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Full Length Research Paper

Comparative anatomical and palynological studies on genus *Ballota* (Lamiaceae) from Egypt

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In this study, the anatomical and palynological features of 5 taxa belonging to the genus *Ballota* of Lamiaceae from Egypt were examined and evaluated by light and scanning electron microscopy (SEM) in order to determine the taxonomic value of the observed peculiarities. Features related to pollen grains (pollen shape and tectum sculpture type) and anatomical characters (shape in outline, dermal, ground and vascular system) were found to be important in separating the examined taxa. Three keys are provided according to stem anatomy, petiole anatomy and pollen grains characters. The light and SEM images of investigated taxa are presented for comparison. Overall, the anatomical and palynological characters are very useful in the separation of *Ballota* species.

Key words: Comparative, anatomy, palynology, *Ballota*, Lamiaceae, Egypt.

INTRODUCTION

The family Lamaiceae has cosmopolitan distribution and includes over 250 genera and close to 7000 species (Thorne, 1992). The family is known for its fine ornamental or culinary herbs like basil, lavender, mint, oregano, rosemary, sage and thyme, and is a rich source of essential oils for the flavoring and perfume industry (Wagstaff et al., 1998). The genus *Ballota* (Lamiaceae) is represented by 33 to 35 species in the world, and are mainly distributed around the Mediterranean and Eurasia (Seidel et al., 1999; Tipirdamaz and Guvene, 2004), In Egypt, it is represented by 5 taxa; two of them (*Ballota kaiseri* and *Ballota saxatilis*) are very rare, threatened species and endemic to St. Catherine Protectorate, Southern Sinai, Egypt (Boulos, 2009).

The name *Ballota* was given to this plant as early as the time of Dioscorides, when the leaves of *Ballota nigra* were used as an antidote for the bite of a mad dog at that time (Gunther, 1959). *Ballota* species are well known in folk medicinal plants for their fresh leaves, fruits and seeds, since they possess calmative and antispasmodic properties and are externally used for colic, asthma, influenza, insomnia and hemorrhoids. They also possess antipruritic and analgesic effects on pain (Davies-Coleman and Rivett, 1990). *Ballota* species are widely used as medicine in Europe and Turkey because of their spasmolitic and sedative effect in (Bezanger-Beauquesne et al., 1990; Garnier et al., 1961).

Thus far, some studies on *Ballota* have been conducted (Tóth et al., 2007; Zaghloul et al., 2006; Tipirdamaz and Guvenc, 2004; Sahpaz et al., 2002), although none of them thoroughly evaluated the taxonomic significance of palynology and anatomy in the taxonomy of this genus. Erdtman (1945) studied the pollen morphology of family Lamiaceae and found that it is composed of two pollen types, the first type present in subfamily Lamioideae; it is usually tricolpate and the second type present in subfamily Nepetoideae, usually hexacolpate. Wunderlich's (1967) after extensive pollen survey lent strong support to Erdtman's groupings through the addition of many new genera to the palynological data base. *Ballota* was placed under the subfamily Stachyoideae/Lamioideae (Erdtman, 1945; Cantino and Sanders, 1986), and divided by Patzak in 1958. Additionally, Zaghloul et al. (2006) investigated the generic diversity of three *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. Osman (2012) also examined trichomes micro-morphology of Egyptian *Ballota*. He showed the considerable variability of the indumentum among different species and therefore, afforded valuable characters in delimitation of species.

Meanwhile, the anatomical and palynological characteristics of the genus *Ballota* have not been studied

Table 1. A list of the investigated species with their origin and collectors.

Species	Locality	Voucher
Ballota damascena	Southern Sinai, St. Katharine, Wadi El-Arbaéen, 14/5/2004	Fayed et al. s.n. (QNA)
Ballota kaiseri	Southern Sinai, St. Katharine, Wadi El-Arbaéen, 18/6/2005	H. Mosallam, K. Abdel Khalik and A.K. Osman, (QNA)
Ballota pseudodictamnus	Al Tamimi, Wadi Derna, Gebel Akhdar: 31/3/1968.	Loutfy Boulos, 2300 (CAI).
Ballota saxatilis	Southern Sinai, St. Katharine, Wadi El-Arbaéen, 18/6/2005	H. Mosallam, K. Abdel Khalik and A.K. Osman, (QNA)
Ballota undulata	Southern Sinai, St. Katharine, Wadi El-Arbaéen, 18/6/2005	H. Mosallam, K. Abdel Khalik and A.K. Osman, (QNA)

CAI, Cairo University Herbarium; QNA, Qena University Herbarium.

before. Therefore, the present study was aimed at providing a detailed account of the anatomical and palynological features of *Ballota* species.

MATERIALS AND METHODS

The specimens for this study were collected during its flowering period from the different localities in Egypt, and were preserved in the Department of Biology, Faculty of Science, South Valley University Herbarium (QNA) and Cairo University Herbarium (CAI) (Table 1). Anatomical studies were carried out on specimens kept in 70% alcohol. The paraffin method was used for the transverse sections of the petioles and stems. The specimens were embedded in the paraffin wax and then sectioned with a Leica RM2125RT rotary microtome. All sections were stained with safranin and crystal violet and then mounted with Canada balsam (Johansen, 1944; Algan, 1981). Measurements for stems and petioles were taken using a Measuring image (ImageJ) computer programme and photographs were taken using a Leica DM1000 binocular light microscope with a Leica DFC280 camera.

For palynological investigations, pollen material was obtained from herbarium samples. The pollen slides were prepared according to Wodehouse (1935) technique. Measurements and observations were made using the Olympus BX50 binocular light microscope with the Leica DFC280 camera. The polar length (P), the equatorial length (E), the colpus length (CLG), the exine and the intine thickness for 20 pollen grains were measured under the light microscope (× 1000) and P/E ratios were calculated. For scanning electron microscopy (SEM), the pollen grains were transferred directly to double-sided tape affixed stubs, and vacuum-coated with gold. Photomicrographs were taken partly with a JEOL-6300 SEM of the Central Laboratory, Faculty of Science, South Valley University, Qena, Egypt, to determine their exine ornamentation. The pollen terminology of Faegri and Iverson (1975) has been used.

RESULTS

Taxa of *Ballota* are arranged alphabetically to facilitate consultation. For each species, the valid name was given, followed by the citation of authority and the date of publication. Synonymy was used at minimum to avoid complications. Full synonymy of the species was given by the following authors, El-Hadidi and Fayed (1994/1995) and Boulos (2002, 2009). A summary of the dimensions of stems and petioles are presented in Tables 2 and 3. The following are descriptions of stems anatomy, petioles anatomy and pollen grains of the studied species.

Anatomical and pollen grains description

Ballota damascena Boiss., Diagn. Pl. Orient., ser. 1, 12: 87 (1853)

Stem anatomy (Figure 1 (plates 1a and b): In transverse section, the stem was quadrangular with large protrusions (stem area = 318732.0 µM). Cuticle was thick and had prickles (cuticle thickness = 8.0 µM). Epidermal cells were large-sized, thick walled, papillose elongated at protrusions and tangentially elongated between protrusions (epidermis thickness = 8.5 µM). Hypodermis was present in the form of one layer. Cortex had three types of cells: the outer annular collenchyma (±10 - 13 layers) only at protrusions (collenchyma area = 57.0 µM), the middle chlorenchyma (± 2 - 12 layers; chlorenchyma area = 21.0 µM) and the inner parenchyma (±1 - 2 layers) which were also present only at protrusions (parenchyma area = 15.0 µM). The vascular tissue was composed of siphonostele (vascular system area with pericycle = 275173.3 µM). Secondary growth is extreme herbaceous, present in both fascicular and interfascicular regions (vascular system area without pericycle = 255341.3 µM). Bast phloem fibers exist as a complete cylinder (±1-4 layers; pericycle cylinder thickness = 23.6 µM). Interfascicular cambium activity formed the phloem (fibers and vessels; ± 1-5 layers; phloem cylinder thickness = 12.4 µM) and xylem (± 2-6 layers; xylem cylinder thickness at corner = 141.5 µM and between

Table 2. Tubular summary showing the dimensions of samples stems.

Taxa	Stem area (µM)	Vas. sys. area * (µM)	Vas. sys. area (µM)	Xylem area (µM)	Pith area (µM)	Cut. thic. (µM)	Epid. thic. (µM)	Coll. thic. (µM)	Chl. thic. (µM)	Par. thic. (µM)	Per. thic. (µM)	Ph. thic. (µM)	X. thic.* (µM)	X. thic. (µM)
Ballota damascena	318732	275173.3	255341.3	231620	122513.3	8.0	8.5	57.0	21.0	15.4	23.6	12.4	141.5	64.4
Ballota kaiseri	662690.3	539535.4	441952.1	375142.4	198029.2	4.9	10.2	29.3	29.7	14.7	25.6	24.1	105.4	50.5
Ballota pseudodictamnus	10664427	1311845.3	766213.7	725918.2	340182.8	2.8	14.2	120.2	23.7	60.9	53.9	43.9	462.1	291.7
Ballota saxatilis	463675.6	360346.7	336688.9	311062.7	190587.1	8.2	12.0	36.5	27.1	16.2	16.0	16.1	108.2	60.1
Ballota undulata	491164.9	386371.1	343546.2	283242.2	115320	4.1	8.6	24.3	21.5	13.1	27.1	33.9	163.1	111.0

Chl. thic: Chlorenchyma thickness; Coll. thic: collenchyma thickness; Cut. thic: cuticle thickness; Epid. thic: epidermis thickness; Par. thic: parenchyma thickness; Per. thic: pericycle thickness; Ph. thic: phloem thickness; X. thic: xylem thickness in between protrusions; X. thic*: xylem thickness at protrusions; Vas. sys. area: vascular system area without pericycle; Vas. sys. area*: vascular system area with pericycle.

Table 3. Tubular summary showing the dimensions of samples petioles.

Taxa	Petiole area (µM)	Vas. sys. area* (µM)	Vas. sys. area (µM)	Xylem area (µM)	Add. bu. area (µM)	Chl. area (µM)	Epid. thic. (µM)	Coll. thic. from down (µM)	Par. thic. from up (µM)	Per. thic. (µM)	Phlo. thic. (µM)	Xylem thic. (µM)	Xylem thic.* (µM)
Ballota damascena	78925.6	26945.2	22919.3	9879.7	3033.1	7969.4	12.2	29.1	37.6	16.1	37.6	41.0	41.0
Ballota kaiseri	4403355.6	93787.2	63873.6	31756.3	7314.6	37347.0	18.0	32.9	98.8	43.0	73.9	108.2	45.1
Ballota pseudodictamnus	1203333	395011	316273	162023	30811.5	45515	43.9	69.9	85.1	59.4	102.6	153.1	78.6
Ballota saxatilis	236736	53527	43009	26794.8	11636.2	23484.4	20	18.2	38.9	21.5	30.6	128.5	65.8
Ballota undulata	203180.9	77289.8	64434.7	19606.2	6654.9	37635.6	13.0	37.0	76.2	20.3	69.5	72.3	33.6

Add. bu. area, Additional bundles area; Chl. area: chlorenchyma area; Coll. thic: collenchyma thickness; Epid. thic: epidermis thickness; Par. thic: parenchyma thickness; Per. thic: pericycle thickness; Phlo. thic: phloem thickness; Xylem thic: xylem thickness at petiole center; Xylem thic.*: xylem thickness in additional vascular bundles; Vas. sys. area: vascular system area without pericycle; Vas. sys. area*: vascular system area with pericycle.

corners = 64.4 µM). Vessels were (diffuse) ring porous (xylem area = 231620 µM). Xylem parenchyma was paratracheal. Pith was solid and heterogeneous of two types, the outer thick walled lignified parenchymatous cells and the inner of thin walled ones (pith area = 122513.3 µM). Simple pits were present in lignified cells. Crystals were raphides and restricted to pith.

Petiole anatomy (Figure 1 (plates 3a and b): In transverse section, the petiole was thickly crescentiform with very deep adaxial furrow (petiole area = 78925.6 µM). Cuticle was medium in thickness (cuticle thickness = 6.5 µM), epidermal cells papillose and tangentially elongated (epidermis thickness = 12.2 µM). Cortex had three types; chlorenchyma with area = 7969.4 µM, annular collenchyma at ad- and abaxial sides and two ridges (collenchyma thickness at adaxial side = 29.1 µM) and parenchyma which exist only at adaxial side (± 3 layers; parenchyma thickness = 37.6 µM). The petiole vasculature of a deep, incomplete crescentric strand was formed of two bundles (vascular system area with pericycle = 26945.2 µM, without pericycle = 22919.3 µM and xylem area = 9879.7; pericycle thickness = 16.1 µM, phloem thickness = 37.6 µM and xylem thickness = 41.0 µM). Two ridge bundles with area = 3033.1 µM were present (xylem thickness in additional bundles = 41.0 µM). Crystals were raphides and restricted to cortical cells.

***Ballota kaiseri* Tɔckh., Svensk Bot. Tidskr. 26: 378 (1932)**

Stem anatomy (Figure 1 (plates 2a and b)): In transverse section, the stem is quadrangular with

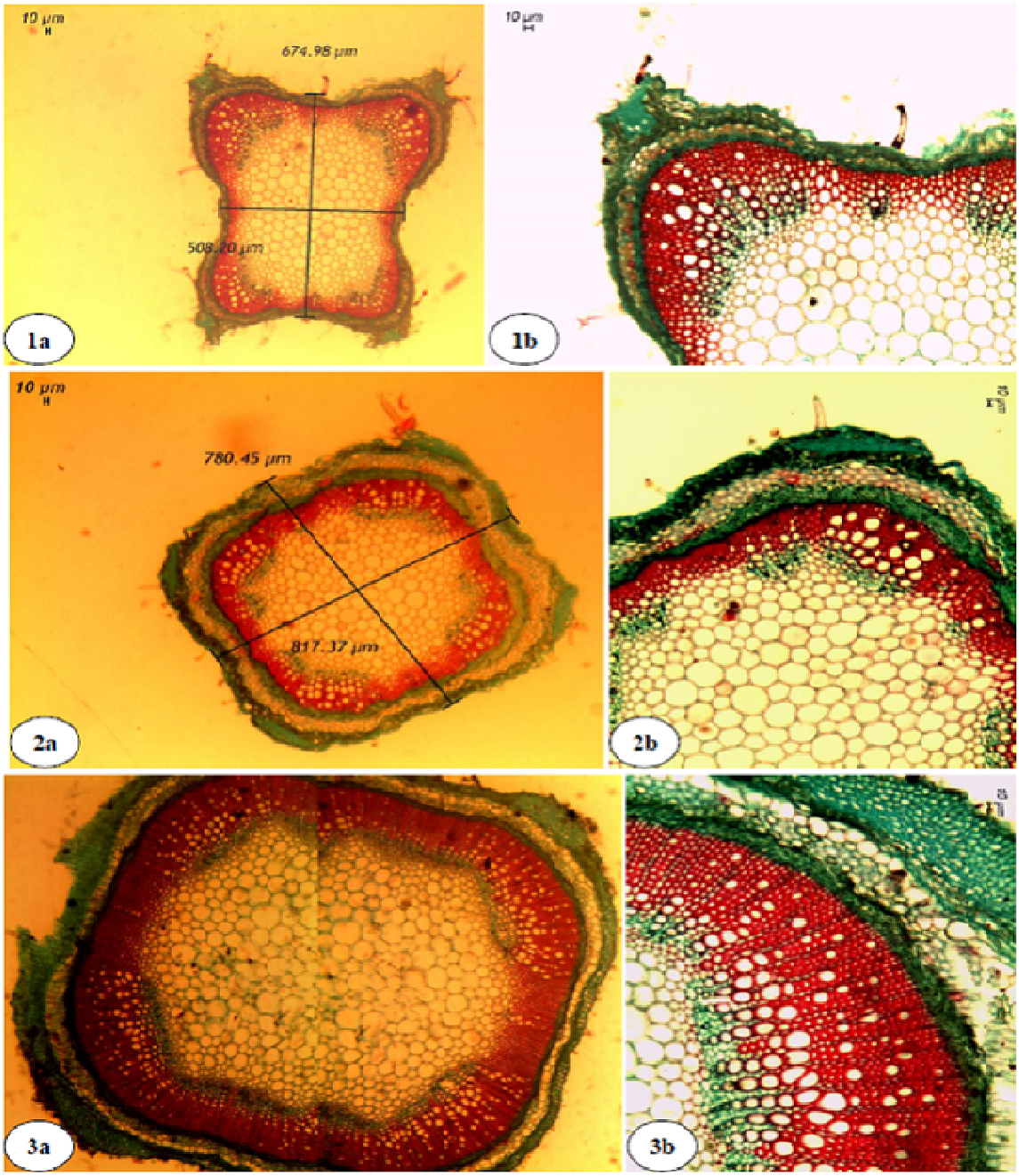

Figure 1. Stem transverse section LM photos; a- outline shape and b- enlarged part. Bar= 10 µM. 1- *Ballota damascena*; 2- *B. kaiseri* and 3- *B. pseudodictamnus*.

small protrusions (stem area = 662690.3 µM). Cuticle medium in thickness (cuticle thickness = 4.9 µM). Epidermal cells small-sized, thick walled, tangentially and papillose elongated at protrusions and tangentially elongated between protrusions (epidermis thickness = 10.2 µM). Hypodermis was present in the form of one layer. Cortex had three types of cells, the outer annular collenchyma (±1-6 layers) only at protrusions (collenchyma area = 29.3 µM), the middle chlorenchyma (±3 - 6 layers; chlorenchyma area = 29.7 µM) and the inner parenchyma (±1 layer; parenchyma area = 14.7 µM). The vascular tissue was composed of siphonostele (vascular system area with pericycle = 539535.4 µM). Secondary growth was extreme herbaceous, present in both fascicular and interfascicular regions (vascular system area without pericycle = 441952.1 µM). Bast phloem fibers existed as a complete cylinder (±1-5 layers; pericycle cylinder thickness = 25.6 µM). Interfascicular

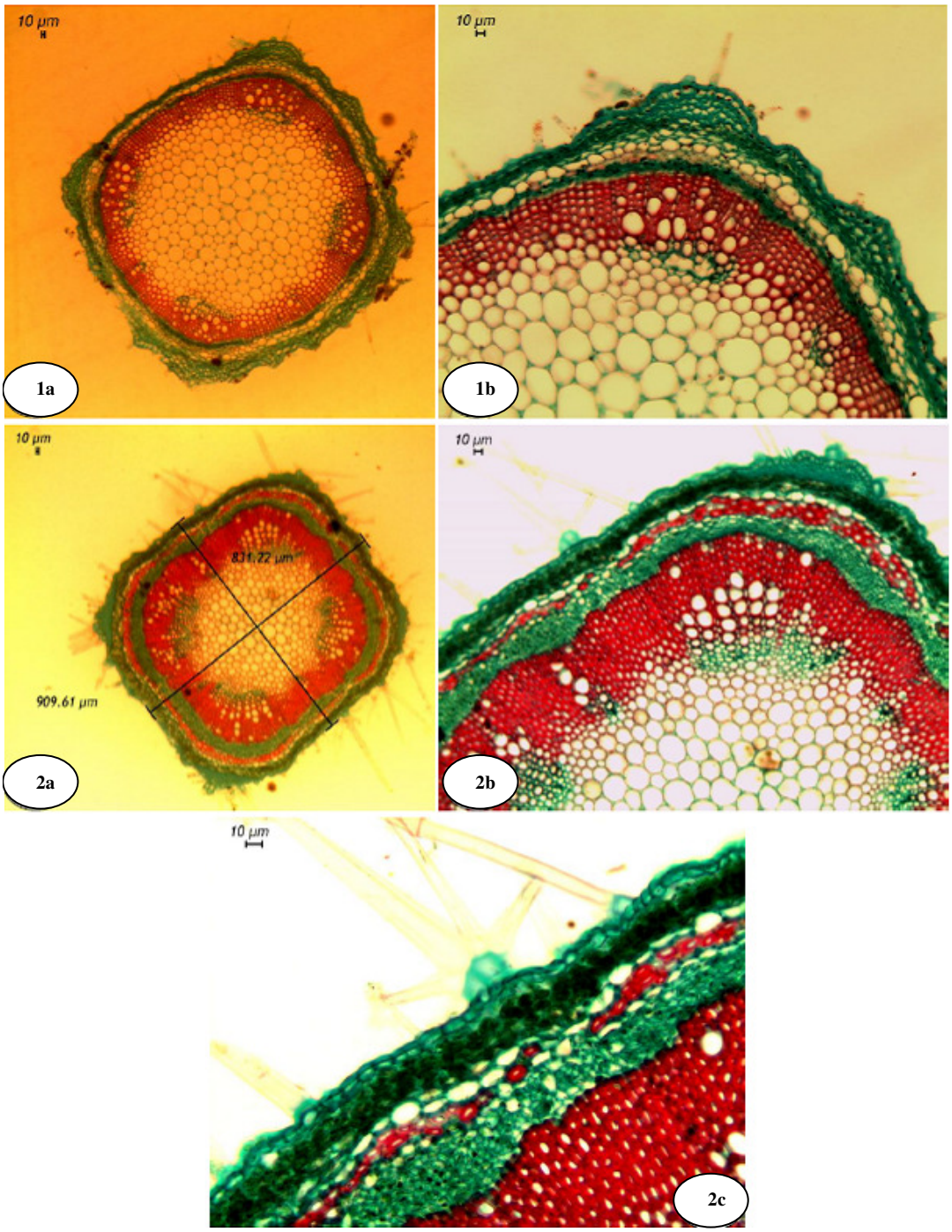

Figure 2. Stem transverse section LM photos; a- outline shape, b and c- enlarged part. Bar= 10 µM. 1- *B. saxatilis*; 2- *B. undulata.*

cambium activity formed the phloem (fibers and vessels) to out (± 3-10 layers; phloem cylinder thickness = 24.1 µM) and xylem to down (± 1 - 5 layers; xylem cylinder thickness at corner = 105.4 µM and between corners = 50.5 µM). Vessels were (diffuse) ring porous (xylem area = 375142.4 µM). Xylem parenchyma was paratracheal. Pith solid and heterogeneous of two types, the outer thick walled lignified parenchymatous cells and the inner of thin walled ones (pith area = 198029.2 µM). Crystals were small raphides restricted to pith.

Petiole anatomy (Figure 3 (plates 2a and b)): In transverse section, the petiole was thickly crescentiform with shallow, broad, adaxial furrow (petiole area =

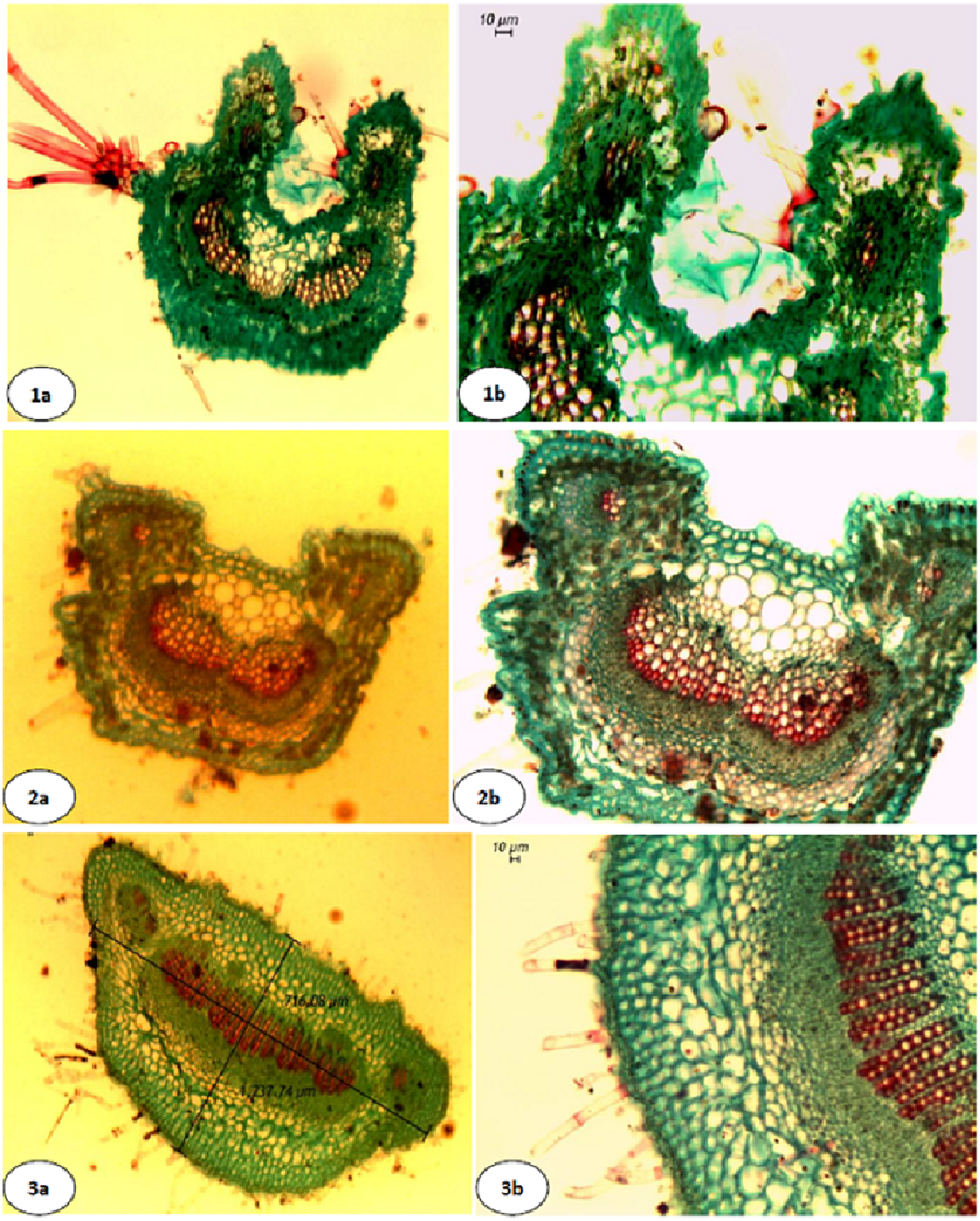

Figure 3. Petiole transverse section LM photos; a- outline shape and b- enlarged part. Bar= 10 µM; 1- *Ballota damascena*, 2- *B. kaiseri* and 3- *B. pseudodictamnus*.

4403355.6 µM). Cuticle was medium in thickness (cuticle thickness = 5.6 µM), Epidermal cells were radially, tangentially, papillose and columnar elongated (epidermis thickness = 18.0 µM). Cortex was four types; chlorenchyma with area = 37347.0 µM, annular collenchyma at ad- and abaxial sides and at the two ridges (collenchyma thickness at adaxial side = 32.9 µM), parenchyma was at both adaxial (± 3 layers) and abaxial sides (± 1-2 layers; parenchyma thickness at adaxial side = 98.8 µM) and fibers at two ridges. The petiole vasculature was of a shallow, incomplete crescentric strand formed of two bundles (vascular system area with pericycle = 93787.2

µM, without pericycle = 63873.6 µM and xylem area = 31756.3; pericycle thickness = 43.0 µM, phloem thickness = 73.9 µM and xylem thickness = 108.2 µM). Two ridge bundles were present (xylem thickness in additional bundles = 45.1 µM). Crystals were rhombic-shaped restricted to cortical cells.

Ballota pseudodictamnus (L.) Benth., Lab. Gen. Sp. 595 (1834) Syn. Marrubium pseudodictamnus L., Sp. Pl., ed. 1, 583 (1753).

Stem anatomy (Figure 1 (plates 3a and b)): In transverse section, the stem was quadrangular with small protrusions (stem area = 10664427 µM). Cuticle was thin and has small prickles (cuticle thickness = 2.8 µM). Epidermal cells were small-sized, thick walled, papillose elongated at protrusions and tangentially elongated between protrusions (epidermis thickness = 14.2 µM). Hypodermis was present in the form of one layer. Cortex was of three types of cells, the outer annular (6-12 layers only at protrusions) and lamellar (3-4 layers between protrusions) collenchyma (collenchyma area at protrusions = 120.2 µM), the middle chlorenchyma (1-5 layers) (chlorenchyma area = 23.7 µM) and the inner parenchyma (±1-3 layers)(parenchyma area = 60.9 µM). The vascular tissue was composed of siphonostele (vascular system area with pericycle = 1311845.3 µM). Secondary growth was extreme herbaceous, present in both fascicular and interfascicular regions (vascular system area without pericycle = 766213.7 µM). Bast phloem fibers existed as an isolated patches (±1-5 layers; pericycle cylinder thickness = 53.9 µM). Interfascicular cambium activity formed the phloem (fibers and vessels; ± 3-6 layers; phloem cylinder thickness = 43.9 µM) and xylem (± 3-13 layers; xylem cylinder thickness at corner = 462.1 µM and between corners = 291.7 µM). Vessels are (diffuse) ring porous (xylem area = 725918.2 µM). Xylem parenchyma was paratracheal. Pith was solid and heterogeneous of two types, the outer thick walled lignified parenchymatous cells and the inner of thin walled ones. Simple pits were present in lignified cells (pith area = 340182.8 µM). Crystals were raphides restricted to pith.

Petiole anatomy (Figure 3 (plates 3a and b)): In transverse section, the petiole was thickly crescentiform without adaxial furrow and had small protrusions at adaxial side (petiole area = 1203333 µM). Cuticle was thin in thickness (cuticle thickness = 2.4 µM), Epidermal cells were radially, tangentially and papillose elongated (epidermis thickness = 43.9 µM). Cortex had three types; chlorenchyma with area = 45515 µM, cartilaginous collenchyma at ad- and abaxial sides and at the two ridges (collenchyma thickness at adaxial side = 69.9 µM) and parenchyma at both adaxial (± 4 layers) and abaxial sides (± 2-3 layers; parenchyma thickness at adaxial side = 85.1 µM). The petiole vasculature of a shallow, incomplete crescentric strand was composed of three bundles (vascular system area with pericycle = 395011 µM, without pericycle = 316273 µM and xylem area = 162023.0; pericycle thickness = 59.4 µM, phloem thickness = 102.6 µM and xylem thickness = 153.1 µM). Two ridge bundles were present (xylem thickness in additional bundles = 78.6 µM). Crystals were absent.

Ballota saxatilis C. Presl in J. & C. Presl, Delic. Prag 81 (1822), subsp. saxatilis.

Stem anatomy (Figure 2 (plates 1a and b): In transverse section, the stem was quadrangular with small protrusions (stem area = 463675.6 µM). Cuticle was thick and had small prickles (cuticle thickness = 8.2 µM). Epidermal cells were large-sized, thin walled, tangentially and papillose elongated at protrusions and tangentially elongated between protrusions (epidermis thickness = 12.0 µM). Hypodermis was present in the form of one layer. Cortex were three types of cells, the outer angular (± 1-6 layers only at protrusions) collenchyma (collenchyma area at protrusions = 36.5 µM), the middle chlorenchyma (± 2 - 5 layers; chlorenchyma area = 27.1 µM) and the inner complete cylinder parenchyma (±1-2 layers; parenchyma area = 16.2 µM). The vascular tissue was composed of siphonostele (vascular system area with pericycle = 360346.7 µM). Secondary growth was extreme herbaceous, present in both fascicular and interfascicular regions (vascular system area without pericycle = 336688.9 µM). Bast phloem fibers existed as an isolated patches (±1 - 4 layers; pericycle cylinder thickness = 16.0 µM). Interfascicular cambium activity formed the phloem (fibers and vessels; ± 1-5 layers; phloem cylinder thickness = 16.1 µM) and xylem (±1 - 4 layers; xylem cylinder thickness at corner = 108.2 µM and between corners = 60.1 µM). Vessels were (diffuse) ring porous (xylem area = 311062.7 µM). Xylem parenchyma was paratracheal. Pith was solid and heterogeneous of two types, the outer thick walled lignified parenchymatous cells and the inner of thin walled ones. Simple pits were present in lignified cells (pith area = 190587.1 µM). Crystals were absent.

Petiole anatomy (Figure 4 (plates 1a and b): In transverse section, the petiole was leafy shaped and had very deep adaxial furrow (petiole area = 236736 µM). Cuticle was very thick (cuticle thickness = 9.4 µM). Epidermal cells were radially, tangentially and papillose elongated (epidermis thickness = 20.0 µM). Cortex had three types of cells; chlorenchyma with area = 23484.4, cartilaginous collenchyma at ad- and abaxial sides and at the two ridges (collenchyma thickness at adaxial side = 18.2 µM) and parenchyma at both adaxial (± 2 layers) and abaxial sides (± 2 layers; parenchyma thickness at adaxial side = 38.9 µM). The petiole vasculature was medium in deepness, complete crescentric strand

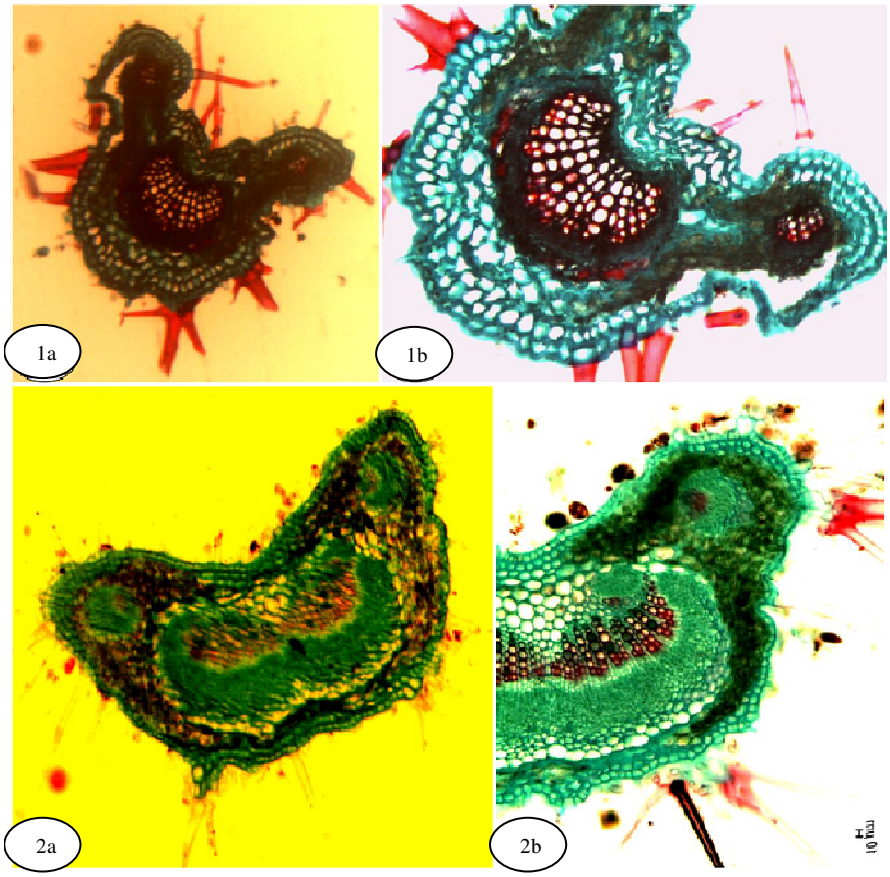

Figure 4. Petiole transverse section LM photos; a- outline shape and b- enlarged part. Bar= 10 µM; 1- *B. saxatilis*, 2- *B. undulata*.

(vascular system area with pericycle = 53527.0 µM, without pericycle = 43009.0 µM and xylem area = 26794.8; pericycle thickness = 21.5 µM, phloem thickness = 30.6 µM and xylem thickness = 128.5 µM). Two ridge bundles were present (xylem thickness in additional bundles = 65.8 µM). On the other hand, crystals were absent.

Ballota undulata (Fresen.) Benth., Lab. Gen. Sp. 595 (1834) Syn. Marrubium undulatum Fresen., Mus. Senkenberg. 1: 92 (1834)

Stem anatomy (Figure 2 (plates 2a and b)): In transverse section, the stem was quadrangular with small protrusions (stem area = 491164.9 µM). Cuticle was medium in thickness (cuticle thickness = 4.1 µM). Epidermal cells were small-sized, thick walled, radially and tangentially elongated at protrusions and tangentially elongated in between protrusions (epidermis thickness = 8.6 µM). Hypodermis was present in the form of one layer. Cortex were three types of cells, the outer annular (± 1-6 layers only at protrusions) collenchyma (collenchyma area at protrusions = 24.3 µM), the middle chlorenchyma (± 2-6 layers) (chlorenchyma area = 21.5 µM) and the inner dissected cylinder parenchyma (±1-2 layers) (parenchyma area = 13.1 µM). The vascular tissue was composed of siphonostele (vascular system area with pericycle = 386371.1 µM). Secondary growth was extreme herbaceous, present in both fascicular and interfascicular regions (vascular system area without pericycle = 343546.2 µM). Bast phloem fibers was present as an isolated patches (±1-3 layers; pericycle cylinder thickness = 27.1 µM). Interfascicular cambium activity formed the phloem (fibers and vessels) to out (± 2-8 layers; phloem cylinder thickness = 33.9 µM) and xylem to down (± 2-8 layers; xylem cylinder thickness at corner = 163.1 µM and between corners = 111.0 µM). Vessels were (diffuse) ring porous (xylem area = 283242.2 µM). Xylem parenchyma was paratracheal. Pith was solid and heterogeneous of two types, the outer thick walled lignified parenchymatous cells and the inner of thin walled ones. Simple pits were present in lignified cells (pith area = 115320.0 µM). Crystals were small raphides restricted to pith.

Petiole anatomy (Figure 4 (plates 2a and b)): In transverse section, the petiole was thickly crescentiform

with shallow, broad, adaxial furrow (petiole area = 203180.9 µM). Cuticle was thick (cuticle thickness = 7.5 µM). Epidermal cells were radially, tangentially, papillose and rare columnar elongated (epidermis thickness = 13.0 µM). Cortex consisted of four types; chlorenchyma with area = 37635.6, cartilaginous collenchyma at ad- and abaxial sides and at the two ridges (collenchyma thickness at adaxial side = 37.0 µM), parenchyma at both adaxial (± 6 layers) and abaxial sides (± 1 layers) (parenchyma thickness at adaxial side = 76.2 µM) and fibers at two ridges.

The petiole vasculature was of a shallow, incomplete crescentric strand of two bundles (vascular system area with pericycle = 77289.8 µM, without pericycle = 64434.7 µM and xylem area = 19606.2; pericycle thickness = 20.3 µM, phloem thickness = 69.5 µM and xylem thickness = 72.3 µM). Two ridge bundles were present (xylem thickness in additional bundles = 33.6 µM). Crystals were small raphides and rhombic-shaped restricted to cortical cells.

Key to studied species according to anatomical characters of stems:

1a. Stem is quadrangular with large protrusions (stem area = 318732 µM): *Ballota damascena*
b. Stem is quadrangular with small protrusions (stem area = 463675.6-10664427 µM): 2
2a. Bast phloem fibers form complete ring (1-5 layers), number of vascular bundles is ten: *Ballota kaiseri*
b. Bast phloem fibers form dissected ring: 3
3a. Pith crystals are absent; number of vascular bundles is eight: *Ballota saxatilis*
b. Pith crystals are present: 4
4a. Number of stem vascular bundles is four: *Ballota pseudodictamnus*
b. Number of stem vascular bundles is six: *Ballota undulata*

Key to studied species according to anatomical characters of petioles:-

1a. Petiole shape is thickly crescentiform with very deep adaxial furrow: *B. damascena*
b. Petiole shape style is otherwise: 2
2a. Petiole shape is thickly crescentiform without adaxial furrow and has small protrusions adaxial side: *B. pseudodictamnus*
b. Petiole shape style is otherwise: 3
3a. Petiole is leafy shaped with very deep adaxial furrow: *B. saxatilis*
b. Petiole shape is thickly crescentiform with shallow, broad adaxial furrow: 4
4a. Petiole is 4403355.6 µM in area: *B. kaiseri*
b. Petiole is 203180.9 µM in area: *B. undulate*

Pollen grains description

Pollen types and main character of pollen types

Pollen types: The careful examination of the available pollen material of the Egyptian species of *Ballota* (Lamiaceae) revealed the presence of 4 pollen types; which can be distinguished through the following key.

Key to the pollen types

1a. Pollen grains with bireticulate exine: *Ballota undulata* type
b. Pollen grains exine otherwise: 2
2a. Pollen grains with reticulate-perforate exine: *Ballota kaiseri* type
b. Pollen grains exine otherwise: 3
3a. Pollen grains with macroreticulate-bireticulate exine: *Ballota damascena* type
b. Pollen grains with reticulate exine: *Ballota saxatilis* type
The following pollen types are recorded among the taxa of *Ballota* represented in the flora of Egypt (Tables 6 and 7).

Main characters of pollen types

B. damascena type (Figure 5 (plates 1 to 4))

Pollen grains tricolpate with area 158.7 (137.9-178.0) µM, oblate-spherioidal to spherioidal in shape (P/E = 0.98), 14.5 (14.1-14.9) x 14.8 (13.4-15.9) µM. Apocolpium diameter 3.4 (3.0-4.3) µM. Colpi were narrow, 11.2 (10.9-11.7) µM long, 3.5 (2.3-5.2) µM wide, with acute ends, colpi costae were present with thickness 1.7 (1.2-3.0) µM. Mesocolpium 8.0 (7.8-8.4) µM wide. Exine thickness 1.7 (1.6 - 1.8) µM thick at centre of mesocolpia. Sexine tectate, tectum macroreticulate-bireticulate in texture (a two layered tectum consisting of a suprareticulate layer supported by a perforate layer), sculpture at pole was reticulate. Lumina diameter is 0.1 (0.04 - 0.3) µM with irregular, thick muri. The following taxon belongs to this type: *Ballota damascena* Boiss.

B. kaiseri type (Figure 5 (plates 5-8))

Pollen grains tricolpate with area 190.7 (173.2-208.3) µM, oblate-spherioidal to spherioidal in shape (P/E = 0.90), 15.6 (14.6-16.4) x 17.4 (16.5-18.4) µM. Apocolpium diameter 2.6 (2.3-3.2) µM. Colpi were wide, 14.2 (13.5-14.9) µM long, 5.0 (4.4-5.6) µM wide, with acute ends, colpi costae were present with thickness 2.9 (2.6-3.2) µM. Mesocolpium 8.8 (7.5-9.4) µM wide. Exine thickness 2.1(1.9-2.3) µM thick at centre of mesocolpia. Sexine

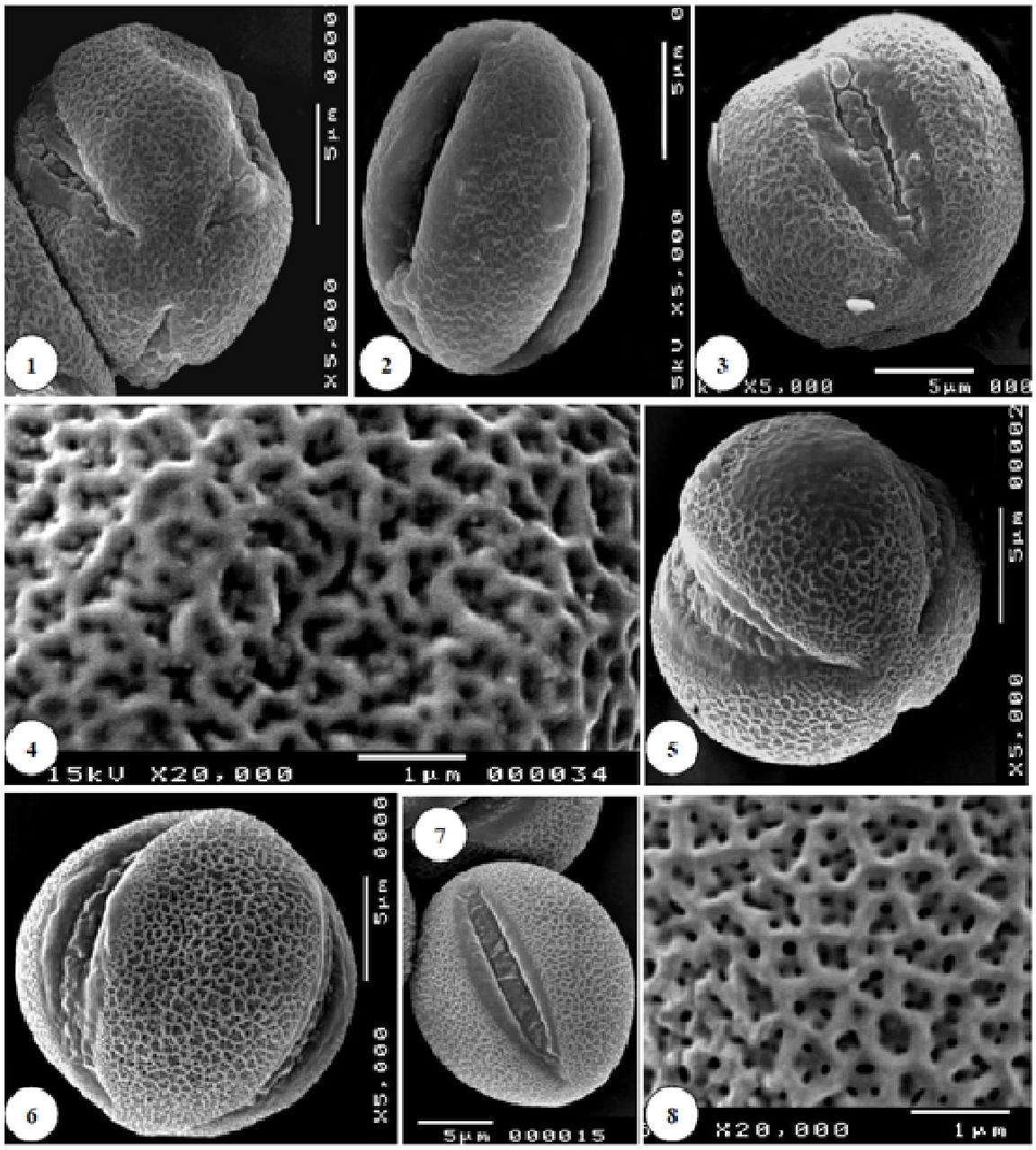

Figure 5. SEM observations of non acetolyzed pollen grains (SEM × 5000-20,000). 1 to 4, Ballota damascena: (1) polar view, (2) equatorial view, (3) equatorial view, (4) magnified part of exine. 5 to 8, Ballota kaiseri: (5) oblique polar view, (6) oblique equatorial view (7) equatorial view showing colpus, (8) magnified part of exine.

tectate, tectum reticulate-perforate in texture, sculpture at pole was reticulate. Lumina diameter is 0.3 (0.1-0.5) µM with regular muri. The following taxon belongs to this type: a. *Ballota kaiseri* Tɔckh.

Ballota saxatilis type (Figure 6 (plates 1 - 4))

Pollen grains tricolpate with area 165.7 (146.5-181.1) µM, oblate-spherioidal to spherioidal in shape (P/E = 0.90), 13.7 (13.4-14.2) x 15.3 (14.9-15.7) µM. Apocolpium diameter 2.3 (2.0-2.9) µM. Colpi were wide, 12.8 (11.2-14.8) µM long, 5.0 (3.7-6.2) µM wide, with acuminate ends, colpi costae were present with thickness 3.1 (2.3-3.7) µM. Mesocolpium 7.6 (5.9-9.0) µM wide. Exine thickness 2.3 (1.7-3.0) µM thick at centre of mesocolpia. Sexine tectate, tectum reticulate in texture, sculpture at pole was reticulate. Lumina diameter is 0.2 (0.1-0.4) µM with irregular muri, upper muri are thick and down ones are thin. The following taxon belongs to this type: a. *Ballota saxatilis* C. Presl in J. & C.

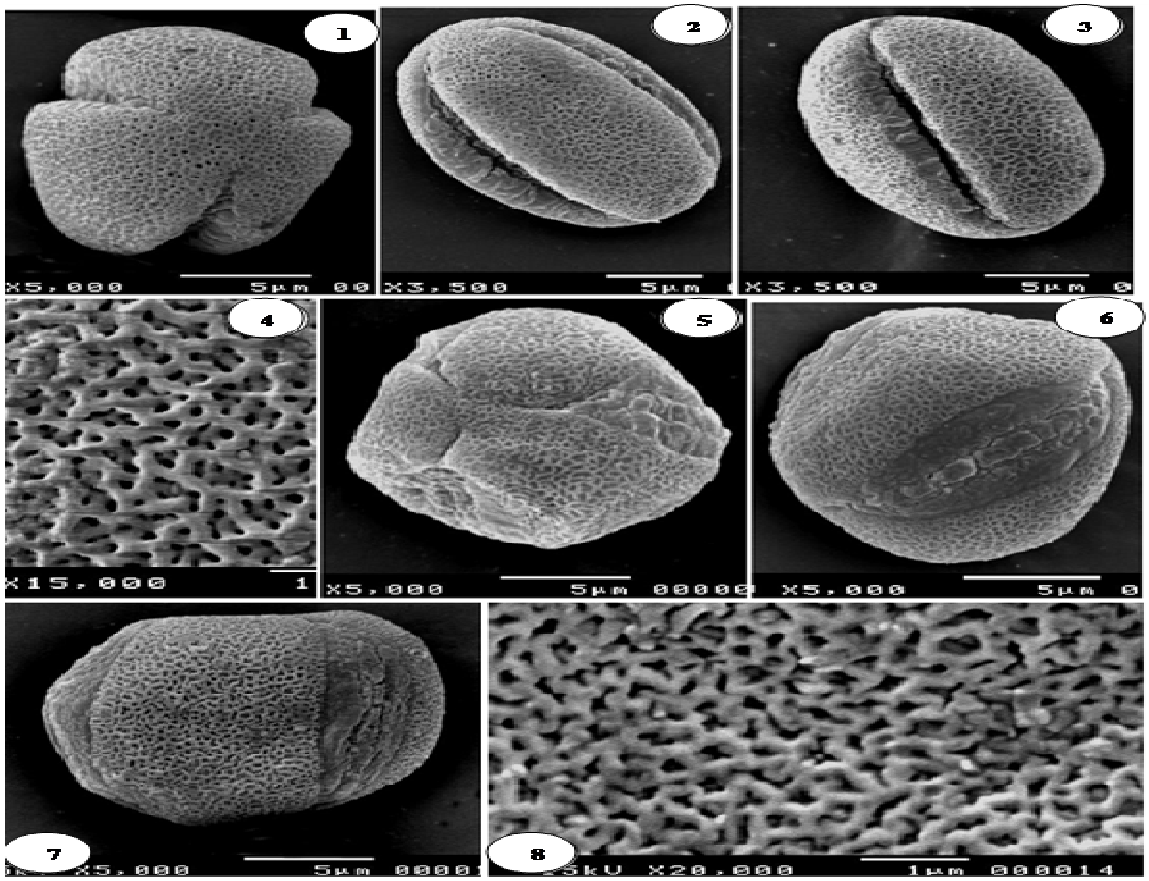

Figure 6. SEM observations of acetolyzed pollen grains (SEM × 5000 – 20000). 1 to 4, Ballota pseudodictamnus: (1) polar view, (2) equatorial view, (3) equatorial view, (4) magnified part of exine. 5 to 8, Ballota saxatilis: (5) polar view, (6) oblique equatorial view, (7) equatorial view showing mesocolpium, (8) magnified part of exine.

Ballota undulata type (Figure 6 (plates 1 to 4) and Figure 6 (plates 1 to 4))

Pollen grains tricolpate with area 153.6 (140.3–216.6) – 190.0 (147.5-160.8) µM, oblate or oblate-spherioidal to spherioidal in shape (P/E = 0.69–0.96), 13.3 (12.6-13.7) – 14.0 (12.8-15.2) x 13.9 (13.2-14.4) – 20.3 (20.1-20.4) µM. Apocolpium diameter 2.8 (2.3-3.4) – 3.6 (3.0 - 4.5) µM. Colpi were narrow or slightly wide, 12.5 (11.4-13.9) – 16.4 (14.0-18.7) µM long, 3.2 (2.8-3.6) - 4.6 (3.6-5.2) µM wide, with acute ends, colpi costae were absent at centre of mesocolpia. Mesocolpium 8.2 (6.8-8.8) – 8.5 (7.3-9.7) µM wide. Exine thickness 1.9 (1.7-2.1) - 2.1 (1.6-2.5) µM thick at centre of mesocolpia. Sexine tectate, tectum bireticulate in sculpture (a two layered tectum consisting of a supermacro reticulate layer supported by a micro-reticulate-perforate), sculpture at pole was microreticulate-reticulate. Lumina diameter was 0.3 (0.1 - 0.7) µM with irregular to regular muri, upper muri were equal to down ones or upper thick and down thin. The following taxa belong to this type: a. *Ballota pseudodictamnus* (L.) Benth; b. *Ballota undulata* (Fresen.) Benth.

Key to species of *B. undulata* pollen type

1a. Equatorial diameter medium is 20.3 (20.1–20.4) µM: *B. pseudodictamnus*
b. Equatorial diameter medium is 13.9 (13.2–14.4) µM: *B. undulata*

DISCUSSION

In this anatomical and palynological investigation of Egyptian representatives of *Ballota* taxa, an additional perspective on the relations among the different *Ballota* taxa studied had been provided.

Stem

Stem anatomy was similar in the examined taxa, but there was difference in the dimensions of stem areas, the nature of dermal system, the character of ground system, the presence and distribution of bast phloem fibers, the thickness of phloem and number of vessels, and the

Table 4. Stem anatomy of the studied taxa of *Ballota*.

Taxa	Shape in outline	Dermal System					Ground System					
		Cuticle	Epidermis				Cortex					Pith
			Type		Size	Wall thickness	Collenchyma			Chloren.	Parenchyma	
			At pro.	Bet. pro.			Angular	Annular	Lamellar			Crystal type
Ballota damascena	1	Thick spinney	P.	T.	Big	thick	-	±(10-13) at pro.	-	+(2-12)	+(1-2) only at pro.	+ raphides
Ballota kaiseri	2	Medium	T. and P.	T.	Small	thick	-	+(1-6) at pro.	-	+(3-6)	+ one only at pro.	+ small raphides
Ballota pseudodictamnus	2	Thin spinney	P.	T.	Small	thick	-	+(6-12) at pro.	+(3-4) bet. pro.	+(1-5)	+ (1-3) only at pro.	+ raphides
Ballota saxatilis	2	Thick spinney	T.&P.	T.	Big	thin	+(1-6) at pro.		-	+(2-5)	+(1-2) complete	-
Ballota undulata	2	Medium	R.&T.	T	Small	thick	-	+(1-6) at pro.	-	+(2-6)	+(1-2) dissected	+ small raphides

Taxa	Vascular system			
	Phloem		Xylem	
	Bast phloem fibers	No. of phloem layers	No. of xylem vessels	No. of vascular bundles
Ballota damascena	Only at pro. (1-12)	(1-5)	2-6	8
Ballota kaiseri	Complete (1-5)	(3-10)	1-5	10
Ballota pseudodictamnus	dissected (1-5)	(3-6)	3-13	4
Ballota saxatilis	dissected (1-4)	(1-5)	1-4	8
Ballota undulata	dissected (1-3)	(2-8)	2-8	6

1= Quadrangular with large protrusions; 2= quadrangular with small protrusions; At pro.= at protrusions; Bet. Pro. = between protrusions.

number of vascular bundles varies among the taxa. Abu-Assab and Cantino (1987), Cantino (1992) and Metcalfe and Chalk (1950) gave information about the general anatomical characteristics of the family Lamiaceae. They stated that in the stem anatomy of *Ballota*, there were multicellular hairs on epidermis. A transverse section taken from the middle part of the stems of the investigated taxa (Figures 1 and 2) revealed the following: In general, the stem outline was quadrangular with small protrusions in all studies taxa, with area 318732-10664427 µM, except in *B. damascena*; it was quadrangular with large protrusions. In the dermal system, the cuticle layer is 2.8 - 8.2 µM in thickness; it was thick spinney in *B. damascena* and *B. saxatilis*, thin spinney only in *B. pseudodictamnus* and medium in both *B. kaiseri* and *B. undulata*. Epidermis consisted of a single layer of papillose, tangentially or radially elongated cells (8.5 - 14.2 µM in thickness), at protrusions the epidermal cells were papillose, tangentially and rarely radially elongated, but in between protrusions all cells were tangentially elongated, there were several types of hairs on it (Osman, 2012). The endodermis was not distinct. Underneath the epidermis, there was ground tissue which was composed of cortex and pith. Cortex (58.9-204.8 µM in thickness) consists of 4-27 layers of usually oval cells with thin walls; it was composed of collenchyma, parenchyma and chlorenchyma cells.

The studied species exhibit obvious differences in number of collenchyma, chlorenchyma and parenchyma layers (Table 4). Collenchyma has 1 - 13 layered. The existence and distribution of collenchyma in cortex of examined species were an additional taxonomic trait for identifying these taxa. There were three forms of collenchymatic tissues: angular, annular and lamellar. Angular collenchyma were recorded only in *B. saxatilis* and number of layers were more than 1 - 6 layers at protrusions, while annular collenchyma were found only in *B. damascena* (number of layers are more or less than 10 - 13 layers at protrusions), *B. kaiseri* (number of layers are more than 1 - 6 layers at protrusions) and *B. undulata* (number of layers are more than 1 - 6 layers at protrusions), and finally both annular and lamellar collenchyma were observed only in *B. pseudodictamnus*, annular collenchyma were present at protrusions (number of layers = 6 - 12) and lamellar collenchyma existed in between protrusions (number of layers = 3 - 4). Chlorenchyma was located under the collenchyma and 1 - 12 layered. The existence of chlorenchyma may be considered as a typical response to the photosynthetic ability of the stem (Koyuncu et al., 2009). Thus, the effect of photosynthesis of the leaf and also the stem was increased (Fahn, 1967).

Table 5. Petiole anatomy of the studied taxa of *Ballota*.

Taxa	Shape type	Cuticle	Epidermal cells				Type of cortical cells			
			Columnar	Tangentially	Radially	Papillose	Chlorenchyma	Parenchyma	Collenchyma	Sclerenchyma
Ballota damascena	1	medium	-	+	-	+	+	+* [3, 0]	Annular	-
Ballota kaiseri	2	medium	+	+	+	+	+	+** [3, 1-2]	Annular	+
Ballota pseudodictamnus	3	thin	-	+	+	+	+	+** [4, 2-3]	Cartilaginous	-
Ballota saxatilis	4	Very thick	-	+	+	+	+	+** [2, 2]	Cartilaginous	-
Ballota undulata	2	thick	+ (rare)	+	+	+	+	+** [6, 1]	Cartilaginous	+

Taxa	Petiole vasculature			
	Form	No. of dissected siphonostele bundles	No. of ridge bundles	Types of crystals
Ballota damascena	I	2 bundles	2	raphides
Ballota kaiseri	II	2 bundles	2	rhombic
Ballota pseudodictamnus	III	3 bundles	2	absent
Ballota saxatilis	IV	1 strand	2	absent
Ballota undulata	II	2 bundles	2	Small raphides and rhombic

1 = Petiole is thickly crescentiform with very deep adaxial furrow, 2 = Petiole is thickly crescentiform with shallow, broad adaxial furrow, 3 = Petiole is thickly crescentiform without adaxial furrow and has small protrusions at adaxial side, 4 = Petiole is leafy shaped with very deep adaxial furrow, - = absent, + = present, +* = parenchyma only at adaxial side, +** = parenchyma at both adaxial and abaxial side. I = The petiole vasculature of a deep, incomplete crescentric strand of two bundles, II = The petiole vasculature of a shallow, incomplete crescentric strand of two bundles, III = The petiole vasculature of a shallow, incomplete crescentric strand of three bundles, IV = The petiole vasculature of a medium in deepness, complete crescentric strand.

Parenchyma were located under the chlorenchyma and consisted of 1-3 layers of usually oval cells and thin walls, they form complete ring in *B. saxatilis*, dissected ring in *B. undulata* and appear only at protrusions in the rest of the studied species. Pith was solid, consisted of parenchymatous, thin walled cells and contained raphides crystals. The crystals were identified only in pith of four of the studied taxa also, they were not found in *B. saxatilis* stem, they were recorded in intensive amounts as large raphides in both *B. damascena* and *B. pseudodictamnus*, and in few amounts as small raphides in both *B. kaiseri* and *B. undulata*. Cambium was not distinguishable.

In the vascular system, the phloem was 12.4 - 43.9 µM in thickness, 1 - 10 layered. The bast phloem fibers form complete ring in *Ballota kaiseri*, exist only at protrusions in *B. damascena* and present as dissected ring in the rest taxa. The xylem thickness in between protrusions was 50.5 - 291.7 µM and at protrusions 105.4 - 462.1 µM. Xylem elements were arranged in parallel rows (1 - 13 layers) and number of vascular bundles exhibit clear differences among the investigated taxa. The number of vascular bundles in *B. kaiseri* was 10, in both *B. damascena* and *B. saxatilis* 8, in *B. pseudodictamnus* 4 and finally in *B. undulata* 6 where the highest and the least respectively (Table 4).

Petiole

Metcalfe and Chalk (1979) pointed out that in many families, especially in Lamiaceae, the structure of the petiole was important in terms of taxonomy. Generally, the petiole outline was thickly crescentiform with very deep adaxial furrow in *B. damascena*, thickly crescentiform with shallow, broad adaxial furrow in both *B. kaiseri* and *B. undulata*, thickly crescentiform without adaxial furrow and had small protrusions at adaxial side in *B. pseudodictamnus* and leafy shaped with very deep adaxial furrow in *B. saxatilis* with area medium 78925.6 - 4403355.6 µM. The cuticle was obviously thick in both *B. saxatilis* and *B. undulata*, thin only in *B. pseudodictamnus* and medium in both *B. damascena* and *B. kaiseri*. On the other hand, epidermis was single layered (12.2-43.9 µM in thickness) of papillose, tangentially, radially or columnar elongated cells (Table 5). Cortex was composed of collenchyma, parenchyma and chlorenchyma. Collenchyma (18.2-69.9 µM in

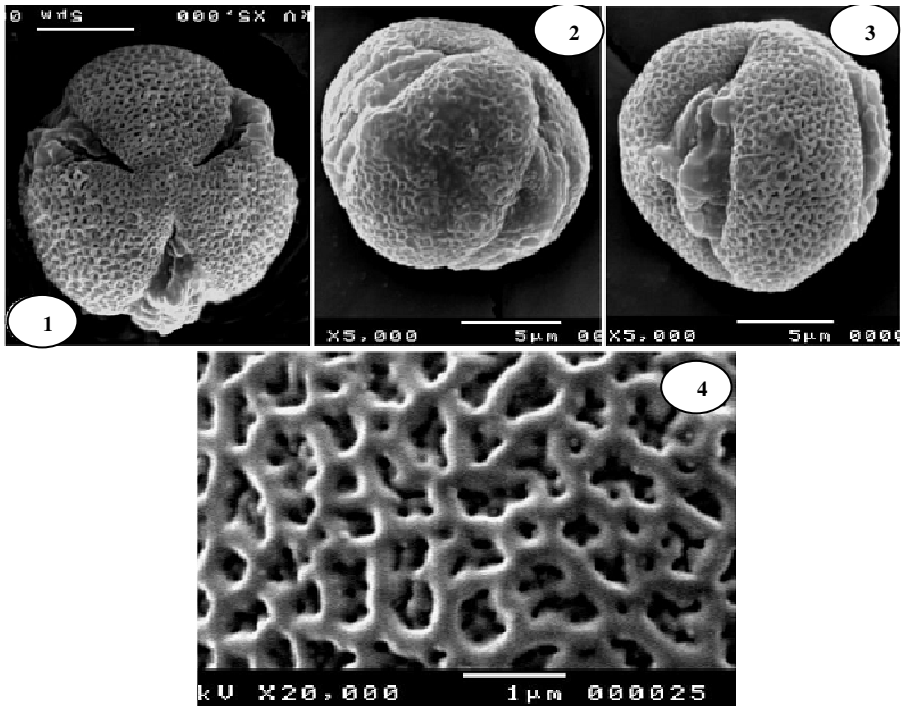

Figure 7. SEM observations of acetolyzed pollen grains (SEM × 5000 – 20000). 1 to 4, Ballota undulata: (1) polar view, (2) equatorial view, (3) equatorial view and (4) magnified part of exine.

thickness from down side) was located under the epidermis and consisted of annular cells in both *B. damascena* and *B. kaiseri* and cartilaginous cells in the rest studied taxa. Parenchyma was large, thin walled (37.6-98.8 µM in thickness from up side) and chlorenchyma (area = 7969.4-45515 µM) were usually restricted into the two petiole edges. In *B. damascena* parenchyma was present only at adaxial side and absent at abaxial side, but in the other studied taxa, it was present in both adaxial and abaxial sides. In both *B. kaiseri* and *B. undulata*, there were some sclerenchymatous cells. In addition, there were raphides crystals in cortex of *B. damascena*, rhombic crystals in *B. kaiseri* cortex, small raphides and rhombic crystals in *B. undulata* cortex. Crystals were not recorded in both *B. pseudodictamnus* and *B. saxatilis*.

In respect to petiole vasculature, there was a large vascular bundle in the median region of the petiole and two small bundles on both sides of this large bundle. Differences were identified in the form and structure of the large vascular bundle among the studied taxa. There was an incomplete crescentric strand of two bundles in *B. damascena*, a shallow incomplete crescentric strand of two bundles in both *B. kaiseri* and *B. undulata*, a shallow, incomplete crescentric strand of three bundles in *B. pseudodictamnus* and finally a medium in deepness, complete crescentric strand in *B. saxatilis*. Palynological characters applied to 5-species of *Ballota* in Egypt provided to be useful in the distinction between four groups. The pollen grains were usually 3-zonocolpate. Four pollen types were distinguished according to ornamentation of the exine: *B. damascena* pollen type, in which the pollen grains had macroreticulate-bireticulate sculpture characteristic of *B. damascena* species only. *B. kaiseri* pollen type in which the pollen grains had reticulate-perforate sculpture was characteristic of *B. kaiseri* species only. *B. undulata* pollen type, in which the pollen grains had bireticulate sculpture, was characteristic of both *B. pseudodictamnus* and *B. undulata* species, and *B. saxatilis* pollen type in which the pollen grains had reticulate sculpture, was characteristic of *B. saxatilis* species only.

The smallest pollen grains were those of *B. undulata* 13.9 (13.2 - 14.4) µM and the largest one were those of *B. pseudodictamnus* 20.3 (20.1 - 20.4) µM. Pollen size of other taxa ranged between 14.8 - 17.4 µM. Pollen grains were more or less similar in shape being oblate-spherioidal to spherioidal or oblate. Colpi were narrow in both *B. damascena* and *B. pseudodictamnus*, wide in both *B. kaiseri* and *B. saxatilis* and slightly wide only in *B. undulata*. Colpi ends contributed to differentiating *B. saxatilis*, which was characterized by acuminate ends from all other studied taxa which had acute ends. On the other side, the costae colpi was present only in three of investigated taxa; it was present in *B. damascena* with scabrate sculpture, in *B. kaiseri* with psilate-scabrate sculpture and in *B. saxatilis* with granulate-scabrate sculpture. The sculpture at pollen pole also could help in

Table 6. Tabular summary showing the pollen grains dimensions.

Species	Polar Axis (µM)	Equatorial Diameter (µM)	P/E.	Exine thickness (µM)	Colpus length (µM)	Colpus width with costae (µM)	Apocolpium diameter (µM)	Mesocolpium diameter (µM)	Pollen area (µM)	Coastae thickness. (µM).	Lumina diameter (µM).	Colpus width without costae (µM).
B. damascena	14.5 (14.1-14.9)	14.8 (13.4-15.9)	0.98	1.7 (1.6-1.8)	11.2 (10.9-11.7)	3.5 (2.3-5.2)	3.4 (3.0-4.3)	8.0 (7.8-8.4)	158.7 (137.9-178.0)	1.8 (1.7-1.9)	0.2 (0.1-0.4)	1.8 (1.1-2.2)
B. kaiseri	15.6 (14.6-16.4)	17.4 (16.5-18.4)	0.90	2.1 (1.9-2.3)	14.2 (13.5-14.9)	5.0 (4.4.-5.6)	2.6 (2.3-3.2)	8.8 (7.5-9.4)	190.7 (173.2-208.3)	1.6 (1.0-2.4)	0.3 (0.1-0.5)	2.1 (1.8-2.4)
B. pseudodictamnus	14.0 (12.8-15.2)	20.3 (20.1-20.4)	0.69	2.1 (1.6-2.5)	16.4 (14.0-18.7)	3.2 (2.8-3.6)	3.6 (3.0-4.5)	8.5 (7.3-9.7)	190.0 (140.3-216.6)	1.7 (1.2-2.2)	0.3 (0.2-0.4)	1.2 (0.9-1.4)
B. saxatilis	13.7 (13.4-14.2)	15.3 (14.9-15.7)	0.90	2.3 (1.7-3.0)	12.8 (11.175-14.8)	5.0 (3.7-6.2)	2.3 (2.0-2.9)	7.6 (5.9-9.0)	165.7 (146.5-181.1)	1.8 (1.2-2.5)	0.1 (0.04-0.3)	1.9 (1.4-2.5)
B. undulata	13.3 (12.6-13.7)	13.9 (13.2-14.4)	0.96	1.9 (1.7-2.1)	12.5 (11.4-13.9)	4.6 (3.6-5.2)	2.8 (2.3-3.4)	8.2 (6.8-8.8)	153.6 (147.5-160.8)	1.6 (1.5-1.8)	0.3 (0.1-0.7)	2.1 (1.6-2.8)

P/E **=** the ratio of the length of the polar axis (P) to the equatorial diameter (E), µM = micrometer.

Table 7. Tabular summary showing the description of LM and SEM samples.

Species	Pollen Shape	Colpi wideness	Colpi ends	Costae	Costae sculpture	Sculpture type	Sculpture at Pole	Muri state	Muri layers
B. damascena	Oblate-spherioidal to spherioidal	Narrow	Acute	+	Scabrate	Macroreticulate-bireticulate	Reticulate	Irregular	Upper thick down thin
B. kaiseri	Oblate-spherioidal to spherioidal	Wide	Acute	+	Psilate-scabrate	Reticulate-perforate	Reticulate	Regular	Upper thick down thin
B. pseudodictamnus	Oblate	Narrow	Acute	-		Bireticulate	Microreticulate	Irregular	Upper and down equal
B. saxatilis	Oblate-spherioidal to spherioidal	Wide	Acuminate	+	Granulate-scabrate	Reticulate	Reticulate	Irregular, thick	One thick layer
B. undulata	Oblate-spherioidal to spherioidal	Slightly wide	Acute	-		Bireticulate	Reticulate	Regular	Upper thick down thin

+ = Present; - = absent.

differentiation of *B. pseudodictamnus*, which was characterized by microreticulate texture from all other taxa which had reticulate texture.

Additionally, the state of muri also contributed to differentiating *B. damascena*, *B. pseudodictamnus* and *B. saxatilis* species, which were characterized by irregular muri from the two other investigated taxa that exhibited regular muri. Also according to thickness of muri layers, we could distinguished *B. damascena*, *B. kaiseri* and *B. undulata*, which had muri with upper thick layer and down thin layer from *B. pseudodictamnus* which had muri with equal upper and down two layers and *B. saxatilis* which had one, thick muri layer. It also pointed to the close relationship between *B. pseudodictamnus* and *B. undulata* species which exhibited very close pollen grains being similar in pollen type, sculpture type and absence of costae colpi.

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