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Anatomical traits of *Artemisia umbelliformis* subsp. *eriantha* (Asteraceae) alpine glacial relict from Mt. Durmitor (Montenegro)

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ABSTRACT:
In this study, the vegetative anatomy of *Artemisia umbelliformis* subsp. *eriantha*, a Central European alpine glacial relict, wild-growing on Mt. Durmitor (Montenegro) was examined for the first time. The aim was to investigate the general anatomy and particular anatomical traits which might have possible taxonomic value. Microscopic slides were prepared according to the standard histological procedures. The adventitious young root showed a primary structure, while the older root showed a secondary structure with a well-developed periderm on its surface. The rhizome showed a primary structure with elements of a secondary structure (periderm). The stem cross section is characterized by a round shape with a well-developed periderm at certain stages, and collateral vascular bundles arranged in a circle. The petiole is concave in shape with a single-layered epidermis and parenchyma tissue with one large and two small vascular bundles. Druses and rhombohedral crystals are observed inside some petiole parenchyma cells. The leaf lobe cross section has an oblong-linear shape and is isolateral and amphistomatous in structure. The anticlinal walls of the leaf epidermal cells are sinuate. Secretory canals are present in the root cortex parenchyma (endodermal secretory canals) and the rhizome cortical parenchyma. The stem, petiole and leaf lack secretory canals. The stem and leaf are covered with T-shaped non-glandular and glandular trichomes. The taxonomic value of the analyzed characteristics is briefly discussed.

Keywords:
anatomy, crystals, secretory canals

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INTRODUCTION

Artemisia L. (Artemisiinae, Anthemideae, Asteraceae) consists of 522 species (Oberprieler *et al.* 2009) mostly distributed in the northern hemisphere (Vallès & Garnatje 2005). This genus has a very complex and unresolved taxonomy (Malik *et al.* 2017), and therefore requires investigation in various fields of botanical science (e.g. morphology and anatomy). Several studies have proved the significance of anatomical data in *Artemisia* taxonomy (Kelsey 1984; Noorbakhsh *et al.* 2008; Hayat *et al.* 2010; Konowalik & Kreitschitz 2012; Janaćković *et al.* 2019).

Artemisia umbelliformis Lam. subsp. *eriantha* (Ten.) Vallès-Xirau & Oliva Brañas is a Central European alpine glacial relict (Stevanović 2011). This rare, herbaceous perennial mountain plant grows on siliceous or, rarely, limestone rock ledges and in crevices (between 1500 and 3400 m a.s.l.) and may be found in small and isolated populations in a very disjunct distributional area:

from the Pyrenees across the south-western Alps and the Apennines to the Carpathians and the Balkan Peninsula (Sanz *et. al.* 2014). In Montenegro, it is present in the high mountain region of Mts. Durmitor, Komovi and Prokletije (Stevanović 2011). In parts of its range, *A. umbelliformis* subsp. *eriantha* faces threats such as collection, trampling, and habitat degradation due to tourism development and recreational activities. However, in the Balkans, these plants inhabit mainly inaccessible habitats that are not subject to any threats (Stevanović 2011). As a result of its rarity and current distribution, *A. umbelliformis* subsp. *eriantha* is included in Annex V of the EC Habitats Directive (European Community 1992) and in the Red Data lists of the Carpathians in Poland and Slovakia (Mirek & Piękoś-Mirkowa 1992; Maglocký & Feráková 1993). In Montenegro, this taxon is included in the national list of protected plant species (Official Gazette of RCG No. 76/06).

Artemisia umbelliformis subsp. *eriantha* has a strong aromatic scent, and its flowers are used in Italy for the preparation of the strong-flavored liqueur 'genepi' with a characteristic bitter taste (Pace *et al.* 2004). The essential oils of this taxon are particularly rich in thujones (Milosavljevic *et. al.* 2001; Mucciarelli & Maffei 2002). Beyond its strong scent, exploited as a flavoring agent, this taxon is also well-known for its usage in traditional medicine, e.g. to counteract respiratory infections, weakness and indigestion (Appendino *et al.* 1982; Mucciarelli & Maffei 2002; Rubiolo *et al.* 2009; Maggio *et al.* 2012).

Artemisia umbelliformis subsp. *eriantha* is completely unexplored from the anatomical point of view. There is only one study related to mucilage formation which has shown that this taxon possesses fruits without mucilage cells (Kreitschitz 2012). Therefore, the aims of our present study are to examine: (1) the anatomy of the vegetative structures of *A. umbelliformis* subsp. *eriantha* and (2) any particular anatomical traits which might have possible taxonomic value.

MATERIAL AND METHODS

Plant material. Plant material (parts of the root, rhizome, leaf, petiole and stem) from five individual species of *Artemisia umbelliformis* subsp. *eriantha* was collected in 2018 during the flowering period in the natural habitat: Mt. Durmitor, Montenegro (N 43°07'8.06''; E 19°02'21.73'') and kept in 50% ethanol. The plants were identified according to Gutermann (1976), while the nomenclature follows the Euro+Med database (2006-). Voucher specimens were deposited in the Herbarium of University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac" (accession number: BEOU 17458).

Anatomical analysis. Paraffin method (Ruzin 1999) was applied for preparing cross-sections (8–10 μm thick) of middle parts of mature roots, rhizomes, petioles, leaf lobes and stems. Sections were double stained in Safranin O (1%, w/v, 50% ethanol) and Alcian blue (1% w/v, aqueous) and mounted using Canada balsam. The fresh plant material was hand sectioned with a razor blade and stained with toluidine blue (0.05 %, w/v, aqueous) (O'Brien *et al.* 1964), phloroglucinol-HCl to detect lignified cell walls (Jensen 1962), and Lugol's solution for the visualisation of starch (Johansen 1940). Leaf epidermal prints were prepared following the procedure described by Wolf (1950). Two methods were combined: paraffin and hand sectioning in order to obtain better slides for precise observations and analysis. Afterwards, the best micrographs for the illustrations were chosen. The permanent slides are stored at the Department of Morphology and Systematics of Plants, University of Belgrade, Faculty of Biology. Observations of the obtained microslides were performed on a light Leica DM2000 microscope equipped with a Leica DFC320 digital camera and a computer with Leica IM 1000 imaging software. The classification of the root secretory canals follows Tetley (1925).

RESULTS

Root anatomy. *Artemisia umbelliformis* subsp. *eriantha* is a rhizomatous perennial plant which develops adventitious roots. The root cross section is round in outline (Fig. 1a). The younger root is characterized by the epidermis on the surface, the parenchyma cortex below the epidermis and parenchyma cells at the center, surrounded by xylem and phloem (Fig. 1a). A visible layer of the endodermis surrounds the central cylinder (Fig. 1a). The older root shows a secondary structure, with a well-developed periderm on its surface (the epidermis is in the process of shedding) (Fig. 1b). Below the periderm is the parenchyma cortex, while secondary xylem and small secondary phloem patches are located in the central cylinder (Fig. 1b). In the root cortex parenchyma secretory canals are present near the endodermis (endodermal secretory canals) (Fig. 1b).

Rhizome anatomy. On the cross-section, the rhizome is round (Fig. 1c). A primary structure is observed with elements of a secondary structure, a well-developed periderm on its surface (the epidermis is in the process of shedding) and the parenchyma cortex below (Fig. 1c). In the central cylinder collateral vascular bundles are observed, while the pith, composed of large parenchyma cells, is located in the central region (Fig. 1c). Adventitious root primordia pushing through the cortical tissue can be seen (Fig. 1c). Large secretory canals are present in the rhizome cortical parenchyma (Fig. 1c, d).

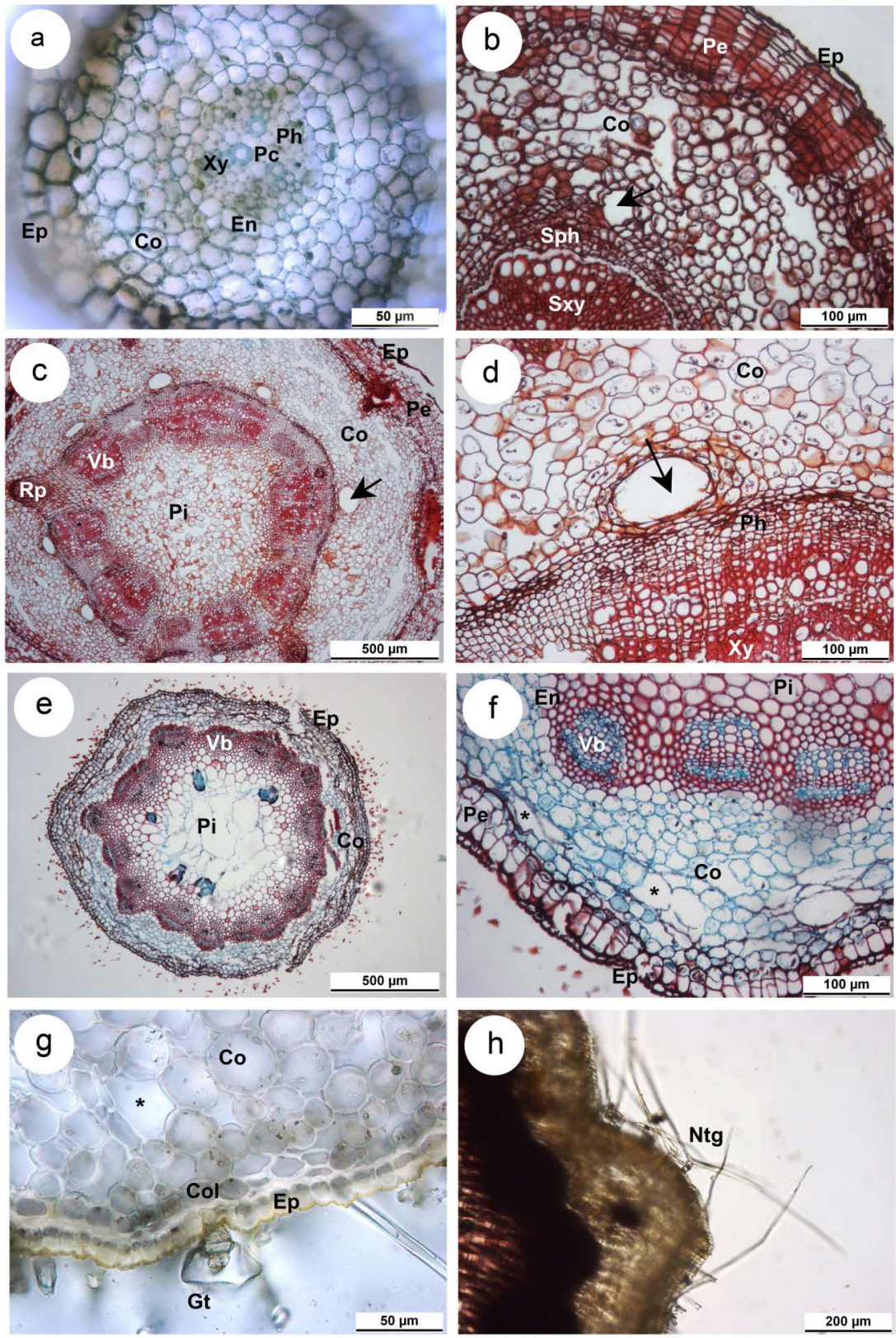

Fig. 1. Micrographs of the root (**a**-**b**), rhizome (**c**-**d**) and stem (**e**-**h**) anatomy of *Artemisia umbelliformis* subsp. *eriantha*. Note the secretory canals in the root and rhizome (*arrows*) and the intercellular canals in the stem (*asterisk*). Abbreviations: Co = cortex; Col = collenchyma; En = endodermis; Ep = epidermis; Gt = glandular trichome; Ngt = nonglandular trichome; Pc = parenchyma cells; Pe = periderm; Ph = phloem; Pi = pith; Rp = adventitious root primordia; Sph = secondary phloem; Sxy = secondary xylem; Xy = xylem; Vb = vascular bundle.

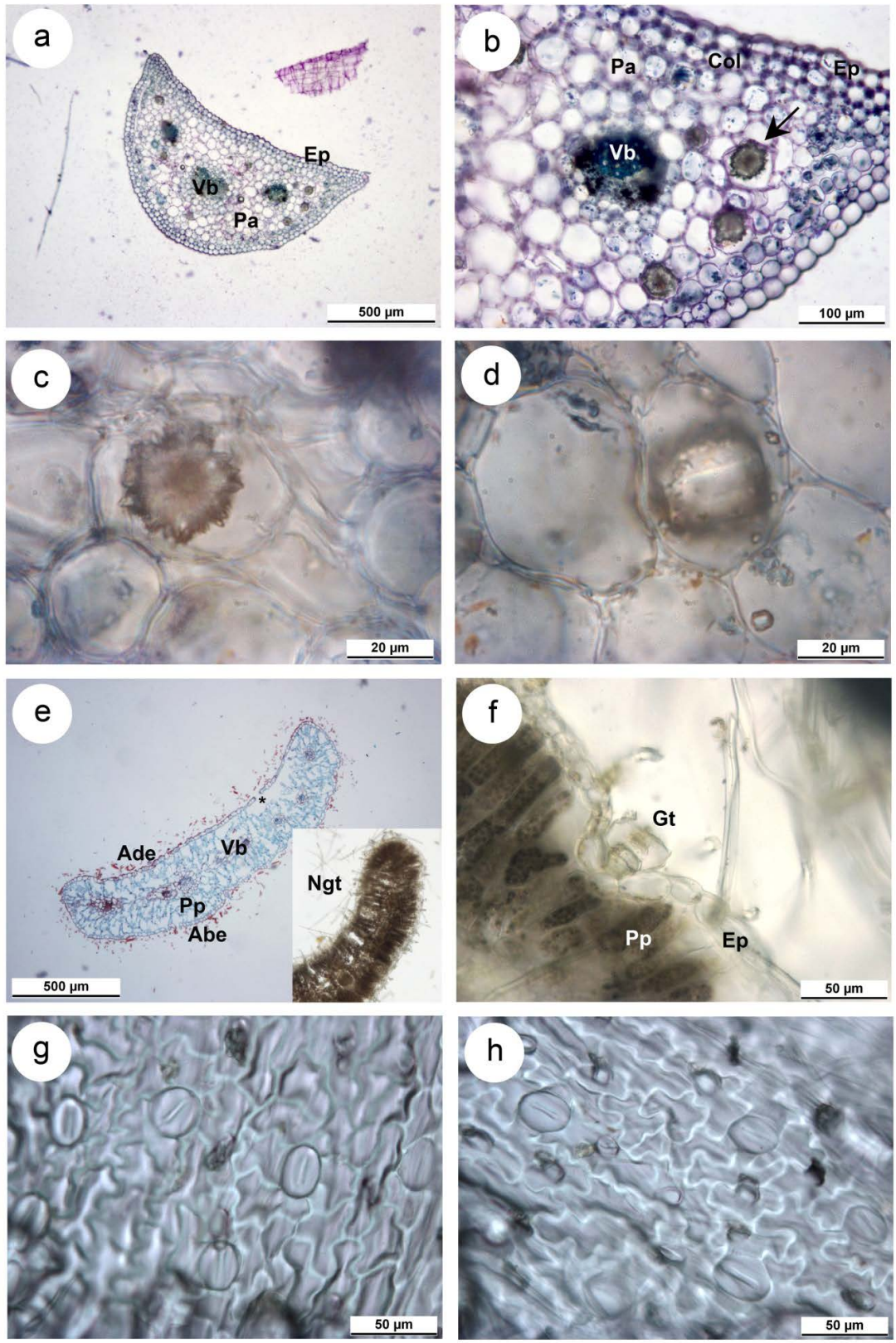

Fig. 2. Micrographs of the petiole (**a-d**) and leaf (**e-h**) anatomy of *Artemisia umbelliformis* subsp. *eriantha*. Note the druses in the petiole (*arrow*) and the large substomatal chamber in the leaf (*asterisk*). Abbreviations: Abe = abaxial epidermis; Ade = adaxial epidermis; Col = collenchyma; Ep = epidermis; Gt = glandular trichome; Ngt = nonglandular trichome; Pa = parenchyma; Pp = palisade parenchyma; Vb = vascular bundle.

Stem anatomy. On the cross-section, the stem is round (Fig. 1e). On the surface is one-layered epidermis and, below, well developed cortex with visible intercellular canals (Fig. 1e-g). In the cortex, collenchyma tissue is seen beneath the epidermis (Fig. 1g). On some parts of the stem, a well-developed periderm is observed below the epidermis (Fig. 1f). The periderm consists of two to three layers of cells arranged in radial rows (Fig. 1f). The vascular bundles are collateral and arranged in a circle (Fig. 1e, f). Well-lignified sclerenchyma tissue surrounds each vascular bundle (Fig. 1f). A clearly visible endodermis separates the cortex from the central cylinder (Fig. 1f). The pith, composed of large parenchyma cells, is located in the central region (Fig. 1e, f). The stem lacks secretory canals (Fig. 1e, f), while the surface is covered with glandular trichomes (Fig. 1g) and T-shaped nonglandular trichomes (Fig. 1h).

Petiole anatomy. The basal leaves of the studied taxon possess a petiole, while the upper leaves are more or less sessile. The petiole is concave (Fig. 2a). There is a single-layered epidermis on the surface (Fig. 2a, b), and a single-layered collenchyma below (Fig. 2b). In the parenchyma tissue one large vascular bundle is located at the centre with two small bundles on the sides (Fig. 2a). The petiole lacks trichomes and secretory canals (Fig. 2a, b). CaOx crystals, druses (Fig. 2b, c) and single block-like rhombohedral crystals (Fig. 2d) can be observed inside some of the parenchyma cells.

Leaf anatomy. The leaf lobe cross-section has an oblong-linear shape (Fig. 2e). On the leaf lobe surface a single-layered epidermis is observed on both leaf sides (Fig. 2e). On the frontal view of both leaf sides, the epidermal cells are irregularly polygonal in shape (Fig. 2g, h). The outer periclinal cell walls are convex, while the anticlinal walls are sinuate (Fig. 2g, h). The leaf blade is amphistomatous and a large substomatal chamber was observed (Fig. 2e). The stomatal type is anomocytic (Fig. 2g, h). The leaf is of an isolateral structure (Fig. 2e, bottom right). Vascular bundles are arranged in a row in the leaf blade plane (Fig. 2e). The leaf lacks secretory canals (Fig. 2e), while the surface is covered with T-shaped nonglandular trichomes (Fig. 2e, bottom right) and glandular trichomes (Fig. 2f).

DISCUSSION

There has been little research or anatomical analyses of the roots of *Artemisia* taxa. Evans & Graney (2018) showed that eccentric growth in the roots of some *Artemisia* species from the western United States was manifested as concavities. However, the cause of concavities in the roots is unknown. Internal secretory structures containing lipid substances in aerial and underground organs are widely distributed among the Asteraceae species (Fahn 1979). Secretory canals found in the roots of Asteraceae members may be both endodermal, with an extra-endodermal position, and non-endodermal, which differ in size and in the presence of surrounding epithelial-like cells (Tetley 1925). In the root of *A. umbelliformis* subsp. *eriantha* endodermal secretory canals are present, which were also documented for *A. campestris* L., *A. absinthium* L., *A. arborescens* L. and related *A. judaica* L. (Janaćković *et al.* 2019). Large secretory canals are also present in the rhizome cortical parenchyma of the studied taxon. These findings are important given that the morphology and localization of secretory structures have been used as diagnostic characters for recognition of the Asteraceae species (da Silva *et al.* 2014; Ivănescu *et al.* 2015; Janaćković *et al.* 2019).

The stem anatomy of the examined taxon is in accordance with the previously described anatomy for other *Artemisia* species (Konowalik & Kreitschitz 2012; Ivănescu *et al.* 2015; Janaćković *et al.* 2019). The presence of a well-developed periderm, also found in *A. campestris* and *A. arborescens* (Janaćković *et al.* 2019), could be considered as an important trait. Konowalik & Kreitschitz (2012) found that *A. absinthium* var. *calcigena* Rehm. formed a continuous periderm layer which might be linked to adaptation to the dry habitat. Furthermore, Zhang *et al.* (2018) showed that in addition to other tissues, the hypodermal periderm of *A. lavandulaefolia* DC. and *A. selengensis* Besser enable the plants to withstand flooding. Secretory canals were not observed in the stem cortex of the investigated taxon, although they were found in the stem cortex of *A. campestris*, *A. absinthium*, *A. arborescens*, *A. herba-alba* Asso and *A. judaica* (Konowalik & Kreitschitz 2012; Janaćković *et al.* 2019).

Artemisia umbelliformis subsp. *eriantha* has a concave petiole on the cross-section while *A. absinthium* is ellipsoidal, *A. arborescens* and *A. santonica* L. trapezoidal and *A. scoparia* Waldst. & Kitam. circular-irregular in shape (Badea & Zamfirache 2011; Janaćković *et al.* 2019). The calcium oxalate druses and rhombohedral crystals found inside the petiole parenchyma cells have not been documented for petioles of other *Artemisia* taxa to date. Rhomboidal crystals were found inside the stem pith parenchyma cells of related *A. arborescens* (Janaćković *et al.* 2019). This feature could be of taxonomic importance as crystals have previously been considered to be genetically controlled by the cell (Meric 2009). Calcium oxalate crystals, in diverse forms, are common in plant structures and appear to play a significance role in the cell physiology, as well as in protection against herbivores

(Franceschi & Nakata 2005). Druses found in species of the tribe Inuleae Cass. (Meric 2009), Vernonieae (Sosa *et al.* 2014) and Cardueae (Gavrilović *et al.* 2020) could be significant for Asteraceae taxonomy.

The leaf lobe on the cross-section of the examined taxon is oblong-linear similar to *A. absinthium*, *A. arborescens*, *A. judaica* and *A. herba-alba* (Janaćković *et al.* 2019). Anatomically, the leaf blade is in accordance with the previously described leaf blade of other *Artemisia* species (Fahmy 1997; Noorbakhsh *et al.* 2008; Bercu & Broască 2012; Bakr 2014; Janaćković *et al.* 2019) in terms of the isolateral and amphistomatous leaf structure with a large substomatal chamber. It has been shown that plants with isolateral and amphistomatic leaves grow in dry, highly lighted habitats. We found the anomocytic stomatal type in the examined taxon, which was also documented for numerous *Artemisia* taxa (Hayat *et al.* 2010). Secretory canals were not observed in the leaf tissue of the investigated taxon, although they were found in the leaves of *A. campestris*, *A. judaica*, *A. herba-alba* (Janaćković *et al.* 2019) and *A. campestris* subsp. *maritima* (DC.) Arcang. (Ascensão & Pais 1988).

We found both T-shaped nonglandular and glandular trichomes on the stem and leaves of the studied taxon, which has been documented and described for numerous *Artemisia* taxa (Ascensão & Pais 1987; Bercu & Broască 2012; Konowalik & Kreitschitz 2012; Bakr 2014; Ivănescu *et al.* 2015; Janaćković *et al.* 2019).

CONCLUSION

The presence and shape of CaOx crystals (druses and rhombohedral crystals), as well as the secretory canals found in the root and rhizome might represent valuable taxonomic characters for this taxon.

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REZIME

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Anatomske osobine vrste *Artemisia umbelliformis* subsp. *eriantha* (Asteraceae) alpskog glacijalnog relikta sa Durmitora (Crna Gora)

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U ovom radu je po prvi put urađena anatomska analiza vegetativnih organa centralno evropske glacijalne reliktne vrste *Artemisia umbelliformis* subsp. *eriantha*, sa planine Durmitor u Crnoj Gori. Cilj je bio da se istraži anatomija i karakteristične anatomske osobine koje mogu imati potencijalni taksonomski značaj. Mikroskopski preparati su napravljeni prema standardnim histološkim procedurama. Mladi adventivni koren ima primarnu građu, dok se stariji koren odlikuje sekundarnom građom sa peridermom na površini. Rizom ima primarnu građu sa elementima sekundarne građe (periderm). Stablo na poprečnom preseku ima okrugli oblik sa dobro razvijenim peridermom, na nekim delovima, i kružno raspoređene kolateralne provodne snopiće. Lisna drška je, na poprečnom preseku, konkavna sa jednoslojnim epidermisom i parenhimom u kome se uočavaju jedan veliki i dva mala provodna snopića. Druze i romboedrični kristali su uočeni unutar pojedinih ćelija parenhima lisne drške. Režanj lista na poprečnom preseku ima duguljasto-linearni oblik, izolateralan je i amfistomatičan. Ćelije epidermisa lista imaju izvijugane antiklinalne zidove. Sekretorni kanali su prisutni u parenhimu kore korena (endodermalni) i parenhimu kore rizoma. Stablo, lisna drška i list ne sadrže sekretorne kanale. Stablo i list su prekriveni nežlezdanim trihomama u obliku slova T, kao i žlezdanim trihomima. Taksonomski značaj analiziranih karaktera je diskutovan u kratkim crtama.

Ključne reči: anatomija, kristali, sekretorni kanali