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Achene and Flower Characteristics of Some *Centaurea* Species Collected from Diyarbakır (Türkiye)

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Abstract
In this study, belonging to the genus *Centaurea* L. collected from Diyarbakır and its surroundings were investigated flower structures and the achene micro and macro morphology of 10 species [*C. solstitialis* L., *C. iberica* Trev. Ex Sprengel, *C. urvillei* ssp *nimrodis* (Boiss. & Hausskn.) Wagenitz, *C. consanguinea* DC. (E), *C. behen* L, *C. rigida* Banks & Sol., *C. balsamita* Lam., *C. kurdica* Reichardt (E), *C. stapfiana* (Hand.-Mazz.) Wagenitz, *C. virgata* Lam.]. Among the ten species studied, five have yellow flowers *C. solstitialis*, *C. behen*, *C. rigida*, *C. balsamita*, *C. stapfiana* and the other five *C. iberica*, *C. urvillei* ssp *nimrodis*, *C. consanguinea*, *C. kurdica*, *C. stapfiana*, *C. virgata* have pink-purple flowers. It has been found that phyllaries on the involucrum bearing the flowers are typically characterized by horn-like spines or protrusions. As it is well-established that seed and fruit structures are key features used in taxonomy, achene and pappus structures were examined in this study, and measurements were taken with a digital caliper. The results of measurement and SEM imaging reveal that the macro structure of achene differs significantly from its micro structure. This suggests that traits are of great importance and can be used in taxonomic diagnosis.

Research Article

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1. Introduction

Centaurea L. is the third most diverse genus of the Asteraceae family in terms of species diversity (Davis, 1975). According to the List of Plants of Türkiye, the genus *Centaurea* is represented by 194 taxa in Türkiye, while this number has reached 221 with the recently added new species and records (Uysal, 2012; Yıldırımlı, 2018; Negaresh and Rahiminejad, 2018; Duman et al., 2021, Özbek, 2021; Şirin et al., 2022; Uysal, 2022). Of these taxa, 133 are endemic and the endemism rate of the genus for Türkiye is 60 %. The size of this species creates significant confusion in taxonomy, and therefore serious problems are encountered in determining species (Susanna and Garcia-Jacas, 2007). Its natural vegetation cover is steppe and 33 % of its territory is covered with forest and heathland, 40 % with cultivated land, and 22 % with meadows and pastures. A protection decision was taken for *Ajuga xylorrhiza* in Çermik (Ertekin, 2013) and *Rosularia blepharophylla* (Hoşgören, 2018) endemic plants growing in Ergani. *Hesperis hedgei*, *Lathyrus trachycarpus*, *Paracaryum kurdistanicum, and Onobrychis silvanensis* are among other endemic plants identified in Diyarbakır (Ertekin, 2002). *C. stapfiana*, *C. urvillei* ssp *nimrodis*, *C. kurdica* and *C. consanguinea* species, which are the main materials of this study, are also endemic to the region (Güner et al., 2012).

Due to its rich species diversity, it presents major challenges when it comes to species identification (Ranjbar et al., 2013). Therefore, numerous studies on morphological, anatomical (Bona and Aras, 2008; Köse et al., 2010; Çelik et al., 2008), palynological (Erkara et al., 2012; Pehlivan, 1995), cytological (Ghaffari, 1999; Gömürgen and Adıgüzel, 2001), and ecological characteristics (Türkoğlu et al., 2009; Çelik et al., 2005) of the genus *Centaurea* L. have been conducted by many researchers for many years. However, it should not be forgotten that fruit and seed characteristics are important not only in the genus *Centaurea* but also in all plant groups (Öztürk, 2020; Dumanoğlu, 2021).

The genus *Centaurea* L. is characterized by a capitulum-shaped flower. It has flowers in white, yellow, pink or purple. The hairy or spiny phyllaries they carry are usually arranged in several rows, usually with spiny or mucronate tips and pinnate margins. Achene-type fruits possess types of hairy pappus that facilitate the transportation of seeds by wind (Chuang and Ornduff, 1992). Achenes have a variety of shapes, sizes, patterns, and surface differentiation that are used to classify taxa (Roque and Funk, 2013; Özcan and Akıncı, 2019). These microstructural details of achenes enable taxa to be differentiated and their level of phylogenetic to be revealed. This practice is particularly suitable for families whose taxa are complex to identify (Bona, 2014). Hence, evaluation of fruit and seed traits has been deemed necessary by many researchers in recent years (Shabestari, 2013). It is particularly in recent years that studies of this type have taken on greater importance and have reached micromorphological depths. This necessity prompted us to undertake such a study (Köstekçi and Arabacı, 2015).

Despite the stereomicroscope alone being used for the traditional identification of plant species, some researchers disagree and rely on micro morphological characteristics to confirm the identification. This is because parameters related to fruit and seed micro-structure are of great importance in the differentiation and evolutionary identification of taxa (Candan et al., 2016). Therefore, in this study, both stereomicroscopy and scanning electron microscopy were used for imaging. Because, while only morphological distinctions are observed in stereomicroscopic examinations of seeds, precise determination of species is made in electron microscopy examinations. Thus, erroneous or suspicious determinations are avoided.

2. Materials and Methods

Plant samples were collected from their natural habitats in Diyarbakır and surrounding roads during the flowering and ripe fruit periods. The collected samples were meticulously dried using herbarium techniques and preserved in the Botanical Herbarium

(DUF) of the Department of Biology, Dicle University (Table 1). The flowers were photographed using a Nikon d7100 body and 18x140 lens. Meanwhile, Achen samples were imaged with a Leica MC179 HD camera and an FEI QUANTA 250 electron microscope connected to a Leica Z16 APO stereomicroscope.

Diyarbakır, the study area, is a city situated in the Southeastern Anatolia Region of Turkey, around a high plateau along the banks of the Tigris River. In the city, where the continental climate prevails, summers are extremely hot and dry, while winters are cold, harsh, and rainy. Steppe, the natural vegetation of the Southeastern Anatolia Region, is also dominant in Diyarbakır (Figure 1).

Table 1. Localities where the species were collected

Plant name	Herbarium number	Locality
C. balsamita	6999	Dicle University-Campus
C. behen	6700	Diyarbakır-Silvan 15. km
C. consanguinea	6998	Diyarbakır-Eğil
C. iberica	6701	Diyarbakır-Bismil 32. km
C. kurdica	6996	Diyarbakır-Çınar 20. km
C. rigida	6702	Silvan-Diyarbakır 41. km
C. solstitialis	6703	Ergani-Çermik 25. km.
C. stapfiana	6997	Ergani
C. urvillei ssp. nimrodis	6704	Ergani-Mountain Ziyaret
C. virgata	6705	Çermik-Sinek stream

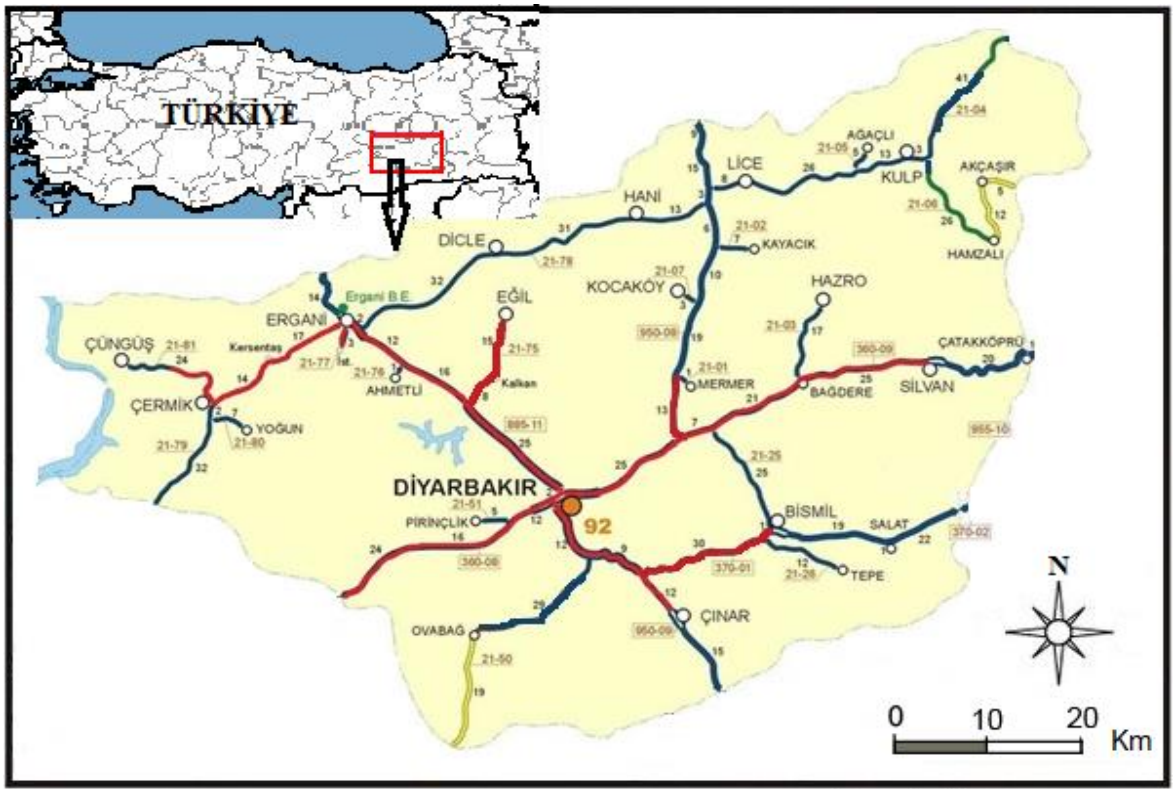


Figure 1. Diyarbakır working area map

The province covers an area of 15.168 km^2 and is 660 meters above sea level. In the city where the continental climate prevails, summers are extremely hot and dry, while winters are cold, harsh, and rainy (www.diyarbakir.bel.tr.).

3. Result and Discussion

3.1. Flowers

The inflorescence is capitulum-shaped, characteristic of the family. Capitulum is the inflorescence bearing numerous sessile flowers on a swollen main axis (Figure 2). It has been observed that the phyllaries of *Centaurea* species end with horn-like protrusions in the form of spines.

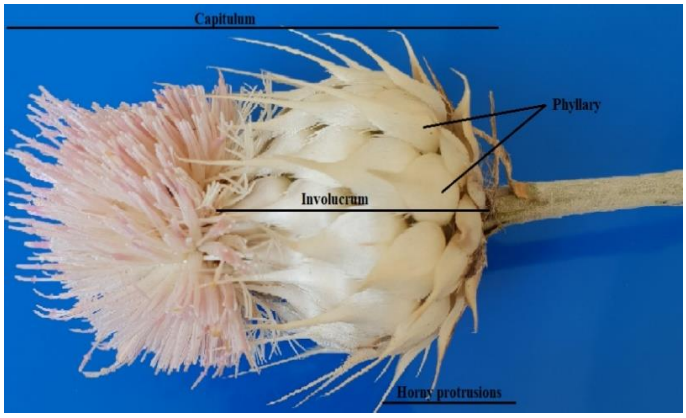


Figure 2. Flower structure of the genus

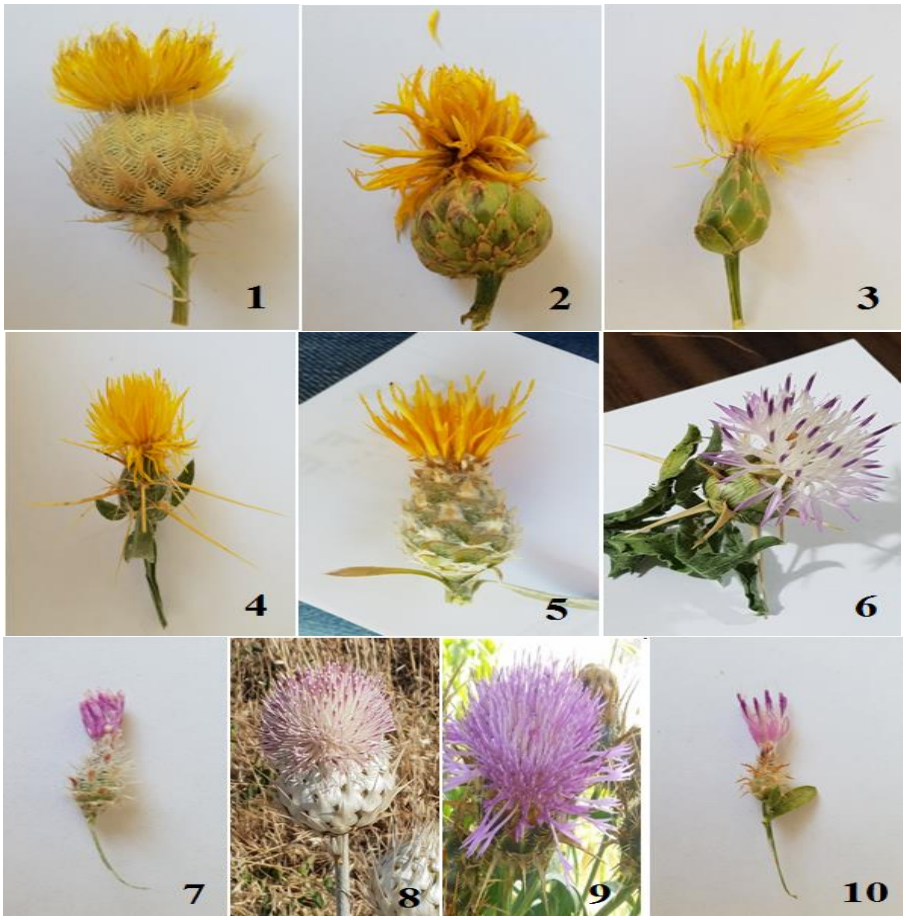


Figure 3. Flowers of 1-*C. balsamita*, 2-*C. behen*, 3-*C. rigida*, 4-*C. solstitialis*, 5-*C. stapfiana* yellow, 6-*C. iberica*, 7-*C. consanguinea*, 8-*C. kurdica*, 9-*C. urvillei* ssp. *nimrodis*, 10-*C. virgata* species have been shown to be pink-purple in color

3.2. Stereomicroscope images of achenes

C. balsamita Lam*:* The phyllaries on the bulging involucrum-bearing yellow flowers are surrounded by stiff, beard-like stinging hairs. The brown-colored achene is decorated with black lines. The pappus is composed of sparse, shiny, and thin to long hairs. The hilum bears a small red additional structure belonging to the calyx called the appendage. This appendage is significant for species identification (Figure 4.1).

C. behen L.: The phyllaries on the yellow-flowered bulging involucrum are pyramids without spines and green in color. Achene is yellowish-white in color and hairless. The pappus bears numerous, long, and soft broom-like bristles. The hilum is in the center and at the base (Figure 4.2).

C. consanguinea DC.: It is endemic. It has a long thin involucrum and pink-purple flowers. The thornless phyllaries are surrounded by brown and soft-textured hairs. The achene is swollen, brown with yellow stripes, and obtuse. The outer papillae are denser and thinner than the inner ones. The hilum is on one side and is small (Figure 4.3).

C. iberica Trev. Ex Sprengel: It bears white-purple flowers. The phyllaries end in thick and long spines that hang outwards. Achene is creamy-yellow with brown spots, obtuse. The pappus is sparse and consists of linear hairs. The hilum is curved inwards (Figure 4.4).

C. kurdica Reichardt: It is an endemic species. The flowers are pink-purple, sometimes white. The bright white involucrum is very large and bears hard, white spines. Achene and papus, brown-yellow, obtuse. The hilum is close to one side (4.5).

C. rigida Banks & Sol.: Its general appearance is very similar to the *C. behen* species. *C. rigida* differs in that the flower-bearing involucrum is smaller and cylindrical. Fillaries, as in *C. behen*, also do not bear thorns or hairs. Achene is very small, brown-red, and densely hairy. Pappus shows a very sparse structure (Figure 4.6).

C. solstitialis L.: Flowers are yellow. Phyllaries bear long, stiff spines. The base of the spines is surrounded by stiff hairs. The achene is brown-yellow in color and hairless. The outer pappus hairs are yellow compared to the inner ones. The hilum is located on one side (Figure 4.7).

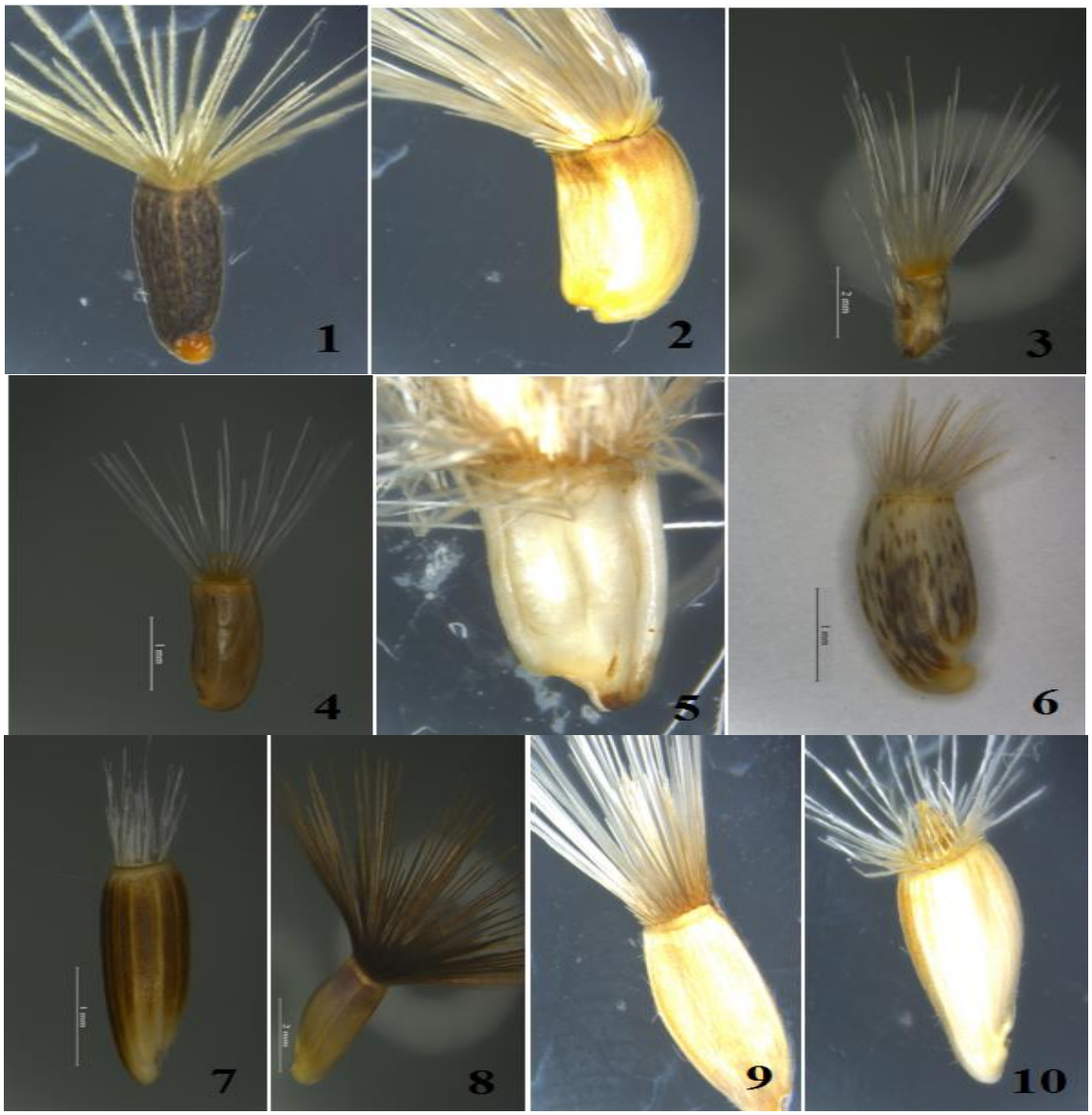


Figure 4. Stereomicroscope microphotographs of achenes: 1-*C. balsamita*, 2-*C. behen*, 3-*C. rigida*, 4-*C. solstitialis*, 5-*C. stapfiana*, 6-*C. iberica*, 7-*C. consanguinea*, 8-*C. kurdica*, 9-*C. urville* issp. *nimrodis*, 10-*C. virgate*

C. stapfiana (Hand.-Mazz.) Wagenitz: It is an endemic species. The flowers are yellow and large. The phyllaries are hard but not spiny. Achenes are yellowish-white, glabrous, and rugose. Pappus are very long. The hilum is located on one edge of the protruding base. The pappus need to be shortened to obtain images when they are long enough to cover the achenes (Figure 4.8).

C. urvillei ssp. *nimrodis* (Boiss. & Hausskn.) Wagenitz: It is endemic. The flowers are pink, purplish red, or whitish. The phyllaries have large hard spines at the ends and hard stinging hairs at the base of these spines. Achenes are yellowish-white, densely hairy, obtuse. The pappus is white and elongated. The hilum is on one side and surrounded by hairs (Figure 4.9).

C. virgata Lam.: It is very similar to the *C. consanguinea* species with its pinkish-purple flowers and involucrum structure. However, the species is distinguished by a more rigid and outwardly curved phyllaries. The achene is yellowish-white with indistinct stripes, obtuse. Pappus hairs are sparse and very short. The hilum is on the side (Figure 4.10).

The achene characteristics of the species were examined and measurements were taken using a digital caliper with 5 samples each. The arithmetic averages of the measurements were calculated and the results were shown in the table (Table 2).

Table 2. Fruit measurements of species

Species	Achene				Pappus	Appendage
	Lenght	Width	Colour	Shape		
C. balsamita	4.38	1.03	Brown-black	Oblong	4.39	+
C. behen	4.60	2.06	Yellowish white	Ovoid-Oblong	6.69	-
C. consanguinea	1.45	0.7	Yellow stripes on brown	Obovoid	3.47	-
C. iberica	3.42	1.80	Mottled brown on creamy yellow	Ovoid-Oblong	1.42	-
C. kurdica	6.88	3.07	Brown-yellow	Oblong	7.25	-
C. rigida	3.62	1.38	Brown	Ovoid	5.58	-
C. solstitialis	2.28	1.09	Brown-yellow	Lanceolate	2.40	-
C. stapfiana	8.12	1.88	White	Oblong	17.13	-
C. urvillei ssp. *nimrodis*	5.06	2.80	Yellowish white	Ovoid-Oblong	13.87	-
C. virgata	3.24	1.25	Yellow	Obovat-Oblong	1.74	-

3.3. Scanning electron microscopy (SEM) images

C. balsamita Lam: The surface of the achene is composed of small, almost cylindrical, short cells interlocking with each other, with the appearance of fish scales. Cells are brightly colored, distinctly circumscribed, and rounded at the tip (Figure 5.1).

C. behen L: The cells covering the surface of the achene are thin, rectangular, elongated, and have wide margins. Because of the width of these margins, the cells appear hollow (Figure 5.2).

C. consanguinea DC.: The cells forming the surface of the achene are elongated oblong and covered with fine hairs. These cells with indistinct margins appear to be intertwined (Figure 5.3).

C. iberica Trev. ex Sprengel: The surface of the achene is covered with sparse hairs, and the cells on it are thin and elongated with indistinct walls (Figure 5.4).

C. kurdica Reichardt: The Achene surface is sparsely hairy. The cells covering the surface are shiny, elongated, and clearly circumscribed (Figure 5.5).

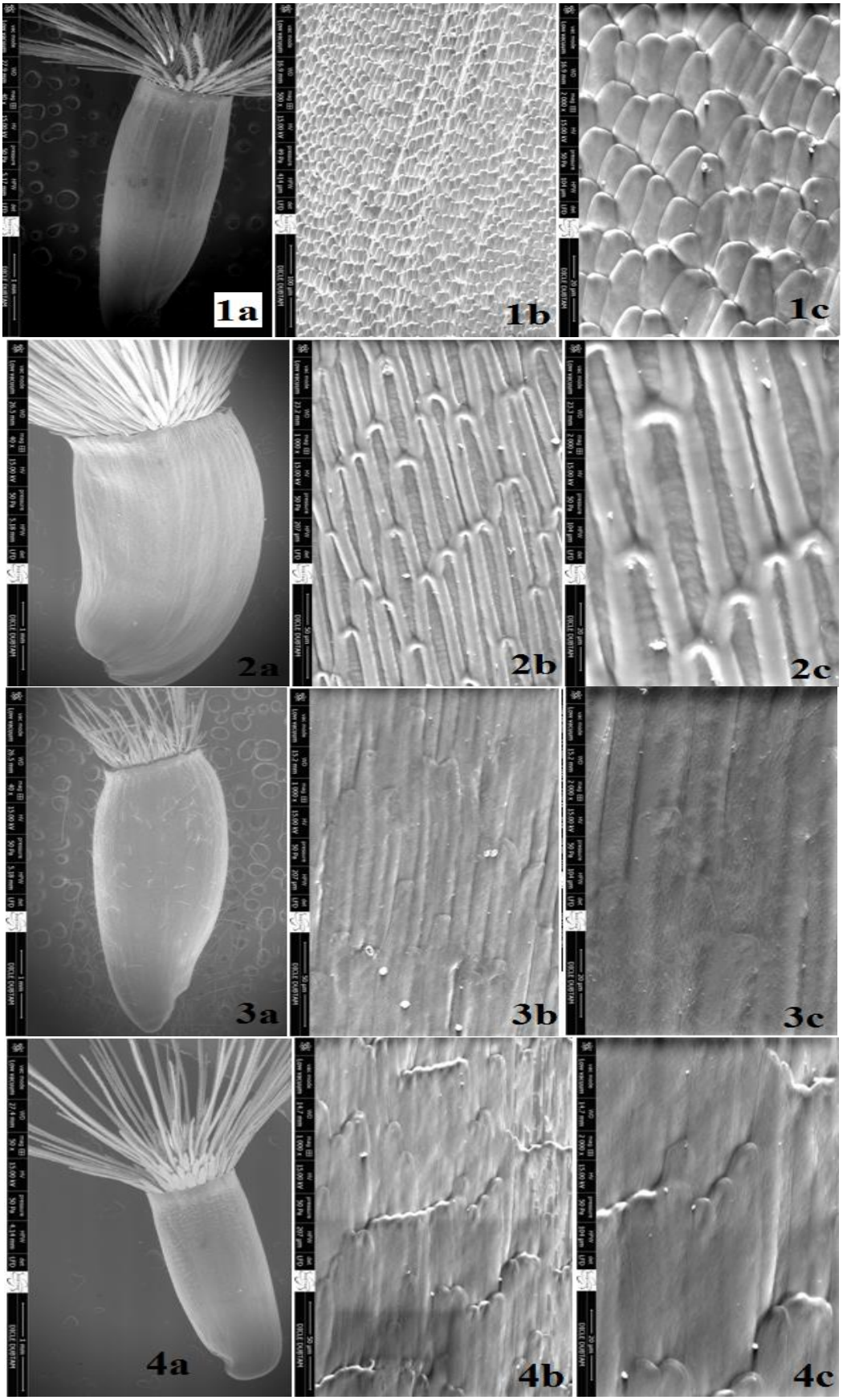
C. rigida Banks & Sol.: The condition of the hairs on the surface varies periodically. The edges of the cells on the surface are quite indistinct. Sometimes it appears as a single layer (Figure 5.6).

C. solstitialis L.: Achene surface cells are covered with cylindrical cells. The arrangement of these cells resembles the waves of the sea. These fluctuations produce irregular stratifications (Figure 5.7).

C. stapfiana (Hand.-Mazz.) Wagenitz: The cells on the achene are shiny and, as in *C. behen*, the cell margins are wide and appear to be higher than the cell center (Figure 5.8).

C. urvillei ssp *nimrodis* (Boiss. & Hausskn.) Wagenitz: The seed surface is covered with hairs dense enough to cover the cells in certain areas. Compared to others, the cells are longer and the cell margins are distinct (Figure 5.9).

C. virgata Lam: The surface is short and sparsely hairy. Cell margins are distinct (Figure 5.10).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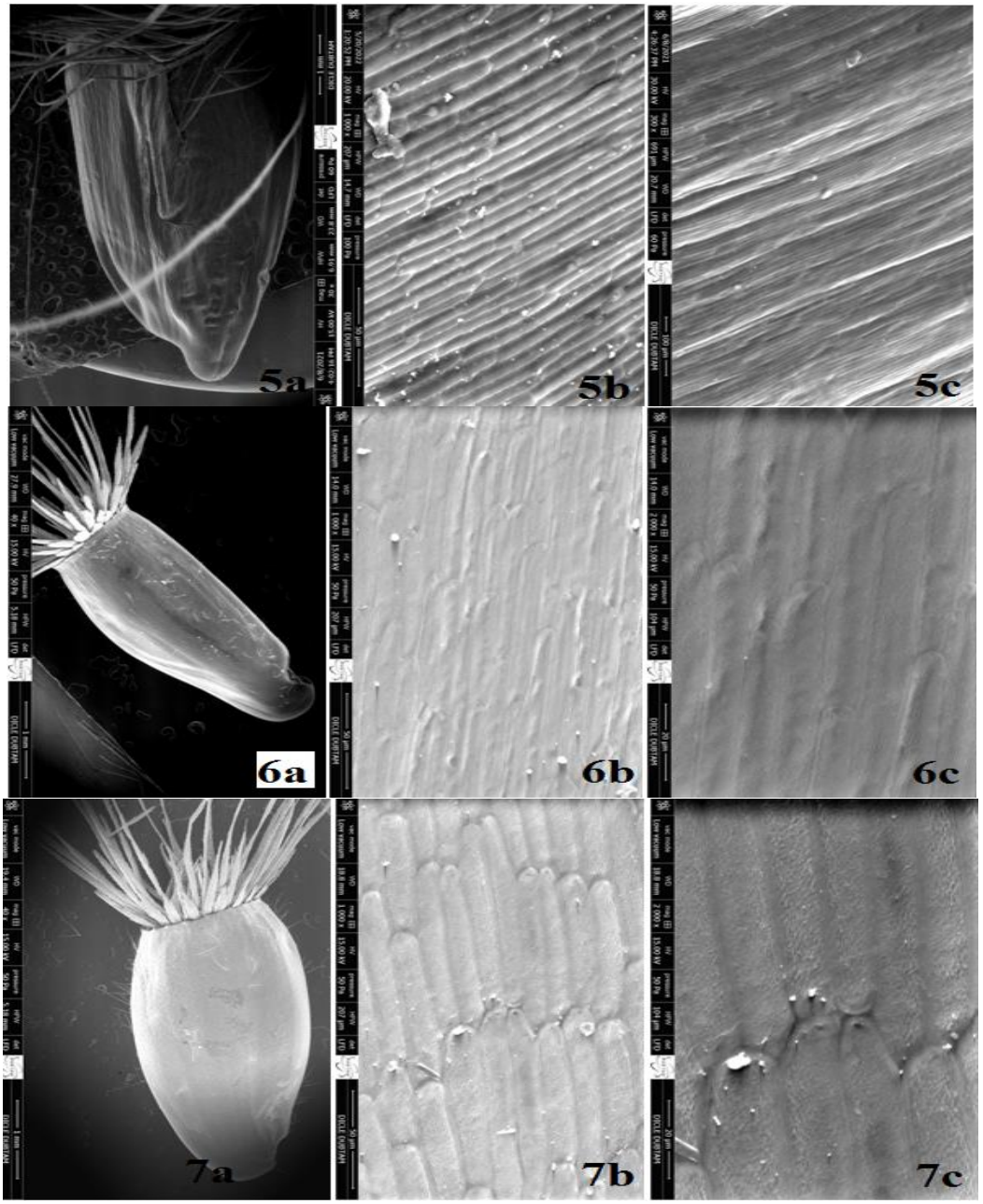
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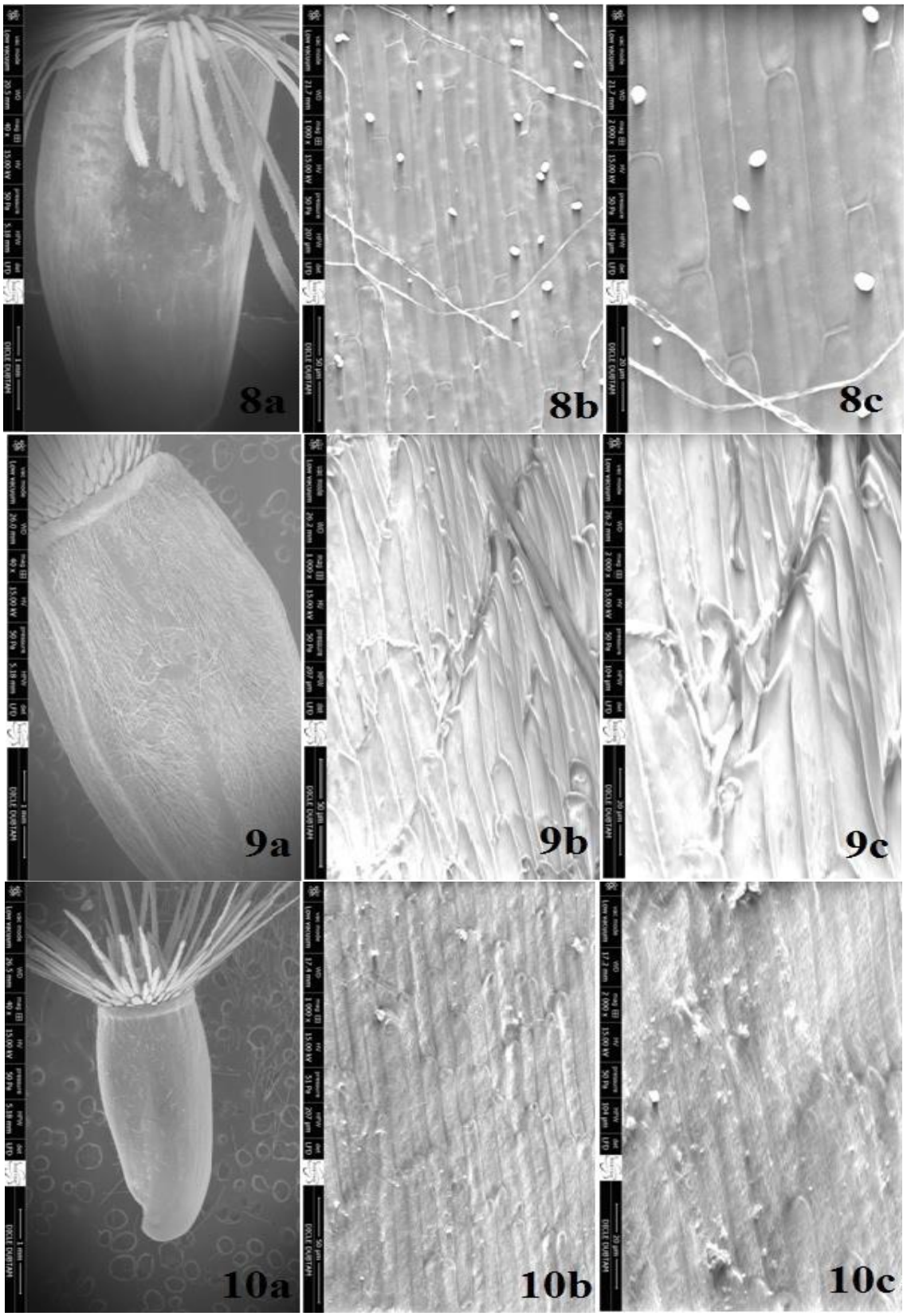

Figure 5. Scanning Electron Microscopy (SEM) microphotos of achenes; 1-*C. balsamita*, 2-*C. behen*, 3-*C. rigida*, 4-*C. solstitialis*, 5-*C. stapfiana*, 6-*C. iberica*, 7-*C. consanguinea*, 8-*C. kurdica*, 9-*C. urvillei ssp. nimrodis*, 10-*C. virgata*

4. Discussion

Numerous studies have been conducted on this genus due to its difficulty in identification. In particular, as more studies have been conducted on achene morphology, it has been observed that there are significant differences between taxa and that these differences can be utilized for taxonomy. The achene morphology of 23 species of the genus *Centaurea* L. was studied. Many qualitative and quantitative differences were observed in the achenes of the species they studied (Aksoy et al., 2010; Bancheva and Gorgorov, 2010; Bona, 2015).

One of the characters used for identification is the presence of pappuses. The presence of a pappus is considered a structural adaptation for wind transport and is of great importance in identification (Kulkarni, 2013). Pappus also can change its morphology in the presence of moisture in various ways that aid germination. The shape change can adjust the rate of abscission, allowing germination to increase or decrease depending on the favorable conditions Greene and David (2005). Loss or significant reduction of the pappus is not only important for identification but also for adaptation to changing environmental factors and habitats. Moreover, the fact that the length of the pappus is less than the length of the achene is considered a result of adaptation to arid environments (Paria ve Chinya, 1998; Abid and Kayzer, 2009).

In this study, pappus was found in all samples. Papuses were sparse and shorter than achene length in *C. virgata*, C. *iberica*, and *C. consanguinea*, close to each other in *C. balsamita* and *C. solstitialis* and longer in the other 4 species. Especially in *C. stapfiana* and *C. kurdica* species, papus lengths were very long, whereas in *C. behen* and *C. urvillei* ssp *nimrodis* they were very dense.

Another taxonomically important character is hairiness (Uysal et al., 2005). In *C. rigida* and *C. urvillei* ssp *nimrodis*, the surface of the achene is covered with dense hairs. Likewise, it has been reported that the hairiness of the species also indicates drought. In *C. iberica*, *C. urvillei* ssp *nimrodis*, *C. kurdica* and *C. solstitialis* the phyllaries end in horn-shaped elongated spines. Sparse trichomes were observed in the *C. iberica* achene (Bıyıklıoğlu et al., 2018).

Furthermore, another important feature in the taxonomy of the genus is the presence of appendages. The appendage is present in the *C. balsamita* species, though the remaining 9 lack appendages. The data obtained in this study prove the significance of achene and pappus size, color, and shape differences in systematics. *C. urvillei* ssp. *nimrodis* and *C. stapfiana* are endemic species and their achene characteristics were reported for the first time in this study.

5. Conclusion

The aim of the study was to demonstrate the significance of the micro and macro morphological characteristics of achenes for systematic differentiation. Similarly, in this study, some of the characteristics used to differentiate taxa, such as achene size, color and shape, pappus length and color, and the presence of appendages or hairs, were used. Based on the results of the investigation, oval or oblong achene shapes were found to be common among the taxa studied. Analysis of the SEM micrographs revealed ornamentation on some achene surfaces and different states of hairiness, and both sides of the achenes were laterally flattened. In conclusion, this study supports the need for the diagnostic use of achene surface patterns.

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