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Leaf and stem anatomy of *Micromeria* species from the Central part of the Balkan Peninsula

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Abstract: The anatomical structure of the leaf and stem of nine *Micromeria* species from the Balkan Peninsula was investigated with the aim to establish the adaptive characteristics and traits that could be useful in the identification of species and subgeneric classification. The species included in the anatomical analysis were: *Micromeria albanica* (Griseb. ex K. Malý) Šilić, *M. thymifolia* (Scop.) Fritch, *M. pulegium* (Rochel) Bentham, *M. dalmatica* Bentham of section *Pseudomelissa* and *M. cristata* (Hampe) Griseb., *M. kosaninii* Šilić, *M. juliana* (L.) Bentham ex Reichenb., *M. croatica* (Pers.) Schott *M. parviflora* (Vis.) Reichenb. of section *Micromeria.* Variability of 17 quantitative characters of the leaf (thickness, height of adaxial and abaxial epidermis, thickness of the palisade and spongy tissue) and stem (radius, cortex thickness, thickness of the vascular cylinder, phloem, xylem, pith radius) was examined. Multivariate analysis of variance (ANOVA), principal component analysis (PCA), canonical discriminant analysis (CDA) and UPGMA clustering method based on Mahalanobis' distances were used to determine the variability structure and level of importance of the anatomical differentiation of the investigated taxa. Qualitative characters were also included in the consideration of the anatomical variability, such as leaf shape, leaf margin, position of sclerenchyma fibers in the leaf and stem. The results showed that investigated *Micromeria* species posses a xeromorphic general structure plan, but each species has achieved distinctive adaptations according to its specific genetic potential. The results have shown a clear difference between the species belonging to the different sections of genus *Micromeria*, so they have a potential diagnostic and systematic value.

Key words: *Micromeria* Benth.; *Pseudomelissa* Benth.; anatomy; Balkan Peninsula.

Introduction

Genus *Micromeria* Benth. belongs to family Lamiaceae, subfamily Nepetoideae and tribe Menthae. This genus comprises perennial suffruticose herbs and shrubs. The species grow on rocks or on walls and in other dry, open habitats, from the Himalayan region to the Macaronesian Archipelago and from the Mediterranean to South Africa and Madagascar. According to Harley et al. (2004), genus *Micromeria* comprises four sections: *Micromeria*, *Pseudomelissa* Benth., *Pineolentia* P. Pérez and *Cymullaria* Boiss. Fifteen species are widespread in the central part of the Balkan Peninsula and they are classified into two sections *Micromeria* and *Pseudomelissa* (Šilić 1979).

In the studies of genus *Micromeria,* the most examined characteristics were morphological (Arabaci et al. 2010; Kremer et al. 2012, 2014a, 2014b; Marin et al. 2013) and chemical (Slavkovska et al. 2005, 2013; Palić et al. 2010; Karousou et al. 2012). The rare anatomical studies of genus *Micromeria* mostly referred to one or two species (Koca 1996, 2002; Ševarda et al. 1997), or were part of a wider study (Doroszenko 1986; Moon et al. 2009). Doroszenko (1986) presented results of the leaf anatomy of different *Micromeria* species, among them those of the central part of the Balkan Peninsula: *M. thymifolia, M. dalmatica, M. pulegium*, *M. cristata*, *M. kerneri* Murbeck.

Although variation in leaf and stem anatomy may be significantly influenced by environmental factors (Lakušić et al. 2007, 2010), it has been shown that comparative anatomy can be useful in the determination and classification of taxa in various Lamiaceae genera (Kaya & Koca 2005; Satil & Kaya 2007; Satil et al. 2011; Salmaki et al. 2011; Dinç & Doğu 2012; Celep et al. 2014). These specific anatomical structures may reflect phylogenetic relationships between species, and could be used as diagnostic characteristics (Moon et al. 2009).

Molecular studies (Bräuchler et al. 2005) have shown that members of section *Pseudomelissa,* genus *Micromeria,* are more closely related to genus *Clinopodium* than to the typical section *Micromeria.* According to the opinion of Bräuchler et al. (2006, 2008) morphological characteristics also support the transfer of the species of section *Pseudomelissa* from genus *Micromeria* to genus *Clinopodium.* But, nrDNA results of Drew & Sytsma (2012) questioned the validity of the tax-

Fig. 1. Locations of the analyzed populations.

onomic transfer suggested by Bräuchler et al. (2006), which were justified largely by their cpDNA phylogenetic analysis (Bräuchler et al. 2005). These authors point out that making taxonomic changes based on only cpDNA data can lead to confusion and further taxonomic problems.

In accordance with this the aim of this study was to: a) examine the anatomical structure of the leaf and stem of *Micromeria* species from the central part of the Balkan Peninsula which would contribute to a better understanding of these species; b) describe species adaptation to ecological conditions of the habitat; c) establish characteristics with potential diagnostic and systematic value and d) determine whether anatomical data supports the separation of the species of section *Pseudomelissa* from genus *Micromeria*.

The species included in the analysis were: *Micromeria albanica, M. thymifolia, M. pulegium, M. dalmatica* of section *Pseudomelissa* and *M. cristata, M. kosaninii, M. juliana, M. croatica* and *M. parviflora* of section *Micromeria*.

Material and methods

Plant material

Taxonomic nomenclature by Chater & Guinea (1972), Šilić (1979) and Harley et al. (2004) was used in the paper. Anatomical structure of the leaf and stem was examined in 9 species from 28 populations (Fig. 1). The investigated species ranges from costal to subalpine zones of the analyzed part of the Balkan peninsula, i.e. at altitudes from sea level to 1700 m in the C and S Dinaric, Scardo-Pindic, Rhodope-Rila and Balkan mountain systems (Horvat et al. 1974). All investigates species grow mostly in open sites but also in canyons and gorges. They occupy predominantly limestone rocky crevices, continental and Mediterranean rocky grounds (Meusel & Jäger 1992; Jäger & Welk 2003). Data about habitats of each investigated population is given in Table 1.

Voucher specimens are deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade (BEOU) and Herbarium of the Department of Botany, Faculty of Pharmacy, University of Belgrade (HFF).

Anatomical analysis

The collected plant material was fixed in 50% alcohol. Anatomical sections (30 per population) of leaves and stems were preserved on permanent slides, prepared by the standard method for light microscopy. Cross-sections of leaves and stems were cut on a Reichert sliding microtome (10–15 μm thick). The sections were stained with safranin (1%, w/v, in 50% ethanol) and alcian blue (1%, w/v, aqueous). All slides were mounted in Canada balsam after dehydration. Anatomical sections of leaves and stems were analyzed on the light microscope (LM) Olympus BX41 with camera Olympus SC30. Observation of the leaf epidermis was studied with SEM (JEOL JSM-6460), for which the samples were covered by gold. All measurements were done with Image Analyzer System Ozaria 2005 and the data was processed

Table 1. Collection data of examined *Micromeria* specimens.

Taxa	Code	Locality	Coordinates	Habitat	Voucher
			Section: *Pseudomelissa*		
M. albanica		Serbia, Prizren, Dušan's city	42°1N 20°7E	rocky grounds, screes, limestone, 400 m a.s.l.	HFF–1886
M. thymifolia	1	Serbia, Trešnjica canyon	44°1N 19°5E	rocky crevices, limestone, 200 m a.s.l.	BEOU–152
	2	Serbia, Ibar gorge, Maglič	43°4N 20°3E	rocky grounds, limestone, 350 m a.s.l.	HFF–1941
	3	Serbia, Derventa canyon	43°9N 19°4E	rocky crevices, limestone, 430 m a.s.l.	HFF–1940
	4	Serbia, Beli Rzav gorge	43°7N 19°3E	rocky grounds, limestone, 600 m a.s.l.	HFF–1933
	5	Serbia, Rugovo gorge	42°4N 20°1E	rocky crevices, limestone, 700 m a.s.l.	BEOU–2230
	6	Montenegro, Morača canyon, Morača Monastery	42°5N 19°4E	rocky grounds, limestone, 287 m a.s.l.	HFF–1938
	7	Montenegro, Mt. Orjen	42°4N 18°5E	rocky crevices, limestone, 1600 m a.s.l.	HFF–1939
M. pulegium		Serbia, Svrljiški Timok gorge	43°3N 22°5E	rocky grounds, limestone, 330 m a.s.l.	HFF–3203
M. dalmatica		Montenegro, near Kotor	42°3N 18°8E	rocky grounds, limestone, 800 m a.s.l.	HFF–1906
			Section: Micromeria		
M. cristata	1	Serbia, Jelašnica gorge	43°2N 22°4E	rocky grounds, limestone, 400 m a.s.l.	HFF–1887
	2	Serbia, Jerma gorge	42°9N 22°6E	rocky crevices, limestone, 550 m a.s.l.	HFF–1889
	3	Serbia, Sićevo gorge	43°2N 22°8E	rocky crevices, limestone, 400 m a.s.l.	HFF–1888
M. kosaninii		FYR Macedonia, Mt. Galičica	41°3N 20°5E	rocky grounds, limestone, 1600 m a.s.l.	HFF–1922
M. juliana	1	Montenegro, Morača canyon, Bioče	42°5N 19°4E	rocky grounds, limestone, 120 m a.s.l.	HFF–1914
	2	Montenegro, Cijevna canyon	42°3N 19°5E	rocky grounds, limestone, 80 m a.s.l.	HFF–1912
	3	Montenegro, Taraboš, on the Ostros–Virpazar road	42°3N 19°2E	rocky grounds, limestone, 200 m a.s.l.	HFF–1910
	4	Montenegro, Virpazar	42°1N 19°6E	rocky grounds, limestone, 55 m a.s.l.	HFF–1915
	5	Montenegro, Mt. Orjen	42°4N 18°5E	rocky grounds, limestone, 300 m a.s.l.	HFF–1916
	6	Montenegro, near Njeguši	42°2N 18°5E	rocky grounds, limestone, 700 m a.s.l.	HFF–1921
	7	Montenegro, Luštica	42°2N 18°3E	rocky grounds, limestone, 90 m a.s.l.	HFF–1909
	8	FYR Macedonia Radožda, Vragudenca	41°6N 20°4E	rocky grounds, limestone, 700 m a.s.l.	HFF–1911
	9	FYR Macedonia Radožda, Klenci	41°6N 20°4E	rocky grounds, limestone, 696 m a.s.l.	HFF–1917
M. croatica	1	Serbia, Beli Rzav gorge	43°7N 19°3E	rocky grounds, limestone, 600 m a.s.l.	HFF–1896
	2	Montenegro, Mt. Durmitor, Boljske grede	43°0N 19°1E	rocky grounds, limestone, 1700 m a.s.l.	HFF–1897
M. parviflora	1	Montenegro,Morača canyon, Bioče	42°5N 19°4E	rocky grounds, limestone, 120 m a.s.l.	HFF–1927
	2	Montenegro, Cijevna canyon	42°3N 19°5E	rocky grounds, limestone, 80 m a.s.l.	HFF–1925
	3	Montenegro, River of Crnojevići	42°3N 19°1E	rocky grounds, limestone, 50 m a.s.l.	HFF–1926

in statistical packages Statistica 5.0 and Statistica 7.0 for Windows.

The following quantitative characters were measured on permanent slides: leaf thickness (WL1), height of adaxial epidermis cells (WEL), height of abaxial epidermis cells (WEN), palisade tissue thickness (WPL), spongy tissue thickness (WS), stem radius at the corners (RSTR), stem radius on the flat sides (RSTS), cortex thickness below the corners (WKOR), cortex thickness on the flat sides (WKOS), vascular cylinder thickness below the corners (WCIR), vascular cylinder thickness on the flat sides (WCIS), phloem thickness below the corners (WFLR), phloem thickness on the flat sides (WFLS), xylem thickness below the corners (WKSR), xylem thickness on the flat sides (WKSS), pith radius below the corners (WSRR), pith radius on the flat sides (WSRS). Leaf area was also measured, however this characteristic was not included in the multivariate statistical analysis. Instead, it was processed by basic statistical method. Qualitative characters were also included in the considera-

Fig. 2. Leaf shape. Section *Pseudomelissa*: A) *M. pulegium*; B) *M. albanica*; C) *M. dalmatica*; D) *M. thymifolia*; Section *Micromeria*: E) *M. croatica*; F) *M. parviflora*; G) *M. cristata*; H) *M. juliana*; I) *M. kosaninii*.

Fig. 3. Leaf cross section. Section *Pseudomelissa*: A) *M. albanica*; B) *M. dalmatica*; C) *M. pulegium*; D) *M. thymifolia*; Section *Micromeria*: E) *M. cristata*; F) *M. croatica*; G) *M. kosaninii*; H) *M. juliana*; I) *M. parviflora*.

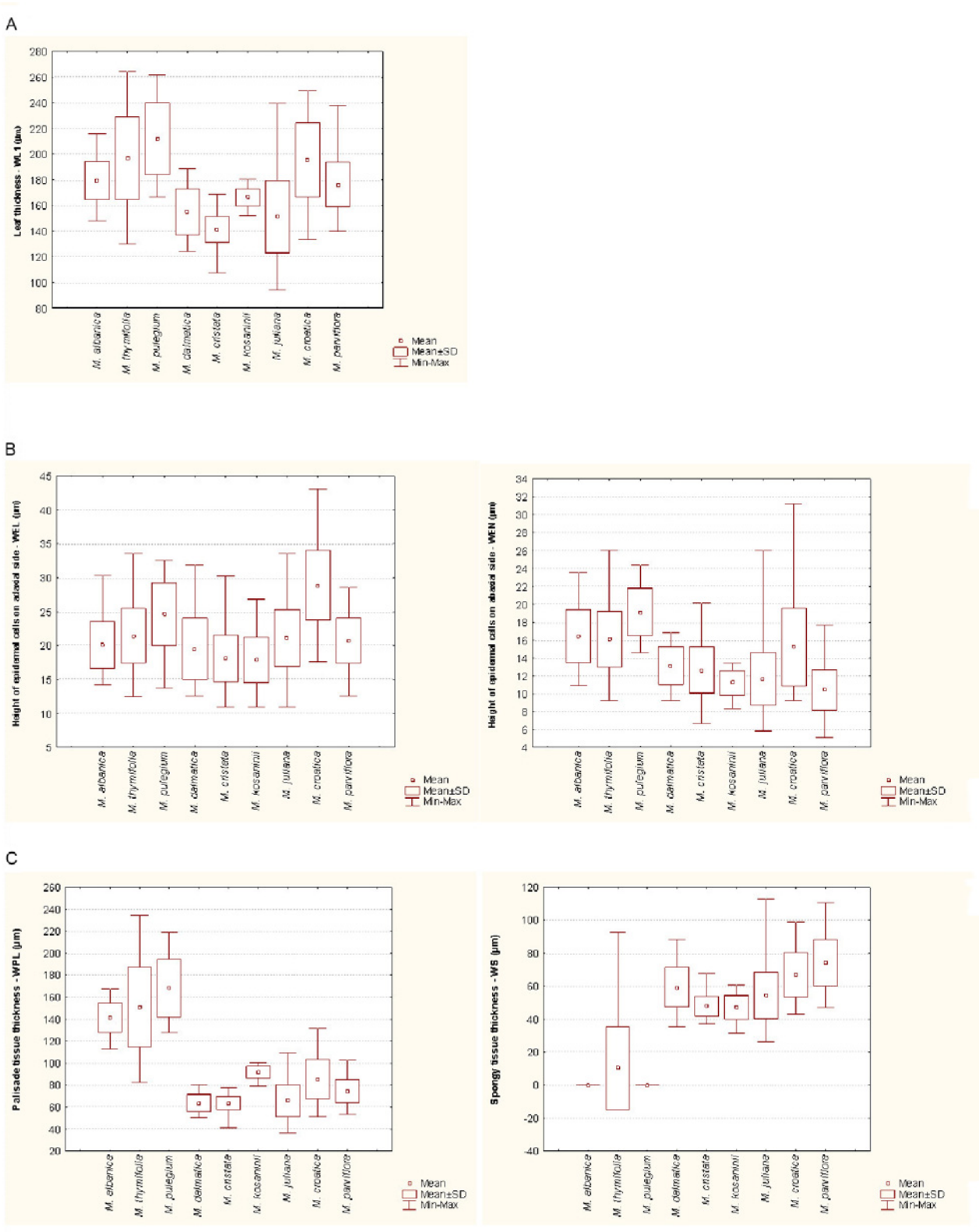

Fig. 4. Box and whisker plots of basic statistical parameters of leaf. A) Leaf thickness; B) Height of adaxial and abaxial epidermal cells; C) Palisade and spongy tissue thickness. Section *Pseudomelissa*: *M. albanica*; *M. thymifolia*; *M. pulegium*; *M. dalmatica*; Section *Micromeria*: *M. cristata*; *M. kosaninii*; *M. juliana*; *M. croatica*; *M. parviflora.*

tion of anatomical variability such as leaf shape, leaf margin, position of sclerenchyma fibers in the leaf and stem.

Statistical analysis

For each of the quantitative characters, a descriptive statistics was done on the basis of the following parameters: average value, minimum, maximum, standard deviation, and standard error. Multivariate analyses were performed to identify the significance of differences between the populations studied (multivariant analysis of variance; ANOVA),

to describe the structure of variability of the analyzed population (principal component analysis; PCA), and to measure the distance between the groups (canonical discriminant analysis; CDA). Characters which have proved to be statistically significant in ANOVA and PCA analysis were included in the CDA analysis. Discriminant function analysis was performed to estimate the contribution of individual characters to overall discrimination. Overall differences between the compared groups are presented by Mahalanobis' distances, which are used for clustering on the basis of UPGMA method.

Results and discussion

Leaf shape and anatomy

The basic form of the leaf in all the analyzed species is ovate or lanceolate. Some species have wide ovate (*M. pulegium, M. albanica, M. dalmatica* and *M. croatica*) and some ovate-lanceolate leaves (*M. thymifolia, M. parviflora, M. cristata, M. juliana* and *M. kosaninii* (Fig. 2). The leaves of section *Pseudomelissa* species are flat while the leaves of section *Micromeria* species are with margins more or less revolute towards the abaxial side (Fig. 3).

Leaf area varies greatly, ranging between 5 and 136 mm^2. Species of section *Micromeria* have small leaves (5–43 mm^2), while the leaves of section *Pseudomelissa* species have bigger surface areas (82–136 mm^2). Leaf area is an important characteristic for the definition of sections within genus *Micromeria* (Fig. 2). Species of section *Pseudomelissa* develop up to ten times broader leaves than the representatives of section *Micromeria,* so that we can define the members of section *Micromeria* as species with narrow leaves, and members of section *Pseudomelissa* as species with broad leaves. *M. croatica*, belonging to section *Micromeria,* differs in leaf size from the other representatives of the section and seems to be a transition toward section *Pseudomelissa*.

The leaves of all the investigated species range in thickness between 94 and 264 μm (Fig. 4A). The thinnest leaves have *M. cristata, M. juliana, M. dalmatica* and *M. kosaninii* (141–166 μm on average). The leaves of *M. parviflora* and *M. albanica* are somewhat thicker (176–180 μm on average). The leaves of *M. croatica* and *M. thymifolia* are 195 and 197 μm (on average) thick, while the leaves of *M. pulegium* are the thickest (212 μm on average).

Epidermis, both adaxial and abaxial, in all the investigated species, is a single-layer (Fig. 5A-F). The height of adaxial epidermis cells ranges from 11 to 43 μm (Fig. 4B). In all the species, the abaxial epidermis cells are smaller, from 5 to 31 μm. In *M. croatica* and *M. parviflora,* the adaxial epidermis cells are almost double the size of the abaxial epidermis cells. The outer cellular wall of the adaxial epidermis has thickened to a greater or a lesser degree in all the species. Species of *Pseudomelissa* section have a thin cuticle layer on both adaxial and abaxial epidermis.The adaxial and abaxial cuticle is smooth (Fig. 5G), or in the form of rare and thin striae (Fig. 5H). Species of *Micromeria* section (Fig. 5I-J) form a thick layer of more or less wrinkled cuticle on both the adaxial and abaxial epidermis.

Most of the investigated species have dorsiventral leaves with single-layer palisade and multilayer spongy tissue. These leaves can be found in all species from *Micromeria* section (Fig. 5E-F) and *M. dalmatica* from *Pseudomelissa* section (Fig. 5D). The *M. thymifolia* plants from the Ibar gorge population have dorsiventral leaves also, but with bilayered palisade tissue (Fig. 5C). Plants of the other populations of *M. thymifolia* (Fig. 5B), *M. pulegium* (Fig. 5A) and *M. albanica* have isobilateral leaves with mesophyll comprising only of palisade parenchyma. In these species, the thickness of palisade parenchyma ranges from 107 to 234 μm (Fig. 4C). In the dorsiventral leaves, the thickness of palisade parenchyma ranged from 36 to 131 μm, and the thickness of spongy parenchyma from 26 to 113 μm. The ratio of palisade to spongy parenchyma is 1–1.5 : 1 except in *M. kosaninii*. In this species the ratio of palisade to spongy parenchyma is approximately 2 : 1.

The leaf vascular system of all the species consists of the dominant central vein and lateral, less developed veins (Fig. 3). The leaves of section *Micromeria* species (Fig. 6A-B) have very pronounced sclerenchyma forming strands under the phloem of the central vein. Species of *Pseudomelissa* section (Fig. 6C-D) don't have sclerenchyma fibers in the central vascular bundle so that their leaves have a somewhat softer consistency. Species of *Micromeria* section (Fig. 6E-F) on the leaf margins, where the leaf bends toward abaxial surface, contain bundles comprised solely of sclerenchyma.

Our results show that sclerenchyma strands under the phloem of the central vein and in the marginal vein, in addition to being an important xeromorphosis (Fahn & Cutler 1992), have diagnostic value in characterizing species of section *Micromeria.* The results yielded by the investigation into the anatomical structure of other species of section *Micromeria* (Doroszenko 1986) also confirm this. The presence of a marginal vein in the species of section *Micromeria* and its absence in the species of section *Pseudomelissa* is of great taxonomic importance because is separates the species of these two sections at the anatomical level.

Stem shape and anatomy

In cross-section, the stem is quadrangular with more or less pronounced corners in species of *Pseudomelissa* section (Fig. 7A). The other species have different stem shapes. Distinctly quadrangular stems can be found in *M. juliana* and *M. croatica* (Fig. 7B), while the other species have an almost round stem (Fig. 7C).

Stem radius in all the investigated species ranged between 222 and 1528 μm (Fig. 8A). Among the species of section *Pseudomelisa, M. thymifolia* had the widest stem (487 to 1528 μm), while *M. pulegium* had the narrowest (410 to 920 μm), and among the species of section *Micromeria* the widest stem was found in *M. juliana* (286 to 957 μm) and narrowest in *M. cristata* (222 to 403 μm). The species of section *Pseudomelissa* have thicker stems than the species of section *Micromeria*.

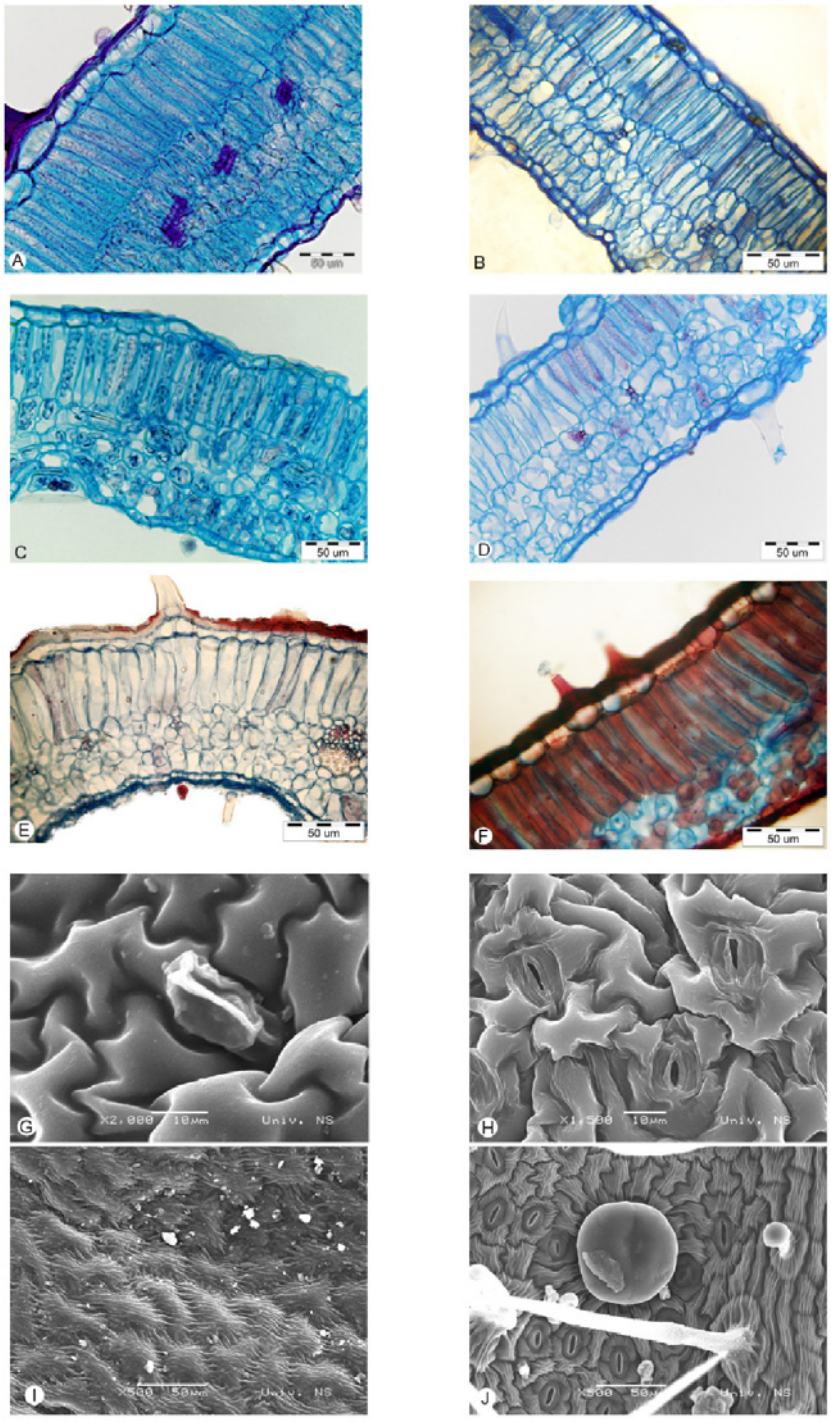

Fig. 5. Leaf cross–sections (LM) and SEM of leaf epidermis. Section *Pseudomelissa*: A) *M. pulegium*; B) *M. thymifolia*; C) *M. thymifolia* (population from the Ibar gorge); D) *M. dalmatica*; Section *Micromeria*: E) *M. cristata*; F) *M. croatica*; Section *Pseudomelissa*: G) *M. thymifolia* (adaxial epidermis); H) *M. dalmatica* (abaxial epidermis); Section *Micromeria*: I) *M. kosaninii* (adaxial epidermis); J) *M. croatica* (abaxial epidermis).

The stems of *Micromeria* section species have a smaller radius so, in accordance with the stem width, all the stem tissues cover a smaller area than in the species of the other section.

The stem structure in the investigated species is fairly uniform. Epidermis has a thickened outer cell wall and thin layer of cuticle on its surface in all the investigated species (Fig. 7D-F).

Subepidermally, in the primary cortex, there are collenchyma and parenchyma. Collenchyma can be found only in the corners of the stem and alternates with parenchyma along the flat sides of the stem. The last layer of parenchyma contains cells bigger than the rest, with thin or slightly thickened walls (Fig. 7D-F). Cortex thickness in all the species ranged from 18 to 218 μm (Fig. 8B). Cortex thickness in the species of section *Pseudomelissa* ranges from 25 (*M. thymifolia*) to 218 μm (*M. pulegium*), and in the species of section

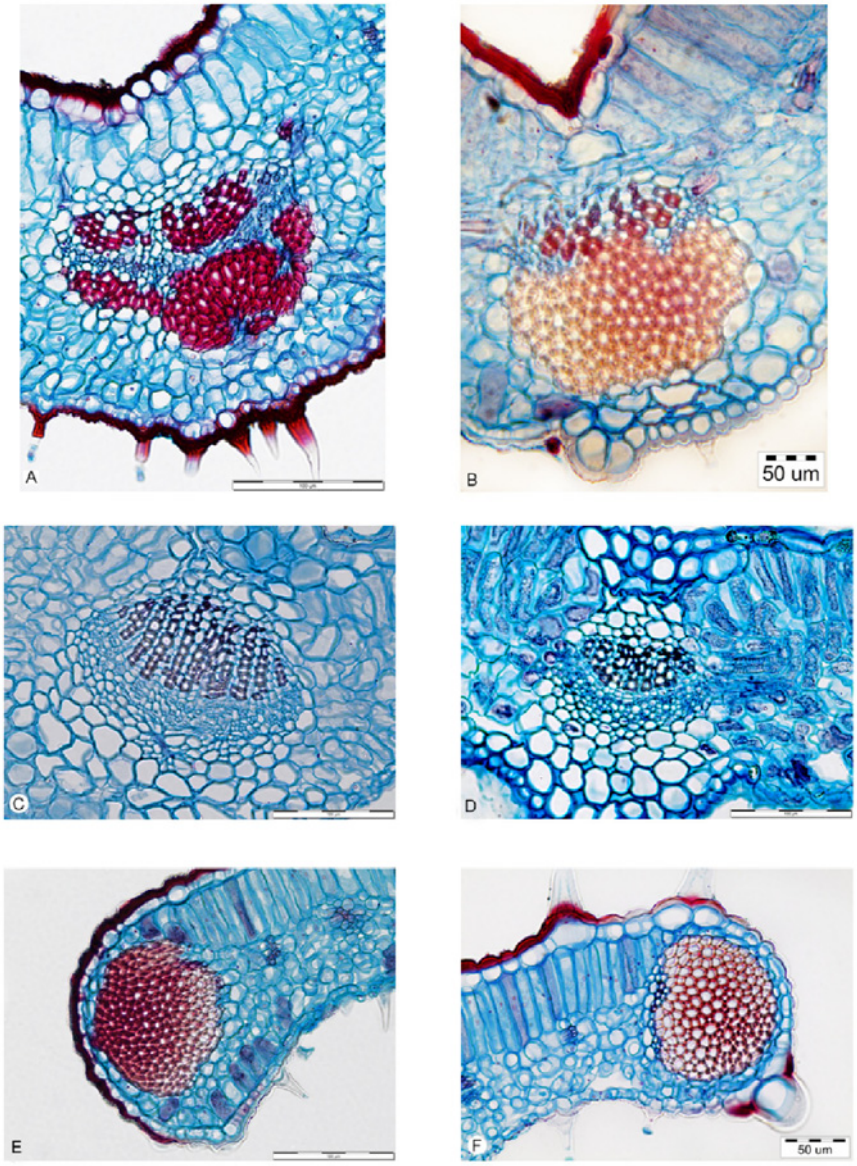

Fig. 6. Central and marginal leaf vein. Section *Micromeria*: A) *M. juliana*; B) *M. parviflora*; Section *Pseudomelissa*: C) *M. albanica*; D) *M. thymifolia*; Section *Micromeria*: E) , *M. juliana*; F) *M. croatica*.

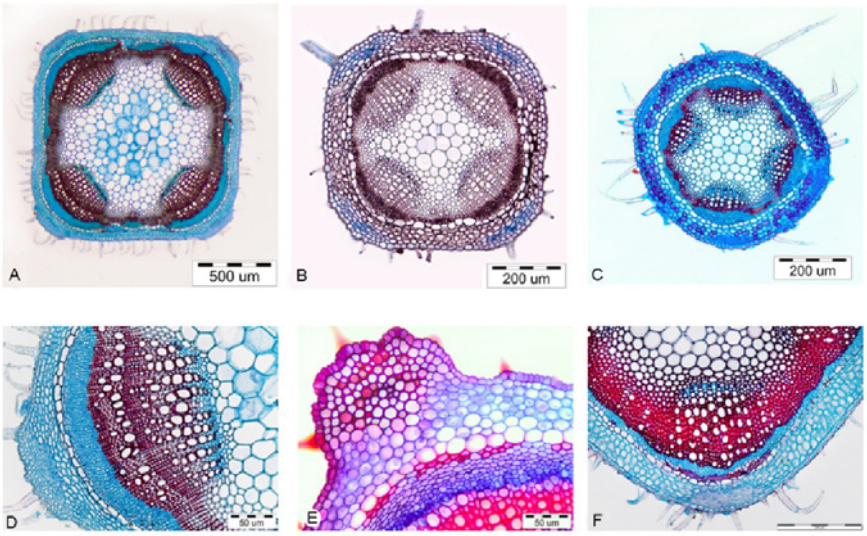

Fig. 7. Stem cross section. Section *Pseudomelissa*: A) *M. albanica*; Section *Micromeria*: B) *M. croatica*; C) *M. cristata*; Section *Pseudomelissa*: D) *M. albanica*; E) *M. pulegium*; Section *Micromeria*: F) *M. juliana*.

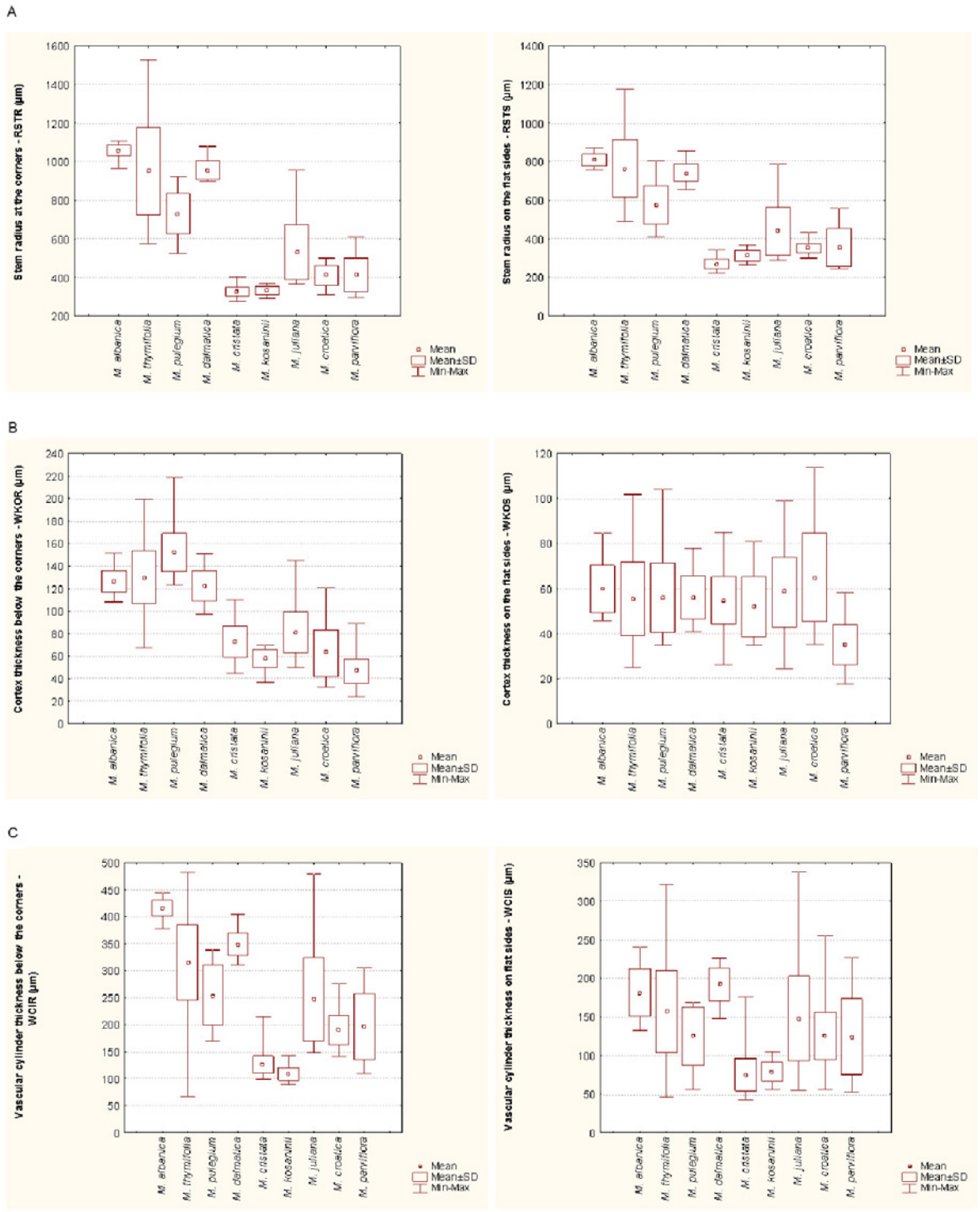

Fig. 8A,B,C. Box and whisker plots of basic statistical parameters of stem. A) Stem radius: B) Cortex thickness; C) Vascular cylinder thickness; Section *Pseudomelissa*: *M. albanica*; *M. thymifolia*; *M. pulegium*; *M. dalmatica*; Section *Micromeria*: *M. cristata*; *M. kosaninii*; *M. juliana*; *M. croatica*; *M. parviflora.*

Micromeria from 18 (*M. parviflora*) to 144 µm (*M. juliana*).

Central cylinder starts with pericycle which has a different structure in different species. *M. cristata, M. kosaninii, M. juliana* (Fig. 7F), *M. croatica, M. parviflora, M. pulegium* (Fig. 7E) and *M. dalmatica* have pericycle comprised of sclerenchyma and parenchyma. Isolated smaller or bigger groups of sclerenchyma fibers connected only to the phloem below the stem corners alternate with parenchyma along the stem sides. In the pericycle of species *M. albanica* (Fig. 7D) and *M. thymifolia,* there is no sclerenchyma. Research done on other species, such as the species of genus *Ajuga, Marrubium, Lamium, Galeopsis, Prunella* (Harley et al. 2004), *Teucrium* (Lakušić et al. 2010), also show that sclerenchyma can be organized in different ways in the pericycle of representatives of family Lamiaceae.

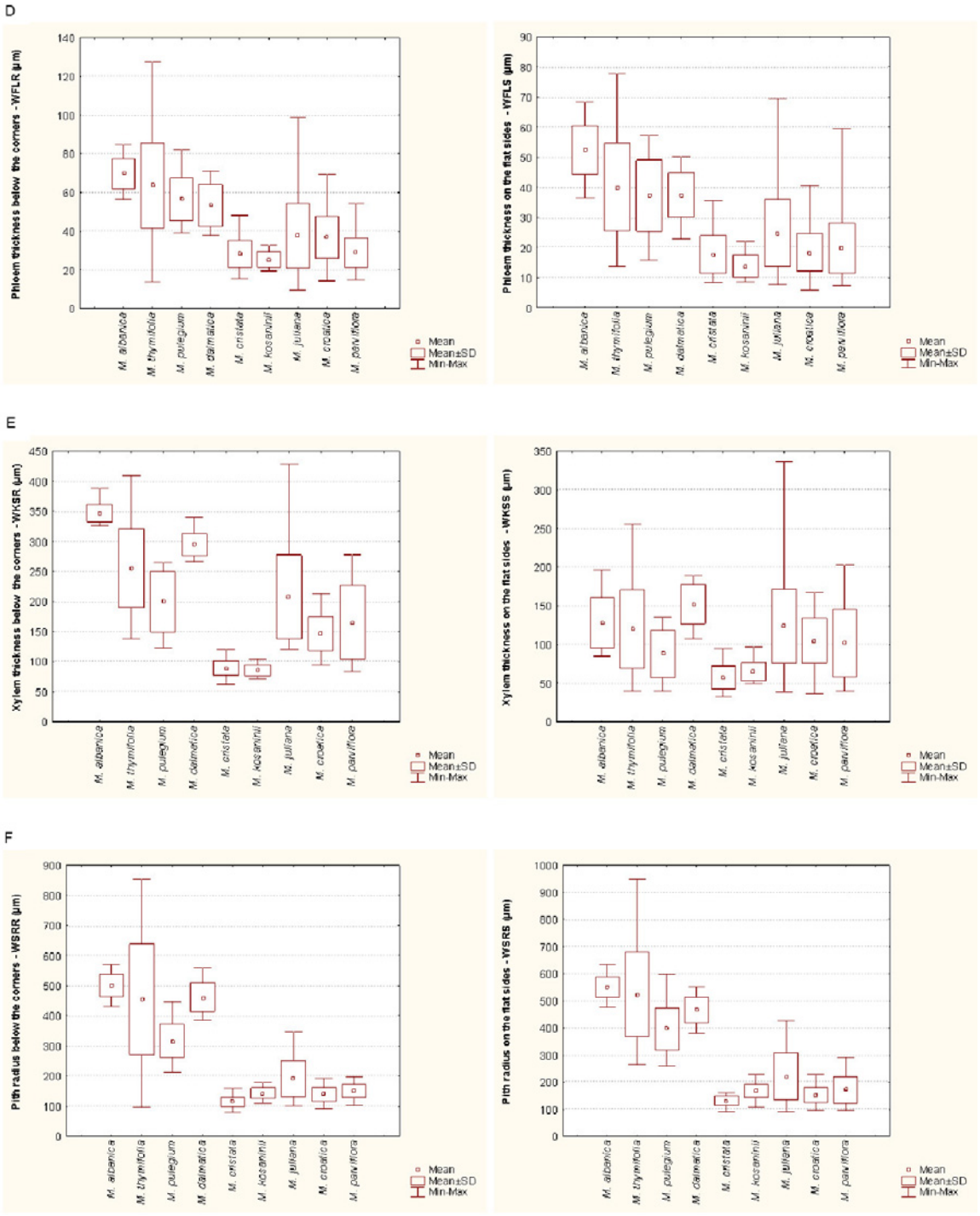

Fig. 8D,E,F. Box and whisker plots of basic statistical parameters of stem. D) Phloem thickness; E) Xylem thickness; F) Pith radius. Section *Pseudomelissa*: *M. albanica*; *M. thymifolia*; *M. pulegium*; *M. dalmatica*; Section *Micromeria*: *M. cristata*; *M. kosaninii*; *M. juliana*; *M. croatica*; *M. parviflora*.

Vascular tissues in most of the species appear as continuous cylinders with particularly well-developed xylem below the stem corners (Fig. 7). In *M. albanica* (Fig. 7A) and *M. croatica* (Fig. 7B) the vascular tissue forms one large vascular bundle below each stem corner. Besides the vascular bundles in the stem corners, *M. albanica* also has one or two smaller bundles on the flat sides of the stem. In all the species, the vascular cylinder thickness ranged between 43 and 482 µm (Fig. 8C). The vascular cylinder thickness in the species of section *Pseudomelissa* ranges between 46 and 482 µm (*M. thymifolia*). In most species of section *Micromeria* the vascular cylinder thickness varies between 43 (*M. cristata*) and 306 µm (*M. parviflora*), only *M. juliana* stands out with a vascular cylinder thickness between 55 and 479 µm. The phloem thickness in all the species ranged between 6 and 127 µm (Fig. 8D). Phloem is of uniform size among the species of section *Pseudomelissa* and among the species of section *Micromeria*. The phloem zone is wider in the species of section

Table 2. Analysis of variance (ANOVA) and principal components (PCA) at the level of individual characters of species of genus *Micromeria* and eigenvectors and cumulative percentages of variances on three principal component axes.

	F	p-level	PCA1	PCA2	PCA3
Leaf thickness (WL1)	80.33	0.00	–0.50	0.34	0.61
Height of adaxial epidermis cells (WEL)	40.38	0.00	–0.13	–0.02	0.74
Palisade tissue thickness (WPL)	337.09	0.00	–0.67	0.50	0.35
Spongy tissue thickness (WS)	234.14	0.00	0.53	–0.43	0.01
Height of abaxial epidermis cells (WEN)	70.23	0.00	–0.48	0.41	0.48
Stem radius at the corners (RSTR)	275.32	0.00	–0.96	0.00	–0.18
Stem radius on the flat sides (RSTS)	275.99	0.00	–0.97	–0.02	–0.15
Cortex thickness below the corners (WKOR)	306.44	0.00	–0.82	0.27	–0.15
Cortex thickness on the flat sides (WKOS)	27.28	0.00	–0.14	0.12	0.01
Vascular cylinder thickness below the corners (WCIR)	145.93	0.00	–0.89	–0.38	0.00
Vascular cylinder thickness on the flat sides (WCIS)	42.32	0.00	–0.61	–0.67	0.25
Phloem thickness below the corners (WFLR)	88.72	0.00	–0.79	0.10	–0.22
Phloem thickness on the flat sides (WFLS)	0.82	0.00	–0.78	–0.01	–0.21
Xylem thickness below the corners (WKSR)	127.16	0.00	–0.83	–0.48	0.07
Xylem thickness on the flat sides (WKSS)	32.80	0.00	–0.48	–0.74	0.31
Pith radius below the corners (WSRR)	208.27	0.00	–0.88	0.15	–0.25
Pith radius on the flat sides (WSRS)	290.63	0.00	–0.92	0.17	–0.24
Principal component axes	1		2		3
Eigenvalue	8.685843		2.236818		1.711731
% Total variance	51.09319		13.15775		10.06901
Cumulative eigenvalue	8.68584		10.92266		12.63439
Cumulative %	51.09319		64.25095		74.31995

Legend: F – distance between individual distributions, p – probability of error, PCA1, PCA2 and PCA3 – factor loadings of principal component axis

Pseudomelissa (14 to 127 μm) than in the species of *Micromeria* section (6 to 98 μm). In most of the species, regardless of section, the xylem (Fig. 8E) is three to five times wider (33 to 429 μm) than the phloem. *M. albanica* and *M. dalmatica* have the widest xylem zone (84 to 388 μm; 108 to 339 μm) of all *Pseudomelissa* species. In most *Micromeria* species the xylem thickness ranged from 33 (*M. cristata*) to 277 μm (*M. parviflora*), except in *M. juliana* whose xylem thickness ranged from 39 to 429 μm.

The species of section *Pseudomelissa* have a wide vascular tissue. In the species of section *Micromeria* vascular cylinder is narrow. However, it is important to keep in mind that the total volume of vascular elements changes in accordance with the general size of the plant (Zimmermann & Brown 1974).

Central part of the stem is comprised of large, round pith parenchyma cells (Fig. 7A-C). Pith radius in the stems of all the species ranged from 79 to 951 μm (Fig. 8F). In the representatives of section *Pseudomelissa,* the pith is almost twice the size (97 to 951 μm) of that in the representatives of section *Micromeria* (79 to 425 μm). In all investigated species of section *Pseudomelissa,* the pith is more developed than the vascular cylinder (Fig. 8C). In contrast, in most species of section *Micromeria* (except *M. kosaninii*) the pith radius is smaller than the vascular cylinder.

Anatomical differentiation of species of genus Micromeria

Multivariate analysis (ANOVA) has shown that quantitative characters are to a higher or lesser degree statistically significant in the formation of differences between the species (Table 2). The characters, as well as palisade tissue thickness (WPL), spongy tissue thickness (WS), stem radius (RSTR, RSTS), pith radius (WSRR, WSRS), cortex (WKOR), vascular cylinder (WCIR) and xylem (WKSR) thickness below the corners stood out as the most important characters for anatomical species differentiation.

Principal component analysis (PCA) has shown that the variability structure of the investigated species is complex considering that the first three axes cover 74.32% of the total variability (Table 2).

The most important characters on the first axis, for the formation of variability are characters relating to stem anatomy: stem radius (RSTR, RSTS), phloem thickness (WFLR, WFLS), pith radius (WSRR, WSRS), cortex (WKOR), vascular cylinder (WCIR) and xylem thickness (WKSR) below the stem corners. Xylem thickness on the flat sides of the stem (WKSS) is a character read on the second axis and is less important for the definition of variability structure.

Canonical discriminant analysis (CDA) was performed with nine taxa defined as a priori groups. The first three discriminant axes explained 94.41 % of variation between groups. For the graphical presentation of CDA, the scores of all specimens were plotted within a two-dimensional space defined by discriminant axis 1 (explaining 85.24 % of variation) and discriminant axis 2 (explaining 5.73 % of variation). CDA has shown the existence of two groups of species which are strongly separated along on the first axis (Fig. 9). One comprises *M. thymifolia*, *M. albanica* and *M. pulegium* of section *Pseudomelissa*, and the other *M. dalmatica* of section

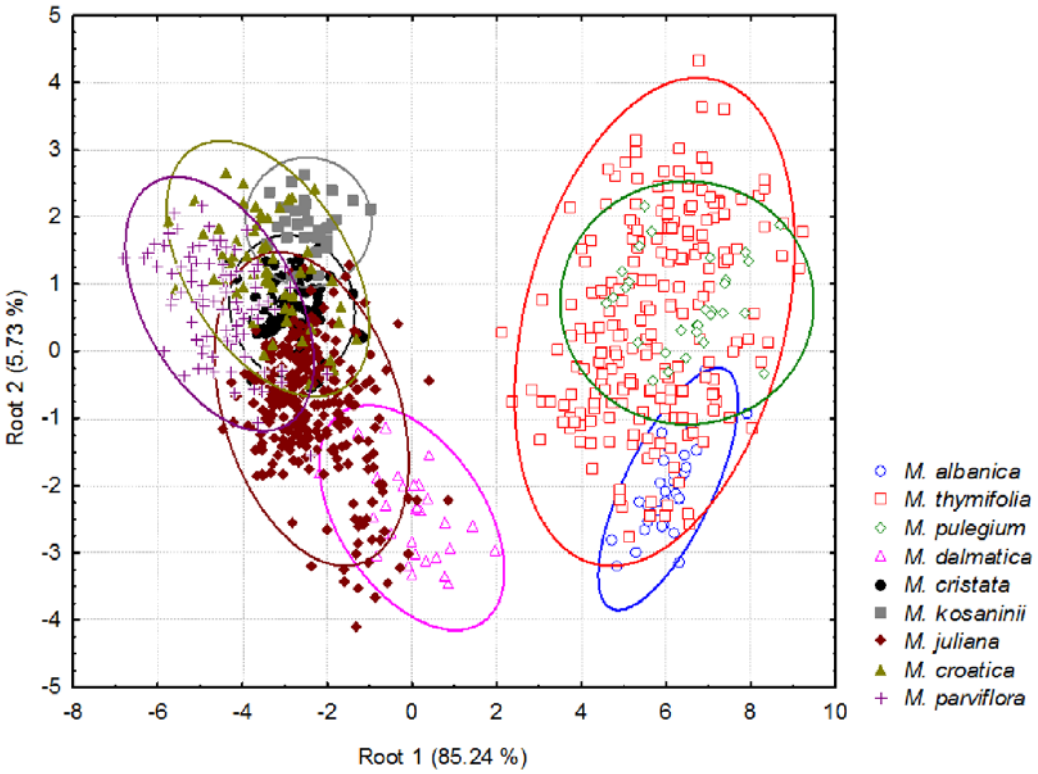

Fig. 9. Canonical discriminant analysis (CDA) of the analyzed species of genus *Micromeria*. Section *Pseudomelissa*: *M. albanica*; *M. thymifolia*; *M. pulegium*; *M. dalmatica*; Section *Micromeria*: *M. cristata*; *M. kosaninii*; *M. juliana*; *M. croatica*; *M. parviflora*.

Table 3. Summary of Discriminant function analysis.

	Wilks' Lambda	F-remove (8,815)	*P*-level
Leaf thickness (WL1)	0.0051	4.9755	**0.0000**
Height of adaxial epidermis cells (WEL)	0.0054	10.9597	**0.0000**
Palisade tissue thickness (WPL)	0.0053	9.0505	**0.0000**
Spongy tissue thickness (WS)	0.0055	12.5448	**0.0000**
Height of abaxial epidermis cells (WEN)	0.0054	10.8213	**0.0000**
Stem radius at the corners (RSTR)	0.0050	2.3970	**0.0147**
Stem radius on the flat sides (RSTS)	0.0052	5.9975	**0.0000**
Cortex thickness below the corners (WKOR)	0.0070	44.7440	**0.0000**
Cortex thickness on the flat sides (WKOS)	0.0061	24.5413	**0.0000**
Vascular cylinder thickness below the corners (WCIR)	0.0051	3.6956	**0.0003**
Vascular cylinder thickness on the flat sides (WCIS)	0.0050	1.6342	0.1112
Phloem thickness below the corners (WFLR)	0.0052	6.4041	**0.0000**
Phloem thickness on the flat sides (WFLS)	0.0053	8.1358	**0.0000**
Xylem thickness below the corners (WKSR)	0.0057	17.3439	**0.0000**
Xylem thickness on the flat sides (WKSS)	0.0051	3.6000	**0.0004**
Pith radius below the corners (WSRR)	0.0051	3.9714	**0.0001**
Pith radius on the flat sides (WSRS)	0.0052	7.0489	**0.0000**

Legend: Wilks's lambda is a multivariate generalization of the univariate F-distribution; F-remove represents a measure of the extent to which a variable makes a unique contribution to the prediction of a group membership; *P*-level values < 0.05 are shown in boldface.

Pseudomelissa and all species of section *Micromeria*. On the second axis, *M. dalmatica* shows slight differentiation from the second group.

Discriminant function analysis revealed that six out of 17 tested characters: height of abaxial epidermis cells (WEN), height of adaxial epidermis cells (WEL), spongy tissue thickness (WS), xylem thickness below the corners (WKSR), cortex thickness on the flat sides (WKOS) and cortex thickness below the corners (WKOR), had a dominant contribution to the overall discrimination (Table 3).

According to Mahalanobis' distances (Fig. 10), section *Micromeria* forms a compact cluster divided into two subclusters: *M. parviflora, M. croatica* and *M. kosaninii* on one, and *M. cristata* and *M. juliana* on the other side. Species *M. thymifolia, M. pulegium* and *M. albanica* of section *Pseudomelissa* also make up a separate compact cluster. As opposed to, species *M. dalmatica* which also belongs to section *Pseudomelissa,* has an intermediate position, showing more similarity to the species of section *Micromeria*.

Interspecies variability on the anatomical level

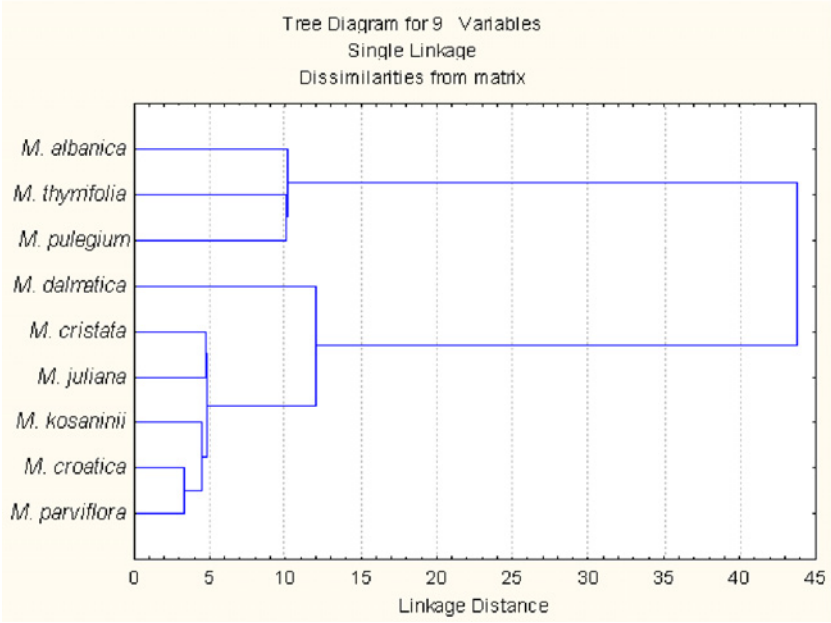

Fig. 10. Mahalanobis' distances between the analyzed species of genus *Micromeria*. Section *Pseudomelissa*: *M. albanica*; *M. thymifolia*; *M. pulegium*; *M. dalmatica*; Section *Micromeria*: *M. cristata*; *M. juliana*; *M. kosaninii*; *M. croatica*; *M. parviflora*.

is less pronounced within section *Micromeria*. The anatomical characteristics according to which species *M. parviflora* and *M. croatica* are connected on the dendrogram at such short distances are: palisade and spongy tissue thickness, stem radius, vascular cylinder thickness, xylem thickness and pith radius. The similarity of these species could also be the result of both their phylogenetic closeness and adaptation to similar environmental conditions of the habitat. Both species inhabit limestone rocks at different altitudes in conditions of physical drought. *M. kosaninii* is connected to these two species by its similar cortex thickness, phloem thickness and pith radius. However, *M. kosaninii* also possesses specific adaptations to the habitat conditions at higher altitudes such as short stem, very small but thick leaves, mesophyll dominated by palisade parenchyma and a wide pith in the stem.

M. juliana and *M. cristata* are similar in: leaf thickness, height of abaxial epidermal cells, palisade and spongy tissue thickness and stem cortex thickness. *M. cristata* grows in stone crevices of the hilly and subalpine belts of temperate continental zones, while *M. juliana* inhabits stony limestone grounds of the Mediterranean and sub-Mediterranean zones. However, *M. juliana* differs from *M. cristata* by its wider stem, wider vascular cylinder, xylem and pith, which can be explained by the height of the plant. The stems of *M. juliana* are taller, and, to ensure the efficiency of the vascular system, it has a bigger total area of vascular elements.

In the cluster analysis, *M. dalmatica* was connected with the species of section *Micromeria* due to its dorsiventral leaves, palisade and spongy tissue thickness, as well as the height of abaxial and adaxial leaf epidermis. The characteristics of the stem are similar to the *Pseudomelissa* species.

The detachment of species *M. thymifolia, M. pulegium* and *M. albanica* into a separate cluster on the dendrogram is the result of specific leaf mesophyll structure, because only the individuals of these species have isobilateral leaves. In addition to this very specific characteristic, these species also have a similar stem structure – their stems all have a wide radius, thick vascular cylinder, xylem and wide pith radius. *M. pulegium* is a species inhabiting rocky grounds of the hilly belt of temperate continental areas, develops isobilateral leaves on a relatively tall stem whose support is assisted by both the well-developed xylem and sclerenchyma fibers forming bilayer arches localized next to the phloem, which are part of the pericycle. *M. thymifolia* inhabits the crevices of mostly limestone rocks on temperate continental and Mediterranean mountains, as well as gorges affected by Mediterranean climate influences reaching deeper inland. On its wide stem, this species develops leaves built only of palisade parenchyma or the mesophyll is differentiated, but in such leaves the palisade tissue takes up more volume. Earlier anatomical investigations of other populations of species *M. thymifolia* (Ševarda et al. 1979) also noted the appearance of isobilateral and dorsiventral leaves in plants from different populations. The noted phenotype plasticity between the populations of the same species is probably caused by different environmental conditions, so populations inhabiting areas with better water supply develop dorsiventral leaves (population from Ibar gorge). *M. albanica* leaf mesophyll consist only of palisade parenchyma and is probably the result of water deficit in the leaf caused by intense lighting in the habitat. The vascular cylinder in the stem, and especially the xylem, are well-developed. The entire structure of the shoot of *M. albanica* ensures its survival on "open" limestone, rocky grounds and screes in sub-Mediterranean climate zones.

The species of section *Pseudomelissa* have larger leaves, isobilateral or dorsiventral, wide vascular tissue, especially xylem, and more developed parenchyma tissue in the pith than the species of *Micromeria* section. All the investigated species of section *Micromeria* have small, lanceolate, dorsiventral leaves, revolute on the margin toward the abaxial side, with sclerenchyma in the shape of islands under the phloem of the central vein and larger vascular bundles, and under the epidermis where the leaf bends toward the abaxial side, as well as on the surface of the stem phloem. Due to the smaller stem radius, all the other stem tissues take up less area than in the species of section *Pseudomelissa.*

According to the presented results we can conclude that the determined anatomical differences between the species belonging to different sections are the adaptive response to habitat conditions, but certainly reflect deeper genetic differences, and in that way have a potential diagnostic and systematic value. Therefore, our anatomical data supports the idea of separation of the two sections into different genera as suggested by Bräuchler et al. (2005). However, in order to confirm that the anatomical data supports the idea of segregation of the two sections into two genera, the analysis needs to include other representatives of genus *Clinopodium* defined on the basis of molecular data by Bräuchler and co-authors.

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