



Comparative anatomical investigation of five *Artemisia* L. (Anthemideae, Asteraceae) species in view of taxonomy

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Abstract

Comparative anatomical analysis of vegetative organs has been conducted on *Artemisia campestris* L., *A. absinthium* L., *A. arborescens* L., *A. judaica* L. and *A. herba-alba* Asso, using light microscopy, in order to examine the most important anatomical features and to find new valid taxonomic characters. Results have shown that general root, stem and leaf anatomical features and nonglandular and glandular trichomes are shared by all species. However, some characters (parenchyma sheath, which surrounded vascular bundle and extended to both epidermises, subepidermal collenchyma and the absence of secretory canals in the leaves) link together *A. absinthium* and *A. arborescens* from the same section. Some characters, as periderm and lignified pith parenchyma cells (*A. campestris* and *A. arborescens*), nonendodermal secretory canals in root cortex (*A. absinthium* and *A. judaica*) and secretory canals in the leaf phloem (*A. judaica* and *A. herba-alba*), connect species belonging to different sections. Moreover, some characters could be considered as species-specific, nonendodermal secretory canals in the root secondary phloem, triangular leaf shape on the cross section and secretory canals in the leaf parenchyma for *A. campestris*, secretory canals in the stem pith for *A. absinthium*, crystals in the pith parenchyma cells for *A. arborescens* and the absence of root secretory canals for *A. herba-alba*. Given results revealed qualitative characters, on the basis of which the studied species are anatomically distinguishable between each other, provide valuable features for better species identification and contribute to the anatomy of the genus *Artemisia*.

Keywords Leaf · Root · Secretory canals · Stem · Trichomes

1 Introduction

One of the largest angiosperm family, Asteraceae, includes hundreds of species of economic interest, e.g., medicinal plants which are used therapeutically, and consequently, their anatomy and morphology have been studied, including the structures which produce and storage specialized metabolites (Werker and Fahn 1981; Ascensão and Pais 1987;

Figueiredo and Pais 1994; Ascensão et al. 2001; Andreucci et al. 2008; Simpson 2009; Camilotti et al. 2014; Gavrilović et al. 2016). These secretory structures in Asteraceae could be found in the form of glandular trichomes, cavities, canals, laticifers, hydathodes, idioblasts and glandular appendices (Metcalf and Chalk 1950; Carlquist 1958; Lestern and Curtis 1985; Castro et al. 1997; Gavrilović et al. 2018a). In addition, an importance of these structures in taxonomic studies was emphasized by some authors (Solereider 1908; Metcalfe and Chalk 1950, 1979; Metcalfe 1983), with the main purpose of distinguishing orders, tribes, genera and even certain species, because various secretory structures may be found in a different parts of the same plant or be only located in certain plant organs.

Likewise, all other vegetative anatomical characters are also useful in taxonomic studies and are important in perceiving phylogenetic relationships of particular plant groups (Lersten and Curtis 2001; Dengler 2002). Anatomical data also may provide useful and valuable characters which may

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help in identification of ambiguous plant taxa, as well as establishing their taxonomic relationships (Metcalf and Chalk 1957; Scatena et al. 2005; Sosa et al. 2014; Karanović et al. 2015; Gavrilović et al. 2018b). Also, micromorphological characteristics, e.g., trichomes, have played an important role in plant taxonomy at generic and species levels (Hayat et al. 2009; Gavrilović et al. 2018b).

Genus *Artemisia* L. (Artemisiinae, Anthemideae, Asteraceae) contains more than 500 taxa (Vallès and Garnatje 2005) mainly distributed in temperate areas of the northern hemisphere, colonizing mainly arid and semiarid habitats, with a few representatives in the southern hemisphere (McArthur and Plummer 1978; Pellicer et al. 2010). Apart from few annuals or biennials, most of the *Artemisia* species are perennial (Vallès et al. 2003). As *Artemisia* species have wide range of geographical distribution and also a variety of habitats on which they occur, it may result in different adaptations to diverse conditions (Kreitschitz and Vallès 2007), e.g., changes in morphology and anatomy, as well in the cell structure and function (increase in ploidy level and nuclear DNA content) (Garcia et al. 2004). Notably, many *Artemisia* species are used as food, medicinal and ornamental plants (Pareto 1985), whereas others are toxic or allergenic and invasive plants (Tan et al. 1998).

Although worldwide research on *Artemisia* taxa is ongoing, it is mainly focused on phytochemistry, as well as investigations of biological activities of different extracts or essential oils, or pure compounds. Still, little is known about anatomical aspects and its value in taxonomy of the genus *Artemisia*. Metcalfe and Chalk (1957) mentioned some anatomical features of *Artemisia*: nonglandular trichomes, medullary canals, secretory cavities and clustered crystals. However, there are several studies on anatomy (Carlquist 1966; Botha and Evert 1981; Fahmy 1997; Marchese et al. 2005; Rabie et al. 2006; Noorbakhsh et al. 2008; Bercu and Broască 2012; Konowalik and Kreitschitz 2012; Bakr 2014; Maghni et al. 2018) and trichome morphology (Kelsey 1984; Ascensão and Pais 1987; Lodari et al. 1989; Ferreira and Janick 1995; Hayat et al. 2009, 2010) of the genus *Artemisia*.

Infrageneric taxonomy of the genus *Artemisia* is ambiguous and still represents a challenging task for taxonomists. Based mainly on the capitula type and florets fertility, five major groups are recognized in the traditional studies: *Absinthium* DC., *Artemisia*, *Dracunculus* Besser, *Seriphidium* Besser and *Tridentate* (Rydb.) McArthur (Torrell et al. 1999). However, latest phylogenetic studies on *Artemisia* s.str., using nrDNA ITS sequences, support the monophyly of the genus and the monophyly of the three main infrageneric groups (*Dracunculus*, *Seriphidium*, *Tridentate*), whereas subgenera *Absinthium* and *Artemisia* are polyphyletic (Watson et al. 2002; Vallès et al. 2003; Sanz et al. 2008).

The objectives of the present study were: (1) to conduct comparative anatomical analysis of root, stem and leaves of *A. campestris* L., *A. absinthium* L., *A. arborescens* L., *A. judaica* L. and *A. herba-alba* Asso in order to evaluate their taxonomic value; (2) to examine the important anatomical features and try to find new valid taxonomic characters, which will contribute to anatomy of the genus and provide characters for better identification of the species.

2 Materials and methods

Plant material – We have chosen five species belonging to different sections, as well as from different origins: *A. campestris*, *A. absinthium*, *A. arborescens*, *A. judaica* and *A. herba-alba* (Table 1). Plant material (parts of root, stem and middle leaves on the stem) (five individuals per species, except for *A. arborescens*, which was only one-old individual) was collected in Serbia, Montenegro and Libya during the flowering period (2012 and 2016) and kept in 50% ethanol. Voucher specimens were deposited in the Herbarium (BEOU) of University of Belgrade—Faculty of Biology, Institute of Botany and Botanical Garden “Jevremovac” (Table 1).

Anatomical analysis – Temporary and permanent slides of mature roots, stems and leaves of adult plants were

Table 1 List of the examined *Artemisia* species

Species	Section	Locality	Coordinates	Date	Voucher numbers (BEOU)
<i>A. campestris</i> L.	<i>Dracunculus</i>	Podgorica (Montenegro)	N 42°24'43" E 19°15'1"	2016	17434
<i>A. absinthium</i> L.	<i>Absinthium</i>	Đerdap (Serbia)	N 44°64'996" E 22°54'674"	2016	17438
<i>A. arborescens</i> L.	<i>Absinthium</i>	Budva (Montenegro)	N 42°16'37.49" E 18°50'19.66"	2016	17432
<i>A. judaica</i> L.	<i>Artemisia</i>	Zintan (Libya)	N29°33'44.97" E10°14'15.13"	2012	AJU04022012
<i>A. herba-alba</i> Asso	<i>Seriphidium</i>	Zintan (Libya)	N31°57'21.19" E12°13'6.35"	2012	AHA28022012

prepared. Plant parts were sectioned fresh or fixed (50% ethanol) before preparation for a standard paraffin method (Ruzin 1999). Handmade cross sections of fresh plant material were performed using sharp razor blades. Some temporary slides (stem and leaf) were stained with toluidine blue (0.05% w/v, aqueous) (O'Brien et al. 1964) or with Lugol solution (Johansen 1940). Paraffin method was applied for preparing cross sections (8–10 µm thick) of middle parts of mature roots, stems and leaves. Sections were double stained in Safranin O (1%, w/v, 50% ethanol) and alcian blue (1% w/v, aqueous) and then were mounted on slides using Canada balsam. The permanent slides are preserved in the Chair of Morphology and Systematics of Plants, University of Belgrade - Faculty of Biology. Observations of the obtained microslides were performed on a light microscope Leica DM2000 with a digital camera Leica DFC320 and a computer with the imaging software Leica IM 1000. Classification of root secretory canals follows Tetley (1925). Investigated species are arranged phylogenetically in all tables and figures.

3 Results

Root – All investigated species are perennial; thus, secondary tissues are noticed in the root cross sections [especially a well-developed xylem (Figs. 1, 3, 5, 7, 9)]. A multilayered exodermis is on the surface of the root of all species (Figs. 2, 4, 6, 8, 10). Below the exodermis, a cortex is present (Figs. 2, 4, 6, 8, 10). Below cortex, secondary phloem and some groups of sclerenchyma fibers could be noticed, especially well developed in *A. campestris*, *A. judaica* and *A. herba-alba* (Figs. 1, 7, 10, respectively). The secondary xylem is the dominant part of the root cross sections, composed of vessels and tracheids (Figs. 3, 5, 7, 9). Endodermal secretory canals are present in the roots of *A. campestris*, *A. absinthium*, *A. arborescens* and *A. judaica* (Figs. 1, 3, 6, 8, respectively). Nonendodermal secretory canals are present in the secondary phloem of the root of *A. campestris* (Fig. 1) and in the cortex of *A. absinthium* (Fig. 4) and *A. judaica* (Fig. 8). Secretory canals in the cortex of *A. absinthium* are with some brownish content (Figs. 3, 4). Roots of *A. herba-alba* lack of secretory canals (Figs. 9, 10).

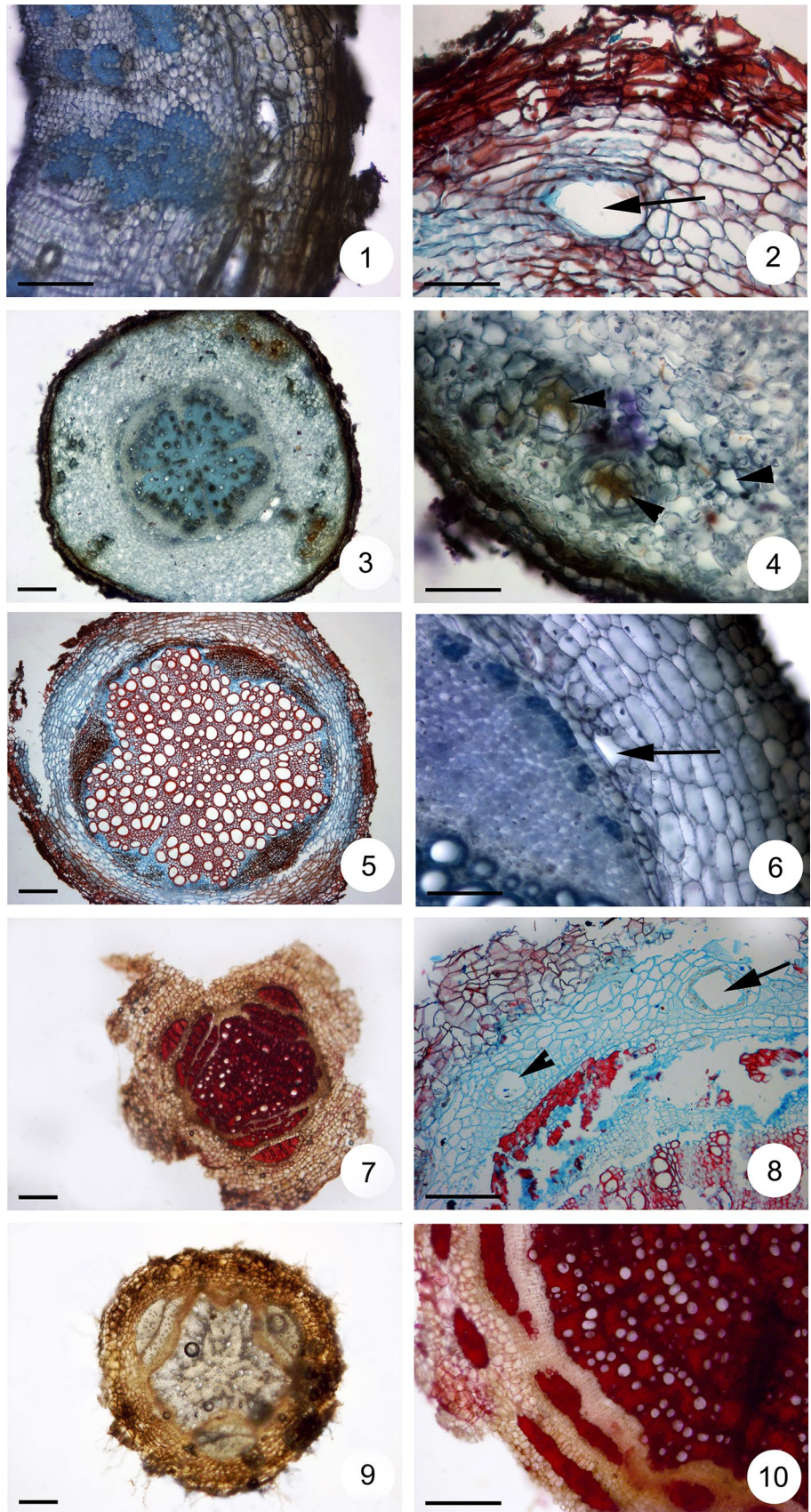
Stem – Young stem cross sections of all studied species have irregular pentagonal shape (Figs. 11, 13, 15, 17, 19), while later become more or less round or polygonal (Figs. 12, 14, 16, 18, 20). One-layered epidermis, composed of oval to isodiametric cells, is present on the surface of all investigated species (Figs. 12, 14, 16, 18, 20). The cortex is composed of collenchyma and chlorenchyma, which are arranged alternately. Prominent ribs in young stems contained collenchyma tissue, whereas chlorenchyma is present

between the ribs (Figs. 11, 13, 15, 17, 19). Below the epidermis, well-developed periderm could be noticed only in the stem of *A. campestris* and *A. arborescens* (Figs. 12, 16). The periderm is continuous in older stems and consisted of a several layers of enlarged cells arranged in radial rows (Figs. 12, 16). The vascular bundles are collateral and arranged in a circle (Figs. 11, 13, 15, 18, 20) and separated from one another by a parenchyma tissue (Figs. 11, 13, 15, 17, 19). The primary xylem consists of four to eight parallel rows of xylem elements; each row comprised 2–5 vessels. The vascular cylinder in a secondary state of growth produces secondary xylem inside and secondary phloem outside, giving to more basal parts of the stem almost cylindrical outline, while medullary rays on xylem side form connective tissue of lignified cells (Figs. 12, 14, 16, 18). Well-lignified sclerenchyma is above the phloem. Sclerification of the central cylinder could be noticed, because well-developed xylem and sclerenchyma tissue form a thick ring (Figs. 11, 13, 15, 18, 19). A large parenchyma cells are in the central region of the stem of all investigated species (Figs. 11, 13, 15, 17, 19) and with lignified cell walls in *A. campestris* and *A. arborescens* (Figs. 11, 15, respectively). Crystals, in the rhomboidal form, could be noticed only inside the pith parenchyma cells of *A. arborescens* (Fig. 16, upper left). Very large secretory canals are present in the cortex of *A. arborescens* (Fig. 16), while small secretory canals could be seen in the cortex of *A. campestris*, *A. absinthium*, *A. judaica* and *A. herba-alba* (Figs. 12, 14, 18, 20, respectively). Small secretory canals in the pith are found only in *A. absinthium* stem (Fig. 14).

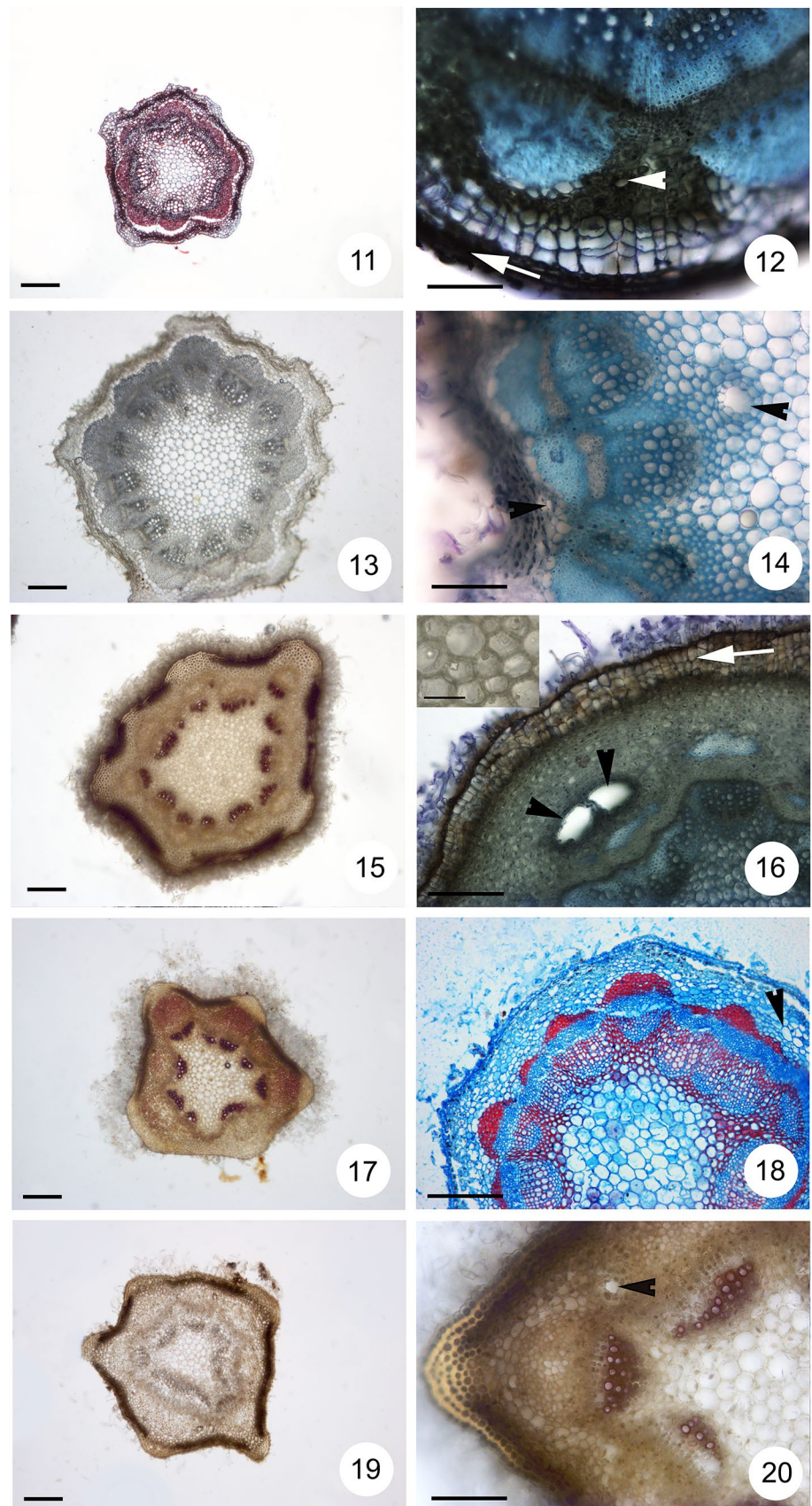
Leaf – Middle leaves of *A. absinthium* and *A. arborescens* possess petioles, while leaves of other investigated species are sessile. Petiole of *A. absinthium* has ellipsoidal shape, with similar anatomy as the stem (Fig. 24). On the surface is one-layered epidermis, below alternately arranged collenchyma and chlorenchyma and vascular bundles, in the center, in a circle (Fig. 24). Petiole of *A. arborescens* has trapezoidal shape, unstratified epidermis, alternately arranged by collenchyma and chlorenchyma, one central and two lateral vascular bundles (Fig. 27).

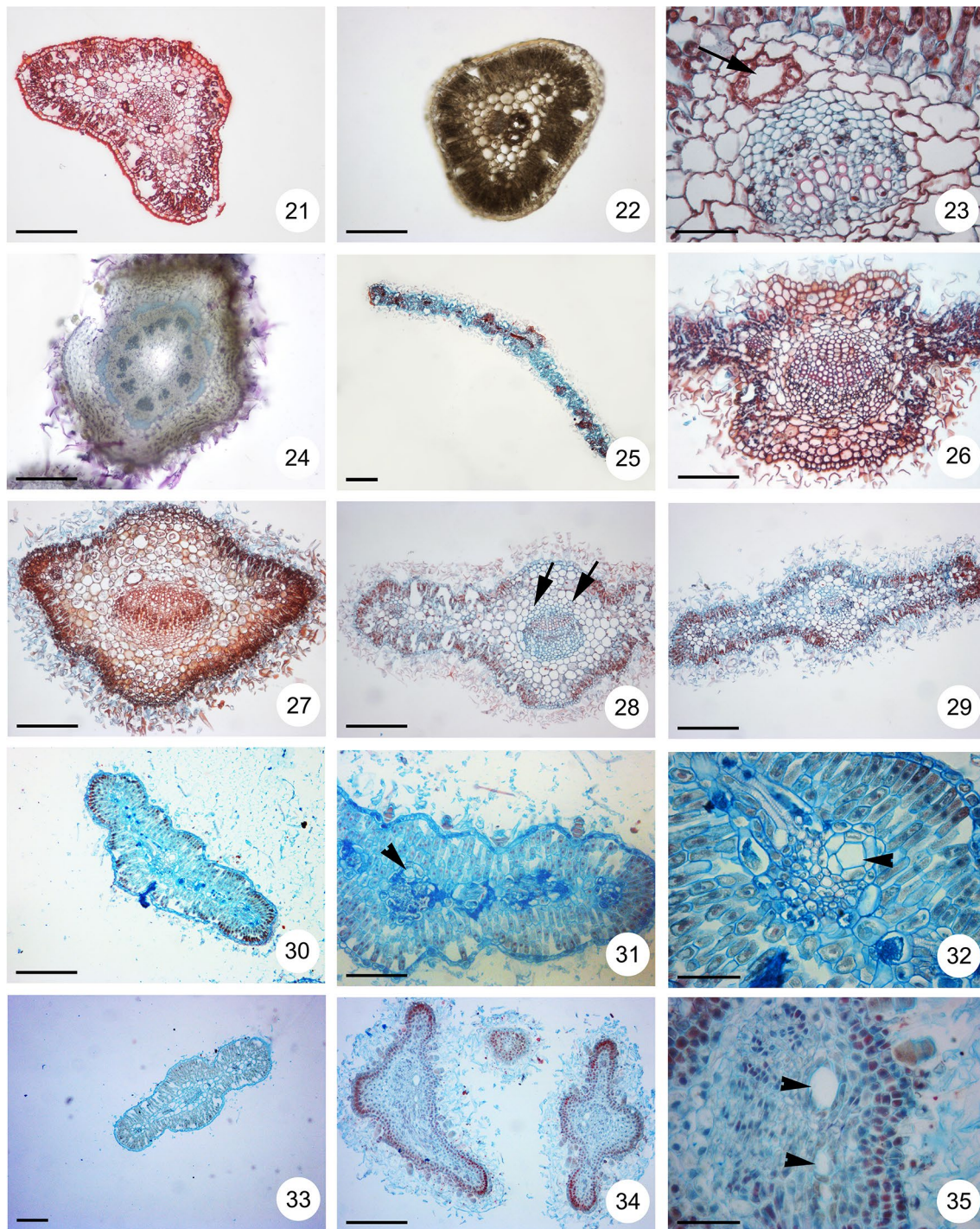
Leaf on the cross section is triangular in *A. campestris* (Figs. 21, 22), while in other studied species, *A. absinthium* (Fig. 25), *A. arborescens* (Figs. 28, 29), *A. judaica* (Fig. 30) and *A. herba-alba* (Fig. 33), is oblong-linear. On the leaf surface a cuticle could be noticed, which is very well developed in *A. campestris* (Figs. 21, 22). On both leaf side, one-layered epidermis could be noticed (Figs. 21, 22, 26, 28, 31, 34). The leaf blade of all investigated species is amphistomatous, and large stomatal chamber could be noticed (Figs. 21, 25, 28, 31, 33). The palisade tissue, on both leaf side, is consisted of large rich in chloroplasts cells, arranged in one or two layers.

Figs. 1–10 Cross sections of the roots of *A. campestris* (1, 2), *A. absinthium* (3, 4), *A. arborescens* (5, 6), *A. judaica* (7, 8) and *A. herba-alba* (9, 10). 1, 3, 5, 7, 9 General root anatomy showing secondary structures and secretory canals. 2, 4, 6, 8, 10 Detail of the root anatomy showing endodermal (2, 6, 8) (arrows) and nonendodermal secretory canals (4, 8) (arrowheads). Bar = 100 μm in 2, 4 and 6; 200 μm in 1, 3, 5, 7, 8, 9 and 10



Figs. 11–20 Cross sections of the stems of *A. campestris* (11, 12), *A. absinthium* (13, 14), *A. arborescens* (15, 16), *A. judaica* (17, 18) and *A. herba-alba* (19, 20). 11, 13, 15, 17, 19 General stem anatomy showing secondary structures. 12, 14, 16, 18, 20 Detail of the stem anatomy showing periderm (12, 16) (arrows) and secretory canals in the cortex (12, 14, 16, 18, 20) (arrowheads) and in the pith (14) (arrowhead). Bar = 50 μ m in 16a and 20; 100 μ m in 12 and 14; 200 μ m in 11, 13, 15, 16, 17, 18 and 19





Figs. 21–35 Cross sections of the leaves of *A. campestris* (21, 22, 23), *A. absinthium* (24, 25, 26), *A. arborescens* (27, 28, 29), *A. judaica* (30, 31, 32) and *A. herba-alba* (33, 34, 35). 21, 22, 25, 28, 29, 30, 33 General leaf anatomy showing amphistomatous leaves with isolateral structure and large stomatal chamber. 24, 27 Petiole anatomy. 23, 28, 31, 32, 35 Detail of the leaf anatomy showing secretory canals in the leaf parenchyma (23) (arrow), leaf base parenchyma (28) (arrows) and beyond the phloem of the vascular bundle (31, 32, 35) (arrowheads). Bar = 50 μ m in 23, 32 and 35; 100 μ m in 31; 200 μ m in 21, 22, 24, 25, 26, 27, 28, 29, 30, 33, 34

In the central leaf blade plane one large collateral closed vascular bundle could be noticed (Figs. 21, 26, 29, 30, 33). On the leaf cross sections of *A. absinthium* (Figs. 25,

26), *A. arborescens* (Figs. 28, 29), *A. judaica* (Figs. 30, 31) and *A. herba-alba* (Figs. 33, 34), the main vein is prominent with two lateral ribs, which are with many

valleculae. Vascular bundle in the main vein of *A. campestris* (Figs. 21, 22), *A. absinthium* (Figs. 25, 26) and *A. arborescens* (Figs. 28, 29) is surrounded with parenchyma sheath, which in *A. absinthium* (Fig. 26) and *A. arborescens* (Figs. 28, 29) extend to both epidermises. In the main vein of *A. absinthium* (Fig. 26) and *A. arborescens* (Fig. 28) a subepidermal collenchyma could be seen on both leaf sides. Secretory canals are noticed in the leaf parenchyma of *A. campestris* (Figs. 21, 22, 23) and beyond the phloem of the vascular bundle of *A. judaica* (Figs. 30, 31, 32) and *A. herba-alba* (Figs. 33, 34, 35). In *A. arborescens* secretory canals are present in the leaf petiole parenchyma (Fig. 27) and in the leaf base parenchyma (Fig. 28).

Trichomes – On the aerial parts of all investigated species numerous nonglandular (Figs. 36, 37) as well as glandular (Figs. 38, 39) trichomes could be seen. Only exception could be noticed for *A. campestris* which possesses very few trichomes (Figs. 11, 21). Stems and leaves of all other species are densely covered with trichomes (Figs. 13, 15, 17, 19, 26, 29, 31, 34). The nonglandular trichomes are T-shaped, with various variable number of cells which form a neck of the trichome, and with long curly or straight arms (Figs. 36, 37). Glandular trichomes are of biseriata type covered with cuticle sheath (Figs. 38, 39).

Major distinguishing qualitative anatomical characters and their states of all studied species are shown in Table 2.

4 Discussion

In the roots of all investigated species secretory canals were found, except of roots of *A. herba-alba*. Endodermal secretory canals were noticed in the roots of *A. campestris*, *A. absinthium*, *A. arborescens* and *A. judaica*, while nonendodermal secretory canals were found only in the secondary phloem of *A. campestris* and in the cortex of *A. absinthium* and *A. judaica*. Morphology and distribution of secretory structures have been used as diagnostic characters in recognition of the species within Asteraceae (Solleder 1908; Metcalfe and Chalk 1979; Castro et al. 1997; da Silva et al. 2014). Ivănescu et al. (2015) also found secretory canals in the cortex and phloem of the roots of *A. absinthium*, but we did not found canals in the phloem of this species. General root anatomy of *A. absinthium* was in accordance with those described by Ivănescu et al. (2015).

Internal secretory spaces of lipid substances occurring both in aerial and in underground organs are widely distributed among the species of the Asteraceae family (Fahn 1979). The various types of secretory canals found in the Asteraceae could be classified into two groups: (1) endodermal canals with an extra-endodermal position, but their formation is closely related to the endodermis and (2) nonendodermal canals, which are not connected with the endodermis and have schizo-lysigenous formation in the older stages of the root (Tetley 1925). Fatty substances are released during the phloem differentiation, and from phloem, it passes outward and deposited in the endodermal canals (Tetley 1925). Nonendodermal canals differed from the endodermal canals

Figs. 36–39 Nonglandular T-shaped (36, 37) and biseriata glandular trichomes, lateral view (38) and frontal view (39) of *A. judaica* (36), *A. herba-alba* (37, 38) and *A. absinthium* (39). Bar = 50 µm

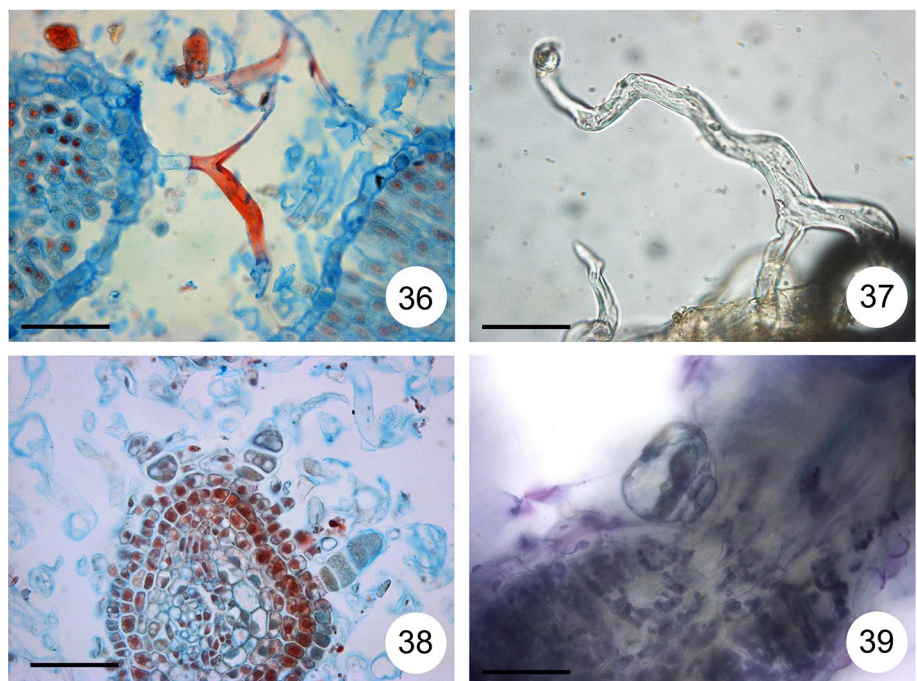


Table 2 Qualitative anatomical characters distinguishing examined *Artemisia* species

Organ	Character		<i>A. campestris</i>	<i>A. absinthium</i>	<i>A. arbore-scens</i>	<i>A. judaica</i>	<i>A. herba-alba</i>	
Root	Secretory canals	Endodermal	+	+	+	+	–	
		Nonendodermal	Cortex	–	+	–	+	–
			Secondary phloem	+	–	–	–	–
Stem	Periderm		+	–	+	–	–	
	Lignified pith parenchyma cells		+	–	+	–	–	
	Crystals in pith parenchyma cells		–	–	+	–	–	
	Secretory canals	Cortex	+	+	+	+	+	
Pith		–	+	–	–	–		
Leaf	Shape on cross section	Triangular	+	–	–	–	–	
		Oblong-linear	–	+	+	+	+	
	Secretory canals	Parenchyma	+	–	–	–	–	
		Phloem	–	–	–	+	+	
	Parenchyma sheath, surround vascular bundle, extend to both epidermises		–	+	+	–	–	
		Subepidermal collenchyma		–	+	+	–	–

–=represents the absence of character

in size, and they are shorter and possess epithelial-like cells which surround these canals (Tetley 1925). Cury and Appezato-da-Glória (2009) have been shown that secretory structures have a significant role in plant defense against herbivores and pathogens. Also, they found cortical canals and cavities in roots, cavities in the roots secondary phloem, cortical cavities in the xylopodium and in the underground stem and canals in the secondary xylem in the xylopodium while investigating the secretory structures of six Asteraceae species belonging to the different tribes: Eupatorieae, Mutisiae and Vernoniaeae. In addition, it was shown that these structures possess lipid substances.

In the stem cortex of all investigated species secretory canals were also recorded, while only in the pith of *A. absinthium* small secretory canals were found additionally. Konowalik and Kreitschitz (2012) also found secretory canals in the stem cortex and pith of two varieties of *A. absinthium*. This character could be considered as specific for *A. absinthium*. Accumulations of resin-like compounds are remarkably abundant in Anthemideae and Ambrosieae (Carlquist 1966). Massive accumulations in at least a few vessels and massive accumulations or droplets in vessels and in other cell types in wood were recorded in *A. absinthium* and *A. arbore-scens* (Carlquist 1966). In addition, secretory canals are noticed in the leaf parenchyma of *A. campestris* and beyond the phloem of the vascular bundle of *A. judaica* and *A. herba-alba*. Secretory canals are found in the stem cortex and leaf mesophyll (Hoffmann et al. 1984;

Ponce 1986), often as a part of the bundle sheath. Most often one canal exists for a bundle, rarely two. Ascensão and Pais (1988) conducted investigation on ultrastructure and histochemistry of secretory canals in *A. campestris* subsp. *maritima* (DC.) Arcang. and found that the resin canals are distributed in the cortical parenchyma, in the proximity of the vascular bundles, and run longitudinally through the petiole and stem. In addition, the canal lumen, which is surrounded by two concentric layers of flattened cells, the epithelial and subepithelial, contains oleoresin. Moreover, plastids inside of canal epithelial cells may be involved in the terpenoids and polyacetylenes synthesis, while smooth endoplasmic reticulum and mitochondria in the steroids synthesis (Ascensão and Pais 1988). Also, the ultrastructure investigations of the secretory canals of *Grindelia pulchella* Dunal were also shown that plastids, mitochondria and endoplasmic reticulum are involved in the secretion (Bartoli et al. 2011). According to Bartoli et al. (2011), special tissue with “transfer cells” secretes the products and transferred to the canal epithelial cells where it accumulates in the vacuoles.

Besides secretory canals inside the organs, glandular trichomes at the organ surfaces are another secreting system present in Asteraceae plants (Bartoli et al. 2011). Indumentum features (shape, occurrence, distribution and density of glandular and nonglandular trichomes and stomata characteristics) represent valuable characters in taxonomy (Hayat et al. 2009). We documented numerous nonglandular T-shaped, as well as biseriate glandular trichomes on the

aerial parts of all investigated species, except for *A. campestris* which was almost glabrous. Such a dense cover of trichomes is a known characteristic in xeromorphic plants, which help them to adapt to dry conditions by limiting transpiration, reflecting radiation or even might helping to absorb water (Ehleringer et al. 1976; Lyshede 1979). Pubescence may also be included in the control of water loss and temperature regulation (Johnson 1975; Ehleringer et al. 1976). Various types of glandular and nonglandular trichomes occur in *Artemisia* species and include multiseriate glandular trichomes, as well as nonglandular filamentous T-trichomes (Ferreira and Janick 1995). These types of trichomes were also found in *A. absinthium* from Romania (Ivănescu et al. 2015) and from Poland (Konowalik and Kreitschitz 2012), in *A. campestris* subsp. *maritima* (Ascensão and Pais 1987), in *A. alba* subsp. *saxatilis* (Willd.) P. Fourn. (Bercu and Broască 2012), in *A. judaica* var. *sinaïtica* Tackholm (Bakr 2014), in *A. judaica* (Fahmy 1997) and in several *Artemisia* species (including *A. absinthium*) examined by Lodari et al. (1989). Hayat et al. (2010) stated that foliar epidermal anatomical characteristics are valuable taxonomic traits, which could help in resolving complex taxonomy within the genus *Artemisia*. Also, glandular trichomes were found as helpful taxonomic character for *A. nova* A. Nelson (Kelsey 1984). However, both nonglandular and glandular trichomes of the examined species were uniform. Glandular trichomes are usually consisted of a biseriate peduncle and a head which is formed of one to many cells. The peduncle is usually formed by five pairs of cells (Ciccarelli et al. 2007). Duke and Paul (1993) and Duke et al. (1994) described in detailed development of glandular trichomes in *A. annua*, the same as Ascensão and Pais (1987) for *A. campestris* subsp. *maritima*. Regarding glandular trichomes of *G. pulchella*, Bartoli et al. (2011) showed that the secretion is accumulated between the cell wall and the cuticle, which released the secretion when it breaks. It is documented that the secretion, of both, canals and trichomes, is composed of essential oils, lipids, sesquiterpene lactones, resins, pectin-like substances, alkaloids, flavonoids and tannins (Ascensão and Pais 1987, 1988; Pagni et al. 2003; Andreucci et al. 2008; Lusa et al. 2016; Gavrilović et al. 2018a).

Stem anatomy of the examined species could be considered as typical for the Asteraceae (Metcalfe and Chalk 1957) and is in accordance with previously described in the papers of Konowalik and Kreitschitz (2012) and Ivănescu et al. (2015) who studied anatomy of *A. absinthium*. Exception from typical anatomy could be seen in the presence of well-developed periderm found only in the stem of *A. campestris* and *A. arborescens*. Also, lignified walls of pith parenchyma cells were recorded only in these two species. Konowalik and Kreitschitz (2012) found that *A. absinthium* var. *calcigena* Rehm. was characterized with a continuous layer of periderm, formed in the early stage of development,

and lignification of pith cells, which could be an effect of the dry calcareous habitat. Furthermore, Zhang et al. (2018) examined anatomy of *A. lavandulaefolia* DC. and *A. selenensis* Besser and demonstrated that barrier layers, like endodermis and exodermis, dilated endodermis and exodermis, air spaces and hypodermal periderm, provide roots, rhizomes and aerial stems with tissues which could enable the plants to withstand flooding. Why, in spite of living in dry habitats, *A. absinthium*, *A. judaica* and *A. herba-alba* do not form such a layer in the stem? We consider that many other *Artemisia* species should be anatomically investigated in order to define whether the presence of periderm in the stem represents a taxonomic character or it only reflects environmental conditions. In addition, Carlquist (1966), in his investigations on wood anatomy of the Anthemideae and three related tribes of Asteraceae, concluded that *A. arborescens* lack of adaptation to extreme ecological conditions in view of wood anatomy.

Rhomboidal crystals were found only inside the pith parenchyma cells of *A. arborescens*. The most often found minerals inside the plant cells are calcium oxalate (Korth et al. 2006), which were documented in many plant families (more than 215) (Molano-Flores 2001; Kartal 2016). These crystals occur in various plant tissues and organs (Horner et al. 2000, 2012; Ilarslan et al. 2001; Meric 2009a, b). The presence of crystals is in relation to many functions: calcium regulation within the cells (Kostman and Franceschi 2000; Volk et al. 2002), against herbivory (Molano-Flores 2001), detoxification of oxalic acid or heavy metals (Franceschi and Nakata 2005), strengthening the mechanical tissue and reflection and gathering of light (Franceschi and Horner 1980; Kuo-Huang et al. 2007). Within the cells, calcium oxalate crystals are described as raphides, druses, styloids, prisms or crystal sand (Franceschi and Horner 1980; Kartal 2016). These crystals were documented in previous studies in Asteraceae plants (Dormer 1961, 1962; Horner 1977; Meric and Dane 2004; Meric 2009a, b; Kartal 2016). The shapes and occurrence of crystals might be used as valid taxonomic characters (Prychid and Rudall 1999; Lersten and Horner 2000; Pennisi and McConnell 2001; Franceschi and Nakata 2005; Horner et al. 2012; Kartal 2016; Gavrilović et al. 2017), as it was considered that the formation of crystals inside the cells is under genetic control and species-specific (Horner et al. 2009; Ilarslan et al. 2001). Thus, these crystals could be specific to the *A. arborescens*.

Leaf on the cross section is triangular in *A. campestris*, while in other studied species is oblong-linear. Very well-developed cuticle was present in *A. campestris*, probably because this species barely possesses trichomes on its surface. Also, parenchyma sheath which surrounded main vascular bundle extended to both epidermises only in *A. absinthium* and *A. arborescens*. In addition, in the main vein a subepidermal collenchyma could be seen on both leaf sides

of these two species. These characters could be of importance for taxonomy. Anatomically, leaf blade is in accordance with previously described leaf blade of *A. judaica* (Fahmy 1997), *A. alba* subsp. *saxatilis* (Bercu and Broască 2012) and *A. judaica* var. *sinaitica* (Bakr 2014) regarding isolateral leaf structure and amphistomatous leaves with large stomatal chamber, but in contrast to leaf anatomy of two varieties of *A. absinthium*, where stomata were found only on the abaxial side and where bifacial leaf structure was described (Konowalik and Kreitschitz 2012). Although leaf mesophyll, in Asteraceae plants, typically contains palisade and spongy tissue (Duarte et al. 2011; Jane et al. 2011; Gavrilović et al. 2018b), it is known that leaf anatomy often reflects environmental factors too. Plants with isolateral leaf structure usually grow on dry habitats with intense solar radiation, shown for the genera *Aster* L., *Galatella* Cass., *Tripolium* Nees. (Karanović et al. 2015), most of *Centaurea* species (Fahn and Cutler 1992; Luković et al. 2013), as well as recorded for our studied species. Also, the amphistomatic leaves are characteristic of plants growing in highly lighted habitats (Fahmy 1997). It is shown that over 90% of the species, in North American deserts, have amphistomatic leaves (Mott et al. 1982). Although leaf anatomical features are usually in relation with the environmental conditions, they are genetically controlled and thus are of taxonomic importance (Anderson and Creech 1975). Noorbakhsh et al. (2008) analyzed leaf anatomy of 28 *Artemisia* species (including *A. absinthium*) and recognized three groups which differ in their midrib shape, its position in relation to lamina and length and number of veins in each lamina half which is correlated with leaf morphology. According to Noorbakhsh et al. (2008) *A. absinthium* belongs to the first group due to the presence of a lot of collenchymatous tissues under epidermis, the midrib is voluminous and each half of lamina is long which is in accordance with our results.

Our results have shown that general root, stem and leaf anatomical features and nonglandular and glandular trichomes are shared by all species. However, some characters (parenchyma sheath, which surrounded vascular bundle and extended to both epidermises, subepidermal collenchyma and the absence of secretory canals in the leaves) link together *A. absinthium* and *A. arborescens* from the same section. Some characters, as periderm and lignified pith parenchyma cells (*A. campestris* and *A. arborescens*), non-endodermal secretory canals in root cortex (*A. absinthium* and *A. judaica*) and secretory canals in the leaf phloem (*A. judaica* and *A. herba-alba*), connect species belong to different sections. Moreover, some characters could be considered as species-specific: nonendodermal secretory canals in the root secondary phloem, triangular leaf shape on the cross section and secretory canals in the leaf parenchyma for *A. campestris*; secretory canals in the stem pith for *A. absinthium*; crystals in the pith parenchyma cells for *A.*

arborescens; and the absence of root secretory canals for *A. herba-alba*. The given results revealed qualitative characters on the basis of which the studied species are anatomically distinguishable between each other, provide valuable features for better species identification and contribute to the anatomy of the genus *Artemisia*.

Some of the anatomical characters obtained could be guideline for future investigations and thus useful in the delimitation of other *Artemisia* taxa. We consider that a comprehensive anatomical analysis, combined with micro-morphological, phytochemical and molecular data, of the remaining *Artemisia* taxa, will certainly help in resolving their taxonomic and phylogenetic relationships.

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Author contributions PJ, MG and AAG conducted the field work. PJ, MG and DR conducted anatomical analysis. PJ, MG and DR wrote the manuscript. PJ, ZDS and PDM supervised the research and gave comments to the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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