and Irano-Turanian regions (Hellwig 2004; Susanna & Garcia-Jacas 2009; Hilpold *et al.* 2014). The first taxonomic revision on *Centaurea* in Turkey was done by Wagenitz (1975), including 204 taxa under 34 sections. The total species number is increasing with the description of many new species (Negaresh & Rahiminejad 2014; Yüzbaşıoğlu *et al.* 2015; Bona 2015, 2016; Kültür *et al.* 2016; Uysal *et al.* 2016; Behçet *et al.* 2017; Oreizi *et al.* 2017; Uysal *et al.* 2017; Uysal & Hamzaoğlu 2017; Şirin *et al.* 2019, 2020; Hamzaoğlu & Koç 2020; Özbek 2021; Duman *et al.* 2021).

The infrageneric classification of *Centaurea* is mainly based on the form of the scarious bract appendages, floret colour, leaf, cypselas and pappus characteristics (Wagenitz 1975; Bona 2014, 2015, 2016; Candan *et al.* 2016; Kültür *et al.* 2016; Şirin *et al.* 2017; Novaković *et al.* 2018; Rakizadeh *et al.* 2019; Adwan *et al.* 2020; Özbek 2021). The genus is annual, biennial, or perennial herbs, less often shrubs, with usually

unarmed leaves and white, yellow, pink or purple flowers; the tips of phyllaries variously spiny or mucronate and margins pinnate or entire; the stems long and erect, rarely short or prostrate; leaves entire, pinnatifid, pinnatisect and generally covered with grey hairs, rarely dense arachnoid or glabrous; cypselas oblong to ovate, rarely triangular; pappus whitish, straw or brown and composed stiff, minutely barbellate or flat scales (Wagenitz 1975, 1980; Susanna & Garcia-Jacas 2007; Hilpold *et al.* 2014).

Several studies showed that cypselas micromorphology is also useful for identification and taxonomic delimitation in different genera in Asteraceae (Bona 2014; Karaismailoğlu 2015; Candan *et al.* 2016; Şirin *et al.* 2017; Özbek *et al.* 2018; Rakizadeh *et al.* 2019; Ayaz *et al.* 2020). Even the genera that were analysed in molecular aspects, still need further research, especially in the focused macro-micromorphological aspect at a regional scale.

In this study, cypselas morphological characteristics of 15 taxa belonging to seven sections were investigated. They have been investigated using the Light Microscope (LM) and Scanning Electron Microscope. Studied taxa are: *C. goksivriensis* M. Bona, *C. calcitrapa* subsp. *calcitrapa* L., *C. calcitrapa* subsp. *cilicica* (Boiss. & Bal.) Wagenitz, *C. glastifolia* L., *C. cataonica* Boiss. & Hausskn. in Boiss., *C. haradjianii* Wagenitz, *C. haussknechtii* Boiss., *C. regia* Boiss. var. *regia*, *C. sclerolepis* Boiss., *C. spicata* Boiss., *C. amanicola* Hub.-Mor., *C. solstitialis* L., *C. rigida* Banks & Sol., *C. behen* L., *C. antitauri* Hayek. They belong to sections *Acrocentron* (Cassini) DC., *Calcitrapa* (Heister ex Fabr.) DC., *Chartolepis* (Cass.) DC, *Cynaroides* Boiss., *Mesocentron* (Cass.) DC, *Microlophus* (Cass.) DC., *Pseudophaeopappus* Wagenitz. The eight taxa namely, *C. goksivriensis*, *C. calcitrapa* subsp. *cilicica*, *C. amanicola*, *C. cataonica*, *C. haradjianii*, *C. haussknechtii*, *C. sclerolepis*,

TABLE 1. — List of the studied *Centaurea* taxa with sections, localities, and herbarium numbers.

Section	Species	Endemic (E)	Locality	Herbarium Number
<i>Acrocentron</i> (Cassini) DC.	<i>C. goksivriensis</i> M. Bona	E	Hatay	ISTE 102727
<i>Calcitrapa</i> (Heister ex Fabr.) DC.	<i>C. calcitrapa</i> subsp. <i>calcitrapa</i> L.		Hatay	ISTE 99215
<i>Calcitrapa</i>	<i>C. calcitrapa</i> subsp. <i>cilicica</i> (Boiss. & Balansa) Wagenitz	E	Adana	ISTE 99173
<i>Chartolepis</i> (Cass.) DC	<i>C. glastifolia</i> L.		Sivas	ISTE 99210
<i>Cynaroides</i> Boiss. ex Walp.	<i>C. amanicola</i> Hub.-Mor.	E	Osmaniye	ISTE 102864
<i>Cynaroides</i>	<i>C. cataonica</i> Boiss. & Hausskn. ex Boiss. & Hausskn.	E	Kahramanmaraş	ISTE 102850
<i>Cynaroides</i>	<i>C. haradjianii</i> Wagenitz	E	Hatay	ISTE 102895
<i>Cynaroides</i>	<i>C. haussknechtii</i> Boiss.	E	Adana	ISTE 102872
<i>Cynaroides</i>	<i>C. regia</i> Boiss. var. <i>regia</i>		Mardin	ISTE 102890
<i>Cynaroides</i>	<i>C. sclerolepis</i> Boiss.	E	Mardin	ISTE 102891
<i>Cynaroides</i>	<i>C. spicata</i> Boiss.		Hatay	ISTE 102894
<i>Mesocentron</i> (Cass.) DC	<i>C. solstitialis</i> L.		Adana	ISTE 99174
<i>Microlophus</i> (Cass.) DC.	<i>C. behen</i> L.		Adana	ISTE 102871
<i>Microlophus</i>	<i>C. rigida</i> Banks & Sol.		Mardin	ISTE 102887
<i>Pseudophaeopappus</i> Wagenitz	<i>C. antitauri</i> Hayek	E	Hatay	ISTE 102869

TABLE 2. — Pappus morphological data of studied *Centaurea* taxa (values in mm; minimum, maximum, mean \pm standard deviation). Abbreviation: **sd.**, standard deviation.

Species	Color	Hair	Length (min-max, mean \pm sd.)	Length of inner row (min-max, mean \pm sd.)
<i>C. goksivriensis</i>	purplish	barbellate	6.28-7.29, 6.78 \pm 0.3	1.56-2.26, 1.92 \pm 0.18
<i>C. calcitrapa</i> subsp. <i>calcitrapa</i>	absent	absent	absent	absent
<i>C. calcitrapa</i> subsp. <i>cilicica</i>	absent	absent	absent	absent
<i>C. glastifolia</i>	whitish to straw	plumose	10.32-13.16, 11.88 \pm 0.84	absent
<i>C. amanicola</i>	brown	barbellate	7.14-9.65, 8.84 \pm 0.39	2.53-2.80, 2.67 \pm 0.08
<i>C. cataonica</i>	brown	barbellate	5.69-7.39, 6.62 \pm 0.45	2.08-2.70, 2.38 \pm 0.22
<i>C. haradjianii</i>	brown	barbellate	6.75-9.55, 8.56 \pm 0.87	1.63-2.50, 2.18 \pm 0.26
<i>C. haussknechtii</i>	brown	barbellate	8.58-9.38, 9.05 \pm 0.23	1.33-2.49, 2.13 \pm 0.32
<i>C. regia</i> var. <i>regia</i>	straw	barbellate	10.58-12.78, 11.38 \pm 0.59	6.07-7.45, 6.60 \pm 0.45
<i>C. sclerolepis</i>	straw	barbellate	9.38-11.49, 10.44 \pm 0.66	1.76-1.97, 1.88 \pm 0.07
<i>C. spicata</i>	tawny brown	barbellate	6.05-7.87, 7.07 \pm 0.58	1.75-2.20, 1.95 \pm 0.14
<i>C. solstitialis</i>	whitish	barbellate	2.21-2.68, 2.44 \pm 0.15	0.52-0.79, 0.66 \pm 0.07
<i>C. behen</i>	coppery	barbellate	6.81-8.98, 7.99 \pm 0.56	1.71-2.13, 1.91 \pm 0.13
<i>C. rigida</i>	pale brown	barbellate	3.51-4.33, 3.93 \pm 0.26	1.46-1.65, 1.54 \pm 0.06
<i>C. antitauri</i>	dark brown	barbellate	4.65-6.23, 5.46 \pm 0.42	1.31-1.74, 1.50 \pm 0.16

C. antitauri are endemic to Turkey. Studied taxa selected due to their limited information on cypselas micro-macro morphology and their limited distribution pattern to provide relevant information to contribute to solving taxonomic problems of the genus. This study aims to provide relevant characteristics of the cypselas micro-macromorphology and the data useful for solving taxonomic problems through scanning electron microscopy in the studied taxa.

MATERIAL AND METHODS

Mature cypselas of 15 *Centaurea* taxa were used in the analysis of cypselas morphological characteristics. The localities and herbarium accession numbers of the studied species were shown in Table 1. The specimens were stored at The Herbarium of the Faculty of Pharmacy (ISTE), in Istanbul University. Cypselas shape, minimum and maximum lengths, minimum and maximum widths, colour, and pappus' minimum and maximum lengths, and its colour were analysed using the

stereomicroscope. For the stereo microscope observations, at least 10 mature cypselas from at least 5 individuals were used for each taxa. The minimum, maximum and average values were determined, and standard deviations were calculated for each measurement. Besides, cypselas surface patterns were analyzed using SEM (Tables 2-3). Mature cypselas were fixed on stubs with carbon tape and coated with gold/palladium for 45 seconds using EmiTech SC7620 Sputter Coater for SEM micrographs. Cypselas micrographs were taken by the JEOL Neoscope JCM5000 Benchtop Scanning Electron Microscope, at 100-300 \times , 1000 \times , and 3000 \times magnification from the middle of the lateral sides of each sample (Figs 1-3). Terminology was adapted from Barthlott (1981), Roth (1977), Stearn (1992) and other studies on closely related groups for surface characterizations (Marzinek *et al.* 2008; Bona 2014, 2020).

Cypselas and pappus characters of the examined taxa coefficients of correlation were determined, and they were grouped using the clustering analysis method (UPGMA, dissimilarity, standardized variable). Twelve characters were selected to

TABLE 3. — Cypselas morphological data of studied *Centaurea* taxa (values in mm; minimum, maximum, mean ± standard deviation).

Species	Color	Shape	Length (min-max, mean±sd.)	Width (min-max, mean±sd.)	Indumentum	Surface	Boundaries	Cell shape	Centres
<i>C. goksivriensis</i>	straw to pale brown, indistinct denticulate margin	elliptic to obovoid	3.93-5.31 4.63 ± 0.44	1.79-2.11 1.94 ± 0.11	scarcely hairy	smooth	straight, thick and slightly raised	elongated	irregularly and slightly raised
<i>C. calcitrapa</i> subsp. <i>calcitrapa</i>	greenish-brown with dark spots, indistinct stripes and denticulate margin	elliptic	2.48-2.94 2.76 ± 0.14	1.21-1.42 1.33 ± 0.06	scarcely hairy	undulate-ruminate	wavy, thick and raised	elongated	slightly grooved, rugose
<i>C. calcitrapa</i> subsp. <i>cilicica</i>	greenish-brown with dark spots, indistinct stripes and cream denticulate margin	elliptic to obovoid	2.22-3.22 2.79 ± 0.29	1.24-1.59 1.47 ± 0.11	scarcely hairy	undulate-ruminate	straight, thin, slightly raised	elongated	irregularly raised, rugose
<i>C. glastifolia</i>	brown with dark spots, cream stripes and paler denticulate margin	elliptic to obovoid	5.95-6.94 6.32 ± 0.30	1.93-2.82 2.42 ± 0.23	glabrous	undulate	straight, thin and slightly grooved	elongated	flat, rugulose
<i>C. amanicola</i>	brown with distinct whitish stripes and margin	elliptic to obovoid	5.22-7.01 6.09 ± 0.5	2.53-2.97 2.75 ± 0.14	glabrous	undulate-ruminate	straight, thin, raised	elongated	raised, rugulose
<i>C. cataonica</i>	brown with distinct whitish stripes and margin	elliptic to obovoid	5.58-6.31 5.99 ± 0.23	2.85-3.26 3.07 ± 0.16	glabrous	smooth	straight, thin, slightly grooved	elongated	irregularly and slightly raised
<i>C. haradjianii</i>	straw to whitish	elliptic to obovoid	5.59-6.07 5.85 ± 0.14	2.39-3.53 3.12 ± 0.32	scarcely hairy (simple and glandular)	reticulate	wavy, thin, raised	elongated	raised, rugulose
<i>C. haussknechtii</i>	straw to whitish	elliptic to obovoid	5.24-5.89 5.59 ± 0.16	2.53-2.96 2.73 ± 0.12	glabrous	smooth	straight, thin, slightly raised	elongated	raised
<i>C. regia</i> var. <i>regia</i>	straw to whitish	narrowly obovoid to elliptic	8.49-9.95 9.13 ± 0.37	3.31-3.61 3.46 ± 0.11	glabrous	smooth-undulate	straight, thin, raised	elongated	raised, rugulose
<i>C. sclerolepis</i>	brown with paler stripes and whitish connection area	obovoid to elliptic	6.11-7.21 6.73 ± 0.31	2.92-3.49 3.16 ± 0.17	scarcely glandular-hairy	smooth-undulate	wavy, thin, slightly raised	elongated	almost flat, rugulose
<i>C. spicata</i>	straw to pale brown with paler stripes	obovoid to elliptic	4.54-5.76 5.20 ± 0.39	2.13-2.69 2.42 ± 0.19	glabrous	reticulate	wavy, thick, raised	elongated	grooved, undulate
<i>C. solstitialis</i>	straw to brown with dark spots	obovoid to elliptic	1.99-2.34 2.15 ± 0.11	1.01-1.11 1.06 ± 0.03	scarcely hairy	smooth	straight, thin, slightly grooved	elongated	slightly grooved
<i>C. behen</i>	straw to pale brown with the whitish connection area	obovoid to elliptic	4.44-5.96 5.00 ± 0.58	2.02-3.01 2.52 ± 0.33	glabrous	reticulate	straight, thick, grooved	elongated	raised, grooved
<i>C. rigida</i>	brown with distinct whitish stripes and whitish connection area	obovoid to elliptic	3.98-5.06 4.58 ± 0.36	2.04-2.34 2.18 ± 0.10	scarcely hairy	smooth	straight, thin and slightly grooved	elongated	slightly grooved
<i>C. antitauri</i>	dark brown with distinct whitish stripes and whitish connection area	oblong to obovoid	5.88-6.67 6.32 ± 0.27	2.87-3.73 3.22 ± 0.25	glabrous	undulate	wavy, thick, grooved	elongated	raised, rugose

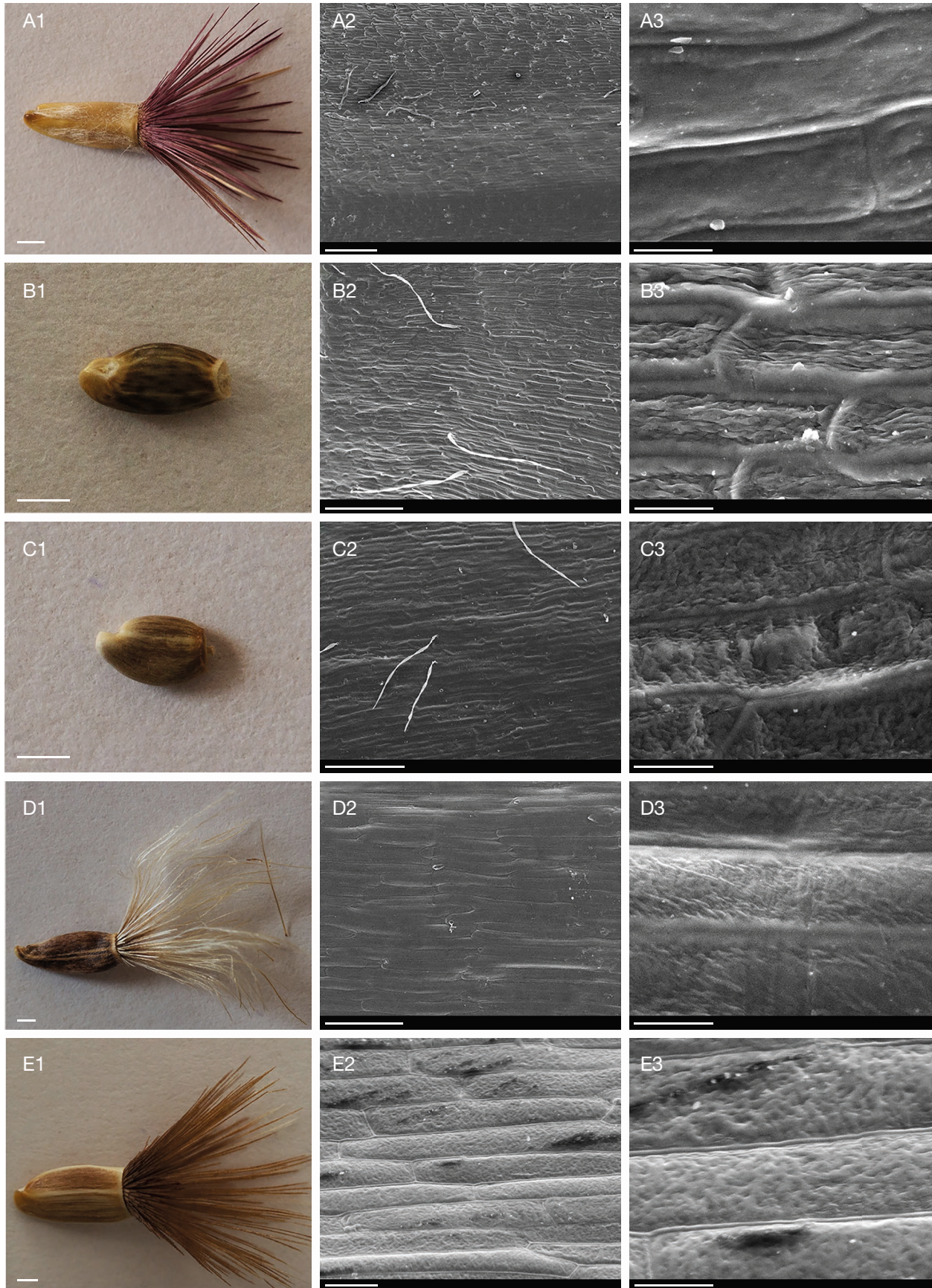


FIG. 1. — SEM micrographs of cypselas of *C. goksivriensis* M. Bona (**A**), *C. calcitrapa* subsp. *calcitrapa* L. (**B**), *C. calcitrapa* subsp. *cilicica* (Boiss. & Bal.) Wagenitz (**C**), *C. glastifolia* L. (**D**), *C. amanicola* Hub.-Mor. (**E**). **A1, B1, C1, D1, E1**, general morphology of cypselas by LM; **A2, A3, B2, B3, C2, C3, D2, D3, E2, E3**, cypselas surfaces by SEM. Scale bars: A1-E1, 1 mm; A2, 200 µm; B2-D2, 100 µm; E2, 20 µm; A3-E3, 10 µm.

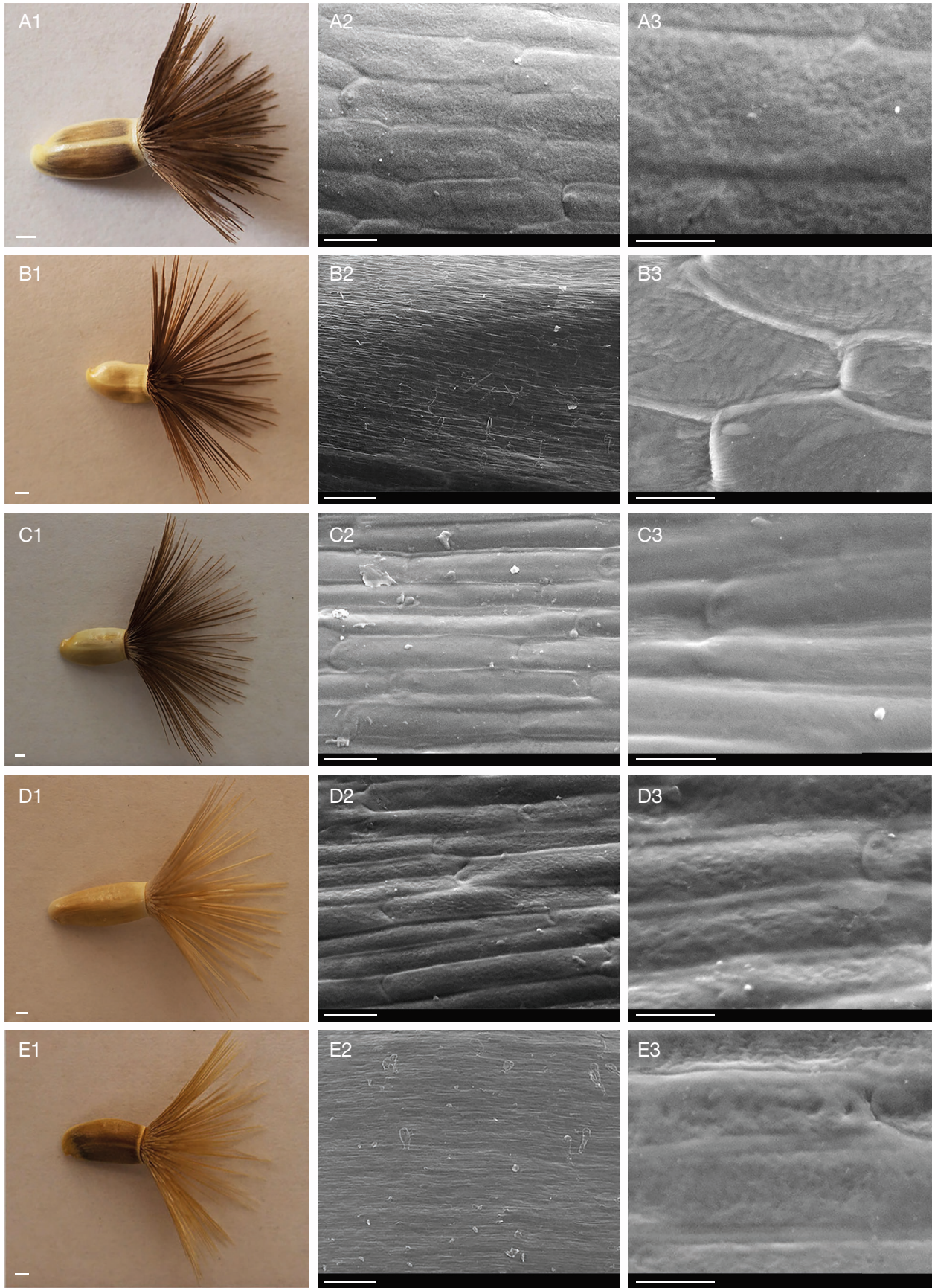


FIG. 2. — SEM micrographs of cypselas of *C. cataonica* Boiss. & Hausskn. *in* Boiss. (A), *C. haradjianii* Wagenitz (B), *C. haussknechtii* Boiss. (C), *C. regia* Boiss. var. *regia* (D), *C. sclerolepis* Boiss. (E). A1, B1, C1, D1, E1, general morphology of cypselas by LM; A2, A3, B2, B3, C2, C3, D2, D3, E2, E3, cypselas surfaces by SEM. Scale bars: A1-E1, 1 mm; A2, C2, D2, 20 µm; B2, E2, 200 µm; A3-E3, 10 µm.

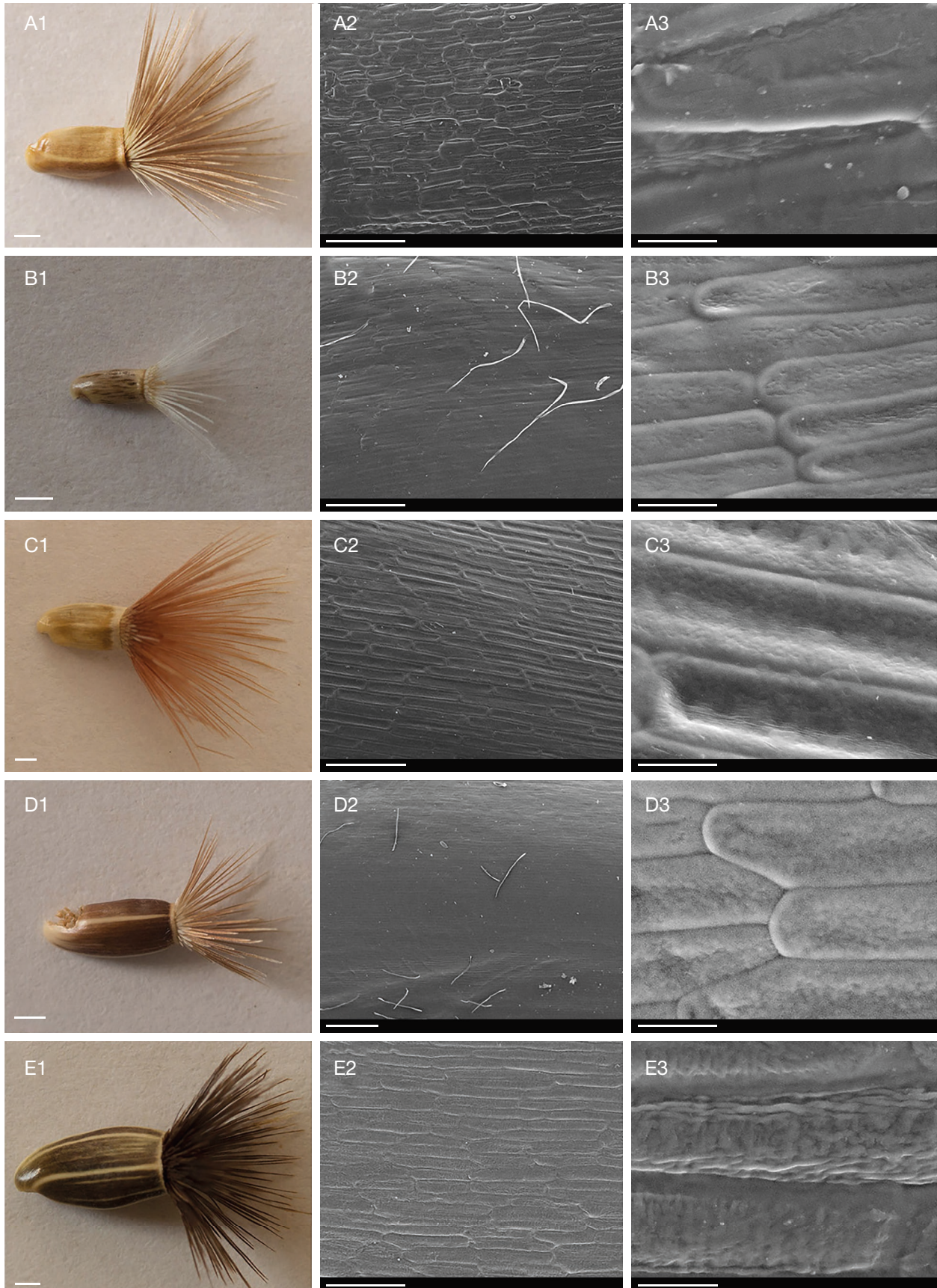


FIG. 3. — SEM micrographs of cypselas of *C. spicata* Boiss. (A), *C. solstitialis* L. (B), *C. behen* L. (C), *C. rigida* Banks & Sol. (D), *C. antitauri* Hayek (E). A1, B1, C1, D1, E1, general morphology of cypselas by LM; A2, A3, B2, B3, C2, C3, D2, D3, E2, E3, cypselas surfaces by SEM. Scaler bars: A1-E1, 1 mm; A2-C2, E2, 100 µm; D2, 200 µm; A3-E3, 10 µm.

TABLE 4. — Morphological character states of examined *Centaurea* taxa.

Taxa/character	Pappus existence	Pappus color	Hair	Pappus length	Inner row existence	Achene pattern	Achene color	Achene length	Achene width	Achene indumentum	Achene surface	Boundaries
<i>C. goksivriensis</i>	1	0	1	1	1	0	1	1	0	1	0	0
<i>C. calcitrapa</i> subsp. <i>calcitrapa</i>	0	0	0	0	0	1	1	0	0	1	1	1
<i>C. calcitrapa</i> subsp. <i>cilicica</i>	0	0	0	0	0	1	1	0	0	1	1	0
<i>C. glastifolia</i>	1	1	0	1	0	1	1	1	1	0	1	0
<i>C. amanicola</i>	1	1	1	1	1	1	1	1	1	0	1	0
<i>C. cataonica</i>	1	1	1	1	1	1	1	1	1	0	0	0
<i>C. haradjianii</i>	1	1	1	1	1	0	0	1	1	1	1	1
<i>C. haussknechtii</i>	1	1	1	1	1	0	0	1	1	0	0	0
<i>C. regia</i>	1	1	1	1	1	0	0	1	1	0	1	0
<i>C. sclerolepis</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>C. spicata</i>	1	1	1	1	1	1	1	1	1	0	1	1
<i>C. solstitialis</i>	1	0	1	0	1	1	0	0	1	0	0	0
<i>C. behen</i>	1	0	1	1	1	0	1	1	1	0	1	0
<i>C. rigida</i>	1	1	1	0	1	1	1	1	1	1	0	0
<i>C. antitauri</i>	1	1	1	0	1	1	1	1	1	0	1	1

distinguish the studied *Centaurea* taxa and analyzed together (Fig. 4). Morphological character states and their values are given in Table 4. The unweighted pair group method uses arithmetic averages (UPGMA) (Mohammadi & Prasanna 2003). NTSYSpc version 2.1 was used for the analysis.

RESULTS

According to analyses by LM and SEM, many differences were observed among the selected species. Differences are mainly in pappus length, colour, structure and the presence of dense inner row, cypsela shape, size, colour, and surface patterns given in Tables 2, 3. The results are discussed below, followed by a key prepared based on the cypsela characteristics.

SECT. ACROCENTRON

C. goksivriensis characterized by purplish, barbellate and 6.78 ± 0.3 mm long pappus. The cypselas were elliptic to obovoid, 4.63 ± 0.44 mm long, straw to pale brown with a scarcely hairy and smooth surface. The boundaries were straight, thick and slightly raised; cell shape is elongated, and cell centres are irregularly and slightly raised.

SECT. CALCITRAPA

C. calcitrapa was characterized by the absence of pappus. The cypselas were elliptic with scarcely hairy and undulate-ruminate surface. The cypselas were 2.76 ± 0.14 mm long in *C. calcitrapa* subsp. *calcitrapa*. The boundaries were wavy, thick and raised, cell shape is elongated, and cell centres are slightly grooved, rugose. The cypselas were 2.79 ± 0.29 mm long in *C. calcitrapa* subsp. *cilicica*. The boundaries are straight, thin, slightly raised, cell shape is elongated, and cell centres are irregularly raised, rugose.

SECT. CHARTOLEPIS

C. glastifolia characterized by whitish to straw, plumose, 11.88 ± 0.84 mm long pappus, which was longer than 6.32 ± 0.30 mm long cypsela. The cypsela is brown with dark spots, cream stripes, elliptic, glabrous and undulate surface patterns. The boundaries were straight, thin and slightly grooved, cell shape was elongated and cell centres are flat and rugulose.

SECT. CYNAROIDES

In this section, seven closely related species have been investigated. The pappus in all investigated taxa of *C. amanicola*, *C. cataonica*, *C. haradjianii*, *C. haussknechtii*, *C. regia* var. *regia*, *C. sclerolepis*, and *C. spicata* was in ray form, barbellate, and longer than cypsela, between 5.69 and 12.78 mm long in two rows. The pappus colour in *C. regia* var. *regia* and *C. sclerolepis* was straw, and the rest of the members of the section was brown. The cypselas were brown with whitish stripes in *C. amanicola* and *C. cataonica*. In *C. sclerolepis* and *C. spicata*, cypsela colour is pale brown with even paler stripes. In *C. haradjianii*, *C. haussknechtii* and *C. regia* var. *regia* the cypselas were straw to whitish. Generally, the cypselas were between 4.54 and 9.95 mm in the sect. *Cynaroides*. In *C. haradjianii* and *C. sclerolepis*, the cypselas were scarcely hairy and the rest of them were glabrous. The surface patterns were in a wide range of diversity from undulate-ruminate, reticulate, and undulate to smooth. The boundaries are straight to wavy, thin and slightly grooved. The cell shapes were elongated and the centres are generally raised and rugulose.

SECT. MESOCENTRON

In *C. solstitialis* pappus was whitish, barbellate 2.44 ± 0.15 mm long, almost equal or slightly longer than cypsela. The cypselas were straw to brown with dark spots, 2.15 ± 0.11 mm long, scarcely hairy, smooth surface, obovoid to elliptic. The boundaries were straight thin, slightly grooved, cell shapes were elongated and cell centres are slightly grooved.

SECT. MICROLOPHUS

In this section, we have investigated two species, namely *C. behen*, *C. rigida*. *C. behen* was characterized by coppery coloured barbellate, 7.99 ± 0.56 mm long pappus, which was longer than 5.00 ± 0.58 mm long cypsela. The cypselas were straw to pale brown, obovoid to elliptic, glabrous and have reticulate surface patterns. The boundaries were straight, thick, grooved, cell shapes were elongated and cell centres were raised and grooved. *C. rigida* was characterized by pale brown, barbellate, 3.93 ± 0.26 mm long pappus, which was shorter than 4.58 ± 0.36 mm long cypsela. The cypselas were brown with whitish stripes, obovoid to elliptic, scarcely hairy and have smooth surface patterns. The boundaries are straight, thin and slightly grooved, cell shape is elongated and centres are slightly grooved.

SECT. PSEUDOPHAEOPAPPUS

C. antitauri was represented by dark brown, barbellate, 5.46 ± 0.42 mm long pappus, which was shorter than 6.32 ± 0.27 mm long cypsela. The cypselas were dark brown with whitish

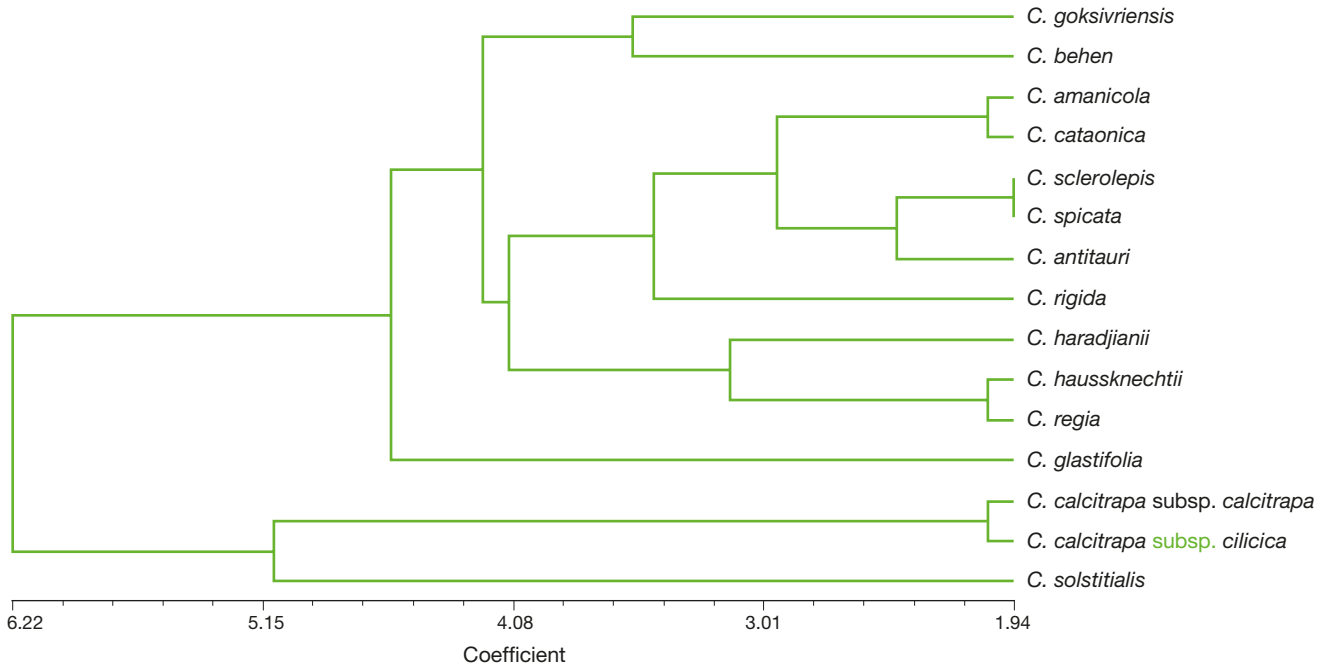


FIG. 4. — The UPGMA dendrogram shows dissimilarity distance of the examined *Centaurea* L. taxa according to combined data of cypselae and pappus morphology.

stripes, oblong to obovoid, glabrous and undulate surface patterns. The boundaries are wavy, thick, grooved, cell shape is elongated and centres are raised and rugose.

DISCUSSION

Previous studies exposed scanning electron microscopy (SEM) as an important tool to identify the micromorphological characteristics for various plant genera (Candan *et al.* 2016; Özüdođru *et al.* 2016; Şirin *et al.* 2017, 2021; Gavrilović *et al.* 2019a, b; Karaismailođlu & Güner 2019; Bona 2020; Erođlu *et al.* 2021; Gavrilović & Janaćković 2022). Cypselae morphology provides diagnostic characters owing to the variation seen in specific and generic levels, therefore, cypselae morphology can provide distinctive data at specific levels (Bona 2014, 2015; Özcan 2018).

According to Herrando-Moraira *et al.* (2019), the cypselae of the subtribe Centaureinae are obovoid, laterally compressed, usually glabrous and smooth, less often hirsute, rugose, pitted or ridged. The pappus is inserted on a ring in the apical plate, biseriate, outer series formed by several rows of long, generally differently pinnulate bristles, basally connate in a ring or free, rarely deciduous, often persistent, sometimes reduced (Herrando-Moraira *et al.* 2019). In our study, the studied taxa have elliptic to obovoid, glabrous or scarcely hairy cypselae.

In our study, cypselae sizes are between 1.99 and 9.95 mm long, the colour of the pappus is straw-coloured, brown, or whitish, barbellate but *C. goksivriensis* is distinguished by its purple pappus and *C. glastifolia* by its plumose pappus.

Cypselae micromorphology of *C. goksivriensis* is investigated for the first time here and macromorphological results are coherent with its description (Bona 2015).

The differences between the examined species are distinct such as *C. calcitrapa* subsp. *calcitrapa* and *C. calcitrapa* subsp. *cilicica* (sect. *Calcitrapa*), which are the only taxa that do not have the pappus. It has been reported that *C. calcitrapa* subsp. *calcitrapa* has 2.7–3.5 mm long, greyish, glabrous, cypselae (Shamso *et al.* 2021). Our SEM results show that *C. calcitrapa* subsp. *calcitrapa* has scarcely hairy and greenish-brown dark-spotted cypselae.

Our results confirm Wagenitz (1975) that *C. glastifolia* has 6 mm long cypselae with 11–13 mm, plumose pappus.

C. amanicola, *C. cataonica* (sect. *Cynaroides*), *C. rigida* (sect. *Microlophus*) and *C. antitauri* (sect. *Pseudophaeopappus*) have a similar appearance by their brown cypselae with distinct whitish stripes and they grouped with other members of the sect. *Cynaroides* (Fig. 4). *C. rigida* differs from *C. amanicola*, *C. cataonica* and *C. antitauri* by its scarcely hairy cypselae surface and paler brown pappus, which is shorter than cypselae. *C. cataonica* differs from *C. amanicola* and *C. antitauri* by smooth cypselae surface pattern. *C. antitauri* differs from *C. amanicola* by thick boundaries. *C. behen* has pappus in coppery colour. *C. solstitialis* (sect. *Mesocentron*) has pale brown cypselae with dark brown spots and whitish pappus.

It has been recorded that *C. amanicola* has oblong, 5.5–6 mm long, cypselae and scabrous, whitish or brownish, 8–9 mm long pappus (Negaresh & Rahiminejad 2018). Cypselae 6 mm; pappus 8–9 mm (Wagenitz 1975). Our results partly confirm previous studies with 5.22–7.01 mm long cypselae and 7.14–9.65 mm long pappus but pappus is barbellate.

Cypselae of *C. cataonica* are oblong, *c.* 4.5–5 mm long, *c.* 2.5 mm wide, smooth and shiny, brown or silver-bronze, glabrous with scabrous, brown to dark brown, *c.* 7–7.5 mm long pappus, which has *c.* 1.5 mm long inner

KEY TO THE STUDIED TAXA ACCORDING TO CYPSELA CHARACTERISTICS

1. Pappus absent	2
— Pappus present	3
2. Boundaries wavy, thick	<i>C. calcitrapa</i> subsp. <i>calcitrapa</i> L.
— Boundaries straight, thin	<i>C. calcitrapa</i> subsp. <i>cilicica</i> (Boiss. & Bal.) Wagenitz
3. Pappus whitish, straw	4
— Pappus brown, purplish, coppery	7
4. Pappus plumose	<i>C. glastifolia</i> L.
— Pappus barbellate	5
5. Cypsela surface glabrous	<i>C. regia</i> Boiss. var. <i>regia</i>
— Cypsela surface scarcely hairy	6
6. Cypsela surface hairs glandular	<i>C. sclerolepis</i> Boiss.
— Cypsela surface hairs not glandular	<i>C. solstitialis</i> L.
7. Pappus purplish	<i>C. goksivriensis</i> M. Bona
— Pappus brownish or coppery	8
8. Cypsela brown with cream stripes	9
— Cypsela straw to whitish with no stripes	12
9. Cypsela surface smooth	10
— Cypsela surface not smooth	11
10. Cypsela glabrous	<i>C. cataonica</i> Boiss. & Hausskn. in Boiss.
— Cypsela scarcely hairy	<i>C. rigida</i> Banks & Sol.
11. Cypsela shorter than pappus	<i>C. antitauri</i> Hayek
— Cypsela longer than pappus	<i>C. amanicola</i> Hub.-Mor.
12. Pappus coppery in colour	<i>C. beben</i> L.
— Pappus colour brownish	13
13. Cypsela scarcely hairy	<i>C. haradjianii</i> Wagenitz
— Cypsela glabrous	14
14. Cypsela surface smooth	<i>C. haussknechtii</i> Boiss.
— Cypsela surface reticulate	<i>C. spicata</i> Boiss.

rows (Negaresh & Rahiminejad 2018). Our *C. cataonica* samples have slightly bigger cypsela (5.58-6.31 × 2.85-3.26 mm) and barbellate pappus.

C. haradjianii has oblong, *c.* 5 mm long, brown, glabrescent cypsela and scabrous, brown or whitish, *c.* 10 mm long pappus with *c.* 2 mm long inner rows (Negaresh & Rahiminejad 2018). Pappus *c.* 8 mm (Wagenitz 1975). Cypsela and pappus length are coherent with our results but cypsela is scarcely hairy with barbellate pappus.

According to previous studies, cypselas and pappus of *C. haussknechtii* are unknown (Wagenitz 1975; Negaresh & Rahiminejad 2018) Cypsela micro and macromorphology of *C. haussknechtii* is investigated for the first time here.

Cypselas oblong, 6-7.5 mm long, 3-4 mm wide, white, or brown, glabrous; pappus scabrous, whitish, purplish or brownish, 10-13-15) mm long, bristles of inner rows 2-5 mm long in *C. regia* var. *regia* according to Negaresh & Rahiminejad (2018) and according to Aslan *et al.* (2010) cypselas 6-9 × 3-4 mm with barbellate, straw-coloured to brownish, 10-13

(-15) mm, inner 2-5 mm long pappus. Our specimens have 8.49-9.95 long, 3.31-3.61 mm wide straw to whitish, glabrous cypselas with barbellate, straw-coloured, 10.58-12.78 mm inner row 6.07-7.45 mm long pappus.

Three different cypsela lengths were reported for *C. sclerolepis* in previous studies: 4-5 mm (Negaresh & Rahiminejad 2018), 8-9 mm (Adwan *et al.* 2020) and 7 mm long (Wagenitz 1975). Our results are similar to Wagenitz (1975) with 6.11-7.21 mm long cypsela. Negaresh & Rahiminejad (2018) also reported that *C. sclerolepis* has glabrous cypsela with scabrous pappus which is different from our samples that have scarcely hairy cypsela with barbellate pappus.

C. spicata has oblong, 4-5 mm long, *c.* 2-2.2 mm wide, smooth, pale brown or whitish cypselas and scabrous, brown to whitish, sometimes dark brown, (6-)7-9 mm long pappus with *c.* 1.5 mm long inner row (Wagenitz 1975; Negaresh & Rahiminejad 2018). Our results also confirm this information about *C. spicata* except for the barbellate pappus of our specimen.

C. solstitialis has 2.5 mm cream with black spot oblong cypselas with 4.6 mm, white, scabrous pappus (Rakizadeh *et al.* 2019). *C. solstitialis* has creamy-brown, elliptic-oblong, 2.7-3.2 mm long cypselas and bright white pappus (Adwan *et al.* 2020). Cypselas smooth, 1.21-2.25 mm, oblong, glabrous; pappus 3.13-5.26 mm, scabrous (Özcan & Akıncı 2019). Cypselas 2-3 mm, dimorphic: marginal dull, blackish, without pappus; central glossy, greyish to brown, with white pappus, 3-4(-5) mm (Wagenitz 1975). Our results are coherent with previous studies, except for the barbellate pappus of *C. solstitialis*.

Macromorphological features of the cypselas of *C. behen* have been reported as cypselas oblong, brownish, 4.5-6.0 mm long, glabrous and pappus scabrous, whitish or brownish, the outer 5-8 mm long, the inner 1.6-2.0 mm long (Negaresh & Rahiminejad 2015), cypselas 4.5 mm, cream, oblong; pappus 6.5 mm, white, scabrous by (Rakizadeh *et al.* 2019), cypselas 4.5-5 mm long, obovate and creamy; pappus grey by (Adwan *et al.* 2020) and cypselas *c.* 5 mm; pappus 5-8 mm by Wagenitz (1975). Our findings for cypselas and pappus length of *C. behen* are similar to previous studies. Our results differ from Negaresh & Rahiminejad (2015) and Rakizadeh *et al.* (2019) by obovoid to elliptic cypselas with barbellate pappus.

Cypselas of *C. rigida* 4-5 mm long, yellowish or brownish, sparsely hairy and pappus scabrous, whitish, the outer 4-5 mm long, the inner 1-2 mm long according to Negaresh & Rahiminejad (2015) and cypselas 3-3.5 mm long mm, lanceolate-oblong, red; pappus white according to Adwan *et al.* (2020) and cypselas *c.* 5 mm; pappus (3-)-4-5 mm according to Wagenitz (1975). Our findings differ from Negaresh & Rahiminejad (2015) with barbellate and pale brown pappus and differ from Adwan *et al.* (2020) with obovoid to elliptic, brown with distinct whitish stripes cypselas and pale brown pappus.

Wagenitz (1975) stated that *C. antitauri* has 5.5-7.7 mm long cypselas and 7.5-8.5 mm, brown, inner row 1.5 mm long pappus. We found slightly shorter, 4.65-6.23 mm long pappus for *C. antitauri*.

Cypselas characteristics of 15 *Centaurea* species belonging to sections *Acrocentron*, *Calcitrapa*, *Chartolepis*, *Cynaroides*, *Mesocentron*, *Microlophus*, and *Pseudophaeopappus*, were investigated in this study. Eight of them are endemic to Turkey. Cypselas micromorphology of *C. goksivriensis*, *C. calcitrapa* subsp. *calcitrapa*, *C. calcitrapa* subsp. *cilicica*, *C. cataonica*, *C. haradjianii*, *C. haussknechtii*, *C. regia* var. *regia*, *C. sclerolepis*, *C. spicata*, *C. amanicola*, *C. rigida*, *C. behen*, and *C. antitauri* were examined for the first time in this study.

The present study shows that shape, size colour and surface structure of cypselas, and length, colour, and structure features of pappus provide significant useful characteristics to distinguish species and subspecies within the genus. Cypselas characteristics with SEM investigation can be a useful tool that helps identification in the genus. The potential systematic importance of the characteristics shown here is specified by the richness of the variation observed in the limited sample of species. Hopefully, this analysis will encourage new research on the cypselas morphology of this genus to elucidate the complex taxonomy of the *Centaurea* further.

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