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Anatomy of four taxa of the Genus *Juniperus* sect. *Juniperus* (Cupressaceae) from the Balkan peninsula

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ABSTRACT: The aim of this research was to describe variability of anatomical characteristics of needles and stems of the taxa: *J. communis* subsp. *communis* var. *communis, J. communis* subsp. *communis* var. *intermedia* Sanio, *J. communis* subsp. *alpina* (Suter) Čelak and *J. deltoides* Adams, from Serbia, Montenegro and Macedonia (Balkan Peninsula), to establish whether anatomical differences are influenced by the phylogenetic or ecological position of individual taxa.

Macroscopic and microscopic analyses were done on samples of 13 populations from Eu-Mediterranean coastal areas of up to 2,000 m a.s.l. in continental mountains. Descriptive statistics were calculated for 23 quantitative characters. Canonical Discriminant Analysis (CDA) and clustering on the basis of the UPGMA method were performed to measure distances between the groups.

At an anatomical level there was fine differentiation between the taxa, at the level of species and subspecies. The species *J. communis* and *J. deltoides* differed in number of stomatal bands, needle cross-sectional shape, dimensions of the needle resin duct, as well as in the structure of the central cylinder in the primary structure of the stem. Within the species *J. communis*, high mountain subspecies *J. communis* subsp. *alpina* was finely differentiated compared with the typical subspecies *J. communis* subsp. *communis*. Basic differences between these two taxa were reflected in the shape and dimensions of the needles. Discriminant and cluster analyses did not show any major differences between *J. communis* subsp. *communis* var. *communis* and *J. communis* subsp. *communis* var. *intermedia*.

Multivariate analysis showed that the level of anatomical differentiation of these taxa were conditioned partially by phylogenetic association of individual taxa, and partially by ecological conditions of the habitat.
Our results showed that anatomical characteristics of needle and primary structures of the stem of these species of genus *Juniperus* have taxonomic significance at the species and infraspecies levels.

Key words: *Juniperus communis*, *J. deltoides,* anatomy, needles, primary and secondary stem.

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INTRODUCTION

The genus *Juniperus* L. consists of 67 species and 34 varieties. All the taxa grow in the northern hemisphere, except *J. procera* Hochst. ex Endl., which grows along the rift mountains in east Africa in the southern hemisphere. The genus is divided into three sections: *Caryocedrus* Endlicher with the only species *J. drupacea* Labill.; *Juniperus* (syn. sect. *Oxycedrus* Spach) with 10 species and *Sabina* (Miller) Spach with approximately 56 species (Adams 2008).

Eight taxa at the species and subspecies level, belong to the section *Juniperus* (taxa with three sharp needles in whorls) within the flora of Europe, of which five taxa are present in the Balkan Peninsula - *J. communis* L. with three subspecies, *J. deltoides* Adams and *J. macrocarpa* Sibth. & Sm. (Franco Amaral 1993, Adams 2008).

Section *Juniperus* is divided into two groups, a northern group associated with *J. communis,* with blue or blue-black mature seed cones and one band of stomata on the adaxial leaf surface; and *J. deltoides* allied with the Mediterranean region that have two stomatal bands and red, reddish-brown to reddish-purple mature cones (Adams 2008). In addition to the clear ecological and geographical differences, molecular research has shown that these two groups within the section *Juniperus* represent separate monophyletic lines (Adams 2008).

***Juniperus communis* L.** is a shrub tree up to 15 m high. This is the species with a rather large area of distribution and wide ecological valence in the regions of Europe, Asia Minor, Asia (Caucasus, Iran, Afghanistan and Himalayas), Northern America and less frequently on the mountains of Northern Africa. Its wide valence in regard to the climate and soil enables it to inhabit, in addition to xerophytic deciduous forests and scrub, xerothermic coniferous forests, acidophilic heaths, as well as the devastated forests of beech and beech-fir regions (Lakušić 1980; Šilić 1990; Jovanović 1992; Vidaković & Franjić 2004; Adams 2008).

In the region of the Balkan Peninsula, the populations of common juniper differentiate on horizontal and vertical profiles into three subspecies and numerous varieties.

J. communis subsp. *communis* var. *communis* and *J. c.* subsp. *communis* var. *intermedia* Sanio are distributed in the lowland, hilly and mountainous regions of internal parts of the peninsula. Typically, the variety is mostly linked with open, insolated and warmer habitats in lowland, hilly and lower parts of the mountain altitude zone, while the variety *J. communis* subsp. *communis* var. *intermedia* is mostly present in the mountain and sub-alpine zones, in habitats with significantly lower temperatures and a shorter vegetative period, i.e., longer period of physiological drought (Lakušić 1980; Jovanović 1992; Lakušić *et al.* 2005). An additional two subspecies are present in the high-mountain regions of the Balkan peninsula, in regions above the upper forest border, namely in vegetational regions of the sub-alpine bushy vegetation: *J. communis* subsp. *hemisphaerica* (J. & C. Presl) Nyman in the Eu-Mediterranean mountains of Southern Greece, and *J. communis* subsp. *alpina* (Suter) Čelak (= *J. sibirica* Burgsdorf, *J. nana* Willd., *J. communis* var. *montana* Ait.) in the Dinarides, northern Scardo-Pindic mountain system, Rhodopes and the Balkan mountains (Lakušić 1980; Christensen 1986; Šilić 1990; Vidaković & Franjić 2004; Lakušić *et al.* 2005).

***J. deltoides* Adams** (= *J. oxycedrus* auct. Apen., Balk. & Turk., non L.) is a cryptic species recently described (Adams 2004; Adams *et al.* 2005) as differing from *J. oxycedrus* in its terpenes, RAPDs, DNA sequences, needle morphology and range. It is a shrub or a low tree up to 14 m high, widely distributed in the central and eastern Mediterranean region, namely in the Apennines and Balkan peninsula, and Asia Minor up to Iran (Adams 2008). On the vertical profile it occupies spaces from the coast up to around 1,000 m a.s.l. In the Eu-Meditarranean region it inhabits the evergreen xerothermic deciduous and coniferous forests, maquis, garigue and open stony regions, while in the Sub-Mediterranean region it is found in xerophytic deciduous oak and hornbeam forests, black pine forests, as well as in various types of scrub and open rocky ground vegetation. Rare, isolated populations occur outside the Mediterranean and Sub-Mediterranean regions, extending to the moderate-continental regions where occasionally they build specific secondary scrub-grasses communities (Lakušić 1980; Christensen 1986; Šilić 1990; Jovanović 1992; Lakušić 1997; Vidaković & Franjić 2004; Lakušić *et al.* 2005).

In the region of the Balkan Peninsula, *J. deltoides* differentiates in morphological and ecological senses into the Mediterranean - Sub-Mediterranean populations belonging to the typical taxon, and oro-mediterranean Adriatic populations belonging to the taxon *J. nanoides* R. Lakušić nomen nudum (Lakušić *et al.* 1983).

***J. macrocarpa* Sibth. & Sm.** is a tree up to 14 m high, distributed in the Eu- Mediterannean region from southern Portugal eastward to Cyprus. It inhabits the evergreen xerothermic deciduous and coniferous forests, maquis, garigue and open Eu-Mediterranean rocky grounds. In fact, this taxon is usually treated as a subspecies of *J. oxycedrus* L. (Christensen 1986; Franco Amaral 1993); however, recent studies have shown that these two taxa, in addition to the morphological level, are also very well differentiated at the molecular level. Therefore in recent

contemporary studies they have been treated as separate species (Vidaković & Franjić 2004; Adams 2008).

Thanks to Adams' several decades of research, it could be said that the genus *Juniperus* is one of the well-studied genera. In his monograph "Juniperus of the World" (Adams 2008) a detailed review of research and literature is presented, showing that this genus has been submitted to detailed systematic-taxonomic, phytochemical-hemotaxonomic and molecular-systematic studies; though the anatomy of these species has been relatively neglected. The literature shows only a few studies of the anatomy of needles and stems of species of the genus *Juniperus* (Thomas 1963; ter Welle & Adams 1998; Vasić *et al.* 2008; Bercu *et al.* 2009).

Therefore, the aim of work presented here was to establish and describe anatomical characteristics of needles and stems of four taxa of the genus *Juniperus* sect. *Juniperus* from the Balkan Peninsula, to establish whether differences amongst these taxa are also associated with the phylogenetic or ecological position of individual taxa, as well as to identify the potential taxonomical importance of anatomical features.

MATERIAL AND METHODS

Plant material. Macroscopic and microscopic analyses were done on samples from 13 populations of taxa *J. communis* subsp. *communis* var. *communis, J. communis* subsp. *communis* var. *intermedia* Sanio, *J. communis* subsp. *alpina* (Suter) Čelak and *J. deltoides* Adams, from Serbia, Montenegro and Macedonia. The collected plant material was either placed in a herbarium or fixed in 50% alcohol and deposited respectively in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade (BEOU) and Herbarium of the Institute of Botany, Faculty of Pharmacy, University of Belgrade (HFF).

Anatomical analyses of the needles and stems were done using permanent slides, prepared by a standard method for light microscopy. Cross-sections of the needles and stems were cut on a Reichert sliding microtome (up to 15 mm thick). The sections were cleared in Parazone and thoroughly washed before staining in safranin (1 % w/v in 50 % ethanol) and alcian blue (1 % w/v, aqueous). All the slides were mounted in Canada balsam after dehydration.

Voucher specimens:

J. communis* L. subsp. *communis* var. *communis

Serbia, Uvac, calcareous *Juniperus communis* scrub, c. 900 m a.s.l. (Lakušić, D., 29.05.2009., HFF 62)

Serbia, Kopaonik, Kukavica, serpentinous *Juniperus communis-intermedia* scrub, c. 1,500 m a.s.l. (Lakušić, B.& D., 13.08.2005. HFF 1609)

Serbia, Gorge of Panjica river, termophilous *Ostrya carpinifolia* open forest, carbonate, c. 400 m a.s.l. (Lakušić, D., 6.12.2009., HFF 3224)

***J. communis* subsp. *communis* var. *intermedia* Sanio**

Serbia, Kopaonik, Kukavica, serpentinous *Juniperus communis-intermedia* scrub, c. 1,500 m a.s.l. (Lakušić, B.& D., 13.08.2005. HFF 1610)

Serbia, Suva planina, mountain meadows, carbonate, c. 1,000 m a.s.l. (Lakušić, B., 23.06.2005., HFF 1608)

***J. communis* subsp. *alpina* (Suter) Čelak**

(= *J. sibirica* Burgsdorf, *J. nana* Willd., *J. communis* var. *montana* Ait.)

Serbia, Kopaonik, Kukavica, serpentinous *Juniperus communis-intermedia* scrub, c. 1,500 m a.s.l. (Lakušić, B.& D., 13.08.2005. HFF 1613)

Serbia, Kopaonik, Nebeske stolice, serpentinous *Juniperus nana* scrub, 1,850 m a.s.l. (Lakušić, D., 27.09.2003., HFF 1611)

Serbia, Kopaonik, Suvo Rudište, silicious *Juniperus nana* scrub, c. 1,900 m a.s.l., 11.08.2005, leg. Lakušić B.& D., HFF 1612)

Montenegro, Durmitor, Suva lokva, calcareous *Juniperus nana* scrub, c. 1,800 m (Lakušić, D., 30.05.2009., BEOU, HFF 61)

Montenegro, Orjen, above Goliševac (42°33.403 N, 18°33.072 E), calcareous *Juniperus nana* scrub, 1,450 m, (Stevanović, V., Lakušić, D., 11.10.2005, BEOU 20258 HFF 58)

Macedonia, Galičica (40°56 37.7 N, 20°49 36.6 E), calcareous *Juniperus nana* scrub, 1,822 m a.s.l. (16.10.2009, BEOU *29797,* HFF 59)

***J. deltoides* Adams**

(= *J. oxycedrus* Auct. Balk. non L.)

Montenegro, Luštica, maquis, carbonate, c. 300 m a.s.l. (Lakušić B.& D., 29.07.2004. HFF 1615)

Montenegro, Valandos, maquis, carbonate, c. 200 m (Lakušić, D., 2.06.2009, BEOU, HFF 60)

Nomenclature of the habitats is based on the EUNIS classification (Davies & Moss 2002; Lakušić *et al.* 2005).

Morpho-anatomical analysis. A total of 23 quantitative characters for statistical analysis were grouped in two categories: I. Needle shape and anatomy characters (14); II. Stem shape and anatomy characters (9).

Needle shape and anatomy characters: 1. Needle shape 2. Needle width 3. Needle thickness; 4. Resin duct area; 5. Resin duct perimeter; 6. Resin duct width; 7. Resin duct height; 8. Number of stomata bands; 9. Upper epidermis

Table 1. Basic statistics of morphometric data of analyzed taxa (all measures in μm)

	J.communis	**J.intermedia**	**J.alpina**	**J.deltoides**
Needle shape	t, t_co, t_ro	t_ro(t)	t, t_co, t_ob	tr
Needle width	(1092-) 1106-1555 (-1679)	(1201-) 1216-1364 (-1377)	(1074-) 1207-1508 (-1518)	(1444-) 1470-1839 (-1839)
Needle thickness	(371-) 388-574 (-648)	(438-) 447-480 (-489)	(391-) 432-543 (-591)	(474-) 504-588 (-589)
Needle resin duct - area	(1833-) 839-13326 (-16806)	(6040-) 6618-15793 (-19720)	(754-) 2774-15139 (-28402)	(229-) 269-1039 (-1437)
Needle resin duct - perimeter	(242-) 261-715 (-877)	(449-) 506-802 (-874)	(124-) 340-762 (-1083)	(74-) 77-133 (-161)
Needle resin duct - width	(54-) 55-145 (-169)	(106-) 111-165 (-184)	(35-) 76-159 (-218)	(21-) 22-40 (-47)
Needle resin duct - height	(50-) 49-132 (-157)	(86-) 93-143 (-164)	(32-) 66-140 (-191)	(20-) 21-37 (-44)
Number of stomata lines	(1-) 1-1 (-2)	(1-) 1-2 (-2)	(1-) 1-2 (-2)	(2-) 2-2 (-2)
Upper epidermis (without stomata) width	(470-) 550-860 (-1120)	(470-) 520-780 (-880)	(320-) 390-920 (1570)	(800-) 870-1060 (-1140)
Stomata line width	(390-) 400-880 (-1320)	(260-) 310-980 (-1040)	(203-) 310-110 (-1290)	(407-) 530-790 (-810)
Needle area	(351718-) 333384-604200 (-729661)	(408969-) 421124-464742 (-478375)	(322550-) 382081-538454 (-570343)	(358077-) 390339-665205 (-657796)
Needle perimeter	(2908-) 2914-4002 (-4311)	(3422-) 3491-3763 (-3827)	(2820-) 3126-3764 (-3850)	(3613-) 3677-4556 (-4570)
Needle width_1	(1104-) 1114-1572 (-1694)	(1273-) 1294-1394 (-1414)	(1094-) 1220-1516 (-1524)	(1453-) 1477-1854 (-1856)
Needle thickness_1	(441-) 449-644 (-721)	(508-) 557-654 (-651)	(434-) 477-588 (-622)	(505-) 530-620 (-625)
Stem_side length	(1018-) 1045-1269 (-1578)	(1260-) 1537-1614 (-1379)	(1031-) 1143-1516 (-1594)	(813-) 812-1130 (-1135)
Stem resin duct - area	(2943-) 4694-11878 (-13266)	(7594-) 6831-29067 (-32019)	(12381-) 16347-60648 (-84734)	(2348-) 3522-8599 (-8785)
Stem resin duct - perimeter	(341-) 431-696 (-775)	(482-) 488-1101 (-1243)	(640-) 802-1519 (-1829)	(310-) 360-560 (-570)
Stem resin duct - width	(72-) 90-141 (-154)	(108-) 113-247 (-269)	(140-) 171-312 (-373)	(65-) 79-125 (-124)
Stem resin duct - heigth	(65-) 82-126 (-133)	(92-) 91-169 (-185)	(124-) 146-264 (-312)	(54-) 65-105 (-106)
Stem - area	(639839-) 692889-1042831 (-1047273)	(797206-) 815536-1044939 (-1091895)	(648411-) 742588-1317765 (-1692929)	(376624-) 380341-812065 (-817468)
Stem - perimeter	(4081-) 4177-4820 (-4898)	(4716-) 4784-5697 (-5842)	(3763-) 4252-5832 (-6045)	(3119-) 3284-4534 (-4538)
Stem - width_1	(1095-) 1136-1396 (-1387)	(1342-) 1349-1470 (-1521)	(1074-) 1220-1620 (-1683)	(839-) 841-1207 (-1214)
Stem - thickness_1	(979-) 1015-1230 (-1239)	(1166-) 1171-1302 (-1579)	(1014-) 1079-1423 (-1583)	(780-) 790-1071 (-1077)

Legend: tr – triangular, t – trapezoidal, t_co - trapezoidal with the concavity on the abaxial side, t_ro - trapezoidal with the rounded edges, t_ob - obtuse triangular

Fig. 1. Forms of needle cross sections: A) basic triangular form in *J. deltoides*; B) basic trapezoidal form in *J. communis*; C) needles with concavity on the abaxial side (*J. communis* subsp. *communis* var. *communis*); D) needles with rounded and bent edges (*J. communis* subsp. *communis* var. *intermedia*); E) needles with obtuse triangular form (*J. communis* subsp. *alpina*).

(without stomata) width; 10. Stomata band width; 11. Needle area; 12. Needle perimeter; 13. Needle width_I; 14. Needle thickness_I.

Stem shape and anatomy characters: 15. Stem side length; 16. Stem resin duct – area; 17. Stem resin duct – perimeter; 18. Stem resin duct – width; 19. Stem resin duct – height; 20. Stem – area; 21. Stem – perimeter; 22. Stem – width_I; 23. Stem – thickness_I.

The characters 2, 3 and 15 were measured manually as the lines, while characters 13, 14, 22 and 23 were automatically calculated from the measured surfaces. All morpho-anatomical measurements were made with Digimizer 3.7.0.; image analysis software

Statistical analysis. Mean, minimum, maximum and standard deviation were obtained for each character. Canonical Discriminant Analysis (CDA) was performed for anatomical characters to test whether species differentiated into discrete groups. Overall differences between groups are presented as Mahalanobius distances, which were used for clustering on the basis of the UPGMA method.

All Statistical analysis were made with the statistical package Statistica 4.5 for Windows.

RESULTS

Needle shape and anatomy. The basic form of cross-sections of needles of *J. communis* was trapezoidal, while in *J. deltoides* it was triangular (Fig. 1). Significant deviations from the basic regular trapezoidal form were present in certain infraspecies taxa, as well as in some populations of the species *J. communis*. Thus, in the taxon *J. communis* subsp. *communis* var. *communis*, in addition to trapezoidal needles, needles with characteristic concavity on the abaxial side were equally present. In the taxon *J. communis* subsp. *communis* var. *intermedia*, needles with rounded and bent edges dominated, while in the taxon *J. communis* subsp. *alpine,* in addition to the dominant trapezoidal needles, there were also needles with a specific obtuse triangular form. Significant variation of the basic triangular form of the needle was not observed in *J. deltoides* (Fig. 1).

The needle surface of these *Juniperus* species was characteristically covered by a thick cuticle layer and a one-layer epidermis composed of cells with rather thickened walls. Sub-epidermally, there was generally a one-layer hypodermis, multi-layered on the needle edges, and absent underneath the stomata. The hypodermis was composed of dead cells with extremely thickened walls (Fig. 2).

On the adaxial side of needles there were stomata distributed in one or two bands in *J. communis* and in two bands in *J. deltoides*. In the typical subspecies *J. communis* subsp. *communis* the distances between stomata were relatively small, composed of only a few epidermal cells, with a well developed hypodermis or completely without it. In *J. communis* subsp. *alpina* those distances were significantly bigger, more pronounced, without or with a well-developed hypodermis. In *J. deltoides*, the distances between stomata were comprised of a large number of epidermal cells under which was a well-developed hypodermis (Fig. 2).

Needle thickness of the *Juniperus* species ranged from 371 to 648 μm, and needle width ranged from 1092 to 1839 μm. Both of these characters had the highest variability in *J. communis* subsp. *communis* var. *communis*, and the smallest in *J. communis* subsp. *communis* var. *intermedia* (Tab. 1).

Abaxially there was one resin duct, with clearly-differentiated secretory cells around the duct's lumen, and around them there was a single layer of cells with thickened walls that presumably had a mechanical function. The diameter of the resin duct varied from 20 to 218 μm, with the highest duct variability in *J. communis* subsp. *alpina* (32-218 μm), and the smallest in *J. deltoides* (20-47 μm) (Tab. 1 Fig. 2, 5).

The mesophyll was composed of one layer of short palisade cells on the adaxial and one to two layers of more pronounced palisade cells on the abaxial side of needles, while between them there was spongy tissue with relatively small intercellular spaces. In the center of the needle there was one vascular bundle. Above the phloem there was an arch composed of several (in all taxa within *J. communis*) or numerous (*J. deltoides*) sclerenchyma cells. Well-developed transfusion tissue was located on the external sides of vascular bundles, rather characteristic for leaves of Gymnospermae plants. Transfusion tissue consisted of traheids and live parenchyma cells (Fig. 2).

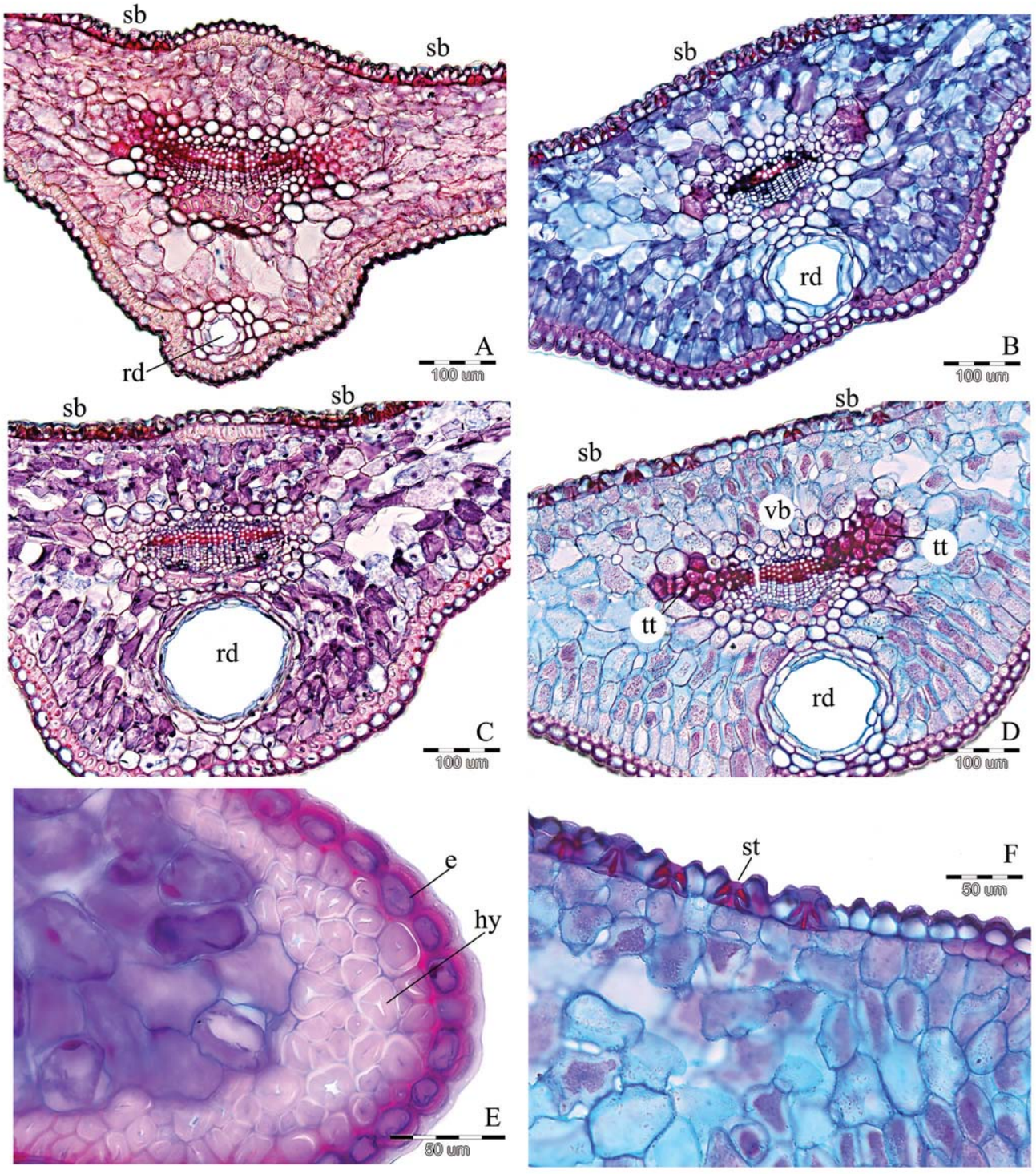

Fig. 2. Anatomical characteristics of needles A) *J. deltoides* with two stomatal bands B) *J. communis* with one stomatal band, C-F) *J. communis* with two stomatal bands (**e** - epidermis with cuticule; **sb** - stomatal band; **st**-stoma; **hy** - hypodermis; **vb** - vascular bundle; **tt** - transfusion tissue; **rd** - resin duct).

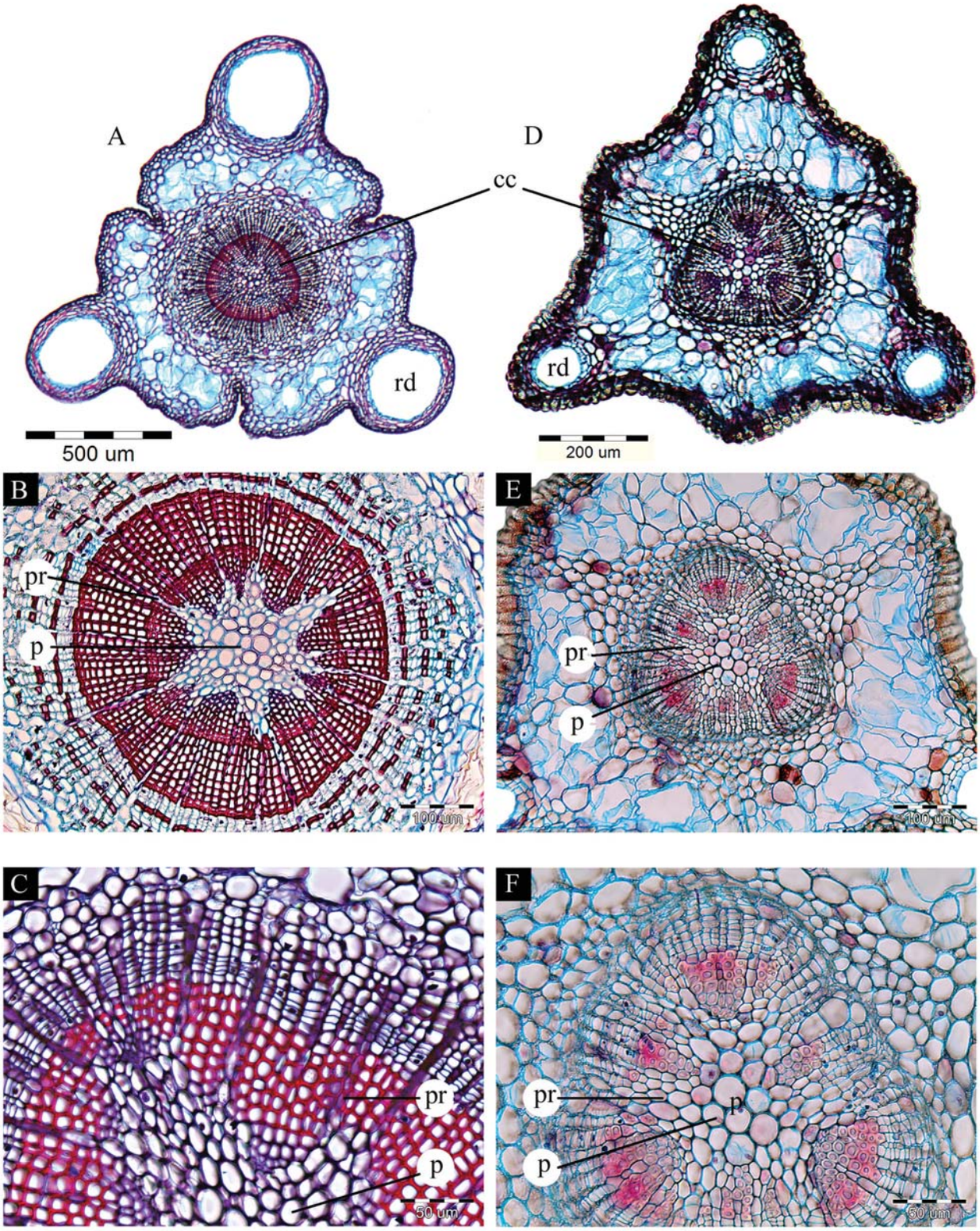

Fig. 3. Cross sections of the primary stem A-C) *J. communis;* D-F) *J. deltoides*. (**rd** - resin ducts; **i** - invaginations; **cc** - central cylinder; **p** - pith; **pr** - pith rays).

Stem Shape and Anatomy

Primary Structure. The stem structure in all these *Juniperus* species was more or less uniform.

The stem cross section was triangular with three pronounced resin ducts, and deep or less deep invaginations on the plane sides of the stem. These invaginations were absent only in plants of the population of the species *J.deltoides* from Luštica (Fig. 3D).

Cells of the singled-celled epidermis had extremely thickened superficial cell walls, and on the surface a thinner or thicker layer of cuticle. Sub-epidermally, there were two to three layers of densely compacted cells with thickened walls, and the rest of the bark was composed of large parenchyma cells, with small intercellular spaces.

Sub-epidermally, in the corners of the stem were three resin ducts, with diameters of 54 to 373 μm. This character showed the highest variability in *J. communis* subsp. *communis* var. *intermedia* (92 – 269 μm), and the lowest in *J. deltoides* (54 – 124 μm) (Fig. 3, 5).

The layers of the bark towards the central cylinder consisted of several layers of densely-compacted cells with more or less thickened cell walls. Invaginations of the stem on the plane sides came down to these cells. An exception was the stem of plants from Luštica of the species *J. deltoides*, where there were no invaginations, and instead there were large parenchyma cells which were connected with the sub-epidermal cells (Fig. 3).

Species were clearly differentiated at the level of the structure of the central cylinder. In *J. communis* the phloem and xylem surrounding the pith in a more or less star-like shape, and the pith rays were narrow, composed of one or two bands of cells. In *J. deltoides* the vascular elements had a triangular shape, the pith rays were wide, with six separate vascular bundles (Fig. 3).

Secondary Stem Structure. In all these taxa, a thinner or thicker layer of periderm was on the surface of the woody stem, under which were parenchyma and a phloem. The woody part consisted of xylem and pith.

This structure had no resin ducts. They had disappeared together with almost the whole primary bark, the phellogen was being formed of parenchyma cells located very close to the phloem. The periderm, replaced the epidermis in the second or the third vegetative season, when already the secondary phloem and xylem can be observed through the existence of the rings – porous wood (Fig. 4).

Multivariate analysis of the anatomical characters. Canonical Discriminant Analysis (CDA) showed that clear anatomical differentiation exists amongst these taxa on the level of species and subspecies (Fig. 6). Samples of *J. deltoides* were located in the negative part of the first discriminant axis (Root1), while samples of *J. communis* were located in the positive and central part of the first axis. Clear discrimination was also observed on the second discriminant axis (Root 2). Samples belonging to the taxon *J. communis* subsp. *communis* were grouped in the negative part of the second axis, while samples belonging to the subspecies *J. communis* subsp. *alpina* were located in the positive part. At this level of analysis, samples of two varieties of the subspecies *J. communis* subsp. *communis* did not show any discrimination (Fig. 6).

At the population level, cluster analysis also showed that the species *J. deltoides* and *J. communis* were clearly discriminated at the anatomical level. Namely, all the samples separated into two distant clusters corresponding to these two species. A trend of differentiation to the subspecies *J. communis* subsp. *communis* and *J. communis* subsp. *alpina* was observed within the cluster *J. communis*, with some variation. These variations were due to samples of *J. communis* subsp. *alpina* from the mountains Kukavica and Galičica that were grouped into the typical "*J. communis*" cluster, while the population of typical *J. communis* from Uvac was grouped into the cluster "*J. alpina*" (Fig. 7).

DISCUSSION

Analyses of basic characters of needle and stem anatomy have shown that among these *Juniperus* taxa there is a fine differentiation at the level of species and subspecies.

The species *J. communis* and *J. deltoides* were clearly differentiated on the basis of number of stomatal bands, basic form of needle cross section, dimensions of the needle resin duct, as well as on the basis of structure of the central cylinder in the primary stem structure.

The basic number of stomatal bands on the adaxial needle surface in *J. communis* was one, while in *J. deltoides* was two. However, in all the analyzed infraspecies taxa of *J. communis* some needles with two stomatal bands were recorded, with the space between the stomatal bands consisting of a small number of epidermal cells. An exception was some needles in *J. communis* subsp. *alpina* where the distance between stomatal bands was much bigger, with a well-developed hypodermis, resembling the needles of *J. deltoides.*

The basic shape of the needle cross-sections in *J. communis* was trapezoidal, while in *J. deltoides* it was triangular. The diameter of the needle resin duct in these infraspecies taxa were within the range for the species *J. communis*, between 90 and 138 μm, while in *J. communis* from Romania (Bercu *et al.* 2009) it was only 6.5 μm. In *J. deltoides* the mean diameter of the needle resin duct was only around 31 μm. The most pronounced differences were in the maximum diameter of the resin duct, which in the taxon *J. communis* subsp. *alpina* was 218 μm, and in *J. deltoides* only 47 μm.

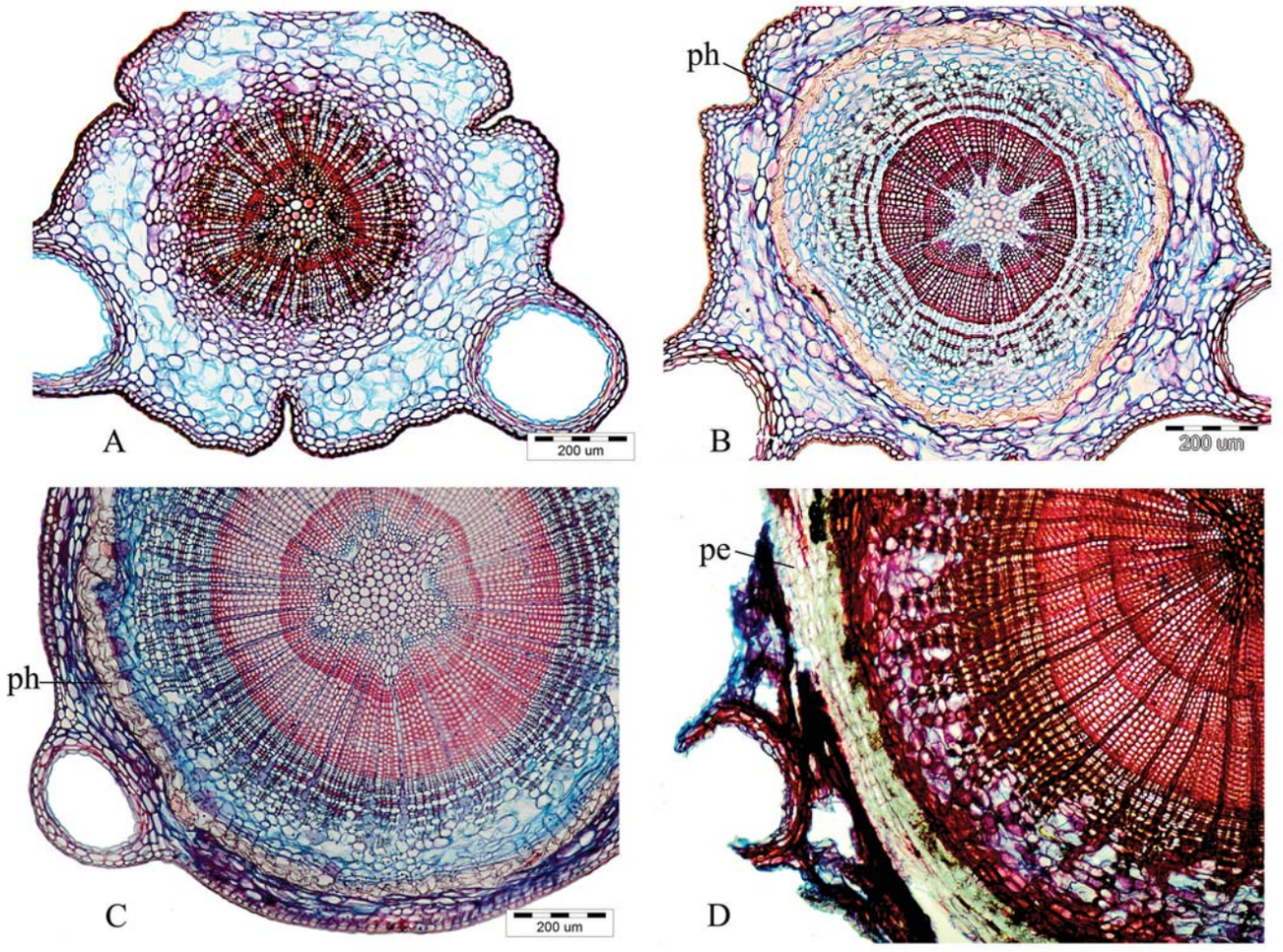

Fig. 4. Cross sections of the stem: A & B) primary structure, C & D) secondary structure (**ph** - phellogen; **pe** - periderm).

The species clearly differed at the level of the structure of the central cylinder. Thus, in *J. communis* the phloem and xylem are located in the cylinder with thin pith rays of one to two lines of cells. In *J. deltoides* the pith rays are wide, so they divide the vascular elements into 6 separate vascular bundles having a triangular shape.

These anatomical differences between the species *J. communis* and *J. deltoids*, that were clearly confirmed in the CDA and cluster analysis, are in complete conformity with results of contemporary molecular and chemical analyses, which divide these two species into separate phylogenetic lines (Adams 2008).

Clear differentiation was also found within the species *J. communis* between the high mountain and lowland-mountain populations at the level of the CDA and partially at the level of the cluster analysis, with the high-mountain taxon *J. communis* subsp. *alpina* being clearly differentiated with regard to the typical subspecies *J. communis* subsp. *communis*.

The basic differences between these two taxa were also reflected in needle shape and dimensions. While in *J. communis* subsp. *communis* the needle cross-section had the shape of a regular trapezoid, trapezoid with a concavity on the abaxial side of the needle, or trapezoid with characteristically rounded and bent edges on the adaxial side of needle, in *J. communis* subsp. a*lpine,* in addition to these shapes, needles with specific obtuse triangular form were also found. In addition, in *J. communis* subsp. a*lpina* all the dimensions of the needle and stem cross sections were slightly greater than those in *J. communis*. These differences were significantly greater for the needle and stem surface volume ratio, which in the case of *J. alpina* were more towards volume increase, in conformity with the ecological conditions of the habitat in which the populations of this subspecies grow. Namely, in many high-mountain plants it was shown that the leaf volume increases with regard to its surface, which represents an adaptive response to the low temperatures and the problem

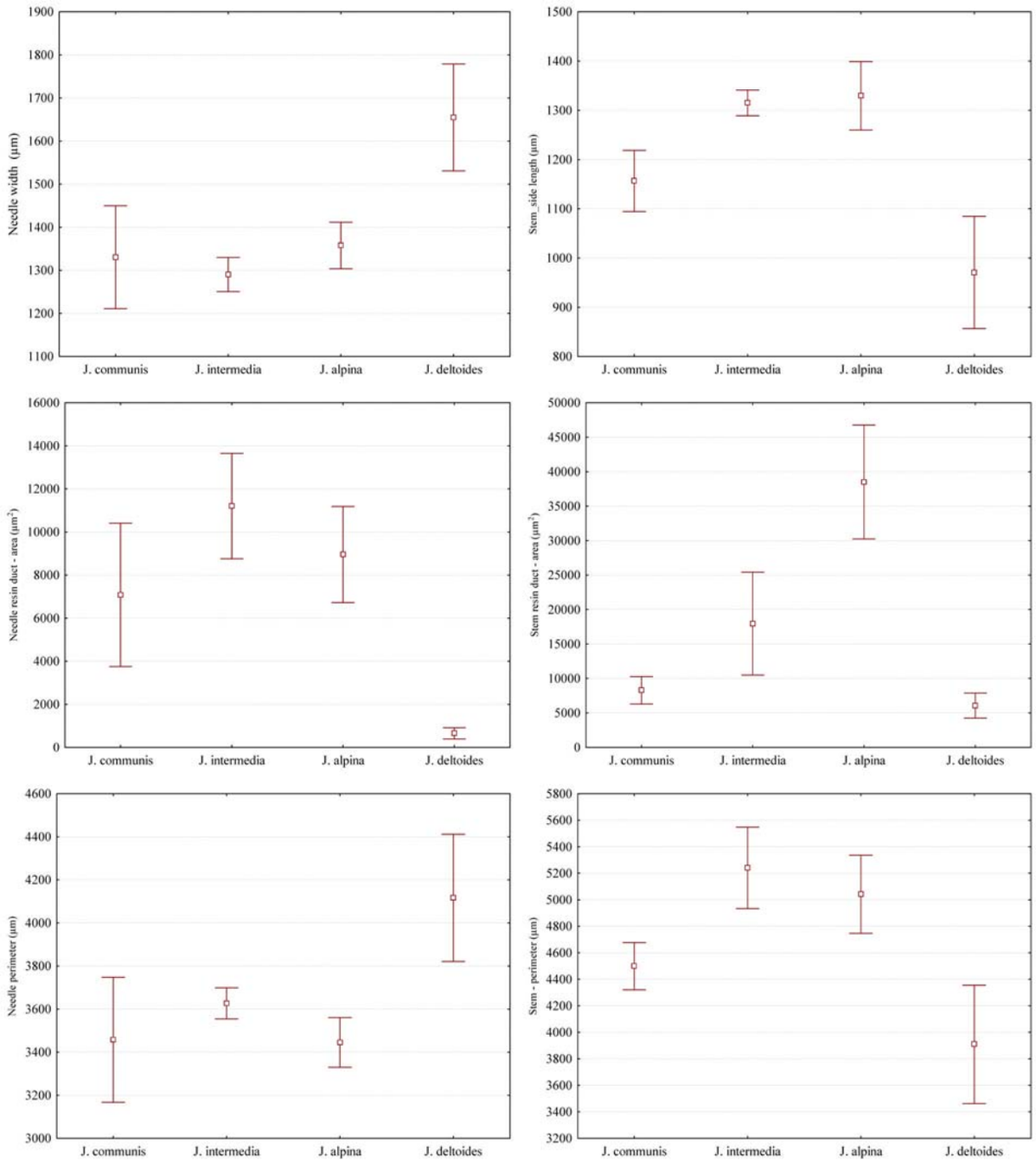

Fig. 5. Means with confidence interval plots of morphometric data of the analyzed taxa (□ Mean Mean±0,95 confidence interval).

of warmth loss that could damage the plant (Lakušić 1999; Kuzmanović *et al*. 2009).

The cluster analysis showed that the level of anatomical differentiation of the *Juniperus* species was only partially conditioned by the phylogenetic association of individual taxa. While the first cluster included species that belong to the species *J. communis* and *J. deltoides*, the second and third clusters included representatives of the remaining three taxa (*J. communis* subsp. *communis* var. *communis*, *J. communis* subsp. *communis* var. *intermedia* and *J. communis* subsp. *alpina*). The organization of the second and third clusters clearly demonstrates that similarities at the anatomical level in this group of taxa were more closely-associated with ecological than with phylogenetic

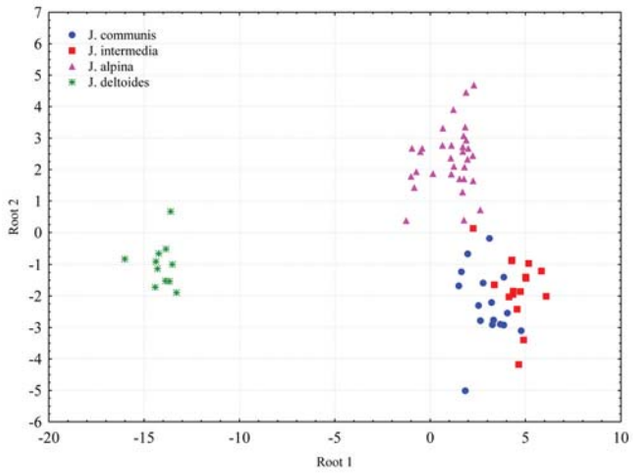

Fig. 6. Canonical Discriminant Analysis (CDA) of morphometric data of the analyzed taxa.

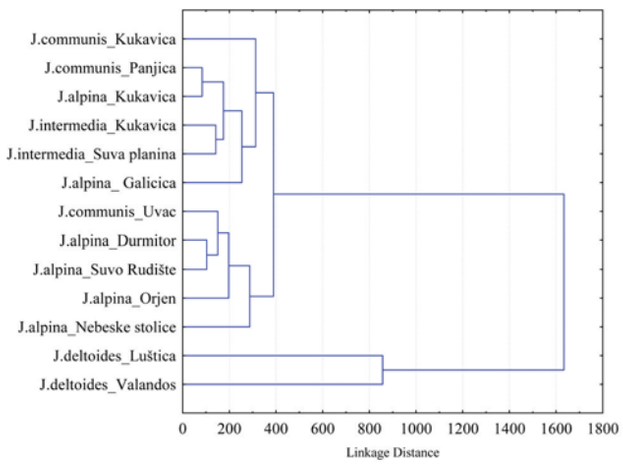

Fig. 7. Cluster analysis (UPGMA) of morphometric data of the analyzed taxa.

characteristics. Namely, the most thermophilous populations of *J. communis* subsp. *alpina* (Galičica and Kukavica) were nested within the clade of thermophilous population of *J. communis* subsp. *communis*. In contrast, the most phrygoriphillous population of *J. communis* subsp. *alpina* formed a separate clade which included a phrygoriphillous population of *J. communis* subsp. *communis* (Uvac). However, discriminant and cluster analyses did not show any difference between *J. communis* var. *communis* and *J. communis* var. *intermedia*.

To conclude, our research showed that needle and stem anatomical characteristics of the primary structure, in the species *Juniperus* sect. *Juniperus* also have taxonomic significance at the species and infraspecies levels.

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Botanica SERBICA

REZIME

Anatomija četiri taksona roda *Juniperus* L. Sect. *Juniperus* (Cupressaecae) sa Balkanskog poluostrva

Branislava Lakušić i Dmitar Lakušić

U radu je opisana varijabilnost anatomskih karaktera četina i primarne i sekundarne građe stabla taksona: *J. communis* subsp. *communis* var. *communis, J. communis* subsp. *communis* var. *intermedia* Sanio, *J. communis* subsp. *alpina* (Suter) Čelak i *J. deltoides* Adams (= *J. oxycedrus* auct. Apen., Balk. & Turk., non L.), sa prostora Balkanskog poluostrva - Srbije, Crne Gore i Makedonije

Makroskopska i mikroskopska istraživanja, koja su urađena na biljkama iz 13 eumediteranskih i kontinentalnih populacija, ukazuju da na anatomskom nivou između istraživanih taksona postoji fina izdiferenciranost na nivou vrsta i podvrsta.

Vrste *J. communis* i *J. deltoides* se na anatomskom nivou potpuno razdvajaju po broju linija stoma, obliku poprečnog preseka četine, dimenziji smonog kanala u četini, kao i na osnovu građe centralnog cilindra u primarnoj građi stabla. Unutar vrste *J. communis* takođe je uočeno da se visokoplaninska *J. communis* subsp. *alpina* fino diferencira u odnosu na tipsku podvrstu *J. communis* subsp. *communis.* Osnovne razlike između ova dva taksona su u obliku i dimenzijama četine. Diskriminantna i klaster analiza nisu pokazale značajne razlike između varijeteta *J. communis* subsp. *communis* var. *communis* i *J. communis* subsp. *communis* var. *intermedia.*

Ključne reči: *Juniperus communis*, *J. deltoides,* anatomija, četine, primarna i sekundarna građa stabla.