Pak. J. Bot., 44(1): 33-46, 2012.

TRICHOME MICROMORPHOLOGY OF EGYPTIAN *BALLOTA* (LAMIACEAE) WITH EMPHASIS ON ITS SYSTEMATIC IMPLICATION

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Abstract

Trichomes of 5 taxa of the genus *Ballota* were examined by using light and scanning electron microscopy. The indumentum shows considerable variability among different species, and therefore, affords valuable characters in delimitation of species. The characters of taxonomic interest were presence of glandular and non-glandular trichomes, number of cells (uni-, bi-, tri- or multi-cellular), presence of multicellular branched trichomes and existence of branched (dendroid and stellate) trichomes. Two basic types of trichomes can be distinguished: glandular and non-glandular trichomes. The glandular trichomes can in turn be subdivided into two subtypes: stalked and sessile. The stalks of the glandular trichomes can be uni-, bi-, tri- or multi-cellular. In addition to presence of epidermal prickles, the non-glandular trichomes are classified also according to number of cells into uni-, bi-, tri-, multicellular, stellate and dendritic trichomes. Some of non-glandular trichomes may be branched or unbranched. Two keys are constituted according to both glandular and non-glandular trichomes. Overall, trichome micro-morphology is more useful in separation of *Ballota* species.

Introduction

The taxonomic value of the indumentum as well as its implication in systematics and phytogenetics are well known in Lamiaceae and in the related families Acanthaceae, Bignonicaceae, Scrophulariaceae and Verbenaceae (Abu-Asab and Cantino, 1987; Ahmad, 1974, 1978; Cantino, 1990; El-Gazzar and Watson, 1968, 1970; Elias and Newcombe, 1979; Gairola *et al*., 2009; Mathew and Shan, 1983; Metcalfe and Chalk, 1950; Rahn, 1992). Trichome micromorphology has been suggested to be useful in the phylogeny reconstruction (Abu-Asab and Cantino, 1987) and systematics of Lamiaceae (Cantino, 1990) as well as specific and subspecific levels (Bruni *et al*., 1987; Giuliani *et al*., 2008; Maleci Bini *et al*., 1992; Sebebe and Harley, 1992; servettaz *et al*., 1992).

Trichomes are widely distributed over the aerial reproductive and vegetative parts of plants of Lamiaceae and are ordinarily distinguished as glandular and non-glandular trichomes (Cantino, 1990; Navarro and El Qualidi, 2000). Glandular trichomes mainly include capitate glandular trichomes, subsessile glandular trichomes, clavate glandular trichomes, and branched glandular trichomes (Bokhari and Hedge, 1971; Huang and Cheng, 1971; Husain *et al*., 1989; Metcalfe and Chalk, 1950; Navaroo and El Qualidi, 2000; Solereder, 1908; Werker *et al*., 1985). They are general features of the family Lamiaceae (Ascensão and Pais, 1998; Bosabalidis 1990; Serrato-Valati *et al*., 1997). Glandular trichomes usually composed of four parts: foot cell, stalk cell, neck cells and head cell (Abu-Asab and Cantino, 1987; Bosabalidis 1990). Non-glandular trichomes are more common than glandular trichomes in Lamiaceae. Based on their morphology and number of cells, Cantino, 1990 divided non-glandular trichomes into four types: simple unicellular, simple multicellular, branched unicellular and branched multicellular.

The genus *Ballota* (Lamiaceae) is a medium sized genus of 33-35 species which are mainly distributed around the Mediterranean and Eurasia (Seidel *et al*., 1999 and Tipirdamaz & Guvene, 2004), in Egypt it is represented by 5 taxa, two of them (*Ballota kaiseri* and *B. saxatilis*) are rare, threatened species and endemic to St. Catherine Protectorate, Southern Sinai, Egypt (Boulos, 2009).

Zaghloul *et al*., (2006) investigated the genetic diversity of 3 *Ballota* species growing in St. Catherine Protectorate, Southern Sinai, They demonstrated that the three *Ballota* species maintain relatively high levels of genetic diversity and that most of their genetic diversity was found within populations.

Up to now some studies of *Ballota* have been conducted (Tóth, 2009, 1992; Zaghloul *et al*., 2006; Şahin *et al*., 2005; Tipirdamaz and Guvenc, 2004). But none of them thoroughly evaluated the taxonomic significance of trichomes in the taxonomy of this genus. Thus, the morphology of trichomes in *Ballota* is still poorly documented. The characters of taxonomic interest in the present study were: glandular or non-glandular; number and length of the cells, unicellular or multicellular trichomes; and presence or absence of dendroid and stellate hairs. In addition, the taxonomic implications of trichome types in elucidating relationships among *Ballota* species were discussed.

Materials and Methods

Trichomes of five taxa of the genus *Ballota* in the flora of Egypt were studied. Generally, more than five different specimens of each taxon were considered for one species to ensure the stability of trichome structure among different specimens of one species. The herbarium material for this study was removed from specimens deposited mainly in the herbarium of Botany department, Faculty of Science, Cairo University (CAI), and herbarium of Botany department, Faculty of Science-Qena, South Valley University (QNA), (Table 1)..

Trichomes were obtained from leaves, stems and petioles and studied with a stereo- or a usual light microscope. For light microscopy, the stems and petioles have been sectioned at 20–30 µm by using hand microtome. For double staining of cross sections, safranin (2%) and light green (1%) combinations were used (Gerlach, 1977) with some modifications. The cross sections were dehydrated through an ethanol gradient and

finally xylol (99.5%), and mounted on slides using Canada balsam. They were studied by using a unit of a LEICA DM1000 light microscope with 400X to 1000X magnifications. Drawing of trichomes of leaves, stems and petioles were made at bench level by aid of KEN-A-VISION Micro-projector. The magnifications are given by a stage micrometer scaled to 0.1 and 0.01mm. For the scanning electron microscopy (SEM), small pieces of leaves, stems and petioles were fixed on aluminium stubs covered by a double-sticky adhesive tape, and were coated by a thin layer (ca.25nm) of gold–palladium with a SPI- Module sputter- coating for (30-60) seconds. The SEM photographs were captured using the JOEL JSM-5500 LV scanning electron microscope; at an acceleration voltage 5-20 KV. The type of indumentum was described and classified. The general classification scheme and the terminology follow Roe (1971), Cantino (1990), as well as Navarro and El-Oualidi (2000).

Table 1. Source of plant material used in the study of *Ballota* trichome micromorphology.

Taxa	Collector and place of collection
Ballota damascens	det. P. Mouterde, leg. Vivi Täckholm, 249, Lebanon, June 1954, (CAI)
Ballota kaiseri	H. Mosallam, K. Abdel Khalik & A.K. Osman, Sinai, St. Katharine, Wadi El-Arbaéen, (QNA)
Ballota pseudodictamnus	Loutfy Boulos, 2300, Al Tamimi, Wadi Derna, Gebel Akhdar, 31/3/1968, (CAI).
Ballota saxatilis	H. Mosallam, K. Abdel Khalik & A.K. Osman, Sinai, St. Katharine, Wadi El-Arbaéen, (QNA)
Ballota undulata	H. Mosallam, K. Abdel Khalik & A.K. Osman, Sinai, St. Katharine, Wadi El-Arbaéen, (QNA)

CAI = Cairo University Herbarium, QNA = Qena University Herbarium, (proposed acronym).

Results

Main types of the investigated trichomes and their distribution among the species studied are summarized in table 2. Selected SEM micrographs and drawn photos of trichome types are presented in plates 1-5. Type of indumentum shows considerable variability among species in different sections or series, and therefore, affords valuable characters in delimitation of species. Two main types of trichomes can be distinguished: non-glandular and glandular trichomes. Non-glandular trichomes can be further subdivided into simple unbranched and branched trichomes subtypes. Based on the shape and cell number, the type "simple unbranched" is again divided into 4 forms: unicellular trichomes, bicellular trichomes, tricellular trichomes and multicellular trichomes. Five kinds of branched trichomes were recognized in the present study based on number of cells and their branching location: bicellular, tricellular, multicellular, stellate and dendroid trichomes.

Glandular trichomes are very common in *Ballota* taxa. Based on the presence or absence of the stalk, glandular trichomes can be subdivided into two subtypes: stalked, or sessile. The stalks of the glandular trichomes can be unicellular, bicellular, tricellular or multi-cellular. Sessile trichomes are mostly not stalked and bladder-like at heads. Stalked glandular trichomes show variation in the size, shape, cell number, presence or absence of neck cell, length of neck cell and position of the stalk and the head.

Synopsis of trichomes types

Simple unbranched non-glandular trichomes

Unicellular trichomes: This kind of trichomes consists of only one cell; they are unicellular epidermal appendages and are common in Lamiaceae (Abu-Asab and Cantino, 1987; Bonzani et al., 2007; Cantino, 1990; El-Gazzar and Watson, 1970). Two forms are recognized in the present study. The first form is small and thick-walled (Plate 2-2, fig. 11; Plate 4-2, figs. 16-17; Plate 5-1, fig. 8), this form is recognized in *B. kaiseri*, *B. saxatilis* and *B. undulata*, the second form is large and thin-walled (Plate 2-2, fig. 13; Plate 3, fig. 8; Plate 4-1 fig. 13; Plate 4-2, figs. 18, 25; Plate 5-1, fig. 9) is present in *B. kaiseri*, *B. pseudodictamnus*, *B. saxatilis* and *B. undulata*.

Bicellular trichomes: This kind of trichomes have two cells but vary in wall thickness and length of the base cell. Two forms were observed, the first form has long base cell and thin-walled cells (Plate 2-2, fig. 14), while the other form has small base cell and thick-walled cells (Plate 5-1, fig. 10). This kind of trichomes observed only in *B. kaiseri* and *B. undulata* and is not common.

Tricellular trichomes: This kind of trichomes has 3 cells that are usually long and thin-walled, present only in *B. saxatilis* (Plate 4-2, fig. 19). Other 4 studied *Ballota* taxa do not have this type of trichomes.

Multi-cellular trichomes: This kind of trichomes has 4 or 5 cells and is not common and occur only in *B. undulata* (Plate 5-1, fig. 12), where in this kind there are two base cells.

Branched non-glandular trichomes

Bicellular trichomes: This kind of trichomes has only two cells. Bicellular branched trichomes were found in *B. kaiseri* and *B. saxatilis* (Plate 2-2, fig. 15; Plate 4-1, fig. 14).

Tricellular trichomes: This kind of trichomes has only three cells, and was observed in both *B. kaiseri* and *B. undulata* (Plate 2-2, fig. 16; Plate 5-1, fig. 11).

Multicellular trichomes: This kind of trichomes has more than three cells (4-10 or more), but the branching here starts after the epidermal cells directly and there are several basal cells. This kind was recorded in petioles of both *B. kaiseri* and *B. undulata* (Plate 2-2, fig. 17; Plate 5-2, figs. 13-15).

Stellate trichomes: This kind of trichomes is multicellular and branched at the tip of the stalk cell to form star-shaped (Xiang et al., 2010). These trichomes are common and occur in three species of the studied *Ballota* taxa (*B. kaiseri*, *B. saxatilis* and *B. undulata* (Plate 2-2, figs. 18-19; Plate 4-2, figs. 22-23, 25; Plate 5-2, figs. 16-19).

Table 2. Distribution of trichomes on the stems and petioles of *Ballota* taxa.

Taxa	Glandular trichomes																
	sessile		Unicellular stalked				Bicellular stalked				Tricellular stalked		Multicellular stalked				
	Number of head cells		Number of head cells				Number of head cells				Number of head cells		Branched	Unbranched			
													Number of head cells	Uniserrate		Bi-triserrate	
														Number of head cells		Number of head cells	
	Uni.	Tri.	Uni.	Bi.	Tri.	Multi.	Uni.	Bi.	Tri.	Multi.	Uni.	Bi.	Uni.	Uni.	Bi.	Uni.	Bi.
Ballota damascens	-	-	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-
Ballota kaiseri	+	-	-	+	+	-	+	+	+	+	+	-	-	-	-	+	+
Ballota pseudodictamnus	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Ballota saxatilis	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	-	-
Ballota undulata	+	-	+	+	-	-	+	+	+	-	+	-	+	-	-	-	-

Uni. = unicellular, Bi. = bicellular, Tri, = tricellular, Multi. = multicellular

Table 2. (Cont'd.).

Taxa	Non-glandular trichomes										
	Prickles	Unicellular	Bicellular		Tricellular		Multicellular		Stellate	Dendritic	
			Branched	Unbranched	Branched	Unbranched	Branched	Unbranched		basally	Longitudinally
Ballota damascens	+	-	-	-	-	-	-	-	-	+	-
Ballota kaiseri	-	+	+	+	+	-	+	-	+	-	-
Ballota pseudodictamnus	+	+	-	-	-	-	-	-	-	-	+
Ballota saxatilis		+	+	-	-	+	-	-	+	-	-
Ballota undulata	-	+	-	+	+	-	+	+	+	-	-

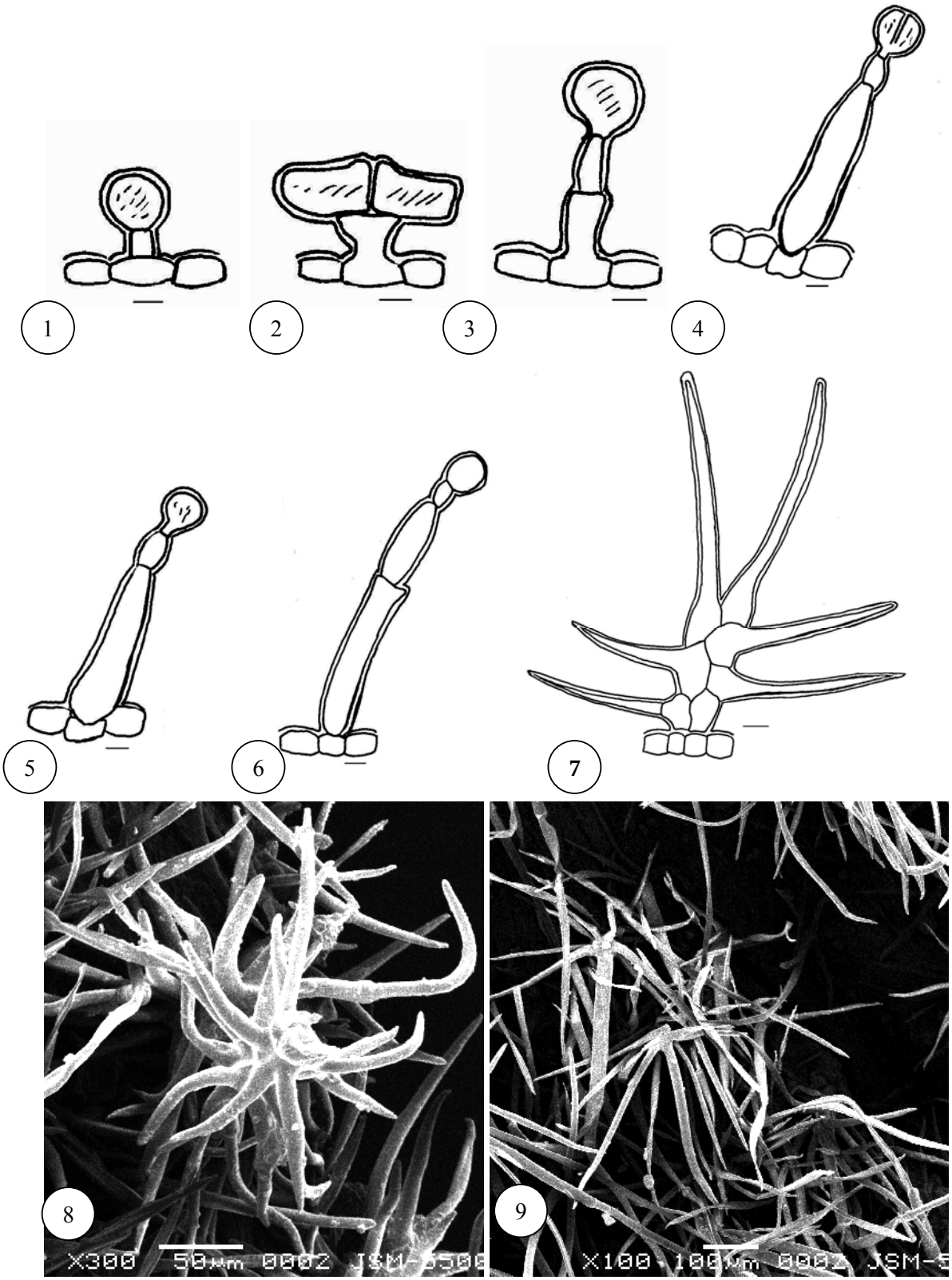

Plate 1. Drawn and SEM micrographs of glandular and non-glandular trichomes in *Ballota damascens*. (1) Glandular short unicellular stalked with unicellular head hair; (2) Glandular short unicellular stalked with bicellular head hair; (3,5) Glandular long bicellular stalked with unicellular head hairs; (4) Glandular long bicellular stalked with bicellular head hair; (6) Glandular long tricellular stalked with unicellular head hair; (7,8,9) dendroid trichomes.

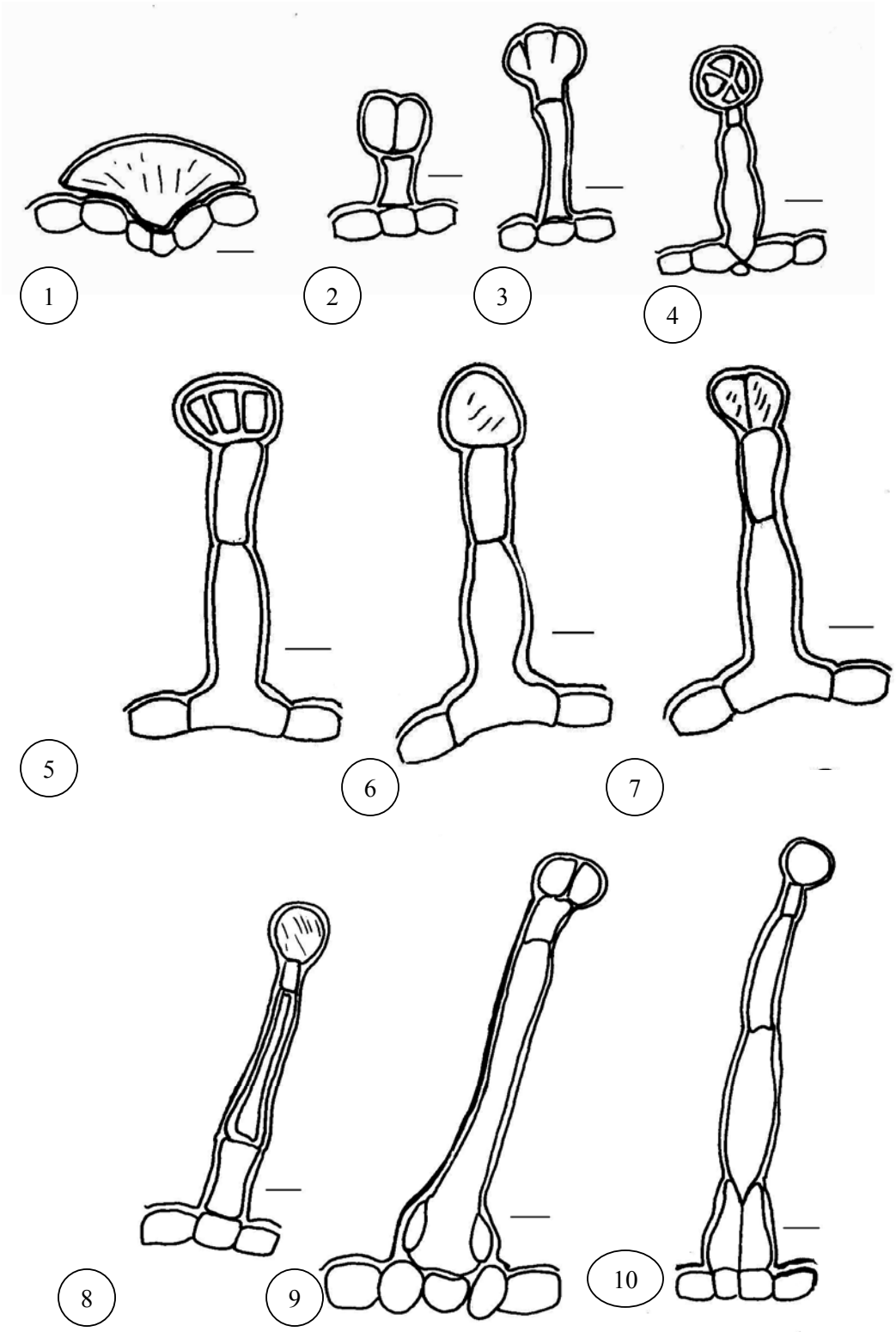

Plate 2-1. Drawn micrographs of glandular trichomes in *Ballota kaiseri*. (1) sessile hair; (2) unicellular stalked with bicellular head hair; (3) unicellular stalked with tricellular head hair; (4) bicellular stalked with multicellular head hair; (5) bicellular stalked with tricellular head hair; (6) bicellular stalked with unicellular head hair; (7) bicellular stalked with bicellular head hair; (8) tricellular stalked with unicellular head hair; (9) multicellular stalked with bicellular head hair; (10) multicellular stalked with unicellular head hair.

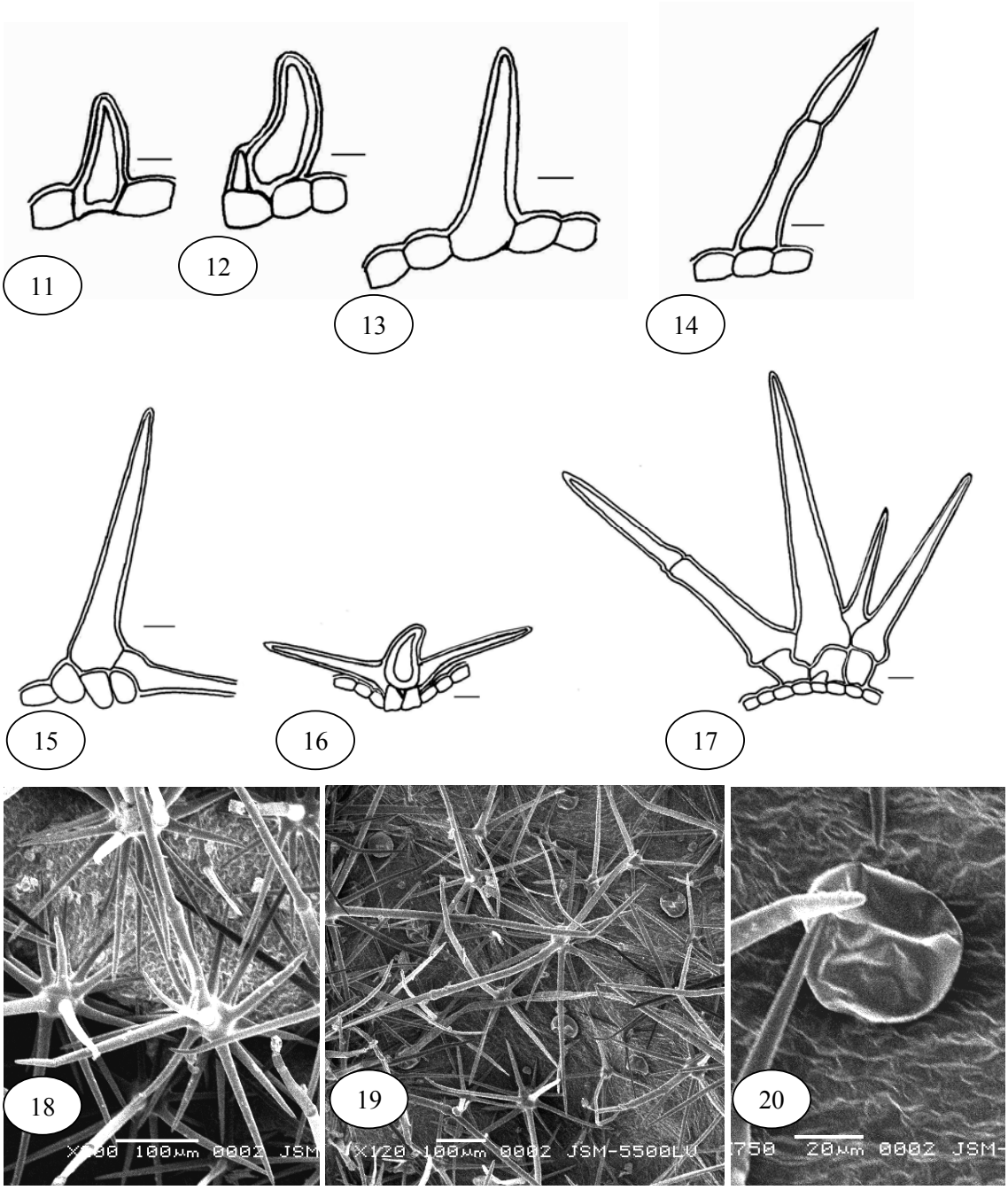

Plate 2-2. Drawn and SEM micrographs of non-glandular and glandular trichomes in *Ballota kaiseri*. (11) unicellular non-glandular hair; (12) bicellular branched non-glandular hair; (13) unicellular non-glandular hair; (14) bicellular non-glandular hair; (15) bicellular branched non-glandular hair; (16) tricellular branched non-glandular hair; (17) multicellular branched non-glandular hair; (18) stellate hair; (19) stellate and sessile hairs; (20) sessile hair.

Dendroid trichomes: This kind of trichome is branched along the stalk cell. Two forms of this kind were observed in the present study. The first form occurs in *B. damascens* (Plate 1, figs. 7-9) where in it the branching is along the biserrate short stalk cells and the another form exists in *B. pseudodictamnus* (Plate 3, figs. 6-7, 9-11) and branched longitudinally at nodes of the uniserrate long stalk cells.

Glandular trichomes: Glandular trichomes are important taxonomic characters in Lamiaceae (Cantino, 1990; Navarro and El Oualidi, 2000). Five subtypes of glandular trichomes were identified in this study. The most important characteristics of each subtype are described below.

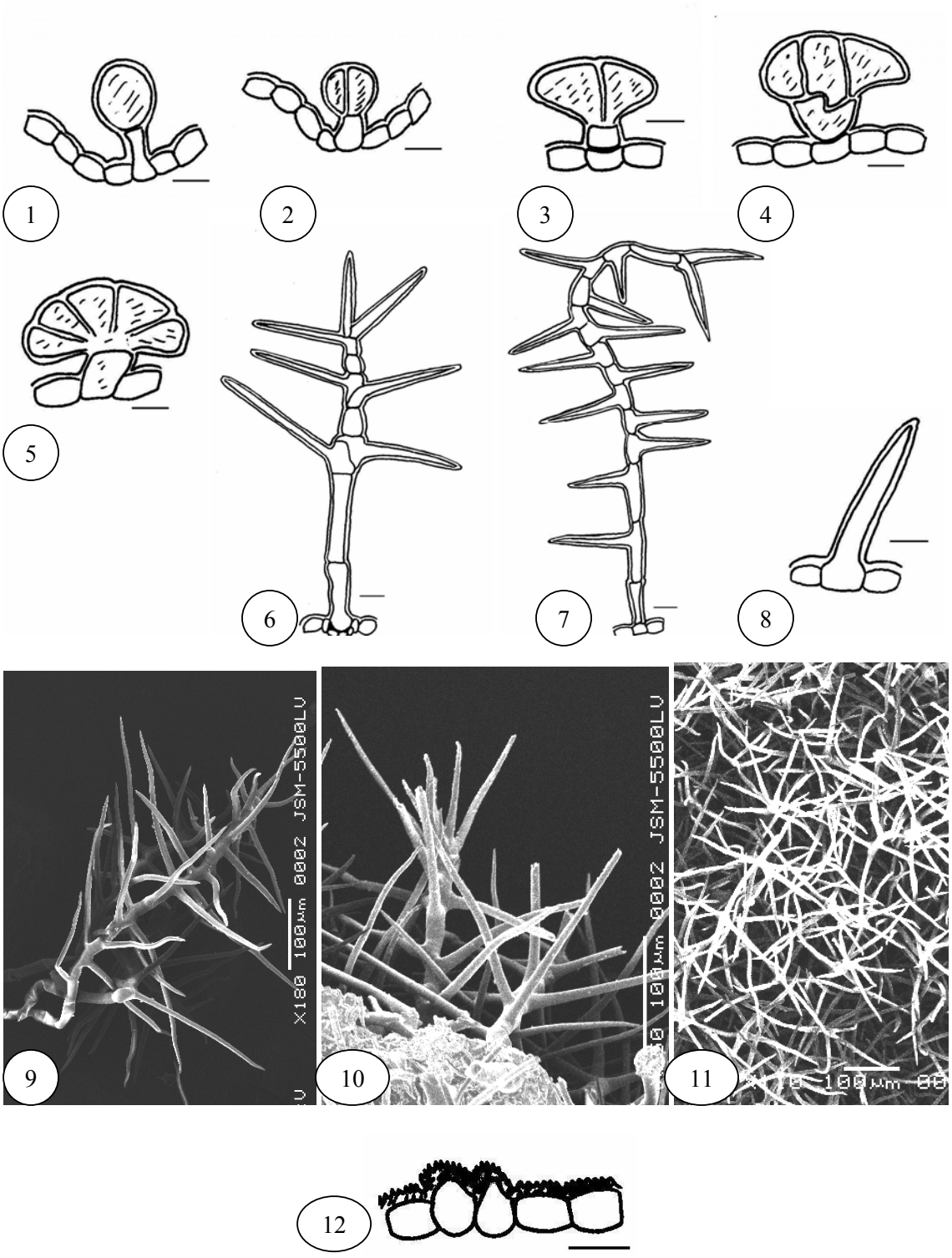

Plate 3. Drawn and SEM micrographs of glandular and non-glandular trichomes in *Ballota pseudodictamnus*. (1) unicellular stalked with unicellular head hair; (2) unicellular stalked with tricellular head hair; (3) unicellular stalked with bicellular head hair; (4) unicellular stalked with tricellular head hair; (5) unicellular stalked with multicellular head hair; (6) dendritic hair; (7) dendritic hair; (8) unicellular hair; (9) dendritic hairs; (10) dendritic hair; (11) dendritic hairs (12) epidermal prickles.

Sessile glandular trichomes: These trichomes have been frequently reported in Lamiaceae and Verbenaceae (Cantino, 1990; Metcalfe and Chalk, 1950; Werker *et al*., 1985; Salmaki *et al*., 2009). Various terms have been used for these trichomes such as short-stalked bladder-like glands, glandular scales, glandular capitate sessile trichomes, peltate hairs or subsessile glandular trichomes (Bruni & Modenesi, 1983; Salmaki *et al*., 2009; Maleci Bini & Servettaz, 1991). These trichomes appear sessile in surface view, nearly flat but with a short, usually

discoid stalk cell in cross-section (Abu-Asab & Cantino, 1987; Fahn, 1979, Xiang *et al*., 2010). In the present study, sessile glands are present in three species (*B. kaiseri*, *B. saxatilis* and *B. undulata*). According to number of head cells, sessile glandular trichomes have two forms, the first form has unicellular head and occur in all the last mentioned three species (Plate 2-1, fig. 1; Plate 2-2, figs. 19-20; Plate 4-1, figs. 1-2; plate 4-2, fig. 23 and Plate 5-2, figs. 17, 19) and the second form with tricellular head and is recorded only in *B. saxatilis* (Plate 4-1, fig. 3).

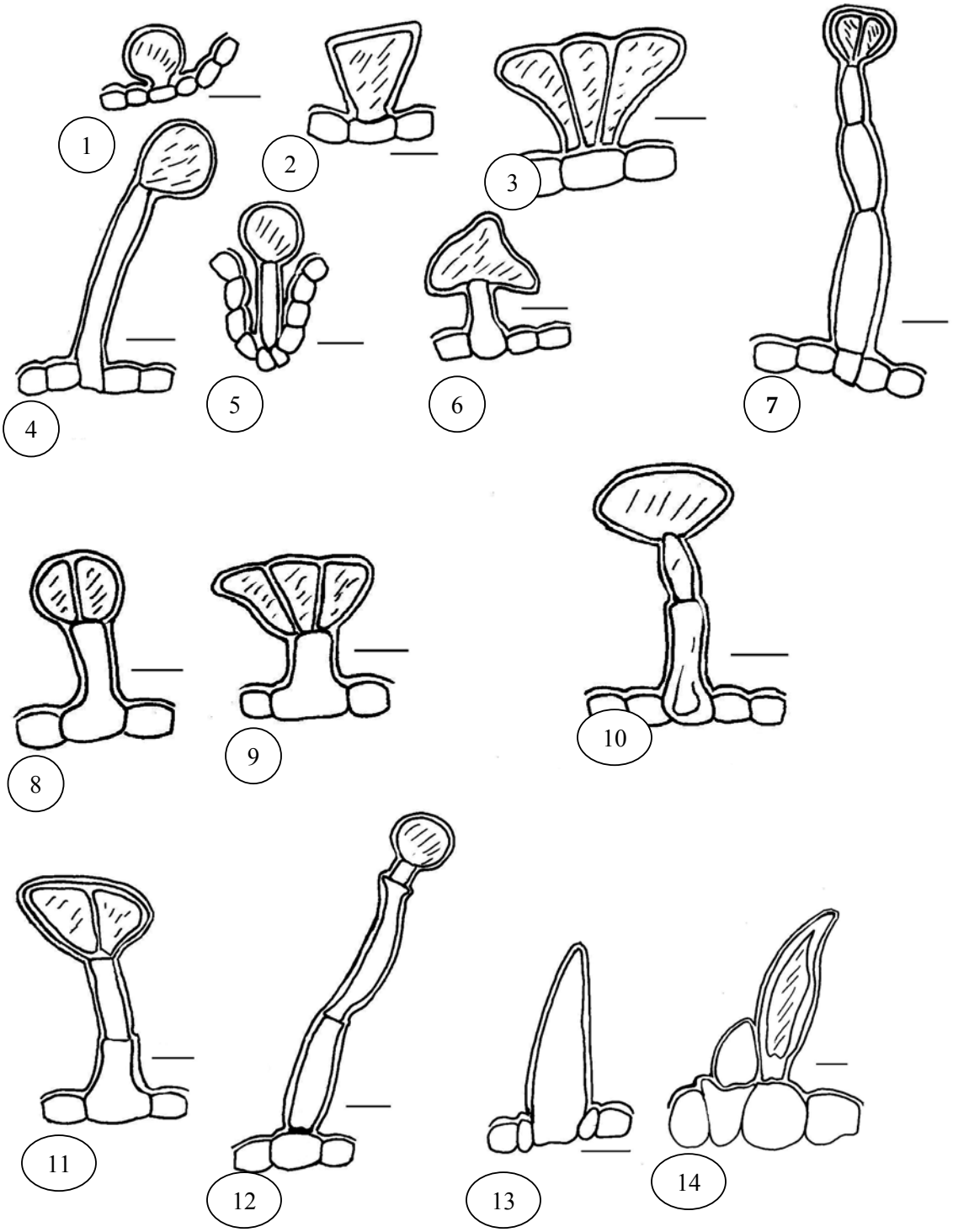

Plate 4-1. Drawn micrographs of glandular and non-glandular trichomes in *Ballota saxatilis*. (1) sessile unicellular hair; (2) sessile unicellular hair; (3) sessile tricellular hair; (4,5,6) unicellular stalked with unicellular head hair; (7) tricellular stalked with bicellular head hair; (8) unicellular stalked with bicellular head hair; (9) unicellular stalked with tricellular head hair; (10) bicellular stalked with unicellular head hair; (11) bicellular stalked with bicellular head hair; (12) tricellular stalked with unicellular head hair; (13) multicellular non-glandular hair; (14) bicellular branched hair.

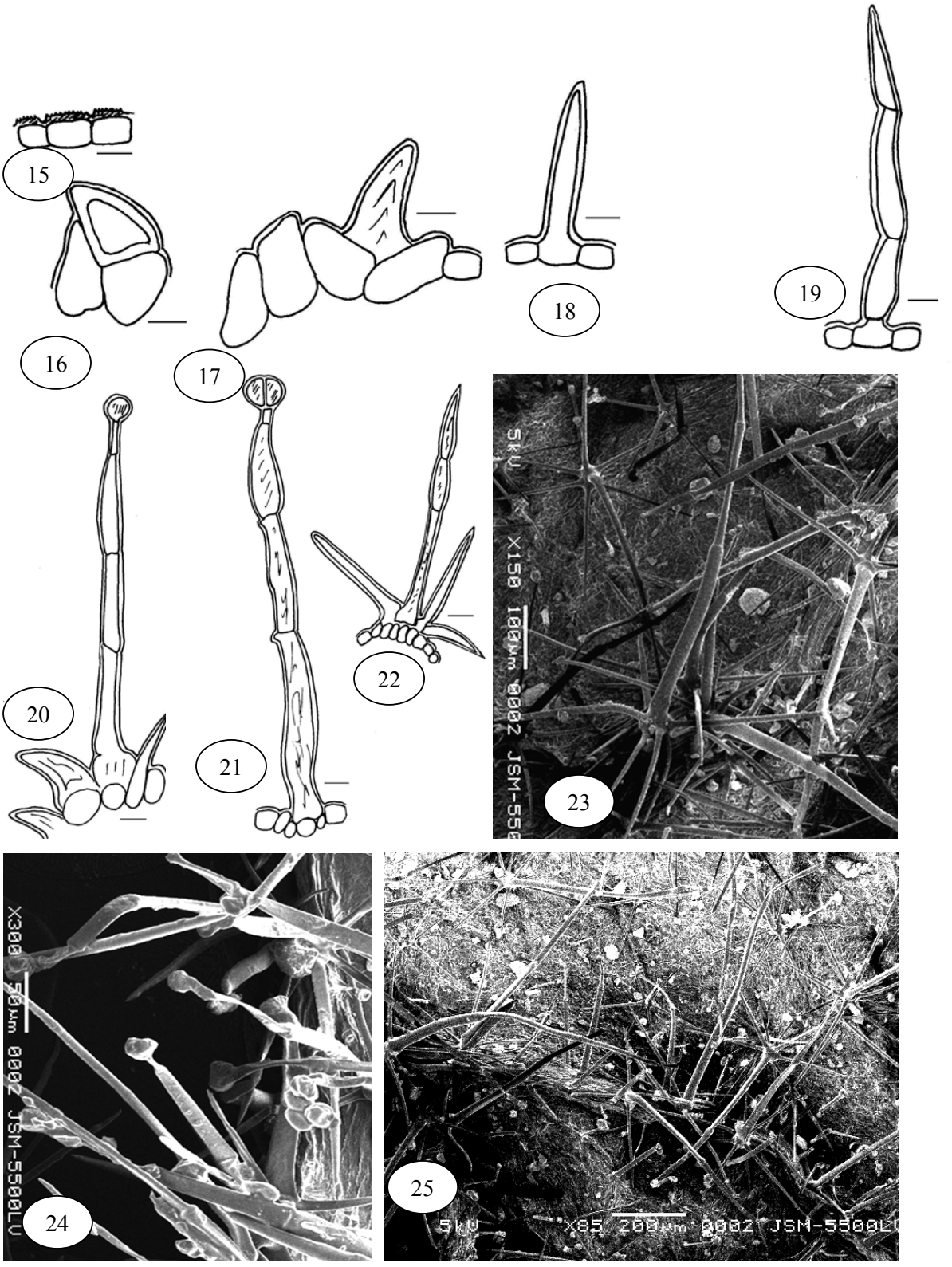

Plate 4-2. Drawn and SEM micrographs of non-glandular and glandular trichomes in *Ballota saxatilis*. (15) epidermal prickles; (16,17,18) unicellular hair; (19) tricellular hair; (20) multicellular stalked with unicellular head hair; (21) multicellular stalked with bicellular head hair; (22,23,25) stellate hair; (23) sessile hairs; (24) glandular hairs.

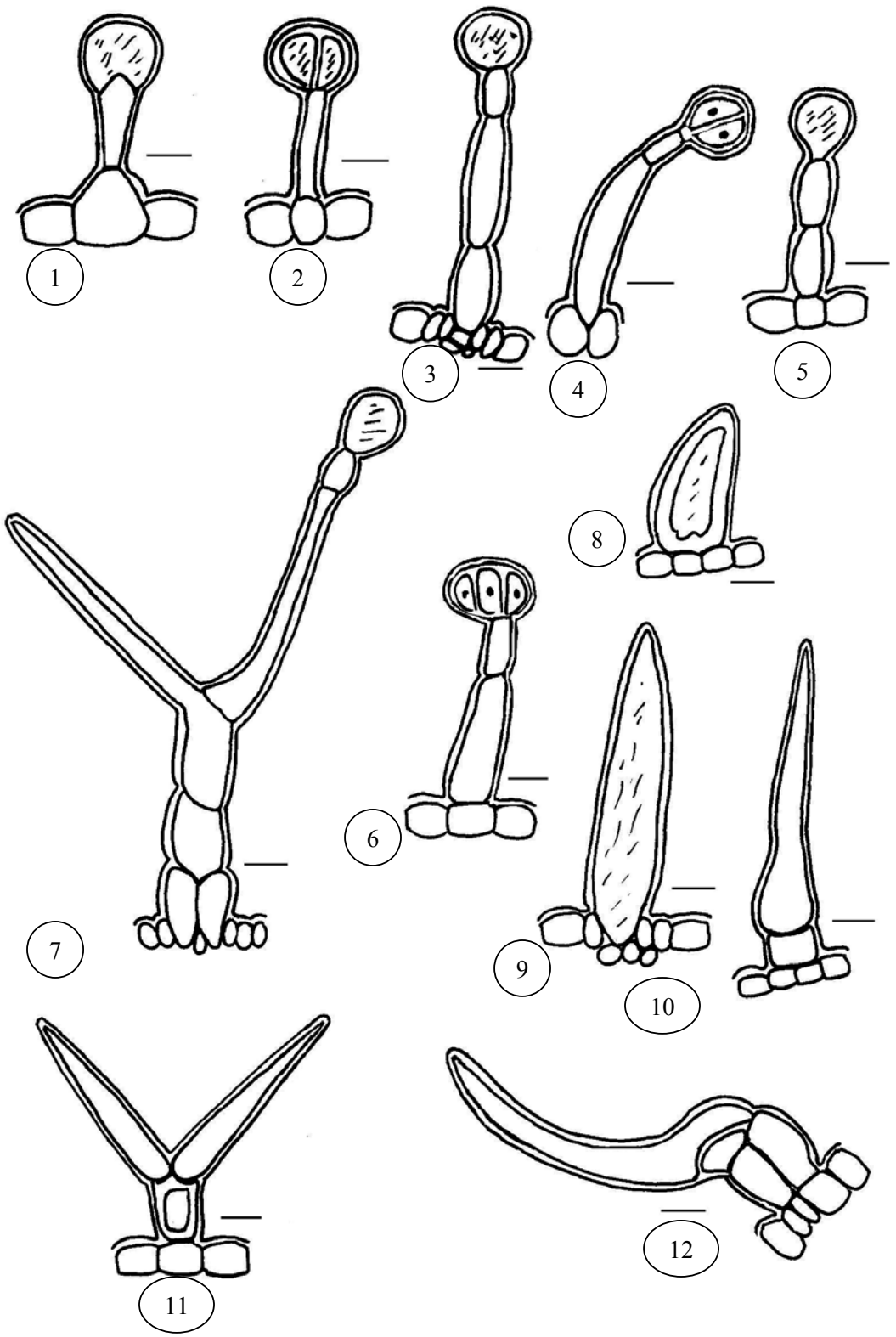

Plate 5-1. Drawn micrographs of glandular and non-glandular trichomes in *Ballota undulata*. (1) unicellular stalked with unicellular head hair; (2) unicellular stalked with bicellular head hair; (3) tricellular stalked with unicellular head hair; (4) bicellular stalked with bicellular head hair; (5) bicellular stalked with unicellular head hair; (6) bicellular stalked with tricellular head hair; (7) multicellular branched stalked with unicellular head hair; (8,9) unicellular non-glandular hair; (10) bicellular non-glandular hair; (11) tricellular branched non-glandular hair; (12) multicellular non-glandular hair.

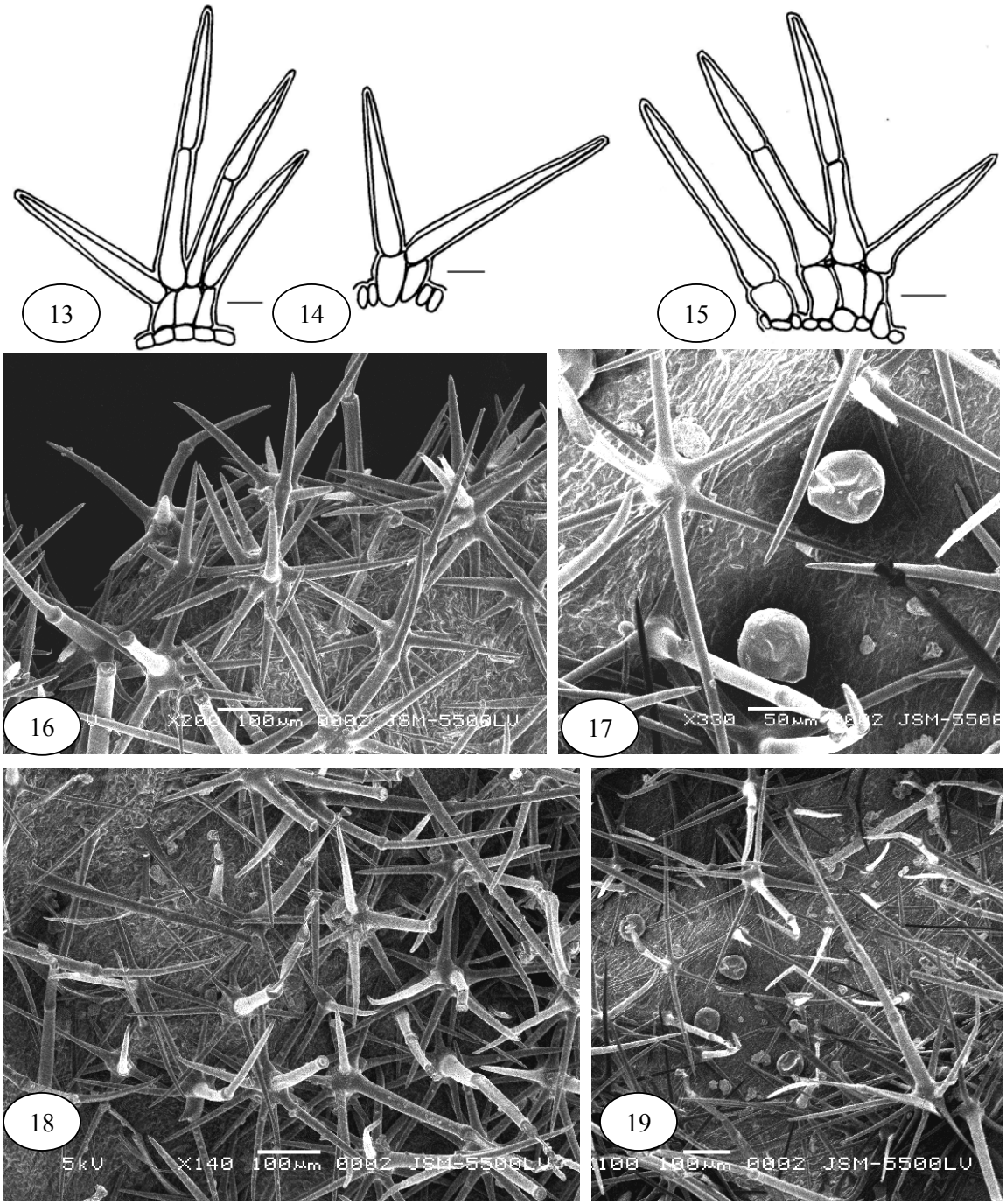

Plate 5-2. Drawn and SEM micrographs of non-glandular and glandular trichomes in *Ballota undulata*. (13,14,15) multicellular branched hairs; (16) stellate hairs; (17) stellate and sessile hairs; (18) stellate hairs; (19) stellate and sessile hairs.

Unicellular long/short stalked glandular trichomes: These have been reported as long/short-stalked glands or capitate trichomes (Maleci Bini and Servettaz, 1991), and were found in fourteen species in Abu-Asab and Cantino's study (1987). The stalk in this kind consists of only one cell, and according to number of head cells and 4 forms are distinguished in this study. The first form has unicellular head and present in *B. damascens*, *B. pseudodictamnus*, *B. saxatilis* and *B. undulata* (Plate 1, fig. 1; Plate 3, fig. 1; Plate 4-1, figs. 4-6 and Plate 5-1, fig. 1), the second form has bicellular head and is recorded in all the studied taxa (Plate 1, fig. 2; Plate 2-1, fig. 2; Plate 3, figs. 2-3; Plate 4-1, fig. 8 and Plate 5-1, fig. 2), the third form possesses tricellular head and is present in *B. kaiseri*, *B. pseudodictamnus* and *B. saxatilis* (Plate 2-1, fig. 3, Plate 3, fig. 4 and Plate 4-1, fig. 9) and the fourth form has multicellular head (more than three cells) and was showed only in *B. pseudodictamnus* (Plate 3, fig. 5). According to length of the stalk cell we can also recognize 2 forms, the first one with short stalk cell and ocean in *B. damascens*, *B. kaiseri*, *B. pseudodictamnus* and *B. saxatilis* (Plate 1, figs. 1-2; Plate 2-1, fig. 2; Plate

3, figs. 1-5 and Plate 4-1, fig. 6), and the another one with long stalk cell and recorded in *B. kaiseri*, *B. saxatilis* and *B. undullata* (Plate 2-1, fig. 3; Plate 4-1, fig. 4 and Plate 5-1, figs. 1-2).

Bicellular stalked glandular trichomes: This kind of glandular trichomes has stalk with two cells. Four forms were observed in this kind. The first form has unicellular head and is found in *B. damascens*, *B. kaiseri*, *B. saxatilis* and *B. undullata* (Plate 1, figs. 3, 5; Plate 2-1, fig. 6; Plate 4-1, fig. 10 and Plate 5-1, fig. 5), while the second form has bicellular head and is recorded in the same last mentioned four taxa (Plate 1, fig. 4; Plate 2-1, fig. 7; Plate 4-1, fig. 11 and Plate 5-1, fig. 4), the third form with tricellular head and is present in *B. kaiseri* and *B. undullata* (Plate 2-1, fig. 5; Plate 5-1, fig. 6), and the last form has multicellular head and occur only in *Ballota kaiseri* (Plate 2-1, fig. 4). According to length of neck cell also 2 forms of trichomes are recognized, the first one has short neck cell and present in *B. damascens*, *B. kaiseri* and *B. undullata* (Plate 1, figs. 4-5; Plate 2-1, fig. 4 and Plate 5-1, fig. 4) and the another one with long neck cell and found only in *B. damascens*, *B. kaiseri*, *B. saxatilis* and *B. undullata* (Plate 1, fig. 3; Plate 2-1, figs. 5-7; Plate 4-1, figs. 10-11 and Plate 5-1, figs. 5-6).

Tricellular stalked glandular trichomes: These trichomes have stalk with three cells and two forms are recorded in this kind. The first form with unicellular head and was observed in all studied taxa except *Ballota pseudodictamnus* (Plate 1, fig. 6; Plate 2-1, fig. 8; Plate 4-1, fig. 12 and Plate 5-1, fig. 3) and the second form has bicellular head and was recorded only in *B. saxatilis* (Plate 4-1, fig. 7). Also there are 2 forms of trichomes according to length of neck cell, the first was present in *B. damascens* and *B. kaiseri* with short neck cell (Plate 1, fig. 6; Plate 2-1, fig. 8) and the second was noticed in *B. saxatilis* and *B. undulata* with long neck cell (Plate 4-1, fig. 7; Plate 5-1, fig. 3).

Multicellular stalked glandular trichomes: Trichomes of this kind may be branched or unbranched. The branched trichomes have unicellular heads and are noticed in *B. undulata* (Plate 5-1, fig. 7), while unbranched trichomes are observed in 4 forms, the first two forms have uniserrate multicellular stalk, occur only in *B. saxatilis*, and their heads may be unicellular (Plate 4-2, fig. 20) or bicellular (Plate 4-2, fig. 21), The second two forms possess bi-triserrate multicellular stalk, are observed only in *B. kaiseri*, and their heads also may be unicellular (Plate 2-1, fig. 10) or bicellular (Plate 2-1, fig. 9).

Key to studied species according to non-glandular trichomes

1. a Dendritic trichomes present ……. 2
   b Stellate trichomes present ……. 3
2. a Branching in dendritic trichomes basally ……. *B. damascens*
   b Branching longitudinally ……. *B. pseudodictamnus*
3. a Tricellular trichomes unbranched ……. *B. saxatilis*
   b Tricellular trichomes branched ……. 4
3. a Multicellular branched and unbranched trichomes present ……. *B. undulate*
   b Multicellular branched trichomes only present ……. *B. kaiseri*

Key to studied species according to glandular trichomes

1. a Tricellular stalked glandular trichomes present ……. 2
   b Tricellular stalked glandular trichomes absent ……. *B. pseudodictamnus*
2. a Multicellular stalked glandular trichomes present ……. 3
   b Multicellular stalked glandular trichomes absent ……. *B. damascens*
3. a Multicellular stalked glandular trichomes branched ……. *B. undulata*
   b Multicellular stalked glandular trichomes unbranched ……. 4
3. a Unbranched stalk uniserrate ……. *B. saxatilis*
   b Unbranched stalk bi-triserrate ……. *B. kaiseri*

Discussion

The value of trichomes in identification of some members of Lamiaceae has already been ligh lighted (Abu-Asab and Cantino, 1992; Cantino, 1990; Doaigey, 1991; Gairola *et al*., 2009; Maleci Bini and Servettaz, 1991; Navarro and El Oualidi, 2000; Salmaki *et al*., 2009; Sebebe and Harley, 1992; Serrato-Valenti *et al*., 1997; Xiang *et al*., 2010). This study shows that the cell numbers of trichomes may be used to classify the trichomes of these five *Ballota* taxa into seven kinds including 12 forms in non-glandular trichomes and 5 kinds including 17 forms in glandular trichomes. However, both glandular and non-glandular trichomes were present on all investigated taxa and the trichomes types appeared on the studied species are mostly similar to those of family Lamiaceae reported by Salmaki *et al*., 2009; Cantino, 1990 and Xiang *et al*., 2010.

Due to the existence of a dense layer of stellate trichomes or dendroid trichomes, the presence of some kinds of non-glandular trichomes were obscured (Xiang *et al*., 2010). The ability to distinguish of non-glandular trichomes becomes difficult because the stellate or dendroid trichomes are crowded.

The taxonomic significance of micromorphology of trichomes in genus *Ballota* is evident. The presence of stellate and dendritic trichomes has a high systematic value for species division. The stellate trichomes were observed in three species (*B. kaiseri*, *B. saxatilis* and *B. undulata*), but were absent in the two remaining species (*B. damascens* and *B. pseudodictamnus*), where the dendritic trichomes were recorded.

The distribution of these two kinds of trichomes is consistent with the macro-morphological classification and provides additional evidence to verify the species delimitation in *Ballota*. There is consistent between macro- and micromorphological characters.

As given in other Lamiaceae genera (Husain *et al*., 1990; Maleci Bini & Servettaz, 1991; Salmaki *et al*., 2009; Xiang *et al*., 2010), some kinds of trichomes are also different in different species or in different sections in *Ballota*. There are differences in kinds of trichomes in different *Ballota* species and in some species the type of trichomes are different in different plant parts..

The morphology and distribution of these kinds of trichomes in accordance with leaf and calyx morphology in species of *Ballota* have valuable taxonomic significance at species level. In this study, three species of *B. kaiseri*, *B. saxatilis* and *B. undulata* are similar to each other, they shar in some characters, e.g., stems green and calyx are distinctly toothed or with crenate-dentate margins. The present results do not exactly confirm the similarity among the three species, also with respect to the distribution and kinds of trichomes that are not totally identical in these species. However, trichome types of these species are identical in some trichome kinds and differ in other kinds (Table 2).

The differences in forms of trichomes among the three morphologically similar species (*B. kaiseri, B. saxatilis and B. undulata*) can be shown as the following, in respect to non-glandular trichomes, presence of bicellular branched and unbranched form in *B. Kaiseri*, while in *B. saxatilis* bicellular branched form only is observed and in *B. undulata* bicellular unbranched form is recorded. Also presence of tricellular branched trichomes in *B. kaiseri* and *B. undulata*, while in *B. saxatilis*, the unbranched form are distinguished. Presence of multicellular branched trichomes in petioles of *B. kaiseri* and multicellular branched and unbranched trichomes in petioles of *B. undulata* and absence them in *B. saxatilis*.

In respect to glandular trichomes, presence of sessile glandular trichomes with bicellular head only in *B. saxatilis* and losing them in the two remained taxa. Existence of unicellular stalked with unicellular head trichomes in *B. saxatilis* and *B. undulata* and losing them in *B. kaiseri*. Absence of unicellular stalked with tricellular head trichomes form in *B. undulata* only and appearing them in the two remained species. Absence of bicellular stalked with tricellular head trichomes only in *B. saxatilis* and showing them in *B. kaiseri* and *B. undulata*. Presence of bicellular stalked with multicellular head trichomes only in *B. kaiseri* and losing them in *B. saxatilis* and *B. undulata*. Presence of tricellular stalked with bicellular head trichomes only in *B. saxatilis* and disappearing them in the two remaining species. Recording of multicellular branched stalked with unicellular head trichomes only in *B. undulata*, while in *B. kaiseri*, the two forms multicellular unbranched, bi-triserrate stalked with uni- and bicellular head trichomes are registered and in *B. saxatilis* multicellular unbranched, uniserrate stalked with uni- and bicellular head trichomes are recorded.

Also in both *B. damascens* and *B. pseudodictamnus* the distribution and variation in trichome kinds provide additional strong character for the separation of these two species from the another three species (*B. kaiseri*, *B. saxatilis* and *B. undulata*) and to distinguish between them. From the recorded differences in forms of trichomes between both *B. damascens* and *B. pseudodictamnus*, in respect to non-glandular trichomes, presence of unicellular trichomes only in *B. pseudodictamnus*, existence of the form of dendritic trichomes that are branched along the stalk cell in *B. damascens* and the form of dendritic trichomes that are branched longitudinally at nodes of the uniserrate long stalk cells in *B. pseudodictamnus*. But in respect to glandular trichomes, in *B. pseudodictamnus* there are four forms of the unicellular stalked trichomes that may be with uni-, bi-, tri- or multicellular heads. On the other hand, in *B. damascens* there are only two forms of unicellular stalked trichomes that may be have uni- or bicellular heads. In addition to unicellular stalked trichome form that is present in *B. damascens* there are two other forms, bicellular stalked with uni- or bicellular head trichomes and tricellular stalked with unicellular head trichomes, these two forms are absent in *B. pseudodictamnus*. All these variations in distribution of trichome forms in *Ballota* species are sufficient to identify among them.

Based on variations mentioned above, *Ballota* can be divided into two groups depending on presence or absence of both stellate and dendritic trichomes. In addition to the stellate and dendritic trichomes, there are other kinds of trichomes that can be useful to identify between two groups of *Ballota*. As in respect to non-glandular trichomes, presence of epidermal prickles only in both *B. damascens* and *B. pseudodictamnus* and absence them in another three similar *Ballota* species. Also with exception of epidermal prickles and dendritic trichomes that are showed in both *B. damascens* and *B. pseudodictamnus* and unicellular trichomes that are found only in *B. pseudodictamnus* all another forms of non-glandular trichomes are not recorded in both *B. damascens* and *B. pseudodictamnus*. On the other hand, in according to glandular trichomes, in addition to unicellular stalked trichomes that are distinguished in both *B. damascens* and *B. pseudodictamnus* and both bicellular stalked and tricellular stalked trichomes that are recognized only in *B. damascens*, both sessile and multicellular stalked trichomes are not observed in both *B. damascens* and **B**. *pseudodictamnus* and appeared in another Ballota group (*B. kaiseri, B. saxatilis and B. undulata*).

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(Received for publication 30 December 2010)