ACTA BOTANICA CROATICA
CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

ACCEPTED AUTHOR VERSION OF THE MANUSCRIPT
80(1), 1st April 2021

Morphological and chemical evidence of Teucrium × rohlenae K.Malý (Lamiaceae), a new hybrid in Croatia.

Miloš Zbiljić, Branislava Lakušić, Mirjana Marčetić, Sandro Bogdanović, Dmitar Lakušić

Please cite this article as: Zbiljić M, Lakušić B, Marčetić M, Bogdanović S, Lakušić D: Morphological and chemical evidence of Teucrium × rohlenae K.Malý (Lamiaceae), a new hybrid in Croatia. Acta Botanica Croatica, DOI: 10.37427/botcro-2020-033

Morphological and chemical evidence of *Teucrium* × *rohlenae* K.Malý (Lamiaceae), a new hybrid in Croatia

Miloš Zbiljić[1*], Branislava Lakušić[1], Mirjana Marčetić[2], Sandro Bogdanović[3,4], Dmitar Lakušić[5]

[1] University of Belgrade, Faculty of Pharmacy, Department of Botany, Vojvode Stepe 450, 11060 Belgrade, Serbia
[2] University of Belgrade, Faculty of Pharmacy, Department of Pharmacognosy, Vojvode Stepe 450, 11060 Belgrade, Serbia
[3]University of Zagreb, Faculty of Agriculture, Department of Agricultural Botany, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[4] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[5] University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", Takovska 43, 11221 Belgrade, Serbia

*Corresponding author e-mail: milos.zbiljic@pharmacy.bg.ac.rs

Running title: *Teucrium* × *rohlenae* in Croatia

Abstract – Several natural hybrids between *Teucrium montanum* L. and *T. polium* L. have been described in certain parts of the area where this two species are in sympatry. A new population with intermediate individuals that share morphological characteristics of both *T. montanum* and *T. polium* is found in Croatia (Bisko near Trilj), and most likely represent a hybridogenous taxon. The aim of this study is to compare morpho-anatomical and phytochemical characteristics of two parental species and their putative hybrid, as well as to determine the most important distinguishing characters between these three groups. In order to describe the variability and significance of morpho-anatomical and phytochemical differentiation of analysed groups several multivariate statistical analysis were conducted (PCA, CDA, DFA, UPGMA-clustering). All analyses confirm the separation of two species, *T. polium* and *T. montanum*, and reveal intermediate position of their putative hybrid. In this way, data of morphological and anatomical analysis together with data of the composition of the essential oils support the assumption of the hybridogenous origin of intermediate individuals. Based on descriptions and original indication of parental species of previously described hybrids *T.* × *castrense* Verg., *T.* × *bogoutdinovae* Melinkov and *T.* × *rohlenae* K.Malý, we found that intermediate specimens collected in Croatia perfectly match with *T.* × *rohlenae*, a hybridogenous taxon discovered by Rohlena in the vicinity of Kotor in Montenegro, and thus prove a presence of a new natural hybrid in the flora of Croatia.

Keywords: Adriatic flora, anatomy, hybridization, morphology, phytochemistry, *Teucrium*

Introduction

The genus *Teucrium* L. is represented by approximately 250 species whose centre of the diversity is in the Mediterranean region (Harley et al. 2004). The genus includes shrubs, subshrubs, and herbaceous perennials, rarely annual or biennial plants, which mostly grow on open exposed rocky grounds, rock crevices and screes. Based on the characteristics of calyx and life forms the genus *Teucrium* is separated into seven sections. The section *Polium* (Mill.) Schreb. comprises of 19 species, where *T. montanum* L. and *T. polium* L. belong to the same phylogenetic lineage (Tutin and Wood 1972, Salmaki et al. 2016). As a result of wide distribution of these two species, chromosome numbers varies greatly within this area. *T. montanum* is diploid or tetraploid with chromosome numbers 2n = 13, 22, 26, 26+0–2B, 26+0–7B, 30, 60. Chromosome numbers for *T. polium* are 2n = 26, 26+3–4B, 52, 78, and it can be found as diploid, tetraploid and hexaploid (Ranjbar et al. 2018).

These two taxa are semi-woody, evergreen small shrubs. *T. polium* is erect up to 45 cm high, whereas *T. montanum* is usually prostrate, growing up to 30 cm. The most prominent morphological differences of this species are observed in the indumentum and leaf shape. *Teucrium polium* is distinguished by a very dense indumentum composed of glandular and non-glandular multicellular, uniseriate, branched hairs, whereas *T. montanum* has a less dense indumentum composed of glandular and non-glandular multicellular, uniseriate and unbranched hairs (Jurišić Grubešić et al. 2007, Lakušić et al. 2010). *Teucrium polium* is characterised by dense, hairy calyx, in contrast to calyx of *T. montanum* whose calyx has less hairs. The calyx teeth of *T. polium* are moderately obtuse, while *T. montanum* has acute often setaceous teeth (Maurer 1967, Diklić 1974).

These two species are found in sympatry in certain parts of their distribution, with several hybridogenous taxa being described as: *T.* × *castrense* Verg., *T.* × *bogoutdinovae* Melinkov and *T.* × *rohlenae* K.Malý. The firstly described hybridogenous taxon between *T. montanum* and *T. polium* was collected in July 1907 and June 1908 near the town Castres in south France by Verguin (Verguin 1908). Verguin named that plant *T.* × *castrense*, with *T. polium* ssp. *polium* Briquet and *T. montanum* L. as supposed parental species. Fifty years later, Maurer found individuals with intermediate morphological characteristics between species *T. montanum* and *T. polium* on dunes near Lignano, North Italy (Maurer 1967). This was the first finding of *T.* × *castrense* in Italy (Maurer 1967). Another hybridogenous taxon has been described by Rohlena in the area near Kotor in Montenegro (Rohlena 1922). However, according to the Shenzhen Code and Art. H.2.1. (Turland et al. 2018) it was invalidly described as *T. montanum* × *polium* (and Malý validly renamed it as *T.* × *rohlenae* (Malý 1950). Subsequently, *T.* × *bogoutdinovae* from Moldova have been described (Melnikov 2014). The species *T. reuticum* Bogoutdinova and *T. polium* were listed as parents for *T.* × *bogoutdinove* which was described on a rocky terrace of the river Reut (Bogoutdinova 1991). Given that *T. reuticum* is considered as a heterotypic synonym of *T. montanum* (Eur+Med 2006), *T.* × *bogoutdinove* can also be treated as a hybrid between *T. montanum* and *T. polium.*

We conducted several field trips, during June 2018 in Bisko near Trilj in Croatia (Fig. 1), where *T. montanum* and *T. polium* grow sympatrycally. There we found individuals with intermediate morphological characteristics between these two species, which represent hybridogenous taxon.

The aim of our study is to compare morpho-anatomical and phytochemical characteristics of parents and their putative hybrid and to determine the most important characters of differentiation.

Materials and methods

Because of the small population sizes of *T. montanum* and the putative hybrid in their narrow hybridization zone (Bisko near Trilj, Croatia), and their presumed endangered status, destructive sampling was limited to a minimum, which resulted in a relatively limited number of analyzed individuals. A total of 29 specimens were selected and scored for analyses. Those include 10 specimens of *T. polium*, 13 specimens of *T. montanum* and six specimens of their putative hybrid. Specimens were deposited in the Herbarium of the Institute of Botany and Botanical Garden of the Faculty of Biology, University of Belgrade (BEOU – herbarium code follows Thiers 2019).

Analysed samples are as follows:

T. montanum

- CROATIA, Trilj, Bisko (43.579400° N ,16.695995° E), pseudomacchia, leg.: D. Lakušić, B. Lakušić, M. Zbiljić, 30.06.2018, (BEOU – 54028)

T. polium ssp. *capitatum*

- CROATIA, Trilj, Bisko (43.579400° N ,16.695995° E), pseudomacchia, leg.: D. Lakušić, B. Lakušić, M. Zbiljić, 30.06.2018, (BEOU – 54027)

Putative hybrid "*T. montanum* × *polium*"

- CROATIA, Trilj, Bisko (43.579400° N ,16.695995° E), pseudomacchia, leg.: D. Lakušić, B. Lakušić, M. Zbiljić, 30.06.2018, (*Teucrium* × *rohlenae* K.Malý, BEOU – 54029)
- CROATIA, Dalmatinska Zagora, Vrgorac, Mt. Matokit, Zavojane, Zekulić-Roč (43.244743° N, 17.264857° E), rocky ground pasture, leg.: M. Vukojević, 13.06.2015, (sub. *T. montanum* L., ZAGR – 40074).

For morphological analysis samples of stem with leaves (without terminal inflorescence), leaves, bracts, calyx and flowers from terminal inflorescences were stored in solution of glycerol and ethanol (1:1). Anatomical sections of leaves were preserved on permanent slides, prepared by the standard method for light microscopy. Cross-sections of leaves were cut on a Reichert sliding microtome (up to 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 (Lakušić et al. 2010). Morphometric analysis included 21 morphological and 23 anatomical characters.

Plant material (3-4 g) was extracted with *n*-hexane (1:10) during 24 hours at room temperature. The extraction was repeated, extracts united and concentrated under the reduced pressure.

The qualitative and quantitative analysis of hexane extracts was done using the GC and GC/MS method. The GC analysis was performed on an Agilent 6890N GC system with a 5975 MSD and FID detectors. The column was HP-5 MS (30 m × 0.25 mm, 0.25 μm film thickness). Two μL were injected and the injector temperature was 200 °C with a 10:1 split ratio. Helium was used as a carrier gas (1.0 mL min^{-1}, constant flow mode). The column temperature was linearly programmed (60–280 °C, rate 3 °C min^{-1}, 280 °C for 5 min). The transfer line was heated at 250 °C and the FID detector at 300 °C. EI mass spectra (70 eV) were obtained in the m/z range of 35–550. The retention indices (Kovat's retention index, RI) of essential oil components were experimentally determined relative to two series of *n*-alkanes (C_8-C_{20} and C_{21}-C_{40}). Their spectra were obtained under the same chromatographic conditions. Identification of compounds was based on comparison of their retention indices (RI) and mass spectra with those from authentic samples and/or the NIST AMDIS software, Wiley, the Adams

database and available literature (Adams 2007). Relative percentages of the identified compounds were computed on the basis of the peak areas obtained by FID detector.

The chemical analysis was performed on all compounds (28) of hexane extracts or only on terpene (17) compounds.

In order to describe the variability and significance of morphoanatomical and phytochemical differentiation of analysed groups Principal component analyses (PCA) and Canonical discriminant analysis (CDA) were used, as well as an UPGMA (unweighted pair group method with arithmetic mean) clustering analysis based on Mahalanobis distances for mopho-anatomical data and Pearson's distances for chemical data in the UPGMA clustering method. Discriminant function analysis (DFA) was used to estimate the contribution of individual characters to the overall discrimination. Statistical analyses were performed using Statistica v.8.0 (StatSoft 2007).

Results

Multivariate analysis of the morpho-anatomical characters

Principal component analysis (PCA) and Canonical discriminant analysis (CDA) performed on morpho-anatomical characters revealed clear separation of *T. polium* and *T. montanum*, as well as clear intermediate position of the putative hybrid (Fig. 2A,B). Both analyses revealed a clear separation of three groups along x-axis, whereas CDA revealed clear separation of putative hybrid along both discriminant axis.

Cluster analysis performed on all morphoanatomical characters classified *T. polium* and the putative hybrid in the first cluster, while *T. montanum* belongs to the second cluster (Fig. 3A). On the other hand, the cluster analysis based on leaf anatomy revealed the hybrid individuals are closer to *T. montanum* (Fig. 3B).

Characters that predominantly contribute to the distinction among the groups are: coverage of adaxial indumentum, thickness of cuticle, number of teeth on the edge of a leaf, bract length, stem length, frequency of lateral wisp on stem's nodes, distance between calyx base and tooth base, length of the narrow part of the calyx teeth and number of terminal inflorescences (Tab. 1).

Chemical analysis of volatile compounds

The chemical analysis of the aerial parts' volatile compounds revealed that total hydrocarbons (saturated and unsaturated) were the main compounds in all investigated samples (82.9–90.7%). Sesquiterpene hydrocarbons and oxygenated sesquiterpenes were present in amounts that did not exceed 10.2% and monoterpene hydrocarbons only about 1% (Tab. 2).

The chemical composition of the extracts of the studied groups was quite similar. Saturated and unsaturated hydrocarbons represented the main compounds (33.2-75.0% and 14.1-49.6%). The saturated hydrocarbons containing 25, 29 and 31 C atoms, pentacosane (33.6% and 46.5%), nonacosane (9.2-17.5), untriacontane (0.2-12.3%) as well as unsaturated untriacontene (11.4-48.4%) were dominant.

The cluster analysis of all compounds of hexane extracts (Fig. 4A) revealed the similarity in the composition of *T. polium* and putative hybrids aerial parts volatiles. The extracts of *T. polium* and the putative hybrid were characterised by pentacosane (46.5 and 33.6%), compound that was not present in the *T. montanum* volatiles. Unsaturated untriacontene (48.4%) was the main compound in the *T. montanum* extract.

On the other hand different results were obtained when only terpene compounds were used for cluster analysis (Fig. 4B). There was a certain similarity between the composition of monoterpenes and sesquiterpenes in extracts of *T. montanum* and the putative hybrid, mainly represented by higher germacrene D content (3.7% and 2.5%).

Discussion

Intermediate position of putative hybrid

According to the leaf shape and the edge of a leaf with one or more teeth, majority of the hybrid individuals resemble *T. polium* (Fig. 5). Indumentum of the leaf of the hybrid is less dense than *T. polium* leaf indumentum, but more dense than indumentum of leaf *T. montanum*. In addition, non-glandular uniseriate branched hairs that dominate in *T. polium* and absent in *T. montanum*, are present in the putative hybrid. In the same time, the hybrid individuals contain many non-gladular uniseriate unbranched trichomes present in *T. montanum*, but absent in *T. polium* (Jurišić Grubešić et al. 2007, Lakušić et al. 2010, Fig. 5).

Also, some hybrid individuals have short dense tufts of the lateral shoots on the stem nodes, features typical for *T. polium*, but absent in *T. montanum* (Fig. 5).

The important indicator of transition in morphological characters of the putative hybrid is the colour and number of flowers and the consistency of the inflorescences. *Teucrium polium* has the compact inflorescence with a large number of small white flowers (between 13 and 51, in average 30); *T. montanum* has uncompact inflorescence with a small number of large pale yellow flowers (between 6 and 16, in average 11), and the hybrid individuals has compact inflorescence with a small number of white flowers (between 7 and 20, in average 13) (Fig. 5). Calyx morphology clearly shows intermediate forms. Based on calyx dimension (calyx tube length, calyx tooth length and width) putative hybrids are between parents: smaller than *T. montanum* and larger than *T. polium*. Additionally, the putative hybrid possesses setae on the top of the acutiform setaceous calyx teeth that are shorter than the setae of *T. montanum*, while setae are missing in *T. polium* (Fig. 5).

Finally, and in their general habitus, putative hybrid has a transitional character. Namely, flowering stems of hybrid individuals are erect like in *T. polium*, while lateral vegetative shoots are decumbent like in *T. montanum.*

A previous work showed that *Teucrium* species from the Balkan Peninsula usually contain small quantities of essential oil (Kovačević et al. 2001). Sesquiterpenes were the main compounds in the essential oils of aerial parts of *T. montanum* and *T. polium*. In the investigated samples from Montenegro germacrene D (15.0%), α-pinene (12.4) and β-eudesmol (10.1%) were the most abundant in *T. montanum*, while β-pinene (19.8%) and germecrene D (11.9%) in the oil of *T. polium* (Kovačević et al. 2001). The essential oil from aerial parts of *T. montanum* from Croatia contained germacrene D (17.2%), β-pinene (12.3%) and β-caryophyllene (7.1%), while β-caryophyllene (52.0%) dominated in the oil of *T. polium* (Bezić et al. 2011). Contrary, germacrene D (31%) was dominant in the essential oil of *T. polium* from Serbia, while δ-cadinene (8.1%) and β-caryophyllene (5.1%) were the main compounds in *T. montanum* (Radulović et al. 2012). Furthermore, the most recent study of the variability of essential oil of different populations of *T. montanum* from central and south Balkan Peninsula (14 populations from Serbia, Greece and Albania), revealed extremely large differences in the chemical composition of essential oils of the aerial parts of *T. montanum* (Marčetić et al. 2018). This study showed that the composition of essential oils was quite variable and the main compounds in almost all oils were germacrene D (trace-45.5%), sabinene (trace-23.1%), α-pinene (trace-20.7%), limonene (trace-20.4%), (*E*)-caryophyllene (2.9-14.5%), γ-cadinene (trace-13.8%) and δ-cadinene (trace-12.0%).

These results show that the composition of the essential oils of the two species is not clearly different, given that they contain similar compounds and similar pattern of the variability. It indicates that only on the basis of the chemical composition of essential oils is not possible to distinguish them, what is in accordance with the results of several recent studies that showed that variation in the composition of essential oils within a species appears to be the rule

rather than the exception, and that the geographical distribution of different types of essential oils of plants is strongly co-related with the environmental conditions including climate, geological, pedological and phytosociological characteristics of the habitats (Kuštrak et al. 1984, Franz 1993, Jug-Dujaković et al. 2012, Lakušić et al. 2012).

However, together with data of morphological and anatomical analysis, as well as the data of the composition of the analysed essential oils also support the assumption of the hybridogenous origin of intermediate individuals.

Taxonomical treatment and distribution

Brief descriptions of hybridogenous taxa *T.* × *castrense* and *T.* × *rohlenae* largely overlap with features of collected hybrid individuals (Verguin 1908, Rohlena 1922, Malý 1950, Maurer 1967). Considering that subspecies *T. polium* ssp. *polium* as one parent of *T.* × *castrense* is distributed only in the western parts of the Mediterranean (Tutin and Wood 1972, Euro+Med 2006) we have excluded the possibility that hybridogenic individuals from Croatia belong to this taxa.

In the same time, given that *T. polium* ssp. *capitatum* (L.) Archangeli and *T. polium* ssp. *vincentinum* (Rouy) D.Wood are distributed in Croatia (Nikolić 2019), as well as that plant from Trilj belongs to subspecies *T. polium* ssp. *capitatum* also distributed in Montenegro (Euro+Med 2006) we suggest to examined the hybrid include in taxon *T.* × *rohlenae*, originally found and described in area near Kotor in Montenegro (Rohlena 1922, Malý 1950).

We have analysed ZAGR herbarium specimens of *T. polium* and *T. montanum* collected in Croatia, and we found specimens that perfectly match the appearance of the hybrid *T.* × *rohlenae*. These specimens were determined as *T. montanum* and were collected in 2015 on the mountain Matokit near Vrgorac in Dalmatian Zagora.

Even though *T.* × *rohlenae* has been registered only in three locations (Montenegro: Kotor, Croatia: Trilj and Matokit, Fig. 1), it is likely that its distribution is much wider, so we can expect hybrid individuals in areas where *T. montanum* and *T. polium* grow in sympatry.

References

Adams, R.P., 2007: Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, Illinois.

Bezić, N., Vuko, E., Dunkić, V., Ruščić, M., Blažević, I., Burčul, F., 2011: Antiphytoviral activity of sesquiterpene-rich essential oils from four Croatian *Teucrium* species. Molecules 16, 8119–8129.

Bogoutdinova, T.R., 1991: A new species of the genus *Teucrium* (Lamiaceae) from Moldova. Botanicheskii Zhurnal (Leningrad) 76, 745–746 (in Russian).

Diklić, N., 1974: *Teucirum* L. In: Josifović, M. (ed.), Flora Srbije 6, 349–357. Srpska akademija nauka i umetnosti, Beograd.

Euro+Med, 2006: Euro+Med PlantBase – the information resource for Euro-Mediterranean plant diversity. Retrieved April 13, 2020 from http://ww2.bgbm.org/EuroPlusMed/

Franz, C.H., 1993: Genetics. In: Hay, R.K.M., Waterman, P.G. (eds.), Volatile oil crops: Their biology, biochemistry and production, 63–96. Longman, Harlow.

Harley, R.M., Atkins, S., Budantsev, A.L., Cantino, P.D., Conn, B.J., Grayer, R., Harley, M.M., De Kok, R., Krestovskaja, T., Morales, R., Paton, A.J., Ryding, O., Upson, T., 2004. Labiatae. In: Kubitzki, K., Kadereit, J.W., Jeffrey, C. (eds.), The families and genera of vascular plants 8, 196–203. Springer, Berlin.

Jug-Dujaković, M., Ristić, M., Pljevljakušić, D., Dajić-Stevanović, Z., Liber, Z., Hančević, K., Radić, T., Šatović, Z., 2012: High diversity of indigenous populations of Dalmatian sage

(*Salvia officinalis* L.) in essential-oil composition. Chemistry and Biodiversity 9, 2309–2323.

Jurišić Grubešić, R., Vladimir-Knežević, S., Kremer, D., Kalođera, Z., Vuković, J., 2007: Trichome micromorphology in *Teucrium* (Lamiaceae) species growing in Croatia. Biologia (Bratislava) 62, 148–156.

Kovačević, N., Lakušić, B., Ristić, M., 2001: Composition of the essential oils of seven *Teucrium* species from Serbia and Montenegro. Journal of Essential Oil Research 13, 163–165.

Kuštrak, D., Kuftinec, J., Blažević, N., 1984: Yields and composition of sage oils from different regions of the Yugoslavian Adriatic coast. Journal of Natural Products 47, 520–524.

Lakušić, D., Ristić, M., Slavkovska, V., Šinžar-Sekulić, J., Lakušić, B., 2012: Environmental-related variations of the composition of the essential oils of rosemary (*Rosmarinus officinalis* L.) from the Balkan Peninsula. Chemistry and Biodiversity 9, 1286–1302.

Lakušić, B., Stevanović, B., Jančić, R., Lakušić, D., 2010: Habitat-related adaptations in morphology and anatomy of *Teucrium* (Lamiaceae) species from the Balkan peninsula (Serbia and Montenegro). Flora 205, 633–646.

Malý, K., 1950: *Teucrium* × *rohlenae*. In: Beck, G., Maly, K. (eds.), Flora Bosnae et Hercegovinae IV – Sympetalae (Gymopetalae) Pars 1, 29. Svjetlost, Sarajevo.

Marčetić, M., Zbiljić, M., Lakušić, D., Lakušić, B., 2018: Variability of essential oil of different populations of *Teucrium montanum* L. (Lamiaceae) from Balkan Peninsula. Botanica Serbica 42 (Suppl. 1), 133.

Maurer, W., 1967: Der Bastard *Teucrium* × *castrense* = *Teucrium montanum* × *polium* bei Lignano in Italien. Phyton (Horn, Austria) 12, 96–101.

Melnikov, D., 2014: A new nothospecies of the genus *Teucrium* L. (Lamiaceae) from Republic of Moldova. Novitates Systematicae Plantarum Vascularium 45, 81–83.

Nikolić, T., 2019: Flora Croatica 4 - Vaskularna flora Republike Hrvatske. Alfa d.d., Zagreb.

Radulović, N., Dekić, M., Joksović, M., Vukičević, R., 2012: Chemotaxonomy of Serbian *Teucrium* species inferred from essential oil chemical composition: the case of *Teucrium scordium* L. ssp. *scordioides.* Chemistry and Biodiversity 9, 106–122.

Ranjbar, M., Mahmoudi, C., Nazari, H., 2018: An overview of chromosomal criteria and biogeography in the genus *Teucrium* (Lamiaceae). Caryologia 71, 63–79.

Rohlena, J., 1922: Additamenta ad floram dalmaticam. Acta Botanica Bohemica 1, 28–34.

Salmaki, Y.S., Kattari, Heub, G., Bräuchler, C., 2016: Phylogeny of non-monophyletic *Teucrium* (Lamiaceae: Ajugoideae): implications for character evolution and taxonomy. Taxon 65, 805–822.

StatSoft. Inc., 2007: STATISTICA, ver. 8.0. Retrieved October 1, 2019 from www.statsoft.com

Thiers, B., 2019: Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's virtual herbarium. Retrieved October 1, 2019 from http://sweetgum.nybg.org

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A. M., Prado, J., Price, M.J., Smith, G.F. (eds.), 2018: International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten.

Tutin, T.G., Wood, D., 1972: *Teucrium*. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D., Valentine, D., Walters, S. (eds.), Flora Europaea 3, 129–135. Cambridge University Press, Cambridge.

Verguin, M.L., 1908: Un *Teucrium* hybride nouveau de la section *Polium* Benth. Bulletin de la Société Botanique de France 55, 607–611.

Tab. 1. Discriminant functional analysis summary of the 18 most important morpho-anatomical characters for 29 specimens of *Teucrium montanum*, *T. polium* and putative hybrid in their narrow hybridization zone in Bisko near Trilj (Croatia). Invariable and highly correlated characters were excluded from analysis.

Anatomy	Wilks' Lambda	Partial Lambda	F-remove (2.8)	P-level
Radius of the central nerve	0.001401	0.990041	0.040235	0.96076
Coverage of adaxial indumentum	0.001605	0.864346	0.627779	0.55815
Number of capitate hairs C	0.001599	0.867574	0.610556	0.56654
Thickness of adaxial epidermal cells	0.001537	0.90247	0.432283	0.66333
Thickness of cuticula	0.001675	0.828184	0.829843	0.47044
Morphology				
Leaf surface	0.002156	0.643504	2.215968	0.17148
Leaf base width	0.001557	0.890808	0.490304	0.62971
Number of teeth on edge of leaf	0.00197	0.704347	1.679023	0.24612
Bract length	0.001626	0.853201	0.688230	0.52991
Average width of bract	0.001747	0.794168	1.036716	0.39779
Stem length	0.001829	0.758577	1.273031	0.33113
Frequency of lateral tuft on stem's nodes	0.001786	0.776835	1.149100	0.36418
Average length of first three internodes	0.002502	0.554452	3.214335	0.09451
Distance between calyx base and tooth base	0.001631	0.850866	0.701094	0.52414
Length of narrow part of tooth	0.001512	0.917384	0.360227	0.70828
Width of tooth	0.001532	0.905726	0.416345	0.67296
Number of flowers in terminal inflorescence	0.001944	0.71378	1.603966	0.25957
Number of terminal inflorescences	0.001763	0.787023	1.082446	0.38366

Tab. 2. The chemical composition (%) of the volatiles of *Teucrium montanum*, *T. polium* and putative hybrid in their narrow hybridization zone in Bisko near Trilj (Croatia). RI – retention index, t – trace (< 0.1%).

Compund	RI	*T. montanum*	putative hybrid	*T. polium*
alpha-Thujene	922	0.39	t	t
alpha-Pinene	929	0.18	0.29	t
Sabinene	968	0.40	0.50	t
beta-Pinene	972	0.13	0.16	0.18
beta-Bourbonene	1381	0.49	0.25	t
(E)-Caryophyllene	1416	t	0.97	2.32
(Z)-beta-Farnesene	1439	0.90	0.00	0.00
alpha-Humulene	1450	0.00	t	0.18
(E)-beta-Farnesene	1452	1.01	0.00	t
Germacrene D	1478	3.74	2.46	1.42
Bicyclogermacrene	1493	0.36	0.30	0.20
alfa-Bisabolene	1498	0.30	t	0.00
beta-Bisabolene	1505	t	0.00	0.27
cis-Sesquisabinene hydrate	1540	1.81	0.00	0.00
trans-Sesquisabinene hydrate	1576	0.48	0.00	0.00
epi-alpha-Cadinol	1637	0.51	0.00	0.00
Hexahydrofarnesyl acetone	1838	0.62	t	0.30
Tetracosane	2397	t	t	0.10
Pentacosene	2489	1.22	2.93	2.74
Pentacosane	2509	0.00	33.56	46.53
Hexacosane	2589	0.31	0.26	0.34
Heptacosane	2689	3.93	2.88	2.61
Octacosane	2789	0.93	0.57	0.70
Nonacosane	2890	17.45	13.43	9.20
Triacontane	2990	3.12	1.47	1.09
Untriacontene	3092	48.43	20.61	11.40
Untriacontane	3125	0.16	11.94	12.32
Dotriacontane	3187	7.31	3.09	2.13
Percentage of identified compounds		94.18	95.67	94.12
Monoterpene hydrocarbons		1.10	0.95	0.18
Sesquiterpene hydrocarbons		6.80	3.98	4.48
Oxygenated sesquiterpenes		3.42	0.00	0.30
Saturated hydrocarbons		33.21	67.20	75.02
Unsaturated hydrocarbons		49.65	23.54	14.14

Fig. 1. Known occurrences of *Teucrium* × *rohlenae* K.Malý based on literature data (A) and recently discovered plants (B, C). A – Kotor in Montenegro, B – Matokit, and C – Trilj, both in Croatia.

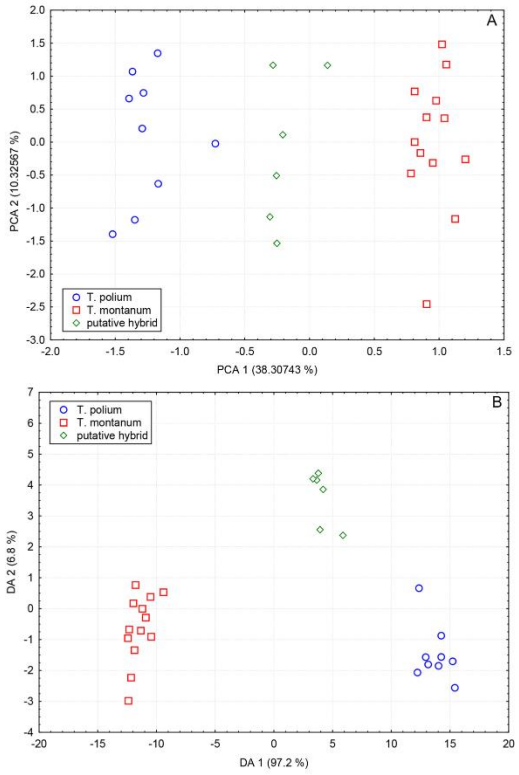

Fig. 2. Multivariate analysis of the morpho-anatomical data for the *Teucrium montanum*, *T. polium* and putative hybrid in their narrow hybridization zone in Bisko near Trilj (Croatia). A – Principal component analysis (PCA), B – Canonical discriminant analysis (CDA).

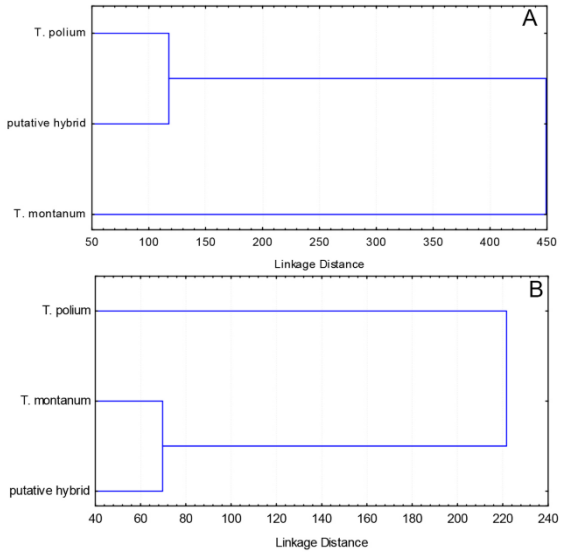

Fig. 3. Cluster (UPGMA) analysis of the morpho-anatomical data for the *Teucrium montanum*, *T. polium* and putative hybrid in their narrow hybridization zone in Bisko near Trilj (Croatia). A – for all morpho-anatomical characters, B – only for the anatomical characters.

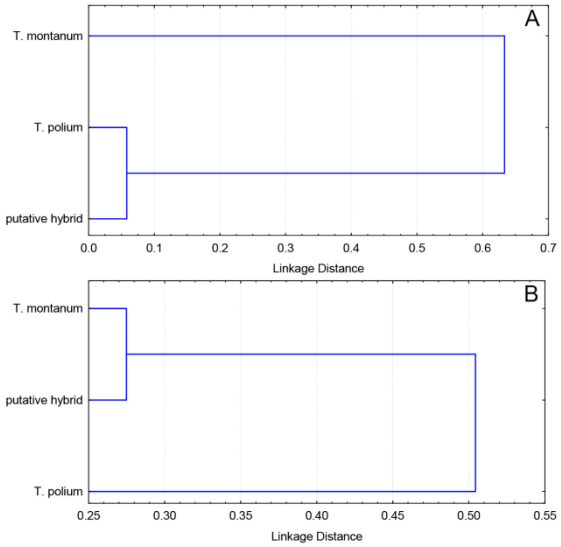

Fig. 4. Cluster (UPGMA) analysis of the chemical data of volatile compounds for the *Teucrium montanum*, *T. polium* and putative hybrid in their narrow hybridization zone in Bisko near Trilj (Croatia). A – for all compounds of hexane extracts (28), B – only of the terpene compounds (17).

Fig. 5. Morphological characteristic of inflorescence, leaves and calyx and anatomical characteristic of leaf cross section of *Teucrium montanum* (m), putative hybrid (×) and *T. polium* (p) in their narrow hybridization zone in Bisko near Trilj (Croatia). 1 – inflorescence, 2 – leaves, 3 – abaxial leaf side, 4 – adaxial leaf side, 5 – calyx, 6 – leaf cross section: m6 – only non-gladular uniseriate unbranched trichomes in *T. montanum*, ×6 – both branched and uniseriate nonglandular trichomes in putative hybrid, p6 – only uniseriat branched nonglandular trichomes on both side of leaf in *T. polium.*