Anatomical properties of wild Turkish *Viburnum* (*Caprifoliaceae*) species

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Abstract. The anatomical peculiarities of *Viburnum lantana*, *V. orientale* and *V. opulus* collected from Trabzon (Turkey) are examined in the present study. As a result, it was observed that the cork structure, existence and distribution of fibers and sclereids in the shoot, the structure of assimilating parenchyma and the shape of epidermal cells in the leaves are important anatomical traits for distinguishing the examined taxa.

Key words: anatomy, systematics, Turkey, *Viburnum*

Introduction

Viburnum L. is a widespread genus of *Caprifoliaceae*. It consists of about 160 species of evergreen, semi-evergreen and deciduous cool-climate shrubs or small trees (Winkworth & Donoghue 2005). Because of their fragrant, showy flowers and colorful berries, or bright fall foliage, many *Viburnum* species are cultivated across the world (Page & Olds 2004). This genus is represented by *V. opulus* L., *V. lantana* L. and *V. timus* L. in Europe (Ferguson 1976a, b) and by *V. lantana, V. orientale* Pallas and *V. opulus* in Turkey (Chamberlain 1972).

The genus *Viburnum* is taxonomically a very difficult group because of the high possibility of hybridization (Lobstein & al. 2003). Floral morphology and anatomy of *V. lantana* and *V. opulus* were investigated by Wilkinson (1948). He underlined that the floral false anatomical characteristics did not provide adequate information to clearly outline the limits of the species, but that they contribute to the phenetic grouping at specific level. Gasson (1979), who examined the root anatomical peculiarities of *V. lantana* and *V. opulus,* reported that spiral thickening in the tracheidal fiber was very important for this genus. Metcalfe & Chalk (1972) reported foliar fibers for *V. prunifolium* and several idioblastic cells containing crystals for the *Caprifoliaceae* members. Many members of *Caprifoliaceae*, including some *Viburnum* species, have been examined in terms of wood anatomical properties by Eom & Chung (1996) and Ogata (1988). Ogata (1988) reported that the number and length of vessel elements and distribution of crystals had distinct taxonomic importance in the genus *Viburnum*. So far, several studies focused on wood anatomy (Ogata 1988; Eom & Chung 1996), palynology (Kollmann & Grubb 2002), pylogeny (Winkworth & Donoghue 2005), chemistry (Lobstein & al. 2003), and cytology (Egolf 1962) have been carried out on this genus, but investigations into the Turkish wild *Viburnum,* especially in anatomical sense, have been very limited. Thus the aim of this study is to explore the anatomical peculiarities of shoot and leaves of wild Turkish *Viburnum* and to compare them, in order to evaluate them taxonomically.

Material and methods

Specimens. *V. lantana*, *V. orientale* and *V. opulus* were collected from A7 Trabzon (Turkey) in 2004 and 2005 (Table 1, Fig. 1). Specimens were dried according to standard herbarium techniques and stored in the Herbarium of Karadeniz Technical University, Department of Biology (KTUB).

Species	Locality
V. lantana	A7 Trabzon: Zigana Mt, ZİTAŞ, 1600 m, 05.06.2004, Odabaş 01, KTUB
V. orientale	A7 Trabzon: Düzköy, Taşocağı village, 700–800 m, 16.05.2004, Odabaş 03, KTUB
V. opulus	A7 Trabzon: Düzköy, Cevizlik , roadside, 650–750 m, 14.05.2005, Odabaş 02, KTUB

Anatomical study. Four – five healthy shoots, about 10 cm long, and 10–15 adult leaves of *V. lantana, V. orientale* and *V. opulus* were fixed in FAA (Formaldehyde: Acetic Acid: Alcohol) for 24 hours in the field, and then preserved in 70 % alcohol for anatomical examination. All observations were performed on transverse sections of shoot and leaves, and surface preparations of leaves taken by hand. All sections were stained with safranine-fast green and mounted on entellene in order to get permanent slides (Vardar 1987). Well-stained sections were photographed with Olympus BX51 from permanent slides. All measurements and observations were repeated three or four times on the basis of selected sections taken from at least two selected specimens.

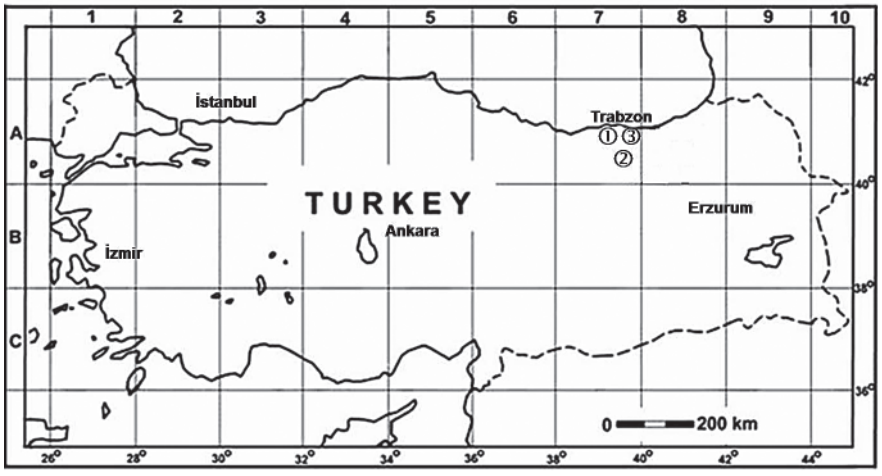


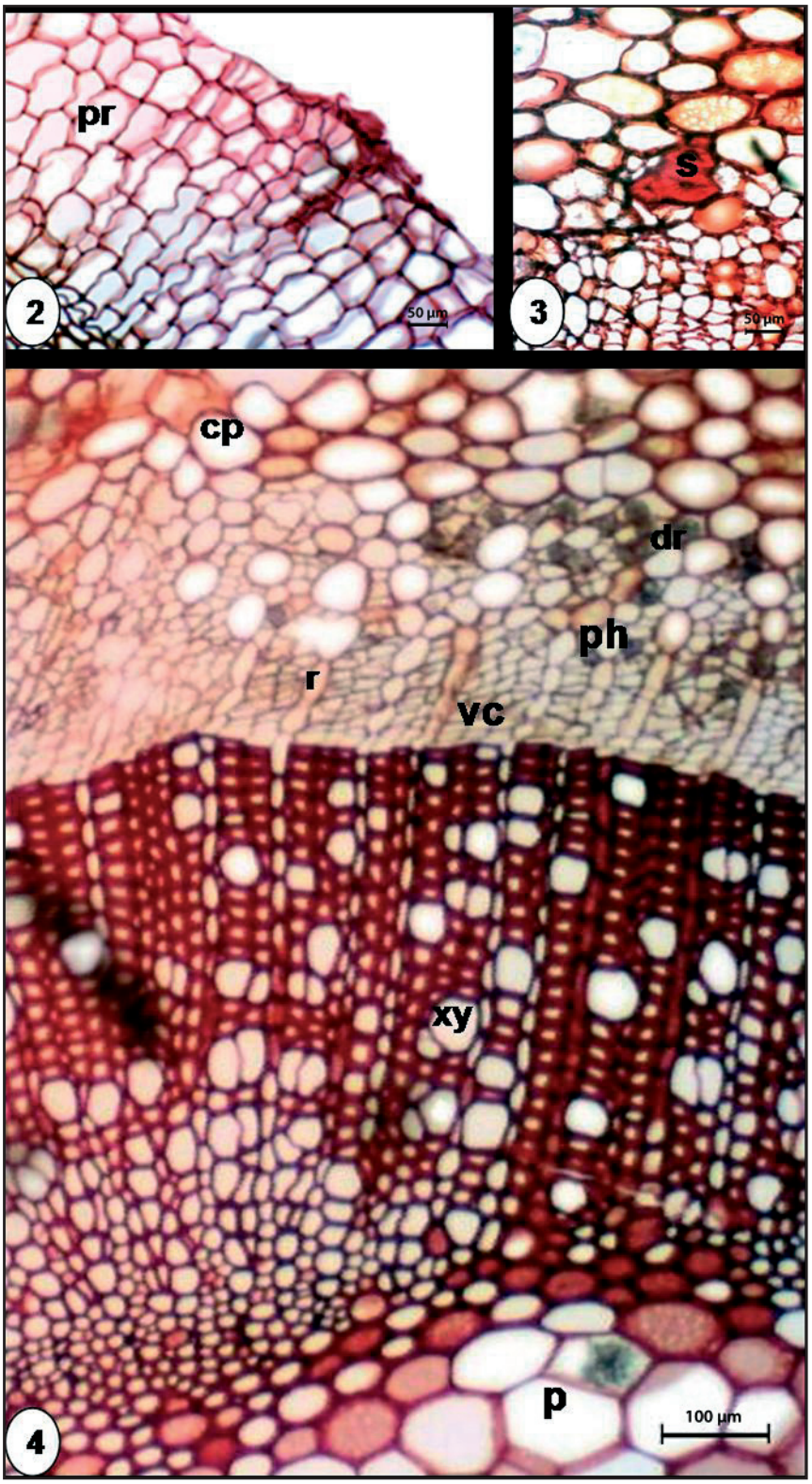
Fig. 1. Distribution map of the investigated species. ①: *V. lantana*;②: *V. orientale*; ③: *V. opulus.*

Results

Viburnum lantana. A cross section of the shoot revealed the following elements (Figs 2–4). Epidermis ultimately crushed. Periderm consisting of 7–10 rows of polygonal or rectangular cells (Fig. 2). Phellogen indistinct but unicellular. Secondary cortex consisting of 8–9 layers of usually oval parenchymatic cells and several single or clustered stone cells (Fig. 3). Vascular cambium distinct (1–2 layered). Phloem surrounded by parenchymatic cells, including druses. Xylem traversed by unicellular rays including solitary or 1–2 clustered vessels (Fig. 4). Pith obviously parenchymatic, including some crystals.

The anatomical features of midrib, lamina and surface preparations of the leaves were analyzed (Figs 5–9). Midrib was semi circular and included 1–2 layers of collenchyma close to the epidermis. Arc-shaped vascular bundles surrounded by orbicular parenchyma consisted of druses (Fig. 5). Mesophyll consisted of a monolayer, conspicuously elongated palisade and 4–5 layers of isodiametric spongy tissue (Fig. 7). There were several druses in the spongy tissue (Fig. 7). Dosiventral leaves had anomocytic stoma located only on the lower surfaces (Figs 8–9). The stoma index was 16.34. The upper epidermal cells were bigger than the lower ones, but both had undulate walls and several branched or simple hairs (Fig. 6).

Viburnum orientale. A cross section of the shoot revealed the following elements (Figs 10–12). Epidermis mostly consisted of monolayer horizontally elongated rectangular cells, occasionally crushed at the sides (Fig. 10). Periderm consisted of three rows of square to rectangular cells (Fig. 10). Phellogen was indistinct. There was an obvious 2–3 layered collenchyma on the outward side of the cortex. Secordary cortex consisted of 8–9 layers of usually oval parenchymatic cells. Vascular cambium was distinct and consisted of 2–3 layers of flattened to rectangular cells. Phloem was surrounded by dense sclerechymatic and few parenchymatic cells including druses. Xylem traversed by unicellular rays included solitary or 1–2 clus-

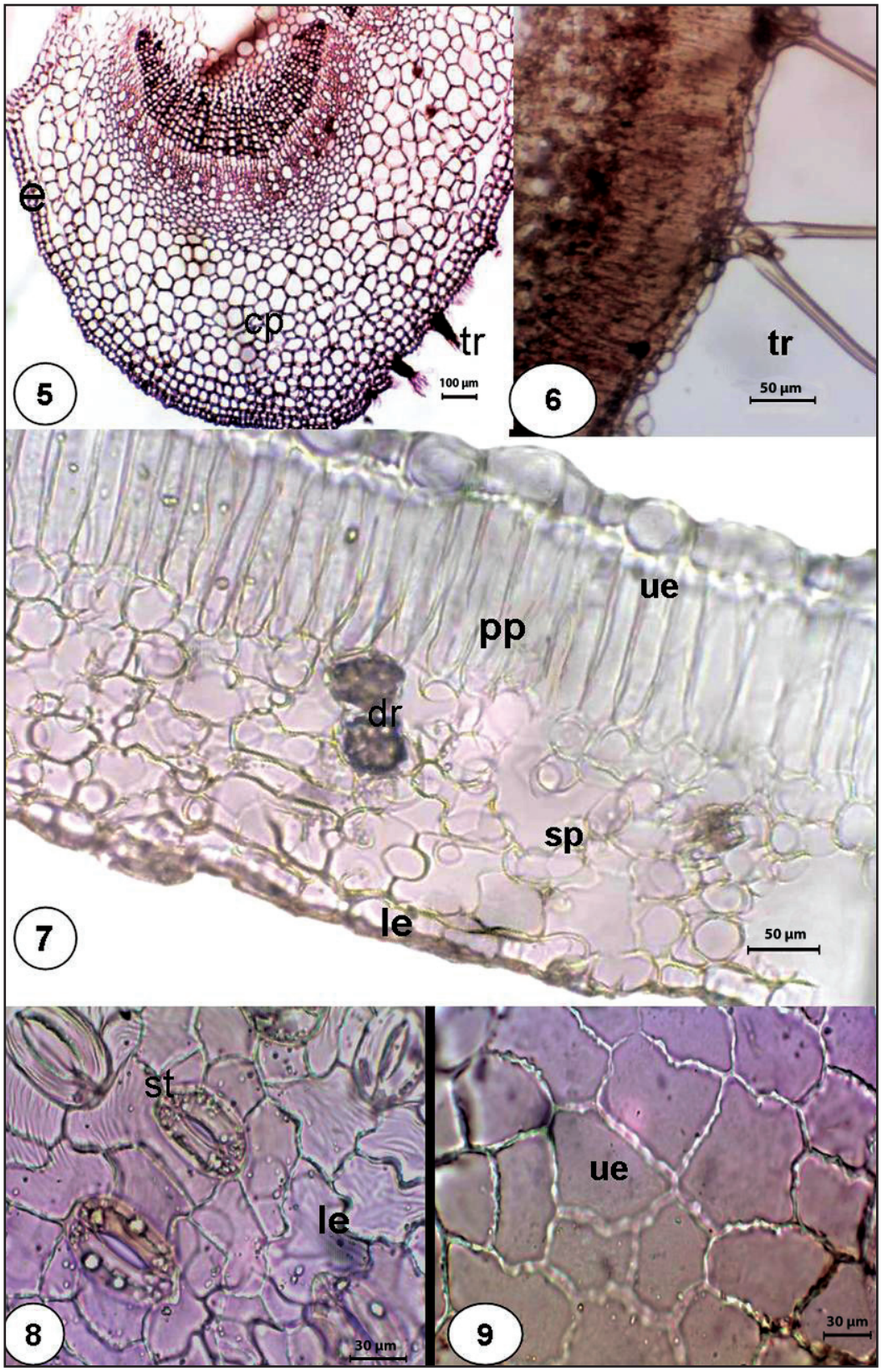


Figs 2–4. *V. lantana*:
2, 4, cross section of the shoot; **3,** cross section of the cork; **pr** – periderm; **s** – sclereid; **cp** – cortex parenchyma; **dr** – druse; **ph** – phloem; **vc** – vascular cambium; **r** – rays; **xy** – xylem; **p** – pith.

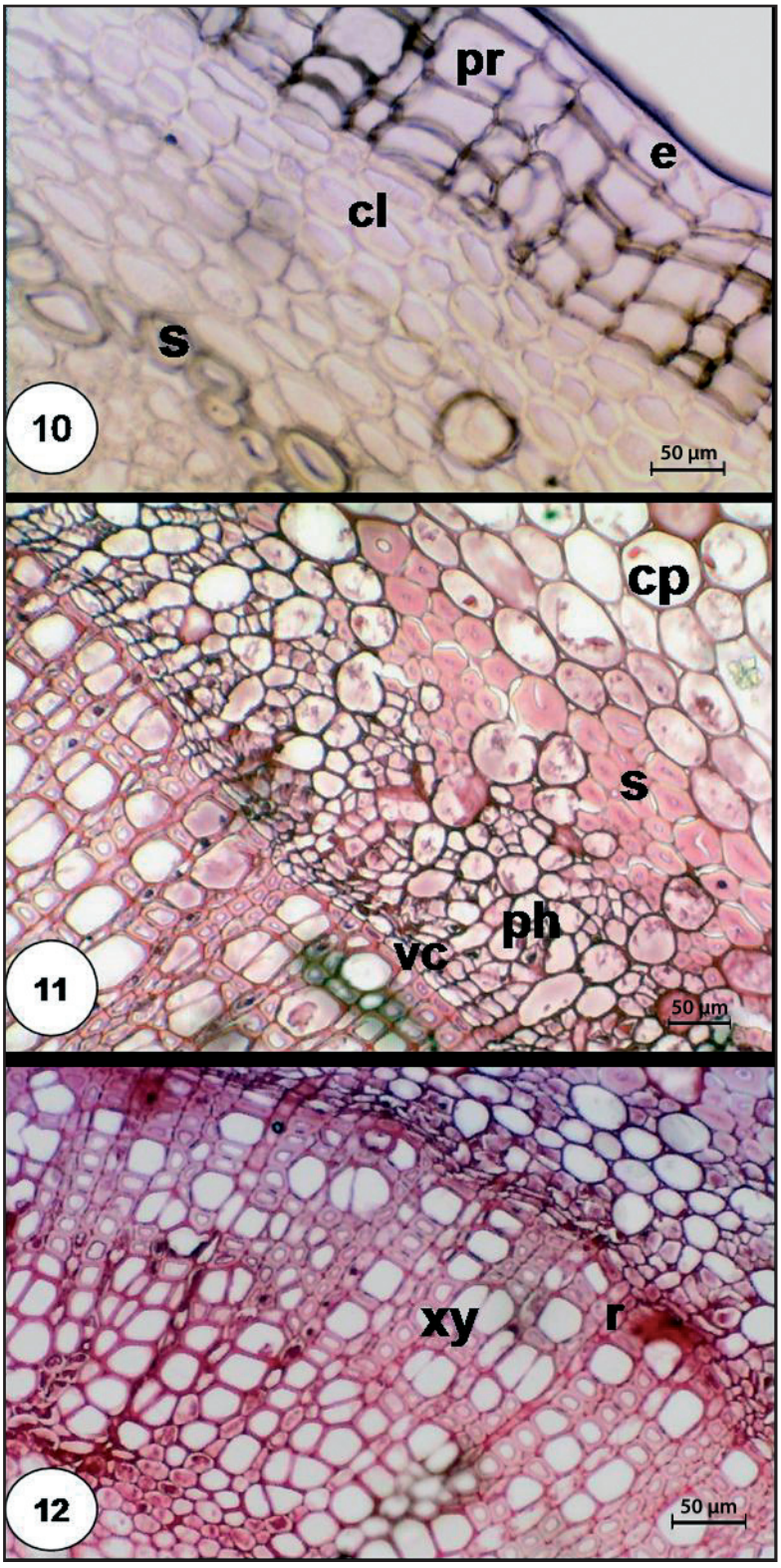
tered vessels (Figs 11–12). Pith was obviously parenchymatic, without crystals.

The anatomical features of midrib, lamina and surface preparations of the leaves were analyzed (Figs 13–16). The semicircular midrib included two-row collenchymatic cells. Arc-shaped vascular bundles were surrounded by orbicular parenchymatous cells containing druses and several intercellular spaces (Fig. 13). Mesophyll consisted of monolayer, oval to rectangular palisade cells and 4–5 layers of isodiametric spongy tissue with several intercellular cavities and druses (Fig. 14). Dosiventral leaves had anomocytic stoma located only on the lower surfaces (Figs 15–16). Stoma index was 18.86. The upper epidermal cells were bigger than the lower ones and had distinctly wavy walls and simple and unicellular trichomes (Fig. 14).

Viburnum opulus. A cross section of the shoot revealed the following elements (Figs 17–19). Epidermis was mostly crushed, but when present, it consisted of a single layer of horizontally elongated rectangular cells. Periderm consisted of 4–5 layered plate cells (Fig. 17). Phellogen was obviously unicellular and formed a continuous cylinder (Fig. 17). Secondary cortex consisted of 8–9 layers of usually oval parenchymatic cells and several single or clustered sclereids (Fig. 18). Vascular cambium was distinct and consisted of 3–4 layers of small flattened cells. Phloem was surrounded by parenchymatic cells including druses. Xylem traversed by unicellular rays included solitary or 1–3 clustered vessels (Fig. 19). Pith was obviously parenchymatic, without crystals.



Figs 5–9. *V. lantana*:
5, cross section of midrib; **6-7,** cross section of leaves; **8–9,** surface section of leaves; **e** – epidermis; **tr** – trichome; **dr** – druse; **ue** – upper epidermis; **le** – lower epidermis; **sp** – spongy palisade; **pp** – palisade parenchyma; **st** – stoma; **cp** – cortex parenchyma.



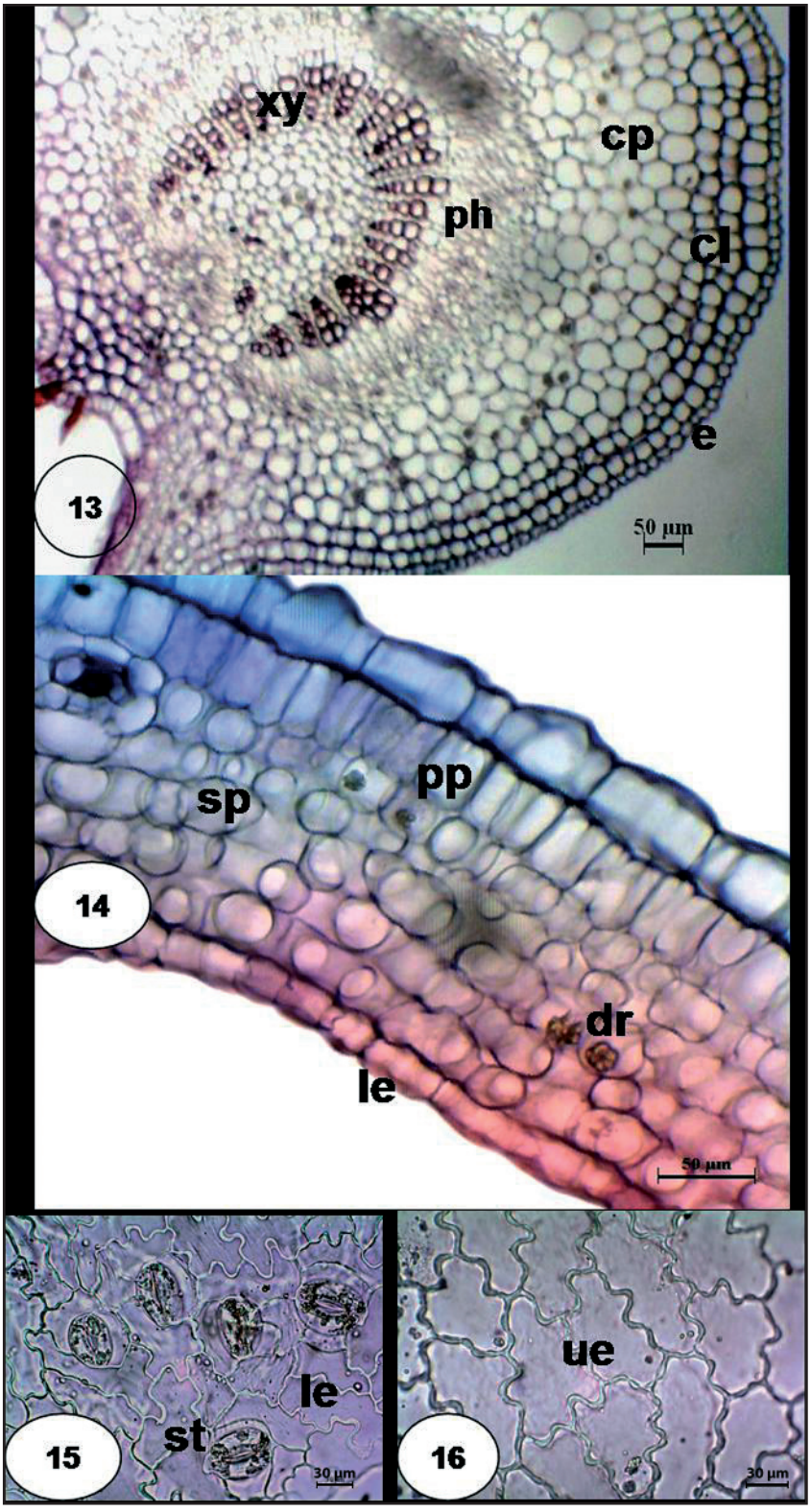
Figs 10–12. *V. orientale*:
10, cross section of the cork; **11–12,** cross section of the shoot; **e** – epidermis; **pr** – periderm; **cl** – collenchyma; **s** – sclereid; **xy** – xylem; **r** – rays; **ph** – phloem; **vc** – vascular cambium; **cp** – cortex parenchyma.

The anatomical features of midrib, lamina and surface preparations of the leaves were analyzed (Figs 20-23). Semicircular midrib included a monolayer collenchyma beneath the epidermis. Arc-shaped vascular bundles were surrounded by orbicular parenchymatous cells (Fig. 20). Mesophyll consisted of one row oval to rectangular palisade cells and 4–5 layers of isodiametric spongy tissue, with several intercellular cavities (Fig. 21). There were no druses in the spongy tissue (Fig. 21). Dorsiventral leaves had anomocytic stoma located only on the lower surfaces (Figs 22–23). Stoma index was 15.69. The upper epidermal cells were bigger than the lower ones and had triangular walls with very sparce simple unicellular hairs.

Discussion

The present investigation has sought to provide additional information on the anatomical characteristics of the wild Turkish *Viburnum* species. Apparently, endomorphic characters can be used in conjunction with morphological ones to distinguish the three species in question.

The naturally found *Viburnum* species in Turkey are generally branched near the base (Chamberlain 1972). Morphologically, they are quite similar (Chamberlain 1972), but some distinct differences could be observed in the structure of the periderm, presence of stone-cells, druses and their distribution in the shoot, and the shape of epidermis and palisade cell in leaves. Periderm is 7–10 layered in *V. lantana* (Fig. 2), 4–5 layered in *V. opulus* (Fig. 17) and 3-layered in *V. orientale* (Fig. 10). Furthermore, the shape of peridermal cells varies from species to species. All anatomical properties observed in the shoots are in agreement with Metcalfe & Chalk (1972), Ogata (1988), Eom & Chung (1996). Fibers have been observed in all examined species. These fibers are not continuous in *V. opulus*

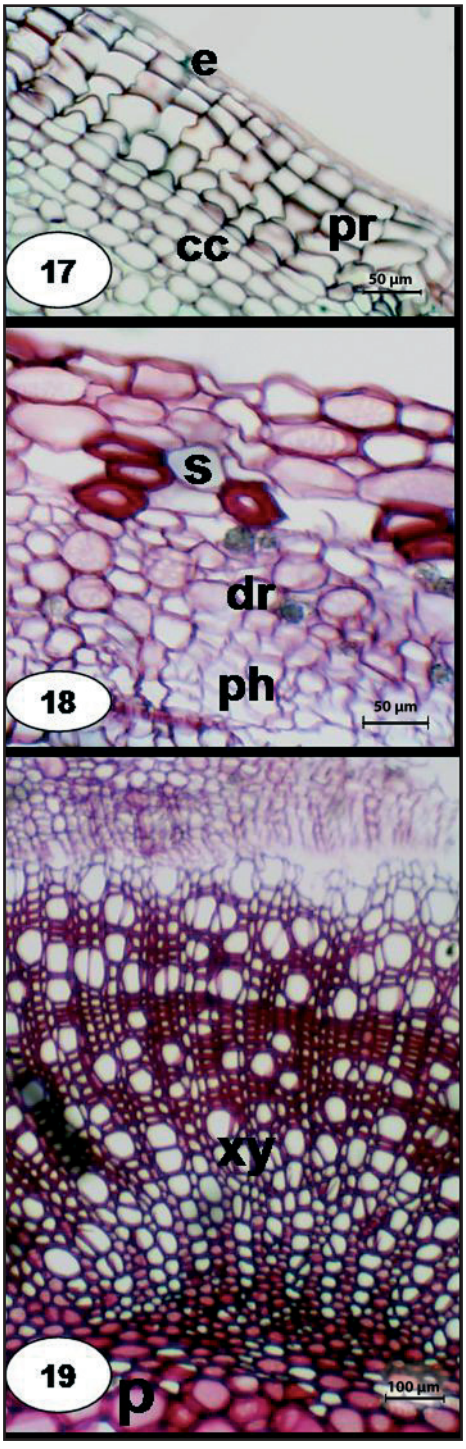


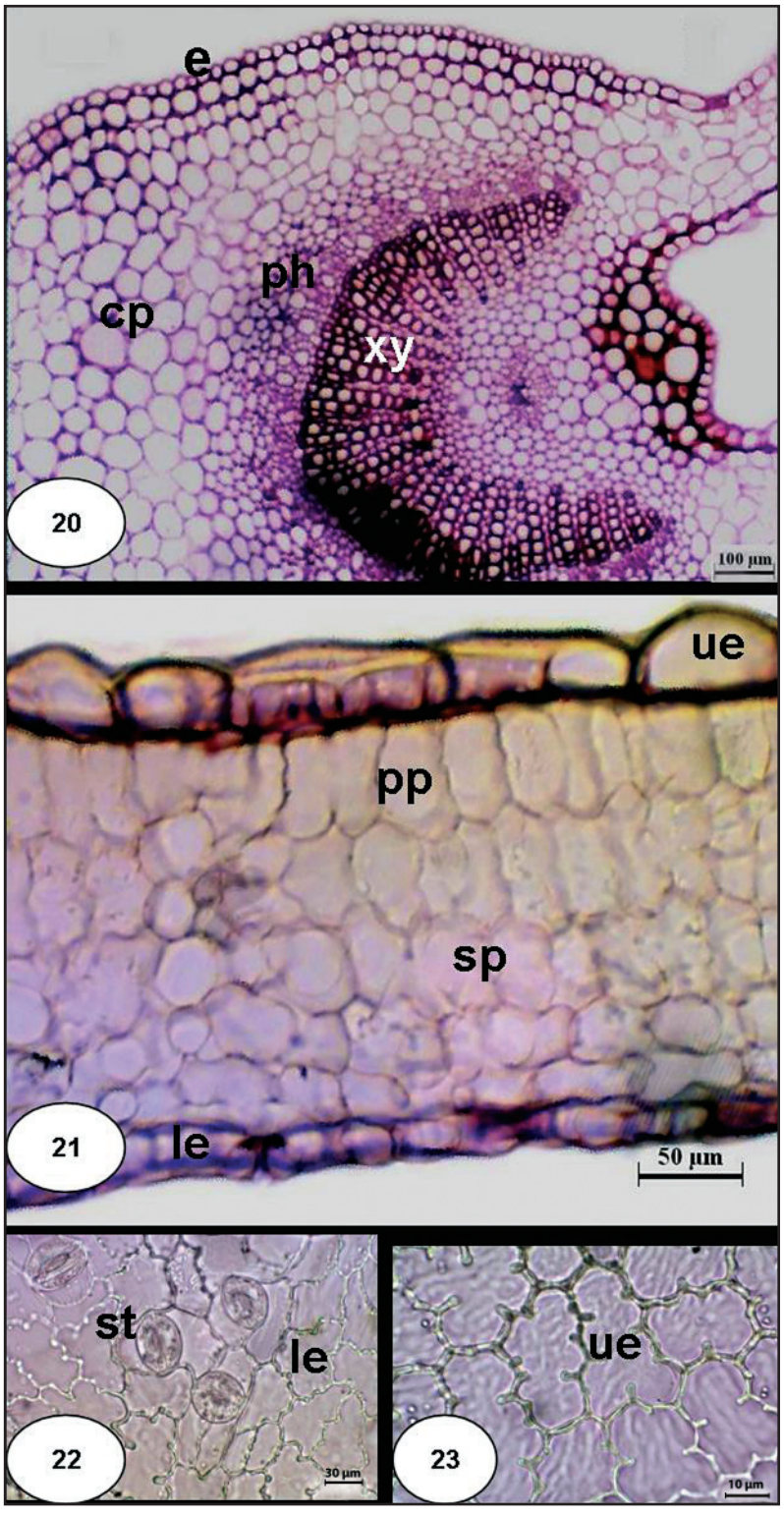
Figs 13–16. *V. orientale*:
13, cross section of the midrib; **14,** cross section of leaves; **15–16,** surface section of leaves; **e** – epidermis; **dr** – druse; **ph** – phloem; **xy** – xylem; **cp** – cortex parenchyma; **ue** – upper epidermis; **le** – lower epidermis; **sp** – spongy palisade; **pp** – palisade parenchyma; **st** – stoma; **cl** – collenchyma.

(Fig. 18) and *V. lantana* (Fig. 3), but are continuous and 2–3 layered in *V. orientale* (Fig. 10). Metcalfe & Chalk (1972) have also mentioned these fibers and explained their systematic value for the *Caprifoliaceae* members. Although yhese authors reported armed palisade cells in genus *Sambucus* and *Viburnum,* they have not been observed during the present investigation. Each of the three species has some cells including druses in the cortex and also the pith, but the distribution varies from species to species. These findings are supported by Ogata (1988) and Metcalfe & Chalk (1972). Vessels in the secondary xylem arranged radially and as several uniseriate rays are present in all examined species.

Vascular bundles in the midrib are crescent-shaped and surrounded by orbicular parenchymatic cells including crystals on some of the sides in all examined species (Figs 5, 13). Radial vessels have 8–10 rows in *V. opulus*, 5–8 rows in *V. lantana* and 3–4 rows in *V. orientale*. One or two layers of distinct collenchyma close to the epidermis are present in all examined leaf midribs.

All leaves are dorsiventral. There is a monolayer rectangle-shaped palisade in the mesophyll of all examined species (Figs 7, 14, 21), but palisade cells are obviously much longer in *V. orientale* than in the other species. Several single or grouped sclereids were reported by Metcalfe & Chalk (1972) in the mesophyll of *V. prunifolium*, but this was not confirmed by the present study in any of the examined species. There is also a distinct difference in the shape and size of the epidermal cells. The upper epidermal cells are always bigger than the lower ones. The outer walls of the upper epidermal cells are undulate in





Figs 17–18. *V. opulus*:
17, cross section of the cork; **18–19,** cross section of the shoot; **e** – epidermis; **pr** – periderm; **cc** – phellogen; **s** – sclereid; **xy** – xylem; **ph** – phloem; **dr** – druse; **p** – pith.

Figs 20–23. *V. opulus*:
20, cross section of the midrib; **21,** cross section of leaves; **22–23,** surface section of leaves; **e** – epidermis; **cp** – cortex parenchyma; **ph** – phloem; **xy** – xylem; **ue** – upper epidermis; **le** -lower epidermis; **sp** – spongy palisade; **pp** – palisade parenchyma; **st** – stoma.

all examined species. Although there are several simple and branched hairs in *V. lantana*, there are very few simple and unicellular hairs in *V. orientale* and *V. opulus*. Stomata are present only in the lower epidermis and are anomocytic in all examined species.

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