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# Morphology and Distribution of Trichomes on Leaves in Seven Croatian Taxa of the Genus *Stachys* (*Lamiaceae*)

By

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With 4 Figures

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Summary

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Trichomes on leaves of *Stachys alpina* L., *S. officinalis* (L.) Trevis., *S. palustris* L., *S. recta* L. subsp. *recta, S. recta* L. subsp. *subcrenata* (Vis.) Briq., *S. salviifolia* Ten., and *S. sylvatica* L. were investigated by light and scanning electron microscopy (SEM). Six types of glandular and three types of non-glandular trichomes were found, including a particular type of glandular trichome that was not described yet for the genus *Stachys*. The investigation of micromorphological characters was the first report on types of trichomes present in *S. palustris* and *S. recta* subsp. *sub-*

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*crenata* and it enabled in particular the characterisation of *S. officinalis* and differentiation among two *S. recta* subspecies. A key for the characterisation of the investigated *Stachys* taxa based on trichome types is presented.

## Zusammenfassung

Bilusic Vundac V., Stabentheiner E., Brantner A.H. & Plazibat M. 2011. Morphology and distribution of trichomes on leaves in seven Croatian taxa of the genus *Stachys* (*Lamiaceae*). [Morphologie und Verteilung der Trichome auf Blättern von sieben kroatischen Taxa der Gattung *Stachys* (*Lamiaceae*)]. – Phyton (Horn, Austria) 51 (1): 161–176, 4 Abbildungen.

Die Trichome auf Blättern von *Stachys alpina* L., *S. officinalis* (L.) Trevis., *S. palustris* L., *S. recta* L. subsp. *recta, S. recta* L. subsp. *subcrenata* (Vis.) Briq., *S. salviifolia* Ten. und *S. sylvatica* L. wurden lichtmikroskopisch und rasterelektronenmikroskopisch untersucht. Es konnten sechs Typen von Drüsenhaaren und drei Typen von Borstenhaaren charakterisiert werden, wobei ein Drüsenhaartyp bisher noch nicht für *Stachys* beschrieben wurde. Erstmalig werden in dieser Arbeit die Trichome von *S. palustris* und *S. recta* subsp. *subcrenata* beschrieben. Eine detaillierte Charakterisierung von *S. officinalis* und eine Unterscheidung der beiden *S. recta* Subspecies auf Grund der Trichomtypen wird präsentiert. Ein Schlüssel für die Charakterisierung der untersuchten *Stachys* Taxa auf Grund der auf den Blättern auftretenden Trichomtypen wird vorgestellt.

## Introduction

*Stachys* L., or woundwort, one of the largest genera of the family *Lamiaceae*, consists of about 300 species with nearly worldwide distribution, but most of them occurring in the warm temperate regions of the Mediterranean and of SW Asia, with secondary centres in N and S America, and southern Africa; it is absent from Australia and New Zealand. The genus comprises nineteen taxa in the Croatian flora. Of these *S. alpina* L., *S. officinalis* (L.) Trevis., *S. palustris* L., *S. recta* L. subsp. *recta, S. recta* L. subsp. *subcrenata* (Vis.) Briq., *S. salviifolia* Ten., and *S. sylvatica* L. were investigated for the presented study. *S. alpina* (alpine woundwort) is a green, hirsute, glandular perennial up to 100 cm hight. In Croatia it grows on limestone soils from the mountainous to the subalpine zone. *S. officinalis* (= *Betonica officinalis* L.; betony) is a perennial of 15–60 cm height, with sparse hairs, a short woody rhizome, well marked basal rosettes and erect, simple or slightly branched stems. Betony occurs in open woods, stony grassy places and dry meadows from the sea level up to the 1600 m. *S. palustris* (marsh woundwort) is a sparsely to densely hairy perennial plant of shady spots in marshes, bogs, ponds, lakes, and stream margins, damp places, roadside verges, and as a weed in cultivated fields growing up to 120 cm from a creeping rhizome. *S. recta* subsp. *recta* (perennial yellow woundwort) is erect or ascending, subglabrous to sparsely hirsute, usually aglandular plant up to 100 cm hight, which grows in Croatia on different dry and stony habitats from the seaside up to the top of the

mountains. *S. recta* subsp. *subcrenata* has a very similar appearance and distribution as compared to the previous subspecies but has narrower leaves and often a glandular calyx with unequal teeth. *S. salviifolia* [= *S. cretica* L. subsp. *salviifolia* (TEN.) RECH. f., *S. germanica* L. subsp. *salviifolia* (TEN.) GAMS] (Mediterranean woundwort) is a densely tomentose or lanate-tomentose perennial growing up to about 80 cm, widely distributed on rocky places in the littoral areas of the country. *S. sylvatica* (hedge woundwort) is an erect and hirsute perennial found in shady spots in woodland, forests, roadsides, alpine meadows and grasslands growing up to 120 cm. It differs from marsh woundwort in habitat preference, the broader leaves and the characteristic, unpleasant smell when crushed, but the two can hybridise where they meet (BALL 1972, BHATTACHARJEE 1980, PIGNATTI 1982, MULLIGAN & MUNRO 1989, FORENBACHER 1990, DOMAC 1994, TURNER 1994, FALCIANI 1997).

BALL 1972 subdivided the genus into four sections and proposed the term "group" for the taxa referred to as *S. recta* and *S. germanica*. BHATTACHARJEE 1980 proposed a new infrageneric classification of *Stachys* and structured it into two subgenera, 21 sections and numerous subsections. PIGNATTI 1982 and GREUTER & al. 1986 suggested a classification of *Stachys* similar to that proposed by BALL 1972, but without his subdivision into sections.

Taxonomy sets a high value on structure and distribution of trichomes – both secretory and non-secretory ones (WERKER & al. 1985a,b, CANTINO 1990, BINI MALECI & SERVETTAZ 1991, KAROUSOU & al. 1992, WERKER 1993, FALCIANI & al. 1995, FAHN 2000).

Trichomes of only few *Stachys* species have been investigated (FALCIANI & al. 1995, BINI MALECI & al. 2002, BINI MALECI & GIULIANI 2006, GIULIANI & al. 2008, GIULIANI & BINI MALECI 2008), and apart from the general observation that some trichomes exist on the leaves, the type of secretory structures and trichome anatomy in most of the taxa remains unknown. The aim of the presented study was to fill these gaps in knowledge, to attain a deeper insight into this difficult genus and to identify some taxonomical characteristics based on micromorphological studies on leaf trichomes of Stachys taxa of the Croatian flora.

## Materials and Methods

Leaves of 10 plants in full flower from each investigated *Stachys* taxon were collected from June 2004 until September 2005. Fresh material was fixed in Carnoy medium (alcohol:acetic acid = 3:1), kept on ice for 4 hours and then stored in the freezer (–20 °C). Some material was also air dried. Voucher specimens (collection numbers 807.1–807.7) were deposited at the Herbarium Croaticum (ZA), Department of Botany, Faculty of Science, University of Zagreb, Croatia. Detailed information on the plant material is presented in Table 1.

Table 1. Origin of the plant material.

| Taxa | Locality | Coordinates |
|---|---|---|
| *Stachys alpina* | Medvednica | N 44° 53′ 00″; E 15° 56′ 30″ |
| *Stachys officinalis* | Maksimir | N 45° 50′ 00″; E 16° 01′ 30″ |
| *Stachys palustris* | Vrbani | N 45° 47′ 30″; E 15° 55′ 00″ |
| *Stachys recta* subsp. *recta* | Velebit | N 44° 31′ 45″; E 15° 11′ 00″ |
| *Stachys recta* subsp. *subcrenata* | Velebit | N 44° 32′ 00″; E 15° 08′ 50″ |
| *Stachys salviifolia* | Vinjerac | N 44° 15′ 15″; E 15° 28′ 00″ |
| *Stachys sylvatica* | Dolje | N 45° 52′ 00″; E 15° 58′ 45″ |

First investigations on the leaves were performed by light microscopy using hand cut sections. For SEM investigations, small pieces of fixed leaves were dehydrated with acetone, critical point dried, sputter coated with gold and investigated by a Philips XL30 ESEM (FEI) in high vacuum mode.

## Results

In the literature terminology of trichome characterisation is diverse and inhomogenous. For the presented work the terminology of WERKER 2000 is used.

The surface of the leaves of all investigated *Stachys* taxa is characterised by various morphologically distinct types of glandular and non-glandular trichomes. The types of trichomes, their distribution on the leaves and the occurrence in the different taxa are summarised in Table 2 and Fig. 1.

Table 2. Characterisation of the trichome types.

| | Type | Description | *Taxa* | Distribution |
|---|---|---|---|---|
| Glandular trichomes | A | short capitate, inclined with the stalk cell nearly parallel to the surface, 1 basal cell, 1 stalk cell and a bicellular secreting head, bearing a small subcuticular space on the apex | *S. recta* subsp. *recta* | adaxial and abaxial, evenly distributed |
| | B | short capitate, erect, 1 basal cell, 1 stalk cell and a secreting head of 4 cells, with a small subcuticular space on the apex | *S. recta* subsp. *subcrenata* | adaxial and abaxial, evenly distributed |
| | | | *S. alpina* | |
| | | | *S. salviifolia* | adaxial: mainly located along the veins of higher order; abaxial: evenly distributed |
| | | | *S. sylvatica* | |
| | | | *S. palustris* | |
| | $C_1$ | sessile capitate, 1 basal cell, 1 short neck cell, and a head of 4 secretory cells, with a small subcuticular space on the apex | *S. officinalis* | adaxial |
| | | | *S. salviifolia* | abaxial |
| | $C_2$ | peltate, 1 basal cell, a short neck cell and a multicellular head, with a large subcuticular space on the apex | *S. officinalis* | abaxial |

| | Type | Description | *Taxa* | Distribution |
|---|---|---|---|---|
| | $D_1$ | long capitate, characterised by a multicellular base, a long uni-bi-cellular stalk and an unicellular head covered by a cuticular sheet | *S. recta* subsp. *subcrenata* | common on abaxial, rare on adaxial |
| | | | *S. salviifolia* | adaxial and abaxial, evenly distributed |
| | | | *S. palustris* | |
| | | | *S. officinalis* | |
| | $D_2$ | long capitate, characterised by 1-2 basal cells, 1 stalk cell, 1 neck cell and a head with 4 secretory cells, covered by thin cuticular sheet | *S. sylvatica* | common on abaxial, rare on adaxial |
| | | | *S. alpina* | adaxial and abaxial, evenly distributed |
| Non-glandular trichomes | E | 1-5 cellular, unbranched, with or without cuticular micropapillae, on a pedestal of slightly enlarged epidermis cells | *S. officinalis* | adaxial and abaxial, evenly distributed |
| | | | *S. alpina* | |
| | | | *S. recta* subsp. *subcrenata* | |
| | | | *S. sylvatica* | |
| | | | *S. palustris* | |
| | F | multi-cellular, unbranched, without cuticular micropapillae, with one slightly swollen basal cell elevated above the epidermis | *S. salviifolia* | adaxial and abaxial, evenly distributed |
| | G | 3-4 cellular, unbranched, with cuticular micropapillae, with one basal cell on epidermis level | *S. recta* subsp. *recta* | adaxial and abaxial, evenly distributed |

Glandular trichomes can be divided into two main types, capitate and peltate. Capitate trichomes are sessile or stalked, with a multicellular or uni-bi-cellular base and a terminal secretory head with 1–4 secretory cells (the head of the capitate trichomes consists of 1–4 more or less rounded secretory cells subtended by a stalk, one to several cells long, and a basal cell; WERKER 2000). Usually they are much smaller than the non-glandular trichomes. The capitate hairs of the investigated *Stachys* taxa displayed some variation (short or long capitate, stalked or sessile, erect, inclined or entirely apressed to the epidermal surface), and five types could be characterised according to the position on the leaf surface, the structure of the trichome base, the stalk length and the head shape (types A, B, $C_1$, $D_1$ and $D_2$) (Fig. 3. I–IV, VI–VIII). Type A trichomes are short capitate, with an oval head composed of two secretory cells subtended by a short stalk and a basal cell. The whole trichome is covered by a smooth cuticle. Unlike all the other glandular trichomes this type is not erect but inclined, with the stalk and the head nearly parallel to the surface. Sometimes the whole trichome is completely apressed to the epidermal surface, with entire trichome body lying on the epidermis. Often a prominent line dividing the head vertically in 2 parts, can be seen (Fig. 3. I).

Type B trichomes are short capitate trichomes consisting of one basal cell, a short stalk cell and a four-celled club-shaped head with a small

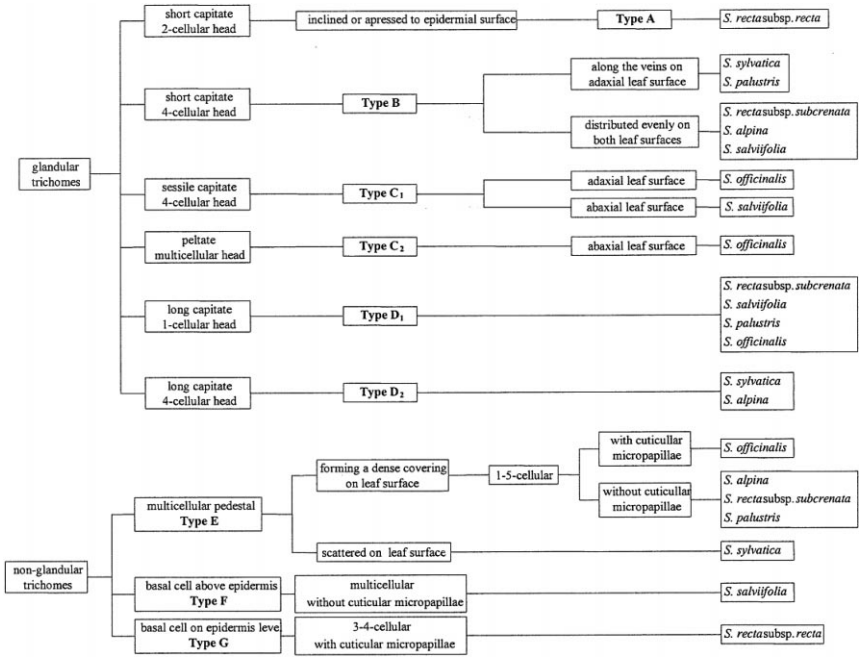


Fig. 1. Key for the investigated *Stachys* taxa based on trichome characteristics.

subcuticular space. These glandular trichomes are erect and completely covered by a smooth cuticle (Fig. 3. VI).

Type $C_1$ trichomes are sessile, capitate, with one basal cell, one short neck cell and a head of 4 secretory cells, bearing a small subcuticular space on the apex (Fig. 3. II–IV).

Type $D_1$ trichomes are long capitate, with an unicellular head subtended by a long uni-bi-cellular stalk. (Fig. 3. VII).

Type $D_2$ trichomes are long capitate, with an uni-bi-cellular base, a long stalk cell, a neck cell and a head consisting of 4 secretory cells, covered by a thin cuticular sheet. The trichome body is slightly elevated by a multicellular pedestal. The diameter of the stalk is equal or smaller than the pedestal and the head is smaller than the stalk (Fig. 3. VIII).

Differing from capitate trichomes, the head of the peltate hairs consists of 4–18 more flattened cells on a horizontal plane, subtended by one stalk cell and a basal cell (Werker 2000). Peltate trichomes are larger than the capitate ones and only one typical peltate trichome (type $C_2$) was observed.

Type $C_2$ peltate trichome constist of one basal cell, a short stalk cell and a large multicellular head bearing a large subcuticular space on the apex. Peltate trichomes are only present on the abaxial leaf surface of *S. officinalis* (Fig. 3. V).

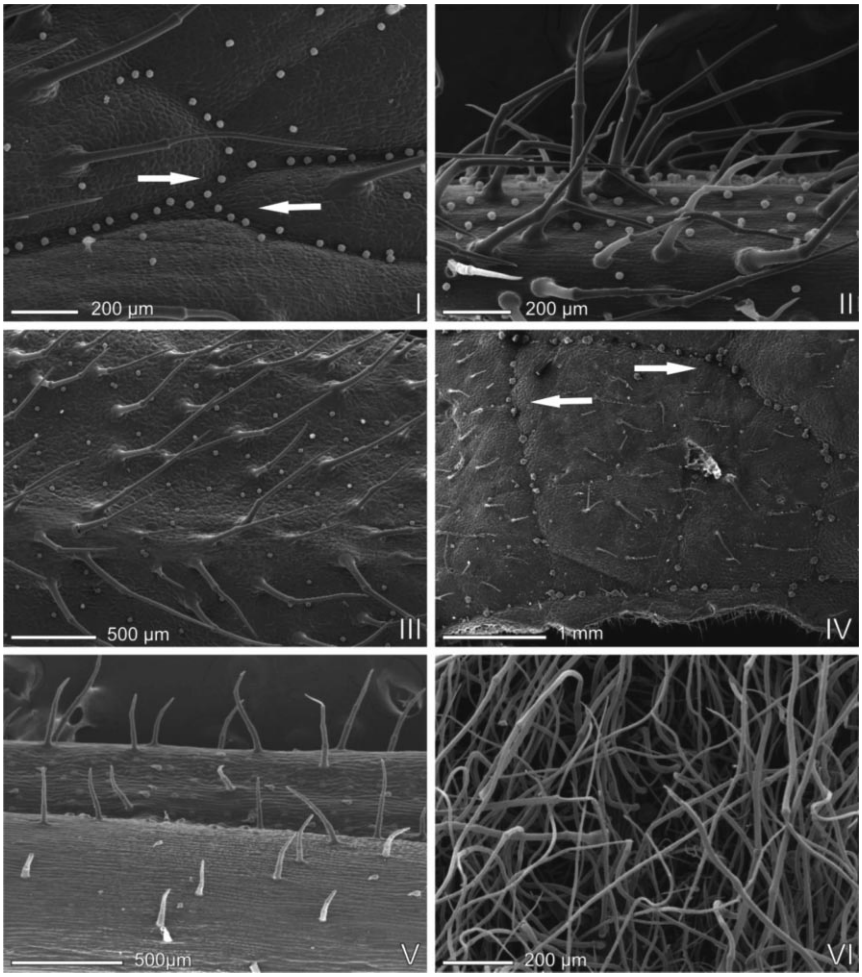


Fig. 2. Trichome distribution (SEM). - I. Glandular trichomes (type B) on adaxial leaf surface of *S. sylvatica* (*arrows*: distribution along the veins). – II. Different trichome types distributed evenly on abaxial leaf surface of *S. sylvatica*. – III. Adaxial leaf surface of *S. alpina* with evenly distributed glandular and non-glandular trichomes. – IV. Glandular trichomes (type B) on upper leaf surface of *S. palustris* (*arrows*: distribution along the veins). – V. Type G non-glandular trichomes and type A glandular trichomes scattered on the adaxial leaf surface of *S. recta* subsp. *recta*. – VI. Dense covering formed by type F trichomes on abaxial leaf surface trichomes of *S. salviifolia*.

The investigated *Stachys* taxa differed in presence of described types of glandular trichomes, as well as in their distribution on the leaf surface. Type A short capitate trichome was found to be a very specific type of trichome, characteristic for *S. recta* subsp *recta*. On the other hand, type B short ca-

pitate trichome, was found to be very common, present on leaves of all the taxa investigated beside *S. recta* subsp. *recta* and *S. officinalis*. Very common was also type $D_1$ long capitate trichome, found on leaves of *S. recta* subsp. *subcrenata, S. salviifolia, S. palustis* and *S. officinalis*. Sessile capitate trichome, type $C_1$, was present in only two of the taxa investigated (*S. officinalis* and *S. salviifolia*), as well as type $D_2$ of long capitate trichome (*S. sylvatica* and *S. alpina*). Presence of the peltate trichome (type $C_2$) was determined only in *S. officinalis*. In most of the investigated species, capitate trichomes were evenly distributed on adaxial and abaxial leaf surfaces, with the exception of *S. officinalis* and *S. salviifolia*, which type $C_1$ capitate trichome were present only on adaxial or abaxial surface, as well as, *S. sylvatica* and *S. palustris*, which type B glandular trichomes were distributed evenly only on the abaxial, while being mainly situated along the veins on the adaxial surface. The difference in distribution was also observed for long capitate trichomes; type $D_1$ was found mostly on abaxial, while being rather rare on adaxial leaf surface of *S. recta* subsp. *subcrenata* and *S. salviifolia*. The same distribution (mostly on abaxial leaf surface) was noticed for type $D_2$ trichomes on leaves of *S. sylvatica* (Table 2, Fig. 2. I-VI).

Unbranched non-glandular trichomes are widespread in the investigated *Stachys* taxa; they are found on both sides of the leaf, always in combination with glandular trichomes. The density of non-glandular trichomes varied from a very dense coverage obscuring the epidermal surface to a very scattered appearance (Fig. 2. V–VI). In the investigated species three types are recognised (type E, F and G), which differ in the structure of the trichome base, in the surface characteristics (with or without cuticular micropapillae) and in the number of cells forming the trichome (Table 2, Fig. 4).

Type E trichomes are usually long and slender, smooth or sculptured with small papillae, 1–5 cellular, on a pedestal of slightly enlarged epidermis cells (Fig. 4. I–II).

Type F trichomes are multi-cellular, smooth, slightly risen above the epidermis by one elevated basal cell. The surrounding epidermis cells show no modification. On both sides of the leaves, this type of trichome is extremely numerous and so long that they are hardly ever straight, but bent and contorted (Fig. 4. III).

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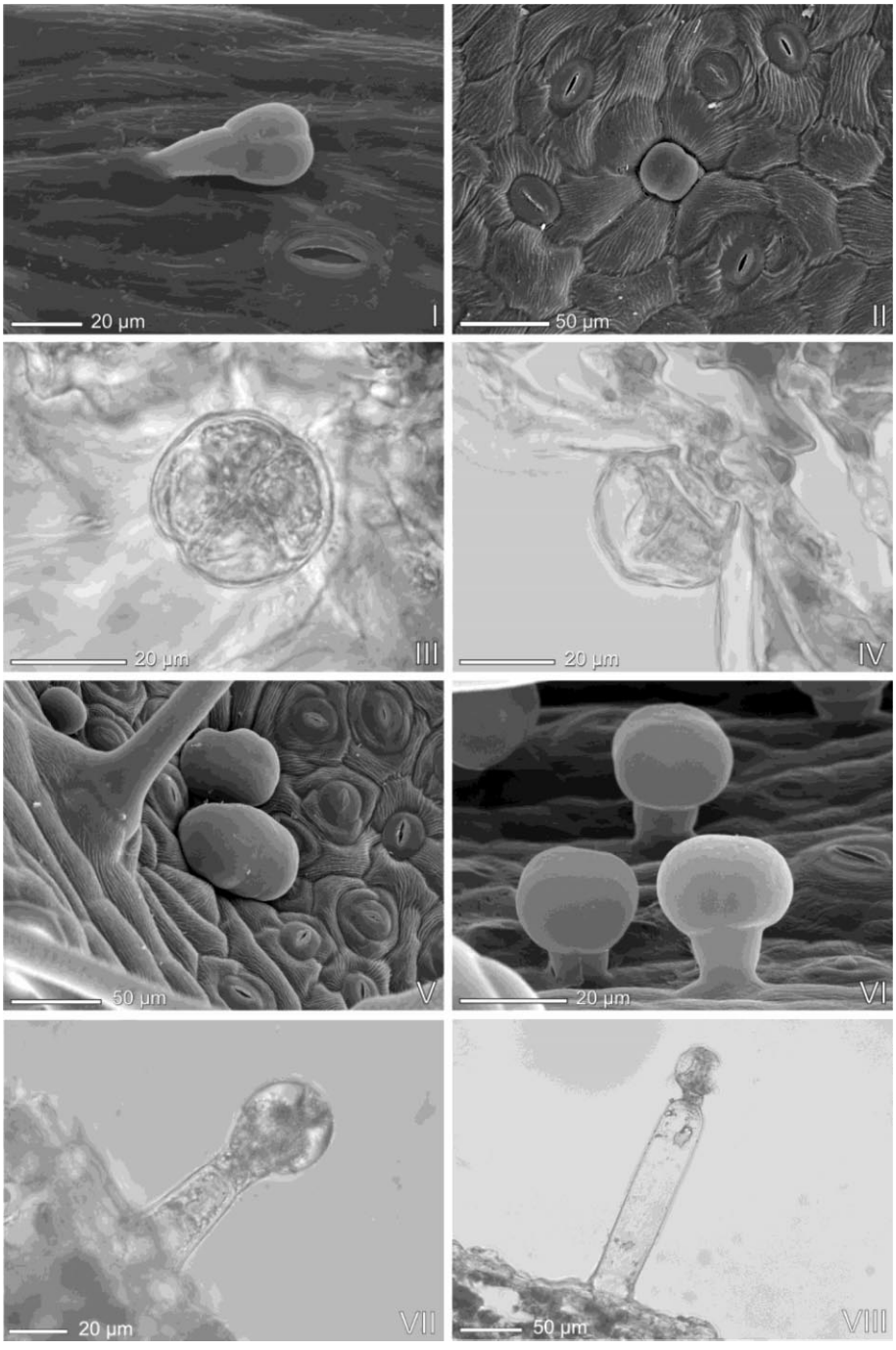
Fig. 3. Secretory (Glandular) trichomes in *Stachys* – I. Short capitate trichome, type A, appressed to the adaxial leaf surface (*S. recta* subsp. *recta*, SEM). – II. Sessile capitate trichome, $C_1$ type (*S. officinalis*, SEM). – III. Type $C_1$ trichome, detail of a secretory head (*S. officinalis*, LM). – IV. Type $C_1$ trichome on transversal cut of *S. officinalis* leaf (LM). – V. Peltate trichome, type $C_2$, on abaxial leaf surface of *S. officinalis* (SEM). – VI. B type short capitate trichome with a 4-celled club-shaped head (*S. sylvatica*, SEM). – VII. Type $D_1$ long capitate trichome with unicellular head (*S. palustris*, LM). – VIII. Type $D_2$ long capitate trichome (*S. alpina*, LM).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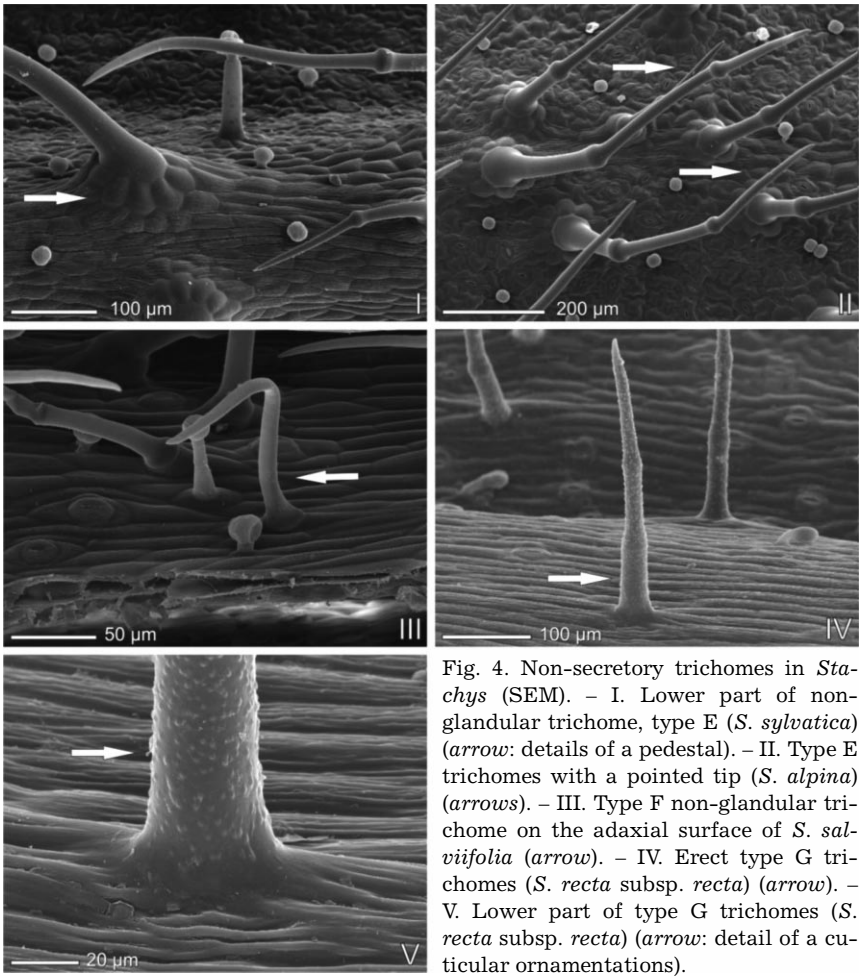


Fig. 4. Non-secretory trichomes in *Stachys* (SEM). – I. Lower part of non-glandular trichome, type E (*S. sylvatica*) (*arrow*: details of a pedestal). – II. Type E trichomes with a pointed tip (*S. alpina*) (*arrows*). – III. Type F non-glandular trichome on the adaxial surface of *S. salviifolia* (*arrow*). – IV. Erect type G trichomes (*S. recta* subsp. *recta*) (*arrow*). – V. Lower part of type G trichomes (*S. recta* subsp. *recta*) (*arrow*: detail of a cuticular ornamentations).

Type G trichomes are erect, 3–4 cellular, covered with small papillae, with one basal cell on epidermis level. The basal cell is not easily distinguishable from other epidermal cells, with the possible exception of being slightly larger in some instances. The surrounding epidermis cells are not modified (Fig. 4. IV–V).

Type E is very common and can be found in 5 of the investigated taxa. However, according to density and occurrence or absence of micropapillae, some differentiation between the investigated taxa is possible (Fig. 1). Type F and G are specific for *S. salviifolia* and *S. recta* subsp. *recta*, respectively. All three types are evenly distributed on both sides of the leaves (Table 2).

## Discussion

The *Lamiaceae* comprise of many commercially important essential oil-accumulating species. Glandular trichomes occurring in this genera are recognized as the site of essential oil biosynthesis, secretion and accumulation (Croteau 1986, Gershenzon & al. 1989) and their structure has been studied extensively (Werker & al. 1985a, b, Fahn 1988, 2000, Werker & al. 1993, Ascensao & al. 1995, Serrato-Valenti & al. 1997, Bisio & al. 1999, Corsi & Botega 1999, Werker 2000). Essential oil analysis of *Stachys* taxa provides a general idea of their essential oil composition, since mono- and sesquiterpens account for the main portion of the essential oils studied (Cakir & al. 1997, Chalchat & al. 2000, Chalchat & al. 2001, Bini Maleci & al. 2002, Tirillini & al. 2004, Bilusic Vundac & al. 2006, Giuliani & al. 2008). Previous investigations of this taxa have shown the presence of some other biologically active compounds, such as flavonoids, tannins and terpenoids (Zinchenko 1972, Ross & Zinchenko 1975, Lehrerr & al. 1984 a,b, Kobzar 1986, Kobzar & Nikonov 1986, Yamamoto & al. 1994, Myase & al. 1996, Bankova & al. 1999, Skaltsa & al. 2003, Grujic-Jovanovic & al. 2004, Bilusic Vundac & al. 2005, Bilusic Vundac & al. 2007).

The surface of the investigated *Stachys* leaves is characterised by glandular and non-glandular trichomes. Three types of non-glandular trichomes were observed (type E, F and G) which differed in the structure of the trichome base, trichome cuticle characteristics and in cell number. The non-glandular hairs on the leaves of *S. alpina, S. officinalis, S. palustris, S. recta* subsp. *subcrenata* and *S. salviifolia* are abundant and long and they certainly play a role in protecting the plant from excessive transpiration and insulation. On leaves of *S. recta* subsp. *recta* and *S. sylvatica*, non-glandular trichomes were more scattered.

Observations on the leaves of the taxa investigated showed the presence of different glandular trichomes and were divided, according to their micromorphological characteristics, into short capitate (type A and B), sessile capitate (type $C_1$), peltate (type $C_2$) and long capitate (type $D_1$ and $D_2$).

Short capitate trichomes found on the leaves of the investigated *Stachys* taxa can be divided into 2 types (type A and B). Type A was found to be a very specific type of short capitate trichome, present only on the leaves of *S. recta* subsp. *recta*. This type of trichome differed from other glandular trichomes in *Stachys* by its structure, as well as its characteristic position on the leaf; it was not erect like the other types, but always inclined or apressed to the epidermal surface, with entire trichome body lying on the epidermis. This is the first report on this type of glandular trichome.

Type B short capitate trichomes, with a 4-celled club-shaped head, which are present in most of the taxa investigated, were not found on the leaves of *S. officinalis* and *S. recta* subsp. *recta*. This type of trichome was

described in literature for some *Stachys* taxa (Bini Maleci & Giuliani 2006, Giuliani & al. 2008, Giuliani & Bini Maleci 2008). In the present study it was not found on leaves of *S. recta* subsp. *recta*, as it was by Giuliani & al. 2008 who proposed it to be the only type of glandular trichome for that taxa. Also, in the present study, peculiar type of peltate trichome, with well developed stalk, described for *S. germanica* subsp. *salviifolia* (= *S. salviifolia*) (Giuliani & Bini Maleci 2008), was not encountered. The reason may be due to the findings that the presence of different types of trichomes on the same species may depend on the particular development stage (Bini Maleci & Giuliani 2006).

Sessile capitate trichomes (type $C_1$), composed of a basal epidermal cell, a short neck cell and a broad head of 4 secretory cells, were found only on leaves of *S. officinalis* (abaxial surface) and *S. salviifolia* (adaxial surface). *S. officinalis*, although widely spread, was not subject of intensive studies (only publication dealing with a particular subspecies of *S. officinalis* was the one by Giuliani & Bini Maleci in 2008). In present study, *S. officinalis* was not only characterized by presence of type $C_1$ trichomes, but was also the only taxa, beside *S. recta* subsp. *recta*, which lacked type B short capitate trichomes. Furthermore, only on *S. officinalis* leaves peltate trichomes (type $C_2$) could be observed. Since not only *S. officinalis*, but also other *Stachys* taxa investigated, contained essential oil (Bilusic Vundac & al. 2006), these findings support the opinion that plants, lacking peltate trichomes, can produce essential oil in particular types of capitate trichomes (Bini Maleci & Giuliani 2006, Giuliani & Bini Maleci 2008, Giuliani & al. 2008). On the other hand, since, in aromatic *Lamiaceae*, the main production of essential oil is attributed to peltate trichomes (Werker 1993, Fahn 2000, Werker 2000), the presence of peltate trichomes in *S. officinalis* could explain why this taxa had the highest essential oil content, among the other taxa investigated (Bilusic Vundac & al. 2006). Nevertheless, in a recent study, Giuliani & al. 2008 have found that peltate trichomes of some of the *Stachys* taxa differ from typical peltate trichomes by producing not only an essential oil, but also a complex mixture of polyphenols and polysaccharides, leaving their complete function yet to be discovered.

Presence of the different types of glandular trichomes, as well their location on either adaxial or abaxial leaf surfaces, presented in this study, can be considered as significant taxonomic characteristics of *S. officinalis*.

Long capitate trichomes observed on the leaves of the investigated taxa can be divided into two types (type $D_1$ and $D_2$). Type $D_1$ trichomes, with unicellular head, were present in *S. salviifolia, S. palustris, S. officinalis* and *S. recta* subsp. *subcrenata*, while on the leaves of *S. alpina* and *S. sylvatica* type $D_2$ trichomes, with head composed of 4 secetory cells, could be observed. The findings of type $D_2$ of long capitate trichomes are consistent with the study of Giuliani & Bini Maleci 2008 who described the

similar type of trichome on abaxial leaf surface of *S. sylvatica*. Microscopical analysis of leaves of *S. recta* subsp. *recta* showed no presence of any type of long capitate trichomes.

Among others, two subspecies of *S. recta* were examined (*S. recta* sub. *recta* and *S. recta* sub. *subcrenata*). These two subspecies were growing under almost identical conditions and were collected at contiguous habitats, in full flower. The types of trichomes found on leaves of these two subspecies were completely different. Type A short capitate trichome, which was found to be the only glandular trichome present on leaves of *S. recta* sub. *recta*, was absent from leaves of *S. recta* sub. *subcrenata* (as well as leaves of other taxa investigated). On the leaves of *S. recta* subsp. *subcrenata* other type of short capitate trichome (type B), as well long capitate trichome ($D_1$ type) were found. Short capitate trichome function is usually related with polysaccharidic or polysaccharidic-proteic secretion (Serrato-Valenti & al. 1997, Bisio & al. 1999, Fahn 2000, Werker 2000), but Giuliani & Bini Maleci 2008 proved that these trichome in some *Stachys* taxa produce not only polysaccharides and proteins, but also essential oil and polyphenols. The same study showed that a certain type of long capitate trichomes can produce essential oil, as well. Taking these findings into account, the presence of different types of glandular trichomes on leaves of these two *S. recta* subspecies, could be directly related to the differences found in their polyphenolic, as well as essential oil composition (Bilusic Vundac & al. 2005, 2006, 2007). The two *S. recta* subspecies differed also in types of non-glandular trichomes; on *S. recta* subsp. *subcrenata* type E was present, while on *S. recta* subsp. *recta* leaves type G could be observed. Furthermore, on *S. recta* subsp. *subcrenata* leaves non-glandular hairs were long and abundant, forming a dense covering, while in *S. recta* subsp. *recta* they were more scattered. The above described differences between these two subspecies, could support the opinion of Visiani 1829, who described *S. subcrenata* as a separate new species.

Very long capitate hairs, characterised by a stalk consisting of three or more cells and a head composed of 4 or more secreting cells, which are considered to be characteristic for some *Stachys* species (Falciani & al. 1995, Bini Maleci & al. 2002, Giuliani & al. 2008, Giuliani & Bini Maleci 2008) were lacking on the leaves. They are usually present on the inflorescence which was not investigated in this study.

This study is the first report on types of trichomes present in *S. palustris* and *S. recta* subsp. *subcrenata*. Furthermore, it shows the types and distribution of glandular trichomes in all the investigated taxa, enabling particularly the characterisation of *S. officinalis* and differentiation among two *S. recta* subspecies (*S. recta* subsp. *recta* and *S. recta* subsp. *subcrenata*). The types B, C and D of the glandular trichomes have already been described for other *Lamiaceae* (Werker & al. 1985 a, b, Werker 1993, Ascensao & al. 1995, Serrato-Valenti & al. 1997, Bisio & al. 1999, Corsi &

Botega 1999, Werker 2000, Tornadore & al. 2004, Bini Maleci & Giuliani 2006, Giuliani & al. 2008, Giuliani & Bini Maleci 2008), while type A has never been recorded previously. This study is also a first report on different types of non-glandular hairs in investigated *Stachys* taxa. Furthermore, in *Lamiaceae*, the presence of non-glandular and glandular trichomes on the calyces is most often used in taxonomy (Ball 1972, Pignatti 1982). Our observations, however, show that the distribution of the trichomes on the leaf is also an important feature that can help to differentiate some *Stachys* taxa. For an unambiguous determination of the trichome type a key for all types found in the investigated taxa is presented (Fig. 1).

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