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Trichomes morphology of six Lebanese species of Stachys (*Lamiaceae*)

Abstract

El Beyrouthy, M., Arnold-Apostolides, N. & Dupont F.: Trichomes morphology of six Lebanese species of *Stachys* (*Lamiaceae*). — Fl. Medit. 19: 129-139. 2009. — ISSN 1120-4052.

The study consists in a micromorphological investigation of the characteristic trichome types in leaves, stems, calyces and corollas in the *Stachys* L. species (*Stachys cretica* L. subsp. *vacillans* Rech. f., *Stachys ehrenbergii* Boiss., *Stachys distans* Benth., *Stachys neurocalycina* Boiss., *Stachys nivea* Labill., *Stachys palaestina* L.) distributed in Lebanon and restricted to Levantine as part of the taxonomical study of the genus *Stachys*. The results showed twenty one different types of trichomes (11 non-glandular, 10 glandular) on the studied species. Fourteen types were characteristic for one species alone. Only four types of trichomes were found on all the 6 studied species. Of the 21 different types of trichomes, 8 non-glandular hairs and six glandular hairs were characteristic of one species. Those results indicate that non-glandular hairs are more important than glandular hairs concerning the separation between *Stachys* species. In addition, the study shows that the corollas and calyces of the studied species have the most important number of typical trichomes (6 of 21). Concerning the species *Stachys neurocalycina* comes first with five typical trichomes (1, 2, 4, 5 and 17), followed by *Stachys palaestina* with four (14, 15, 20 and 21), *Stachys ehrenbergii* with two (12 and 13), and one typical trichome for *Stachys cretica* subsp. *vacillans* (19), *Stachys distans* (18) and *Stachys nivea* (7).

Key words: *Labiateae*, Lebanon, micromorphology.

Introduction

The species belonging to the *Lamiaceae* family are characterized by the presence of glandular trichome. This epidermal appendice is not only in species having a characteristic scent, but also in species which lack fragrance. The literature concerning these trichomes is very rich as regards to morphology, differentiation, ultrastructure and mode of secretion (Werker 2000; Fahn 2000).

Stachys L., one of the largest genera of the *Lamiaceae*, is a sub-cosmopolitan genus centered in the warm temperate regions of the Mediterranean as well as Southwest Asia, with secondary centers in the Americas and southern Africa. It contains about 300 species (Bhattacharjee 1980; Hickey & King 1997; Mabberley 1997; Lindqvist & Albert 2002) and around 487 species according to Govaerts. In Lebanon, they are used in folk medicines to treat genital tumors, inflammatory tumors, coughs and ulcers. Teas prepared from the

plant or its leaves are used in phyto-therapy, possessing carminative, sedative, laxative, antispasmodic, diuretic and emmenagogue activities (El Beyrouthy 2008).

As reported by Bhattacharjee (1980), these plants present a wide range of variability leading to several infrageneric classifications proposed during the nineteenth century (Reichenbach 1830; Bentham 1834, 1848; Boissier 1879). Ball (1972) subdivided the genus into four sections and proposed the term 'group' for the taxa referred to *S. germanica* and *S. recta.* Bhattacharjee (1980) proposed a new infrageneric classification of *Stachys*, structured into two subgenera, 21 sections and numerous subsections. Pignatti (1982), Greuter & al. (1986) suggested a classification of *Stachys* similar to that proposed by Ball (1972), but without his subdivision into sections. They referred several species to the *S. germanicus* group. Ball (1972) and Greuter & al. (1986) considered that *S. alpina* should be referred to this group; however, Pignatti (1982) did not agree with this.

The new taxonomy ascribes great value to the structure and distribution of trichomes, both secretory and non-secretory (Werker & al. 1985a, b; Cantino 1990; Maleci Bini & Servettaz 1991; Karousou & al. 1992). Indeed Ball (1972) and Pignatti (1982) classifying the *S. germanica* group, utilized glandular trichomes present on stems and calyces as distinctive characters in dichotomic keys and species descriptions. The taxonomic value of the indumentum and its importance in systematic and phylogenetic relationships are well known in *Lamiaceae* (Abu-Assab & Cantino 1987). Trichomes are among the most useful taxonomic characters in some genera of the *Lamiaceae* family. Their absence or presence and their typology can be used as taxonomic markers in the infrageneric classification of the genus *Teucrium* L., while the infrasectional classification of section *Polium* (Mill.) Schreb. is based almost totally on the typology of the trichomes (Navarro & El Oualidi 2000). Trichome morphology is an essential taxonomic character for delimitation of the sections *Ambleia* Bentham and *Zietenia* (Gled.) Bentham in the genus *Stachys* as well the section *Ambleia* is characterized by dendroid indumentum and it is isolated from other sections of *Stachys* by this feature (Bhattacharjee 1980, 1982).

Previous work on the 6 studied *Stachys* was only on the chemistry of *S. distans* (Piozzi & al. 2002). In order to obtain a deeper insight into this difficult genus and to identify further distinctive characters at different taxonomic levels, we carried out for the first time, a micromorphological study on trichomes of 6 from the 11 *Stachys* of the Lebanese flora referred to 3 different sections following the Greuter & al. classification (1986). The taxonomical objective of the study is to differentiate between the species of *Stachys* using the trichomes.

Material and methods

The studied species of *Stachys* were collected from different locations in Lebanon (Tab. 1) and identified according to the flora of Lebanon (Mouterde 1966, 1970 & 1983). All voucher specimens of the species are deposited at the Herbarium of the Faculty of Agronomy of the Holy Spirit University of Kaslik (Lebanon) (Tab. 1). For the anatomical study; the corollas, calyces, stems and leaves were observed in cross-sections. We also obtained lower and upper cross-sections from the fresh materials and the trichomes were observed in different spots in every studied organ (e.g. in the case of the leaves, abaxial

Tab. 1. Introduction of the 6 studied *Stachys* species.

Voucher specimen	Taxa	Section	Distribution (Med Checklist)	Place and date of collection
MNV147	*Stachys cretica* subsp. *vacillans* Rech. f.	Eriostomum (Hoffmanns. & Link) Dumort.	Endemic: Asiatic Turkey, Israel, Jordan, Lebanon and Syria	El Arez (the Cedars), 25/06/2005, 1700m
MNV146	*Stachys ehrenbergii* Boiss.	Eriostomum (Hoffmanns. & Link) Dumort.	Endemic: Asiatic Turkey, Lebanon and Syria	Makmel, 20/08/2005, 1900m
MNV149	*Stachys distans* Benth.	Olisia Dumort.	Endemic: Asiatic Turkey, Israel, Jordan, Lebanon and Syria	Jbeil, 10/03/2005, 50m
MNV152	*Stachys neurocalycina* Boiss.	Olisia Dumort.	Endemic: Israel, Jordan, Lebanon and Syria	Nahr-el-Kalb, 23/04/2005, 150m
MNV150	*Stachys nivea* Labill.	Ambleia Benth.	Endeic: Lebanon and Syria, Sinai (doubtfully present)	Ras Baalbeck, 06/04/2006, 1000m
MNV151	*Stachys palaestina* L.	Ambleia Benth.	Endemic: Israel, Jordan, Lebanon and Syria	Kosba, 25/05/2006, 400m

and adaxial surface and in the case of the stems, vegetative and floral stems were studied). In our results (Tab. 2 and 3) we have summarized the localization of the trichome in the organs of the studied species. From the Herbarium sample, the species detailed morphological characteristics were established. For the anatomical investigations, samples were investigated under light microscope and microscope photographs were taken with a Spot In-SIGHT Color Digital camera and an Olympus type microscope.

Results

We used the morphology to differentiate between the types of the non glandular trichomes. Concerning the glandular trichomes we followed the morphological classification described by Fahn (1979) and completed by Werker & al. (1985a, b).

Capitate hairs (Fig.1)

Capitate hairs were different in size and of three types on the basis of their structure:
Type I consisted of a short unicellular stalk and a round secretory head of one or two cells

Type II consisted of a short unicellular or bicellular stalk and a secretory head of two or four cells with a small subcuticular space
Type III consisted of a long pluricellular stalk and a secretory head of one or two cells

Peltate hairs (Fig. 2)

Peltate hairs were different in size and of two types on the basis of their structure:
Type I consisted of a short unicellular stalk and a round secretory head of twelve uniform cells
Type II consisted of a long unicellular or pluricellular stalk and a secretory head of eight to twelve cells

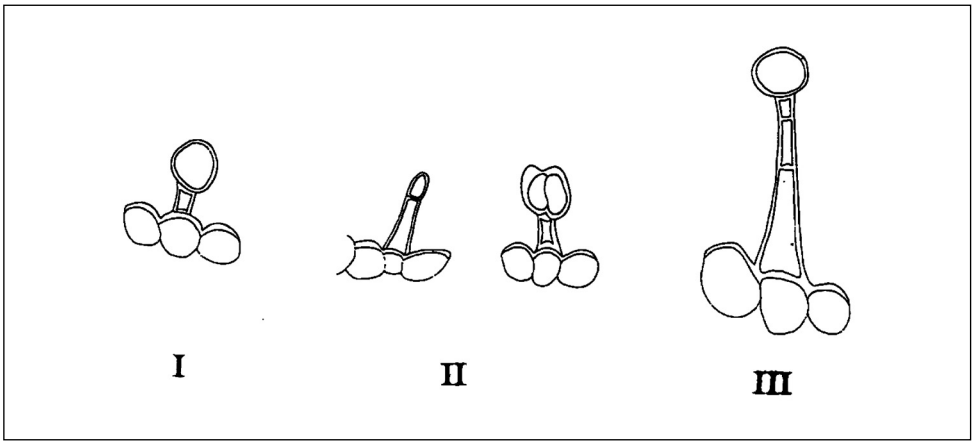


Fig. 1. glandular capitate hairs of the *Lamiaceae* (Arnold & Bellomaria 1993).

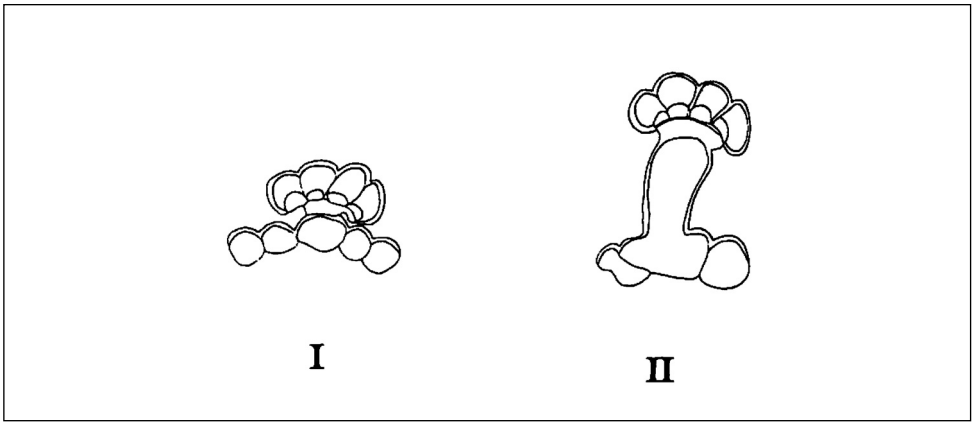


Fig. 2. glandular peltate hairs of the *Lamiaceae* (Arnold & Bellomaria 1993).

Type of trichomes:

21 diverse types of trichomes were observed on different organs of the six examined species. Eleven of the twenty-two were non glandular (non secretory) (Tab. 2) and ten were glandular (secretory) (Tab. 3). The identified types of trichomes are described briefly below.

1- Non glandular bicellular or pluricellular warted trichome with pointed apex and thick base.
2- Long capitate trichome of type III with one, or more, basal epidermal cell, a multicellular stalk, one neck cell and a secretory head.
3- Peltate trichome of type I.
4- Non glandular unicellular warted trichome, unbranched uniseriate with obtuse apex.
5- Non glandular unicellular warted trichome, unbranched uniseriate with pointed apex.
6- Type II short capitate trichome.
7- Non glandular multicellular branched trichome.
8- Non glandular unicellular or multicellular uniseriate filamentous trichome.
9- Short capitate hairs of type II with an apex composed of two secretory cells.
10- Peltate trichome of type II with a triangular uni or bicellular base.
11- Non glandular long multicellular unbranched uniseriate trichomes with pointed apex and thick base. Those hairs are often broken.
12- Long capitate hairs of type II with a multicellular apex.
13- Type I short capitate trichome, with a unicellular apex and base.
14- Capitate trichome of type III with a pluricellular long narrow stalk and a pluricellular apex.
15- Special non glandular hair of the calyx and corolla of *Stachys palaestina* L.
16- Non glandular pluricellular long trichome, unbranched uniseriate with pointed apex.
17- Non glandular bicellular trichome with a hooked apex.
18- Capitate trichome of type III with a pluricellular stalk and apex.
19- Capitate trichome of type III with a pluricellular stalk and an ovate pluricellular apex.
20- Non glandular unicellular warted trichome.
21- Stellate non glandular branched trichome.

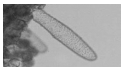
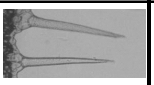
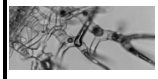
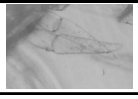
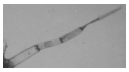
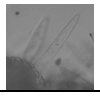
Distribution of trichomes:

Tables 2 and 3 reports the distribution of the different trichome types on stems, leaves, corollas and calyces of the studied taxa. It shows that first, the trichomes 3, 6, 9, 11 can be found on the majority of the studied species. Second, eight trichomes (1, 7, 12, 13, 14, 17, 21 and 19) are characteristic for all the organs in only one species. On the other hand, two trichomes (4, 20) are found on the corollas and two (15, 18) on the calyces and corollas of one species.

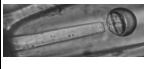
Trichomes 2 and 5 are typical respectively of the leaves and stems, calyces, stems of one species.

In addition, many trichomes are found in different organs of one species and are considered typical of this species. *Stachys neurocalycina* precedes with 5 typical trichomes (1, 2, 4, 5 and 17), followed by *Stachys palaestina* with 4 (14, 15, 20 and 21), *Stachys ehrenbergii* with 2 (12 and 13), and 1 typical trichome for *Stachys cretica subsp. vacillans* (19), *Stachys distans* (18) and *Stachys nivea* (7).

Tab. 2. Distribution of the 11 types of non glandular hairs in the 6 studied *Stachys* species.

	Species	objectif	*Stachys cretica* L. *subsp. vacillans* Rech. f.				*Stachys ehrenbergii* Boiss.				*Stachys distans* Benth.				*Stachys neurocalycina* Boiss.				*Stachys nivea* Labill.				*Stachys palaestina* L.			
	Organ		Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix
1		x200													+	+		+								
4		x200															+									
5		x100																+								
7		x100																	+	+	+	+				
8		x100									+	+	+	+											+	
11		x200	+	+	+	+	+	+	+	+	+	+	+	+												
15		x200																							+	+
16		x40								+				+	+											
17		x100													+	+		+								
21		x40																					+	+	+	+
20		x100																							+	

Tab.3. Distribution of the 10 types of glandular hairs in the 6 studied *Stachys* species.

	Species		*Stachys cretica* L. *subsp. vacillans* Rech. f.				*Stachys ehrenbergii* Boiss.				*Stachys distans* Benth.				*Stachys neurocalycina* Boiss.				*Stachys nivea* Labill.				*Stachys palaestina* L.			
	Organ		Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix	Stem	Leaf	Corolla	Calix
2		x100													+	+										
3		x200	+	+	+	+	+	+	+	+	+	+			+	+			+	+	+	+				
6		x200	+	+	+	+					+	+					+		+	+	+	+	+	+	+	+
9		x200	+	+	+	+	+	+	+	+	+	+			+	+			+	+	+	+				
10		x200					+	+	+	+															+	
12		x100					+	+	+	+																
13		x200					+	+	+	+																
14		x100																					+	+		+
18		x100											+	+												
19		x100	+	+	+	+																				

Discussion

The study allows illustrating that using trichomes to separate between species from a same genus is possible and reliable. Generally, of the 21 described trichomes uncovered on the 6 studied species, only 4 trichomes are found in all the species, 4 trichomes on 2 species and the remaining 13 trichomes are typical of one species or of some organs in one species. The trichomes that are found in all the species (3, 6, 9 and 11) are also found in other *Lamiaceae* in Lebanon (El Beyrouthy 2008; El Beyrouthy & al. 2008). These may be trichomes typical of the *Lamiaceae* family, but this requires further research to be proven.

On the other hand, the study demonstrates that in the *Stachys* genera, the non glandular with 8 typical trichomes, are more characteristic of one species than the glandular with only 6. Note that there were no peltate trichomes typical of a species. This result suggests that the non glandular trichomes are a better choice than the glandular trichome to separate between species of *Stachys*. Note also that there are no trichomes that are typical for a section. Actually, in this study, 2 species were investigated from the section *Ambleia*; there was no particular trichome typical for this section, but separating the two species using trichomes was a simple task. However the branched trichomes (24 and 25), are only present in this section. Those results are in accordance with those of Dinç & Öztürk (2008), for other 2 *Stachys* in the section *Ambleia* in Turkey having a different shape, branched trichomes. Those results can show that branched trichome morphology is an essential taxonomic character for delimitation of the sections *Ambleia* in the genus *Stachys.* Another is example is about the section *Olisia* Dumort. In separating *S. distans* and *S. neurocalycina*, both from the section *Olisia* Dumort, the non-glandular trichomes played an excellent role. In fact, the trichome 1, 4, 5, 16 and 17 were only present on *S. neurocalycina* and the trichomes 8, 11 and 19 only on *S. distans*. Those results demonstrate that trichomes play an essential role in separation species from the same section.

The most interesting observations for taxonomic purposes were those regarding the inflorescence; actually, leaves and stems were very similar in every species observed. Six of the 21 trichomes are found only at the corollas and calyces of one species; these results are similar to those of Cantino (1990) as they both indicate that the morphology of the glandular trichomes - particularly that of the calyces - can be useful in characterizing the different plant species. The distribution of trichomes on the calyces is particularly interesting since the calyx characters are often essential in the taxonomic determination of *Lamiaceae*, as indicated also by Ball (1972) and Pignatti (1982). The latter utilized the non-glandular or glandular calyx as a character to differentiate the various taxa in the *S. germanica* group. The observations made throughout the study, however, show that calyces of all the *Stachys* species examined have glandular and non-glandular trichomes. In previous studies the trichomes served to differentiate between *Stachys.* The differences in trichomes, observed between *S. recta subsp. recta* and *S. recta var. serpentini* allowed to better characterize the two subspecies (Maleci Bini & Giuliani 2006).

In addition, ten different glandular (8 capitate and 2 peltate) trichomes were observed in the 6 studied species. Concerning the section *Eriostomum* (Hoffmanns. & Link) Dumort, two species (*Stachys ehrenbergii* and *Stachys cretica subsp. vacillans*) were studied. In fact in this section, the glandular trichomes played an excellent role in separation the two studied species. The hairs 10, 12 and 13 (also present on the corol-

las of *S. palaestina*) were typical for *S. ehrenbergii* and the hair 19 for *S. cretica subsp. vacillans*. Those results can show that glandular trichome can play a role in separation species from the same section.

There were no peltate trichomes typical of a species. Those hairs probably play another role than to serve as a taxonomical feature in the *Stachys*. In fact, numerous species belonging to the genus *Stachys*, *S. plumosa* Griseb., *S. sylvatica* L., *S. germanica* L. and some related italian species (*S. alpina* L., *S. tymphea* Hausskn., *S. heraclea* All., *S. byzantina* C. Koch, *S. tirkey* C. Koch), *S. recta* L. *subsp. recta* and *S. recta var. serpentini* (Fiori) Arrigoni, lack peltate hairs, having only capitate hairs (Maleci Bini & Giuliani 2006). In *S. plumosa*, species characterized by a strong fragrance, only large capitate hairs, containing essential oil, are present. *S. recta subsp. recta* and *S. recta var. serpentini* are plants with a scarce scent, but containing a certain amount of essential oil. Concerning the calyx, both subspecies present the same glandular trichomes; however, in the *subsp. recta*, also non glandular hairs are present, while in the *subsp. serpentini*, besides the previous ones, large capitate hairs, typical of the *Stachys* inflorescences, have been observed. The difference probably depends on the different environment in which the two subspecies grow: calcareous soils the first, serpentine soils, rich in heavy metals, the second (Maleci Bini & Giuliani 2006).

Conclusions

The data presented here shows that trichome micromorphology is more useful in separation of species in one genus rather than characterizing large genera and families, except for few cases. In fact, 21 different types of trichomes (11 non-glandular, 10 glandular) were found on the 6 studied species. There was no typical trichome for a section or for the *Stachys* genus. But separating the species specially those of the same section, using trichomes was very easy. Nevertheless, numerous and repeated observations are necessary, because the plant can present different types of trichomes, depending on the particular moment of its life. It is likely that, inspecting more species, other types of trichomes can be observed. The observations here recorded have allowed characterizing better the Lebanese *Stachys*.

Although there is few one reliable molecular systematic study on *Stachys* including few species distributed in Lebanon, this work can provide strong supports for evolution of hair characters in *Stachys*. The results about the genus *Stachys* in Lebanon, although not complete, seem to be promising. The above findings confirm the observations made by taxonomists using an ordinary lens or stereoscope, who had noticed the visible differences between species of *Stachys* in respect of their glandular trichomes. This research on the *Stachys* shows that the presence or absence of a trichome in a certain organ of a species can be used to separate between species and genera from a taxonomical point of view.

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