



## Anatomical Remarks of Some Species of *Silene* and *Bufonia* (Caryophyllaceae), Endemic to Sinai, Egypt

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**A**NATOMICAL features of stem and leaf have been studied on four endemic species collected from St. Catherine area, S. Sinai, Egypt. viz. *Silene leucophylla* Boiss., *S. oreosinaica*, *S. schimperiana* and *Bufonia multiceps* which is provided for the first time. Comparative study was conducted using both light microscope (LM) and scanning electron microscopy (SEM), based on the anatomical structure of the leaf and stem. Druses were found abundantly in both leaf and stem tissue in studied *Silene* taxa, absent in *Bufonia multiceps*. Stomata were mostly diacytic or both diacytic and anomocytic were also recorded. Surface view of epidermal cells were straight or undulated. Epicuticular wax platelets have been observed on epidermal and guard cells of *S. schimperiana* and *S. leucophylla*. The leaf epidermal and stem anatomical characters were found distinct in each species. Artificial key was provided for the identification of the studied species.

**Keywords:** Anatomy, *Bufonia*, Caryophyllaceae, Endemic, Flora of Egypt, Leaf, *Silene*, Stem.

### Introduction

Caryophyllaceae A. Juss. is a large family represented by 81 genera and 2625 cosmopolitan species (Christenhusz & Byng, 2016). The family is represented in Egypt by 87 species, belonging to 24 genera of these 61 are known from Sinai (El Hadidi & Hosny, 2000; Boulos, 2009). The family is represented in the present study by 4 species endemics to St. Catherine area viz.: *Bufonia multiceps* Decne., *Silene leucophylla* Boiss., *S. oreosinaica* Chowdhuri and *S. schimperiana* Boiss. The genus *Silene* comprises approximately 700 to 750 species in 44 sections of which about half occur in the Mediterranean region (Melzheimer 1988, Oxelman et al., 2001, Rautenberg et al., 2012). In Egypt *Silene* is represented by 29 species, showing great morphological variation of these 11 species known from Sinai (Täckholm, 1974; Hosny et al., 1993; Hosny & Hadidi, 2000; Boulos, 2009).

Many groups in the genus *Silene* have high taxonomic complexity especially concerning the macro-morphology (Cood & Cullen, 1967). The *Silene* circumscription has been controversial for a long time and several treatments have taxonomically revised the genus (Chowdhuri, 1957a, b; McNeill, 1978; Greuter et al., 1984; Greuter, 1995; Oxelman et al., 2001).

The genus *Bufonia* includes about 30 annual or perennial herbaceous to small sub shrub by species growing mostly on dry gravelly slopes in mountainous regions (Bittrich, 1993; Chrtek & Křisa, 1999; Boulos, 2008) and is distributed in the Mediterranean and Irano-Turnian regions. In Egypt only one species namely *Bufonia multiceps* Decne. was recorded (El Hadidi & Hosni, 2000; Täckholm, 1974; Boulos, 1999, 2009). A species is restricted to montane wadis with granite rocky ground of mountain areas (Moustafa & Klopatek, 1995; Abd El-Wahab et al., 2006; Salama et al.,

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Received 24/6/ 2020; Accepted 11/10/ 2020

DOI: 10.21608/ejbo.2020.32998.1512

Edited by Prof. Dr. Monier M. Abd El-Ghani, Faculty of Science, Cairo University, Giza 12613, Egypt.

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2018; Fouad et al., 2019) the species is describing as Critically Endangered (Omar, 2017).

The anatomical characters, for example, trichome types and stomatal kinds of Egyptian endemic taxa of Caryophyllaceae, have not been concentrated previously. In this manner, the objective of the current study is to explore their anatomical attributes utilizing both light (LM) and scanning electron microscopy (SEM) to study the various kinds of tissues, trichomes, and stomata to evaluate the value of these characters for deliberate purposes and to fill the gaps in our insight of *Silene* and *Bufonia* in Egypt.

This study aims to provide primary documentation of anatomical characters of stem and leaf of the endemic taxa *Silene* and *Bufonia*

### Materials and Methods

Fresh materials were collected from various localities (Musa gorge N: 28.32303 E: 33.960, Wadi Meserdy, Wadi Alarbeen, Wadi Abo Kasaba and Ain Shekia N: 28.55321 E: 33.93482 in Saint Catherine protected area during April and May 2016; the specimens were deposited in the Herbaria of Cairo University (CAI) and Al-Azhar University. list of the material studied, with information was shown in Table 1 and Fig. 1.



Map of Saint Catherine protected area

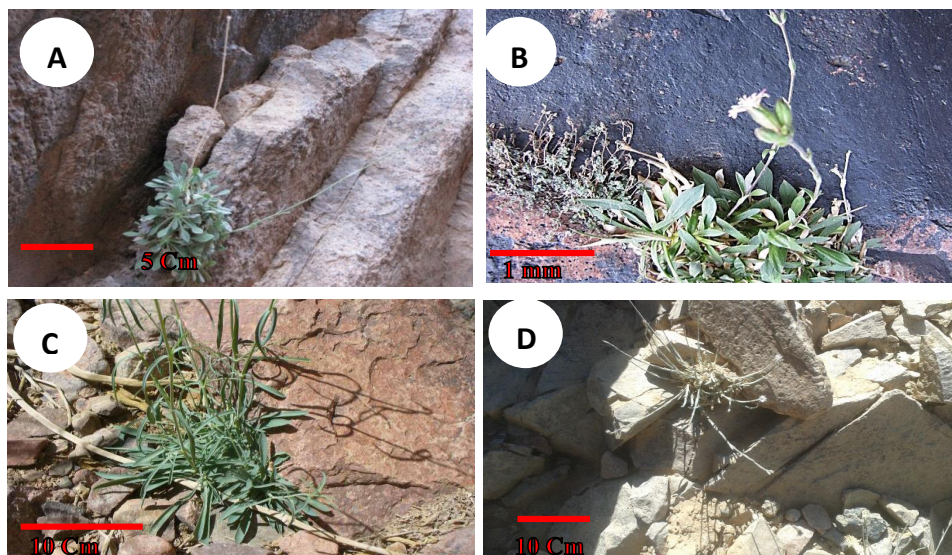


Fig. 1. A: *Silene leucophylla*, B: *Silene oreosinaica*, C: *Silene schimperiana*, D: *Bufonia multiceps*

**TABLE 1. Species of *Silene* and *Bufonia* used in this study and their sectional delimitation, indicate the place and date for each taxon**

No.	Species	Collection	Habitat
1-	<i>Silene leucophylla</i> Boiss.	- Wadi Meserdy, 4/5/2016, A. M. Sadek; (Al-Azhar Univ.) -Ain Shekia, 2/5/2016, A. M. Sadek. (Al Azhar Univ.) -Wadi Abo Kasaba 5/5/2016, A. M. Sadek. (Al-Azhar Univ.)	Rocky habitat
2-	<i>Silene oreosinaica</i> Chowdhuri.	- Musa gorges 30/4/2106; A. M. Sadek.(Al-Azhar Univ.)	Rocky-crevicesand slopes
3-	<i>Silene schimperiana</i> Boiss.	- Musa gorges, 30/4/2016; A. M. Sadek. (Al-Azhar Univ.) -Wadi Al-Arbaen 30/4/2016, A. M. Sadek. (CAI, Al Azhar Univ.)	Rocky slopes and crevices
4-	<i>Bufonia multiceps</i> Decne.	Wadi Al Ferah 30/4/2016; A. M. Sadek. (Al-Azhar Univ.) Wadi Al-Arbaen 30/4/2016, A. M. Sadek. (Al-Azhar Univ.) Ain Shekia 2/5/2016; A. M. Sadek. (CAI, Al-Azhar Univ.)	Rocky slopes.

For light microscopic study (LM), 3–5 specimens of internode and mature blade were taken from basal leaflets. The specimens processed according to paraffin wax method of Johansen (1944) to prepare samples for microtome sectioning at 10–15µm thickness. Sections were fixed on glass slides by means of Haupt's adhesive (1gm gelatin dissolved in 50mL warm distilled water then 7.5mL glycerol added+ small phenol crystal then kept in refrigerator for 24hrs. till solidification) and left to dry for 24hrs. Then sections were stained with Safranin-Fast green standard double stain and mounted in Canada balsam (Sass, 1961).

For epidermal examination, three pieces of lamina were embedded in KOH 5% for 24–48 hrs.; stomata and trichomes of upper and lower epidermis were examined. Terminology followed Barthlott (1981, 1990), Barthlott et al. (1998), and Stearn (1996).

For scanning electron microscopy (SEM), leaves were mounted on stubs using double-sided adhesive tape, then coated with Nano gold then examined and photographed with JIOL JSM SEM at the Electron Microscope unit at The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. For measurements and calibration stage micrometer was used as well as image J software

and photographed by using stereomicroscope equipped with Premiere (MA88–900) digital camera.

## Results

### *Silene leucophylla* Boiss

Stem circular in cross section, epidermis uniseriate with oblong cells. Cortex with 3-4 layers of chlorenchyma tissue followed by 6 layers of pericyclic fibers. Vascular cylinder consists of many collateral vascular bundles arranged in a cycle. Each bundle contains few phloem and xylem elements. Large pith of thin parenchyma present. (Plate 1, Figs. 1-2). Leaf transection revealed wavy epidermis, uniseriate with cubic cells. Midrib arc shaped, penetrated with small collateral vascular bundle with arc shape. The bundle consists of few phloem elements, many xylem elements. Mesophyll dorsiventral, with two continuous palisade layers followed by 4-5 layers of spongy tissue. Druses present in leaf and stem. (Plate 1, Figs. 3 & 4). Stomata anomocytic, diacytic (Plate 1, Fig. 5). Trichomes unicellular, un-branched and non-glandular (Plate 1, Fig. 6). In SEM, epidermal cells with undulated cell wall; stomata are semi-depressed and cuticle ultrastructure with highly dense epicuticular wax platelets on epidermal and guard cells (Plate 1, Figs. 7 & 8), (Table 2).



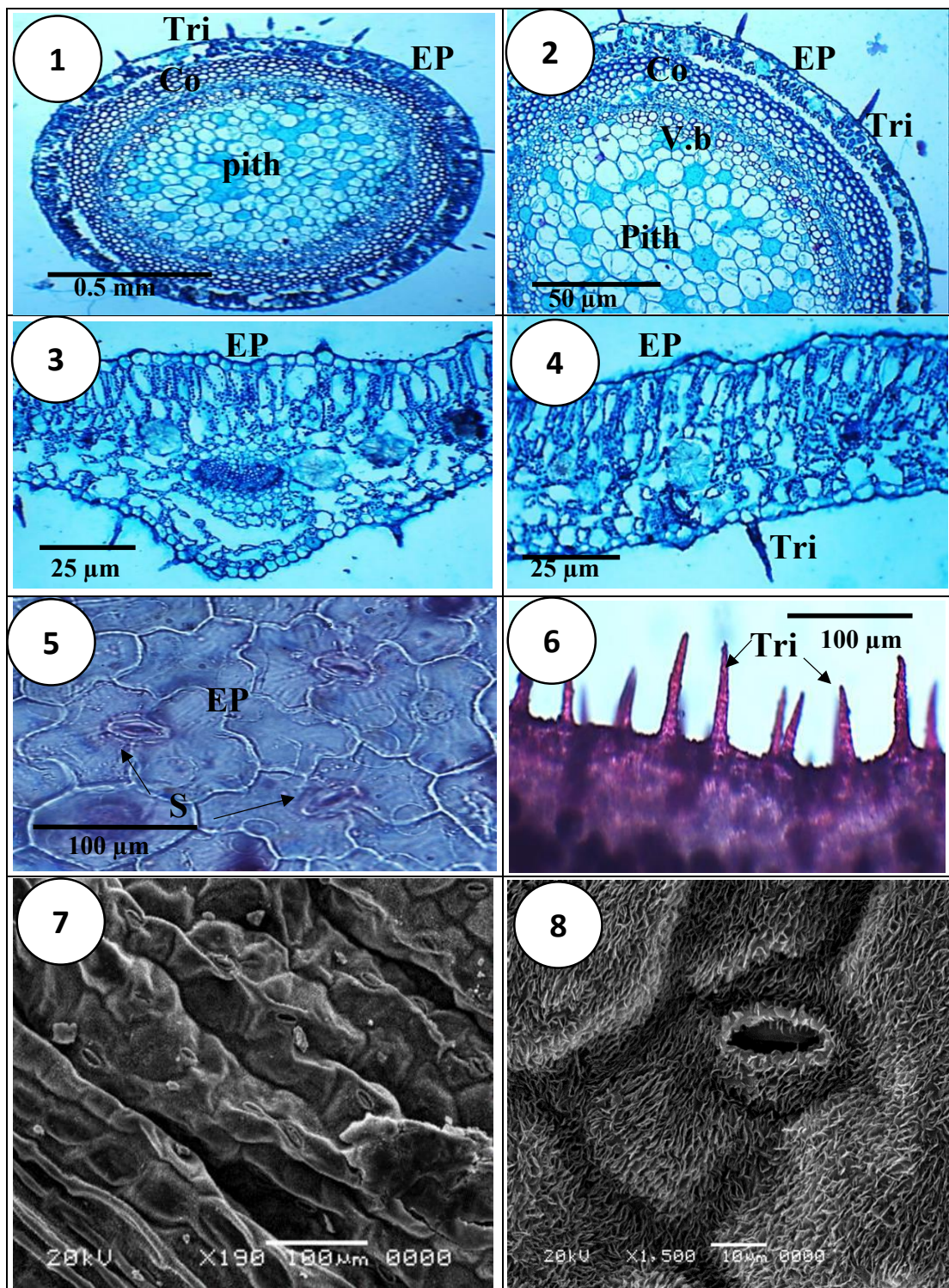


Plate 1. (Figs. 1-8). *Silene leucophylla*: Micrograph stem and leaf anatomy, 1-2: Stem TS; 3 - 4: Leaf TS; 5, 7, 8: Stomata; 6: Trichome on L.M and SEM [Tri= Trichome, EP= Epidermis, Co= Cortex, V.b= Vascular bundles, S= Stomata]

TABLE 2. Anatomical characters of the stem and leaf of the studied taxa.

Characters	Studied taxa			
	<i>S. leucophylla</i>	<i>S. oreosinaica</i>	<i>S. schimperiana</i>	<i>B. multiceps</i>
<b>Stem</b>				
Cross section	Circular	Fusiform	Circular	Rounded
Epidermal cell shape	Uniseriate- oblong	Uniseriate- oblong	Uniseriate- oblong	Uniseriate- oblong
Cortex layers	3-4	5-7	3-4	5-7
Parenchyma layers	6	7-8	8-11	1-2
Vascular bundle No.	Many	6	7	10
Pith	Large	Large	Medium	Medium
Calcium oxalate	Druses	Druses	Druses	Absent
<b>Leaf</b>				
Epidermal cell shape	Uniseriate- cubic	Uniseriate- oblong	Uniseriate- cubic	Uniseriate- oblong
Midrib vascular bundle	Arc- shaped	Ovate	Ovate	Ovate
Calcium oxalate	Druses	Druses	Druses	Druses
Spongy palisad tissue	4-5	4-5	4-5	4-6
Stomata type	Diacytic and anomocytic	Diacytic	Diacytic	Diacytic and anomocytic
Trichomes type	-Unicellular -Unbranched -Non glandular	-Multicellular -Unbranched -Non glandular	-Tiny -Unicellular -Unbranched -Non glandular	-Multicellular -Unicellular -Unbranched -Non glandular

*Silene oreosinaica* Chowdhuri

Stem fusiform in cross section, epidermis uniseriate with oblong cells, Cortex 5–7 layers of thin parenchyma followed by 7-8 layers of pericyclic sclerenchyma tissue. Vascular cylinder consists of 6 collateral vascular bundles, with elliptic shape; each consists of many xylem, phloem elements. Pith contains large thin parenchyma cells. (Plate 2; Figs. 1 & 2). Leaf transection revealed that epidermis uniseriate with oblong cells. Midrib penetrated with small collateral vascular bundle with ovate shape. The bundle consists of few phloem elements, many xylem elements. Midrib ground tissue palisade, thin parenchyma and angular collenchyma. Mesophyll dorsiventral, two continuous palisade layers followed by 4-5 layers of spongy tissue (Plate 2; Figs. 3 & 4). Druses present in leaf and stem (Plate 2; Figs. 2, 3 & 5); epidermal cells with straight walls; stomata diacytic only

(Plate 3; Fig. 1). In SEM stomata semi-depressed and cuticle ultrastructure without epicuticular wax platelets (Plate 3; Figs. 3 & 4). Trichomes multicellular, unbranched, non-glandular (Plate 3; Fig. 2), (Table 2).

*Silene schimperiana* Boiss

Stem outline circular, epidermis uniseriate with oblong cells. Cortex with 3-4 layers of chlorenchyma tissue followed by 8-11 layers of pericyclic fibers. Vascular cylinder consists of 7 collateral vascular bundles arranged in a cycle. Each bundle contains few phloem elements, many xylem elements. pith with thin parenchyma cells (Plate 4; Figs. 1 & 2). Leaf Transection revealed that epidermis uniseriate with cubic, elongated cells. Midrib rounded, penetrated with small collateral vascular bundle with ovate shape. The bundle, consists of few phloem elements, many xylem



elements. Mesophyll dorsiventral, two continuous palisade layers followed by 4-5 layers of spongy tissue. (Plate 4; Figs. 3, 4, 5 & 6). Druses present in leaf and stem. Epidermal cells straight wall; stomata diacytic only (Plate 4; Fig. 7), Trichomes

tiny unicellular, unbranched, non-glandular. (Plate 4; Fig. 8) on SEM stomata semi-depressed with slightly dense epicuticular wax platelets on both epidermal cells and guard cells. Epidermis papillate (Plate 4; Figs. 9 & 10), (Table 2).

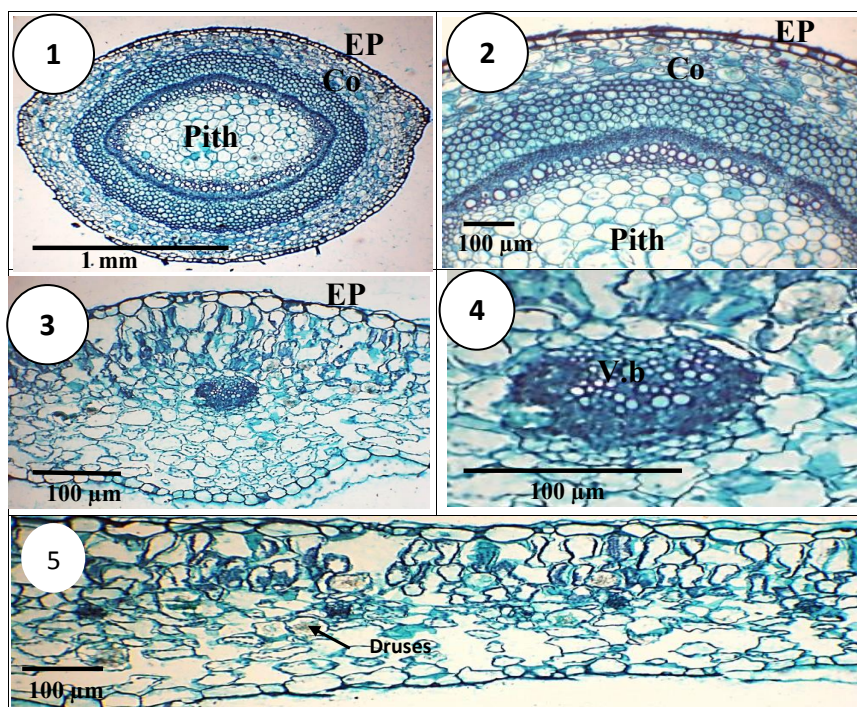


Plate 2. (Figs. 1-5). *Silene oreosinaica*: Micrograph stem and leaf anatomy, 1-2: Stem TS; 3-5: Leaf [EP= Epidermis, Co= Cortex, V.b= Vascular bundles]

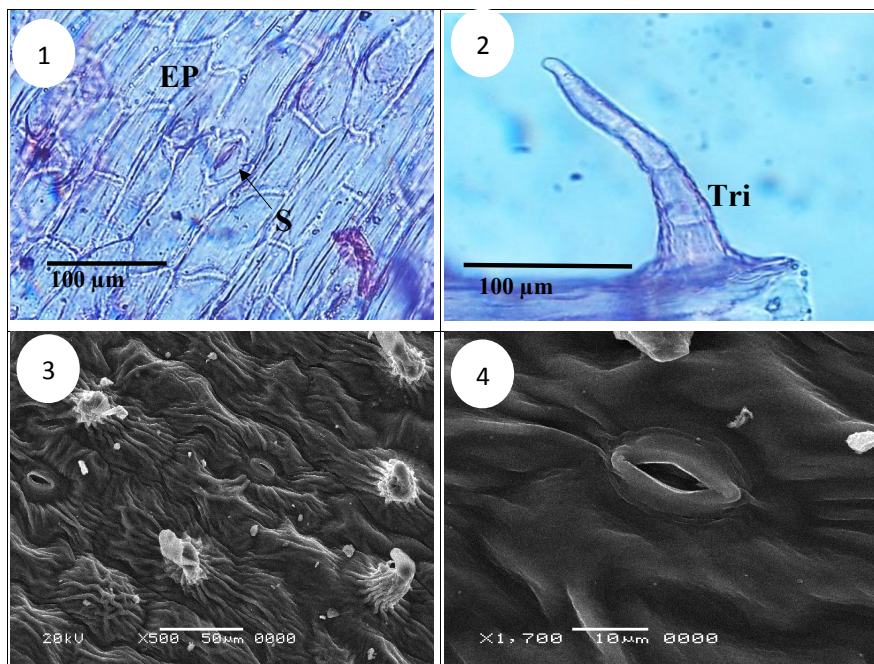


Plate 3. Figs. 1-4. *Silene oreosinaica*, 1: Stomata; 2: Trichome on L.M and 3,4: SEM (Dorsal view of stomata and trichomes) [Tri= Trichome, EP= Epidermis, S= Stomata]



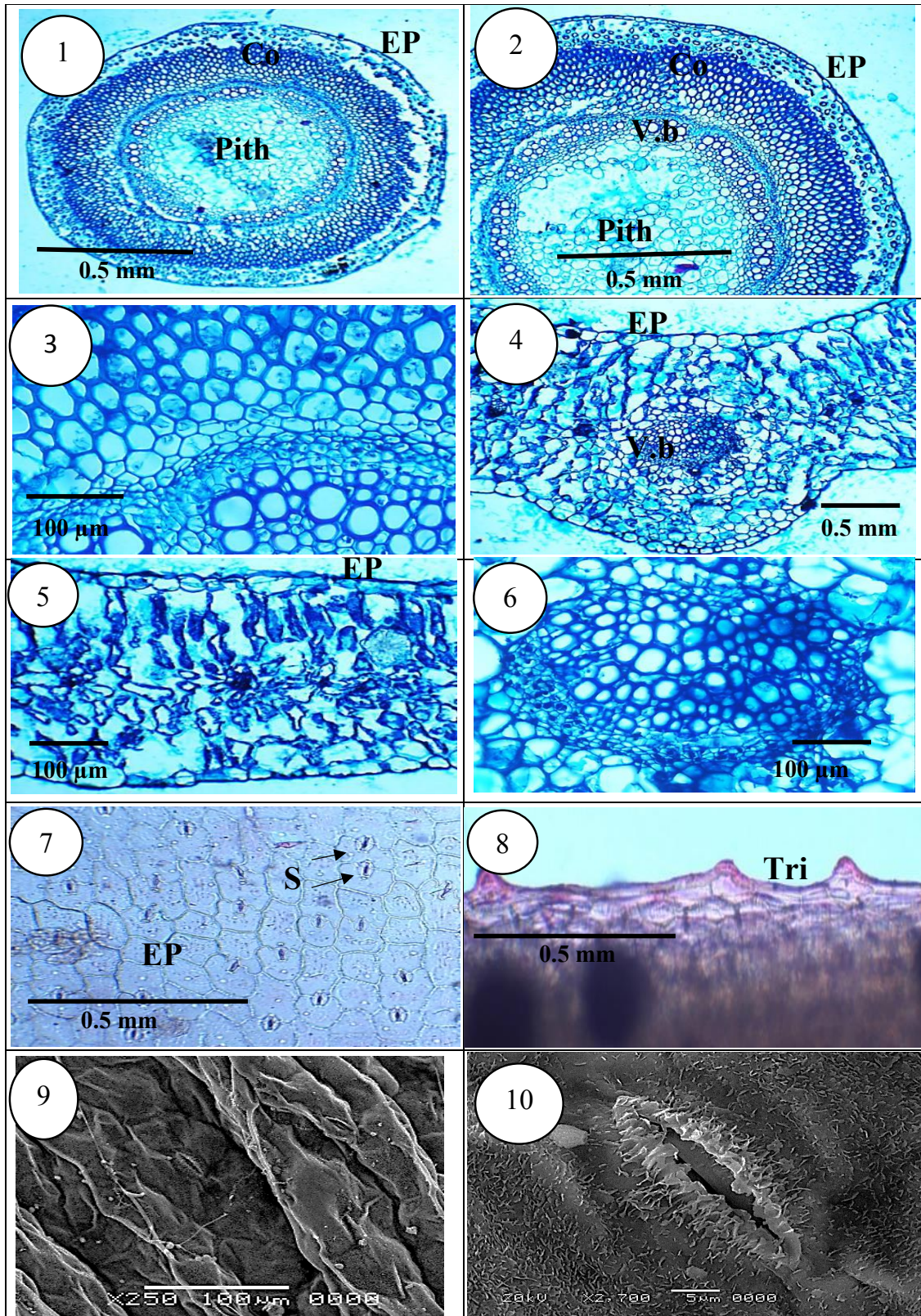


Plate 4. Figs. 1-10. *Silene schimperiana* Micrograph stem and leaf anatomy, 1-2: Stem TS.; 3-6: Leaf TS.; 7: Stomata; 8: Trichome (unicellular) on L.M.; 9-10: SEM. Dorsal view of stomata and trichome [Tri= Trichome, EP= Epidermis, Co= Cortex, V.b= Vascular bundles, S= Stomata]



*Buфонia multiceps* Decne

Stem circular in cross section. Epidermis uniseriate with oblong cells. Cortex consists of 5–7 layers of chlorenchyma. Pericycle of 1-2 discontinuous layers of fibers. Vascular cylinder consists of 10 collateral vascular bundles arranged in a ring. Pith with thin parenchyma cells. Epidermis of leaves uniseriate with oblong, cubic cells in transection. Midrib arc shaped with one large, ovate, collateral vascular bundle with 4 layers of angular collenchyma above and 6 layers below. The vascular bundle consists of few phloem elements and many xylem elements. Mesophyll isobilateral consists of 4 – 6 layers of palisade tissue. (Plate 5; Figs. 1-6). Epidermal cells with undulated wall, Stomata diacytic and anomocytic (Plate 6, Figs. 1 & 3). In SEM stomata semi-depressed and cuticle ultrastructure without epicuticular wax platelets (Plate 6; Figs. 5 & 6). Trichomes unicellular and multicellular, unbranched, glandular and non-glandular. (Plate 6, Figs., 2 & 4) (Table 2).

**Discussion**

The present study aims to investigate anatomical

features of 4 taxa belonging to Caryophyllaceae endemic to St. Catherine, S Sinai, to determine the various types of tissues, trichomes, stomata and epidermal features to evaluate the usefulness of these characters for systematic purposes. The anatomical characteristics in this work give the first detailed description of 3 *Silene* species and *Buфонia multiceps*.

The anatomical structure of the leaves of some *Silene* species showed a significant variation in anatomical structure and the importance of the shape and size of the epidermal cells, hairs and crystals in the separation of species (Keshavarzi et al., 2014). In the studied taxa, stem cross section was mostly circular or fusi form in *S. oreosinaica* with multi-layered parenchymatous cortex, presence of an outer sclerenchyma tissue and an inner pericyclic ring each consisting of at least of two rows of fibers, a large central pith and continuous xylem vessels with variable thickness surrounding the central pith. Stomata were mostly diacytic, although anomocytic stomata were also recorded in *Silene leucophylla* and *Buфонia multiceps*.

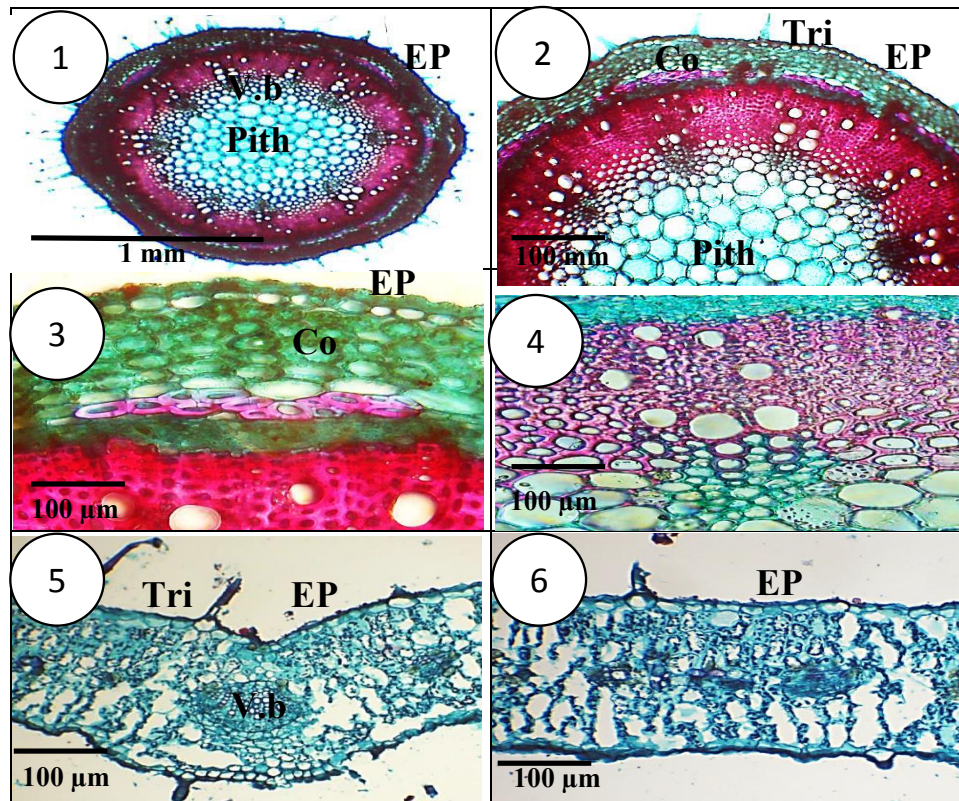


Plate 5. (Figs. 1-6). *Buфонia multiceps* Micrographs of stem and leaf anatomy, 1-2: Stem TS.; 3-4: Cortex and vascular bundles; 5: Lower leaf midrib TS; 6: Mesophyll [Tri= Trichome, EP= Epidermis, Co= Cortex, V.b= Vascular bundles]



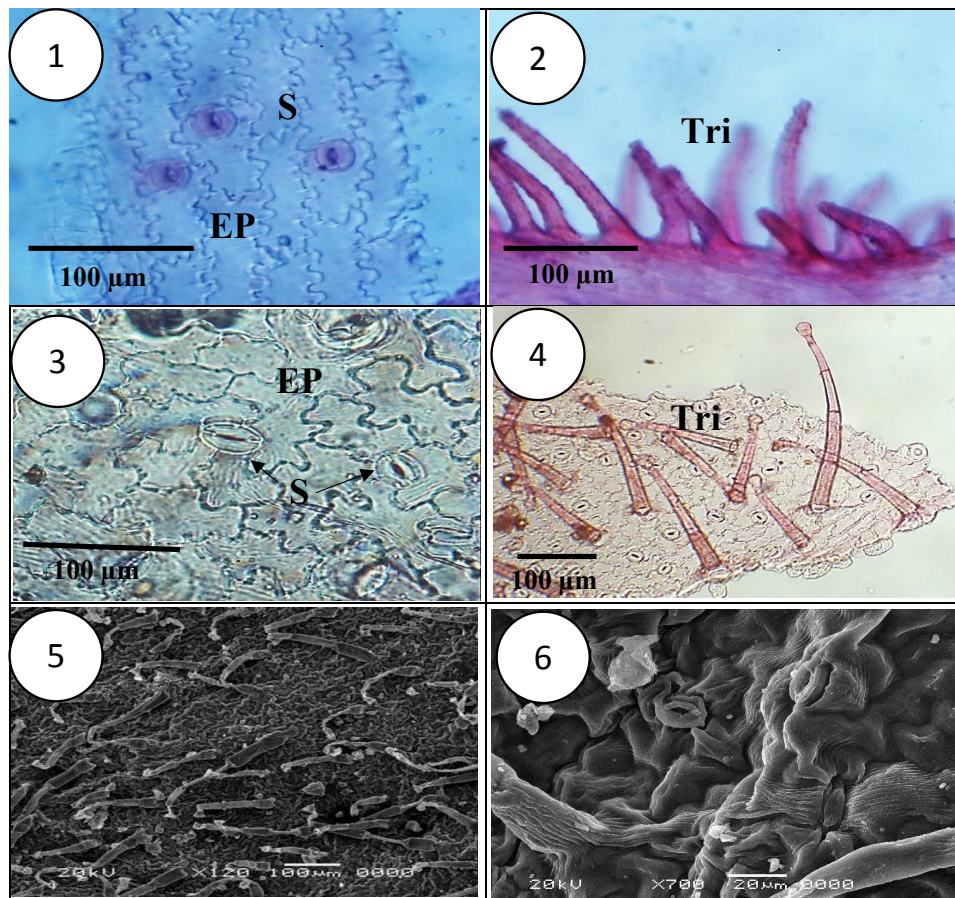


Plate 6. (Figs. 1-6). *Bufofonia multiceps* Surface view of upper and lower leaves, 1-2: LM of stomata and trichomes of the upper leaves; 3-4: Stomata and trichomes of the lower leaves; 5- 6: SEM of stomata and trichomes of the lower leaves [Tri= Trichome, EP= Epidermis, S= Stomata]

Metcalf & Chalk (1950) described several types of trichomes unicellular simple, long or short uniseriate, uniseriate with glandular cell at the apex, and branched in different genera of Caryophyllaceae. In the present study, unicellular and multicellular, unbranched, non-glandular or glandular hairs were recorded in addition to papillae in *Silene schimperiana*. Druses crystals were observed in stem and leaves of studied taxa of *Silene*; while in *Bufofonia multiceps* no druses were recorded although Mousavi et al. (2019) recorded the presence of druses in all studied taxa of *Bufofonia* from Iran. Dense epicuticular wax was observed on epidermal and guard cells of *S. leucophylla* whereas the wax was moderate in *S. schimperiana* and absent in the rest of the studied taxa.

An artificial key is prepared based on anatomical characters for identification purposes as follows:

1. a. Stem circular, epidermis uniseriate with oblong cells; cortex with 3-4 layers of chlorenchyma tissue followed by 6 layers of pericyclic fibers..... *S. leucophylla*
- 1.b. Stem fusiform, epidermis uniseriate with oblong cells; cortex with 5-7 layers of thin parenchyma tissue followed by 7-8 layers of pericyclic sclerenchyma tissue ..... 2
- 2.a. Vascular cylinder consists of 6 collateral vascular bundles, with elliptic shape, each consists of many xylem, phloem elements... .....*S. oreosinaica*
- 2.b. Vascular cylinder consists of 7 collateral vascular bundles, each consists of many xylem elements, few phloem elements. .... 3
3. a. Stomata diacytic only, semi-depressed with slightly dense epicuticular wax platelets,

epidermal cell straight wall.....*S. schimpeiana*

3.b. Stomata diacytic and anomocytic, semi-depressed without epicuticular wax platelets, epidermal cells with undulated wall....*B. multiceps*

### **Conclusion**

The anatomical characteristics of stem and leaf, including epidermal cells, and stomata type were important for the identification and taxonomy of the four endemic species collected from the area of St. Catherine, S Sinai, Egypt. In this analysis, 3 species of *Silene* and one species of *Bufonia multiceps* were assessed. To build a dichotomous key for easy identification of each of the studied taxa, the most accurate stem and leaf anatomical attributes have been used.

*Acknowledgement:* We would like to express our gratitude to Prof. Dr. Hasnaa A. Hosni, The Herbarium, Faculty of Science, Cairo University for revision and valuable discussions in this manuscript.

*Conflict of interest:* The authors reported no potential conflict of interest.

*Authors contribution:* This work was carried out in collaboration between all authors. Abbas A. El-Ghamery and Ahmed, M. Sadek conceived of the presented idea. Ahmed, M. Sadek and Ali Gaafar performed the measurements and processed the experimental data. Abbas A. El-Ghamery and Ali Gaafar discussed the results and contributed to the final manuscript.

*Ethical approval:* Not applicable.

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## ملاحظات تشريحية لبعض أنواع السيلين والعدمة الفصيلة القرنفلية ، المتوطنة في سيناء ، مصر

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تمت دراسة الصفات التشريحية للساق والأوراق على أربعة أنواع متوطنة تم جمعها من منطقة سانت كاترين، جنوب سيناء، مصر. وهي: السيلين ليكوفيليا أو ذو الأوراق البيضاء (*Silene leucophylla* Boiss.)، السيلين أوريسينيكيا أو سيلين جبال سيناء (*S. oreosinica*)، والليصيق أو الوسبية (*S. schimperiana*) والعدمة أو الحلاو (*Bufonia multiceps*) والتي يتم تقديمها لأول مرة. أجريت دراسة مقارنة باستخدام كل من المجهر الضوئي (LM) والمجهر الإلكتروني الماسح (SEM)، وبناءً على التركيب التشريحي للورقة والساق. ظهرت البلورات النجمية أو الوردية بكثرة في كل من أنسجة الأوراق والساق في أنواع السيلين المدروسة، ولكنها غير موجودة في جنس العدمة (*Bufonia multiceps*) كانت الثغور في الغالب من النوع العمودي أو تم تسجيل كلاهما عمودي وشاذ. وكان منظر سطح خلايا البشرة مستقيماً أو متموجاً. كذلك لوحظ وجود صفائح شمعية على خلايا البشرة والخلايا الحارسة في الليصيق أو الوسبية (*S. schimperiana*) والسيلين ليكوفيليا (*S. leucophylla*) ولقد تم وصف كل من الصفات التشريحية لبشرة الأوراق والساق المميزة لكل نوع. وتم عمل مفتاح اصطناعي لتحديد الأنواع المدروسة.