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Comparative study of *Salvia limbata* C.A. and *S. palaestina* Bentham (sect. Aethiopis Bentham, Labiatae) from East Anatolia, Turkey

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Morphological characteristics of stems, leaves, bracts, calyces and corollas are taxonomically discriminating characters in *Salvia*. In this paper we present morphological, anatomical and ecological features of *S. limbata* C.A. Meyer and *S. palaestina* Bentham as well as micromorphological characteristics of their pollen grains and nutlets using scanning electron microscopy. Anatomical characters such as size of cortex and vascular tissue, number of palisade parenchyma rows and vascular bundles are found to be important species specific characters. Pollen grains in the species are different in shape and size, but they look similar in their exine sculpturing; shape, size and ornamentation of nutlets are found to be different. The two investigated *Salvia* species grow on clayey-loamy and loamy soils, with pH 7.6–7.9, with 0.4–2.1% of organic matter, 0.006–0.026% of total salt content, 4.2–21.0 mg kg^{-1} phosphorus and 87.0–445.8 mg kg^{-1} potassium.

Key words: Morphology, anatomy, ecology, palynology, nutlet, *Salvia limbata*, *Salvia palaestina*

Introduction

The family Labiatae includes 250 genera and about 7000 species distributed all over the world (THORNE 1992). It is the third largest family in Turkey with 45 genera and 574 species, 256 of which are endemic. The rate of endemism is 44.5% in this family (DAVIS 1965–1985, GÜNER et al. 2000).

The genus *Salvia* L. represents a cosmopolitan assemblage of nearly 1000 species, distributed in three regions of the world: Central and South America (500 species), western Asia (200 species) and eastern Asia (100 species) (WALKER and SYTSMA 2007). The lever mechanism regulates pollination, selection and evolution in the group (CLAßEN-BOCKHOFF et al. 2004).

The genus has been the subject of a number of studies on morphology (HEDGE 1982), anatomy (METCALFE and CHALK 1972, KAHRAMAN et al. 2009a, 2010a, b), palynology (ERDTMAN 1945, CANTINO et al. 1992) and nutlet micromorphology (MARIN et al. 1996).

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As many as 107 species of *Salvia* has been recognized in the *Flora Orientalis*, 75 of which were recorded from Turkey (BOISSIER 1875). These species have been placed under seven sections using BENTHAM's (1833) sectional delimitation. These sections are as follows: *Eusphace* Benth., *Hymenosphace* Benth., *Aethiopis* Benth., *Plethiosphace* Benth., *Horminum* Benth., *Drymosphace* Benth. and *Hemisphace* Benth. After that sect. *Eusphace* was changed to sect. *Salvia* (HEDGE 1972). *Salvia limbata* and *S. palaestina* were placed in the section *Aethiopis* (BENTHAM 1833, BOISSIER 1875). Section *Aethiopis* comprises biennial or perennial herbs or chamaephytes. The characteristic features of the section are tubular or campanulate calyx, more or less falcate upper lip of corolla and not annulate corolla tube. Staminal connectives are longer than filaments, arms are unequal, the sterile shorter and more or less flattened distally.

The first revision of *Salvia* in Turkey was made by HEDGE (1982), who recognized 87 species, one of them however being doubtful. Between 1982 and 2005, four more species, *S. nydeggeri* (HUBER-MORATH 1982), *S. aytachii* (VURAL and ADIGÜZEL 1996), *S. hedgeana* (DÖNMEZ 2001), *S. anatolica* (HAMZAOĞLU et al. 2005), were published from Turkey. Since 2005, as a part of a revision of the genus *Salvia* in Turkey, the authors have carried out extensive field studies and collected a large number of specimens. Population size, habitat, phenological and ecological properties of the species were observed and their GPS coordinates and photographs were taken in the field. The studies have revealed two new species, *S. marashica* (İLÇİM et al. 2009) and *S. ekimiana* (CELEP and DOĞAN 2010) and new records of *S. macrosiphon* Boiss. (KAHRAMAN et al. 2009b) and *S. viscosa* Jacq. (CELEP et al. 2009).

Salvia limbata and *S. palaestina* grow in eastern Anatolia and have similar morphological features and confused taxonomy. In this paper for the first time we give descriptions of *S. limbata* and *S. palaestina,* their comparative root, stem, leaf and petiole anatomy, palynology and nutlet micromorphology.

Materials and methods

Specimens of *S. limbata* and *S. palaestina* were collected from six localities in Turkey (Tab. 1, Fig. 1). The specimens have been stored in the Middle East Technical University (METU) Department of Biological Sciences and Ankara University Herbarium (ANK).

Morphological studies were carried out on living as well as herbarium materials. 25 individuals of each species and their 25 characters were studied and measured so as to determine their morphological characteristics using a Leica S8AP0 stereomicroscope. Minimum and maximum ranges of measured characters are presented.

Anatomical studies were carried out on specimens kept in 70 % alcohol. The paraffin method was used for the transverse sections of the root, the stem, the leaf and the petiole, and surface sections of leaves. The specimens were embedded in paraffin and then sectioned with a Leica RM2125RT rotary microtome. All sections were stained with Safranin and Fast Green and then mounted in Canada Balsam or Entellan (JOHANSEN 1944). Measurements and photographs were taken using a Leica DM1000 binocular light microscope and a Leica DFC280 camera.

For palynological investigations, pollen material was obtained from herbarium samples. The pollen slides were prepared according to WODEHOUSE (1935) technique. Measurements and observations were made using the Leica DM1000 binocular light micro-

Tab. 1. Collection data of *Salvia limbata* and *S. palaestina* from Turkey.

	Collection data and collector number
S. limbata	**B8** Erzurum: Ilica to Erzurum, 5 km to Erzurum, 38° 55' 00'' N 41° 12' 42'' E, 1817 m, 10.7.2006, AKahraman 1293.
	B9 Van: Bahçesaray to Van, near Naren village, 38° 09' 568'' N 43° 01' 452'' E, 2281 m, 9.7.2007, AKahraman 1437.
	C10 Hakkari: Hakkari to Van, 1 km to Van city border, 37° 48' 015'' N 44° 05' 274'' E, 1839 m, 7.6.2008, Akahraman 1571.
S. palaestina	**B6** Malatya: Kangal to Hekimhan road, 39° 02' 132'' N 37° 44' 240'' E, 1460 m, 2.6.2008, AKahraman 1528B.
	B8 Diyarbakir: Lice to Diyarbakir, 65 km to Diyarbakir, 38° 18' 675'' N 40° 31' 895'' E, 789 m, 4.6.2008, AKahraman 1533A.
	C9 Şirnak: 5 km from Şirnak to Cizre, 37° 30' 891'' N 42° 25' 613'' E, 1090 m, 5.6.2008, AKahraman 1544.

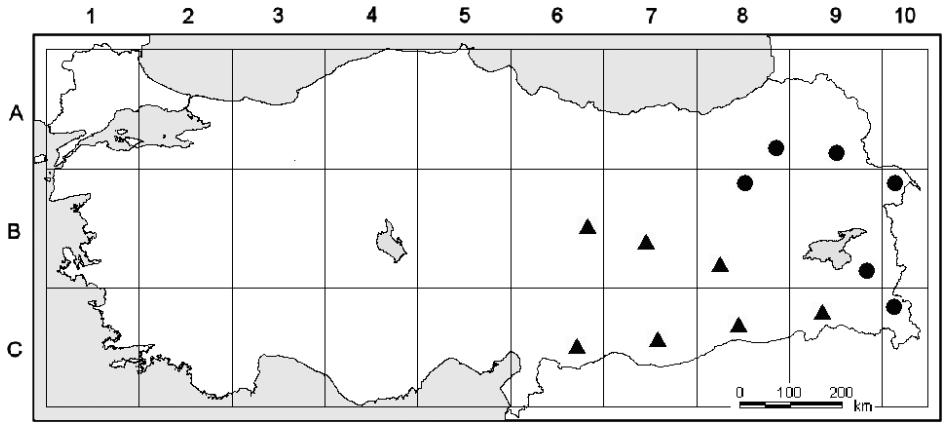

Fig. 1. Distribution map of *Salvia limbata* (●) and *S. palaestina* (▲) in Turkey.

scope and Leica DFC280 camera. Polar length, equatorial length, colpus length, exine and intine thickness for 30 pollen grains were measured under the light microscope (magnification of 1000×). Pollen grain exine sculpturing was observed using a JEOL-6060 scanning electron microscope. The pollen terminology of FAEGRI and IVERSON (1975) has been used.

The nutlets were examined using the Leica S8AP0 stereomicroscope to ensure their size and maturity. In order to determine the average seed sizes, 30 mature nutlets were measured. For SEM, the mature nutlets were placed on stubs directly and covered with gold (DOĞAN 1988). After that, they were observed and photographed with a JEOL JSM-6400 SEM.

For ecological studies, soil samples were taken from suitable habitats of *S. limbata* and *S. palaestina*. All soil samples were analyzed at the Soil, Fertilizer and Water Resources Central Research Institute, Ankara. Soil texture, organic matter, total salt, pH, $CaCO_3$, P and K analysis were made using standard techniques (BAYRAKLI 1987) and the results have been evaluated according to KAÇAR (1972).

Results

Morphological characteristics

Stems of *Salvia limbata* C.A. Meyer (synonym: *S. chrysadenia* Freyn) are 30–120 cm. The stem indumentum is retrorsely scabridulous below and with sessile glands above. Leaves are (3.5–) 5–16 × (2–) 4–10 cm, ovate-oblong and erose-dentate. The leaf indumentum is glabrous and glandular-punctate below, and sparsely pilose above. Petiole is 2–12 cm. Inflorescence is 15–80 cm. Verticillasters are 2–6 (–8)-flowered. Bracts are 2–7 mm, pale green. Pedicels are 2–6 mm. Calyces are ovate-campanulate, 6–10 mm in flower and 11–13 mm in fruit. The calyx indumentum is eglandular scabrid or densely pilose with hairs with many sessile glands. Its upper lip is shortly tridentate and recurved. Corolla is 15–25 mm and white with pale yellow lip. Corolla tube is 5–9 mm and squamulate. Filaments are 3–4 mm. Fertile anthers are 3–4 mm. Upper thecae are 15–20 mm and lower thecae are 1–2 mm. Style is 35–40 mm (Fig. 4).

Stems of *Salvia palaestina* Bentham (synonym: *S. lorentii* Hochst.) are 20–65 cm. The stem indumentum is hirsute with long flattened eglandular hairs below and densely glandular pilose (sometimes with long flattened eglandular hairs) above. Leaves are 4.5–15 (–20) × 1.5–7 (–9) cm, oblong to ovate and erose. The leaf indumentum is tomentose. Petiole 2.5–15 cm. Inflorescence is 10–50 cm. Verticillasters are (2–) 3–6-flowered. Bracts are 15–25 × 10–20 mm, often tinged pink or purple. Pedicels are 2–5 mm. Calyces are almost tubular, often pink or purple (rarely green), 12–16 mm in flower and 17–25 mm in fruit. The calyx indumentum is papillose-glandular with some longer hairs. Its upper lip is equally tridentate and spinulose. Calyx teeth are 2–4 mm, corolla 20–35 mm long, lilac or whitish-lilac. Corolla tube is 10–20 mm long and not squamulate. Filaments are 2–3 mm long. Fertile anthers are 3–4 mm. Upper thecae are 12–15 mm and lower thecae are 1–2 mm. Style is 25–45 mm (Fig. 5).

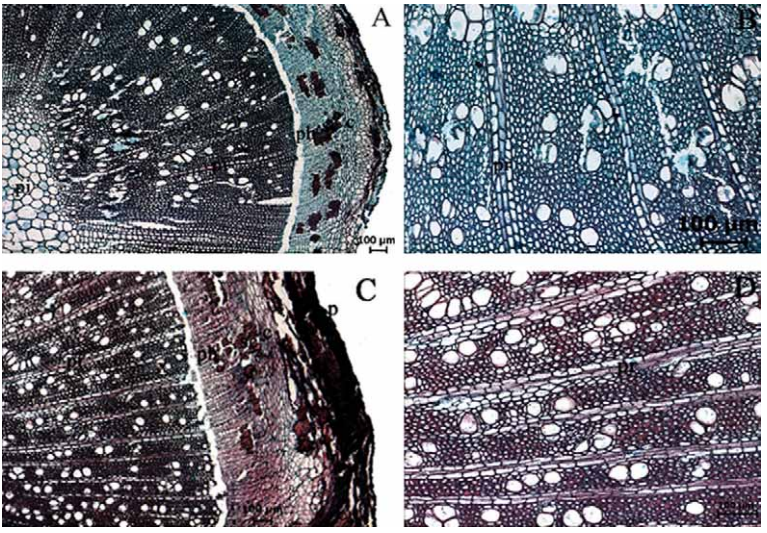

Fig. 2. Cross-sections of root. **A–B.** *S. limbata*, **C–D.** *S. palaestina*. p – periderm, c – cortex, sc – sclerenchyma, ph – phloem, x – xylem, pr – pith ray, pi – pith.

Anatomical characteristics

Root anatomy: Cross-sections taken from the root of *Salvia limbata* have revealed that the periderm layer on the outermost surface of the root is thin and its cells are irregular. A multilayered parenchymatic cortex is present under the periderm. There are several sclerenchyma groups above the phloem. The xylem (800–1300 μm) is composed of vessels and tracheids. Those cells underlying the xylem are thick-walled and narrower. Pith rays comprise 2–6-rowed rectangular cells. The pith consists of polygonal or orbicular parenchymatous cells (Fig. 2A–B, Tab. 2).

Cross-sections taken from the root of *Salvia palaestina* have showed that the multilayered periderm layer on the outermost surface of the root is thick and its cells are squashed or breaking up. Under the periderm, there is a parenchymatic cortex with a few layers. Phloem and xylem are distinguished from one another by sclerenchyma and ground tissue. Phloem is embedded in sclerenchyma tissue as several groups. The xylem (1500–2000 μm) consists of regular vessels and tracheids. Pith rays are composed of 1–8(–10)-rowed rectangular cells. The pith comprises polygonal, oval or orbicular parenchymatous cells (Fig. 2C–D, Tab. 2).

Stem anatomy: Cross-sections taken from the stem of *Salvia. limbata* have exhibited a monolayer epidermis covered by an undulate cuticle. The epidermis is composed of oval,

Tab. 2. Comparative anatomy of root, stem, leaf and petiole of *S. limbata* and *S. palaestina*.

	S. limbata		*S. palaestina*	
	Width (μm)	Length (μm)	Width (μm)	Length (μm)
	Min. – Max.	Min. – Max.	Min. – Max.	Min. – Max.
Root				
Periderm cell	15–50	10–45	30–100	30–70
Cortex cell	30–50	10–20	25–50	15–20
Pith ray	15–20	15–40	10–35	15–60
Stem				
Cuticle	4–7		5–7	
Epidermis cell	15–55	10–20	20–45	10–20
Cortex cell	30–120	25–80	30–105	20–65
Trachea cell	20–40	30–50	25–85	25–110
Pith cell	50–180	50–150	45–150	50–150
Leaf				
Cuticle	3–7		1.5–4	
Upper Epidermis Cell	25–65	15–35	15–55	10–25
Lower Epidermis Cell	10–30	7–25	15–50	10–25
Palisade Parenchyma	11–16	25–60	10–15	20–50
Spongy Parenchyma	10–16	15–20	8–20	10–20
Petiole				
Abaxial Epidermis	15–25	10–20	15–25	15–20
Adaxial Epidermis	10–20	10–20	15–30	15–20
Cortex Cell	30–105	30–115	30–125	30–130

ovate or rectangular cells. Underneath the epidermis, multilayered collenchyma cells (60–90 μm) are located at the corners and there are 1–2 rows of chlorenchyma cells between them. The cortex tissue (250–400 μm) consists of 7–10 layers of oval, ovate or orbicular parenchymatous cells. The phloem (80–120 μm) is surrounded by more or less sclerenchymatous fibers. Cambium is distinguishable. The xylem (500–1150 μm) considerably bulges at ridges. The pith comprises hexagonal or orbicular parenchymatous cells with intercellular spaces (Fig. 3A, Tab. 2).

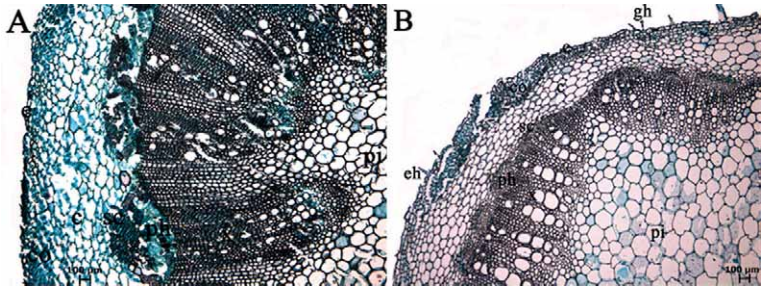

Fig. 3. Cross-sections of stem. **A.** *S. limbata,* **B.** *S. palaestina*. e – epidermis, gh – glandular hair, eg – eglandular hair, co – collenchyma, c – cortex, sc – sclerenchyma, ph – phloem, x – xylem, pi – pith.

Fig. 4. General appearance of *S. limbata*. Scale bar: 2 cm.

Fig. 5. General appearance of *S. palaestina*. Scale bar: 2 cm

Cross-sections taken from the stem of *S. palaestina* display a monolayer epidermis covered by an undulate cuticle. The epidermis consists of oval or rectangular cells. Multilayered collenchyma cells (70–130 μm) are located at the corners. There are 1–3 rows of chlorenchyma (20–30 μm) with a large number of chloroplasts between the corners. The cortex (150–200 μm) is composed of 4–7 layers of oval or orbicular parenchymatous cells. The phloem with less or more sclerenchymatic fibers measures 25–55 μm. Cambium is not clearly distinguishable. Size of the xylem is 90–300 μm and it immediately bulges at the ridges. The pith region comprises large hexagonal, polygonal or circular parenchymatous cells (Fig. 3B, Tab. 2).

Leaf anatomy: Cross-sections of the lamina and surface sections of upper and lower epidermises of *S. limbata* have showed that both epidermises are covered with eglandular hairs and sessile glands and they consist of uniseriate rectangular or oval cells with undulate cuticles. Cells of the upper epidermis are clearly larger than the lower. The leaf is equifacial and amphistomatic, with diacytic stoma type. Stomata on the lower surface show higher frequencies than those on the upper surface. On the upper surface, the length of stomata varies from 26–29 μm while the width of stomata ranges from 15–20 μm. On the lower surface, the length of stomata varies from 22–30 μm whereas the width of stomata ranges from 16–20 μm. Mesophyll (135–220 μm) comprises elongated palisade and isodiametric spongy parenchyma cells. Palisade parenchyma is 2–3-rowed under the upper and lower epidermis. Spongy parenchyma is 1–2-rowed in the middle. The midrib region, which forms a projecting part, comprises 1–3 layers of collenchyma adjacent the epidermal cells. A single large vascular bundle is located in the center (Fig. 6A–D, Tab. 2).

Cross-sections of the lamina and surface preparations of both epidermises of *S. palaestina* have revealed that the upper and lower epidermises are covered with glandular and eglandular hairs and they are composed of uniseriate rectangular or oval cells with undulate cuticles. Cells of the upper epidermis cells are nearly equal to the lower. The equifacial leaf is amphistomatic, with diacytic stoma type. Number of stomata on the lower surface is nearly equal to that of stomata on the upper surface. On the upper surface, the length of stomata varies from 38–45 μm while the width of stomata ranges from 30–36 μm. On the lower surface, the length of stomata varies from 34–43 μm whereas the width of stomata ranges from 23–30 μm. Mesophyll region (60–180 μm) consists of 1–2 layers of elongated palisade parenchyma cells found on both sides of 1–2 layers of isodiametric spongy parenchyma cells, occupying a small part in the middle. The midrib region, which forms a projecting part, comprises 2–3 collenchyma layers below the epidermal cells. There is a single large vascular bundle in the center (Fig. 6E–H, Tab. 2).

Petiole anatomy: In cross-sections taken from the petiole of *S. limbata* it has been observed that the epidermal cells of both surfaces are oval, rectangular or squarish epidermal cells. The adaxial epidermis cells are slightly larger than the abaxial epidermis cells. There are several layers of collenchyma cells under the epidermis. Four broad vascular bundles are located in the middle. Also, there are four small vascular bundles in each of the petiolar wings. Vascular bundles are of collateral type. The sclerenchyma tissue is well developed outside of the phloem and the xylem (Fig. 7A–B, Tab. 2).

Cross-sections taken from the petiole of *S. palaestina* showed that the epidermal cells of both surfaces are oval, rectangular or squarish epidermal cells. Adaxial and abaxial epidermis cells are equal in size. 2–5-layered collenchyma are located under the epidermis.

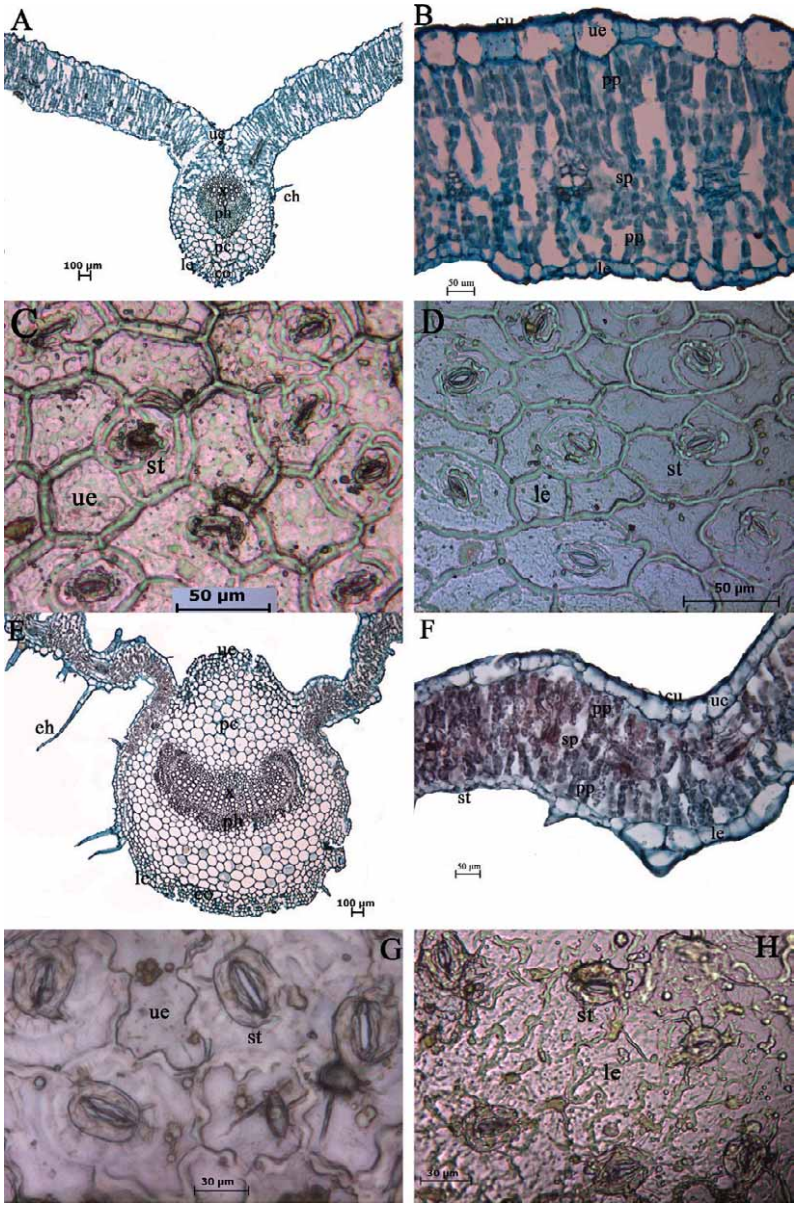

Fig. 6. Cross and surface sections of leaf. **A–D.** *S. limbata,* **E–H.** *S. palaestina*. cu – cuticle, eg – eglandular hair, ue – upper epidermis, le – lower epidermis, pc – parenchyma cell, x – xylem, ph – phloem, pp – palisade parenchyma, sp – spongy parenchyma, st – stomata.

Two or three broad vascular bundles are located in the middle. Moreover, there are two small vascular bundles in each of the petiolar wings. The vascular bundle tissue surrounded by parenchymatic cells seems as a shallow crescent and collateral. There are several sclerenchymatous cells on the phloem (Fig. 7C–D, Tab. 2).

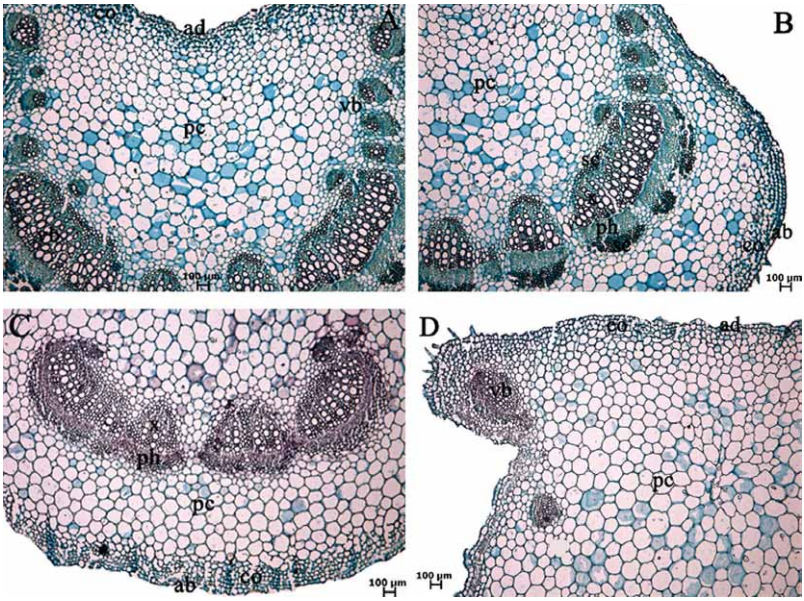

Fig. 7. Cross-sections of petiole. **A–B.** *S. limbata,* **C–D.** *S. palaestina*. ad – adaxial epidermis, ab – abaxial epidermis, co – collenchyma, pc – parenchyma cell, vb – vascular bundle, x – xylem, ph – phloem, sc – sclerenchyma.

Ecological characteristics

Both *Salvia limbata* and *S. palaestina* prefer clayey-loamy and loamy soils that are slightly alkaline. The organic matter and $CaCO_3$ content of the soil of *S. limbata* are 2.07–2.10% (a middle level) and 0.94–16.90% (a very small to a high level), whereas the organic matter and $CaCO_3$ content of the soil of *S. palaestina* are 0.44–2.04% (a very low to middle level of organic matter) and 3.89–72.84% (a small to a very high level) (Tab. 3).

Tab. 3. Soil features of *S. limbata* and *S. palaestina*.

Parameter	*S. limbata*	*S. palaestina*
Sand (%)	43.9–46.6	23.0–42.8
Silt (%)	25.9–30.5	31.3–38.2
Clay (%)	22.9–30.2	25.9–38.8
Texture	Clayey-loamy and loamy	Clay loam and loamy
pH	7.56–7.93	7.60–7.82
Organic matter (%)	2.07–2.10	0.44–2.04
Total salt (%)	0.014–0.016	0.006–0.026
Total $CaCO_3$ (%)	0.94–16.90	3.89–72.84
P (ppm)	9.36–16.00	4.21–21.01
K (ppm)	113.55–296.31	87.00–445.84

Salvia limbata shares its habitat with mainly herbaceous plants including *S. nemorosa* L., *S. poculata* Náb*, S. xanthocheila* Boiss. ex Bentham*, S. macrochlamys* Montbret et Aucher ex Bentham*, S. atropatana* Bunge, *S. syriaca* L.*, S. multicaulis* Vahl, *S. aethiopis* L., *S. virgata* Jacq.*, S. verticillata* L. subsp. *verticillata* and *amasiaca* (Freyn et Born.) Bornm., *Senecio* sp., *Euphorbia* sp., *Vicia* sp., *Dactylis* sp., *Verbascum* sp., *Hordeum* sp. and *Centaurea* sp. Other species growing in association with *S. palaestina* are *S. cryptantha* Montbret et Aucher ex Bentham, *S. euphratica* Montbret et Aucher ex Bentham var. *euphratica* and var. *leiocalycina* (Rech. fil.) Hedge, *S. candidissima* Vahl subsp. *candidissima, S. ceratophylla* L.*, S. syriaca* L.*, S. multicaulis* Vahl, *S. aethiopis* L.*, S. virgata* Jacq.*, S. verticillata* L. subsp. *amasiaca* (Freyn et Born.) Bornm.*, Tripleouspermum* sp., *Crupina* sp., *Psorolea* sp., *Phlomis* sp., *Crepis* sp., *Tragopogon* sp., *Avena* sp., *Trigonella* sp., *Torillis* sp., *Aegilops* sp., *Mellilotus* sp., *Alopecurus* sp., *Scabiosa* sp. and *Alkanna* sp.

Pollen characteristics

Shape of pollen grains of *S. limbata* is oblate-spheroidal (Fig. 6A–B). Polar axis is 52.41 ± 4.69 μm and its equatorial axis is 59.82 ± 4.89 μm. The ratio of P/E is 0.88. Colpus length is 49.25 ± 4.78 μm and colpus width is 7.57 ± 1.61 μm. The exine thickness is 1.33 ± 0.20 μm and the intine thickness is 0.55 ± 0.09 μm (Tab. 4). The exine sculpturing is bireticulate-perforate (Fig. 8C–D).

Shape of pollen grains of *S. palaestina* is prolate-spheroidal (Fig. 9A–B). Polar axis is 47.72 ± 3.85 μm and equatorial axis is 45.40 ± 3.73 μm. The ratio of P/E is 1.05. Colpus length is 42.07 ± 3.99 μm and colpus width is 3.79 ± 0.72 μm. The exine thickness is 1.37 ± 0.21 μm and the intine thickness is 0.75 ± 0.03 μm (Tab. 4). The exine sculpturing is bireticulate-perforate (Fig. 9C–D).

Tab. 4. Pollen morphological data of *S. limbata* and *S. palaestina*.

Parameter	*S. limbata*	*S. palaestina*
Polar axis*	41.52–65.03	40.00–51.96
	52.41 ± 4.69	47.72±3.85
Equatorial axis*	50.16–74.10	38.35–48.99
	59.82 ± 4.89	45.40±3.73
P/E	0.88	1.05
Colpus length*	39.61–59.15	33.08–46.24
	49.25 ± 4.78	42.07±3.99
Colpus width*	5.25–11.78	3.00–5.21
	7.57 ± 1.61	3.79±0.72
Exine thickness*	1.03–1.67	1.07–1.70
	1.33 ± 0.20	1.37±0.21
Intine thickness*	0.42–0.70	0.71–0.81
	0.55 ± 0.09	0.75±0.03
Shape	oblate-spheroidal	prolate-spheroidal
Exine surface	bireticulate-perforate	bireticulate-perforate

* range, mean and standard deviation

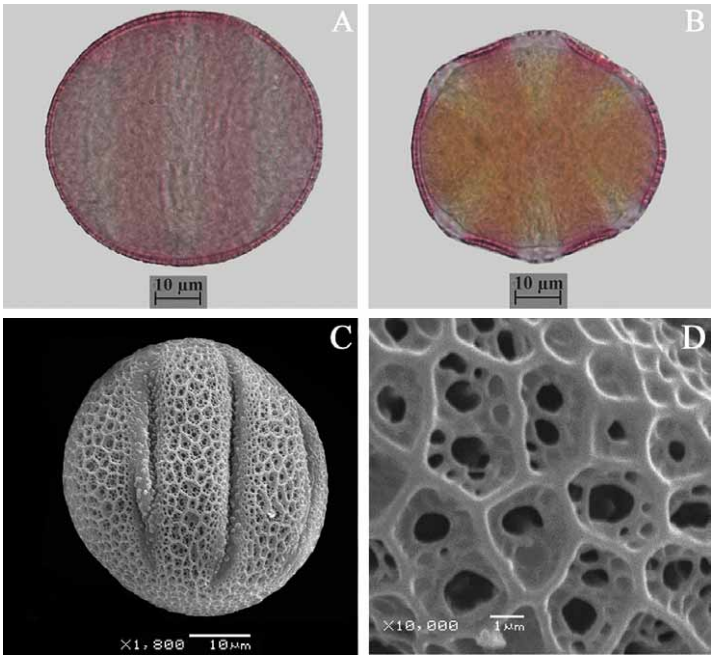

Fig. 8. LM and SEM micrographs of the pollen grains of *S. limbata*. **A–B** Equatorial and polar view in LM micrographs, **C–D** Equatorial view and exine sculpturing SEM.

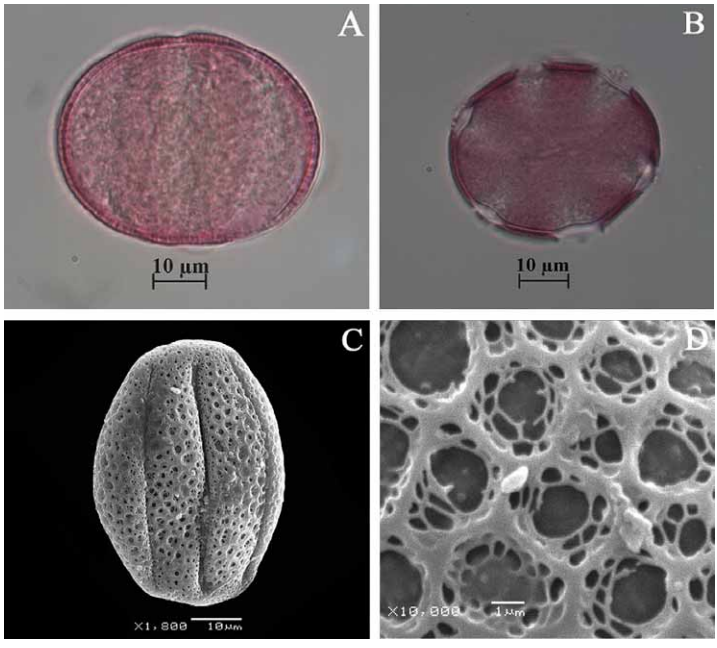

Fig. 9. LM and SEM micrographs of the pollen grains of *S. palaestina*. **A–B** Equatorial and polar view in LM micrographs, **C–D** Equatorial view and exine sculpturing in SEM.

Nutlet micromorphology

The nutlets of *Salvia limbata* are ovate to oblong in their outline. Mature nutlets of *S. limbata* are 2.5–3.0 mm long and 1.8–2.1 mm wide. Their hilum diameter is 0.40–0.55 mm. The nutlet surface is glabrous and has irregular protuberances and undulating ridges (Fig. 10 A–B). The mature nutlet is light brown.

The nutlets of *Salvia palaestina* are spherical in their outline. Mature nutlets of *S. palaestina* are 2.4–2.8 mm long and 2.2–2.6 mm wide. Their hilum diameter is 0.23–0.38 mm. The nutlet surface is glabrous and smooth to slightly papillaes (Fig. 10 C–D). The mature nutlet is light brown.

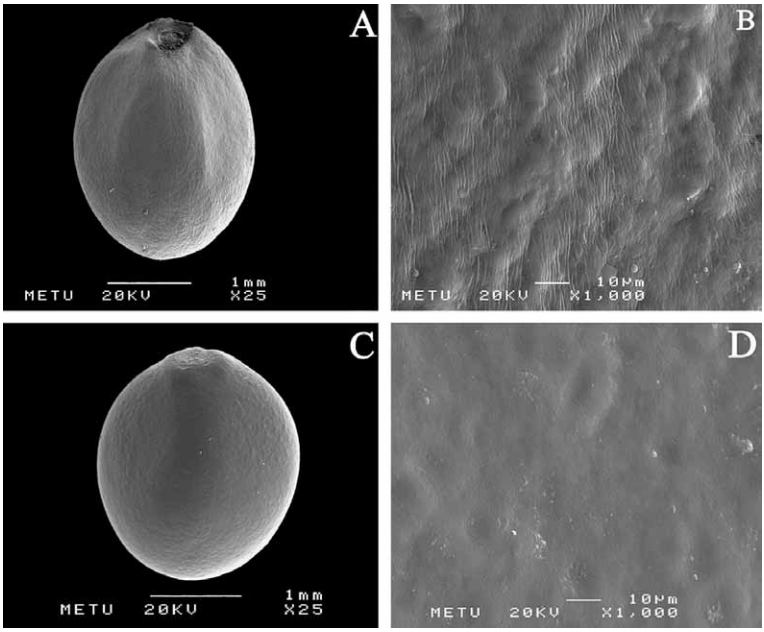

Fig. 10. SEM photos of the nutlets of *Salvia limbata* and *S. palaestina*. **A–B** General appearance and surface of the nutlets of *S. limbata*, **C–D** General appearance and surface of the nutlets of *S. palaestina*.

Habitat and phenology

Salvia limbata grows on stony slopes, roadsides, steppe and cornfields between 1060–2400 m. It flowers in June to August. *Salvia palaestina* grows on limestone and igneous rocky slopes, in *Quercus* scrub, vineyards, fallow fields and roadsides between 300 and 1460 m. It flowers in April to July.

Discussion

The main morphological characters useful for *Salvia* species determination are stem, leaf and calyx indumentum, bract and calyx length, bract and corolla colour. The stem texture of *S. limbata* is retrorsely scabridulous below and with sessile glands above, whereas

that of *S. palaestina* is hirsute with long flattened eglandular hairs below and often glandular pilose above. In *S. limbata* the leaf texture is glabrous and glandular-punctate below and sparsely pilose above, but in *S. palaestina* the leaf indumentum is tomentose. The calyx texture is eglandular scabrid or densely pilose in *S. limbata* but papillose-glandular in *S. palaestina*. The length of bracts and calyces in *S. limbata* are shorter than that of bracts and calyces in *S. palaestina*. In *S. limbata* bract is pale green and corolla is white with pale yellow lip whereas in *S. palaestina* bract colour is pink or purple and corolla colour is lilac or whitish-lilac. *S. limbata* has taller corolla tube, upper thecae and filaments than *S. palaestina*. Morphological characters of *S. limbata* and *S. palaestina* are compared in Tab. 5.

Tab. 5. Diagnostic morphological features of *Salvia limbata* and *S. palaestina*.

Character	*S. limbata*	*S. palaestina*
Stem indumentum	retrorsely scabridulous below and with sessile glands above	hirsute with long flattened eglandular hairs below and densely glandular pilose above
Leaf indumentum	glabrous and glandular-punctate below and sparsely pilose above	tomentose
Bract length (mm)	2–7	15–25
Bract colour	pale green	pink or purple
Calyx length (mm)	6–10 in flower, 11–13 mm in fruit	12–16 in flower, 17–25 in fruit
Calyx indumentum	eglandular scabrid or densely pilose hairs	papillose-glandular
Corolla colour	white with pale yellow lip	lilac or whitish-lilac
Corolla length (mm)	15–25	20–35
Corolla tube length (mm)	5–9	10–20
Corolla tube shape	Squamulate	Not squamulate
Upper theca length (mm)	15–20	12–15
Filament length (mm)	3–4	2–3

Our findings usually agree with the description of the Flora of Turkey (HEDGE 1982), but some differences were found here and so their descriptions were emended and expanded. It is reported that the stem length of *S. limbata* was 30–100 cm, its leaf size was 10–16 cm in length and 6–10 cm in width, its bract size was 2–6 mm, its corolla length was c. 25 mm, its calyx length was 8–10 mm in flower and to c. 12 mm in fruit while the stem length of *S. palaestina* was 30–60 cm, its leaf size was 5–13 (–20) cm in length and 1.5–7 (–8.5) cm in width, its bract size was c. 15 × 18 mm, its corolla length was c. 25 mm, its calyx length was c. 15 mm in flower and to c. 17 mm in fruit (HEDGE 1982). According to our study, the stem length of *S. limbata* was 30–120 cm, its leaf size was (3.5–) 5–16 cm in length and (2–) 4–10 cm in width, its bract size was 2–7 mm, its corolla length was 15–25 mm, its calyx length was 6–10 mm in flower and 11–13 mm in fruit, but the stem length of *S. palaestina* was 20–65 cm, its leaf size was 4.5–15 (–20) cm in length and 1.5–7 (–9) cm in width, its bract size was c. 15–25 × 10–20 mm, its corolla length was 20–35 mm, its calyx length was 12–16 mm in flower and 17–25 mm in fruit. Additionally, inflorescence, corolla tube, filament, fertile anther, style, upper thecae and lower thecae features are presented the first time in this study.

Root anatomy of the family Labiatae (METCALFE and CHALK 1972) is characterized by pith rays of roots composed of 2–12 or more rowed cells. Our study shows that pith rays of *S. limbata* consist of 2–6 rowed cells while those of *S. palaestina* comprise 1–8 (–10) rowed cells. It was reported that *S. macrochlamys* Boiss. et Kotschy (sect. *Salvia*) has mainly 1–4 rays of cells (KAHRAMAN et al. 2010a) and *S. ballsiana* (Rech. fil.) Hedge has 1–3 (–4) rays of cells (KAHRAMAN 2010b). The number of pith ray rows may be used to distinguish *Salvia* species at the sectional level. In addition, xylem size is a diagnostic character in the two species. In *S. limbata* xylem size in is 800–1300 μm while in *S. palaestina* it is 1500–2000 μm.

The stems of Labiatae are rectangular, have well-developed collenchyma covering a broad area at the corners and have a sclerenchymatic tissue surrounding the vascular bundle tissue (METCALFE and CHALK 1950). We found the same anatomical features in the stems of *S. limbata* and *S. palaestina* and also detected a few layers of chlorenchyma cells below the epidermis between the corners. However, vascular and cortex size are different in the two species. In *S. limbata* phloem is 80–120 μm in width while in S. *palaestina* phloem is 25–55 μm in width. Xylem is 500–1150 μm in *S. limbata*, however, xylem is 90–300 μm in *S. palaestina*. The cortex tissue of *S. limbata* (250–400 μm) is larger than that of *S. palaestina* (150–200 μm). In addition, *S. limbata* has a 7–10-layered cortex while *S. palaestina* has a 4–7-layered cortex.

Mesophyll is completely parenchymatic and midrib is surrounded by collenchyma cells in *Salvia* species (METCALFE and CHALK 1972). We observed the same characteristics in the leaves of *S. limbata* and *S. palaestina*. However, the number of palisade parenchyma rows in the two species is different. The mesophyll of *S. limbata* is composed of 2–3 layers of palisade parenchyma cells on both sides while that of *S. palaestina* consists of 1–2 layers of palisade parenchyma cells. Cells of the upper epidermis of *S. limbata* are clearly larger than the lower epidermis, but those of the upper epidermis of *S. palaestina* are almost equal to the lower epidermis. Additionally, stomata of *S. limbata* (22–30 μm in length and 15–20 μm in width) are smaller than stomata of *S. palaestina* (34–45 μm in length and 23–36 μm in width) on both surfaces of leaves.

The structure of the vascular bundles in the petiole structure of the family Labiatae is a taxonomically significant character (METCALFE and CHALK 1950). In the petiole of *Salvia limbata*, there are four broad vascular bundles in the middle and also there are four small vascular bundles in each of the petiolar wings. In the petiole of *S. palaestina*, there are two or three large vascular bundles in the middle and also there are two small vascular bundles in each of the petiolar wings. *S. ballsiana* (KAHRAMAN 2010b) has a broad vascular bundle in its middle part of the petiole and four or six small bundles on its wings, and *S. macrochlamys* (KAHRAMAN et al. 2010a) has a single large vascular bundle in the center of the petiole and two small bundles its wings. To sum up, number of vascular bundles in the petiole can be used to distinguish the species.

Salvia limbata and *S. palaestina* grow on clayey loamy and loamy soils with slightly alkaline (pH 7.56–7.93) and a low or medium level of organic matter (0.44–2.10%). The amounts of P, K and total salt present vary between 4.21–21.01 ppm (enough to very much), 87.00–445.84 ppm (very much) and 0.006–0.026% (saltless to slightly salty), respectively. However, the soils of *S. limbata* (0.94–16.90%) are less limy than that of *S. palaestina* (3.89–72.84%). Both the species generally shares their habitats with herbaceous plants.

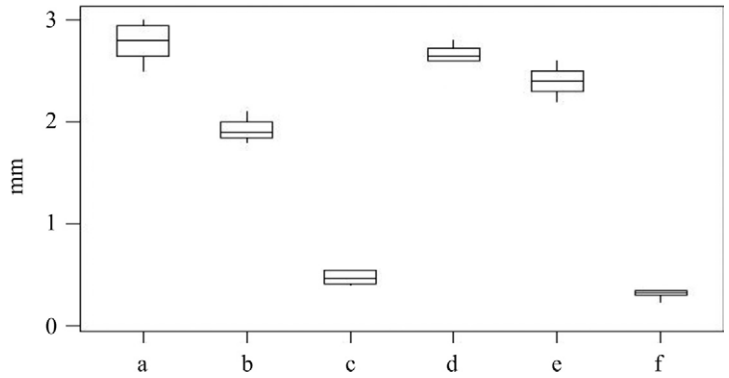

Fig. 11. Boxplots of the nutlet length, width and hilum diameter of *Salvia limbata* and *S. palaestina*. Nutlet length (a), width (b) and hilum diameter (c) of *S. limbata*. Nutlet length (d), width (e) and hilum diameter (f) of *S. palaestina*.

Tab. 6. Comparison of anatomical, palynological and nutlet characters of *Salvia limbata* and *S. palaestina*.

Character	S. limbata	S. palaestina
Root		
Xylem size in (μm)	800–1300	1500–2000
Stem		
Phloem size (μm)	80–120	25–55
Xylem size (μm)	500–1150	90–300
Cortex size (μm)	250–400	150–200
Cortex layer	7–10-layered	4–7-layered
Leaf		
Number of palisade parenchyma rows	2–3	1–2
Size of stomata (μm)	22–30 × 15–20	34–45 × 23–36
Upper epidermis versus lower epidermis	Large	Equal
Petiole		
Number of vascular bundles	4 large in the center and 4 small in each wing	2–3 large in the center and 2 small in each wing
Pollen		
Size (μm)	52.41± 4.69 × 59.82 ± 4.89	47.72 ± 3.85 × 45.40 ± 3.73
Shape	oblate-spheroidal	prolate-spheroidal
Nutlet		
Size (mm)	2.5–3.0 × 1.8–2.1	2.4–2.8 × 2.2–2.6
Shape	ovate to oblong	spherical
Hilum diameter (mm)	0.40–0.55	0.23–0.38
Ornamentation	irregular protuberances and undulating ridges	smooth to slightly papillaes

Pollen characteristics of the family Labiatae have considerable taxonomic importance (ERDTMAN (1945). The classification of genera in Labiatae has been revised (CANTINO et al. (1992), with *Salvia* placed within the subfamily Nepetoideae because it had hexacolpate pollen. Light microscope observations in this study show pollen size and shape in different species is different. However, scanning electron micrographs reveal that their exine sculpturing is similar to each other. *Salvia limbata* has a pollen size of 52.41 ± 4.69 × 59.82 ± 4.89 µm while *S. palaestina* has a pollen size of 47.72 ± 3.85 × 45.40 ± 3.73 µm (Tab. 4). In addition, pollen shape is oblate-spheroidal in *S. limbata* but it is prolate-spheroidal in *S. palaestina*. The exine sculpturing of the two species are bireticulate-perforate. Pollen size, shape and sculpturing of *S. indica* L. (section *Aethiopis*) are 44.98 ± 4.22 × 52.24 ± 4.41 µm, suboblate and bireticulate-perforate, respectively (KAHRAMAN 2009a). *S. anatolica* Hamzaoglu et A. Duran and *S. bracteata* Banks et Sol. were observed to be oblate--spheriodal shaped pollen grains. The sculpturing in *S. anatolica* is euryreticulate, however the sculpturing in *S. bracteata* is suprareticulate (HAMZAOĞLU et al. 2005).

Seed surface micromorphology was found to have systematic value at generic and specific levels (HEDGE 1970, MARIN et al. 1996). The nutlets of *S. sclarea* were observed to be rounded-trigonous, c. 3 × 2 mm (HEDGE 1982) and its surface had protuberances (MARIN et al. 1996). The nutlets of *S. verticillata* L. (sect. *Hemisphace*) were analyzed to be c. 2.2 × 1.3 mm (HEDGE 1982) and its surface had reticulo-papillosae (MARIN et al. 1996). According to our study, the nutlets of *S. limbata* are ovate to oblong, 2.5–3.0 mm long and 1.8–2.1 mm wide while the nutlets of *S. palaestina* are spherical, 2.4–2.8 mm long and 2.2–2.6 mm wide. Additionally, hilum diameter in the former species is 0.40–0.55 mm, however in the latter species it is 0.23–0.38 mm. The nutlet surface of *S. limbata* has irregular protuberances and undulating ridges in *S. palaestina* it is smooth to slightly papillaes. Their seed length, width and hilum diameter are illustrated in figure 11. Consequently, seed size, shape and ornamentation characters appear to have significant taxonomic value in distinguishing *S. limbata* and *S. palaestina*. Diagnostic anatomical, palynological and nutlet characteristics of the two species are summarized (Tab. 6).

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