

MORPHOLOGICAL AND ANATOMICAL STUDIES ON SOME SCROPHULARIA L. (SCROPHULARIACEAE) TAXA FROM NE ANATOLIA, TURKEY

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In this study, morphological and anatomical features of *Scrophularia chrysantha* Jaub.& Spach, *Scrophularia scopolii* [Hoppe ex] Pers. var. *adenocalyx* Somm.& Lev., *Scrophularia olympica* Boiss., *Scrophularia sosnowskyi* Kem.-Nath., *Scrophularia canina* L., collected from NE Anatolia, were described and illustrated. Features related to morphologic peculiarities of the leaf, staminod, bracteol, alar pedicel, and corolla were found to be important for separating the taxa morphologically. Presence and distribution of the phloem fibers were seen crucial differences among examined species in terms of stem anatomical properties. All leaves are bifacial and stomata cells are anomocytic. It is determined that distribution of idioblasts is important anatomic character in the examined taxa.

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Scrophularia (Scrophulariaceae)

ویژگیهای ریختارشناختی و آناتومی گونه های زیر که از شمال شرق آناتولی جمع آوری شده اند به همراه تصویر شرح داده می شوند.

Scrophularia chrysantha Jaub.& Spach, *Scrophularia scopolii* [Hoppe ex] Pers. var. *adenocalyx* Somm. & Lev., *Scrophularia olympica* Boiss., *Scrophularia sosnowskyi* Kem.-Nath., *Scrophularia canina* L.

خصوصیات ریختی برگ، نا پرچی ها، برگک، دمگل گل میانی و جام گل جهت تفکیک آرایه ها اهمیت دارند. حضور و انتشار فیبر های آبکشی ساقه در بین آرایه های مورد مطالعه اختلاف بارزی را نشان می دهد. تمامی برگها دارای تیپ مزوفیل پشته شکمی و در هر دو سطح، تیپ روزنه ها آنوموسیتیک است. توزیع اید یوبلاست ها در آرایه های مطالعه شده ویژگی آناتومی مهمی است.

Introduction

Scrophularia L. (*Scrophulariaceae*), as one of the most important genera of the flowering plants, consists of about 300 species. It is mainly Holarctic, and the species are found in both the Old and New Worlds (Lersten & Curtis 1997). The genus is represented with 59 species in Turkey (Lall and Mill 1978). Their primary diversity center is located in south-east Asia (Stiefelhagen 1910). Many species belonging to this genus have been used as folk remedies for some medical treatments since ancient times (Heather & Henderson 1994).

Anatomical studies performed on *Scrophularia* are mainly focused on leaf features. The first brief description of idioblast in *Scrophularia* was carried out by Volkens (1887) and then Metcalfe and Chalk

(1950). Pennel (1929, 1935) determined important differences in the leaf, stem and root anatomy of 25 *Scrophulariaceae* species and explained their taxonomic importance. In the recent paper by Lersten and Curtis (1997, 2001), distribution of idioblast and internal secretory structures of leaf were investigated in different *Scrophulariaceae* taxa and many crucial differences were found among the examined taxa. Another description of the idioblasts in the genus was made by Makbul et al. (2006). They investigated anatomical features of some *Scrophularia* taxa and distribution of idioblasts and found many important differences between the examined taxa. Intercellular inclusions such as idioblasts and cellular cavities are

Table 1. Locality information of the examined *Scrophularia* taxa.

No.	Taxa	Locality
1	<i>S. chrysantha</i> Jaub.& Spach	A8 Rize: Ardeşen, Eskiarmutluk 700m, 14.04.2001, Makbul 04, KTUB
2	<i>S. scopolii</i> Hoppe ex Pers. var. <i>adenocalyx</i> Somm.& Lev.	A8 Rize: Kalkandere, 150 m, 23.04.2001, Makbul 05, KTUB; A8 Trabzon: 50 m, 25.04.2001, Makbul 07, KTUB
3	<i>S. olympica</i> Boiss.	A8 Rize: Çat, 1200m, 09.06.2001, Makbul 14, KTUB
4	<i>S. sosnowskyi</i> Kem.-Nath.	A8 Rize: Çat, 1100m, 09.06.2001, Makbul 15, KTUB
5	<i>S. canina</i> L.	A7 Gümüşhane: 800-2000m, 21.06.2002, Makbul 19 KTUB

new useful microcharacter used in taxonomic and systematic treatments.

Although anatomic properties are very important characters in the genus *Scrophularia*, there is no sufficient data related to the Turkish species. Therefore, the main object of this study is to explore the anatomical and morphological features of *Scrophularia* taxa distributed in NE Anatolia and to explain their systematic importance.

Materials and Methods

The plants were collected from northeast Anatolia in 2002. The collection data of the examined specimens are given in Table 1. Specimens for morphological studies were dried according to standard herbarium techniques and stored in the Herbarium of Karadeniz Technical University, Department of Biology (KTUB). The materials for anatomical studies were firstly fixed in formaldehyde-acetic acid-alcohol (FAA) for 24 h and then preserved in % 70 alcohols in the field.

Anatomic observations were performed on the cross-sections of stem and leaves, and surface sections of leaves cut by free-hand. The materials were stained with safranin/fast green for 24 h and mounted with glycerine-gelatine to make permanent slides (Vardar 1987). Well-staining sections were photographed on camera Olympus BX51 from permanent slide. All measurements and observations were determined on three or four staining sections.

Results

I- Morphological Results

Scrophularia chrysantha Jaub.& Spach: Plant perennial or biennial; stem c. 15–75 cm, branched (Fig. 1A). Leaves decussate and petiolate; lamina entire, ovate to reniform, margin crenate to dentate. Inflorescence aphyllous, alar pedicel 4–6 mm with glandular hairs (Fig. 1B). Calyx lobes are glabrous without scarious margin. Corolla yellow. Stamens clearly exerted; staminod absent. Capsule ovoid (Fig. 1C).

S. scopolii [Hoppe ex] Pers. var. *adenocalyx* Somm. & Lev.: Plant perennial or biennial; stem c. 50–80 cm, glabrous at base, glandular above (Fig. 2A). Leaves

decussate and petiolate; lamina entire, oblong to ovate, base cordate, margin bicrenate to dentate. Inflorescence subfoliate to aphyllous, alar pedicel 5–20 mm with glandular hairs (Fig. 2B). Calyx lobes are glabrous with scarious margin. Corolla purplish-red. Stamens included; staminod retuse (Fig. 2D). Capsule ovoid glabrous (Fig. 2C).

S. olympica Boiss.: Plant variable perennial; stem c. 40–60 cm, glabrous below, glandular above (Fig. 3A). Leaves opposite and petiolate; lamina deeply pinnatifid, oblong to ovate. Inflorescence aphyllous; alar pedicel 2–4 mm with glandular hairs (Fig. 3B). Calyx lobes are glabrous with scarious margin. Corolla brownish; upper lobes purple. Pedicel glandular. Stamens shortly exerted; staminod reniform (Fig. 3D). Capsule glabrous, ovoid to globose (Fig. 3C).

S. sosnowskyi Kem.-Nath.: It is similar to *S. olympica* (Fig. 4A). Stems and veins green. Leaves with more pinnatisect, decussate, scarious-white margined. Pedicel glandular. Calyx lobes are narrower than *S. olympica*. Corolla greenish-yellow with upper lip purple (Fig. 4B). Pedicel glandular. Staminod suborbiculate to reniform (Fig. 4D). Capsule glabrous (Fig. 4C).

S. canina L.: Plant perennial; stem c. 40–110 cm, woody at base, many, glabrous (Fig. 5A). Leaves opposite, in lower part petiolate, uppers sessile; lamina clearly pinnatisect; segments lanceolate. Inflorescence aphyllous; alar pedicel 1–2 mm with glandular hairs (Fig. 5B). Calyx lobes are glabrous with white scarious margin. Corolla maroon; upper lobes white bordered. Stamens exerted; staminod oblong to lanceolate (Fig. 5D). Capsule globose, completely glabrous (Fig. 5C).

II- Anatomical Results

S. chrysantha:

STEM TRANSVERSE SECTION (TS) (Fig. 6A). Epidermis consists of single layer of rectangular cells. Lamellar collenchyma is one or two layered located close to the epidermis, but 2 or 3 layers of collenchyma can be seen at the stem ridges. Cortex (180–200 µm) consists of 5–6 layers of usually oval cells with thin



Fig. 1. *Scrophularia chrysantha*. A. General appearance, B. Inflorescence and leaf shape, C. Fruit.

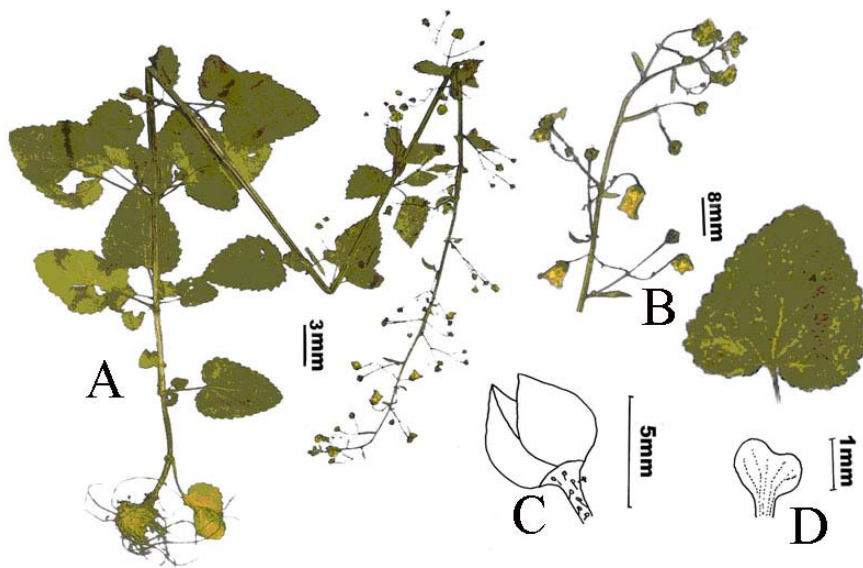


Fig. 2. *Scrophularia scopolii* var. *adenocalyx*. A. General appearance, B. Inflorescence and leaf shape, C. Fruit, D. Staminod.

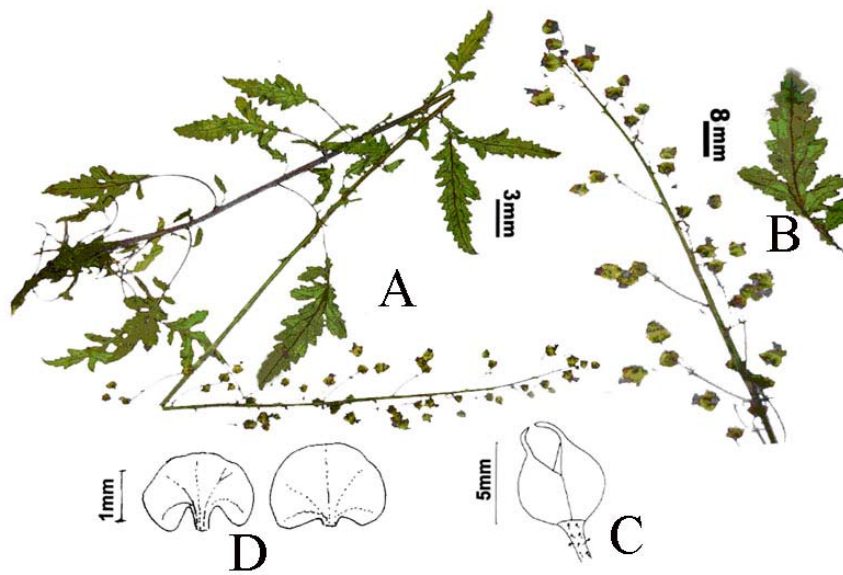


Fig. 3. *Scrophularia olympica*. A. General appearance, B. Inflorescence and leaf shape, C. Fruit, D. Staminod.

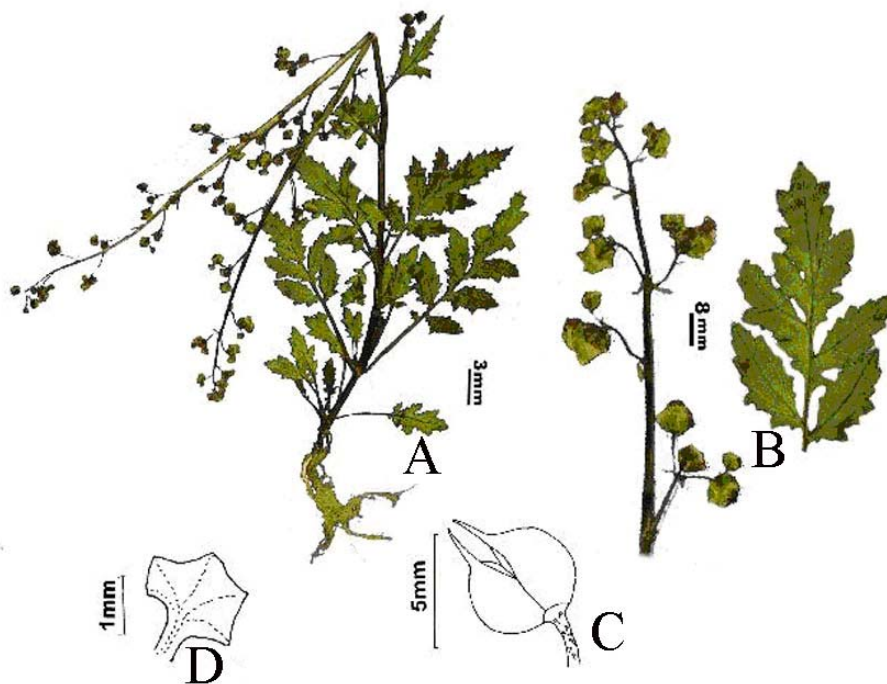


Fig. 4. *Scrophularia sosnowskyi*. A. General appearance, B. Inflorescence and leaf shape, C. Fruit, D. Staminod.

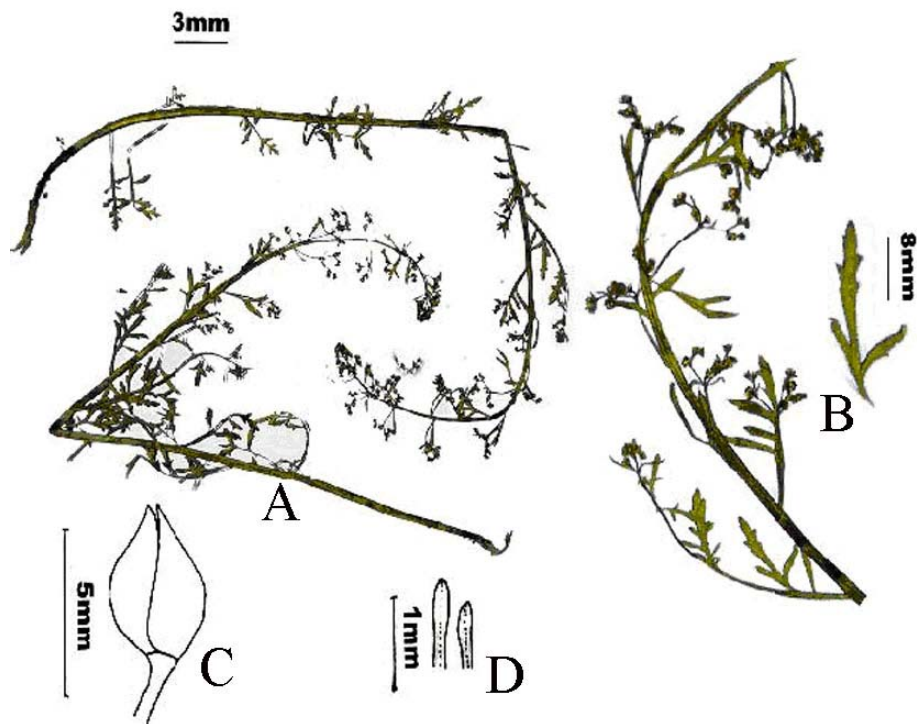


Fig. 5. *Scrophularia canina*. A. General appearance, B. Inflorescence and leaf shape, C. Fruit, D. Staminod.

Table 2. Stomata features on the upper and lower epidermis of *Scrophularia* taxa.

Taxa	<i>S. chrysantha</i>	<i>S. scopolii</i> var. <i>adenocalyx</i>	<i>S. olympica</i>	<i>S. sosnowskyi</i>	<i>S. canina</i>
Width/length of upper epidermal stomata cells ($\mu\text{m}/\mu\text{m}$)	26x43	-	26x32	28x34	18x28
Width/length of lower epidermal stomata cells ($\mu\text{m}/\mu\text{m}$)	20x28	19x21	23x28	21x28	19x30
Average number of upper epidermal stomata (1mm^2)	60	-	131	136	185
Average number of lower epidermal stomata (1mm^2)	163	494	234	278	216
Stomata index for upper epidermis	23.6	-	28.5	25.7	28.08
Stomata index for lower epidermis	31.6	31.7	27.7	29.3	25.2

walls, and occupies 20-23 % of the stem radius. Cambium is not distinguishable. Phloem is 80-90 μm thickness and sclerenchyma fibers spread among the phloem cells in groups. Xylem tissue including solitary or clustered vessels makes up 30-35 % of stem cortex. Pith cells parenchymatous, large and cylindrical, and occupy % 35 of the stem radius.

MIDRIB and LAMINA TS, also LEAF SURFACE (Fig. 6B-D). Only dorsal zone of the midrib is covered with short hairs. Triangular or reniform-shaped midrib has a monolayer of lamellar collenchyma located close to the epidermal cells. Arc-shaped vascular bundle is

surrounded by orbicular and thin-walled parenchymatous cells. Upper epidermal cells are larger than the lower ones. Mesophyll tissue (200 μm) consists of 1 or 2 layers of elongated palisade cells and 4 or 5 layers of isodiametric spongy parenchymatous cells with large intercellular cavities. Lower epidermal cells are more undulate than upper ones. Bifacial leaf has anomocytic stomata occurring on both surfaces. Stomatal measurements of the species are given in Table 2.

S. scopolii var. *adenocalyx*:

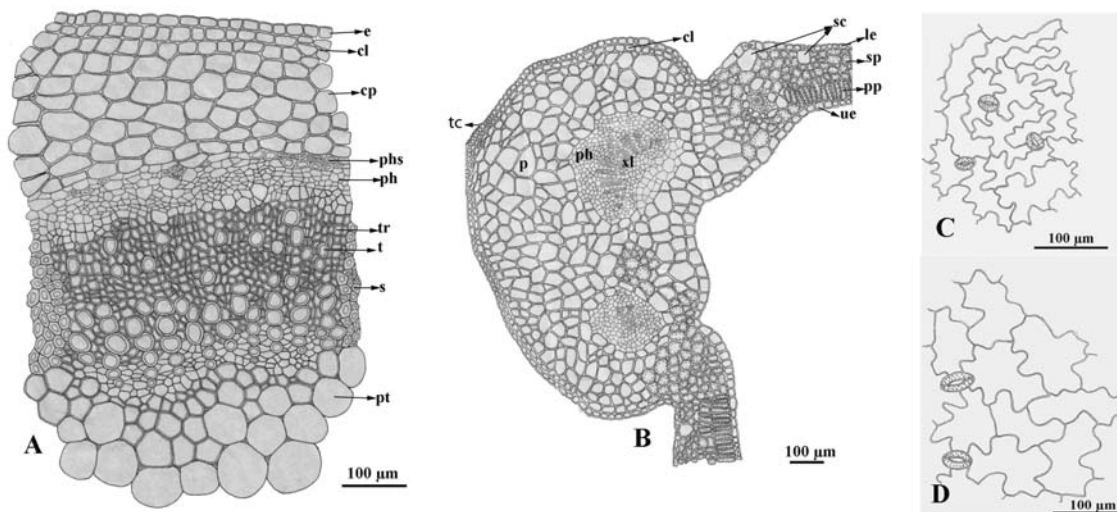


Fig. 6. *Scrophularia chrysantha*. A: Transverse section of the stem, B: Transverse section of the leaf, C: Surface section of the lower epidermis, D: Surface section of the upper epidermis. e: epidermis, cl: collenchyma, cp: cortex parenchyma, phs: phloem sclerenchyma, ph: phloem, tr: tracheid, t: trachea, s: sclerenchyma, pt: pith, tc: trichome, p: parenchyma, xl: xylem, sc: stomata cavity, le: lower epidermis, ue: upper epidermis, sp: spongy parenchyma, pp: palisade parenchyma.

STEM TS (Fig. 7A). Epidermis consists of a single layer of rectangular or orbicular cells. A monolayer of lamellar collenchyma occurs close to the epidermis. Cortex (120-150 μm) consists of 4 or 5 layers of usually oval cells with several intercellular spaces and occupies 20-25 % of the stem radius. Phloem without sclerenchyma fibres is 30-40 μm . Xylem expanded at the site of ridges makes up 30-35 % of the stem. Stem pith with large and thin-walled parenchymatous cells occupying % 40 of the stem.

MIDRIB and LAMINA TS, also LEAF SURFACE (Fig. 7B-D). Triangular midrib has arc-shaped vascular bundle is surrounded by orbicular parenchymatous cells and 1-2 layers of lamellar collenchyma located under the lower epidermis. Upper epidermal cells are bigger than the lower ones without trichomes. Mesophyll consists of a monolayer of elongated palisade cells, 2 or 3 layers of isodiametric spongy parenchymatous cells with large intercellular cavities. Idioblastic cells with empty lumens are seen clearly between the palisade cells. It occurs scarcely on both surfaces of the palisade (Fig. 7B). Leaf is bifacial and both epidermal cells have undulate walls. Anomocytic stomata occur only on lower epidermis. Measurements related to the stoma of the species are given in Table 2.

S. olympica:

STEM TS (Fig. 8A). Uniseriate epidermis consists of flattened, rectangular or orbicular cells. There is a

monolayer lamellar collenchyma under the epidermis. Stem cortex (120-140 μm) consists of 5 or 6 layers of usually oval cells and occupies 10-15 % of the stem radius. Phloem contains grouped scleranchymatous fibers, xylem makes up 25 % of the stem. Bundles connected with sclerenchymatic sheath (120-150 μm). Pith including large and cylindrical cells occupies % 50-60 of the stem radius.

MIDRIB and LAMINA TS, also LEAF SURFACE (Fig. 8B-D). Midrib is semicircular in shape and consists of monolayer lamellar collenchyma adjacent to lower epidermal cells. Upper epidermal cells are larger than the lower ones. Trichomes occur along the all lower epidermis. Solitary arc-shaped vascular bundles are surrounded by parenchymatous cells. Mesophyll consists of 2 or 3 layers of elongated palisade cells and 3 or 5 layers of isodiametric, spongy parenchymatous cells having large intercellular cavities. Leaf is bifacial, both epidermal cells have undulate wall and anomocytic stomata occur in both surfaces. Stoma features of the species are given in Table 2.

S. sosnowskyi:

STEM TS (Fig. 9A). Epidermis consists of a monolayer of flattened, rectangular or orbicular cells. Lamellar collenchyma is a single layer under the epidermis. Stem cortex (140-150 μm) consists of 6 or 7 layers of usually oval cells and occupies 13-15 % of the stem radius. Phloem with grouped or solitary scleranchyma fibers is

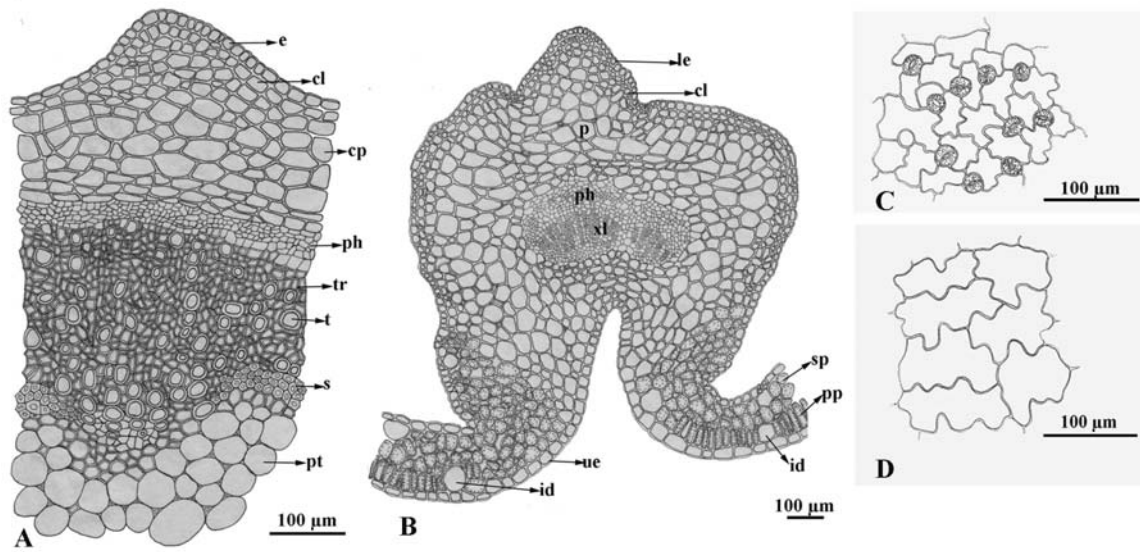


Fig. 7. *Scrophularia scopolii* var. *adenocalyx*: A: Transverse section of the stem, B: Transverse section of the leaf, C: Surface section of the lower epidermis, D: Surface section of the upper epidermis. e: epidermis, cl: collenchyma, cp: cortex parenchyma, ph: phloem, tr: tracheid, t: trachea, s: sclerenchyma, pt: pith, p: parenchyma, xl: xylem, id: idioblast, le: lower epidermis, ue: upper epidermis, sp: spongy parenchyma, pp: palisade parenchyma.

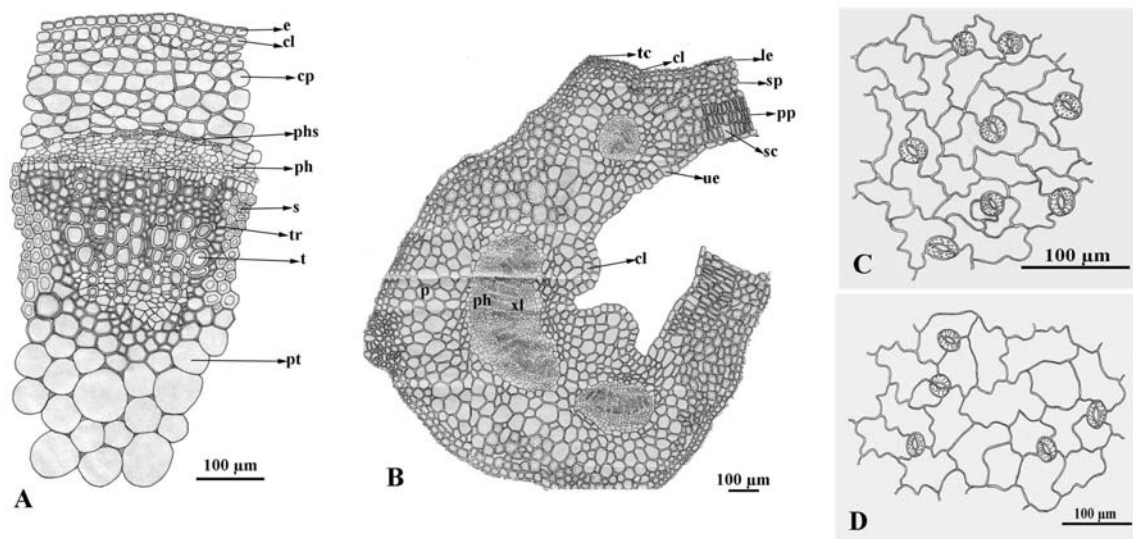


Fig. 8. *Scrophularia olympica*. A: Transverse section of the stem, B: Transverse section of the leaf, C: Surface section of the lower epidermis, D: Surface section of the upper epidermis. e: epidermis, cl: collenchyma, cp: cortex parenchyma, phs: phloem sclerenchyma, ph: phloem, tr: tracheid, t: trachea, s: sclerenchyma, pt: pith, tc: trichome, p: parenchyma, xl: xylem, sc: stomata cavity, le: lower epidermis, ue: upper epidermis, sp: spongy parenchyma, pp: palisade parenchyma.

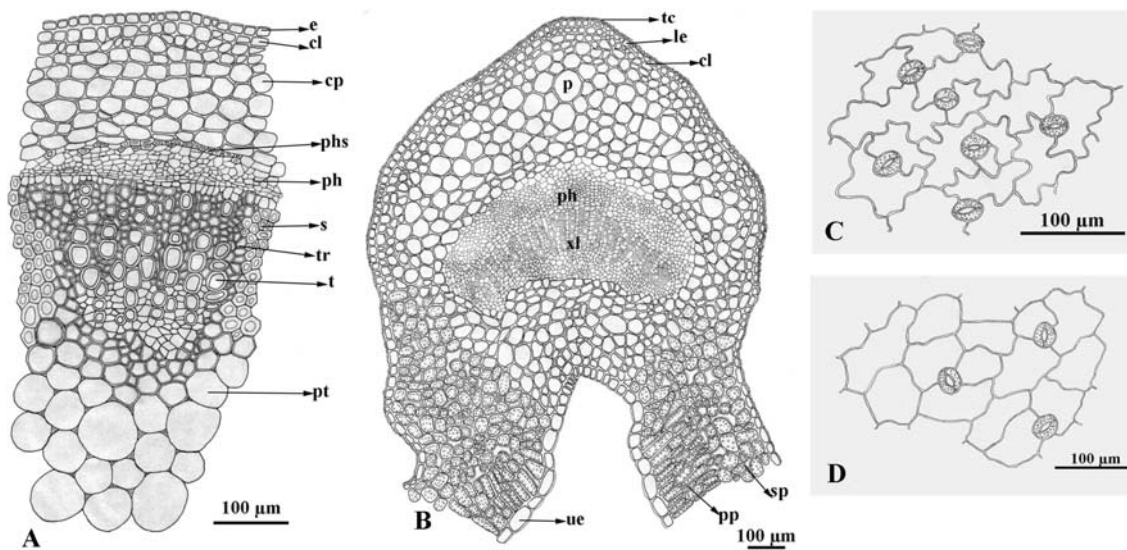


Fig. 9. *Scrophularia sosnowskyi*. A: Transverse section of the stem, B: Transverse section of the leaf, C: Surface section of the lower epidermis, D: Surface section of the upper epidermis. e: epidermis, cl: collenchyma, cp: cortex parenchyma, phs: phloem sclerenchyma, ph: phloem, tr: tracheid, t: trachea, s: sclerenchyma, pt: pith, tc: trichome, p: parenchyma, xl: xylem, sc: stomata cavity, le: lower epidermis, ue: upper epidermis, sp: spongy parenchyma, pp: palisade parenchyma.

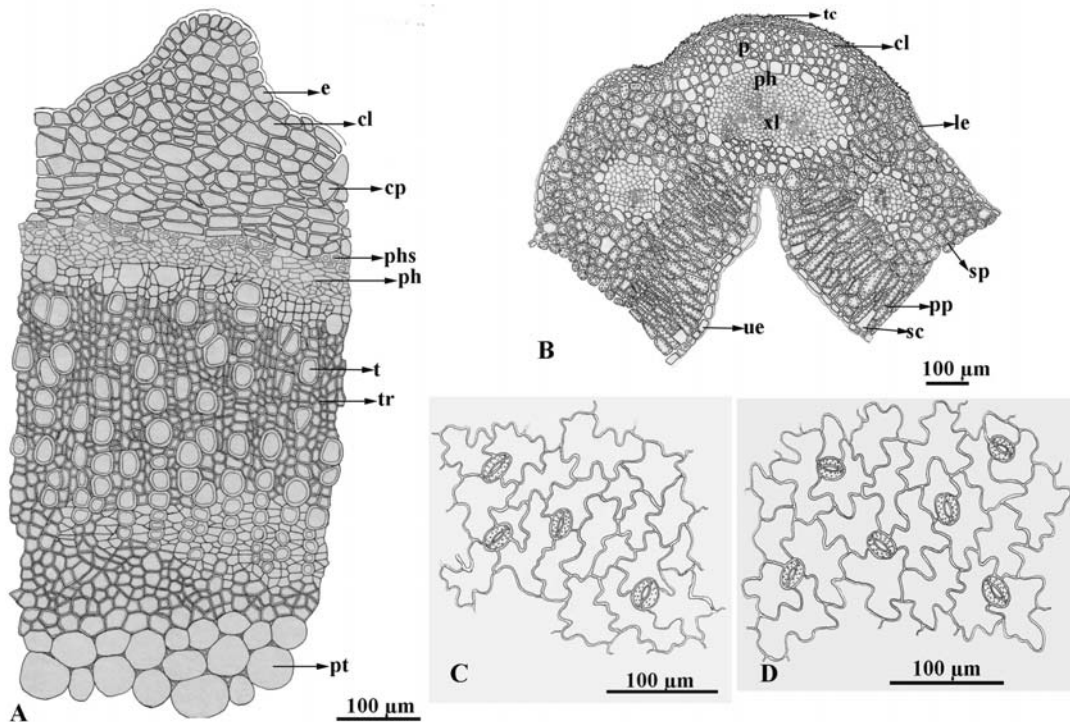


Fig. 10. *Scrophularia canina*. A: Transverse section of the stem, B: Transverse section of the leaf, C: Surface section of the lower epidermis, D: Surface section of the upper epidermis. e: epidermis, cl: collenchyma, cp: cortex parenchyma, phs: phloem sclerenchyma, ph: phloem, tr: tracheid, t: trachea, pt: pith, tc: trichome, p: parenchyma, xl: xylem, sc: stomata cavity, le: lower epidermis, ue: upper epidermis, sp: spongy parenchyma, pp: palisade parenchyma.

60 µm. Xylem occupying 30-35 % of stem cortex is large. A continuous sclerenchymatous sheath (200 µm) occurs between the bundles. Pith cells are large and cylindrical and occupy % 25-30 of the stem radius.

MIDRIB and LAMINA TS, also LEAF SURFACE (Fig. 9B-D). Reniform-shaped midrib consist a monolayer of lamellar collenchyma under the both epidermises. Solitary arc-shaped vascular bundle is surrounded by thin-walled and orbicular parenchymatous cells. Upper epidermal cells are larger than the lower ones in transverse sections of the lamina. Trichomes continuously occur along the lower surface of the leaf. Mesophyll (380-400 µm) consists of 3 layers of elongated palisade cells and 3 or 4 layers of isodiametric, spongy parenchymatous cells with large intercellular cavities. Leaf is bifacial and lower epidermal cells are more undulating than upper ones. Anomocytic stomata occur on both surfaces at the same level with neighboring cells. Stomatal measurements of the species are given in Table 2.

S. canina: A transverse section taken from the stem was investigated (Fig. 10A). Epidermis consists of a monolayer of flattened, rectangular or orbicular cells. Lamellar collenchyma is single layer under the epidermis, but 2 or 3 layers of collenchyma can be seen below the epidermis at the stem ridges. Stem cortex (110-130 µm) consists of 5 or 6 layers of usually oval cells and occupies 15-20 % of the stem. Vascular bundles arrange continuously along the stem. Cambium is not distinguishable. Phloem with grouped sclerenchymatous fibers is 70-80 µm. Xylem expanded at the ridges occupies 35-40 % of stem radius. Tracheas arranged radially and numerous are large. Pith cells are large and cylindrical and occupy % 35-40 of the stem radius.

A transverse section taken from midrib and lamina, and surface sections from both epidermises were studied (Figs. 10B-D). Midrib is triangular and consists of 1-2 layers of lamellar collenchyma adjacent to lower epidermis. Solitary arc-shaped vascular bundle is surrounded by orbicular parenchymatous cells. Upper epidermal cells are larger than the lower ones in transverse sections of the leaf. Trichomes occur only on the midrib epidermal cells of lower surface. Mesophyll (440-460 µm) consists of generally 4 or 5 layers of elongated palisade cells and 2 or 3 layers of isodiametric, spongy parenchymatous cells with large intercellular spaces. Leaf is bifacial and both epidermal cells have undulate wall. Anomocytic stomata occur on upper and lower surface of the leaf. Stoma features of the species are given in Table 2.

Discussion

This study is aimed to provide useful anatomical and morphological information and an additional

perspective to the systematic of examined *Scrophularia* taxa. This is the first anatomical report on the five *Scrophularia* taxa. Among the morphological characters, systematically leaf properties are the most important (Lall & Mill 1978). In our study, we found that lamina shape is pinnatifid in *S. olympica*, *S. sosnowskyi* and *S. canina*, and entire in the others. While glands occur only on the pedicel in *S. olympica*, *S. sosnowskyi*, and *S. scopolii* var. *adenocalyx*, pedicel and whole fruit surface of *S. chrysantha* covered with glands. There are no glands on *S. canina* fruit. The presence or absence, and shapes of staminods are important in separating related taxa (Lall & Mill 1978). In a study (Makbul & al. 2006), performed on some Turkish *Scrophularia* taxa, staminods and glands on the capsule and pedicel were investigated. The staminod is linear to ovate in *S. libanotica* Boiss. var. *pontica* R. Mill., and reniform, cordate to emarginated, cordate to reniform or oblong to ovate in *S. ihwensis* C. Koch, *S. lucida* L., *S. cinerascens* Boiss., *S. capillaries* Boiss., and *S. nodosa* L. Nevertheless, except for *S. capillaris*, all taxa have glands on capsule or pedicel (Makbul & al. 2006). In our study, it is determined that there are no staminod in *S. chrysantha*. But, staminod is retuse in *S. scopolii* var. *adenocalyx*, reniform in *S. olympica*, suborbiculate to reniform in *S. sosnowskyi*, and oblong to lanceolate in *S. canina*.

Stamens are included in *S. scopolii* var. *adenocalyx*, and are exerted in the others. Especially, *S. chrysantha* has clearly exerted stamens. Corolla is yellow in *S. chrysantha*, but it is brownish, purplish-maroon or red in the rest of the other taxa. All our morphological observations are similar to those reported by Lall and Mill (1978).

A foliar endodermis with a casparian strip is rarely present in angiosperms (Dickson & Weitzman 1996). These structures and crystals were previously described by Metcalfe and Chalk (1950) in *Scrophulariaceae*, but we didn't observe such features in this study.

Trichomes are important trait for identifying these taxa and vary their presence on lower epidermis. There is no trichome in *S. scopolii* var. *adenocalyx*. Trichomes occur only on the midrib epidermal cells in *S. canina* and *S. chrysantha* and along the all lower surface in *S. sosnowskyi* and *S. olympica*.

The distribution of scleranchymatic tissue in the cortex and phloem has taxonomic value (Makbul & al. 2006, Canne-Hilleker & Kampny 1990). Fibers were not seen among the phloem cells of *S. scopolii* var. *adenocalyx*. Scleranchymatic cells occur as solitary or grouped in the others. Grouped fibers consists of 15-20 cells in *S. sosnowskyi*, 6-10 cells in *S. canina*, 20-25 cells in *S. olympica*, and 5-15 cells in *S. chrysantha*.

Although taxa including idioblasts are present in all sections and subsections of the genus, but the state, distribution and shapes of idioblasts are significant characters in *Scrophularia* (Stiefelhaven 1910, Murbeck 1933). The presence of idioblasts in *S. scopolii* var. *adenocalyx* is an additional taxonomic character. Lersten and Curtis (2001) stated that strictly subepidermal idioblasts with primary cell walls are empty at cell maturity. It is indicated that idioblast lumens are empty in some *Scrophularia* taxa by Makbul et al. (2006). No substances were observed in any of the idioblasts in this study. Number of rows of palisade parenchyma varies in examined taxa. Mesophyll consists of 4 layers of palisade in *S. canina*, 3 layers in *S. sosnowskyi* and *S. olympica*, and monolayer in *S. chrysantha* and *S. scopolii* var. *adenocalyx*. The other anatomical features of leaves and stem determined in this study are also important characters. These findings supplement the information about the genus and family given by Metcalfe and Chalk (1950), and should be useful in features studies.

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