



Research Article

Ultrastructural features of cypsela in selected *Senecio* L. (Asteraceae) species

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Abstract – In this study, the cypselae features of eight *Senecio* species in Türkiye (*Senecio olympicus* Boiss., *S. paludosus* L., *S. tauricolus* VA. Matthews, *S. salsuginea* H. Duman & Vural, *S. castagneanus* DC., *S. fluviatilis* Wallr., *S. pseudo-orientalis* Schischk., and *S. racemosus* (M. Bieb.) DC.) were investigated in detail using light microscopy (LM) and transmission electron microscopy (TEM). A very thick and darkly stained cuticle layer was observed on the epidermal cells of all the species examined. Papillae were observed only in epidermal cells of *S. olympicus* Boiss., and sclerenchyma cells were less developed in the cypselae. In the other seven species, well-developed sclerenchyma tissue was found along the pericarp wall in the form of a semicircle, in bundles, or around the secretory ducts. Although secretion ducts were observed along the cypselae wall in the mid-layer cells of *S. olympicus*, *S. tauricolus*, *S. castagneanus*, and *S. fluviatilis*, no secretion ducts were observed in the other four species. Although prismatic calcium oxalate crystals were observed in the intermediate (mid) layer of *S. tauricolus*, *S. castagneanus*, and *S. pseudo-orientalis*, no crystals were found in the other five species. Phytomelanin accumulation was found in the schizogenic spaces between the outer and intermediate layers of *S. olympicus*, *S. paludosus*, *S. tauricolus*, *S. castagneanus*, *S. pseudo-orientalis*, and *S. racemosus*, but not in *S. salsuginea* and *S. fluviatilis*.

Keywords: anatomy, cypselae, cytology, *Senecio*, transmission electron microscopy (TEM)

Introduction

The genus *Senecio* L. belongs to the Asteraceae (Compositae) family and the Senecioneae Cass tribe. Senecioneae, the largest tribe of the Asteraceae family, has a worldwide distribution with 150 genera and approximately 1200 species (Nordenstam 2007, Pelser et al. 2007). Additionally, described is the genus *Turanecio*, originating from Türkiye (Hamzaoglu and Çetin 2016). It has been very difficult to classify the species within this widely distributed genus and its taxonomic positions have been revised multiple times.

Comprehensive and consistent morphological studies contribute to both a better understanding of the biology of the species and a correct taxonomic and phylogenetic classification of species with similar characters (Reis et al. 2016). Phylogenetic studies attempt to determine the boundaries of the genus and the relationships between species by including sister groups (Bozkurt et al. 2024). Many researchers have reported that the morphological and anatomical features of fruits and seeds are important criteria in taxonomic, phylogenetic, and ecophysiological studies (Soares et al. 2019). These data were also used in the classification of the Asteraceae (Zarembo and Boyko 2008,

Leszek and Marek 2013, Jana and Mukherjee 2014, Talukdar 2015a, b, Sukhorukov and Nilova 2015, Karanović et al. 2016, Shil et al. 2016, Zareh et al. 2016, Paul 2017, Paul and Mukherjee 2017, Özcan 2017, Kürşat et al. 2023, Naderifar et al. 2023).

There are many published studies on achene, cypselae (pericarp+testa), and seed morphology or micromorphology in different Asteraceae taxa. Findings based on anatomical and ultrastructural features of cypselas, such as the organization of sclerenchyma tissue in cypselae, the presence of papillae or trichomes in epidermal cells, the presence of secretion ducts along the cypselae wall, the localization of prismatic-shaped crystals in the mid-layer cells, and the presence of phytomelanin in the intercellular spaces in the mesocarp and mid-layer are valuable data for taxon classification. According to a literature survey, there is no record regarding the seed anatomy, histology, and cytology of the *Senecio* genus. This study aimed to contribute to the taxonomic problems of the genus by examining the cypselae features of eight *Senecio* species, using light microscopy (LM) and transmission electron microscopy (TEM), and revealing their histological and cytological features.

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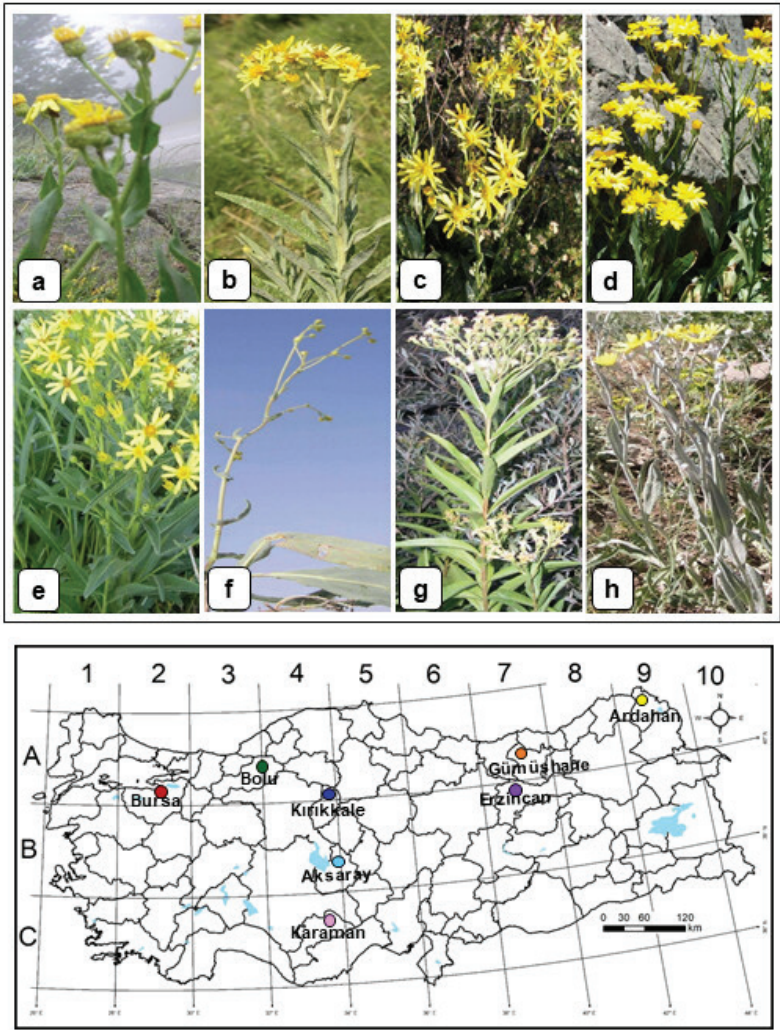


Fig. 1. Map of studied populations of *Senecio* taxa in Türkiye: a – *Senecio olympicus* (●), b – *Senecio paludosus* (●), c – *Senecio tauricolus* (●), d – *Senecio salsuginea* (●), e – *Senecio castagneanus* (●), f – *Senecio fluviatilis* (●), g – *Senecio pseudo-orientalis* (●), h – *Senecio racemosus* (●).

Material and methods

Plant materials belonging to *Senecio* species that were used in the study were collected from different regions of

Türkiye (Fig. 1). Regions, collectors, and collection data are listed in Tab. 1.

Mature cypselas of each taxon were pre-fixed with 3% glutaraldehyde for three hours. After the pre-fixation pro-

Tab. 1. Data on examined *Senecio* specimens.

Taxa	Collector and/or Locality Collection No.
<i>S. olympicus</i> Boiss.	Hamzaoglu 4399 Bursa: Uludağ, upper of Kırkpınar valley, 2100-2200 m, Hamzaoglu 4399, Aksoy & Budak (Bozok Hb.).
<i>S. paludosus</i> L.	Budak 1994 Bolu: The edges of the channels around the Abant lake, 1300 m, Budak 1994, Hamzaoglu & Aksoy (Bozok Hb.).
<i>S. tauricolus</i> V.A.Matthews	Budak 1735 Karaman: Between Ermenek-Karaman, 16 km, under the steep rocks to the left of the road,1670 m, Budak 1735, Hamzaoglu & Aksoy (Bozok Hb.).
<i>S. salsuginea</i> H.Duman & Vural	Hamzaoglu 5377 Aksaray: Eskil, Tersakan location, 900 m, Hamzaoglu 5377, Aksoy & Budak (Bozok Hb.).
<i>S. castagneanus</i> DC.	Hamzaoglu 3774 Kırkkale: Dinek Mountain is about 1600 m from the exit of Uzunlar village to Mamikkaya hill, Hamzaoglu 3774, (Bozok Hb.).
<i>S. fluviatilis</i> Wallr.	Budak 2123 Erzincan: Between Aşkale-Tercan 20 km, 1550 m, Budak 2123, Hamzaoglu & Aksoy (Bozok Hb.).
<i>S. pseudo-orientalis</i> Schischk.	Budak 1900 Gümüşhane: Between Kelkit Akdağ village and Kuzuçimeni plateau, Kaz Mountain, 2340 m, Budak 1900, Hamzaoglu & Aksoy (Bozok Hb.).
<i>S. racemosus</i> (M.Bieb.) DC.	Budak 1956 Ardahan: Between Çamlıbel and Ardahan, 2000 m, Budak 1956, Hamzaoglu & Aksoy (Bozok Hb.).

Tab. 2. Anatomical features of the cypsela of the studied *Senecio* species.

Taxa	Papillae	Sclerenchyma tissue or /bundles	Secretory ducts	Crystals	Phytomelanin accumulation
<i>S. olympicus</i> Boiss.	+	+	+	-	+
<i>S. paludosus</i> L.	+	+	-	-	+
<i>S. tauricolus</i> V.A.Matthews	+	+	+	+	+
<i>S. salsuginea</i> H.Duman & Vural	-	+	-	-	-
<i>S. castagneanus</i> DC.	-	+	+	+	+
<i>S. fluviatilis</i> Wallr.	-	+	+	-	-
<i>S. pseudo-orientalis</i> Schischk.	-	+	-	+	+
<i>S. racemosus</i> (M.Bieb.) DC.	-	+	-	-	+

cess, the materials were washed with 0.1 M Na-P buffer (pH 7.2) and kept at 4 °C overnight, then post-fixed in 1% osmium tetroxide (Os₂O₄) for two hours. The materials were dehydrated through an ethanol series. After dehydration and saturation, the samples were placed in the embedding medium Epon 812 (Luft 1961). Semi-thin sections were taken from the prepared blocks using an ultramicrotome and these sections were stained with methylene and toluidine blue. Thin sections (ultrathin) were placed on copper grids and stained first with uranyl acetate (Stempak and Ward 1964), then with lead citrate (Sato 1967). They were exam-

ined with a JEOL CX II TEM and micrographs were taken. For LM, semi-thin sections of 1.5 µm or 2 µm were taken from the samples embedded in epoxy and stained with 1% toluidine blue. The sections were examined under a light microscope, and photographs were taken.

Results

Eight *Senecio* species were examined, cypsela anatomical characters and various diagnostic anatomical characters of these species were determined (Tab. 2). The cypsela

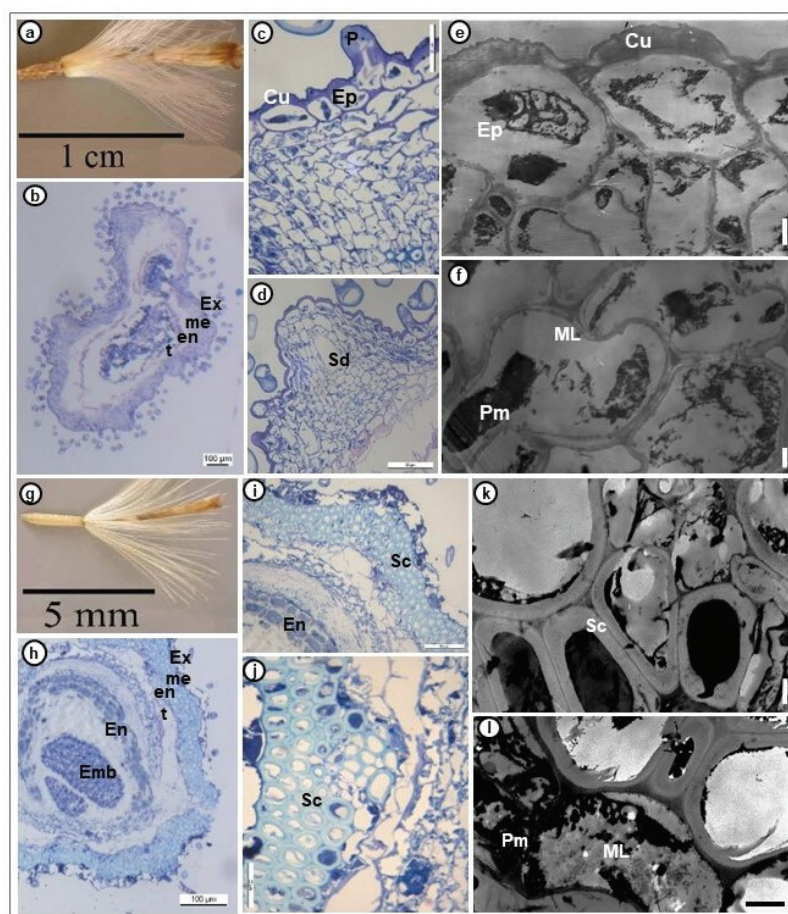


Fig. 2. *Senecio olympicus*: a – cypsela, b – general view of cypsela structure, scale bar = 100 µm, c – epidermal cells and papilla, scale bar = 20 µm, d – secretory duct in sclerenchyma bundle, scale bar = 50 µm, e – electron micrograph of epidermal cells, scale bar = 1 µm, f – electron micrograph of mid-layer cells, scale bar = 1 µm; *Senecio paludosus*: g – cypsela, h – general view of cypsela structure, scale bar = 100 µm, i – sclerenchyma cells, scale bar = 50 µm, j – sclerenchyma cells with large lumens, scale bar = 20 µm, k – electron micrograph of sclerenchyma cells, scale bar = 5 µm, l – electron micrograph of mid-layer cells, scale bar = 5 µm.

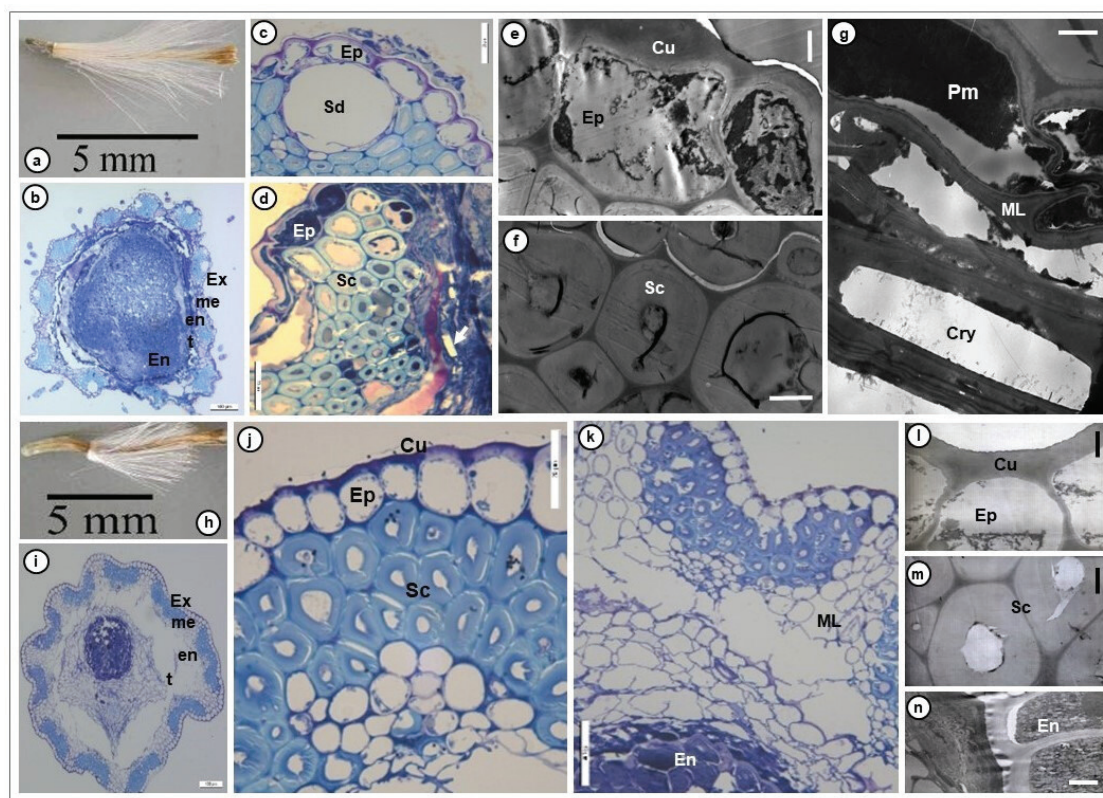


Fig. 3. *Senecio tauricolus*: a – cypselas, b – general view of cypselas structure, scale bar = 100 µm, c – epidermal cells and secretory duct, scale bar = 20 µm, d – sclerenchyma bundle, scale bar = 20 µm, e – electron micrograph of epidermal cells, scale bar = 1 µm, f – electron micrograph of sclerenchyma bundle, scale bar = 1 µm, g – electron micrograph of mid-layer cells, scale bar = 1 µm; ***Senecio salsuginea*:** h – cypselas, i – general view of cypselas structure, scale bar = 100 µm, j – epidermal cells and sclerenchyma bundle, scale bar = 20 µm, k – mid-layer cells, scale bar = 50 µm, l – electron micrograph of epidermal cells, scale bar = 5 µm, m – electron micrograph of sclerenchyma cells, scale bar = 5 µm, n – electron micrograph of endosperm cells, scale bar = 1 µm.

consists of the pericarp, testa, endosperm, and cotyledons, and the mature pericarp consists of three regions: the exocarp (outer epidermis), mesocarp, and endocarp (inner epidermis).

***Senecio olympicus* Boiss.**

Cypselas wall is wavy (Fig. 2a) and the epidermis is papillary (Fig. 2b, c). The cuticle on the epidermal cells is darkly stained. There are 1-3 sclerenchyma cells among the parenchymatic cells under the epidermal cells (Fig. 2c). Below the exocarp, secretory ducts line the cypselas wall (Fig. 2d). Below the epidermis, the mesocarp contains 4-5 layers of mid-layer cells. In the innermost part, there are endosperm cells. Ultrastructural examinations revealed that epidermal cells are rectangular and that thick cuticle covers them (Fig. 2e). Mid-layer cells are wavy-walled and electron opaque. There are electron-dense phytomelanin deposits in some mid-layer cells (Fig. 2f).

***Senecio paludosus* L.**

In the exocarp, the epidermis is papillary and thick-walled (Fig. 2g). The cuticle layer was darkly stained (Fig. 2h). Under the epidermal cells, there are 4-6 layers of sclerenchyma cells, which are located along the pericarp

wall (Fig. 2i). The lumens of sclerenchyma cells are wide. In some cells, the lumen is filled and the cell walls are electron-opaque (Fig. 2j, k). Beneath the sclerenchymatous cells, there are 3-5 layers of cells with large vacuoles and thin walls, which are parenchymatous cells. The schizogenous spaces of the mid-layer cells are filled with electron-dense phytomelanin aggregates (Fig. 2l).

***Senecio tauricolus* V.A. Matthews**

The structure of the cypselas has a wavy wall and an oval shape (Fig. 3a, b). The cuticle layer is stained dark. Secretory ducts were found under the epidermal cells in the sclerenchyma bundles across the entire cypselas wall (Fig. 3c). Sclerenchyma cells are thick-walled. The interiors of some secretory ducts are empty. Under the sclerenchyma bunches, there are 2-3 layers of wavy-walled mid-layer cells. Prismatic and quadrangular crystals were observed in the mid-layer cells. In the innermost region, endosperm cells are present (Fig. 3d). Cypselas ultrathin sections have a thick cuticle layer on the epidermal cells (Fig. 3e). The walls of sclerenchyma cells are quite thick, and others are less electron-dense (Fig. 3f). The phytomelanin in the cypselas is observed in mid-layer cells with thick walls (Fig. 3g).

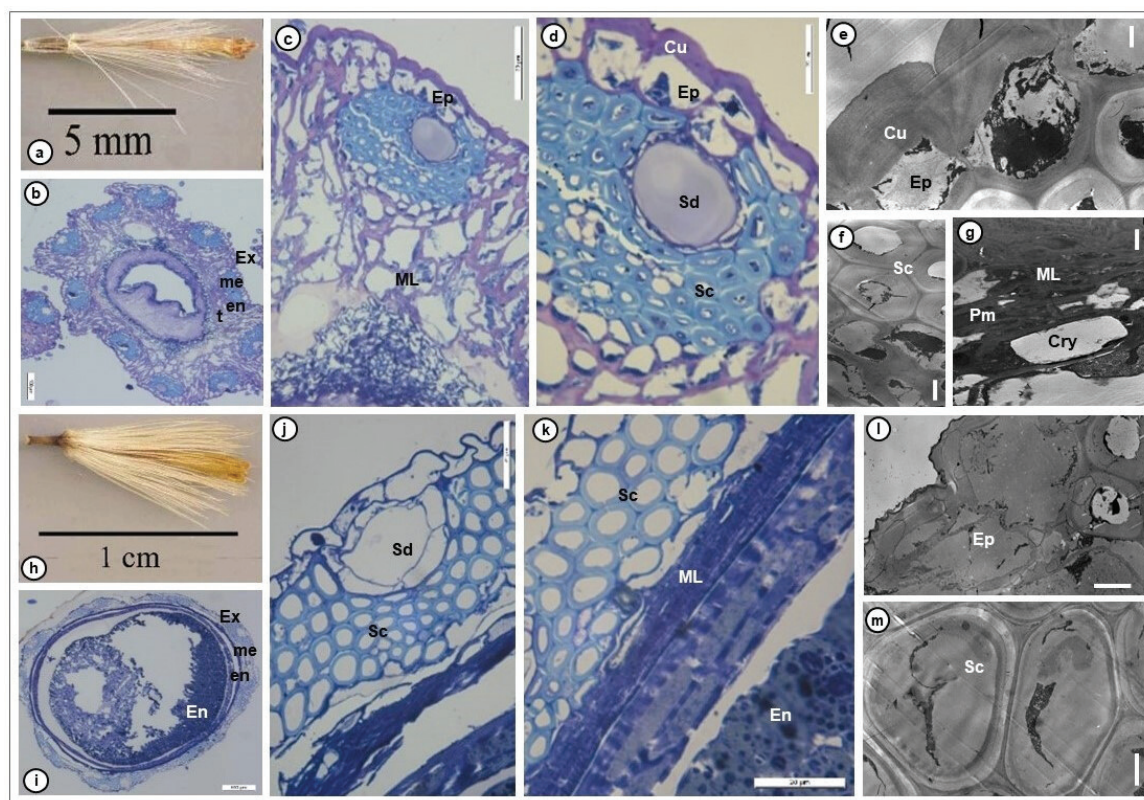


Fig. 4. *Senecio castagneanus*: a – cypselas, b – general view of cypselas structure, scale bar = 100 μ m, c – epidermal cells and mid-layer cells, scale bar = 50 μ m, d – secretory duct in sclerenchyma bundle, scale bar = 20 μ m, e – electron micrograph of epidermal cells, scale bar = 5 μ m, f – electron micrograph of sclerenchyma bundle, scale bar = 5 μ m, g – crystals in mid-layer cells, scale bar = 1 μ m; *Senecio fluviatilis*: h – cypselas, i – general view of cypselas structure, scale bar = 100 μ m, j – sclerenchyma bundle and secretory duct, scale bar = 20 μ m, k – mid-layer cells and endosperm, scale bar = 20 μ m, l – electron micrograph of epidermal cells, scale bar = 5 μ m, m – electron micrograph of sclerenchyma bundle, scale bar = 5 μ m.

Senecio salsuginea H. Duman & Vural

Cypselas has a wavy wall and oval shape (Fig. 3h, i). The cuticle layer on the epidermal cells was thick and dark-stained (Fig. 3j). Sclerenchyma bundles were observed in the wavy regions of the cypselas. The sclerenchyma cells are thick-walled, and their lumen is narrow. Under the sclerenchyma bundles are 2-3 layers of wavy-walled mid-layer cells. This layer consists of thin-walled, large parenchymatic cells (Fig. 3k). In the innermost part, there are endosperm cells. In the cypselas ultrathin sections, the epidermal cells are oval and have a thick layer of cuticle (Fig. 3l). Sclerenchyma cell walls are very thick and electron-opaque (Fig. 3m). Underneath these cells are mid-layer cells with wavy walls. Under the mid-layer are long rectangular endosperm cells with thickened walls (Fig. 3n).

Senecio castagneanus DC.

In this cypselas, sclerenchyma cells located in the form of bundles along the walls were observed (Fig. 4b, c). Under the epidermal cells, secretory ducts were observed along the pericarp wall. There are some thin parenchymatic cells around the secretory ducts (Fig. 4d). Below the sclerenchyma bundles, there are 2-3 layers of wavy walled mid-layer cells. In electron microscopy images, epidermal cells were

observed to have dense cytoplasm (Fig. 4e). Sclerenchyma cell walls are very thick, and their lumens are filled in most cells. The walls of sclerenchyma cells are electron-opaque (Fig. 4f). The walls of the mid-layer cells are very thick, and crystals were observed in some cells. Additionally, these mid-layer cells have wavy walls beneath them. The crystals appear to be typically prismatic or rectangular and are electron-transparent. Phytomelanin accumulates inside the intercellular spaces in mid-layer cells (Fig. 4g).

Senecio fluviatilis Wallr.

Cypselas is circular (Fig. 4h, i). Under the epidermis, there are large secretory ducts surrounded by sclerenchymal cells (Fig. 4j). There are some thin-walled parenchymatic cells around the secretory ducts. Wavy wall mid-layer cells were observed under the sclerenchyma cells. Mid-layer cells are darkly stained. Inside, there are endosperm cells (Fig. 4k). Cypselas ultrathin sections have a thick cuticle layer on the epidermal cells (Fig. 4l). Sclerenchyma cell walls are thin and electron-transparent (Fig. 4m).

Senecio pseudo-orientalis Schischk.

In semithin sections, the exocarp is wavy and oval (Fig. 5b). In dicotyledons, the embryo occupies a significant part

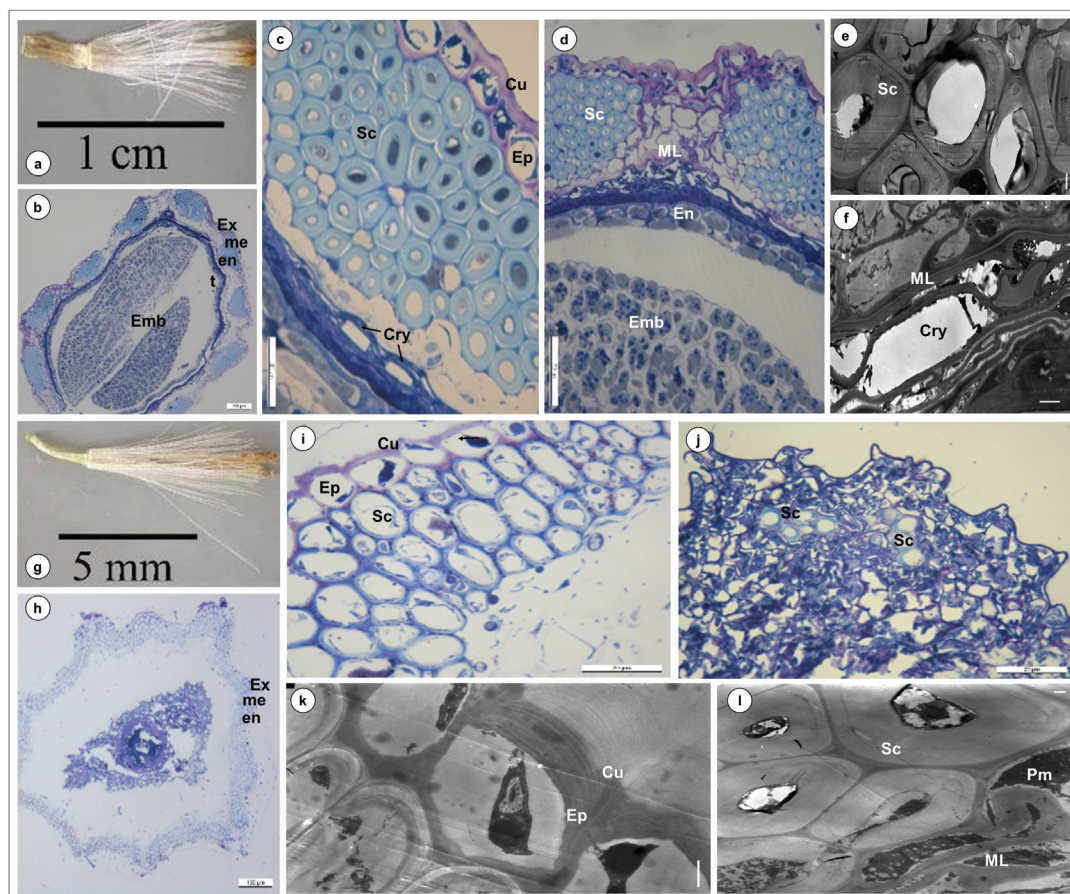


Fig. 5. *Senecio pseudo-orientalis*: a – cypselus, b – general view of cypselus structure, scale bar = 100 µm, c – sclerenchyma cells, scale bar = 20 µm, d – mid-layer cells, scale bar = 50 µm, e – electron micrograph of sclerenchyma cells, scale bar = 5 µm, f – electron micrograph of mid-layer cells and crystals, scale bar = 1 µm; *Senecio racemosus*: g – cypselus, h – general view of cypselus structure, scale bar = 100 µm, i – sclerenchyma cells, scale bar = 20 µm, j – mid-layer cells, scale bar = 20 µm, k – electron micrograph of epidermal cells, scale bar = 5 µm, l – electron micrograph of sclerenchyma bundle, scale bar = 5 µm.

of the seed. The cuticle of the epidermal cells is stained dark. Sclerenchyma bundles were observed in the wavy regions of the pericarp. Sclerenchyma cells have thick walls and narrow lumens. In some cells, the lumen is full. There are irregularly shaped mid-layer cells under the sclerenchyma bundles. Crystals in these cells are prismatic and rectangular, arranged in regular layers (Fig. 5c). Inside are polygonal endosperm cells (Fig. 5d). In ultrathin sections, the walls of sclerenchyma cells are electron opaque (Fig. 5e). Electron-dense phytomelanin was observed in mid-layer cells (Fig. 5f).

Senecio racemosus (M.Bieb.) DC

The pericarp and seed membrane are partially fused with each other, and the cuticle layer on the epidermal cells appears darkly stained (Fig. 5h, i). Below the epidermis, in the mesocarp, sclerenchyma cells are present with 3-4 layers of dark-stained and thickened walls. The sclerenchyma is uninterrupted, and the lumens of its cells are large. In some cells, the lumen is full. Cells stained dark in cypselus sections are mid-layer cells (Fig. 5j). Cytoplasm is dense in these cells. 2-4 sclerenchyma cells were observed at certain locations. In the cypselus ultrathin sections, the epidermal

cells are rectangular and have a thick layer of cuticle (Fig. 5k). It has been observed that sclerenchyma cell walls are thick, as in other species, and lumens are filled in some cells (Fig. 5l). The walls of sclerenchyma cells are electron-opaque. The cytoplasm was observed to be dense in the phytomelanin-containing mid-layer cells, which were located under the sclerenchyma cells.

Discussion

In this study, the cypselus structures of eight *Senecio* were described ultrastructurally and anatomically. Although ultrastructural studies can be used as a powerful tool in species delimitation, detailed studies on the cypselae of *Senecio* taxa have not been conducted. However, in our previous studies (Büyükkartal et al., 2017, 2018), 14 *Senecio* taxa were also examined.

Many studies have reported that the cypselus in the Asteraceae family is a single-chambered, dry fruit that does not open (Judd et al. 2002, Marzinek et al. 2008, Funk et al. 2009, Jeffrey 2009, Mandel et al. 2019). It has been stated that the characters of pericarp layer cells, the presence or absence of calcium oxalate crystals in these layer cells, phytomelanin

accumulation, secretory ducts, and different cell structures have taxonomic value (Roth 1977, Jana and Mukherjee 2014). In *Senecio*, the cypselae comprise the pericarp, testa, endosperm, and cotyledons. The mature pericarp is divided into three regions: the exocarp (outer epidermis), mesocarp, and endocarp (inner epidermis). There is a thick and dark cuticle layer on the epidermal cells of all species examined. Papillae were observed in the epidermal cells of *S. olympicus*, *S. paludosus*, and *S. tauricolus*. In our previous studies, trichomes were found in the epidermis of *S. grandidentatus* Ledeb., *S. trapezuntinus* Boiss., and *S. cilicius* Boiss. In *S. hypochionaeus* Boiss. subsp. *hypochionaeus*, papillae were observed in epidermal cells (Büyükkartal et al. 2017, 2018). Roth (1977) and Jana and Mukherjee (2014) reported that cypselae are generally variable in length, glabrous in structure, with or without pappus, and are ribbed or smooth. In this study, it was observed that sclerenchyma cells were less developed in the pericarp in *S. olympicus*, while they were highly developed in the other seven species. Others have reported that the presence of sclerenchyma tissue at the genus level in the Asteraceae family is taxonomically important (Wiklund 1983, Anderberg 1991, Zhu et al. 2006, Biswas et al. 2014).

Secretory ducts were observed along the pericarp wall in the mid-layer cells of *S. olympicus*, *S. tauricolus*, *S. castagneanus*, and *S. fluviatilis*. No secretory ducts were found in the other four species (*S. paludosus*, *S. salsuginea*, *S. pseudo-orientalis*, and *S. racemosus*). In our previous studies, secretory ducts along the pericarp wall were observed only in *S. grandidentatus* and *S. eriospermus* DC. subsp. *eriospermus* (Büyükkartal et al. 2017, 2018). Robinson (1981) reported that resin or secretory canals are commonly found in vegetative tissues and floral units in Asteraceae.

In this study, long prismatic calcium oxalate crystals were detected in the intermediate cells of *S. tauricolus*, *S. castagneanus*, and *S. pseudo-orientalis*. No crystals were found in the intermediate layer cells of the other five species. In our previous studies, calcium oxalate crystals were observed in the mid-layer cells of *S. aquaticus* Hill subsp. *erraticus* (Bertol.) VA. Mathews, *S. mollis* Willd., and *S. trapezuntinus* (Büyükkartal et al. 2017), *S. eriospermus* subsp. *eriospermus*, *S. eriospermus* subsp. *lorentii* (Hochst), and *S. hypochionaeus* subsp. *hypochionaeus* (Büyükkartal et al. 2018). Similarly, in a study conducted on the determination of fruit morphology and anatomy in Cardueae (Asteraceae), it was reported that the epidermal cell structure in the pericarp, the localization of calcium oxalate crystals, and the formation and location of secretion ducts in the mesocarp are important features for distinguishing taxa (Zarembo and Boyko 2008). Paul (2017) studied the external and internal characteristics of cypselae of *Emilia sonchifolia* (L.) DC. and *Calendula officinalis* L. species belonging to the tribes Senecioneae and Calenduleae. It was stated that the distribution and shape of the crystals are genetically controlled and thus represent taxonomically useful traits.

Electron-dense phytomelanin accumulation was observed in the schizogenous space between the outer and mid-layer cells in all studied species, except in *S. salsuginea* and *S. fluviatilis*. Phytomelanin is highly resistant to degradation and is secreted by fibers in the pericarp (De-Paula et al. 2013, Pandey et al. 2014). In addition, phytomelanin has been detected in many taxa belonging to the Asteraceae family (Freitas et al. 2015, Marques et al. 2021). It has been reported that phytomelanin accumulation in Asteraceae is of taxonomic importance (Freitas et al. 2015, Marques et al. 2022).

Previous studies have pointed out the need to consider cypselae anatomical and cytological features in resolving some taxonomic issues between species and genera. In this study, the anatomical and cytological differences among some *Senecio* species were revealed. The findings regarding the ultrastructural characteristics of cypselae in *Senecio* species appear to be consistent with the data obtained for other members of the Asteraceae family. Data based on anatomical and ultrastructural characteristics of cypselae, such as the organization of sclerenchyma tissue, the presence of papillae or trichomes in epidermal cells, the presence of secretory ducts along the cypselae wall, the localization of prismatic crystals in the middle layer cells, and phytomelanin accumulation in the intercellular spaces of the mesocarp and middle layer cells, can aid in classifying and identifying *Senecio* taxa. This study will further emphasize the value of the cypselae substructure for plant systematics and encourage future research on *Senecio* species. Gaps in our knowledge of *Senecio cypselae* substructure and differentiation make detailed studies essential.

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Author contribution statement

The plant materials were secured and collected by ÜB. Plants were photographed by ÜB and systematic revision work was carried out by the research team. HNB and HÇ conducted LM and TEM observations. EK and HNB wrote the first version of the manuscript. BK contributed to the sectioning and preparation processes. All authors have read and approved the final version of the manuscript.

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