Article

Micromorphological Traits of Balcanic Micromeria and Closely Related Clinopodium Species (Lamiaceae)

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Abstract: A study of the trichomes types and distribution and pollen morphology was carried out in nine Micromeria taxa (M. cristata ssp. cristata, M. cristata ssp. kosaninii, M. croatica, M. graeca ssp. graeca, M. graeca ssp. fruticulosa, M. juliana, M. kernerii, M. longipendulcata and M. microphylla) and five closely related Clinopodium species (C. dalmaticum, C. frivaldszkianum, C. puteugium, C. spargillifolium and C. thymifolium) from the Lamiaceae family of the Balkan Peninsula. By scanning electron microscope, non-glandular trichomes, peltate and capititate trichomes were observed on the calyx, leaves and stem of the studied species. Two subtypes of capititate trichomes were observed in Micromeria species: subtype 1 (consisting of a basal epidermal cell and an elliptically shaped head cell) and subtype 2 (consisting of a basal epidermal cell, two to three stalk cells and a round head cell). In Clinopodium species, three types of capititate trichomes were observed: subtype 1, subtype 3 (consisting of a basal epidermal cell, a short peduncle cell, and a single round head cell), and subtype 4 (consisting of a basal epidermal cell, a stalk cell, and an elongated head cell). These results support the recent transfer of Micromeria species from the section Pseudomelissa to the genus Clinopodium.

Keywords: Balkan Peninsula; capititate trichomes; glandular trichomes; peltate trichomes; pollen; SEM
1. Introduction

The family Lamiaceae is one of the largest families among the eudicots, consisting of more than 240 genera and more than 7200 species [1]. Many of species belonging to this family are aromatic and medicinal plants due to the presence of glandular trichomes that produce essential oil. One of the plant groups in this family is the genus Micromeria Bentham and closely related species from the genus Clinopodium L. The genus Micromeria belongs to subtribe Menthae and tribe Mentheae (subfam. Nepetoideae). According to Briquet [2], Killick [3] and Greuter et al. [4], it is considered part of the indistinctly defined “Satureja” complex. However, this opinion is slowly being abandoned today. According to Bentham [5], Micromeria species form a separate genus and this opinion prevails today [6,7]. The genus Micromeria includes 54 [8] to 70/20 [9] perennial herbs, subshrubs and shrubs, rarely annual herbs. According to Bräuchler et al. [8,10], the range of Micromeria species extends from India and China to the Macaronesian Archipelago and from the Mediterranean to South Africa and Madagascar. Bräuchler et al. [10] showed that the genus Micromeria is polyphyletic and that a revision of this genus is necessary. In accordance with this opinion, Bräuchler et al. [11] transferred the taxa included to the section Pseudomelissa from the genus Micromeria to the genus Clinopodium.

Micromeria species are more or less aromatic plants with opposite leaves and thyrsoid or sometimes racemelike, dense or loose, spikelike inflorescences. The leaf blade is entire or with a few indistinct teeth, often with a thickened and revolute margin. The petiole is distinct, short or minute. The calyx is tubular in the lower part and five-lobed in the upper part, almost actinomorphic to distinctly two-lipped, mostly hairy in the mouth. The upper lip is three-lobed; the lower lip is two-lobed, divided to the base and their lobes are mostly narrower and longer than those of the upper lip. The teeth are ciliate or not. The corolla is strongly two-lipped, with a straight tube. The upper lip is emarginated; the lower lip is three-lobed with middle lobe broader than lateral [8,10,12]. Clinopodium species are also aromatic plants with opposite leaves and flowers arranged in opposite, axillary, dense, many-flowered cymes. The calyx is tubular with a curved tube and more or less two-lipped in the upper part, hairy in the mouth. The teeth are almost never ciliate. The corolla is two-lipped, with straight tube. The upper lip is entire or emarginated, while the lower lip is three-lobed with middle lobe wider than lateral [8,10].

The type and distribution of secretory and non-secretory trichomes play an important role in taxonomic study. The study of trichomes in Micromeria and Clinopodium species has been reviewed by several authors [13–19]. Palynology also provides valuable taxonomic data and information on the origin and evolution of species [20]. The pollen of Micromeria and Clinopodium species has also been studied by several authors [15,16,21–24].

This study represents a continuation of our investigations of Balkan Micromeria and Clinopodium species. Instead of studying additional species individually, we included all available Micromeria and Clinopodium species from Balkan Peninsula in this paper. Of the genus Clinopodium, only species transferred from Micromeria sect. Pseudomelissa were included in this study. So, the aim of this study is to gain insight into the micromorphological traits of Micromeria species as well as the closely related Clinopodium species recently transferred from the Micromeria sect. Pseudomelissa. The main objective is to determine whether micromorphological traits support the transfer of species from section Pseudomelissa to the genus Clinopodium.
2. Results and Discussion

2.1. Trichomes

The non-glandular and glandular trichomes were observed in the studied *Micromeria* and *Clinopodium* species. Non-glandular (NG) trichomes have been observed on the leaves, calyces, and stem of both genera. These trichomes are unbranched, uniseriate, bi- to multicellular and most often bent on different sides. They could be called attenuated trichomes because they gradually taper from base to tip [25]. The length of the NG trichomes is highly variable and ranges from very short trichomes, especially on the adaxial leaf surface, to very long hairs on the calyx and stem (Figures 1 and 2). The surface of these trichomes is warty (Figure 1). The main function of NG trichomes is to protect the plant from water loss. The same type of NG trichomes is present in both *Micromeria* and *Clinopodium* species.

The occurrence and frequency of NG trichomes on the adaxial and abaxial sides of the leaf, the outer side of the calyx and the stem are shown in Tables 1 and 2. All *Micromeria* species studied have a more or less abundant coverage of NG trichomes on the adaxial and abaxial leaf surface (Figure 1). A certain exception is *M. longipedunculata*, in which the upper and lower leaf surfaces are less hairy. The adaxial and abaxial leaf surfaces and stem of *M. graeca* ssp. *fruticulosa* and the stem of *M. cristata* have the highest number of long NG trichomes forming an abundant covering.
In general, *Micromeria* species have a denser indumentum of attenuated trichomes on leaves than *Clinopodium* species. *Clinopodium serpyllifolium* is an exception as it has the densest NG trichomes cover of all *Micromeria* and *Clinopodium* species examined (Figure 2). Studies by Firat et al. [22] also showed very dense indumentum on the leaves and stem of *Clinopodium serpyllifolium* ssp. *sirnakense*. The frequency of NG trichomes on the calyx of *Clinopodium* species is similar to this coverage of NG trichomes in *Micromeria* species. A slightly lower frequency of NG trichomes was observed on the calyx of *C. frivaldszkyanum* and *C. pulegium*, and on the stem of *C. frivaldszkyanum*. The presence of NG trichomes has been previously well documented in *Micromeria* [13,15,16] and *Clinopodium* [13,14,17,18] species, as well as in other Lamiaceae [13,14,26].

Glandular trichomes were observed on all plant parts of the *Micromeria* and *Clinopodium* species examined. These trichomes can be further divided into two main subtypes, namely, peltate and capitate trichomes. Peltate trichomes consist of a basal cell, a very short unicellular stalk and a multicellular head with a large subcuticular space (Figure 1). These trichomes are also known from some other Lamiaceae species [13,26–32]. The distribution and frequency of peltate trichomes on the studied plant parts are shown in Tables 1 and 2. Although peltate trichomes are present on all plant parts studied, they are more abundant on the abaxial leaf surface and on the calyx. They are completely absent on the adaxial side of the leaf in *M. croatica* and *M. longipedunculata* (Table 1). Peltate trichomes were previously reported in *Micromeria fruticosa* [13], *Micromeria myrtifolia* Boiss. et Hohen. [33], *M. croatica* [15], *M. kernerii*, *M. juliana* [16] and *M. longipedunculata* [34]. Husain et al. [14] showed the presence of peltate trichomes on the leaves of *Clinopodium serpyllifolium*, *C. dalmaticum* and *C. thymifolium*. Peltate trichomes were also detected in micrographs of *C. thymifolium* leaves presented by Marin et al. [35]. Mladenova et al. [19] observed these trichomes in the leaves of *C. frivaldszkyanum*, although they did not describe them as peltate.

The capitate trichomes can be further divided into several subtypes. Subtype 1 (C1) capitate trichomes consist of a basal epidermal cell and an elliptically shaped head cell with a larger subcuticular space. C1 trichomes are not erect but can be described as adherent to the surface (Figure 1). This type was observed on both the abaxial and adaxial sides of the leaves, on the stem and on the outer side of the calyx. They are present in all *Micromeria* and *Clinopodium* species studied (Tables 1 and 2). These trichomes were described in detail in *Micromeria croatica* by Kremer et al. [15].
Figure 2. SEM micrographs with different types and distribution of trichomes in *Clinopodium dalmaticum* (Cd) including *Micromeria bulgarica* (CdB), *C. frivaldzkyanum* (Cf), *C. pulegium* (Cp), *C. serpyllifolium* (Cs), and *C. thymifolium* (Ct). Peltate trichome (P), Subtype 1 capitate trichomes (C1), Subtype 3 capitate trichomes (C3) and Subtype 4 capitate trichomes (C4) on the adaxial (1) and abaxial (2) leaf surface, calyx (3), and stem (4).

C1 trichomes have also been observed in *M. kernerii* and *M. juliana* [16]. Although Husain et al. [14] did not mention C1 trichomes in *M. longipedunculata*, unclear SEM micrographs probably show just the C1 type of trichomes. On the other hand, SEM micrographs of *M. fruticosa* presented by Werker et al. [13] did not show C1 trichomes. According to drawings made by Koca [33], it seems that C1 trichomes are also present in *Micromeria myrtifolia*. C1 trichomes were also observed in *Clinopodium dalmaticum*, *C. pulegium*, *C. serpyllifolium* and *C. thymifolium* [17].
Table 1. Occurrence and frequency of trichomes on aerial parts of *Micromeria* taxa.

| Taxon/ Locality       | Trichomes | Leaf | Calyx | Stem |
|-----------------------|-----------|------|-------|------|
|                       | Adaxial   | Abaxial |       |      |
| *M. cristata*          | NG        | +     | ++    | ++   |
| ssp. cristata          | peltate   | +     | +     | ++   |
| Humsko                | capitate C1 | +/++ | +     | +    |
| Brdo                  | capitate C2 | –     | ±/+   | +    |
| *M. cristata*          | NG        | +     | ++    | ++   |
| ssp. cristata          | peltate   | +     | +     | +    |
| Vitosha Mt            | capitate C1 | +     | +     | +    |
|                       | capitate C2 | –     | ±/+   | ±    |
| *M. cristata*          | NG        | ++    | ++    | ++   |
| ssp. cristata          | peltate   | +     | +     | ±/+  |
| Pirin Mt              | capitate C1 | ±     | ±/+   | ±/±  |
|                       | capitate C2 | –     | ±     | ±    |
| *M. cristata*          | NG        | ++    | +     | ++   |
| ssp. cristata          | peltate   | +     | +     | +    |
| Demir                 | capitate C1 | ±/+   | ±/±   | ±/±  |
| Kapija                | capitate C2 | –/±   | ++/±  | ±/+  |
| *M. cristata*          | NG        | ++    | ++    | ++   |
| ssp. cristata          | peltate   | +     | +     | +    |
| Nomos                 | capitate C1 | ±     | ±     | ±    |
| Serron                | capitate C2 | –     | +     | +    |
| *M. croatica*          | NG        | +     | ++    | ++   |
| Rossijev              | peltate   | –     | ++/±  | ±/±  |
| kuk                   | capitate C1 | –     | ±/+   | ±    |
|                       | capitate C2 | ±/+   | ++    | ±/±  |
| *M. croatica*          | NG        | +     | ±/+   | ±/±  |
| Stupinašćevo          | peltate   | –     | ±/+   | ±    |
| capitate C1           | –     | +/±   | ±/±   |
| capitate C2           | –     | ±/+   | ±/±   |
| *M. croatica*          | NG        | +     | ++    | ++   |
| Dubočani             | peltate   | –     | ++/±  | ±/±  |
| capitate C1           | –     | ±/+   | ±    |
| capitate C2           | –     | ±/+   | ±/±   |
| *M. croatica* (syn. *M. pseudocroatica*) | NG | + | + | + |
| Pijavčino             | capitate C1 | ±     | ++/±  | ±/±  |
| capitate C2           | ±     | ±/+   | ±/±   |
| *M. croatica* (syn. *M. pseudocroatica*) | NG | + | + | + |
| Prapratno             | capitate C1 | ±/+  | ++/±  | ±/±  |
| capitate C2           | ±     | ±/+   | ±/±   |
| *M. graeca*            | NG       | ++    | ++    | ++   |
| ssp. graeca           | peltate   | +     | +     | +    |
| Malo                  | capitate C1 | ±     | ++    | +    |
| zlo polje             | capitate C2 | ±     | ±     | +    |
| *M. graeca*            | NG       | ++    | ++    | ++   |
| Plant Species | Trichome Type | Frequency |
|---------------|---------------|-----------|
| ssp. graeca | peltate | + ++ + + |
| Komiža | capitate C1 | ± ++ + + |
| | capitate C2 | ± + ++ ± |
| M. graeca | NG | ++ + +/+/+ + |
| ssp. fruticulosa | peltate | ±/++ + + + |
| Sušac | capitate C1 | ± + + + |
| | capitate C2 | ± + + + |
| M. juliana | NG | + + ++ ++ |
| Grude | peltate | + + ++ |
| | capitate C1 | ± + + + |
| | capitate C2 | - ++ ++ + |
| M. juliana | NG | ++ ++ ++ |
| Cijevna | peltate | + + + + |
| | capitate C1 | ± + + + |
| | capitate C2 | - + ++ + |
| M. juliana | NG | ++ ++ ++ |
| Rajec Reka | peltate | + + + + |
| | capitate C1 | ± + + + |
| | capitate C2 | - + ++ + |
| M. juliana | NG | + +/++ +/++ +/++ |
| Cholomon Mt | peltate | + + ±/+ + |
| | capitate C1 | ± + + + |
| | capitate C2 | - ±/ + ± |
| M. kernerii | NG | +/++ + ++ |
| Zavratnica | peltate | + + +/++ + |
| | capitate C1 | ± + ± |
| | capitate C2 | - + ++ + |
| M. kernerii | NG | +/++ + ++ |
| Mostar | peltate | ± + + |
| | capitate C1 | ± + ±/ + ± |
| | capitate C2 | - ± ++ ± |
| M. longipedunculata | NG | + + + |
| Jazina | peltate | - + + |
| | capitate C1 | ± +/++ + + |
| | capitate C2 | - + + ± |
| M. longipedunculata | NG | + + + |
| Krivošije Mt | capitate C1 | ± +/++ + + |
| | capitate C2 | - + + ± |
| M. microphylla | NG | + + ++ |
| Svetac Island | peltate | + + + |
| | capitate C1 | ±/± +/± ± ± |
| | capitate C2 | - + ++/++ + |

Note: NG = non-glandular trichomes; trichomes frequency: −, trichomes completely missing; ±, trichomes are present in small numbers; +, trichomes are moderately present; ++, trichomes are present in large numbers.

Hanlidou et al. [36] described trichomes comparable to C1 trichomes in *Calamintha menthifolia* Host as short and usually curved trichomes. These trichomes are regularly present in all studied *Micromeria* and *Clinopodium* species, but they cannot be considered specific only to these two genera. In addition, the SEM micrographs presented by Werker et al. [13] show C1 trichomes in *Majorana syriaca* (L.) Rafin., *Satureja thymbra* L., and *Thymus capitatus* (L.) Hoffmanns. et Link. However, it is evident that C1 trichomes are not as common as peltate trichomes in Lamiaceae species.

Subtype 2 capitate trichomes (C2) are composed of a basal epidermal cell, two to three stalk cells and a rounded head cell with subcuticular space (Figure 1). C2 trichomes are...
upright while the height of these trichomes varies from short trichomes, mainly on the abaxial side of the leaf, to quite long trichomes on the calyx and stem. However, they are often absent on the adaxial leaf side. C2 trichomes are observed only in *Micromeria* species studied.

**Table 2.** Occurrence and frequency of trichomes on aerial parts of *Clinopodium* taxa.

| Taxon/ Locality | Trichomes | Leaf | Calyx | Stem |
|----------------|-----------|------|-------|------|
|                |           | Adaxial | Abaxial |       |       |       |       |
| *C. dalmaticum* Orjen NG | + | +/- | + | +/- |
| capitate C1 | + | +/- | + | +/- |
| capitate C3 | ± | ++ | +/++ | - |
| capitate C4 | - | ± | ± | ± |
| *C. dalmaticum* NG | + | +/- | +/++ | + |
| (syn. *M. bulgarica*) peltate | ± | + | +/- | + |
| Mesta | capitate C1 | + | +/- | ±/+/++ | ++ |
| River | capitate C3 | - | -/± | ±/+ | ± |
| Valley | capitate C4 | - | - | ±/+ | ±/± |
| *C. dalmaticum* NG | + | + | +/- | + |
| (syn. *M. bulgarica*) peltate | ± | ++ | +/- | + |
| Vlahina Mt | capitate C1 | + | +/- | + | ++ |
| capitate C3 | - | - | - | - |
| capitate C4 | - | - | -/± | -/± |
| *C. frivaldskyanum* NG | ± | ± | + | -/+ |
| Malusha peak peltate | ±/+ | + | +/++ | -/+ |
| capitate C1 | + | ++ | + | + |
| capitate C3 | - | - | - | - |
| capitate C4 | - | - | -/± | - |
| *C. frivaldskyanum* NG | ± | ±/+ | + | + |
| Vikanata Skala peltate | ± | + | + | + |
| Nature | capitate C1 | + | + | + |
| Monument | capitate C3 | - | - | -/± | - |
| capitate C4 | - | - | + | - |
| *C. pulegium* NG | ± | + | + | + |
| Mededa peltate | + | ++ | ++ | + |
| capitate C1 | ± | +/- | +/++ | + |
| capitate C3 | - | - | ± | - |
| capitate C4 | - | - | ± | - |
| *C. serpyllifolium* NG | +++ | +++ | ++ | ++ |
| Prizren peltate | + | + | + | + |
| capitate C1 | + | ++ | ++ | +/++ |
| capitate C3 | - | ± | ± | ± |
| capitate C4 | - | ± | ± | - |
| *C. thymifolium* NG | ± | + | + | + |
| Učka Mt peltate | + | +/- | +/++ | + |
| capitate C1 | +/- | +/- | +/++ | +/++ |
| capitate C3 | - | - | ± | - |
| capitate C4 | - | - | ± | - |
| *C. thymifolium* NG | + | + | + | +/++ |
| Dokozina peltate | + | +/- | +/++ | + |
| plan | capitate C1 | + | ++ | ++ | + |
capitate C3  −  −  −  −  −
capitate C4  −  −  −  −  −

C. thymifolium  NG  +  +  +  +
Šušanj  peltate  +  ++  +/++  +
capitate C1  +  ++  +/++  +
capitate C3  −  −  −  −
capitate C4  −  −  ±  −

C. thymifolium  NG  +  +  +  +
Blinanje  peltate  +  ++  +/++  +
capitate C1  +  ++  +/++  +
capitate C3  ±  −  −  −
capitate C4  −  −  −  −

C. thymifolium  NG  ±  +  +  ±/+  −
Manastir  peltate  + /+  +  ++  +
capitate C1  +/+  ++  ++  +/+  +
capitate C3  −  −  −  −
capitate C4  −  −  ±  −

Note: NG = non-glandular trichomes; trichomes frequency: −, trichomes completely missing; ±, trichomes are present in small numbers; +, trichomes are moderately present; ++, trichomes are present in large numbers; ++++, trichomes completely cover the surface.

So far, these trichomes have been observed in Micromeria fruticosa [13], M. myrtifolia [34], M. croatica [15], M. kerner, M. juliana [16], and M. longipedunculata [34]. These trichomes are more common than C1 trichomes in Lamiaceae species and trichomes comparable to C2 trichomes have also been described in Salvia L. [30] and Satureja L. [37–39] species.

Subtype 3 capitate trichomes (C3) are also erect and consist of a basal epidermal cell, a relatively short stalk cell, and a single roundish cell head with subcuticular space. Although C3 trichomes were observed in all investigated Clinopodium species, they were almost absent in C. frivaldszyjanum, C. pulegium and C. thymifolium (Table 2). Marin et al. [35] did not mention C3 trichomes, but they are visible in the presented SEM micrographs of C. thymifolium leaves. They are also present in the micrographs of Satureja montana L., S. subspicata Vis. and S. kitaibelii Wierzb. ex Heuff. presented by Dunkić et al. [38] and Dodoš et al. [39].

The capitate trichomes of subtype 4 (C4) are upright and consist of a basal epidermal cell, a stalk cell and an elongated head cell. In these trichomes, the head cell is as narrow as the stalk cells, and only slightly enlarged above, with a subcuticular space. They resemble a finger (Figure 2). Although C4 trichomes are present in all Clinopodium species studied, they are relatively rare trichomes (Table 2). The presence of C4 trichomes was previously observed in Clinopodium dalmaticum, C. pulegium, C. serpyllifolium and C. thymifolium by Dunkić et al. [17]. These trichomes are also visible in SEM micrographs of leaves of Micromeria fruticosa and Clinopodium thymifolium presented by Werker et al. [13] and Marin et al. [35], respectively. C4 trichomes are visible in SEM micrographs of Calamintha menthifolia, and in micrographs of Satureja montana and S. subspicata presented by Hanlidou et al. [36] and Dunkić et al. [38], respectively. Unclear micrographs presented by Al-Zubaidy et al. [40] probably show just C4 trichomes in Clinopodium vulgare L. ssp. vulgar and C. vulgar ssp. arundanum Boiss. In addition, they can be observed in SEM micrographs of Majorana syriaca, Salvia fruticosa Mill., S. officinalis L. [13], and S. divinorum Epling et Jářiva [30]. Although data from the literature [13] suggest that C4 trichomes may sometimes be present in Micromeria species, our results show that they are characteristic for Clinopodium species.

The results presented here show that NG trichomes, peltate trichomes and capitate trichomes of subtype 1 are present in both Micromeria and Clinopodium taxa. On the other
hand, the capitate trichomes of subtype 2 are present only in studied *Micromeria* taxa, while subtypes 3 and 4 are present only in *Clinopodium* taxa. Since the investigated taxa of the genus *Clinopodium* belong to the former *Micromeria* sect. *Pseudomelissa*, it can be concluded that micromorphological traits also support the recent transfer of *Micromeria* sect. *Pseudomelissa* to the genus *Clinopodium*.

2.2. Pollen

The pollen of all *Micromeria* and *Clinopodium* species examined is single (monad pollen) and isopolar with an elliptical equatorial outline (Figures 3 and 4).

![Figure 3. SEM micrographs of pollen grains and exine surface after critical point drying in *Micromeria cristata* ssp. cristata (Mc), *M. cristata* ssp. kasaninii (McK), *M. croatica* (Mcr) including *M. pseudocroatica* (McrP), *M. graeca* ssp. graeca (Mg), *M. graeca* ssp. fruticulosa (MgF), *M. juliana* (Mj), *M. kerneri* (Mk), *M. microphylla* (Mm), and *M. longipedunculata* (Ml).](image-url)
The polar view shows a circular shape with visible ends of apertures. The pollen has six apertures (hexacolporate pollen) located in the equatorial pollen belt (zonocolporate pollen). The apertures are long and rather narrow, widest in the middle and gradually narrowing towards the poles. The margins of the apertures are clear and sharp, while the ends are narrow and pointed. The membranes are ornamented. The apocolpium is relatively small, while the mesocolpium is quite large. The pollen exine is semitectate with medium reticulate ornamentation. The reticulum meshes are unequal (heterobrochate reticulum type) with more or less smooth surfaces of muri. Only the pollen exine of *M. cristata* ssp. *cristata* and *M. cristata* ssp. *kasaninii* show uneven surfaces of the muri (Figure 3). The lumina vary in size and are narrower or about the same width as the muri, rarely wider than muri as in *M. croatica* (Figure 3). The shape of the lumina is irregular with obtuse angles. So far, the pollen of *Micromeria marginata*, *M. croatica*, *M. longipedunculata*, and *M. imbricata* (Forssk.) C. Chr. have been described in detail [15, 21, 23]. In general, there is no visible difference in pollen shape between the *Micromeria* and *Clinopodium* species studied.

The size of pollen grains according to polar and equatorial axis is shown in Table 3. According to Erdtman [20], the pollen of *M. cristata* ssp. *kasaninii*, *M. longipedunculata*, *M. microphylla*, and *C. thymifolium* belong to the small pollen, while the pollen of the other investigated species belong to the medium pollen. *Micromeria longipedunculata* and *M. microphylla* have the smallest pollen (according to the length of the longer axis, 21.10 and 21.80 μm, respectively). On the other hand, *M. graeca* ssp. *graeca* and *M. graeca* ssp. *fruticulosa* have the largest pollen (by the longer axis, 34.65 and 33.86 μm, respectively). The size of pollen grains of *M. croatica* in this study was similar to the results of Kremer et al. [15], who studied the pollen of this species from another locality. Comparable results were also obtained for the pollen of *M. juliana*, *M. kernerii*, and *M. longipedunculata* [16, 34]. *Micromeria marginata* has middle-large pollen with a polar axis of 26.6 μm and an equatorial diameter of 35.2 μm [21]. According to Doaigey et al. [23], the pollen of *Micromeria imbricata* has a length of 34.57 and 31.33 μm (polar and equatorial axis, respectively). The smallest pollen among the investigated *Clinopodium* species was in *C. thymifolium* (23.52 μm), while the largest pollen (according to longer axis 32.47 μm) was in *C. dalmaticum*. The measure of polar length of *Clinopodium foliosum* pollen ranges from 20.45–28.6 μm and for *C. menthifolium* from 25.7–31.4 μm [24]. On the other hand, the
equatorial width is 20.46–28.57 μm for *C. foliosum* and 20.9–31.43 μm for *C. menthifolium* [24].

Table 3. Descriptive statistics for length of *Micromeria* and *Clinopodium* pollen grain according to polar (P) and equatorial (E) axis. Mean, Stdev, Min, and Max are in μm; CV is in %.

| Taxon                        | Polar Axis (P) | Equatorial Axis (E) | Pollen Size | P/E Ratio |
|------------------------------|----------------|---------------------|-------------|-----------|
| *M. cristata* ssp. cristata* |                |                     |             |           |
| Mean                         | 21.65          | 25.90               |             |           |
| Stdev                        | 1.10           | 2.28                |             |           |
| Min                          | 19.40          | 21.30               | Medium      | 0.84      |
| Max                          | 23.60          | 28.60               |             |           |
| CV                           | 5.08           | 8.80                |             |           |
| *M. cristata* ssp. kosaninii*|                |                     |             |           |
| Mean                         | 21.00          | 22.94               |             |           |
| Stdev                        | 0.91           | 1.10                |             |           |
| Min                          | 19.00          | 21.30               | Small       | 0.92      |
| Max                          | 22.70          | 25.00               |             |           |
| CV                           | 4.33           | 4.80                |             |           |
| *M. croatica*                |                |                     |             |           |
| Mean                         | 25.97          | 28.22               |             |           |
| Stdev                        | 1.86           | 1.93                |             |           |
| Min                          | 23.90          | 25.70               | Medium      | 0.92      |
| Max                          | 30.50          | 32.40               |             |           |
| CV                           | 7.16           | 6.84                |             |           |
| *M. croatica* (syn. *M.* pseudocroatica) | | | | |
| Mean                         | 26.35          | 29.65               |             |           |
| Stdev                        | 2.38           | 2.80                |             |           |
| Min                          | 22.10          | 25.40               | Medium      | 0.89      |
| Max                          | 30.00          | 23.90               |             |           |
| CV                           | 9.03           | 9.44                |             |           |
| *M. graeca* ssp. graeca*     |                |                     |             |           |
| Mean                         | 28.65          | 34.65               |             |           |
| Stdev                        | 2.42           | 2.69                |             |           |
| Min                          | 24.60          | 31.20               | Medium      | 0.83      |
| Max                          | 33.00          | 44.00               |             |           |
| CV                           | 8.45           | 7.76                |             |           |
| *M. graeca* ssp. fruticulosa*|                |                     |             |           |
| Mean                         | 26.73          | 33.86               |             |           |
| Stdev                        | 3.16           | 2.74                |             |           |
| Min                          | 22.40          | 28.10               | Medium      | 0.79      |
| Max                          | 35.20          | 38.00               |             |           |
| CV                           | 11.82          | 8.09                |             |           |
| *M. juliana*                |                |                     |             |           |
| Mean                         | 23.37          | 27.10               |             |           |
| Stdev                        | 1.21           | 1.29                |             |           |
| Min                          | 21.30          | 25.10               | Medium      | 0.86      |
| Max                          | 26.30          | 29.40               |             |           |
| CV                           | 5.18           | 4.76                |             |           |
| *M. kerner*                 |                |                     |             |           |
| Mean                         | 23.92          | 27.92               |             |           |
| Stdev                        | 1.40           | 1.02                |             |           |
| Min                          | 21.90          | 26.50               | Medium      | 0.86      |
| Max                          | 26.20          | 29.90               |             |           |
| CV                           | 5.85           | 3.65                |             |           |
| *M. longipedunculata*        |                |                     |             |           |
| Mean                         | 18.18          | 21.10               |             |           |
| Stdev                        | 0.74           | 1.67                | Small       | 0.86      |
| Min                          | 17.10          | 18.30               |             |           |
The results of the ANOVA show the significant difference between most of the studied taxa in terms of pollen size (Tables 4 and 5). Although there is a significant difference between most of *Micromeria* and *Clinopodium* taxa, this difference is not clear. There is a significant difference between the closely related taxa *M. cristata* ssp. *cristata* and *M. cristata* ssp. *kosaninii* in terms of equatorial diameter. However, there is a significant difference between the closely related taxa *M. graeca* ssp. *graeca* and *M. graeca* ssp. *fruticulosa* according to the polar axis. There is no significant difference between *M. croatica* and *M. pseudocroatica*, which are one species according to Bräuchler et al. [8]. On the other hand, there is a significant difference between *C. dalmaticum* from Orjen Mt (Montenegro) and *C. dalmaticum* from Mesta River Valley (Bulgaria) for both polar and equatorial axes.
Table 4. Results of Tukey HSD post hoc test at the 0.05 level for the polar length of studied *Micromeria* and *Clinopodium* taxa. Presence of asterisk (*) indicates significance at $p \leq 0.05$.

| Taxon  | Mc4  | McK  | Mcr2 | McrP1 | Mg1  | MgF  |
|--------|------|------|------|-------|------|------|
| Mc4    | 0.996467 |      |      |       |      |      |
| Mcr2   | 0.000029 * | 0.000029 * |      |       |      |      |
| McrP1  | 0.000029 * | 0.000029 * |      | 0.999995 |      |      |
| Mg1    | 0.000029 * | 0.000029 * |      | 0.000041 * | 0.000605 * |      |
| MgF    | 0.000029 * | 0.000029 * |      | 0.981839 | 0.999995 | 0.013724 * |
| Mj1    | 0.054533 | 0.000335 * | 0.000059 * | 0.000030 * | 0.000029 * | 0.000029 * |
| Mk1    | 0.000813 * | 0.000303 * | 0.005354 * | 0.000199 * | 0.000029 * | 0.000032 * |
| Ml1    | 0.000029 * | 0.000032 * | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * |
| Mj1    | 0.000030 * | 0.0000389 | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * |
| Cd     | 0.000029 * | 0.000029 * | 0.992281 | 1.000000 | 0.008540 * | 1.000000 |
| CdB1   | 1.000000 | 0.999997 | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * |
| Cf1    | 0.000029 * | 0.000029 * | 0.999806 * | 0.930688 | 0.000029 * | 0.464403 |
| Cp     | 0.000029 * | 0.000029 * | 0.919882 * | 0.999711 | 0.037175 * | 1.000000 |
| Cs     | 0.998023 | 0.469331 | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * |
| Ct4    | 0.999848 | 0.649443 | 0.000029 | 0.000029 * | 0.000029 * | 0.000029 * |

| Taxon  | Mj1  | Mk   | Ml1  | Mm   | Cd   |
|--------|------|------|------|------|------|
| Mj1    |      |      | 0.999474 |      |      |
| Ml1    | 0.000029 * |      | 0.000029 * |      |      |
| Mm     | 0.000029 * | 0.000029 * |      | 0.999931 |      |
| Cd     | 0.000029 * | 0.000035 * | 0.000029 * | 0.000029 * |      |
| CdB1   | 0.007514 * | 0.000077 * | 0.000029 * | 0.000037 * | 0.000029 * |
| Cf1    | 0.003785 * | 0.151169 | 0.000029 * | 0.000029 * | 0.559547 |
| Cp     | 0.000029 * | 0.000030 * | 0.000029 * | 0.000029 * | 1.000000 |
| Cs     | 0.711451 | 0.081441 | 0.000029 * | 0.000029 * | 0.000029 * |
| Ct4    | 0.534318 | 0.038831 * | 0.000029 * | 0.000029 * | 0.000029 * |

| Taxon  | Cd   | CdB1 | Cf1  | Cp   | Cs   |
|--------|------|------|------|------|------|
| CdB1   | 0.000029 * |      |      |      |      |
| Cf1    | 0.559547 | 0.000029 * |      |      |      |
| Cp     | 1.000000 | 0.000029 * | 0.272970 |      |      |
| Cs     | 0.000029 * | 0.924334 | 0.000029 * |      |      |
| Ct4    | 0.000029 * | 0.917758 | 0.000029 * |      | 1.000000 |

Table 5. Results of Tukey HSD post hoc test at the 0.05 level for the equatorial diameter of pollen grain of studied *Micromeria* and *Clinopodium* taxa. Presence of asterisk (*) indicates significance at $p \leq 0.05$.

| Taxon  | Mc4  | McK  | Mcr2 | McrP1 | Mg1  | MgF  |
|--------|------|------|------|-------|------|------|
| McK    | 0.000032 * |      |      |       |      |      |
| Mcr2   | 0.001274 * | 0.000029 |      |       |      |      |
| McrP1  | 0.000029 * | 0.000029 * | 0.324744 |       |      |      |
| Mg1    | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * |      |      |
| MgF    | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * | 0.983544 |      |
| Mj1    | 0.638719 | 0.000029 * | 0.763081 | 0.000190 * | 0.000029 * | 0.000029 * |
| Mk1    | 0.012601 * | 0.000029 * | 1.000000 | 0.080933 | 0.000029 * | 0.000029 * |
| Ml1    | 0.000029 * | 0.44219 * | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * |
| Mj1    | 0.000029 * | 0.725134 | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * |
| Cd     | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * | 0.000029 * |
| CdB1   | 1.000000 | 0.000031 * | 0.001974 * | 0.000029 | 0.000029 * | 0.000029 * |
### 3. Materials and Methods

#### 3.1. Plant Material

The survey included, depending on the point of view [8,9,12,41–46], nine to ten *Micromeria* taxa from the Balkan Peninsula (Table 6, Figures 5–7): *M. cristata* (Hampe) Griseb. ssp. cristata, *M. cristata* ssp. *kosaninii* (Šilić) Bräuchler et Govaerts (syn. *M. kosaninii* Šilić), *M. croatica* (Pers.) Schott (including *M. pseudocroatica* Šilić), *M. graeca* (L.) Benth. ex Rchb. ssp. *graeca*, *M. graeca* ssp. *fruticulosa* (Bertol.) Guinea (syn. *M. fruticulosa* (Bertol.) Šilić), *M. julianna* (L.) Benth. ex Rchb., *M. kernerii* Murb., *M. longipedunculata* Bräuchler, (syn. *M. parviflora* Rchb.), and *M. microphylla* (d’Urv.) Benth. In addition, five to six, depending on point of view [8,12,19,41–46], taxa recently transferred from the genus *Clinopodium* to the genus *Micromeria* were studied, namely *Clinopodium dalmaticum* (Benth.) Bräuchler et Heubl (syn. *Micromeria dalmatica* Benth.) including *M. bulgarica* (Velen.) Vandas, *C. frioida* (Degen) Bräuchler et Heubl (syn. *M. frioida* (Degen) Velen.), *C. pulegium* (Rochel) Bräuchler (syn. *M. pulegium* (Rochel) Benth.), *C. serpyllifolium* (M. Bieb.) Kuntze (syn. *M. albanica* (Griseb. ex K. Malý) Šilić), and *C. thymifolia* (Scop.) Kuntze (syn. *M. thymifolia* (Scop.) Fritsch.). Each locality was described with GPS coordinates and elevation. Voucher specimens of the plant material were deposited in the Herbarium “Fran Kušan” (HFK-HR), Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia (Tables 6 and 7).

Samples of the stems, leaves, and flowers of ten plants per population were fixed in FAA (formalin/96% ethanol/acetic acid/water: 5/70/5/20). After three days the samples were transferred from the fixation medium to 70% (v/v) ethanol and stored in the refrigerator until analysis.

| Taxon | Mj1 | Mk | Ml1 | Mm | Cd |
|-------|-----|----|-----|----|----|
| Mk1   | 0.977496 |     |      |    |    |
| Ml1   | 0.000029 | 0.000029 |     |    |    |
| Mm    | 0.000029 | 0.000029 | 0.995845 |    |    |
| Cd    | 0.000029 | 0.000029 | 0.000029 | 0.000029 |    |
| CdB1  | 0.711996 | 0.018248 | 0.000029 | 0.000029 | 0.000029 |
| Cf1   | 0.566977 | 0.999982 | 0.000029 | 0.000029 | 0.000029 |
| Cp    | 0.055158 | 0.897260 | 0.000029 | 0.000029 | 0.000029 |
| Cs    | 0.950327 | 0.090359 | 0.000029 | 0.000029 | 0.000029 |
| Ct4   | 0.000029 | 0.000029 | 0.000029 | 0.000029 | 0.000029 |

| Taxon | Cd | CdB1 | Cf1 | Cp | Cs |
|-------|----|------|-----|----|----|
| Cd    | 0.000029 |     |     |    |    |
| CdB1  | 0.000029 |     |     |    |    |
| Cf1   | 0.000029 | 0.000598 |     |    |    |
| Cp    | 0.000029 | 0.000032 | 0.999755 |    |    |
| Cs    | 0.000029 | 1.000000 | 0.004970 | 0.000068 |    |
| Ct4   | 0.000029 | 0.000505 | 0.000029 | 0.000029 | 0.000068 |

* indicates significant differences (p ≤ 0.05).
Table 6. Details on origin and collection data of investigated *Micromeria* (M.) and *Clinopodium* (C.) taxa.

| Taxa—According to | Balkan Literature | Collecting Site | Voucher no. |
|-------------------|-------------------|-----------------|-------------|
| Bräuchler et al., 2008 (Code) | | | |
| *M. cristata* ssp. *cristata* (Mc1) | *M. cristata* | Humsko brdo Mt (Serbia) | HFK-HR-51126 |
| *M. cristata* ssp. *cristata* (Mc2) | *M. cristata* | Vitosha Mt (Bulgaria) | HFK-HR-51132 |
| *M. cristata* ssp. *cristata* (Mc3) | *M. cristata* | Pirin Mt (Bulgaria) | HFK-HR-51133 |
| *M. cristata* ssp. *cristata* (Mc4) | *M. cristata* | Demos Serron (Greece) | HFK-HR-51141 |
| *M. cristata* ssp. *cristata* (Mc5) | *M. cristata* | Paikon Mt (Greece) | HFK-HR-51146 |
| *M. cristata* ssp. *cristata* (Mc6) | *M. cristata* | Pletvar (N. Macedonia) * | HFK-HR-51142 |
| *M. graeca* ssp. *graeca* (McK) | *M. graeca* | Rossijev kuk (Croatia) * | HFK-HR-51016 |
| *M. croatica* (Mcr1) | *M. croatica* | Stupačinovo (Croatia) * | HFK-HR-51017 |
| *M. croatica* (Mcr2) | *M. croatica* | Dubočani (BIH) | HFK-HR-51020 |
| *M. croatica* (Mcr3) | *M. croatica* | Pijavičino (Croatia) * | HFK-HR-51032 |
| *M. croatica* (McrP1) | *M. pseudocrocutea* | Prapratno (Croatia) | HFK-HR-51033 |
| *M. croatica* (McrP2) | *M. pseudocrocutea* | Malo zlo polje (Croatia) * | HFK-HR-51036 |
| *M. graeca* ssp. *graeca* (Mg1) | *M. graeca* | Komiža (Croatia) | HFK-HR-51037 |
| *M. croatica* ssp. *fruticulosa* (Mg2) | *M. fruticulosa* | Sušac Island (Croatia) * | HFK-HR-51038 |
| *M. juliana* (Mj1) | *M. juliana* | Grude (BIH) * | HFK-HR-51043 |
| *M. juliana* (Mj2) | *M. juliana* | Cijevna Canyon (Montenegro) | HFK-HR-51045 |
| *M. juliana* (Mj3) | *M. juliana* | Rajec Reka (N. Macedonia) | HFK-HR-51168 |
| *M. juliana* (Mj4) | *M. juliana* | Cholomon Mt (Greece) | HFK-HR-51147 |
| *M. kerner* (Mk1) | *M. kerner* | Zavratnica (Croatia) * | HFK-HR-51018 |
| *M. kerner* (Mk2) | *M. kerner* | Mostar (BIH) | HFK-HR-51044 |
| *M. longipesdnculata* (Ml1) | *M. parvisflora* | Jazina (BIH) * | HFK-HR-51046 |
| *M. longipesdnculata* (Ml2) | *M. parvisflora* | Krivošije Mt (Montenegro) | HFK-HR-51047 |
| *M. microphylla* (Mm) | *M. microphylla* | Svetac Island (Croatia) * | HFK-HR-51039 |
| *C. dalmaticum* (Cd) | *C. dalmaticum* | Orjen Mt (Montenegro) * | HFK-HR-51051 |
| *C. dalmaticum* (CdB1) | *C. dalmaticum* | Mesta River Valley (Bulgaria) * | HFK-HR-51134 |
| *C. dalmaticum* (CdB2) | *C. dalmaticum* | Vlahina Mt (Bulgaria) | HFK-HR-51135 |
| *C. frivaldshkyana* (Cf1) | *C. frivaldshkyana* | Malusha peak (Bulgaria) * | HFK-HR-51136 |
| *C. frivaldshkyana* (Cf2) | *C. frivaldshkyana* | Vikanata Skala Nature | HFK-HR-51137 |
| *C. pulegium* (Cp) | *C. pulegium* | Mededa (BIH) * | HFK-HR-51050 |
| *C. serpylliifolium* (Cs) | *C. serpylliifolium* | Prizren (Kosovo) * | HFK-HR-51074 |
| *C. thymifolium* (Ct1) | *C. thymifolium* | Učka Mt (Croatia) | HFK-HR-51077 |
| *C. thymifolium* (Ct2) | *C. thymifolium* | Dokozina plan (Croatia) | HFK-HR-51082 |
| *C. thymifolium* (Ct3) | *C. thymifolium* | Šušanj (Croatia) | HFK-HR-51081 |
| *C. thymifolium* (Ct4) | *C. thymifolium* | Blidinje (BIH) * | HFK-HR-51042 |
| *C. thymifolium* (Ct5) | *C. thymifolium* | Manastir Morača (Montenegro) | HFK-HR-51053 |

Note: BIH = Bosnia and Herzegovina; N. = North; * = site of pollen collecting.
| Taxa—According to | Bräuchler et al., 2008 (Code) | Balkan Literature | Latitude | Longitude | Altitude a.s.l. (m) |
|-------------------|-------------------------------|------------------|----------|-----------|-------------------|
| M. cristata (Mc1) | M. cristata                    | 43°22'25.0"      | 21°53'50.1" | 387        |
| M. cristata (Mc2) | M. cristata                    | 42°29'33.4"      | 23°11'43.1" | 980        |
| M. cristata (Mc3) | M. cristata                    | 41°46'11.4"      | 23°25'19.1" | 1970       |
| M. cristata (Mc4) | M. cristata                    | 41°24'18.1"      | 22°15'47.0" | 111        |
| M. cristata (Mc5) | M. cristata                    | 41°15'10.3"      | 23°24'49.8" | 190        |
| M. cristata (Mc6) | M. cristata                    | 40°57'21.2"      | 22°20'02.0" | 1650       |
| M. cristata ssp. kosaninii (McK) | M. kosaninii | 41°22'09.0"      | 21°39'06.1" | 1020       |
| M. croatica (Mcr1) | M. croatica                    | 44°45'51.1"      | 4°59'17.1"  | 1641        |
| M. croatica (Mcr2) | M. croatica                    | 44°32'37.5"      | 15°10'04.7" | 1058       |
| M. croatica (Mcr3) | M. croatica                    | 43°35'10.1"      | 18°04'44.0" | 715        |
| M. croatica (McrP1) | M. pseudocroatica              | 42°56'58.8"      | 17°22'02.1" | 436        |
| M. croatica (McrP2) | M. pseudocroatica              | 42°49'28.1"      | 17°39'57.9" | 167        |
| M. graeca (Mg1) | M. graeca                      | 43°03'43.3"      | 16°12'55.9" | 137        |
| M. graeca (Mg2) | M. graeca                      | 43°02'17.4"      | 16°05'49.5" | 43         |
| M. graeca ssp. fruticulosa (MgF) | M. fruticulosa | 43°02'13.4"      | 16°05'50.5" | 10         |
| M. juliana (Mj1) | M. juliana                     | 43°23'18.5"      | 17°23'27.8" | 495        |
| M. juliana (Mj2) | M. juliana                     | 42°25'44.6"      | 19°28'53.5" | 157        |
| M. juliana (Mj3) | M. juliana                     | 41°26'12.2"      | 21°52'06.4" | 199        |
| M. juliana (Mj4) | M. juliana                     | 40°27'30.0"      | 23°31'04.6" | 1100       |
| M. kerner (Mk1) | M. kerner                      | 44°42'02.2"      | 14°54'45.6" | 161        |
| M. kerner (Mk2) | M. kerner                      | 43°20'27.7"      | 17°48'53.2" | 61         |
| M. longipedunculata (Ml1) | M. parviflora | 42°42'11.7"      | 18°30'37.1" | 347        |
| M. longipedunculata (Ml2) | M. parviflora | 42°32'51.8"      | 18°42'41.1" | 624        |
| M. microphylla (Mm) | M. microphylla                 | 43°01'07.8"      | 15°45'08.2" | 28         |
| C. dalmaticum (Cd) | M. dalmatica                   | 42°33'45.1"      | 18°37'36.6" | 1074       |
| C. dalmaticum (CdB1) | M. bulgarica                  | 41°40'46.6"      | 23°43'29.7" | 580        |
| C. dalmaticum (CdB2) | M. bulgarica                  | 41°50'30.6"      | 22°59'27.8" | 1140       |
| C. frivaldszkyanum (Cf1) | M. frivaldszkyanuna          | 42°45'01.0"      | 25°16'53.7" | 1311       |
| C. frivaldszkyanum (Cf2) | M. frivaldszkyanana          | 42°45'57.6"      | 25°30'08.1" | 1040       |
| C. pulegium (Cp) | M. pulegium                    | 43°44'01.2"      | 19°17'07.3" | 563        |
| C. serpyllifolium (Cs) | M. albanica                   | 42°12'01.6"      | 20°45'57.7" | 376        |
| C. thymifolium (Ct1) | M. thymifolia                 | 45°17'08.1"      | 14°12'02.4" | 1189       |
| C. thymifolium (Ct2) | M. thymifolia                 | 44°39'04.3"      | 15°02'39.1" | 1441       |
| C. thymifolium (Ct3) | M. thymifolia                 | 44°31'33.8"      | 15°06'45.1" | 604        |
| C. thymifolium (Ct4) | M. thymifolia                 | 43°31'07.8"      | 17°23'09.8" | 1195       |
| C. thymifolium (Ct5) | M. thymifolia                 | 42°45'50.9"      | 19°23'34.6" | 301        |
Figure 5. Collection sites of studied *Micromeria* and *Clinopodium* taxa: *M. cristata* ssp. *cristata* (Mc), *M. cristata* ssp. *kosaninii* (McK), *M. croatica* (Mer) including *M. pseudocroatica* (MerP), *M. graeca* ssp. *graeca* (Mg), *M. graeca* ssp. *fruticulosa* (MgF), *M. juliana* (Mj), *M. kernerii* (Mk), *M. microphylla* (Mm), *M. longipedunculata* (Ml), *C. dalmaticum* (Cd) including *M. bulgarica* (CdB), *C. frivaldszkyanum* (Cf), *C. pulegium* (Cp), *C. serpyllifolium* (Cs), and *C. thymifolium* (Ct).
Figure 6. Photographs of Micromeria cristata ssp. cristata (Mc), M. cristata ssp. kosaninii (McK), M. croatica (Mcr) including M. pseudocroatica (McrP), M. graeca ssp. graeca (Mg), M. graeca ssp. fruticulosa (MgF), M. juliana (Mj), M. kernerii (Mk), M. longipedunculata (Ml) and M. microphylla (Mm).
The samples for scanning electron microscopic (SEM) investigation were transferred from 70% (v/v) ethanol to 70% (v/v) acetone. Then, the samples were dehydrated from 70% (v/v) to 90% (v/v), and 100% (v/v) acetone. The dehydrated samples were subjected to critical point drying using CO₂ as the drying medium (CPD030; Bal-tec, Balzers, Liechtenstein). The samples were then sputter coated with gold (Sputter Coater, Agar Scientific Ltd., Essex, UK) and examined under the scanning electron microscope XL30 ESEM (FEI) with an acceleration voltages of 20 kV in high vacuum mode. The presence and abundance of the different trichome types was qualitatively assessed (~ missing, ± rare, + present, ++ abundant, +++ extremely abundant). Pollen was collected from the anthers of several flowers per plant after critical point drying. A total of pollen from ten

**Figure 7.** Photographs of Clinopodium dalmaticum (Cd) including Micromeria bulgarica (CdB), C. frivaldszkyanum (Cr), C. thymifolium (Ct), C. pulegium (Cp) and C. serpyllifolium (Cs).
plants per population studied was mixed and examined under a scanning electron microscope. The length of 30 pollen grains per population was measured. Pollen size is expressed as the length of the longest axis according to Erdman [20]. Common terminology was used in describing trichomes and pollen [25,47].

3.2. Statistical Analysis

Descriptive statistics: minimum (Min), maximum (Max), mean, standard deviation (Sdev) and coefficient of variation (CV) for length and diameter of pollen grains were calculated. Polar end equatorial axis lengths of pollen grains were subjected to One-way Analysis of Variance (ANOVA). Differences between taxa were tested with Tukey’s HSD post hoc tests [48].

4. Conclusions

Non-glandular trichomes, peltate trichomes, and four subtypes of capitulate trichomes were observed on the aerial parts of the Micromeria and Clinopodium taxa examined. The NG trichomes, peltate trichomes and capitulate trichomes of subtype 1 (bent trichomes consisting of a basal epidermal cell and an elliptically shaped head cell) were observed in both Micromeria and Clinopodium taxa. Capitate trichomes of subtype 2 (erect trichomes consisting of a basal epidermal cell, two to three stalk cells and a rounded head cell) were observed only in Micromeria taxa, while capitulate trichomes of subtype 3 (upright trichomes consisting of a basal epidermal cell, a relatively short stalk cell and a rounded celled head) and subtype 4 (upright trichomes consisting of a basal epidermal cell, a stalk cell, and a narrow and elongated head cell) were observed only in Clinopodium taxa. Such a distribution of capitulate trichomes subtypes in Micromeria and Clinopodium taxa shows that, on the basis of micromorphological traits, it is possible to distinguish taxa from the former Pseudomelissa section from other Micromeria taxa. In this way, micromorphological traits support the recent transfer of Micromeria species from the section Pseudomelissa to the genus Clinopodium.

On the other hand, the pollen studies have shown that there are no significant differences between pollen of the Micromeria and Clinopodium taxa.

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