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Comparative anatomical study of some gymnospermae species leaves

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ABSTRACT: The paper is a part of a complex study of some Gymnospermae leaf species and presents a comparative study concerning the leaf structure of six Gymnospermae species which belong to two families. All of them are also popular ornamental trees in parks and large gardens. Four of them were introduced in Romania after 1990 and acclimatized in different regions: *Abies alba* Miller, *Abies nordmanniana* subsp. *nordmanniana* (Stev.) Spach, *Cedrus atlantica* (Endl.) G. Manetti ex. Carriére var. *glauca* Carriére, *Cedrus deodara* (Roxb.) G. Don (Pinaceae), *Juniperus communis* L. and *Juniperus chinensis* L. (Cupressaceae). Anatomically, the leaves of the studied coniferous species are quite similar in the basic structure but differ somewhat in terms of details. The paper presents anatomical information concerning the epidermal cells, the hypodermal cells, the type of mesophyll, the number, size and arrangement of the resin ducts and the distribution of the vascular system elements. The leaf anatomical features of the six studied coniferous species are comparatively discussed.

Key words: anatomy, Gymnospermae, epidermis, leaf, mesophyll, resin ducts, vascular system

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INTRODUCTION

The studied species belong to the large Gymnospermae group of plants, being evergreen trees or shrubs. *Abies alba* Miller (fir tree) is native to Europe and measures up to 45-55 m in height. In Romania it forms large forests in the Carpathian Mountains: Bucovina and North Moldavia regions and South Carpathians. The needle-shaped leaves are thick, flattened, measuring 1.8-3 cm in height and 2 mm in width. The needle base is twisted with a notched or rounded apex. The surface of the leaf is shiny dark green above and grooved and whitish-green below (Beldie 1952, Silba 1986; Jaworski 1995). The Nordmann fir (*Abies nordmanniana* subsp. *nordmanniana*) is a large coniferous tree growing to height of 40 or 60 m and with a trunk diameter of up to 2 m. The needles are flattened as well, 1.8-3.5 cm long and 2 mm wide. The leaf upper surface is dark green. The tip of the leaf is usually blunt.

Cedrus atlantica var. *glauca* (Atlas Cedar), is native to Atlas Mountains of northwestern Africa where can grow up to 40 m high and up to 2 m in diameter but in Romania it is smaller and decorates parks and large gardens. It can live in the sunny hot summers and sometimes dry winters (Till 1987). The needles are silvery bluish or green, usually not longer than 2.5 cm, between 19 and 28 in a whorl (Nicolson 1986; Vidaković 1991). *Cedrus deodara* (Deodar Cedar) is native to the western Himalayas in the eastern part of Afganistan. It is up to 40-50 m tall and 3 m in diameter. The leaves are needle-like (2.5-5 cm long) and 1 mm thick, grouped in dense clusters of 20-30 on short shoots and isolated on long shoots. The leaves are soft, grey-green in color and 0.5-1.5 cm wide.

Juniperus communis L. (Common Juniper) is distributed in temperate zones in Europe, Asia and America. In Romania it grows in different zones of the Carpathian mountains: Caliman and Rodnei mountains (Maramures County), Gilau mountains (Cluj County), Bucegi mountains (Prahova County), Semenic (Banat Mountains), Vrancea and Ceahlau mountains (Moldavia County). It is a dioecious multistemmed small shrub (height = 4 m) or tree (height = 10 m). It has spreading or ascending branches and green scale-like mature

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leaves, 5-20 mm long (Beldie 1952). Grown widely as an ornamental plant in Europe (including Romania) and North America, the Chinese Juniper (*Juniperus chinensis* L.) is native to China, Mongolia and Japan. It is a shrub (height = 2-3 m) with dark green juvenile leaves (5-10 mm long) and adult scale-shaped leaves (1.5-3 cm long) (Fu *et al.* 1999, Zsolt & RÁcz 2000).

Little information is available about the leaf anatomy of most coniferous species, while the comparative studies lack almost entirely. The results of the study on leaf anatomy were obtained after a period of one year. The present investigations on the leaf features of the studied species Pinaceae and Cupresaceae may contribute to the knowledge of this group of plants.

MATERIAL AND METHODS

The mature leaves of the studied species were collected from the Rodnei Mountains – The Horse Waterfall in Maramures County (North of Romania) (*Abies alba* and *Juniperus communis*), while the rest of the coniferous species leaves were collected from the trees brought from the nursery Pistoia mountainous region (Italy) and aclimatized by S.C. IRIS International S.R.L., Constanta. Small pieces of mature leaves were fixed in FAA (formalin:glacial acetic acid:alcohol 5:5:90). Cross sections of the vegetative organs were performed with a rotary microtome (Bercu & Jianu 2003). The samples were stained with alum-carmine and iodine green. Anatomical observations and micrographs were performed with a BIOROM–T bright field microscope, equipped with a TOPICA 6001A video camera. The microphotographs were obtained from the video camera through a computer.

RESULTS AND DISCUSSION

The cross sections of leaves of *Abies alba* and *Abies nordmanniana* subsp. *nordmanniana* exhibit an elliptical shape with a prominence in the abaxial position and a slight groove above (Fig.1 a, b). The epidermis consists of a single layer of highly lignified cells, covered with wax. However, the epidermal cells of the second species of *Abies* are slightly elongated and less lignified. Below the epidermis is a single-layered hypodermis with highly lignified cells. The hypodermal cells of *Abies alba* are placed on a large area, whereas those of *A. nordmanniana* consist of one layer of either isolated cells or grouped in 3 or 4, placed especially in the area of the vascular system of the vein. At the leaf margins they are continuously arranged, protecting the resin ducts as well. The continuity of the epidermis and hypodermis is broken by the presence of numerous engrossed stomata with small substomatal cavities for both species. The epidermis of *Abies alba* and

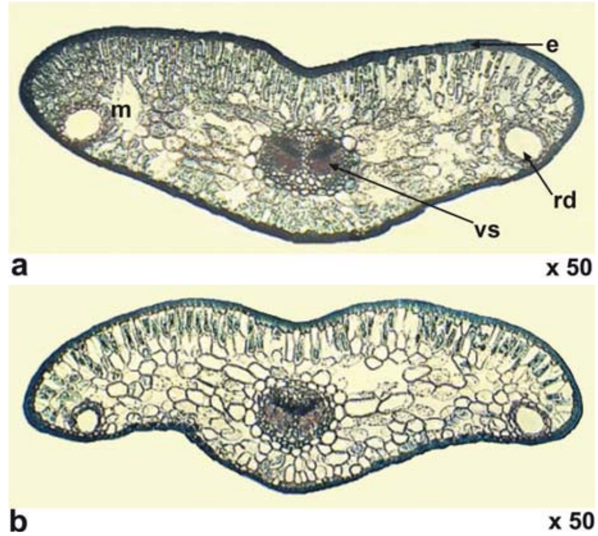

Fig. 1. Cross sections of the leaf – ansamble – (a) *Abies alba* (b) *A. nordmanniana* subsp. *nordmanniana*: e- epidermis; m- mesophyll; rd- resin duct; vs- vascular system.

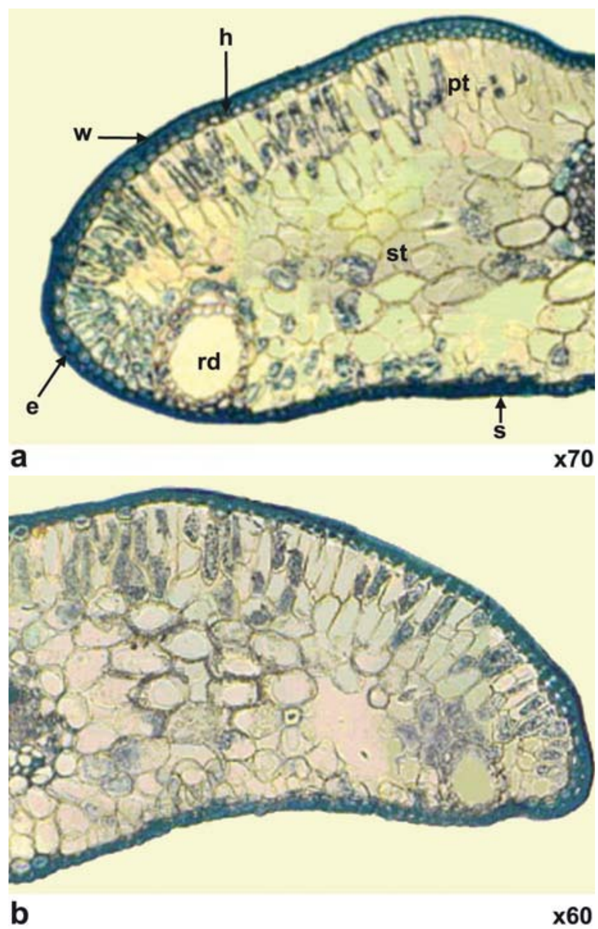

Fig. 2. Partial view of the mesophyll with a resin duct (a) *Abies alba* (b) *A. nordmanniana* subsp. *nordmanniana*: e- epidermis; h- hypodermis; pt- palisade tissue; rd- resin duct; s- stoma, st- spongy tissue; w- wax.

A. nordmanniana possesses numerous stomata, placed abaxialy, and interrupted in the vascular system region (Fig.2 a, b).

As Lambers *et al.* (1998) report for *Abies alba* leaves exposed to the sun, similarly to our studied species, the mesophyll is well developed, differentiated into a high palisade and spongy parenchyma, possessing numerous chloroplasts (Fig.2 a, b). *Abies alba* and *Abies nordmanniana* subsp. *nordmanniana* possesses the same type of mesophyll. Abaxialy, just below the hypodermis, in the mesophyll, in lateral position, two large resin ducts (*A. alba* – 15 µm and *A. nordmanniana* subsp. *nordmanniana* 10 µm in diameter) are present, formed as in all coniferous species, by the disintegration of the cells (lysigenous cavities) (Raven 1994; Bavaru & Bercu 2002). The small gland (secretive) cells are protected, to the exterior, by a layer of slightly thick-walled cells (Fig.3 a, b).

The vascular system of the vein in both *Abies* species is embedded in the diffusion tissue, represented by two open collateral bundles. Adaxially, the xylem elements, placed

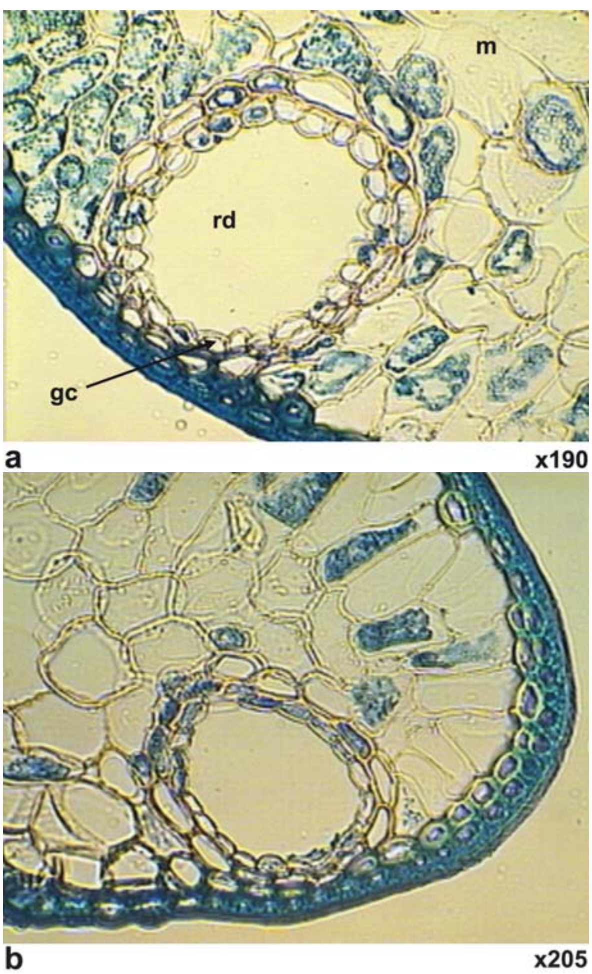

Fig. 3. Portions of the leaf margins with resin ducts (a) *Abies alba* (b) *A. nordmanniana* subsp. *nordmanniana*: gc- glandular cell; m- mesophyll; rd- resin duct.

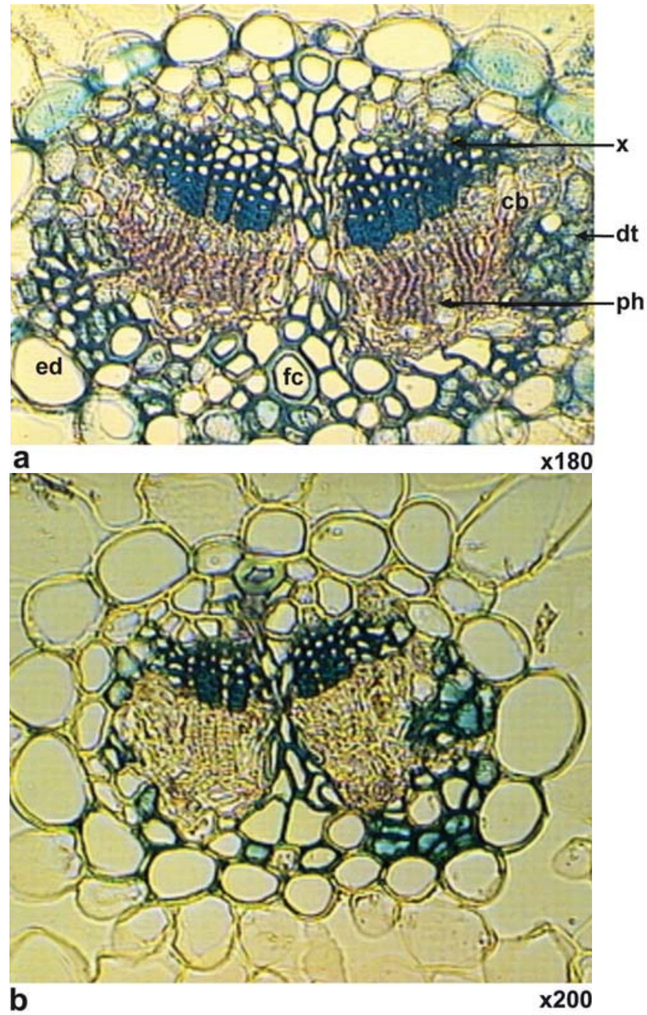

Fig. 4. The vascular system of the vein (a) *Abies alba* (b) *A. nordmanniana* subsp. *nordmanniana*: cb- cambium; dt- diffusion tissue; ed- endodermis; fc- fibre cell; ph- phloem; x- xylem.

in a radial arrangement, are represented by tracheids, whereas the phloem elements are in abaxial position. The diffusion tissue consists of diffusion tracheids and diffusion parenchyma. In the vascular system, a number (*Abies alba*) or few (*A. nordmanniana* subsp. *nordmanniana*) fiber cells are present. The vascular system is surrounded by evidently endodermis (Fig.4 a, b).

The cross sections on leaves of *Cedrus atlantica* var. *glauca* and *C. deodara* exhibit a more or less elliptical shape with a large prominence, in adaxial position (Fig.5 a, b). The epidermis consists of a single layer of highly lignified cells, covered by wax. The hypodermis of both *Cedrus* species consists of one-layered highly lignified cells. The *Cedrus deodara* hypodermis is interesting as it is two-layered to the leaf margins. The continuity of the epidermis and hypodermis is interrupted by the presence of few (2, 3) engrossed stomata with large substomatal cavities (Fig.6 a, b).

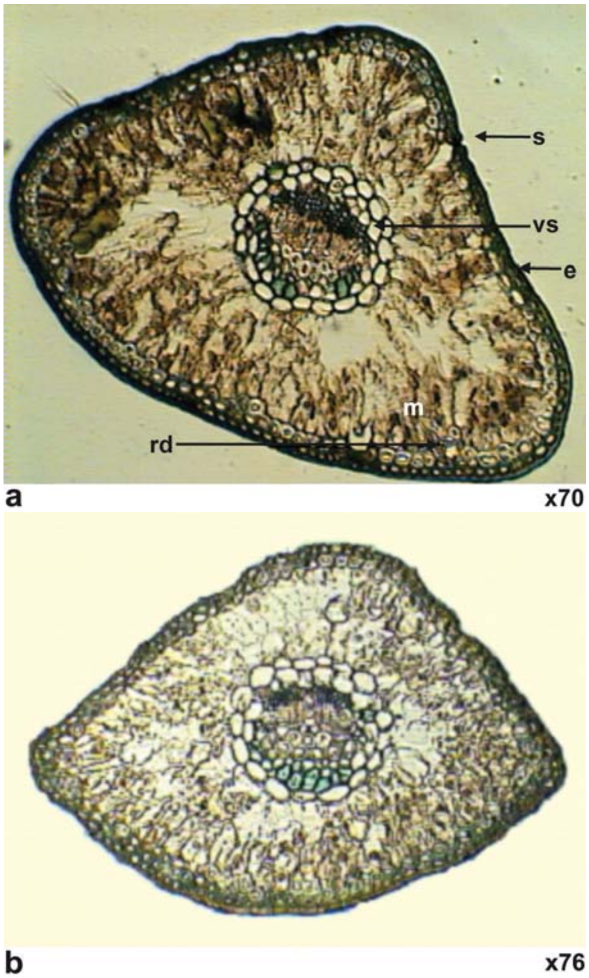

Fig. 5. Cross section of the leaf – ansamble – (a) *Cedrus atlantica* var. *glauca* (b) *C. deodara*: e- epidermis; m- mesophyll; rd- resin duct; s- stoma; vs- vascular system.

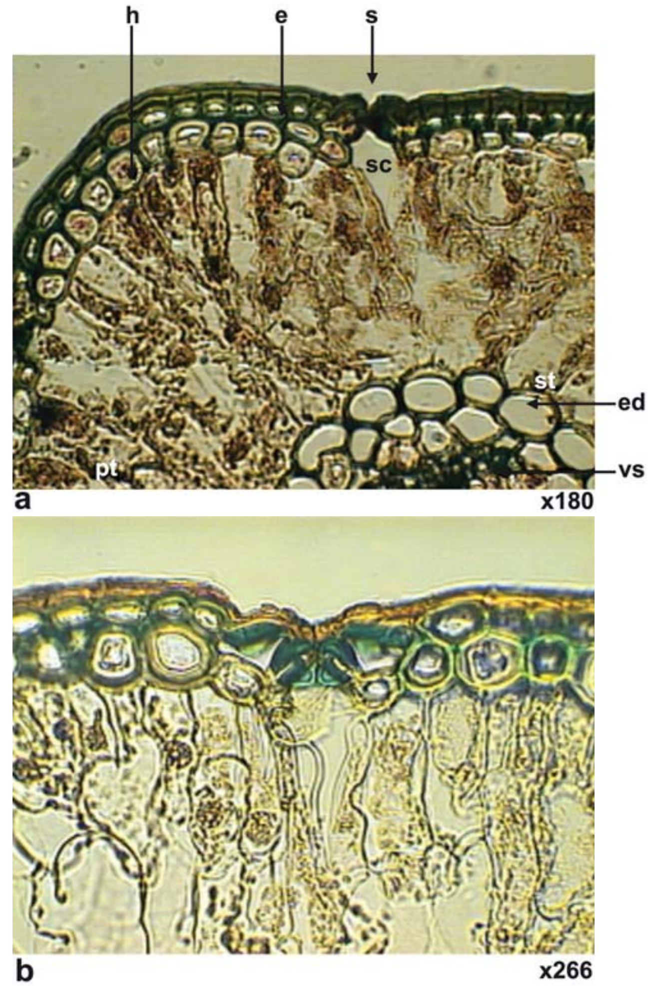

Fig. 6. Partial view of the mesophyll (a) *Cedrus atlantica* var. *glauca* (b) *C. deodara*: e- epidermis; ed- endodermis; h- hypodermis; pt- palisade tissue; s- stoma; sc- substomatal cavity; st- spongy tissue, vs- vascular system.

Characteristically, the mesophyll is differentiated into palisade and spongy parenchyma, possessing numerous chloroplasts (Fig.6 a, b). Just below the hypodermis there are two small circular resin ducts (*Cedrus atlantica* var. *glauca* - 2.5 µm and *C. deodara* - 0.5 µm in diameter). They are placed in abaxial position in *Cedrus atlantica* var. *glauca* and on laterals in the mesophyll for the leaf of the second *Cedrus* species. The small secretive cells are protected, to the exterior, by a layer of sclerenchymatous cells, some of them with hypodermal value. Additionally to the sclerenchyma sheath, 3-4 isolated fibre cells occur in *Cedrus atlantica* var. *glauca* (Fig.7 a, b) (Bercu *et al.* 2009).

The centrally located vascular system of the vein is surrounded by a one-layered evidently endodermis. As other authors mentioned for other cedar species, below the endodermis there is the diffusion tissue (Batanouny 1992; Andrei & Predan 2001), more developed in *Cedrus deodara* than that of *C. atlantica* var. *glauca*. The vascular system of the vein is represented by two collateral bundles. The xylem string, distributed adaxially, is represented by tracheids, whereas the phloem elements are in abaxial position (Fig.8 a, b). The fiber cells are in a larger number in *Cedrus deodara*, in comparison with *C. atlantica* var. *glauca*.

In transverse sections, the leaves of *Juniperus communis* and *J. chinensis*, have the same elliptical shape as the rest of the species. Abaxially, a large prominence (*Juniperus communis*) and a smaller one in *J. chinensis*, is present (Fig.9 a, b). The epidermis is one-layered with highly elongated cells. The epidermis consists of thick-walled cells (especially the outer walls). The epidermal cells, as in other species of *Juniperus* (Thomas 1963), are covered by a thick cuticle with loose flakes of wax. Below the epidermis there is the one-layered hypodermis. The hypodermal cells are highly lignified. Adaxially, the continuity of the epidermis is broken by the presence of numerous stomata interrupted in the region of the vascular system in leaves of both *Juniperus* species, with small substomatal cavities (Fig.10 a, b).

The mesophyll is slightly divided into palisade and spongy tissue (Fig.10 a, b). Abaxially, there is one resin duct (*J. communis* - 6.5 µm and *J. chinensis* – 5 µm in diameter), placed between the vascular bundle and epidermis. The elongated secretive cells are small and surrounded by thick-walled cells, some of them with hypodermal value (Fig.11 a, b). The vascular system of *Juniperus communis* and *J. chinensis* is represented by a single vascular bundle and the diffusion tissue elements, the latter placed on both lateral bundle sides. It is surrounded by a more or less obvious endodermis. The xylem is poorly developed,

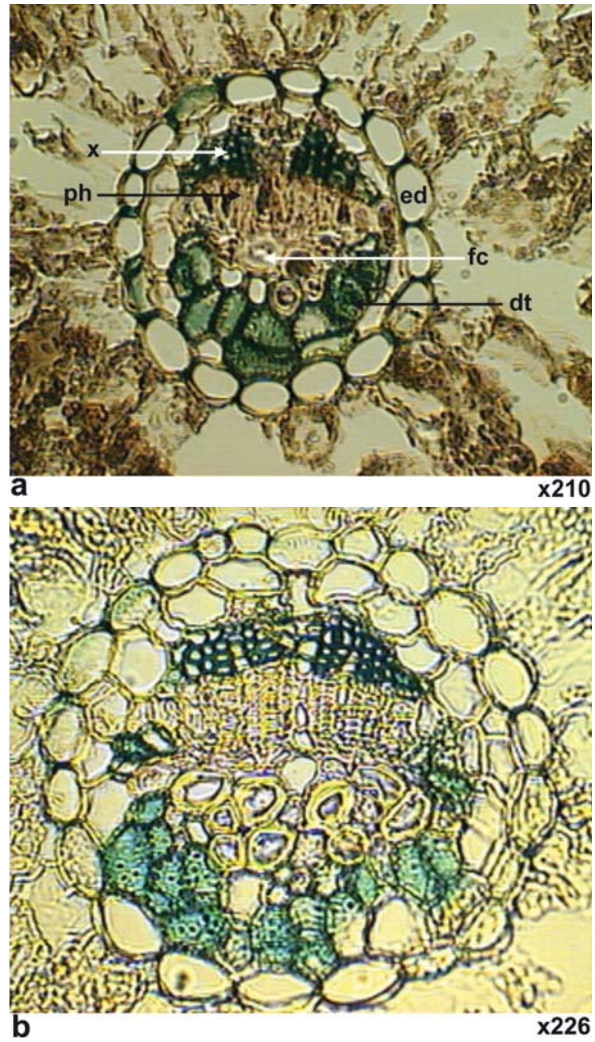

Fig. 8. The vascular system of the vein. (a) *Cedrus atlantica* var. *glauca* (b) *C. deodara*: dt- diffusion tissue; ed- endodermis; fc- fibre cell; m- mesophyll; ph- phloem; x- xylem.

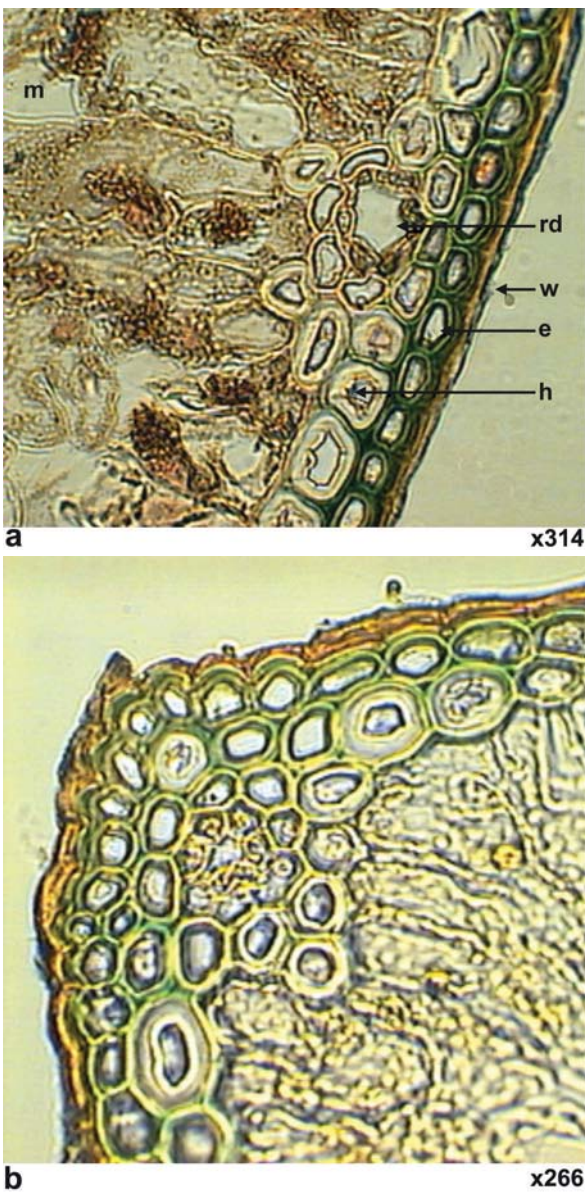

Fig. 7. Portions of the leaf margins with resin ducts. (a) *Cedrus atlantica* var. *glauca* (b) *C. deodara*: e- epidermis; h- hypodermis; m- mesophyll; rd- resin duct; w-wax.

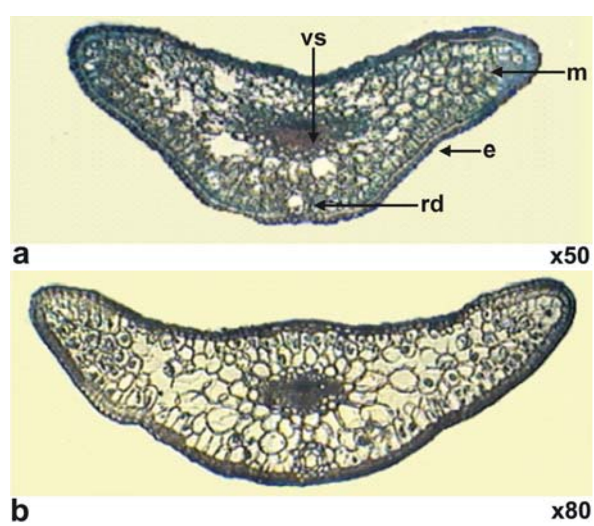

Fig. 9. Cross section of the leaf – ansamble – (a) *Juniperus communis* (b) *J. chinensis*: e- epidermis; m- mesophyll; rd- resin duct; vs- vascular system.

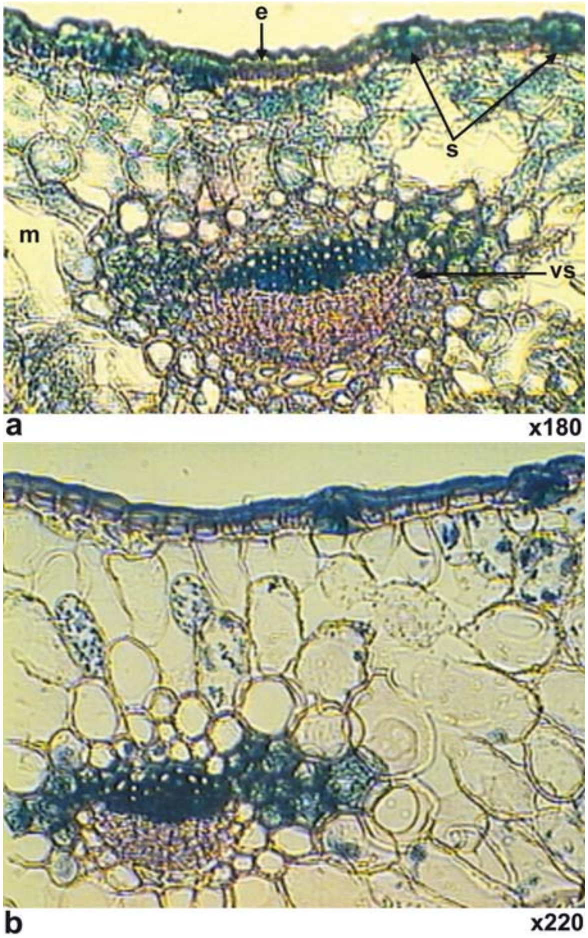

Fig. 10. Partial view of the mesophyll (a) *Juniperus communis* (b) *J. chinensis*: e- epidermis; m- mesophyll; s- stomata; vs- vascular system.

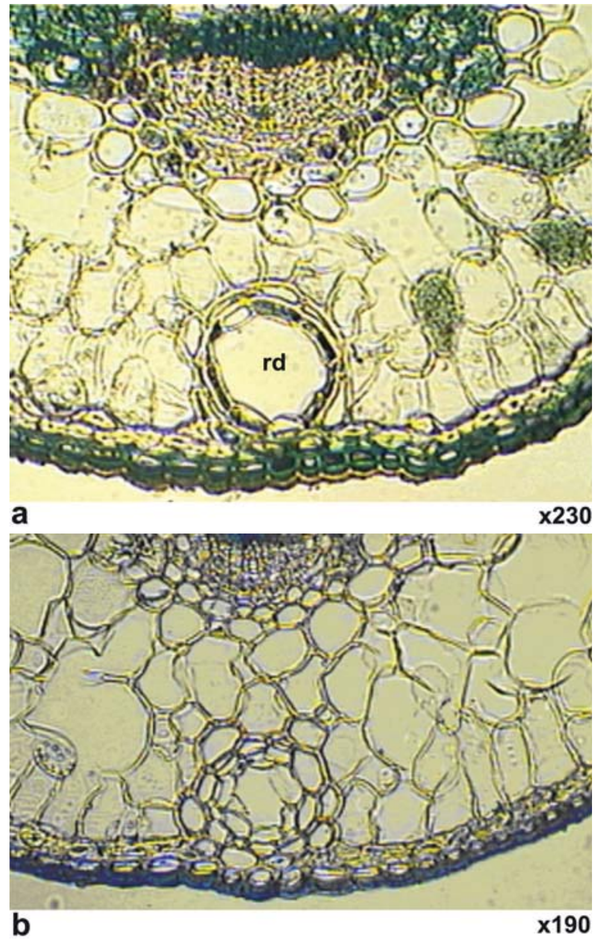

Fig. 11. Portions of the leaf margins with resin ducts (a) *Juniperus communis* (b) *J. chinensis*: rd- resin duct.

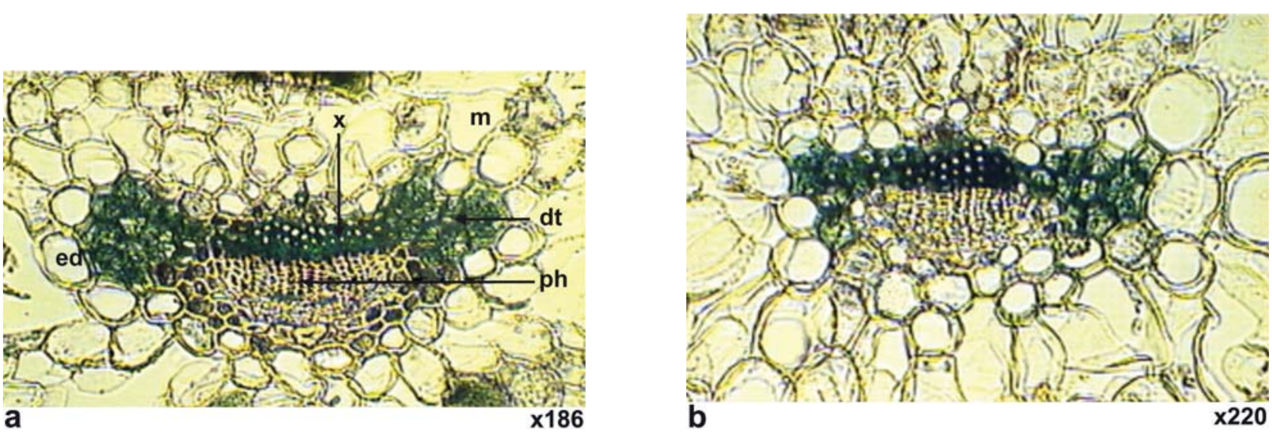

Fig. 12. The vascular system of the vein (a) *Juniperus communis* (b) *J. chinensis*: dt- diffusion tissue; ed- endodermis; m- mesophyll; ph- phloem; x- xylem.

whereas the phloem has a normal development. The diffusion tissue consists of a large number of diffusion traheids. In *Juniperus communis*, few fiber cells, placed below the phloem, occur (Fig.12 a, b).

CONCLUSIONS

The results indicate that:

1. The cross sections of the leaves of all six species exhibit a single layer of more or less lignified epidermal cells, covered by wax. The hypodermis is highly lignified with cells arranged characteristically for each species. Engrossed stomata, with large (*Cedrus* species) or small (*Abies* and *Juniperus* species) substomatal cavities occur. They are numerous for the leaves of *Abies* and *Juniperus* species and few for *Cedrus* species.
2. In the heterogenous mesophyll, the resin ducts are present with differences concerning their number, size and position.
3. The vascular system of the single vein is represented by collateral vascular bundles, two for *Abies* and *Cedrus* species and one for *Juniperus* species, and the diffusion tissue. The vascular system and the diffusion tissue are surrounded by a more or less obvious endodermis. Differences appear in terms of the development of the xylem and phloem elements as well as in what concerns the diffusion tissues.

We may mention that the anatomical features of *Cedrus deodara* are almost similar with *C. libani* previously studied (Bercu *et al.* 2009).

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Botanica SERBICA

REZIME

Komparativno anatomska studija listova odabranih četinara

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Ovaj rad je deo komleksne studije četina I bavi se komparatinmo-anatomskom analizom četina šest vrsta iz dve familije četinara *Abies alba* Miller, *Abies nordmanniana* subsp. *nordmanniana* (Stev.) Spach, *Cedrus atlantica* (Endl.) G. Manetti ex. Carriére var. *glauca* Carriére, *Cedrus deodara* (Roxb.) G. Don (Pinaceae), *Juniperus communis* L. i *Juniperus chinensis* L. (Cupressaceae). Sve ove vrste su popularno ukrasno drveće u parkovima i baštama, od kojih je četri uneto u Rumuniju posle 1990 i aklimatizovane su u raznim oblastima. Anatomski posmatrano, četine su slične u osnovnoj strukturi ali se razlikuju u detaljima. Ovaj rad daje anatomske podatke koji se tiču epidermalnih ćelija, hipodermalnih ćelija, tipa mezofila, broja, veličine i rasporeda smolnih kanala te rasporedom elemenata vprovodnog sistema. Anatomske odlike četina šest izučavanih vrsta su diskutovane.

Ključne reči: anatomija, četinari, epidermis, četine, mezofil, smolni hodnici, provodni sistem