Turkish Journal of Forest Science
Available online at dergipark.gov.tr/turkjforsci

MICROMORPHOLOGICAL STUDIES (ACHENE / CYPSELA) ON SELECTED ASTERACEAE TAXA NATIVE TO TÜRKİYE

Alper UZUN[1], Seyran PALABAS UZUN[1,*]

[1]Department of Forest Botany, Kahramanmaras Sutcu Imam University, Kahramanmaras

*Corresponding author: seyran@ksu.edu.tr

Alper UZUN: https://orcid.org/0000-0002-2577-7460
Seyran PALABAS UZUN: https://orcid.org/0000-0001-7090-4804

Please cite this article as: Uzun, A. & Palabas Uzun, S. (2024) Micromorphological studies (Achene / Cypsela) on selected Asteraceae taxa native to Türkiye, *Turkish Journal of Forest Science*, 8(2), 218-229.

ESER BİLGİSİ / ARTICLE INFO
Araştırma Makalesi / Research Article
Geliş 15 Ağustos 2024 / Received 15 August 2024
Düzeltmelerin gelişi 6 Eylül 2024/ Received in revised form 6 September 2024
Kabul 3 Ekim 2024 / Accepted 3 October 2024
Yayımlanma 31 Ekim 2024 / Published online 31 October 2024

ABSTRACT: With this study fruit width-length measurements, weights and surface morphologies of some taxa belonging to Asteraceae family distributed in Türkiye were studied. The aim of the study was to reveal the fruit morphological characteristics and differences between the taxa of *Tragopogon buphthalmoides, Dittrichia viscosa, Dittrichia graveolens, Tripleurospermum caucasicum, Kemulariella caucasica, Leontodon hispidus* subsp. *hispidus, Jurinea consanguinea* and *Jurinea moschus.* Widths and lengths of the fruits were measured with Leica EZ4 microscope and digital caliper. Fruit weights were weighted out with Dikomsan precision scales. The stereomicroscope to determine the morphological characteristics of the fruits was also used. Scanning Electron Microscopy (SEM) in USKIM (Center for University and Industry Collaboration) was used to determine the surface morphology of the fruits. The smallest fruits were determined in *Dittrichia graveolens* (0.40 mm wide and 1.23 mm long), while the largest fruits were determined in *Tragopogon buphthalmoides* (25.88 mm long and 1.62 mm wide). The heaviest fruits were measured in *Jurinea consanguinea* (0.0065 gr) and the slightest fruits in *Dittrichia graveolens* (0.000062 gr) taxa.

Keywords: Morphological measurements, SEM, fruit, surface ornamentation, Asteraceae

TÜRKİYE İÇİN DOĞAL, BAZI ASTERACEAE TAKSONLARI ÜZERİNDE MİKROMORFOLOJİK ARAŞTIRMALAR (AKEN / SİPSELA)

ÖZET: Bu çalışma ile Türkiye'de yayılış gösteren Asteraceae familyasına ait bazı taksonların meyve en-boy ölçümleri, ağırlıkları ve yüzey morfolojileri çalışılmıştır. Çalışmada; *Tragopogon buphthalmoides, Dittrichia viscosa, Dittrichia graveolens, Tripleurospermum caucasicum, Kemulariella caucasica, Leontodon hispidus* subsp. *hispidus, Jurinea consanguinea* ve *Jurinea moschus* taksonlarına ait meyve morfolojik özellikleri ve birbirleri

arasındaki farklılıkları ortaya koymak amaçlanmıştır. Meyvelerin en ve boyları Leica EZ4 mikroskop ve dijital kumpas kullanılarak ölçülmüştür. Dikomsan hassas terazi ile meyvelerin ağırlıkları tartılmıştır. Meyvelerin morfolojik özelliklerinin belirlenmesine yardımcı olmak için stereomikroskop ile de çalışılmıştır. Meyvelerin yüzey morfolojisinin belirlenmesinde ÜSKİM (Üniversite-Sanayi-Kamu İşbirliği Geliştirme Uygulama ve Araştırma Merkezi) bünyesinde bulunan Taramalı Elektron Mikroskobu (SEM) kullanılmıştır. Çalışılan taksonlar içerisinde en küçük meyveler *Dittrichia graveolens* taksonunda (1.23 mm uzunluğunda ve 0.40 mm genişliğindedir), en büyük meyveler ise *Tragopogon buphthalmoides* taksonunda tespit edilmiştir (25.88 mm uzunluğunda ve 1.62 mm genişliğindedir). En ağır meyveler *Jurinea consanguinea*'da (0.0065 gr.) ve en hafif meyveler *Dittrichia graveolens*'de (0.000062 gr.) ölçülmüştür.

Anahtar kelimeler: Morfolojik ölçümler, SEM, meyve, yüzey ornemantasyonu, Asteraceae

INTRODUCTION

Asteraceae is one of the largest plant families distributed on all continents. According to current information, it is represented by more than 23,000 plant taxa (Jeffrey, 2007). This cosmopolitan family consists of annual, biennial, or perennial plants in the form of herbs, shrubs, climbers, or occasionally trees, and some taxa also contain latex. Their leaves are alternate, opposite or in the form of rosette; rarely auriculate, from simple leaf blade to crenate-toothed, lobed or otherwise dissected. The flowers (florets) are in a capitulum, and each capitulum is surrounded by a calyx-like series of phyllaries (involucral bracts) consisting of one to several rows, the whole called involucre. It resembles the calyx. The surface of the receptacle to which fruits attach may be naked or paleaceous (Matthews et al., 1975).

Plant morphology studies examine the internal and external structures of plants and reveal their important differences, making it easier to both identify plant species and distinguish them from each other with prominent characters. This is critically important in identification and classification of species and understanding their relationships with each other. Although genetic studies have progressed a little further in solving taxonomic problems today (Zhao et al., 2024), detailed micromorphological studies still provide remarkable data in reaching important conclusions (Zarre et al., 2024). The importance of seed morphological traits in taxonomic delimitation emerged after the mid-20th century, and until this time they were not even considered as taxonomic character. The increasing use of technologically advanced scanning electron microscopes, especially in micromorphological studies on seed, pollen, leaf, trichome and other plant organs, has increased the ability to provide advanced information and interpretation at the micro level (Abid & Qaiser, 2002; Yigit, 2016).

The main idea of this study is to reveal the achene (cypsela) characteristics of Asteraceae taxa, some of which have not been subject to micromorphological studies before. The fruits of Asteraceae are technically called achene or cypsela, and the fruit is mostly dry, indehiscent, unilocular, one-seeded. Most achene have an appendage called a pappus (Hussein & Eldemerdash, 2017; Gabr, 2019). This study includes measurements of fruit dimensions and examination of the fruit surfaces with stereomicroscope and scanning electron microscopy (SEM) in order to increase botanical knowledge about the micromorphological structures of the fruit surface and advance systematic evaluations. With this study, it is also aimed to make contributions to the Flora of Türkiye in terms of achene micromorphology.

MATERIAL AND METHOD

In this study, especially the less studied taxa that are Colchis flora elements and species belonging to similar genera were studied mutually. It was examined whether the species shape, color and ornamentation were different. The fruits belonging to Asteraceae taxa were obtained from the plant collections of Dr. Seyran Palabaş-Uzun and Dr. Alper Uzun in the herbaria KATO and KASOF (Thiers, 2022). The list of herbarium specimens and voucher data are listed in Table 1. The fruits were examined morphologically using a Leica EZ4 stereomicroscope, and fruit length and width are measured with a digital standard caliper. Measurements were carried out on 30 seeds for each taxon. The characteristics of the hilum part of the fruits were determined by examining them under the stereomicroscope. The weights of the fruits were measured using Dikomsan precision scales. Leica S8-APO stereomicroscope was used for morphological examination and colour determination of the fruits, and micro-photographs of the fruit samples were obtained with a digital photography system and fruit colours were determined. Fruit samples were also placed directly on the stabs with the help of double-sided adhesive tape and coated with gold to make them conductive and display images on the screen of electron microscope (EVO LS10) in Kahramanmaraş Sütçü İmam University Microscopic Analysis Laboratory (USKİM). Then, the micrographs of fruit samples were taken at different magnifications.

Table 1. List of Species Analyzed in The Study

Taxa	Voucher locations	Collection Number
Tragopogon buphthalmoides (DC.) Boiss.	C6: Kahramanmaraş, Ahir Mountain, Keklikdere Valley, 1905 m, 19.07.2019	[1] KASOF 1177
Dittrichia viscosa (L.) Greuter	C6: Kahramanmaraş, Andırın, roadside, 950 m, 22.09. 2017	KASOF: 1835
Dittrichia graveolens (L.) Greuter	C6: Kahramanmaraş, Ahir Mountain, 750 m, 18.09.2017.	KASOF: 1829
Tripleurospermum caucasicum (Willd.) Hayek	A7: Trabzon: Şalpazarı, Sisdağı, 1900 m, 11.06.2005	KATO: 17013
Kemulariella caucasica (Willd.) Tamamsch.	A7: Trabzon, Çamlıdüz plateau, forest edge, 1769 m, 22.07.2008	KATO: 18429
Leontodon hispidus L. subsp. *hispidus*	A7: Trabzon, Ormanüstü plateau, open place, 1800 m, 05.08.2005	KATO: 18494
Jurinea consanguinea DC.	A7: Trabzon, Çeşmeler district, stony shrubby place, 700 m, 01.06.2008	[2]KATO: 18475
Jurinea moschus (Hablitz) Bobrov	A7: Trabzon, Altındere V., Mezere hill., roadside, 2400 m, 19.06.2001	KATO: 15620

[1] KASOF: Herbarium of Faculty of Forestry, Kahramanmaraş Sütçü İmam University
[2] KATO: Herbarium of Faculty of Forestry, Karadeniz Technical University

Fruit Ornamentation Forms and Distribution Areas of Species

In determining the surface ornamentation of the fruits, atlas of seeds and fruit of central and East-European flora (Bojnanský & Fargašová's, 2007) was used. The surface shapes and surface ornamentations of the fruits seen in this study are as follows (Table 2);

Table 2. Fruit Shape and Surface Ornamentations

Fruit shapes	**Fruit surface ornamentations**
Linear-lanceolate	Muricate
Oblong-elliptic	Rugulose
Oval	Undulate-Rectangular
Longitudinal ellipsoid	Furrowed
Ellipsoid	Favulariate
Obovoid	Rectangular

In the result section, plant distribution information is given according to the grid system of Türkiye (Davis et al., 1965).

RESULTS AND DISCUSSION

Tragopogon buphthalmoides (DC.) Boiss.

The genus *Tragopogon* L. consists of approximately 150 plant taxa, mostly distributed in the semi-arid and mountainous regions of Eurasia. The number of *Tragopogon* taxa distributed in Türkiye is 25, but three of them are doubtful (*T. balcanicus* Velen, *T. pusillus* M.Bieb and *T. vaginatus* Ownbey & Rech.f.), not clear to be in the country (Coşkunçelebi & Gültepe, 2012). In most species of the genus, the achene differentiates into a broad (seed-containing) body and a thinner beak. The length of the beaks (without pappus) varies between 1 and 5.5 cm, and the ratio between the body and the beak varies depending on the species (Sukhorukov & Nilova, 2015). According to results of the study of the achene body have a linear-lanceolate shape (Figure 1A). Achene colour is greyish brown. The achene surface is muricate. According to the SEM images, it was determined that the achene body had a ribbed structure in 5 rows, the ornamentation on the ribs was slightly protruding (muricate) and also decurrent and the ornamentation between the ribs was reticulate-scale like. The average achene dimensions were found as 25.88 mm (22.10-27.68) in length and 1.62 mm (1.12-2.37) in width. Achenes weigh 0.0011 g. In Türkiye, it is distributed in the squares A5, A7, A8, B5, B6, B7, B8, B9, C4, C5 and C6.

***Dittrichia viscosa* (L.) Greuter**

The genus *Dittrichia* L. belongs to the Inuleae tribe of the Asteraceae family and is distributed mainly in the Mediterranean region and extending to the Middle East (Brullo & de Marco). Fruit characters are of great importance for the taxonomy and phylogeny of the tribe Inuleae (Karanović et al., 2016). *Dittrichia* genus includes two taxa in Türkiye. *Dittrichia viscosa* achenes have a slightly oblong-elliptic shape with smooth edges and rounded tips. Achene colour is straw yellow and the surface has indumentum. According to Karanovic et al. (2016), there is no significant difference between achenes consisting of ray and disc florets and achenes are homomorphic. However, they state that achenes consisting of ray florets have a denser indumentum than disc achenes. According to the SEM images, it was determined that the ornamentation on the achenes was rugulose (slightly wrinkled) (Figure 1B). Calcium oxalate crystals are observed on the surface. The average achene dimensions were found as 3.07 mm

(2.47-3.37) in length and 0.59 mm (0.40-0.79) in width. Achenes weigh 0.00031 g. It is distributed in the squares A1, A2, A3, A4, B1, C1, C2, C3, C4 and C5.

Dittrichia graveolens (L.) Greuter

In this taxon, fruits have an oblanceolate-elliptic shape, narrowing slightly towards the base. Achene colour is straw yellow and has an indumentum. According to the SEM images of the achenes in the study, the ornamentation was rugulose (slightly wrinkled) and extended parallel to the length of the fruit (Figure 1C). As noted by Karanović et al. (2016), no distinct longitudinal ribs can be seen on the achene surface. There are also calcium oxalate crystals on the outer surface of achene. The average achene dimensions were found as 1.23 mm (1.03-1.43) in length and 0.4 mm (0.23-0.54) in width. Achenes weigh 0.000062 g. It is distributed in the squares A2, A3, A6, B1, C1, C3, C4 and C6.

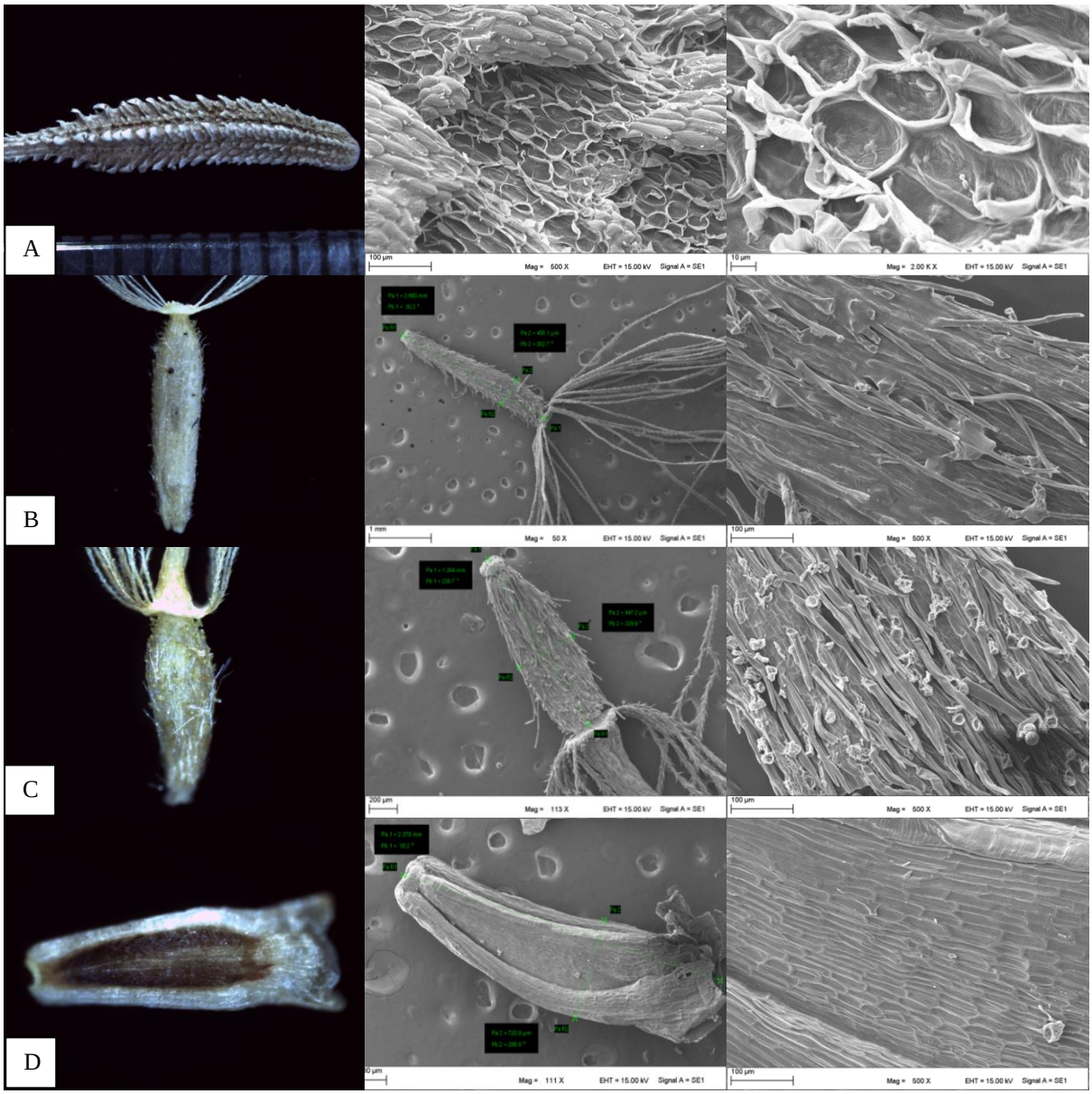

Figure 1. From left to right; fruit colour, fruit length & width, surface ornamentation (the close-up SEM image). A) *Tragopogon buphthalmoides*, B) *Dittrichia viscosa*, C) *Dittrichia graveolens*, D) *Tripleurospermum caucasicum*

***Tripleurospermum caucasicum* (Willd.) Hayek**

The genus *Tripleurospermum* Sch. Beep. belongs to Anthemideae tribe of the Asteraceae family and consists of approximately 38 species distributed mainly in the Mediterranean Basin, including Europe, temperate Asia and North Africa (İnceer & Beyazoğlu, 2004; İnceer & Özcan, 2021). In Türkiye the genus comprises ca 33 taxa (İnceer, 2012) and most of these are distributed in the Black-Sea Region. In the present study the mature achenes were examined in stereomicroscope and seen that the achenes have an oblong-elliptic shape. Achene colour is dark brown. The SEM images of the achenes revealed that the surface ornamentation was rectangular (Figure 1D). The average size of the achenes was determined as 1.93 mm (1.58-2.26) in length and 0.56 mm (0.38-0.78) in width. Achene weight is 0.00017 g. It is distributed in the squares A1, A4, A5, A6, A7, A8, A9, B4, B6, B7, B8, B9, C5, C6 and C7.

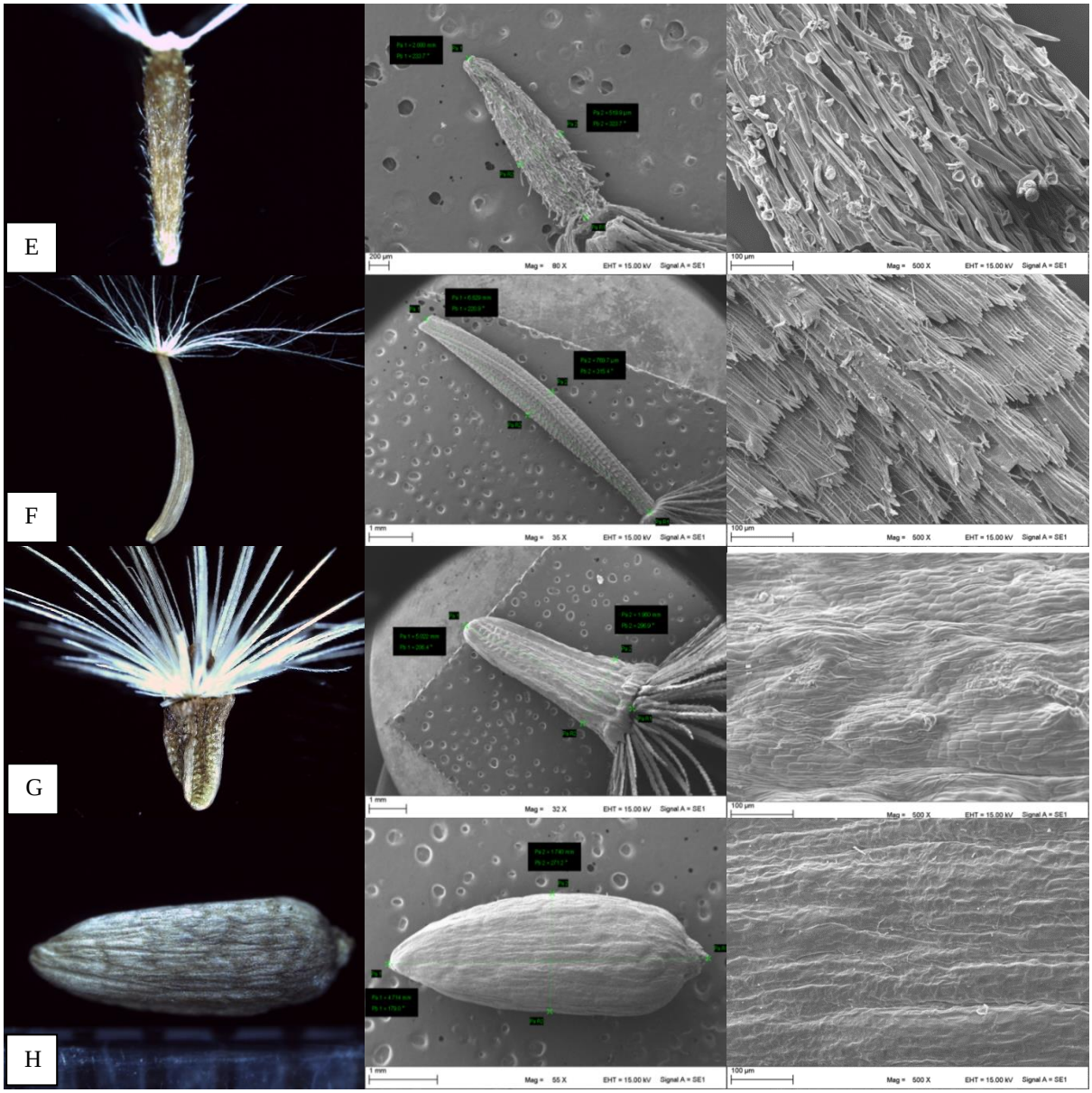

Figure 2. From left to right; fruit colour, fruit length & width, surface ornamentation (the close-up SEM images). E) *Kemulariella caucasica,* F) *Leontodon hispidus* subsp. *hispidus,* G) *Jurinea consanguinea,* H) *Jurinea moschus*

***Kemulariella caucasica* (Willd.) Tamamsch.**

The genus *Kemulariella* Tamamsch. is almost completely endemic to the Caucasus and hitherto and has six plant taxa classified in two sections (Fırat, 2016). In Türkiye, it is represented by three taxa (Ekim, 2012). The genus mainly distinguished from Aster by its biseriate pappus (Tamamschian, 1959). No study has been found in the literature regarding the achene characteristics of this taxon. The edges of the achenes are smooth, slightly angular, and have a longitudinal ellipsoid shape. Achene colour is light brown and surface has an indumentum. According to the SEM images of the achenes, it was determined that the ornamentation was slightly furrowed longitudinally (Figure 2E). The average size of the achenes was found as 1.78 mm (0.56-2.20) in length and 0.40 mm (0.24-0.53) in width. Achenes weigh 0.00021 g. It is distributed in the north-east part of Türkiye (A7 and A8 squares).

Leontodon hispidus* L. subsp. *hispidus

The genus *Leontodon* is native to Eurasia and North Africa and is represented by approximately 79 plant taxa in the world and 6 species (8 taxa) in Türkiye (Aslan, 2012). According to micro-morphological examinations, the achenes have a straight-edge and linear-lanceolate shape. Colour of the achene is yellowish-brown. The achene surface has a rough structure. According to the SEM images of the achenes, the ornamentation was longitudinally furrowed (cracked, with depressed areas, opposite of rib) (Figure 2F). The average achene dimensions were found as 6.56 mm (5.58-7.52) in length and 0.55 mm (0.30-0.83) in width. Achenes weigh 0.00046 g. It is distributed in the A2, A3, A4, A5, A6, A7, A8, A9, B1, B4, B6, B9, B10, C2 and C9.

***Jurinea consanguinea* DC.**

The genus *Jurinea* Cass., which is included in the Saussureinae subtribe of the Asteraceae family, has 180 plant taxa distributed mainly in Central Asia, the Mediterranean basin, Iran and Türkiye (Bona, 2020). In this study, the mature achenes were examined and it was understood that the achenes had an oval shape. Achene colour is yellowish brown. The achene surface has a longitudinally striate structure. SEM analyses showed that the ornamentation on the achenes was undulate-rectangular (Figure 2G). The average achene dimensions were found as 4.48 mm (4.24-4.77) in length and 1.79 mm (1.27-2.14) in width. Achenes weigh 0.0065 g. It is distributed in the squares A1, A2, A3, A4, A5, A7, A8, A9, B1, B2, B3, B4, B5, B7, B9, C1, C2, C3, C4 and C5.

***Jurinea moschus* (Hablitz) Bobrov**

We initially identified the collected seeds as *Jurinella moschus* subsp. *pinnatisecta*. However, this taxon was later accepted as *Jurinea moschus* based on phylogenetic analyses (Szukala et al., 2019). We followed this change in here. In previous evaluations, the genus *Jurinella* Jaub. & Spach was distinguished by its ecoronulate, conical-tipped, round-shouldered achene and readily deciduous pappus (Matthews et al., 1975), which formerly represented by a single species and two taxa in Türkiye. This species has a large sessile capitula and is a perennial herb that is generally distributed on alpine slopes. According to stereomicroscope examination the

edges of the achenes are smooth and have an obovoid shape. Achene colour is greyish brown. SEM analyses showed that the achene surface has a smooth structure. The ornamentation was in the form of favulariate (thin longitudinal veins) (Figure 2H). The average achene dimensions

Table 3. Comparison of The Fruit Traits

Taxa	FL ± std.d.	min-max	FW ± std.d.	min-max	FS	SO	FC	FW
Tragopogon buphthalmoides	10.33 ± 0.52 *25.88 ± 1.99	9.67 - 11.18 *22.1 - 27.68	1.62 ± 0.42	1.12 - 2.37	linear-lanceolate	muricate and reticulate-scale like	greyish brown	0.0011
Dittrichia viscosa	3.07 ± 0.17	2.47 - 3.37	0.59 ± 0.10	0.40 - 0.79	oblong-ellipsoid	rugulose	straw yellow	0.00031
Dittrichia graveolens	1.23 ± 0.11	1.03 - 1.43	0.40 ± 0.09	0.23 - 0.54	oblanceolate-elliptic	rugulose	straw yellow	0.000062
Tripleurospermum caucasicum	1.93 ± 0.17	1.58 - 2.26	0.56 ± 0.10	0.38 - 0.78	oblong- cylindrical	rectangular	dark brown	0.00017
Kemulariella caucasica	1.78 ± 0.31	0.56 - 2.20	0.40 ± 0.09	0.24 - 0.53	ellipsoid	furrowed	light brown	0.00021
Leontodon hispidus subsp. *hispidus*	6.56 ± 0.48	5.58 - 7.52	0.55 ± 019	0.30 - 0.83	linear-lanceolate	furrowed	tawny	0.00046
Jurinea consanguinea	3.36 ± 0.04	4.24 - 4.77	1.37 ± 0.05	1.27 - 2.14	tetragonal	undulate-rectangular	tawny	0.0065
Jurinea moschus	4.39 ± 0.15	4.08 - 4.60	1.69 ± 0.13	1.38 - 1.89	obovoid	favulariate	tawny	0.00644

Legend. The measurements (mm); FL: fruit length (mean std.d. / min-max), FW: fruit width (mean std.d. / min-max), FS: fruit shape, SO: surface ornamentation, FC: fruit colour, FW: fruit weight (g).
*values with beak

were found as 4.39 mm (4.08-4.60) in length and 1.69 mm (1.38-1.89) in width. Achenes weigh 0.00644 g. It is distributed in the A6, A7, A8, A9, B7, B9, and B10.

It has been revealed that the achene shapes and characteristics differ significantly among both genera and taxa. The achene shapes of *Dittrichia viscosa* and *Dittrichia graveolens* were oblong-elliptic and oblanceolate-elliptic, respectively, and their surface ornamentations were rugulose. *Dittrichia graveolens*, which is distributed in Pakistan and Kashmir region, was also studied by Abid and Qaiser (2002) and with this previous study, the fruit shape was defined as oblanceolate-elliptic, but the surface ornamentation as smooth. The achene shape of *Jurinea consanguinea* in the present study was determined as oval and its surface ornamentation was undulate-rectangular. Bona (2020)'s results, the achene ornamentation as undulate and achene shape narrowly ovate, are also consistent with our results. Dogan et al. (2010) also found tetragonal achene shape in *Jurinea turcica*, a new taxon described from Türkiye, but the surface ornamentation was longitudinally striate. The achene shape of *Tripleurospermum caucasicum* was determined as oblong-elliptic, and the ornamentation of the fruit lobes adjacent to the endosperm was rectangular. These results are also compatible with the study conducted by İnceer et al. (2012) in which the achene characteristics of *Tripleurospermum* taxa specific to Türkiye were examined.

In terms of fruit length (Table 3), the longest fruit is measured for *Tragopogon buphthalmoides* (25.88 mm). The shortest fruit is seen in *Dittrichia graveolens* (1.23 mm). The widest fruit was measured in *Jurinea consanguinea* (1.79 mm). The narrowest fruits are seen in *Dittrichia graveolens* (0.23 mm). The lightest fruits measured in *Dittrichia graveolens* (0.000062 gr). The heaviest fruits were defined in *Jurinea consanguinea* (0.0065 gr).

CONCLUSION

In this study, fruit characteristics of eight taxa belonging to the Asteraceae family, some of which have not been subject to achene morphology studies before, were examined using stereomicroscope and SEM. As a result of the examinations, it was revealed that taxa may contain significant differences in terms of morphological and micromorphological characteristics. Although the size of the fruits is affected by environmental conditions, their shape, type and surface ornamentations offer more stable features. In this sense, surface ornamentations are often considered to be more important in taxonomic delimitation. Fruit colors may vary slightly depending on the species that have reached maturity, providing very limited discrimination between species. It is also known that the flower, fruit and seed characteristics are important in taxonomic delimitation, but genetic studies are still one step ahead in today's taxonomy. However, it is an obvious fact that genetic differences / similarities should also be supported morphologically. Otherwise, taxonomy may become even more complicated. To prevent this, it is recommended to add micromorphological studies to genetic studies.

AUTHOR CONTRIBUTIONS

Alper Uzun: Conceptualization, Methodology, Data curation, Formal analysis, Writing - original draft. **Seyran Palabaş Uzun:** Conceptualization, Methodology, Data curation, Formal analysis, Writing - original draft.

ACKNOWLEDGEMENT

Preliminary results of this study were presented in III. International Mediterranean Forest and Environment Symposium (IMFES2019). Authors thank to staff of USKIM Central Laboratory (Kahramanmaraş Sütçü İmam University) for taking the micrographs of the fruits.

FUNDING STATEMENT

The study received no financial support.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS COMMITTEE APPROVAL

This study does not require any ethics committee approval.

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