



Research Article

Stem and leaf anatomy and achene structure in some species of *Senecio* (Asteraceae).

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Abstract: The genus *Senecio* L. belongs to the tribe Senecioneae Cass. of the family *Asteraceae* which is the richest plant family of Turkey in terms of endemic species. The study was conducted on a total of 8 taxa belonging to *Senecio*. 4 taxa were collected from West and 4 were collected from East Anatolia. Stem and leaf anatomy of the taxa were studied by light microscopy; achene structure was examined by light microscopy and transmission electron microscopy in order to identify morphological, anatomical and histological modifications and resolve taxonomic problems of the genus.

Keywords: *Senecio*, anatomy, achene, TEM

Introduction

Asteraceae (*Compositae*) is the richest plant family of Turkey in terms of endemic species (Erik and Tarıkahya, 2004). The genus *Senecio* L. belongs to the tribe Senecioneae Cass. of the family *Asteraceae* (*Compositae*). *Senecioneae* is the largest tribe of the *Asteraceae* comprising 150 genera and 3,500 species distributed worldwide (Nordenstam, 2007). The tribe *Senecioneae* is represented in Turkish flora by three subtribes and a total of eight genera including subsequent additions of *Ligularia* Cass., *Crassocephalum* Moench and *Adenostyles* Cass. (Matthews, 1975; Kit, 2000; Akıncı and Özhatay, 2004). The newly described genera *Jacobaea* L., *Iranecio* B.Nord. and *Caucasalia* B.Nord. which also have taxa in Turkey are not recognized due to their taxonomic characters being insufficient and transitive. As a result of taxonomic studies, Turkish *Senecio* species were suggested to be transferred to the genera *Senecio* and *Tephrosieris* (Rchb.) Rchb. (Hamzaoglu *et al.*, 2009).

The genus *Senecio* is represented by 102 species in Flora of the U.S.S.R, 67 species in Flora Europaea, 29 species (including *Iranecio*) in Flora Iranica and five species in Flora Palaestina (Shishkin, 1995; Chater and Walters, 1976; Nordenstam, 1989; Fernbrun-Dothan, 1978). The genus is divided into sections and even series in the papers such as Flora Orientalis, Flora Europaea and Flora of the U.S.S.R. (Boissier, 1875; Chater and Walters, 1976; Shishkin, 1995). However, no sectional classification is made in Flora of Turkey (Matthews, 1975). *Senecio* is represented in Flora of Turkey by 52 taxa (43 species, 3 subspecies and 6 varieties). 21 of these taxa are endemic for Turkey and the rate of endemism is 40%. Many morphological, anatomical (Adedeji and Jewoola, 2008; Noorbakhsh, *et al.*, 2008; Akçin and Akçin, 2010; Garcia Brejio *et al.*, 2011; Bona, 2014; Özbek *et al.*, 2014; Sosa *et al.*, 2014; Selvi *et al.*, 2014; Karaismailoğlu, 2015; Özcan *et al.*, 2015; Candan *et al.*, 2016; Norouzi *et al.*, 2016; Ivashchenko and Ivarenko, 2017), palynological (Kandemir *et al.*, 2006; Ghahreman *et al.*, 2007; Qureshi *et al.*, 2008a,b Özmen *et al.*, 2009; Nbagwu *et al.*, 2009;

Türkmen *et al.*, 2010; Akyalcın *et al.*, 2011; De Abreu *et al.*, 2015), taxonomic and genetical (Karanovic *et al.*, 2015; Hoda Lova' *et al.*, 2015; Andre'S-Sa'Nchez *et al.*, 2015; Ruvimbo *et al.*, 2016) studies have been conducted to identify relationships among *Asteraceae* taxa.

This study was conducted on a total of 8 taxa belonging to *Senecio*; 4 of which were collected from West Anatolia (*Senecio jacobaea* L. subsp. *jacobaea*, *Senecio aquaticus* Hill subsp. *erraticus* (Bertol.) V.A.Matthews, *Senecio maritimus* (L.) Rchb, *Senecio sandrasicus* P.H.Davis) and 4 collected from East Anatolia (*Senecio erucifolius* L. subsp. *erucifolius*, *Senecio grandidentatus* Ledeb., *Senecio mollis* Willd., *Senecio trapezuntinus* Boiss.). Stem and leaf anatomy of the taxa were studied by light microscopy; seed coat (achene+testa) was examined by light microscopy and transmission electron microscopy (TEM) in order to identify morphological, anatomical and histological modifications and contribute to taxonomic and phylogenetic assessment of the genus.

Materials and Methods

Stem and leaf anatomy and achene histology were examined in the taxa of *Senecio* some species (Table 1) (Fig. 1a-h). Upper, middle and lower leaf samples and young stem samples were taken from each taxon. These were prepared for anatomical studies using paraffin method (Algan, 1981). 8-10 µm sections from leaves and 12-15 µm sections from stems were taken and stained with safranin-fast green. For surface-section analyses, a sample of 20 surface-sections was taken separately from lower and upper surfaces of each of 5 randomly selected leaves per each taxon and stomatal index and stomatal index rates were calculated by the formula based on number of epidermal cells and stomata per mm². For cross-section analyses, 5 cross-sections were taken from each of 5 randomly selected leaves per each taxon and average leaf blade thickness (µm) was measured (Table 2).

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Table 1. Examined taxa of *Senecio* and collection localities

Taxon Name	Collector	Collection Locality
<i>Senecio jacobaea</i> L. subsp. <i>jacobaea</i> (<i>S. aquaticus</i> Hill subsp. <i>aquaticus</i>)	Budak- 2192	Kırklareli: Kırklareli-Edirne direction, 14th km, 100 m, 23.07.2007, Budak 2192, Hamzaoğlu & Aksoy (Bozok Hb.).
<i>S. aquaticus</i> Hill subsp. <i>erraticus</i> (Bertol.) V.A.Matthews	Budak- 2191	Bolu-Yedigöller direction, entrance of Yukarı Soku village, 740 m, 22.07.2007, Budak 2191, Hamzaoğlu & Aksoy (Bozok Hb.).
<i>Senecio maritimus</i> (L.) Rchb (<i>S. bicolor</i> (Willd.) Tod. Subsp. <i>bicolor</i> var. <i>bicolor</i>)	Hamzaoğlu- 4378	Isparta: Süleyman Demirel University Campus, Faculty of Science and Arts locality, 27.07.2006, Hamzaoğlu 4378, Aksoy & Budak (Bozok Hb.).
<i>Senecio sandrasicus</i> P.H.Davis	Hamzaoğlu - 4379	Muğla: Köyceğiz Yaylaköy (Ağla) village, Sandras Mountain locality, 1545 m, 28.07.2006, Hamzaoğlu 4379, Aksoy & Budak (Bozok Hb.);
<i>Senecio erucifolius</i> L. subsp. <i>erucifolius</i>	Hamzaoğlu- 4997	Ağrı: Doğubayazıt Tendürek Passage, 2590 m, 30.07.2007, Hamzaoğlu 4997, Aksoy & Budak (Bozok Hb.).
<i>Senecio grandidentatus</i> Ledeb	Hamzaoğlu- 5368	Ardahan: Posof, Yurtbekler village, 1300 m, 23.08.2008, Hamzaoğlu 5368, Budak & Aksoy (Bozok Hb.).
<i>Senecio mollis</i> Willd.	Budak-2122	Sivas: Taşlıdere, iron-steel factory junction locality, 1330 m, 18.07.2006, Budak 2122, Hamzaoğlu & Aksoy (Bozok Hb.).
<i>Senecio trapezuntinus</i> Boiss.	Budak-2011	Trabzon: Esentepe quarter direction, Maşatlık locality, 120 m, 21.04.2006, Budak 2011, Hamzaoğlu & Aksoy (Bozok Hb.).

Table 2. Mesophyll size, number of stomata per mm², number of epidermal cells, stomatal index and stomatal index rates in *Senecio* taxa

<i>Senecio</i> taxa	Mesophyll size (µm)	Surface of the section	Stomata Number (number/mm ²)	Number of Epidermal Cells (number/mm ²)	Stomatal Index	Stomatal Index Rate
<i>Senecio jacobaea</i> L. subsp. <i>jacobaea</i>	266.6±6.5	Adaxial surface	24.34±1.7	172.96±4.0	12.33±3.2	0.67
		Abaxial surface	37.15±3.8	164.09±5.9	18.46±1.5	
<i>S. aquaticus</i> Hill subsp. <i>erraticus</i> (Bertol.) V.A.Matthews	135.6±4.6	Adaxial surface	42.2±0.2	368.1±0.8	10.29±0.8	0.36
		Abaxial surface	185.4±0.3	463.4±0.4	28.58±0.1	
<i>Senecio maritimus</i> (L.) Rchb	229.9±12.0	Adaxial surface	88.1±0.6	440.9±0.0,	16.65±0.1	0.64
		Abaxial surface	300.5±6.0	851.4±1.3	26.08±0.6	
<i>Senecio sandrasicus</i> P.H.Davis	246.7±4.8	Adaxial surface	42.64±0.1	483.2±0.2	8.11±0.5	0.59
		Abaxial surface	76.0±0.0	485.4±5.9	13.54±0.2	
<i>Senecio erucifolius</i> L. subsp. <i>erucifolius</i>	135.6±4.6	Adaxial surface	42.2±0.2	368.1±0.8	10.29±0.8	0.36
		Abaxial surface	184.4±0.3	462.4±0.4	26.58±0.1	
<i>Senecio grandidentatus</i> Ledeb	228.9±12.0	Adaxial surface	86.1±0.6	448.9±0.0,	16.64±0.1	0.64
		Abaxial surface	300.5±6.0	851.4±1.3	26.08±0.6	
<i>Senecio mollis</i> Willd.	269±7.7	Adaxial surface	112.3±1.6	458.3±3.1	19.68±0.6	0.89
		Abaxial surface	118.8±0.0	415.8±0.0	22.22±0.2	
<i>Senecio trapezuntinus</i> Boiss.	111.1±6.5	Adaxial surface	51.2±0.1	385.2±1.0	11.73±0.7	0.45
		Abaxial surface	105.5±0.0	350.3±3.3	26.27±0.5	

For TEM studies, achene samples per each taxon were first fixed with 3% glutaraldehyde and then with 1% osmium tetroxide. Following dehydration and saturation, samples were embedded in Epon 812 (Luft, 1961). Semi-thin sections were taken from these blocks and the sections were stained with methylene blue and toluidine blue. Ultrathin sections were first stained with uranyl acetate (Stempak and Ward, 1964) and lead citrate (Sato, 1967) then examined with Jeol CX-100 TEM.

Results

In leaf cross-sections of *Senecio jacobaea* L. subsp. *jacobaea* collected from Kırklareli-Edirne locality, two layers of cylindrical palisade parenchyma cells are observed in mesophyll tissue below upper epidermis (Fig. 2a). Sponge parenchyma cells are observed below mesophyll tissue. Leaves are bifacial. Leaf thickness is 266.6. ±6.5 µm (Table 2) and several layers of collenchyma cells are found in the midrib located between the upper and lower epidermis. Small bundles are collateral. The leaves are amphistomatic (Fig. 2b). Stomata are placed at the same level as the epidermal cells. In adaxial surface, amaryllis type stomata are observed in epidermal cells. The number of stomata is 24.34±1.7 per mm² (Table 2). Palisade parenchyma cells are oval and circular in shape (Fig.

2b-c). Stomata are of anomocytic type and average number of stomata in abaxial surface is 37,15±3.8 per mm² (Table 2). In abaxial surface, wavy-walled epidermal cells (Fig. 2d) have amaryllis type stomata (Fig. 2e). Sponge parenchyma cells have large intercellular spaces (Fig. 2f).

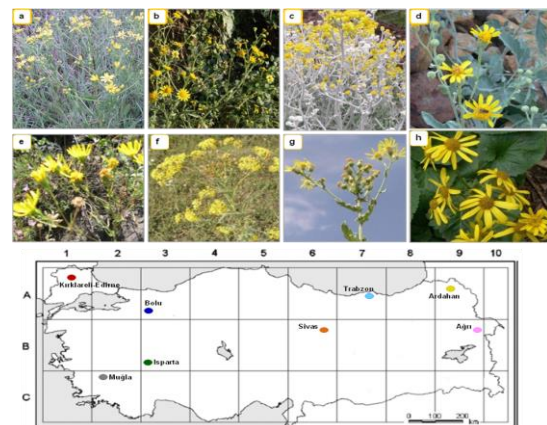


Figure 1. Habitat views and collection localities of *Senecio* taxa in Turkey. **a.** *Senecio jacobaea* L. subsp. *jacobaea* (●) **b.** *Senecio aquaticus* Hill subsp. *erraticus* (Bertol.) V.A. Matthews (●) **c.** *Senecio maritimus* (L.) Rchb (●) **d.** *Senecio sandrasicus* (●) **e.** *Senecio erucifolius* L. subsp. *erucifolius* (●) **f.** *Senecio grandidentatus* Ledeb. (●) **g.** *Senecio mollis* Willd. (●) **h.** *Senecio trapezuntinus* Boiss. (●)

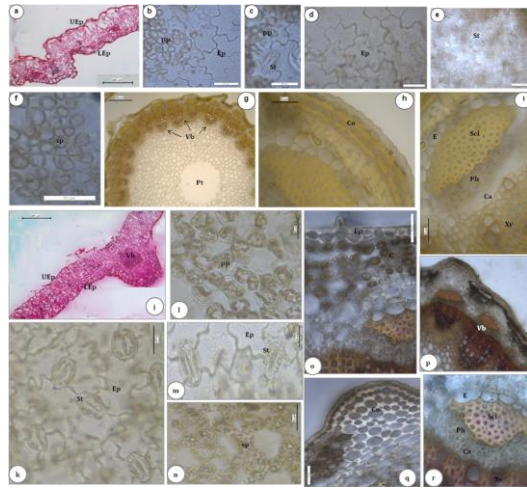


Figure 2. Leaf and stem anatomy of *Senecio jacobaea* L. subsp. *jacobaea* and *Senecio aquaticus* Hill subsp. *erraticus* (Bertol.) V.A. Matthews.

a. Leaf cross section of *S. jacobaea* L. subsp. *jacobaea* Bar = 200 μ m **b.** Epidermis in upper leaf surface Bar = 50 μ m. **c.** Stomata in upper leaf surface Bar = 50 μ m. **d.** Epidermis in lower leaf surface **e.** Stomata in lower leaf surface Bar = 50 μ m. **f.** Sponge parenchyma in lower leaf surface Bar = 50 μ m. **g.** General view of stem Bar = 500 μ m **h.** Collenchyma Bar = 50 μ m **i.** Appearance of a vascular bundle Bar = 50 μ m **j.** Leaf cross-section of *S. aquaticus* Hill subsp. *erraticus* (Bertol.) V.A. Matthews Bar = 200 μ m **k.** Epidermis in upper leaf surface and stomata Bar = 50 μ m **l.** Palisade parenchyma in upper leaf surface Bar = 50 μ m. **m.** Epidermis and stomata in lower leaf surface Bar = 50 μ m. **n.** Sponge parenchyma in lower leaf surface Bar = 50 μ m. **o.** Chlorenchyma Bar = 50 μ m. **p.** General view of stem Bar = 100 μ m **q.** Collenchyma Bar = 50 μ m **r.** Appearance of a vascular bundle Bar = 50 μ m

In stem cross-sections, vascular bundles are of open-collateral type, xylem is located towards the center of the stem and phloem is located towards the epidermis (Fig. 2g). 4-5 layers of lamellar collenchyma cells are seen below epidermis in the wavy parts of the stem (Fig. 2h). 5-7 layers of sclerenchyma bundles are observed above vascular bundles. Sclerenchyma cells are thick-walled (Fig. 2i). The cambium is composed of 2-3 layers of thin-walled cells. Pith tissue is parenchymatic.

In leaf cross-sections of *Senecio aquaticus* Hill subsp. *erraticus* (Bertol.) V.A. Matthews collected from Bolu-Yedigöller locality, leaves are bifacial (Fig. 2j). There are glandular hairs on both leaf surfaces (Fig. 2k). One layer of palisade parenchyma cells is observed in mesophyll tissue below upper epidermis composed by large cells. Below mesophyll tissue, there are 3-4 layers of sponge parenchyma with large intercellular spaces. 2-3 layers of collenchyma cells are found in the midrib located between the upper and lower epidermis. Small bundles are collateral. Average mesophyll size is measured as $135.6 \pm 4.6 \mu$ m (Table 2). There are wavy-walled epidermal cells in abaxial and adaxial surfaces (Fig. 2l). Amaryllis type stomata are observed in surface-sections. Stomata cells are placed at the same level as the epidermal cells. Stomata neighbouring cells are of the same shape as epidermal cells and their number ranges between 3-

5. Stomata are of anomocytic type and average numbers of stomata in adaxial and abaxial surfaces are 42.2 ± 0.2 and 185.4 ± 0.3 per mm^2 , respectively (Table 2). In adaxial surface, palisade parenchyma cells are oval and circular in shape (Fig. 2m). In abaxial surface, amaryllis type stomata are observed in epidermal cells. (Fig. 2n). Sponge parenchyma cells have large intercellular spaces (Fig. 2o).

The stem is surrounded by a single layer of epidermis with a thick cuticle at the outermost layer (Fig. 2p). 2-3 layers of lamellar collenchyma are seen below epidermis concomitant with a significant decrease in wall thickness at the corner where they connect with the cortex (Fig. 2q). Parenchymatic cells with abundant chloroplast (Chlorenchyma) exist just below epidermis around large vascular bundles (Fig. 2r). Large groups of phloem sclerenchyma bundles are located between cortex parenchyma and phloem. 2-3 layers of vascular cambium are located below phloem (Fig. 2r).

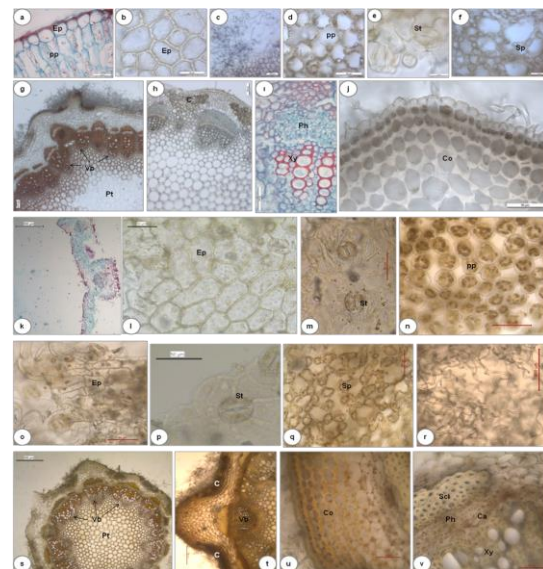


Figure 3. Leaf and stem anatomy of *Senecio maritimus* (L.) Rchb (*Senecio bicolor* (Willd.) Tod. ssp. *bicolor* var. *bicolor*) and *Senecio sandrasicus* P.H.Davis

a. Palisade parenchyma in leaf of *S. maritimus* (L.) Rchb Bar = 50 μ m **b.** Epidermis in upper leaf surface Bar = 50 μ m. **c.** Cover hairs in upper leaf surface Bar = 100 μ m. **d.** Palisade parenchyma in upper leaf surface Bar = 50 μ m. **e.** Epidermis and stomata in lower leaf surface Bar = 50 μ m. **f.** Sponge parenchyma in lower leaf surface Bar = 50 μ m. **g.** General view of stem Bar = 100 μ m **h.** Chlorenchyma Bar = 100 μ m **i.** Appearance of a vascular bundle Bar = 50 μ m **j.** Collenchyma Bar = 50 μ m. **k.** General view of leaf cross-section of *S. sandrasicus* P.H.Davis Bar = 500 μ m **l.** Epidermis in upper leaf surface Bar = 50 μ m. **m.** Stomata in upper leaf surface Bar = 50 μ m. **n.** Palisade parenchyma in upper leaf surface Bar = 50 μ m. **o.** Epidermis and cover hairs in lower leaf surface Bar = 50 μ m. **p.** Epidermis and stomata in lower leaf surface Bar = 50 μ m. **q.** Sponge parenchyma in lower leaf surface Bar = 50 μ m. **r.** Cover hairs in lower leaf surface Bar = 50 μ m. **s.** General view of stem Bar = 500 μ m. **t.** Chlorenchyma Bar = 200 μ m. **u.** Collenchyma Bar = 50 μ m. **v.** Appearance of a vascular bundle Bar = 50 μ m.

In leaf cross-sections of *Senecio maritimus* (L.) Rehb collected from Isparta: Süleyman Demirel University Campus, Faculty of Science and Arts locality, 2-3 layers of cylindrical palisade parenchyma cells are observed in mesophyll tissue below upper epidermis (Fig. 3a). Below mesophyll tissue is sponge parenchyma. Leaves are bifacial. Leaf thickness (average mesophyll size) is $229.9 \pm 12.0 \mu\text{m}$ (Table 2) and several layers of collenchyma cells are found in the midrib located between the upper and lower epidermis. Arc-shaped sclerenchyma bundles are seen around vascular bundles. Small bundles are of collateral type. The leaves are amphistomatic. Stomata are placed at the same level as the epidermal cells. Stomata are of anomocytic type and average numbers of stomata in adaxial and abaxial surfaces are 88.1 ± 0.6 and 300.5 ± 6.0 per mm^2 , respectively (Table 2). In adaxial surface, amaryllis type stomata and abundant glandular hairs are observed in polygonal-shaped epidermal cells (Figs. 3b-c). Palisade parenchyma cells are oval and circular in shape. Chloroplasts are abundant and aligned along the cell wall (Fig. 3d). Amaryllis type stomata are observed in abaxial surface (Fig. 3e). Sponge parenchyma cells have large intercellular spaces (Fig. 3f).

In stem cross-sections, vascular bundles are of open-collateral type (Fig. 3g). The number of vascular bundles in stem primary structure ranges between 20-24. 5-7 layers of sclerenchyma bundles are seen above vascular bundles. Sclerenchyma cells have thick walls (Fig. 3h). The cambium is composed of 1-2 layers of thin-walled cells. Trachea in xylem are formed from a row of cylindrical cells arranged in radial series (Fig. 3i). 4-5 layers of lamellar collenchyma cells are seen below epidermis (Fig. 3j). Angular collenchyma cells exist in some parts. Like the leaf cross-sections, stem cross-sections have cover and glandular hairs. The head of glandular hairs has about 2-3 and the stalk has about 2-7 cells. Pith tissue is comprised of parenchymatic cells.

In leaf cross-sections of *Senecio sandrasicus* P.H.Davis collected from Muğla: Köyceğiz Yayla (Ağla) village, Sandras Mountain locality, leaves are bifacial. There are glandular hairs on both leaf surfaces. 1-2 layers of palisade parenchyma cells are observed in mesophyll tissue below upper epidermis. Sponge parenchyma cells with large intercellular spaces are seen below mesophyll tissue. 2-3 layers of collenchyma cells are found in the midrib located between the upper and lower epidermis. Small bundles are collateral (Fig. 3k). Average mesophyll size is measured as $246.7 \pm 4.8 \mu\text{m}$ (Table 2).

Epidermal cells in lower and upper leaf sections have flat walls perforated by simple pits (Fig. 3l). Amaryllis type stomata are observed in surface-

sections (Fig. 3m). Stomata are placed at the same level as the epidermal cells. Stomata neighbouring cells are of the same shape as epidermal cells and their number ranges between 3-5. Stomata are of anomocytic type and average numbers of stomata in adaxial and abaxial surfaces are 42.64 ± 0.1 and 76.0 ± 0.0 per mm^2 , respectively (Table 2). Palisade parenchyma cells are oval and contain chloroplasts (Fig. 3n). Average numbers of epidermal cells in adaxial and abaxial surfaces are 483.2 ± 0.2 and 485.4 ± 5.9 per mm^2 , respectively (Table 2). Lower leaf surface has stomata and leaves are amphistomatic (Figs. 3o-p). Sponge parenchyma cells have large intercellular spaces (Fig. 3q). Lower leaf surface has plenty of cover hairs (Fig. 3r).

The stem is surrounded by a single layer of epidermis with a thick cuticle at the outermost layer (Fig. 3s). Parenchymatic cells with abundant chloroplast (Chlorenchyma) exist just below epidermis around large vascular bundles. The stem has abundant cover and glandular hairs (Fig. 3t). 2-3 layers of lamellar collenchyma cells are seen below epidermis (Fig. 3u). In stem cross-sections, vascular bundles are of open-collateral type (Fig. 3v). Large groups of phloem sclerenchyma bundles are located between cortex parenchyma and phloem. 2-3 layers of vascular cambium are located below phloem. An internal gland with apparent intercellular spaces exists just above the phloem sclerenchyma. The layer just above vascular bundles separating the outer cortex from the central cylinder is endodermis.

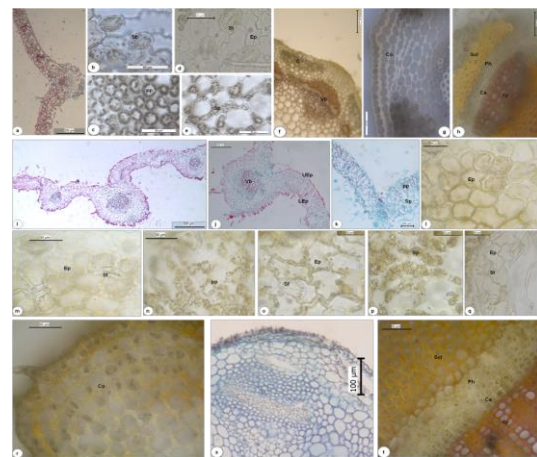


Figure 4. Leaf and stem anatomy of *Senecio erucifolius* L. subsp. *erucifolius* and *Senecio grandidentatus* Ledeb.

a. Main rib in leaf cross-section of *S. erucifolius* L. subsp. *erucifolius* Bar = 200 μm . **b.** Epidermis and stomata in upper leaf surface Bar = 50 μm . **c.** Palisade parenchyma in upper leaf surface Bar = 50 μm . **d.** Epidermis and stomata in lower leaf surface Bar = 50 μm . **e.** Sponge parenchyma in lower leaf surface Bar = 50 μm . **f.** General view of stem Bar = 200 μm . **g.** Collenchyma Bar = 50 μm . **h.** Appearance of a vascular bundle Bar = 50 μm . **i-j.** General view of leaf cross-section and midrib i-Bar = 500 μm , j-Bar = 200 μm . **k.** Mesophyll tissue in leaf cross-section of *S. grandidentatus* Ledeb. Bar = 50 μm . **l.** Epidermis in upper leaf surface Bar = 50 μm . **m.** Stomata in upper leaf surface Bar = 50 μm . **n.** Palisade parenchyma in upper leaf surface Bar = 50 μm . **o.** Epidermis and stomata in lower leaf surface Bar = 50 μm . **p.** Sponge parenchyma in lower leaf surface Bar = 50 μm . **q.** Cover

hairs in lower leaf surface Bar = 50 μm . r. Collenchyma in stem Bar = 500 μm . s-t. Appearance of a vascular bundle s-Bar = 100 μm , t-Bar = 50 μm .

In leaf cross-sections of *Senecio erucifolius* L. subsp. *erucifolius* collected from Ağrı: Doğubayazıt Tendürek Passage, leaves are bifacial (Fig. 4a). One layer of palisade parenchyma cells is observed in mesophyll tissue below upper epidermis. Below mesophyll tissue, there are 3-4 layers of sponge parenchyma with large intercellular spaces. 2-3 layers of collenchyma cells are aligned in the midrib located between the upper and lower epidermis. Small bundles are collateral. Epidermal cells in abaxial and adaxial sections have wavy walls perforated by simple pits. Amaryllis type stomata are observed in surface-sections (Fig. 4b). Stomata are placed at the same level as the epidermal cells. Stomata are of anomocytic type and average numbers of stomata in adaxial and abaxial surfaces are 42.2 ± 0.2 and 184.4 ± 0.3 per mm^2 , respectively. Average numbers of epidermal cells in adaxial and abaxial surfaces are 368.1 ± 0.8 and 462.4 ± 0.4 per mm^2 , respectively. In upper leaf surface, palisade parenchyma cells are oval and circular in shape (Fig. 4c). Lower leaf surface has stomata and leaves are amphistomatic (Fig. 4d). Sponge parenchyma cells have large intercellular spaces (Fig. 4e). Average mesophyll size is 135.6 ± 4.6 μm (Table 2).

The stem is surrounded by a single layer of epidermis with a thick cuticle at the outermost layer (Fig. 4f). Parenchymatic cells with abundant chloroplast (Chlorenchyma) exist just below epidermis around large vascular bundles (Fig. 4f). 2-3 layers of lamellar collenchyma are seen below epidermis concomitant with a significant decrease in wall thickness at the corner where they connect with the cortex (Fig. 4g). In stem cross-sections, vascular bundles are of open-collateral type (Fig. 4h). Large groups of phloem sclerenchyma bundles are present between cortex parenchyma and phloem. 2-3 layers of vascular cambium are located below phloem.

In leaf cross-sections of *Senecio grandidentatus* Ledeb. collected from Ardahan: Posof, Yurtbekler Village, leaves are bifacial (Fig. 4i). One layer of palisade parenchyma cells is observed in mesophyll tissue below upper epidermis composed by large cells. Below mesophyll tissue, there are 2-3 layers of sponge parenchyma with large intercellular spaces. 2-3 layers of collenchyma cells are located in the midrib located between the upper and lower epidermis. Vascular bundles are of collateral type (Figs. 4j, k). Average mesophyll size is measured as 228.9 ± 12.0 μm (Table 2). Rectangular, pentagonal, hexagonal epidermal cells in lower and upper leaf sections have flat walls perforated by simple pits (Figs. 4l, m). Amaryllis type stomata are observed in surface-sections (Figs. 4n, o). Stomata are placed at the same level as the epidermal cells. Stomata are of

anomocytic type and average numbers of stomata in adaxial and abaxial surfaces are 86.1 ± 0.6 and 300.5 ± 6.0 per mm^2 , respectively. Lower leaf surface has stomata and leaves are amphistomatic. Average numbers of epidermal cells in adaxial and abaxial surfaces are 448.9 ± 0.0 and 851.4 ± 1.3 per mm^2 , respectively. In upper leaf surface, palisade parenchyma cells are oval and circular in shape (Fig. 4p). Sponge parenchyma cells have large intercellular spaces and contain less amount of chloroplast compared to that of palisade cells (Fig. 4q).

The stem is surrounded by a single layer of epidermis with a thick cuticle at the outermost layer. There are large vascular bundles that bulge outward. 2-3 layers of lamellar collenchyma are seen below epidermis concomitant with a significant decrease in wall thickness at the corner where they connect with the cortex (Fig. 4r). Parenchymatic cells with abundant chloroplast (Chlorenchyma) exist just below epidermis around large vascular bundles. In stem cross-sections, vascular bundles are of open-collateral type (Figs. 4s, t). Large groups of phloem sclerenchyma bundles are present between cortex parenchyma and phloem. 2-3 layers of vascular cambium are located below phloem.

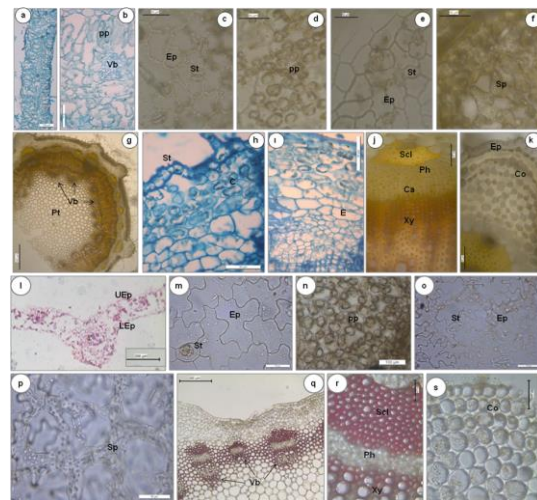


Figure 5. Leaf and stem anatomy of *Senecio mollis* Willd. and *Senecio trapezuntinus* Boiss.

a. Leaf cross-section of *S. mollis* Willd. Bar = 200 μm . b. Mesophyll in leaf cross Bar = 200 μm . c. Epidermis and stomata in upper leaf surface Bar = 50 μm . d. Palisade parenchyma in upper leaf surface Bar = 50 μm . e. Epidermis and stomata in lower leaf surface Bar = 50 μm . f. Sponge parenchyma in lower leaf surface Bar = 50 μm . g. General view of stem Bar = 200 μm . h. Chlorenchyma Bar = 50 μm . i. Endodermis Bar = 50 μm . j. Appearance of a vascular bundle Bar = 50 μm . k. Collenchyma Bar = 50 μm . l. Leaf cross-section and main rib of *S. trapezuntinus* Boiss. Bar = 200 μm . m. Epidermis and stomata in upper leaf surface Bar = 50 μm . n. Palisade parenchyma in upper leaf surface Bar = 100 μm . o. Epidermis and stomata in lower leaf surface Bar = 50 μm . p. Sponge parenchyma in lower leaf surface Bar = 50 μm . q. General view of stem Bar = 200 μm . r. Vascular bundle in stem Bar = 50 μm . s. Collenchyma Bar = 50 μm .

Abbreviations : **C**: Chlorenchyma, **Ca**: Cambium, **Co**: Collencyhna, **E**: Endodermis, **Ep**: Epidermis, **Gh**: Glandular hair, **LEp**: Lower epidermis, **Ph**: Phloem, **pp**: Palisade parenchyma, **Pt**: Pith tissue, **Sd**: Secretory duct, **Scl**: Sclerenchyma, **sp**: Sponge parenchyma, **St**: Stomata, **UEp**: Upper epidermis, **Vb**: Vascular bundle, **Xy**: Xylem.

In leaf cross-sections of *Senecio mollis* Willd. collected from Sivas: Taşlıdere, iron-steel factory junction locality, leaves are bifacial. There are glandular hairs on both leaf surfaces. 1-2 layers of palisade parenchyma cells are observed in mesophyll tissue below upper epidermis composed by large cells. Below mesophyll tissue, there are 2-3 layers of sponge parenchyma with large intercellular spaces. 2-3 layers of collenchyma cells are arranged in the midrib located between the upper and lower epidermis. Small bundles are collateral (Fig. 5a-b). Epidermal cells in upper leaf section have wavy walls perforated by simple pits (Figure 5c). Amaryllis type stomata are observed in surface-sections. Stomata are placed at the same level as the epidermal cells. Stomata neighbouring cells are of the same shape as epidermal cells and their number ranges between 3-5. Stomata are of anomocytic type and average numbers of stomata in adaxial and abaxial surfaces are 112.3 ± 1.6 and 118.8 ± 0.0 per mm^2 , respectively. Lower leaf surface has stomata and leaves are amphistomatic. Average numbers of epidermal cells in adaxial and abaxial surfaces are 458.3 ± 3.1 and 415.8 ± 0.0 per mm^2 , respectively. In upper leaf surface, palisade parenchyma cells are oval and circular in shape (Fig. 5d). In lower leaf surface are wavy-walled epidermal cells (Fig. 5e). Sponge parenchyma cells have large intercellular spaces (Fig. 5f). Average mesophyll size is 269 ± 7.7 μm (Table 2).

The stem is surrounded by a single layer of epidermis with a thick cuticle at the outermost layer (Fig. 5g). Parenchymatic cells with abundant chloroplast (Chlorenchyma) exist just below epidermis around large vascular bundles (Fig. 5h). In stem cross-sections, vascular bundles are of open-collateral type (Figs. 5i-j), xylem is located towards the center of the stem and phloem is located towards the epidermis. Large groups of phloem sclerenchyma bundles are located between cortex parenchyma and phloem. 2-3 layers of vascular cambium are located below phloem. The stem has abundant glandular hairs. An internal gland with apparent intercellular spaces exists just above the phloem sclerenchyma. The layer just above vascular bundles separating the outer cortex from the central cylinder is endodermis. In some samples, secondary thickening is observed in stem. Around large vascular bundles, 2-3 layers of lamellar collenchyma are seen below epidermis concomitant with a significant decrease in wall thickness at the corner where they connect with the cortex (Fig. 5k).

In leaf cross-sections of *Senecio trapezuntinus* Boiss. collected from 60 m east of Trabzon: Boztepe transmitters, leaves are bifacial (Fig. 5l). One layer of palisade parenchyma cells is observed in mesophyll tissue below upper epidermis composed by large cells. Below mesophyll tissue, there are 3-4 layers of sponge parenchyma with large intercellular spaces. 2-3 layers of collenchyma cells are located in the midrib located between the upper and lower epidermis. Small bundles are collateral. Epidermal cells in upper leaf section have wavy walls perforated by simple pits (Fig. 5m). In upper leaf surface, palisade parenchyma cells are oval and circular in shape (Fig. 5n). Chloroplasts are abundant and aligned along the cell wall. Amaryllis type stomata are observed in surface-sections (Figs. 5m, o). Stomata are placed at the same level as the epidermal cells. Stomata neighbouring cells are of the same shape as epidermal cells and their number ranges between 3-5. Stomata are of anomocytic type and average numbers of stomata in adaxial and abaxial surfaces are 51.2 ± 0.1 and 105.5 ± 0.0 per mm^2 , respectively. Lower leaf surface has stomata and leaves are amphistomatic. Average numbers of epidermal cells in adaxial and abaxial surfaces are 385.2 ± 1.0 and 350.3 ± 3.3 per mm^2 , respectively. Sponge parenchyma cells have large intercellular spaces (Fig. 5p). Average mesophyll size is 111.1 ± 6.5 μm (Table 2).

The stem is surrounded by a single layer of epidermis with a thick cuticle at the outermost layer. Parenchymatic cells with abundant chloroplast (Chlorenchyma) exist just below epidermis around large vascular bundles (Fig. 5q). In stem cross-sections, vascular bundles are of open-collateral type (Fig. 5r) Large groups of phloem sclerenchyma bundles are located between cortex parenchyma and phloem. 2-3 layers of vascular cambium are located below phloem. An internal gland with apparent intercellular spaces exists just above the phloem sclerenchyma. The layer just above vascular bundles separating the outer cortex from the central cylinder is endodermis. 2-3 layers of lamellar collenchyma are present below epidermis (Fig. 5s).

In achene fruit of *S. jacobaea* L. subsp. *jacobaea*, the cuticle above epidermal cells is thick and dark-stained (Figs. 6a-b). Below epidermis are 3-4 layers of sclerenchyma bundles among thin-walled cells (Fig. 6c). Sclerenchyma cells are thick-walled and electron-opaque (Fig. 6d). Mid-layer cells have wavy and thick walls and tannin is detected in some of the cells (Fig. 6e). Achene of *S. aquaticus* Hill ssp. *erraticus* (Bertol.) V.A. Mathews. (*Senecio erraticus* Bertol.) is circular (Fig. 6f). Epidermal cells are long and wavy-walled. The cuticle is dark stained. Below epidermis are 3-4 layers of thick-walled sclerenchyma cells (Figs. 6g-h). Below sclerenchyma cells are mid-layer cells, some having

prismatic crystals (Fig. 6i). In electron microscopy images, a thick cuticle was detected above epidermal cells (Fig. 6j). Sclerenchyma cells have thick and electron-opaque walls (Fig. 6k). Prismatic crystals are seen in mid-layer cells (Fig. 6l).

In achene fruit of *S. maritimus* (L.) Rchb., there is a thin cuticle above epidermal cells (Fig. 6m). Below epidermis are 5-6 layers of sclerenchyma cells (Fig. 6n). Below sclerenchyma cells, there are 3-5 layers of thin-walled mid-layer cells. Ultrathin sections show that sclerenchyma cells have thick walls and lumens are full in some of the cells (Fig. 6o). The walls of sclerenchyma cells are electron-transparent. Mid-layer cells are thick-walled (Fig. 6p).

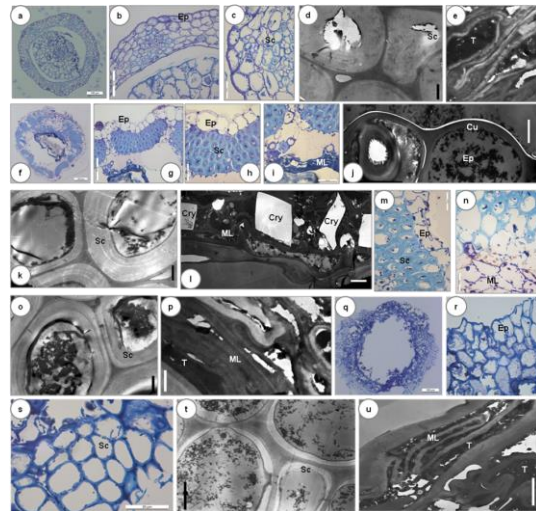


Figure 6a. General view of achene fruit structure of *S. jacobaea* L. subsp. *Jacobaea* Bar = 100 μ m. **b.** Epidermal cells Bar = 50 μ m. **c.** Sclerenchyma cells Bar = 20 μ m. **d.** Electron micrograph of sclerenchyma cells Bar = 2 μ m. **e.** Mid-layer cells in achene fruit Bar = 1 μ m. **f.** General view of achene fruit structure of *S. aquaticus* Hill ssp. *erraticus* (Bertol.) V:A. Mathews. Bar = 100 μ m. **g.** Epidermal cells Bar = 50 μ m. **h.** Sclerenchyma bundle Bar = 20 μ m. **i.** Mid-layer Bar = 20 μ m. **j.** Electron micrograph of epidermal cells Bar = 5 μ m. **k.** Sclerenchyma cells Bar = 3 μ m. **l.** Crystals in mid-layer cells Bar = 3 μ m. **m-n** Epidermis and sclerenchyma cells in achene fruit of *S. maritimus* (L.) Rchb Bars = 20 μ m. **o.** Electron micrograph of sclerenchyma cells Bar = 2 μ m. **p.** Electron micrograph of mid-layer cells Bar = 2 μ m. **q.** General view of achene fruit structure of *S. sandrasicus* P.H.Davis Bar = 100 μ m. **r.** Epidermal cells Bar = 20 μ m. **s.** Sclerenchyma cells in achene fruit Bar = 20 μ m. **t.** Electron micrograph of sclerenchyma cells Bar = 5 μ m. **u.** Electron micrograph of mid-layer cells Bar = 3 μ m.

Achene fruit of *S. sandrasicus* P.H.Davis has a thin-walled cuticle above epidermal cells (Figs. 6q-r). Below epidermis are 3-5 layers of thick-walled sclerenchyma cells (Fig. 6s). Lumens of sclerenchyma cells are wide (Fig. 6t). Wavy-walled, dark-stained mid-layer cells are observed below sclerenchyma cells (Fig. 6u).

In achene fruit of *S. erucifolius* L. subsp. *erucifolius*, epidermis consists of long, oval-shaped cells (Fig. 7a). Small sclerenchyma bundles are detected in dark-stained mid-layer cells (Fig. 7b). In achene electron micrographs, epidermal cells have vacuoles (Fig. 7c). The walls of mid-layer cells are wavy, considerably thick and some are electron-dense (Fig. 7d).

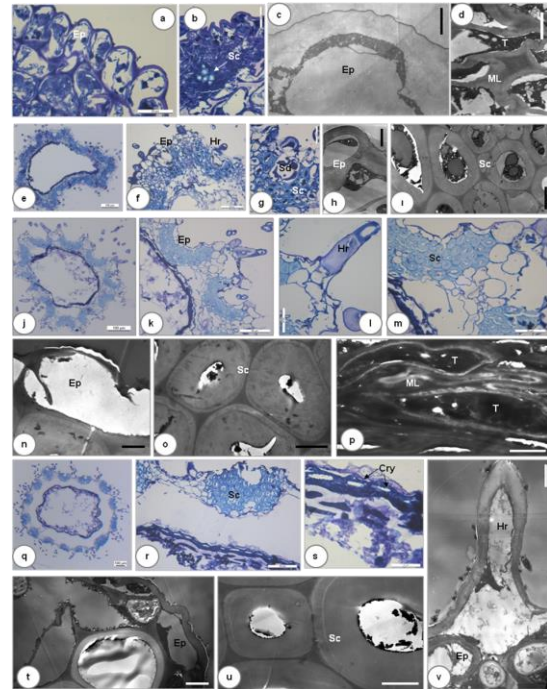


Figure 7a. Epidermal cells in achene fruit of *S. erucifolius* L. subsp. *erucifolius* Bar = 20 μ m. **b.** Small sclerenchyma bundles in mid-layer cells (arrow). Bar = 20 μ m. **c.** Electron micrograph of epidermal cells Bar = 3 μ m. **d.** Electron micrograph of mid-layer cells. Bar = 3 μ m. **e.** General view of achene fruit structure of *S. grandidentatus* Ledeb. Bar = 100 μ m. **f.** Epidermal cells and hairs Bar = 50 μ m. **g.** Secretory duct in sclerenchyma bundle Bar = 20 μ m. **h.** Electron micrograph of epidermal cells Bar = 5 μ m. **i.** Electron micrograph of sclerenchyma cells Bar = 3 μ m. **j.** General view of achene fruit structure of *S. mollis* Willd. Bar = 100 μ m. **k.** Epidermal cells Bar = 50 μ m. **l.** Hair in achene fruit Bar = 20 μ m. **m.** Sclerenchyma bundle Bar = 20 μ m. **n.** Electron micrograph of epidermal cells Bar = 3 μ m. **o.** Electron micrograph of sclerenchyma cells Bar = 3 μ m. **p.** Electron micrograph of mid-layer cells. Bar = 2 μ m. **q.** General view of achene fruit structure of *S. trapezuntinus* Boiss. Bar = 100 μ m. **r.** Sclerenchyma bundles Bar = 50 μ m. **s.** Crystals in mid-layer cells (arrows). Bar = 20 μ m. **t.** Electron micrograph of epidermal cells Bar = 5 μ m. **u.** Electron micrograph of sclerenchyma cells Bar = 3 μ m. **v.** Hair Bar = 3 μ m.

Abbreviations: **Cry:** Crystal, **Cu:** Cuticula, **Ep:** Epidermis, **Hr:** Hair, **ML:** Mid-layer cells, **Sd:** Secretory duct, **Sc:** Sclerenchyma, **T:** tannin.

In achene fruit of *S. grandidentatus* Ledeb., short hairs are seen partly in epidermal cells (Figs. 7e-f). Below epidermis are 3-5 layers of sclerenchyma cells with dark-stained and thickened walls. Secretory ducts were observed partly along the achene wall (Fig. 7g). In achene electron

micrographs, epidermal cells have vacuoles (Fig. 7h). Lumens of sclerenchyma cells are full (Fig. 7i).

Achene fruit of *S. mollis* Willd. has a dark-stained cuticle above epidermal cells (Fig. 7j). Below epidermis are 3-5 layers of thick-walled sclerenchyma cells (Fig. 7k). Epidermis has cover hairs (Fig. 7l). Dark-stained mid-layer cells are observed below sclerenchyma cells (Fig. 7m).

In electron microscopy images, epidermal cells are long, oval-shaped and the cuticle is dark-stained (Fig. 7n). Sclerenchyma cells have narrow lumens (Fig. 7o). Wavy-walled, dark-stained mid-layer cells are detected below sclerenchyma cells (Fig. 7p). Some cells have tannin.

Achene fruit of *S. trapezuntinus* Boiss. is oval-shaped (Fig. 7q). In wavy parts of the fruit, epidermal cells develop indents from the outside inward. Below epidermis, there are partly seen 4-5 layers of sclerenchyma bundles (Fig. 7r). Below sclerenchyma bundles are wavy-walled mid-layer cells some of which have prismatic crystals (Fig. 7s). Short hairs comprised of epidermal cells are thick-walled (Fig. 7t). Epidermal cells are long, rectangular-shaped and have thick cuticles (Fig. 7u). Sclerenchyma cells have thick walls (Fig. 7v).

Discussion

This study was conducted on a total of 8 taxa belonging to *Senecio*; 4 of which were collected from West Anatolia (*S. jacobaea* L. subsp. *jacobaea*, *S. aquaticus* Hill subsp. *erraticus* (Bertol.) V.A. Matthews, *S. maritimus* (L.) Rchb., *S. sandrasicus* P.H. Davis) and 4 collected from East Anatolia (*S. erucifolius* L. subsp. *erucifolius*, *S. grandidentatus* Ledeb., *S. mollis* Willd., *S. trapezuntinus* Boiss.). Leaf and stem anatomy and achene histology of the taxa were studied in order to identify similarities and differences among examined species.

Leaf anatomy among species was not significantly different. The leaves of the examined species are amphistomatic. In species of section *Jacobaeae*, stomata are amaryllis type and anomocytic as indicated by neighbouring cells. Leaves are bifacial. Walls of epidermal cells on leaf upper and lower surfaces are slightly wavy in species of *S. jacobaea* L. subsp. *Jacobaea*, *S. aquaticus* Hill subsp. *erraticus* (Bertol.) V.A. Matthews, *S. erucifolius* L. subsp. *erucifolius* and *S. trapezuntinus* Boiss. and are polygonal in species of *S. maritimus* (L.) Rchb., *S. sandrasicus* P.H. Davis and *S. mollis* Willd. The highest leaf thickness was measured in species of *S. mollis* Willd. and the smallest leaf thickness was measured in species of *S. aquaticus* Hill subsp. *erraticus* (Bertol.) V.A. Matthews and *S. erucifolius* L. subsp. *erucifolius* (Table 2).

As a result, while there are differences among 8 species of *Senecio* in mesophyll thickness and numbers of epidermal cells and stomata, the shapes and structures of these cells are similar.

Metcalf and Chalk (1950) reported that species of *Asteraceae* (*Compositae*) exhibit differences as regards stomata distribution on leaf surface, positions of guard cell and epidermal cells, hypoderm development on leaf upper surface, mesophyll and vascular system and waxy coating on leaves

The anatomical features that can be observed in *Asteraceae* family are: presence of various types of glandular or covering trichomes; papillae on epidermis; anomocytic, anisocytic and rarely heliocytic stomata; presence of hypoderm; homogeneous or heterogeneous mesophyll; and vascular bundles with parenchymatic sheath composed by large cells (Metcalf and Chalk, 1979).

In the stem cross-sections of all 8-species studied, epidermis consists of a single row of cells. In young stems, stomata are detected in epidermis. In species of *S. maritimus* (L.) Rchb., *S. sandrasicus* P.H. Davis and *S. mollis* Willd., the stem has more abundant glandular and cover hairs compared to those of other studies species. Multiple layers of lamellar collenchyma cells are seen below epidermis and changes are observed in cell walls as the collenchyma approaches vascular bundles. The cortex is parenchymatic. In the stem cortex, below collenchyma are thin-walled parenchyma cells. Vascular bundles are of open-collateral type. The cambium is composed of multiple layers of thin-walled cells.

In achene fruit of species *S. erucifolius* L. subsp. *erucifolius* and *S. jacobaea* L. subsp. *Jacobaea*, sclerenchyma cells are in the form of small bundles, whereas in other species, they occur as strongly developed structures. In species *S. grandidentatus* Ledeb. and *S. mollis* Willd., hairs are detected in epidermis along the achene wall while no hairs are identified in other studied species. Prismatic crystals are seen in mid-layer cells in species of *S. aquaticus* Hill ssp. *erraticus* (Bertol.) V.A. Matthews., *S. mollis* Willd. and *S. trapezuntinus* Boiss. whereas no crystals are detected in mid-layer cells of the other species.

Occurrence of secretory ducts and calcium oxalate crystals in the inner parenchyma layer of pericarp were reported in some East Asian representatives of the tribe *Cardueae* (*Asteraceae*) (Zarembo and Boyko, 2008).

Genus *Senecio* was described by Matthews in 5th Volume of Flora of Turkey (1975) and two species were subsequently described in the 11th Volume (annex volume 2) (Kit Tan, 2000). Species *Senecio salsuginea* and *Tephrosia cladobotrys* (Synonym: *Senecio cladobotrys*) were also added to the

Turkish flora as of 2006 and 2007, respectively (Vural et al., 2006; Budak et al., 2007). All taxa formerly placed in *Senecio* in Flora of Turkey were then transferred to the genera *Senecio* and *Tephrosieris* (Hamzaoglu et al., 2009).

The *Tephrosieris* is represented in Turkish flora by 2 species. The *Senecio*, according to the latest records, is represented in Turkish flora by 52 taxa (43 species, 3 subspecies and 6 varieties). 21 of these taxa are endemic for Turkey and the rate of endemism is 40% (Matthews, 1975; Kit Tan, 2000).

The present study seeks to contribute to the resolution of taxonomic problems of genus *Senecio* based on identification of morphological, anatomical and histological modifications by light microscopy and TEM study of stem and leaf anatomy and seed coat (achene-testa). Derived from the data that set the basis for classification of genus, the subject taxa were reevaluated phylogenetically, doubtful taxonomical validity of some species in Turkey has been clarified and distribution areas and threat categories were updated.

Conclusions

In all the taxa, leaves are bifacial (dorsiventral) and amphistomatic, stomata are of the anomocytic and amaryllis type. In young stems, stomata are observed in epidermis. In the taxa *S. maritimus* (L.) Rchb., *S. sandrasicus* P.H.Davis and *S. mollis* Willd., the stem has intensive cover and glandular hairs.

In the taxa *S. grandidentatus* Ledeb. and *S. mollis* Willd., hairs are observed in the epidermis along the achene wall while no hairs are identified in the epidermises of other species. In the taxa *S. aquaticus* Hill ssp. *erraticus* (Bertol.) V:A. Mathews., *S. mollis* Willd. and *S. trapezuntinus* Boiss., prismatic crystals are seen in mid-layer cells whereas no crystals are detected in mid-layer cells of the other taxa.

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