

The leaf anatomy of naturally distributed *Juniperus* L. (Cupressaceae) species in Turkey

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Abstract: In this study, the anatomical characteristics of the leaves of the *Juniperus* section of the *Juniperus* L. species [*Juniperus drupacea* Lab.; *J. communis* L. var. *communis*; *J. communis* var. *saxatilis* Pall.; *J. oxycedrus* L. subsp. *oxycedrus*; and *J. oxycedrus* subsp. *macrocarpa* (Sibth. & Sm.) Ball] are reported for those samples naturally distributed in Turkey. Microscopic views of the transverse and surface sections from the leaves of each species were examined and described. Differentiations were made between 3 species as a result of observations of the arrangement of the palisade parenchyma and the location of the secretory canals.

Key words: *Juniperus*, leaf, anatomy, Cupressaceae

Türkiye’de yetişen *Juniperus* L. (Cupressaceae) türlerinin yaprak anatomisi

Özet: Bu çalışmada, Türkiye’de doğal olarak yetişen *Juniperus* seksiyonunda yer alan *Juniperus* L. türlerinin [*Juniperus drupacea* Lab., *J. communis* L. var. *communis*, *J. communis* var. *saxatilis* Pall., *J. oxycedrus* L. subsp. *oxycedrus*, ve *J. oxycedrus* subsp. *macrocarpa* (Sibth. & Sm.) Ball] yapraklarının anatomik özellikleri değerlendirilmiştir. Her bir türün yapraklarından alınan enine ve yüzeyel kesitler ışık mikroskobu ile incelenmiş ve tanımlanmıştır. Palizat parenkimasının durumu ve salgı kanallarının yerlerinin bu 3 türde farklılık gösterdiği görülmüştür.

Anahtar sözcükler: *Juniperus*, yaprak, anatomi, Cupressaceae

Introduction

The genus *Juniperus* L. (Cupressaceae) is represented by about 70 species in the northern hemisphere and extends south, especially in mountainous regions, into the Himalayas, Taiwan, tropical Africa, and the West Indies (Townsend &

Guest, 1966; Nakanishi et al., 2004; Seca & Silva, 2007). This genus, known locally as “Arđıç”, is represented by 7 species in Turkey (Coode & Cullen, 1965; Townsend & Guest, 1966; Baytop, 1994). The genus of *Juniperus* is divided into 2 sections: *Juniperus* (identified by leaves that are all acicular, rigid, and jointed at the base) and *Sabina* Spach. (identified by

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leaves that are generally scale-like or, if acicular, not jointed at the base). The section *Juniperus* contains 3 species (*J. drupacea* Lab., *J. communis* L., and *J. oxycedrus* L.); the section *Sabina* has 4 species (*J. phoenicia* L., *J. foetidissima* Willd., *J. sabina* L., and *J. exelsa* M.Bieb.) (Coode & Cullen, 1965; Farjon, 2000).

Leaves from *J. communis* are commonly sold in herbalist shops in Turkey as well as being collected for export as a resource native to Turkey (Özhatay et al., 1997). In Turkish traditional medicine, decoctions made from leaves and fruits of *J. oxycedrus* have been used as diuretics, to aid in the passing of kidney stones, and in the treatment of haemorrhoids (Sezik et al., 1992; Yeşilada et al., 1993). However, there is no record of using the leaves of *J. drupacea* in herbal medicine. There are many studies regarding the essential oil of the *Juniperus* species and their antimicrobial activities (Adams 1997; Pepeljnjak et al., 2005; Ünlü et al., 2008). Further biological activities have also been reported for several species of *Juniperus*, including uses such as antitumoral, antifertility, abortifacient, diuretic, vasorelaxing (Seca & Silva, 2007), anti-inflammatory, and antinociceptive (El-Sayed, 1998; Seca & Silva, 2007; Akkol et al., 2009). There have been 2 botanical reports on the plant in Turkey: the first report was on the morphological and anatomical structures of the wood of *J. communis* subsp. *communis*, *J. communis* subsp. *hemisphaerica* (Persl.) Nyman, *J. communis* subsp. *nana* Syme, *J. oblonga* M.Beib., *J. oxycedrus* subsp. *oxycedrus*, *J. oxycedrus* subsp. *macrocarpa* (Sibth. et Sm.) Ball., *J. phoenicia*, *J. foetidissima*, *J. sabina*, and *J. exelsa* (Eliçin, 1977); the second report described a new variety, *J. oxycedrus* subsp. *oxycedrus* var. *spilinanus* Yalt. (Yaltırık et al., 2007). Classical reviews of the species have focused on the stem anatomy of the *Juniperus* species rather

than the leaf anatomy (Esau, 1977; Mauseth, 1988; Evert, 2006). An exploration of the epicuticular waxes of *J. communis* was published recently based on an extensive scanning electron microscopy (SEM) study (Ensikat et al., 2006). Another study used 4 co-occurring, related species to explore links between species distributions, xylem anatomy, and vulnerability to both drought- and freezing-induced cavitation (Willson & Jackson, 2006).

Anatomical characters are not always as useful as morphological characters for plant identifications; however, they are well-established criteria in the identification of herbal drugs (Güvenç & Duman, 2010). Anatomical studies offer significant assistance in the identification of medicinal products in a powdered or extremely broken form (Jacson & Snowdon, 1968). The aim of our study was to report on the anatomical properties of the leaf of *J. drupacea* (Syn. *Arceuthos drupacea* (Lab.) Ant. & Kotschy); *Juniperus communis* var. *communis* (Syn. *J. oblonga* M.Bieb.; *J. communis* subsp. *hemisphaerica* (J. & C.Presl) Nyman); *Juniperus communis* var. *saxatilis* (Syn. *J. nana* Willd.; *J. communis* susp. *nana* (Willd.) Syme); *Juniperus oxycedrus* subsp. *oxycedrus* (Syn. *J. rufescens* Link); and *Juniperus oxycedrus* subsp. *macrocarpa* (Syn. *J. macrocarpa* Sibth. & Sm.) that are used in traditional medicine in Turkey.

Material and methods

The plant materials were collected in different seasons from a variety of localities in Turkey. A voucher specimen was deposited at the Herbarium of Ankara University, Faculty of Pharmacy (AEF), in Ankara, Turkey. The location from which each of the investigated plant samples was taken is shown in Table 1.

Table 1. Description of the collection locations of the studied *Juniperus* species.

Species	Location Description
<i>Juniperus drupacea</i>	C4 Antalya: Antalya-Konya road, around Akseki, 1150 m, 10 09 2004, <i>Ayşegül & Uğur Güvenç</i> . AEF 23610.
<i>J. communis</i> var. <i>communis</i>	B9 Ağrı: Doğubeyazıt, Korhan Y, 2400 m, 16 10 2004, <i>Mehmet Koyuncu & Ali Erdem</i> . AEF 23854.
<i>J. communis</i> var. <i>saxatilis</i>	A4 Ankara: Kızılcahamam, Işık Dağı, ca. 1300 m, 22 05 2004, <i>Ayşegül Güvenç & Erdoğan Şatır</i> . AEF 23801.
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i>	A4 Ankara: Kızılcahamam, Işık Dağı, ca. 1300 m, 22 05 2004, <i>Ayşegül Güvenç & Erdoğan Şatır</i> . AEF 23799.
<i>J. oxycedrus</i> subsp. <i>macrocarpa</i>	B1 İzmir: Çeşme, around Çiftlikköy, 10-20 m, 25 09 2004, <i>Ali Erdem & Erdoğan Şatır</i> . AEF 23855.

For anatomical studies, the aerial parts of the *Juniperus* species were preserved in 70% ethanol. The cutting of transverse (mid-parts of the leaves) and surface (upper and lower faces of the leaves) sections of the leaves was performed by hand from material preserved in chloral hydrate solution R. and Sartur reagent. The Sartur reagent was comprised of KI-I, aniline, sudan III, lactic acid, alcohol, and water (Çelebioğlu & Baytop, 1949). Characteristic elements were determined, their figures were drawn (Olympus BX50 microscope attachment to Olympus U-DA 2K 17149 drawing tube), and microphotographs were taken (Leica DM 4000 B).

Results

Whole leaves of the plant are linear-lanceolate. In the transverse section, however, these leaves are seen to be semi-circular or triangular. The following anatomical structures and features were observed in transverse and surface sections of the leaves of 5 *Juniperus* taxa.

Epidermis: Cuticle is thick and penetrates toward the border between the cells. Upper epidermal cells are usually square-shaped and single layered, with thick walls and usually small lumen. In the surface sections of the leaves, epidermal cells are long, with thick walls, and abundant plasmodesma pores are seen. The stomata are deeply sunken and suspended within the epidermis. Upper epidermis has abundant stomata cells and is composed of small cells with 4-6 angled, irregular form which are similar appearance to those in the lower epidermis. The outstanding feature of these stomata is that the subsidiary cells are partly lignified. Stomata are present in the upper and lower epidermis of *J. drupacea*; however, in *J. communis* and *J. oxycedrus* these cells have been seen only in the upper epidermis (Figures 1-5).

Hypodermis: The hypodermis is beneath the epidermis layer. It is usually single-layered, with sclerified fibrous, very small lumen (smaller than the epidermal lumen) and cells that are multi-layered in the corners. Hypodermis cells occasionally penetrate toward the border between the palisade cells and the hypodermis is seen as multi-layered in these zones. While the hypodermis is shown beneath the whole lower epidermis, this layer is seen in the corners and

midrib sections in the upper surface (except under the rows of stomata). However, the hypodermis layer is not located in the mid-part of upper surface of *Juniperus communis* var. *communis* (Figures 1-5).

Mesophyll: The mesophyll is differentiated into palisade and spongy parenchyma. The palisade parenchyma consists of 1-2 layers of thin-walled and compactly arranged cylindrical cells. The first layer of the palisade consists of long cells while the second layer consists of small cells. These cells contain dense starch granules. Spongy parenchyma usually consists of circular or oval cells with intercellular space. This parenchyma is located underneath the palisade parenchyma (Figures 1-5).

Vascular bundle: The vascular bundle is located in the leaf centre and is usually one bundle and semicircular. Vascular and transfusion tissues are clearly seen and are to the inside of the endodermis. Transfusion tissue is seen to the right and left of the vascular bundle. The vascular bundle is the collateral type; the xylem is located on the upper side and the phloem on the lower side (Figures 1-5).

Secretory canals: There is only one large secretory canal for all of the 5 taxa and it is located between the vascular bundle and the lower epidermis. Secretory cells are thin-walled and one-layered. These cells are surrounded by a one-layer fibrous sheath with lignified walls. Species can be differentiated according to the location of secretory canals (Figures 1-5).

Discussion

In this study, we have investigated the anatomical structure of leaves of the 3 *Juniperus* species (*J. drupacea*, *J. communis* var. *communis*, *J. communis* var. *saxatilis*, *J. oxycedrus* subsp. *oxycedrus*, *J. oxycedrus* subsp. *macrocarpa*) that are sold in herbalist shops and naturally distributed throughout Turkey. The fruit, leaves, tar, and resin of the *Juniperus* species, called "Ardıç" locally, are commonly used both internally and externally in traditional medicine in Turkey (Coode & Cullen, 1965; Townsend & Guest, 1966; Sezik et al., 1992; Yeşilada et al., 1993; Baytop, 1994). In addition to such medicinal uses, the leaves of some of *Juniperus* species are also used as herbal tea (Özhatay et al., 1997). For this reason, the *Juniperus* species growing in Turkey are economically valuable.

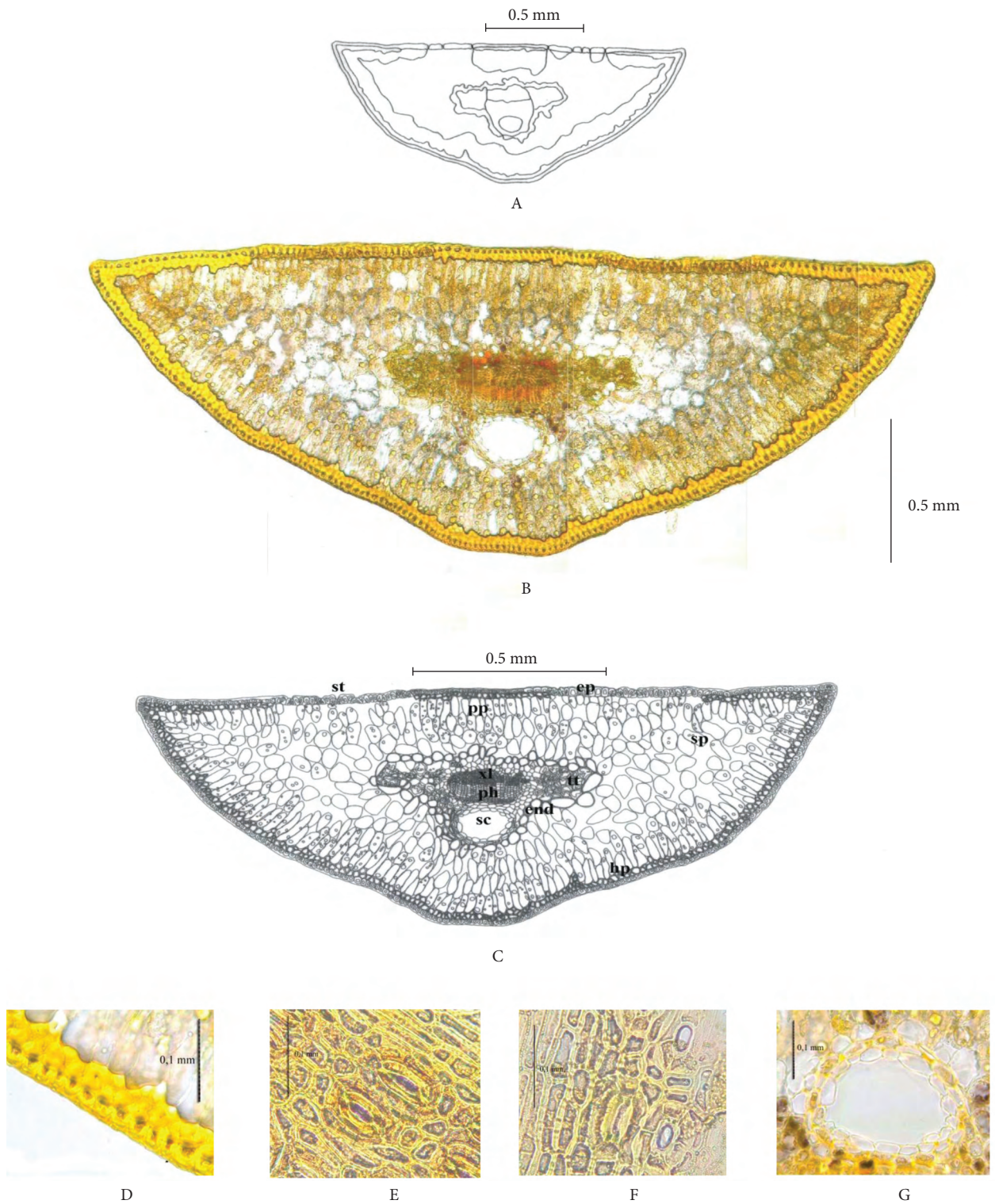


Figure 1. *Juniperus drupacea* – cross section of leaf blade. **A** and **B** - general view, **C** - anatomic properties, **D** - epidermis and hypodermis, **E** - upper surface of epidermis, **F** - lower surface of epidermis, **G** - secretory canal. Abbreviations are as follows: **ep** - epidermis, **hp** - hypodermis, **end** - endodermis, **sc** - secretory canal, **pp** - palisade parenchyma, **sp** - spongy parenchyma, **ph** - phloem, **xl** - xylem, **tt** - transfusion tissue, **st** - stoma.

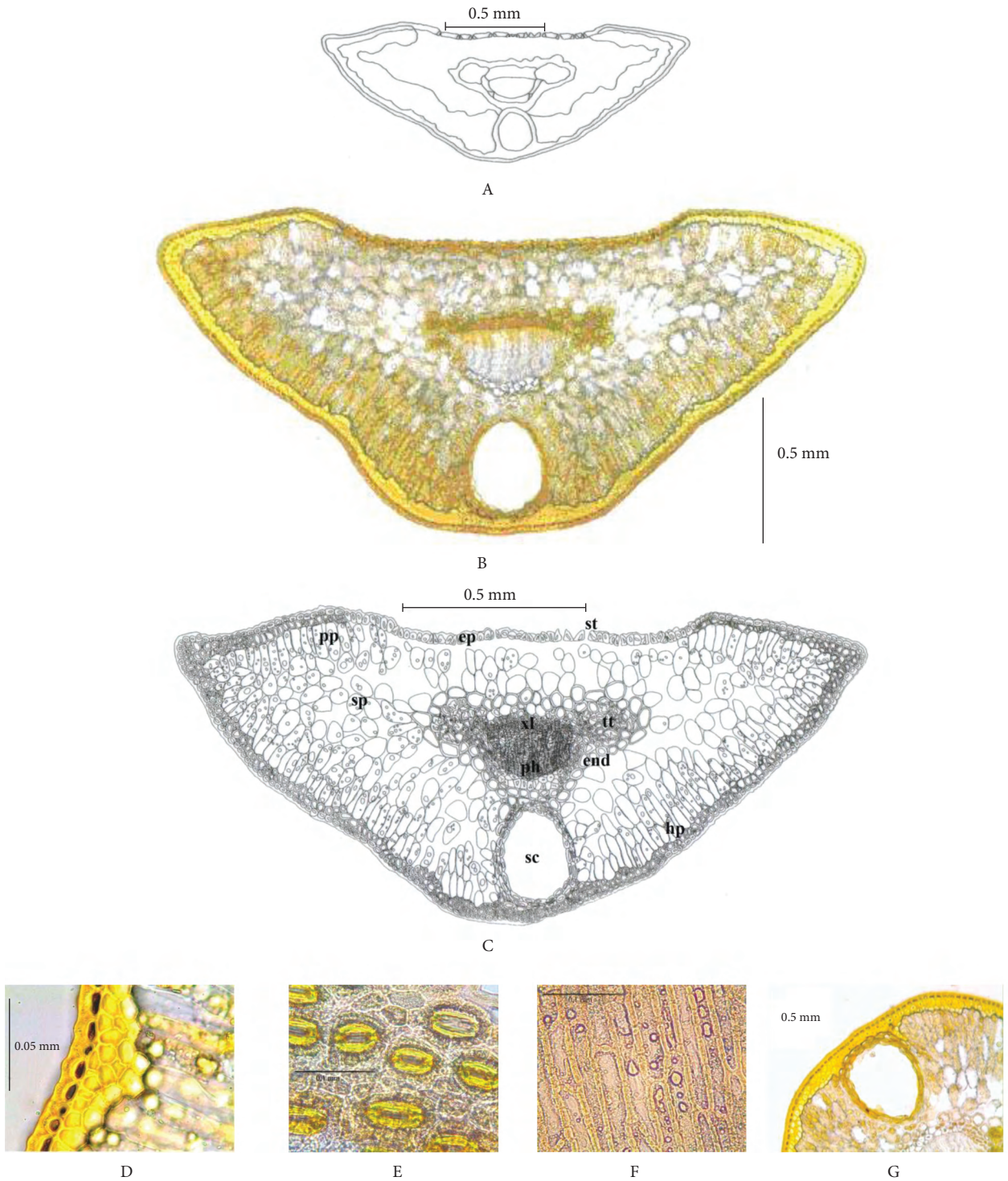


Figure 2. *Juniperus communis* var. *communis* – cross section of leaf blade. **A** and **B** - general view, **C** - anatomic properties, **D** - epidermis and hypodermis, **E** - upper surface of epidermis, **F** - lower surface of epidermis, **G** - secretory canal. Abbreviations are as follows: **ep** - epidermis, **hp** - hypodermis, **end** - endodermis, **sc** - secretory canal, **pp** - palisade parenchyma, **sp** - spongy parenchyma, **ph** - phloem, **xl** - xylem, **tt** - transfusion tissue, **st** - stoma.

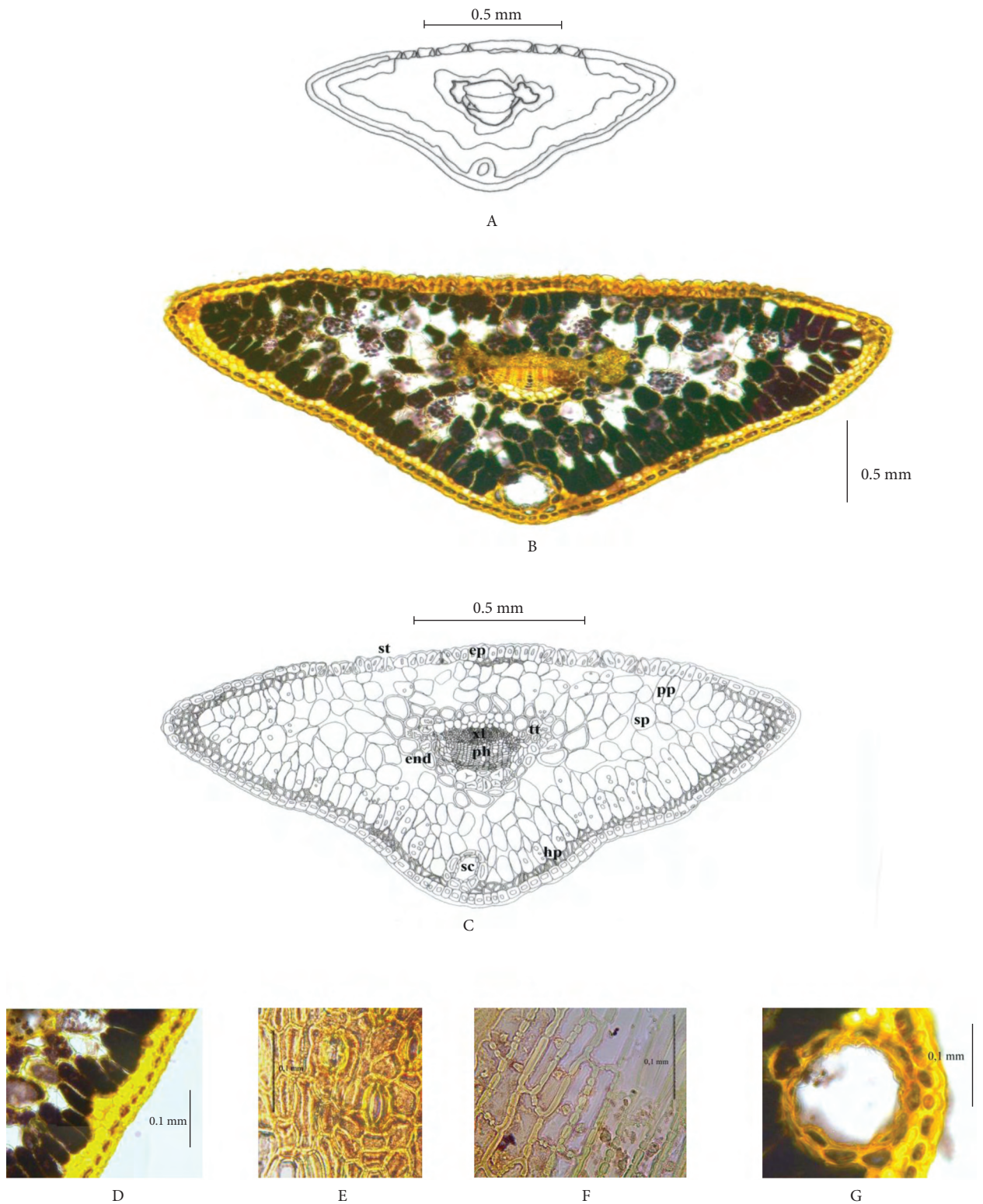


Figure 3. *Juniperus communis* var. *saxatilis* – cross section of leaf blade. **A** and **B** - general view, **C** - anatomic properties, **D** - epidermis and hypodermis, **E** - upper surface of epidermis, **F** - lower surface of epidermis, **G** - secretory canal. Abbreviations are as follows: **ep** - epidermis, **hp** - hypodermis, **end** - endodermis, **sc** - secretory canal, **pp** - palisade parenchyma, **sp** - spongy parenchyma, **ph** - phloem, **xl** - xylem, **tt** - transfusion tissue, **st** - stoma.

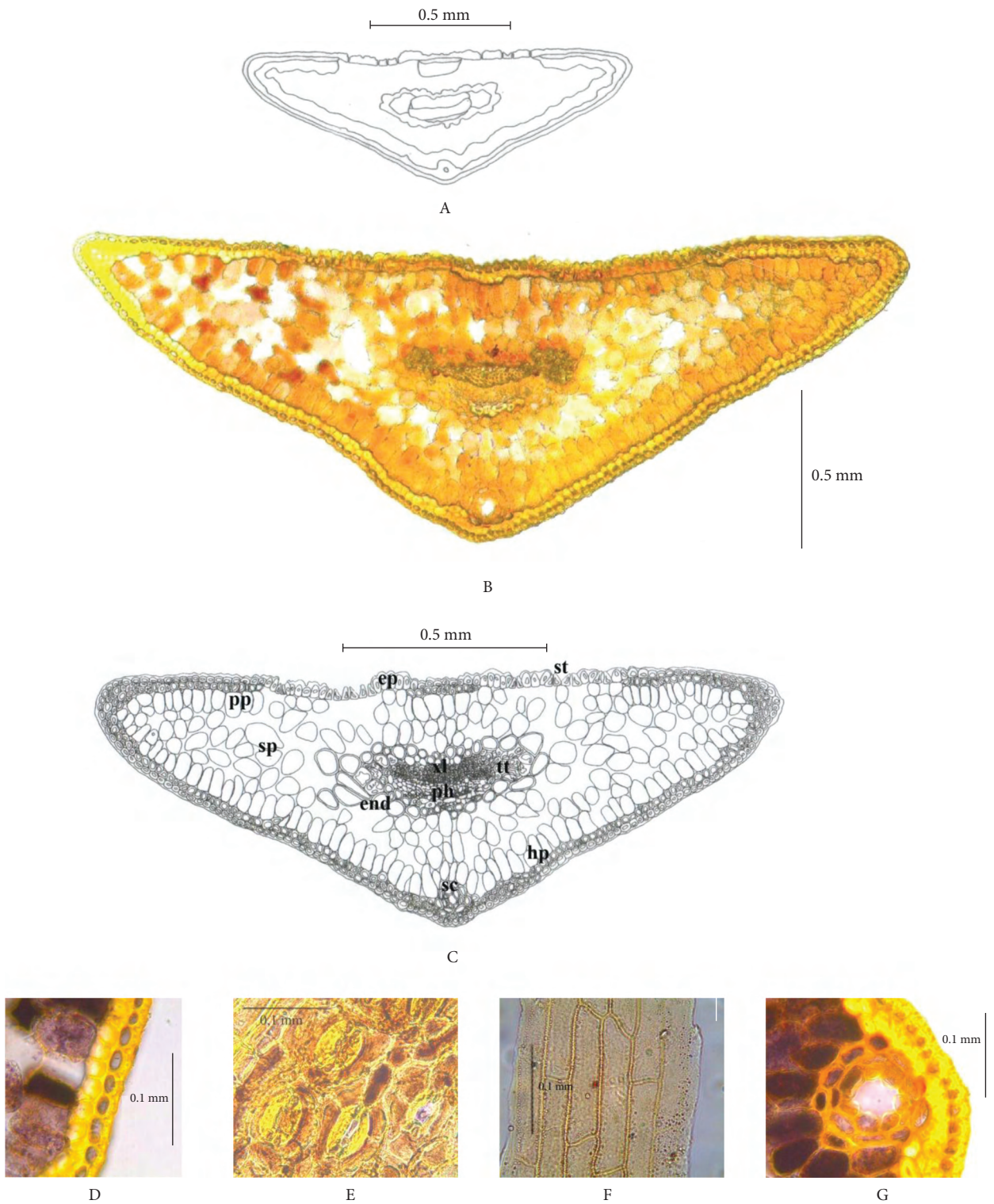


Figure 4. *Juniperus oxycedrus* subsp. *oxycedrus* – cross section of leaf blade. **A** and **B** - general view, **C** - anatomic properties, **D** - epidermis and hypodermis, **E** - upper surface of epidermis, **F** - lower surface of epidermis, **G** - secretory canal. Abbreviations are as follows: **ep** - epidermis, **hp** - hypodermis, **end** - endodermis, **sc** - secretory canal, **pp** - palisade parenchyma, **sp** - spongy parenchyma, **ph** - phloem, **xl** - xylem, **tt** - transfusion tissue, **st** - stoma.

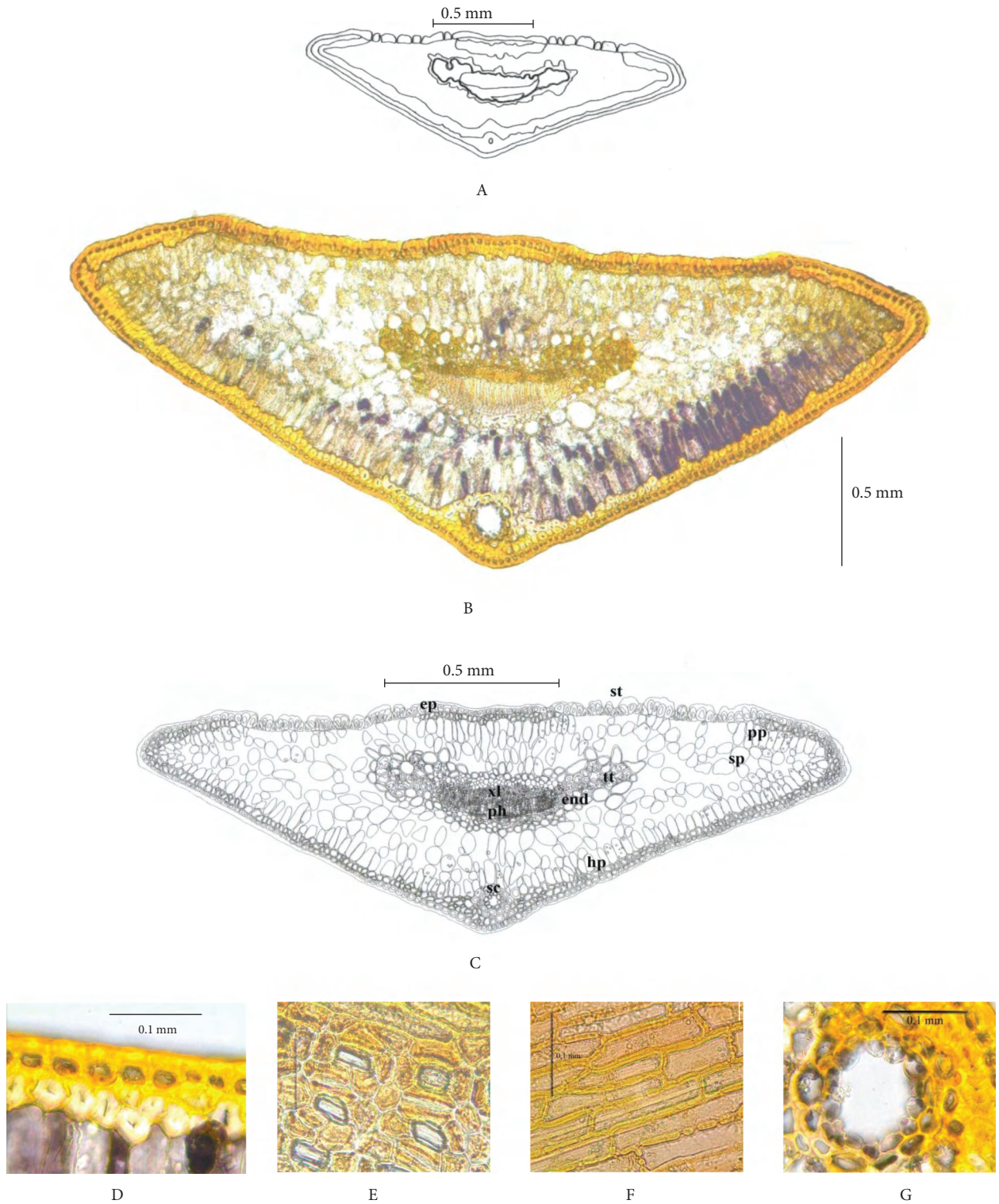


Figure 5. *Juniperus oxycedrus* subsp. *macrocarpa* – cross section of leaf blade. **A** and **B** - general view, **C** - anatomic properties, **D** - epidermis and hypodermis, **E** - upper surface of epidermis, **F** - lower surface of epidermis, **G** - secretory canal. Abbreviations are as follows: **ep** - epidermis, **hp** - hypodermis, **end** - endodermis, **sc** - secretory canal, **pp** - palisade parenchyma, **sp** - spongy parenchyma, **ph** - phloem, **xl** - xylem, **tt** - transfusion tissue, **st** - stoma.

Table 2. Comparison of leaf cross sections from naturally distributed *Juniperus* species in Turkey.

Species	Stomata	Palisade parenchyma	Secretory canals	Starch*
<i>Juniperus drupacea</i>	both surfaces	all surfaces of the leaf, 1-2 layers	big, into the spongy parenchyma	±
<i>J. communis</i> var. <i>communis</i>	upper surface	interrupted on the upper surface, 1-2(-3) layers	bigger than the other taxa, into the mesophyll	++
<i>J. communis</i> var. <i>saxatilis</i>	upper surface	usually continuous on the upper surface, 1(-2) layer	small, into the palisade parenchyma	+++
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i>	upper surface	interrupted on the upper surface, usually 1 layer	smaller than the other taxa, into the palisade parenchyma	++
<i>J. oxycedrus</i> subsp. <i>macrocarpa</i>	upper surface	interrupted on the upper surface, usually 1 layer	small, into the palisade parenchyma	++

*±: rare, ++: dense, +++: abundant.

The epidermal cells are thick-walled. Similar to the leaves of other gymnosperm, a subepidermal layer of hypodermal sclerenchyma is present in these 3 species. Like some other genera of gymnosperms, such as *Abies* Miller, *Araucaria* Juss., *Pseudotsuga* Carrière, *Sequoia* Endl. and *Taxus* L., the mesophyll of leaves of these species have been divided into palisade and spongy parenchyma. The palisade parenchyma are usually located on the lower surface, however, while the stomata are usually found on the upper surface, except in *J. drupacea*. A single vascular bundle is positioned centrally and enclosed by endodermis. Transfusion tissue is common in the gymnosperms and this tissue, which is shown in the 5 *Juniperus* taxa, is similar to other genera such as *Cupressus* L., *Thuja* L., *Sequoia*, and *Taxus*. The results of this study, provided by the anatomical study of leaves of the *Juniperus* species, were found to be in agreement with previous anatomical studies (Esau, 1977; Mauseth, 1988; Dickison, 2000).

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