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Full Length Article

Authentication, Micromorphology and Ultrastructure of Pollen Grains and Seeds of Endemic Taxa in Saint Katherine Protectorate, South Sinai, Egypt

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Abstract

The endemic taxa were restricted to a specific geographic region and they are essential for setting conservation priorities. This study aimed to update the endemic taxa list in Saint Katherine Protectorate (SKP) depending on literature reviews, field trips and herbaria consultation. Other characters also recorded *viz*, sex forms, dispersal types and flowering time. Also, the morphological characters of the pollen grains and seeds were examined and photographed using light microscope (LM) and scanning electron microscope (SEM). In addition, the mineral composition of pollens and seeds was detected using energy dispersive X-ray (EDX). The updated list included 13 taxa belonging to 11 genera and 8 families. All the recorded taxa were bisexual; ballochores were the most represented dispersal type. There was a gradual increase in the endemic taxa from March to August while decreasing from October to February. Pollens were isopolar and medium in size. They possessed colpate, colporate, or porate aperatus, as well as reticulate exine sculpture. Furthermore, operculum and margo were absent in most of the pollens. The seed colour ranged from light brown to black; elliptic; basal hilum; polygonal and irregular-shaped seeds were the most represented. All previous characters were diagnosed at generic and specific levels, which helped in the construction of artificial keys to facilitate the differentiation between the studied taxa. The present study has the priority in describing pollens and seeds of *Astragalus fresenii* and *Micromeria serbaliana*, in addition to the description of the seeds of *Ballota kaiseri*. The presence and percentage of twelve elements detected by EDX differed significantly within the investigated pollen grains and seeds. The present data indicated that pollen grains and seeds of studied taxa had high percentages of carbon, oxygen, phosphorous, magnesium, nitrogen and calcium. This study is the first attempt using EDX technique with these taxa.

Keywords*:* Authentication; EDX; Endemic; Pollen morphology; Saint Katherine; Seed morphology

Introduction

The endemic taxa inhabit particular habitats restricted to a specific area due to factors such as isolation, climatic changes or urban development. Such species were often endangered, so it is essential to conserve them (Brooks *et al.* 2006; Lima *et al*. 2020). Egypt is divided into four geographic regions; Nile, Western Desert, Eastern Desert and Sinai Peninsula. The latter possesses a unique triangular shape and all the geologic formations, structures, and landforms of Egypt are nearly represented in it. Moreover, Saint Katherine Protectorate (SKP) encloses most of the mountainous area of South Sinai and occupies about 4350 km^2 (Embabi 2018).

The SKP is extremely arid with long, hot, rainless summers and cold, rainy winters and lies in the low rain belt of Egypt with an annual rainfall of 57 mm/year. However, its high mountains receive higher amounts of precipitation (100 mm/year) as rain and sometimes snow. Nonetheless, rainfall is not an annual characteristic rather two to three consecutive years without rainfall is common. Rain takes the form of sporadic flash floods or limited local showers; thus, high spatial heterogeneity in receiving moisture is also common (Ibraheem 2012).

Danin (1986), reported 28 endemic taxa in Sinai, 25 of them occur in the mountainous district. Täckholm (1974) reported 34 taxa restricted to Sinai out of 69 endemic taxa in Egypt. According to El-Hadidi and Fayed (1994/1995), 27 taxa endemic to South Sinai were reported, while El-Hadidi and Hosni (2000) recorded 30

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endemic taxa to Sinai as a whole. Boulos (2009) reported 20 endemic taxa in SKP out of 31 taxa in Sinai. Hosni *et al*. (2013) reported 17 taxa in SKP out of 27 taxa in Sinai. Zahran *et al*. (2015) reported 17 endemic taxa in SKP out of 28 taxa in Sinai. At the same time, Abdelaal *et al*. (2018) reported 14 taxa in SKP out of 26 taxa in Sinai. Generally, the endemic taxa in SKP are growing in four habitats; Wadi bed, gorge, slope and basin. The gorge habitat is the most suitable for the growth of endemic taxa, while the basin is the lowest (Zahran *et al.* 2015).

Palynology is a fascinating science with a multidimensional approach covering almost botanical science branches and is considered a helpful tool in taxonomic and evolutionary studies. Many successful trials taken pollen characters as a basis for classification (Erdtman 1952; Takhtajan 1980). Pollen morphology helped botanists and ecologists to reconstruct the past assemblages of plants and identify periods of environmental change (Krzywinski *et al.* 1989; Moore *et al.* 1991; Yao *et al.* 2017).

Seed morphological characters are considered a powerful tool for the classification of various plant groups. These characters, especially the seed shape and details of the outer seed coat's sculpturing can be quite variable and important compared to other organs, to cite but a few, we can refer to (Barthlott 1981, 1984; Svetlova 2008; Lomonosova 2009; Kaya *et al.* 2016). This is because the seed and fruit characteristics are less affected by changing the environmental conditions (Zoric *et al.* 2010).

The research studies on the morphology of pollen grains and seeds of endemic taxa in Saint Katherine are few. There is no previous complete data available, despite that we can refer to some studies dealt with these taxa either in the context of the endemic flora of Egypt or as separate taxa within special groups (Shehata and Kamel 2007; Rabei *et al.* 2016; El-Ghamery *et al.* 2018; Shiha 2020).

Energy descriptive X-ray spectroscopy (EDX) provides a quantitative and qualitative element analysis of the samples. One advantage of this technique is that tiny specimens (such as pollen grains or seeds) can be analyzed under electron beam control. EDX depends on the type of X-ray called the characteristic X-ray; produced from samples due to the electron beam interaction. The released X-rays exhibit a pattern of peaks at energy corresponding to the element. An EDX analysis system combined with a SEM allows investigating of the elemental composition of biological samples. The elemental composition of pollen can be measured without destroying the organic matrix (Lott *et al.* 1982; Lott 1984; Ockenden and Lott 1988). Pocock and Vasanthy (1986) think that elements detected in the pollen grains with EDX depend on the percentage of the component in the soil where the plants were grown. Wolter and Nilsson (1990) used the EDX investigations on the pollen of some taxa belonging to Apocynaceae family for systematic purposes. Rehman *et al.* (2008) used EDX to detect the potassium accumulation in the sulcus area in pollen of *Pinus densiflora*. The present study is the first attempt to use this technique with these taxa.

Most of the endemic taxa have become closer to extinction within the last years due to environmental conditions and human activities. Hence, the goal of the present study was to share the knowledge of endemic taxa in SKP including investigation of pollen grains and seeds morphological characters (macro- and micro-characters), and the element detection by using EDX, constructing artificial keys based on the most prominent characters for identifying and discriminating between the studied taxa.

Materials and Methods

Field trips

Twenty field trips were conducted from summer 2017 to spring 2020 to SKP for collecting the studied taxa as fresh materials from their natural habitats (Fig. 1). Regarding *Astragalus fresenii*, we depended on a herbarium specimen from Cairo University Herbarium (CAI). Samples of flowers and seeds were collected for laboratory investigations. Identification and synonyms followed Täckholm (1974), Boulos (1999-2005, 2009) and International Plant Names Index (http://www.ipni.org). The collected taxa were compared with holotypes preserved at different herbaria in Egypt. The affiliation of taxa to their families followed APG (2016). Voucher specimens were preserved in the herbarium of Botany and Microbiology Department, Faculty of Science, Kafrelsheikh University and the herbarium of Botany Department, Faculty of Science, Tanta University (TANE), Egypt.

Authentification and verification of the endemic taxa in SKP were carried out according to: Täckholm and Täckholm (1941); Täckholm and Drar (1950-1969); Täckholm (1974); Boulos (1999-2005, 2009); El-Hadidi and Hosni (2000); Taifour and El-Oqlah (2014); Ibrahim *et al*. (2016); El-Khalafy (2018); Shaltout *et al.* (2018) and Abdelaal *et al.* (2018). Some websites were consulted to collect more information about the recorded taxa (Table 1). Gaps of information collected from the herbaria of Cairo University (CAI), Ain Shams University (CAIA), Agriculture Museum (CAIM), Tanta University (TANE), Assiut University (ASTU), Aswan University (ASW) and Alexandria University (ALEX).

Sex form, flowering time and dispersal types

Sex forms were determined directly from the flowers in the field. Flowering time was determined according to field observations and data from preserved herbarium sheets. Dispersal types were recorded according to description of dispersal units depending on the available diaspores and the studies of Täckholm and Täckholm (1941); Täckholm and Drar (1950-1969) and Boulos (1999-2005).

Table 1: Global online databases used for authentication of the endemic taxa in the SKP

No.	Database	Source
1	International Plant Names Index (IPNI)	http://www.ipni.org
2	The Plant List (TPL)	http://www.theplantlist.org
3	Plants of the World Online (POWO)	http://www.plantsoftheworldonline.org
4	Catalogue of Life	http://www.catalogueoflife.org/annual-checklist/2010
5	Tropicos	http://www.tropicos.org
6	Global Biodiversity Information Facility (GBIF)	http://www.gbif.org/occurence
7	JSTOR Global Plants	http://plants.jstor.org
8	Euro+Med PlantBase	http://ww2.bgbm.org/EuroPlusMed/query.asp
9	Kew World Checklist of Selected Plant Families (WCSP)	http://wcsp.science.kew.org
10	King Saud University	http://faculty.ksu.edu.sa
11	Asian Flora Database	http://www.asianflora.com
12	Flora of Israel Online	http://flora.org.il
13	India Biodiversity Portal	https://indiabiodiversity.org/species/show/245963

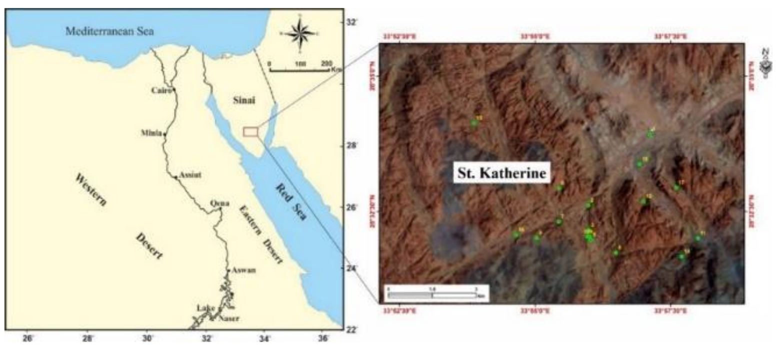

Fig. 1: Map of Saint Katharine Protectorate showing the visiting sites

Pollen grain morphology

Flowers of the studied taxa were fixed in 70% ethanol. For LM investigation, mature anthers from the collected flowers were left to dry, carefully opened using sharp needles and sputtered onto glass slides. The pollen grains (five to ten pollen grains per taxa) were examined, measured and photographed using Canon power-shot A470, 7.1 megapixels. The polar axis (P) and equatorial diameter (E) were measured, pollen size, shape and aperture type were also assessed. For SEM investigation, non-acetolyzed pollen grains were transferred onto a metallic stub using a double-sided cellotape and coated with a thin layer of gold in a sputtering chamber, then scanned and photographed using JEOL JSM-IT100 SEM for exine and aperture ornamentations. The terminology used for describing pollen grains morphology was followed as given by Erdtman (1952); Punt *et al.* (2007) and Hesse *et al.* (2009).

Seed morphology

Mature seeds (five to ten seeds from each taxon) were examined using stereo-microscope and photographed using Canon power-shot A470, 7.1 mega pixels digital camera. Stage micrometer in addition to ImageJ software was used for seed measurements and calibration. Seed color, length and width, as well as hilum position were studied by stereomicroscope. For SEM investigation, the mature seeds were mounted onto SM stubs, coated with gold and examined and photographed using JEOL JSM-IT100 SEM. In *Rosa arabica* the achene must be opened and its wall shed off to allow the seed's appearance. Terminology was followed as given by Barthlott (1981) and Stearn (1992). The SEM photographs were carried out in the Institute of Nanoscience and Nanotechnology, Kafrelsheikh University.

Energy descriptive X- ray technology (EDX)

The non-acetolyzed pollen grains and mature seeds were placed in a JEOL JSM-IT100 scanning electron microscope equipped with an EDX detector at the Institute of Nanoscience and Nanotechnology, Kafrelsheikh University and coated with a very thin film of gold. The beam was focused on pollen and seed at 20 KV and analyzed for approximately 20 seconds and the machine dead time was not included. The count rate per second (CPS) reached 1845.

Results

Recorded taxa

Thirteen endemic taxa (11 species, one subspecies and one variety) belonging to 11 genera and eight dicotyledonous families were recorded. Caryophyllaceae was the most represented family (four taxa), followed by Lamiaceae (three taxa). *Silene* was the most represented genus (three taxa), while the remaining genera were represented by only

Table 2: Collection data, dispersal type and flowering time of the studied taxa in SKP

No.	Taxa	Family	Dispersal Type	Flowering time
1	***Anarrhinum pubescens*** Fresen	Scrophulariaceae	Ballochore	March - May
	= *Anarrhinum duriminium* (Brot.) Pers			
	= *Anarrhinum forsskaohlii* (J. F. Gmel.) Cufod. subsp. *pubescens* (Fresen.) D. A. Sutton			
	=*Anarrhinum orientale* Benth. var. *pubescens* (Fresen.) Rouy			
	=*Cardiotheca pubescens* (Fresen.) Ehrenb. ex Steud.			
	=*Simbuleta pubescens* Kuntze			
2	***Astragalus fresenii*** Decne	Fabaceae	Pogonochore	March- May
	=*Tragacantha fresenii* (Decne.) Kuntze			
3	***Ballota kaiseri*** Täckh	Lamiaceae	Microsclerochore	March-May
4	***Buffonia multiceps*** Decne	Caryophyllaceae	Ballochore	March - June
5	***Hyoscyamus boveanus*** (Dunal) Asch. & Schweinf	Solanaceae	Ballochore	March - June
	= *Scopolia boveana* Dunal			
6	***Micromeria serbaliana*** Danin & Hedge	Lamiaceae	Microsclerochore	April- August
	=*Satureja serbaliana* (Danin & Hedge) Greuter & Burdet			
7	***Origanum syriacum* L. subsp. *sinaicum*** (Boiss.) Greuter & Burdet	Lamiaceae	Microsclerochore	April- October
	= *Origanum maru* L. var. *sinaicum* Boiss.			
	= *Majorana nervosa* Benth.			
	= *Origanum syriacum* var. *sinaicum* (Boiss.) Ietsw.			
	=*Origanum nervosum* (Benth.) Vogel			
	=*Majorana syriaca* (L.) Raf., *nom. illegit.*			
8	***Polygala sinaica*** Botsch. **var. *sinaica***	Polygalaceae	Pogonochore	April- August
	= *Polygala spinescens* Decne			
9	***Primula boveana*** Decne. ex Duby	Primulaceae	Ballochore	March - June
	= *Primula verticillata* subsp. *boveana* (Decne.) W.W. Sm. & Forrest			
	=*Primula verticillata* Forssk.			
10	***Rosa arabica*** Crép.	Rosaceae	Ballochore	June- August
	= *Rosa rubiginosa* var. *arabica* (Crepin) Boiss			
	=*Rosa rubiginosa* L.			
	=*Rosa agrestis* Savi			
11	***Silene leucophylla*** Boiss	Caryophyllaceae	Ballochore	May- June
12	***Silene oreosinaica*** Chowdhuri	Caryophyllaceae	Ballochore	March- April
	=*Silene sinaica* Boiss			
13	***Silene schimperiana*** Boiss	Caryophyllaceae	Ballochore	March- August

one taxon (Table 2). All the studied taxa were bisexual (hermaphrodites). Ballochore (seven taxa = 53.8% of the total taxa) was the most represented dispersal type, followed by microsclerochore (three taxa = 23.1%) and pogonochore (two taxa = 15.4%). There was a gradual increase in the frequency of flowered taxa from March till reaching a maximum in April and May (12 taxa = 92.3%). In general, the period from March to May was characterized by the highest, while the period from September to February was characterized by the lowest flowering activity (Table 2).

Pollen morphology

The examined pollen grains showed considerable variations in their characteristics as they represented different genera and families. The polar length ranged from 14.72 to 44.51 µm, while the equatorial length ranged from 9.31 to 45.04 µm. In general, medium size was the most represented. Most of the pollen grains were isopolar. The apertures were colpate, colporate or porate. Operculum and margo were absent in most of the studied taxa. Eight types of exine sculpture were described; reticulate type was the most represented (four taxa). The annulus was absent in most of the studied taxa. The pollen morphological characters (LM and SEM) were summarized in Table 3, and some of the specific structures (micro-photographs) were arranged and illustrated in Fig. 2. So far as the data of the present work are concerned, the subsequent artificial key based on the pollen morphological characters is provided to enable the different endemic taxa to SKP to be distinguished.

Distinguishing features	Character/taxa
1a. Apolar pantoporate pollen	2
1b. Isopolar colpate or colporate	5
2a. Operculum present	3
2b. Operculum absent	4
3a. aculeate exine sculpture	*Buffonia multiceps*
3b. Foveate- micropapilate exine sculpture	*Silene oreosinaica*
4a. 25- 30 pore/ grain, tectate	*Silene leucophylla*
4b. 15- 20 pore/ grain, tectum indistinct	*Silene schimperiana*
5a. Colpate	6
5b. Colporate	9
6a. Stephanocoplate (22-27) pore/pollen, psilate exine sculpture	*Polygala sinaica*
6b. Tri- or hexacolpate	7
7a. Tricolpate, reticulate perforate pollen	*Ballota kaiseri*
7b. Hexacolpate	8
8a. Medium- sized, prolate grain	*Micromeria serbaliana*
8b. Small- sized, subprolate grain	*Origanum syriacum* subsp. *sinaicum*
9a. Subprolate, reticulate exine sculpture	*Astragalus fresenii*
9b. Prolate or prolate- spheroidal pollen	10
10a. Prolate, reticulate- foveolate exine sculpture	*Anarrhinum pubescens*
10b. Prolate- spheroidal pollen	11
11a. Large- sized pollen, striate- perforate exine sculpture	*Hyoscyamus boveanus*
11b. Medium- sized pollen	12
12a. Reticulate exine sculpture	*Primula boveana*
12b. Striate exine sculpture	*Rosa arabica*

Seed morphology

The examined seeds showed considerable variations in their characteristics as they represented different genera and families. The color of the seeds ranged from light brown to black. Seven shapes of seeds were described; elliptic type was the most represented (four taxa). The hilum position for most taxa was basal, while its level may be raised, flat,

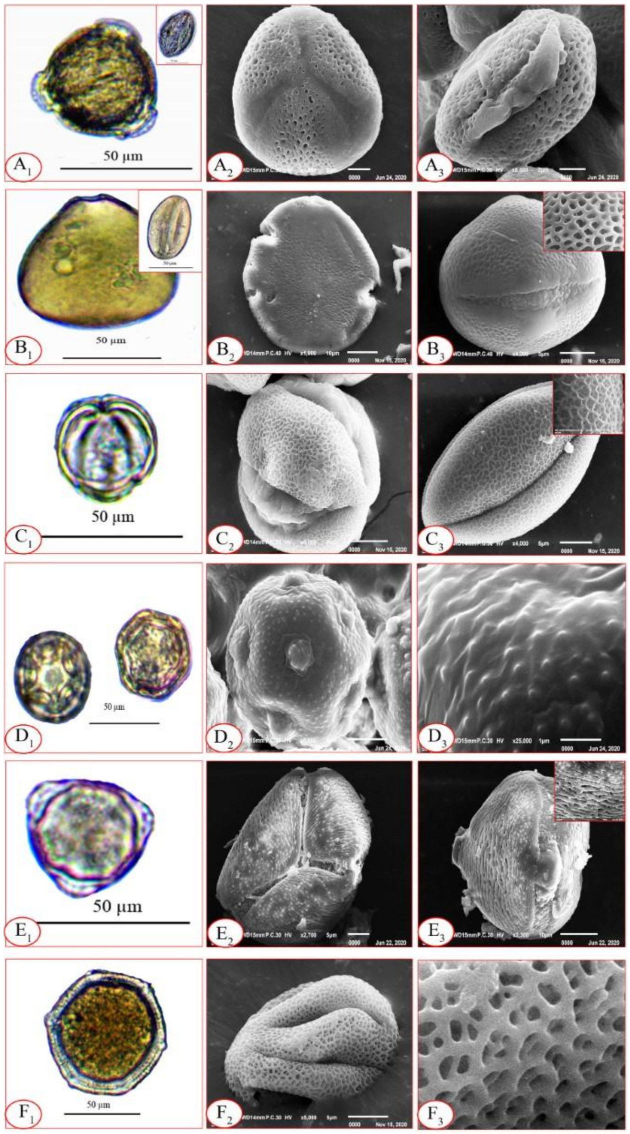

Fig. 2: A-F. Micro-photographs of pollen grains of the studies taxa (LM & SEM). (A) *Anarrhinum pubescens*; prolate, trizonocolporate, semi-tectate, reticulate- foveolate sculpture, (B) *Astragalus fresenii*; subprolate, trizonocolporate, tectate, reticulate-sculpture, (C) *Ballota kaiseri*; prolate, trizonocolpate, tectate, reticulate- perforate sculpture, (D) *Buffonia multiceps*; prolate-spheroidal, pantoporate, semi-tectate, distinct operculum, aculeate sculpture, (E) *Hyoscyamus boveanus*; prolate-spheroidal, trizonocolporate, striate- perforate sculpture, (F) *Micromeria serbaliana*; prolate, hexazonocolpate, tectate, reticulate sculpture

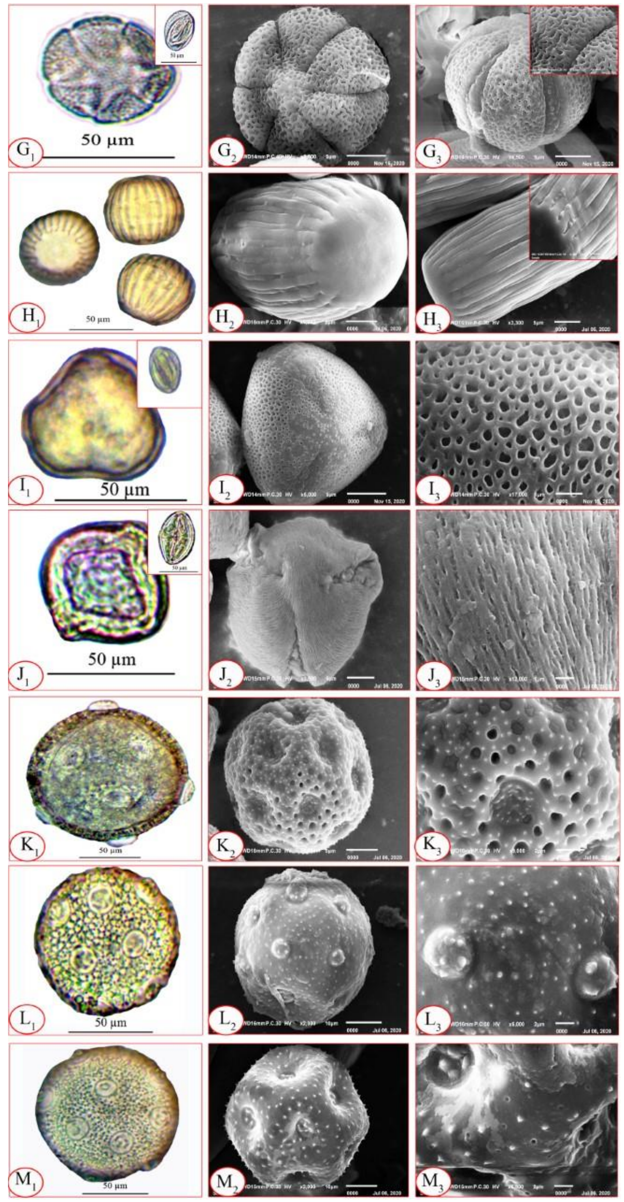

Fig. 2: Cont. G-M. Micro-photographs of pollen grains of the studies taxa (LM & SEM). (G) *Origanum syriacum* subsp. *sinaicum*; subprolate, hexazonocolpate, tectate, reticulate sculpture, (H) *Polygala sinaica*; prolate, stephanozonocolpate, psilate sculpture, (I) *Primula boveana*; prolate-spheroidal, trizonocolporate, tectate, reticulate sculpture, (J) *Rosa arabica*; prolate-spheroidal, trizonocolporate, semi-tectate, striate sculpture, (K) *Silene leucophylla*; prolate-spheroidal, pantoporate, tectate, foveate- micropapilate sculpture, (L) *S. oreosinaica*; prolate-spheroidal, pantoporate, semi-tectate, distinct operculum, foveate- micropapilate sculpture, (M) *S. schimperiana*; prolate-spheroidal, pantoporate, foveate- micropapilate sculpture

depressed or semi-depressed. Eleven different types of surface sculpture patterns were described. Four types of epidermal cell shapes were described; polygonal and irregular shapes were the most represented. The anticlinal wall may be raised or depressed; its shape may be striate, sinuate or undulate. The seed morphological characters (LM and SEM) were summarized in Table 4 and some of the specific structures (micro-photographs) were arranged and illustrated in Fig. 3. The seed morphology (macro- or micro-characters) is an additional tool to taxa delimitation as they have a diagnostic value and sometimes the seed characters alone are satisfactory, so an artificial key based on the most

Table 3: Quantitative and qualitative pollen grains micro-morphological characteristics of the studied taxa (LM & SEM)

Attributes	Dimension (µm)		Pollen	Pollen shape		Polarity	Aperture				Exine sculpture	Tectum	Annulus	Collumella visibility
Taxa	Polar = length (P)	Epuatorial = width (E)	size (P+E / 2)	Amb (LM)	Shape class (P/E X 100)		Shape	Number	Operculum	Margo				
1	14.173-16.262 (14.717)	8.876- 9.756 (9.3115)	Small	Triangular	Prolate	Isopolar	Zonocolporate (elliptic)	3	Absent	Present	Reticulate-foveolate	Semi-tectate	Absent	Indistinct
2	22.508- 23.409 (22.958)	17.26-18.181 (17.720)	Medium	//	Subprolate	//	//	//	//	//	Reticulate	Tectate	//	Distinct
3	30.854-32.747 (31.8)	17.113-19.576 (18.344)	//	//	Prolate	//	Zonocolpate (slit- like)	//	//	Absent	Reticulate-perforate	//	//	//
4	18.523-19.436 (18.979)	17.642-18.612 (18.127)	//	Circular	Prolate-spheroidal	Apolar	Pantoporate (circular)	10- 15	Present	//	Aculeate	Semi-tectate	Present	Indistinct
5	37.141- 39.26 (38.2)	33.02- 35.056 (34.038)	Large	Triangular	//	Isopolar	Zonocolporate (elliptic)	3	Absent	//	Striate- perforate	Indistinct	Absent	//
6	24.472-25.380 (24.9)	16.521-17.320 (16.9)	Medium	Hexagonal	Prolate	//	Zonocolpate (slit- like)	6	//	//	Reticulate	Tectate	//	Distinct
7	14.620-15.590 (15.105)	11.785-12.820 (12.3)	Small	Circular-hexagonal	Subprolate	//	//	//	//	//	//	//	//	//
8	34.897-37.562 (36.229)	18.114-21.041 (19.577)	Large	Semi-circular	Prolate	//	//	22- 27	//	//	Psilate	Indistinct	//	Indistinct
9	16.112-18.928 (17.52)	15.125-17.963 (16.544)	Medium	Triangular	Prolate-spheroidal	//	Zonocolporate (elliptic)	3	//	//	Reticulate	Tectate	//	Distinct
10	24.851-25.431 (25.141)	23.565-24.162 (23.863)	//	Semi-circular	//	//	//	//	//	//	Striate	Semi-tectate	//	Indistinct
11	23.165-24.080 (23.622)	22.541-24.323 (23.432)	//	Circular	//	Apolar	Pantoporate (circular)	25- 30	//	//	Foveate-micropapilate	Tectate	Present	Distinct
12	38.564-39.377 (38.970)	37.641-38.392 (38.016)	Large	//	//	//	//	20- 25	Present	//	//	Semi-tectate	//	Indistinct
13	43.652-45.375 (44.513)	44.748-45.338 (45.043)	//	//	//	//	//	15- 20	Absent	//	//	Indistinct	//	//

(//) = as pervious; E= equatorial diameter or width; P= polar axis or length, LM: Light microscope, SEM: Scanning electron microscope.

Table 4: Quantitative and qualitative seed micro-morphological characteristics of the studied taxa (LM & SEM)

Attributes	Color	Shape	Dimension	Hilum		Surface sculpture	Epidermal cell	Anticlinal wall				Periclinal wall	
Taxa			(L x W) mm	Position	Level	pattern	shape	Elevation	Width	Shape	Surface sculpture	Elevation	Surface sculpture
1	Dark brown	Elliptic	1.5- 1.8 X 0.8 – 1	Sub-basal	Semi-depressed	Tuberculate-ruminate	Polygonal	Raised	Narrow	Straight	Smooth	Depressed	Smooth
2	//	//	1.9- 2.1 X 0.8-1	Basal	Flat	Reticulate	//	//	Wide	//	Tuberculate	//	Obscure
3	Brown	Oblong ovate	0.6- 0.8 X 0.3-0.5	//	//	Ruminate	Irregular	Depressed	Narrow	Sinuate	Obscure	Raised	Striate to microreticulate
4	Light brown	Orbicular	0.6- 0.8 X 0.5-0.6	//	Depressed	Scalariform	Isodiametric	Raised	Wide	Straight	Smooth	Depressed	Smooth
5	Black	Obovate to orbicular	1- 1.5 X 0.9- 1.3	//	Flat	Cerebelloid	Polygonal	//	//	Sinuate	//	//	Glebulate
6	Light brown	Oblong ovate	0.4- 0.8 X 0.1-0.3	//	Raised	Rugose	Irregular	//	//	Straight	Ribbed	//	Smooth
7	Yellowish brown	Subglobose	0.9- 1.3 X 0.8-1.2	//	//	Ruminate	//	//	//	Undulate	Smooth	//	//
8	Black	Elliptic	2.9- 3.9 X 0.5-1.1	Sub-basal	//	Reticulate, hairy	Polygonal	Depressed	Narrow	Straight	Obscure	Raised	//
9	//	Cuboid	0.6- 0.8 X 0.4-0.8	//	Flat	Reticulate - verrucate	Polygonal	Raised	//	//	Smooth	Depressed	Verrucate
10	Light brown	Elliptic	1.8- 4 X 1.6- 1.8	Basal	Raised	Reticulate-scalariform	//	//	Wide	//	//	//	Smooth
11	Dark brown	Reniform to elliptic	2.9- 3.5 X 1.6-1.9	//	//	Colliculate	Elongated polygonal	Depressed	Narrow	Sinuate	Smooth	Raised	Granulate
12	//	//	1.5- 1.8 X 1.1-1.4	//	Depressed	Aculeate- verrucate	Irregular	//	//	Undulate	//	//	//
13	Light brown	//	1.1- 1.5 X 1- 1.4	//	//	Colliculate	Elongated polygonal	//	//	Sinuate	//	//	//

(//) = as pervious; (L)= length; (W)= width. LM: Light microscope, SEM: Scanning electron microscope

obvious seed characters can be used as a confirmatory key that ensure the identification of the studied taxa as follow:

Distinguishing features	Character/taxa
1a. Black colored seed	2
1b. Brownish colored	4
2a. Seed covered with long hairs	*Polygala sinaica*
2b. Seed glabrous	3
3a. Obovate to orbicular shape, cerebelloid surface sculpture	*Hyoscyamus boveanus*
3b. Cuboid shape, reticulate- verrucate surface sculpture	*Primula boveana*
4a. Reniform shape	5
4b. Not as above	7
5a. Aculeate- verrucate surface sculpture	*Silene oreosinaica*
5b. Colliculate surface sculpture	6
6a. Dark brown seed	*Silene leucophylla*
6b. Light brown	*Silene schimperiana*
7a. Subglobose shape, yellowish brown, ruminate surface sculpture	*Origanum syriacum* subsp. *sinaicum*
7b. Ovate, oblong ovate, orbicular or elliptic shape	8
8a. Orbicular shape, light brown color, scalariform surface sculpture	*Buffonia multiceps*
8b. Oblong ovate or elliptic shape	9
9a. Oblong ovate shape	10
9b. Elliptic shape	11
10a. Rugose surface sculpture	*Micromeria serbaliana*
10b. Ruminate surface sculpture	*Ballota kaiseri*
11a. Tuberculate- ruminate surface sculpture	*Anarrhinum pubescens*
11b. Reticulate surface sculpture	*Astragalus fresenii*
11c. Reticulate- scalariform surface sculpture	*Rosa arabica*

Energy dispersive X-ray (EDX) investigation

The mineral composition of endemic taxa pollen grains and seeds in SKP indicated that 12 elements were represented in both of them (Table 5). Pollen grains and seeds constituted a rich source of mineral elements. Mineral composition and relationship among them for pollen grains indicated that the predominant minerals were carbon (C) and oxygen (O) (in both pollen and seeds of all endemic

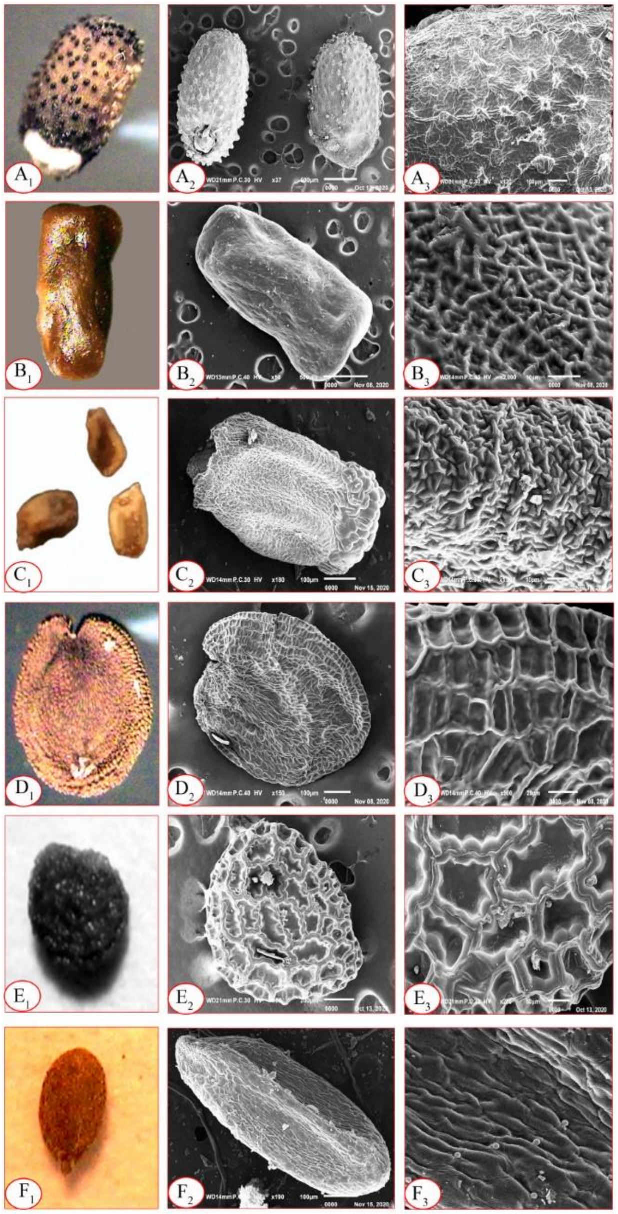

Fig. 3: A-F. Micro-photographs of seeds of the studies taxa (LM & SEM). (A) *Anarrhinum pubescens*; dark brown, elliptic, polygonal cell shape, tuberculate- ruminate surface sculpture, (B) *Astragalus fresenii*; dark brown, elliptic, polygonal cell shape, reticulate surface sculpture, (C) *Ballota kaiseri*; brown, oblongovate, irregular cell shape, ruminate surface sculpture, (D) *Buffonia multiceps*; light brown, orbicular, isodiametric cell shape, scalariform surface sculpture, (E) *Hyoscyamus boveanus*; black, obovate to orbicular, polygonal cell shape, cerebelloid surface sculpture, (F) *Micromeria serbaliana*; light brown, oblong ovate, irregular cell shape, rugose surface sculpture

taxa in SKP) followed by phosphorous (P), magnesium (Mg) and chloride (Cl), while sodium (Na), silicon (Si) and aluminum (Al) are less represented (they represented only in three studied taxa). C ranged from 54.0% for pollens of *Silene. leucophylla* to 73.4% for pollens of *Silene*

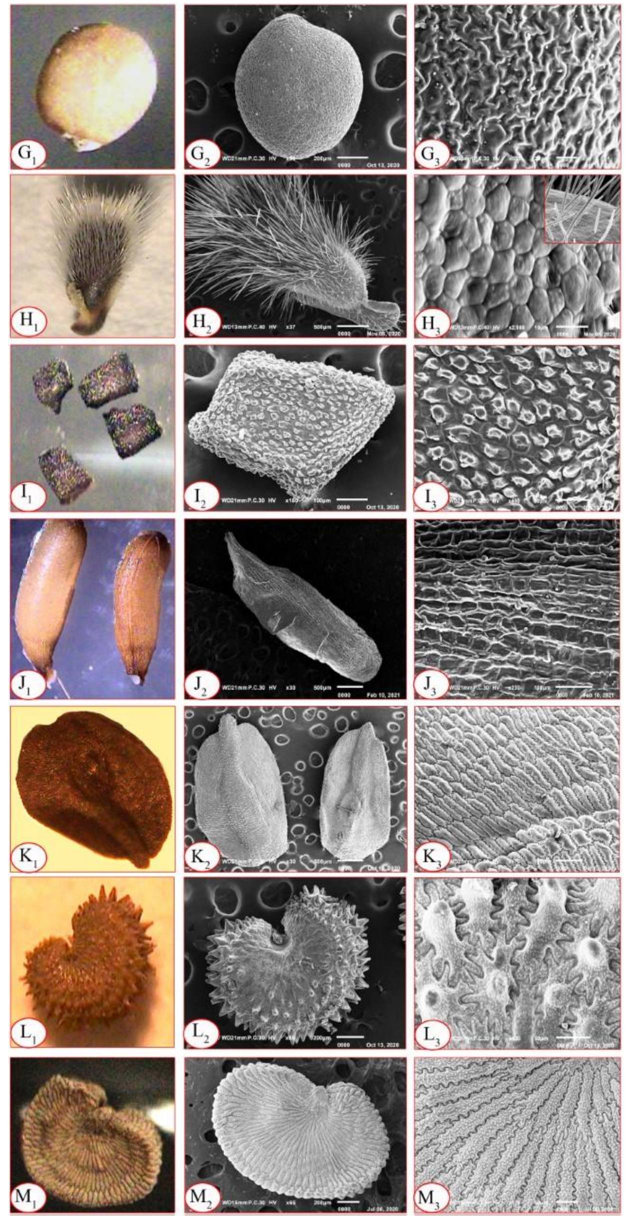

Fig. 3: Cont. G-M. Micro-photographs of seeds of the studies taxa (LM & SEM). (G) *Origanum syriacum* subsp. *sinaicum*; yellowish brown, subglobose, irregular cell shape, ruminate surface sculpture, (H) *Polygala sinaica*; black, hairy, elliptic, polygonal cell shape, reticulate surface sculpture, (I) *Primula boveana*; black, cuboid, polygonal cell shape, reticulate- verrucate surface sculpture, (J) *Rosa arabica*; light brown to brown, elliptic, polygonal cell shape, reticulate- scalariform surface sculpture, (K) *Silene leucophylla*; dark brown , reniform to elliptic, elongated polygonal cell shape, colliculate surface sculpture, (L) *S. oreosinaica*; dark brown , reniform, irregular cell shape, aculeate- verrucate surface sculpture, (M) *S. schimperiana*; light brown, reniform, elongated polygonal cell shape, colliculate surface sculpture

schimperiana, while O ranged from 17.9% for pollens of *Silene oreosinaica* to 31.3% for pollens of *Rosa arabica*. The percentage for nitrogen (N) varied between 18.4 to

Table 5: Element presence and their percentage in pollen grains and seeds of the studied taxa by EDX

Element Taxa	C	N	O	Mg	Al	P	S	Cl	K	Ca	Si	Na
Pollen												
1	55.3	20.4	23.3	0.12	0.12	0.32	0.15	0.08	0.19	0.07	-	-
2	68.1	-	30.2	0.19	-	0.23	0.12	0.60	0.10	0.47	-	-
3	52.9	21.2	24.9	0.13	-	0.25	0.14	0.18	0.24	0.06	-	-
4	72.4	-	26.5	0.10	0.17	0.09	-	0.13	0.24	0.14	0.24	-
5	67.8	-	29.7	0.30	0.49	0.20	0.12	0.52	0.15	0.13	0.27	0.34
6	68.8	-	29.9	0.21	-	-	-	0.49	0.04	0.36	-	0.16
7	72.9	-	25.3	0.17	-	0.26	0.29	0.15	0.37	0.35	-	0.16
8	56.2	21.1	22.0	-	-	0.24	0.11	-	0.38	-	-	-
9	55.9	18.4	24.8	0.13	-	0.13	0.14	0.30	0.07	0.13	0.04	-
10	68.0	-	31.3	0.13	-	0.16	0.12	-	0.26	0.08	-	-
11	54.0	20.3	24.2	0.17	-	0.37	0.15	0.17	0.63	-	-	-
12	80.6	-	17.9	0.20	-	0.39	0.19	0.21	0.44	-	-	-
13	73.4	-	26.1	-	-	0.10	-	0.13	0.30	0.07		
Seed												
1	61.8	-	35.5	0.32	0.69	0.18	0.12	-	-	1.40	-	-
2	40.1	15.3	43.3	0.14	-	-	0.11	0.06	0.96	-	-	-
3	41.5	13.4	43.2	0.48	-	0.24	0.11	0.15	0.90	-	-	-
4	56.6	-	42.5	-	-	-	-	0.07	0.63	0.15	-	-
5	54.3	-	44.3	0.18	0.18	-	-	0.17	0.13	0.38	0.14	-
6	55.0	-	43.3	0.28	0.19	-	-	0.08	0.11	0.58	0.32	0.15
7	54.8	-	44.2	-	0.39	-	-	0.03	-	0.57	-	-
8	55.4	-	43.4	0.13	-	-	-	-	0.31	0.74	-	-
9	58.2	-	40.2	-	0.47	-	-	0.13	0.41	0.27	-	0.26
10	68.2	-	31.7	-	-	-	-	-	-	0.14	-	-
11	58.3	-	40.6	0.17	-	-	-	-	0.23	0.74	-	-
12	45.5	18.2	35.0	-	0.15	-	0.05	0.13	0.28	0.66	-	-
13	42.1	23.9	33.0	0.31	-	-	-	-	0.34	0.32	-	-

(C) carbon, (N) nitrogen, (O) oxygem, (Mg) magnesium, (Al) aluminum, (P) phosphorus, (S) Sulphur, (Cl) chloride, (K) potassium, (Ca) calcium, (Si) silicon, (Na) sodium

Table 6: The updated list of endemic taxa in SKP in the present study compared to the four previous related studies; 1- Boulous, 2009; 2- Hosni *et al*. (2013); 3- Zahran *et al.* (2015); 4- Abdelaal *et al*. 2018 and 5- present study. (×) refers to exclusion from the endemics while (√) refers to being in the endemics

Taxa	1	2	3	4	5
Anarrhinum pubescens, Astraglus fresenii, Ballota kaiseri, Buffonia multiceps, Hyoscyamus boveanus, Micromeria serbaliana, Origanum syriacum L. subsp. *sinaicum, Primula boveana, Rosa arabica, Silene leucophylla, Silene oreosinaica*	√	√	√	√	√
Silene shimperiana	√	×	√	×	√
Polygala sinaica var. *sinaica*	√	√	√	×	√
Euphorbia obovata Decne.	√	√	√	√	×
Phlomis aurea Decne.	√	√	√	√	×
Pterocephalus arabicus Boiss.	√	√	√	×	×
Veronica kaiseri Täckh.	√	√	√	×	×
Silene odontopetala Fenzl var. *congesta* (Boiss.) Melzh.	√	√	×	×	×
Teucrium leucocladum Boiss. subsp. *sinaicum* Danin	×	√	×	√	×
Teucrium leucocladum Boiss. subsp. *leucocladum* var. *glandulosum* Danin	×	√	×	×	×
Phagnalon nitidum Fresen.	√	×	×	×	×
Plantago sinaica (Barn.) Decne.	√	×	×	×	×
Euphorbia sanctae-catharinae Fayed	×	√	×	×	×
Kickxia macilenta	×	√	×	×	×
Nepeta septemcrenata Benth.	×	√	×	×	×
Veronica musa Täckh. & Hadidi	×	√	×	×	×

21.2% and calcium (Ca) from 0.06 to .47%. On the other hand, mineral composition and relationship among them for seeds indicated the predominant minerals were C and O (represented in pollen and seeds of all endemic taxa in SKP) followed by potassium (K) and Ca, while P, Na and Si are less represented (they represented by only two taxa). The carbon ranged from 42.1% for *S. schimperiana* to 68.2% for *R. arabica*, while O ranged from 31.7% for seeds of *R. arabica* to 44.3% for seeds of *Hyoscyamus boveanus*. N percentage varied between 13.4 and 23.9% and Ca from 0.14 to 1.4%.

Discussion

Endemism is the occurrence of a taxon to a restricted defined country, while the narrow-distributed taxa restricted to a limited area in the country are steno-endemics (Gaston 1994). Thirteen taxa of vascular plants, belonged to 11 genera and eight families, were recorded in SKP in the present study comparing with the previous survey of Boulos (2009) who reported 20 endemic taxa, Hosni *et al.* (2013) and Zahran *et al.* (2015) recorded 17 taxa, while Abdelaal *et al.* (2018) recorded 14 taxa. In the present study, eleven taxa were common between the four previously mentioned studies. In addition, *Polygala sinaica* var. *sinaica* and *S. schimperiana* were only recorded by Boulos (2009) and Zahran *et al.* (2015). As shown in Table 6, the variation in the number of endemic taxa from those of the previous studies was based on the presence of endemic taxa in a country other than Egypt. Additional records were collected from literature, scientific websites and global databases with specific localities, coordinates, photos or herbarium specimens. Two taxa were excluded from the endemism of SKP for the first time; *Euphorbia obovata* was recorded in Iran, Palestine, Syria and India (Websites 6 and 13; Barnet 2002) and *Phlomis aurae* in Jordan and Saudi Arabia (Websites 7 and 6; Al-Eisawi and Al-Khader 2007).

In angiosperms, sex is a quantitative phenomenon that can be measured on a continuous scale between strictly male and female extremes (Lloyd 1980). All endemic taxa in SKP were hermaphrodite. The preponderance of the hermaphroditic species (bisexual) is a common feature in most world floras. The ecological and evolutionary significance of bisexuality was emphasized by Baker and Hurd (1968). They suggested that the co-evolution of hermaphroditic flowers with animal pollination might be an important advancement by early angiosperms since pollen-producing and pollen-receiving organs in the same flower allowed for efficient simultaneous deposition and removal of pollen (Baker and Hurd 1968). Moreover, most dioecious species are animal pollinated (Bawa 1980); though in temperate and arid zones many dioecious species are wind-pollinated (Freeman *et al.* 1980; Hultine *et al.* 2007).

In general, the period from March to May (spring season) was characterized by the highest number of flowering endemic taxa in SKP. In Egypt, the highest humid period of the year extends from November to April, associated with low temperature and evaporation; therefore, much favorable soil moisture. During this period, the plants start their growth, reaching the flowering and fruiting stages in March, April and May, respectively **(**Burnie *et al*. 2004; Heneidy 2010). In the present study, the dispersal types of the endemic were assessed using the system of Dansereau and Lems (1957), which distinguished dispersal types primarily by the morphology of the diaspora. Wide distribution of ballochoric plants may be due to their explosive nature, which is often related to rapid desiccation, and hence efficient local seed dispersal.

Pollen morphological characters in some members of endemic and near-endemic taxa in Sinai have been recorded by Shehata and Kamel (2007), Ibrahim (2015) and El-Ghamery *et al.* (2018). From the foregoing data, the pollen morphological characters are considered diagnostic at the specific level among the studied taxa.

All the examined pollen grains are radially symmetric. All pollen grains are isopolar, except *Buffonia multiceps*, *S. leucophylla*, *S. oreosinaica*, *S. schimperiana* (belonging to Caryophyllaceae) which are a polar. Such result is in accordance with Rabei *et al*. (2016) and other previous studies on this family (Yildiz 2005), but disagrees with El-Ghamery *et al.* (2018) since they reported that all endemic taxa in their study are isopolar. The shape class of the examined pollen grains is subprolate in *S. oreosinaica* and *Origanum syriacum* subsp. *sinaicum*; prolate in *Anarrhinum pubescens*, *Ballota kaiseri*, *Micromeria serbaliana* and *P. sinaica*; while prolate-spheroidal in the rest of the studied taxa. They took generally triangular, hexagonal, circular-hexagonal, semi- circular or circular in polar view.

The present pollen data showed considerable variations in their measurable characters *viz.* polar axis (14.717 µm to 44.513 µm) and equatorial axis (9.3115 µm to 45.043 µm) in *A. pubescens* and *S. schimperiana*, respectively. Pollen grains apertures showed variations among the studied taxa. The pantoporate grain with 10- 30 circular pores were observed in *B. multiceps*, *S. leucophylla*, *S. oreosinaica* and *S. schimperiana* (operculum detected in *B. multiceps* and *S. oreosinaica* only and considered a diagnostic character) and the trizonocolporate grain in *A. pubescens*, *A. fresenii*, *H. boveanus* and *R. arabica* (margo detected in *A. pubescens* and *A. fresenii* only and considered a diagnostic character). However, *M. serbaliana* and *O. syriacum* subsp. *sinaicum* can clearly be delimited from the other studied taxa by having hexacolpate grain and *B. kaiseri* by trizonocolpate grain. Moreover, stephanocolpate pollen grain with 22-27 colpi recorded only in *P. sinaica* which is considered a diagnostic character in contrast with El-Ghamery *et al.* (2018) who described it as polycolporate pollen. The present finding has been previously reported in some endemic taxa or generally of their families in some studies such as Yildiz (2005); Pinar *et al.* (2009); Banks *et al.* (2008); Bazarragchaa *et al.* (2012); Osman (2012); El-Ghamery *et al.* (2018) and Shiha (2020).

Out of the present pollen morphological characters, seven types of exine sculpture have been observed and considered diagnostic at specific level *viz*, foveate-micropapillate in three studied *Silene* species that distinguished them from other studied taxa, reticulate exine in *A. fresenii*, *M. serbaliana*, *O. syriacum* subsp. *sinaicum* and *P. boveana*, reticulat foveolate in *A. pubescens*, reticulate-perforate in *B. kaiseri*, striate in *R. arabica*, striate-perforate in *H. boveanus*. However, *B. multiceps* is delimited from other taxa by aculeate exine sculpture. Moreover, *P. sinaica* is distinguished by psilate exine sculpture. Four taxa belonging to Caryophyllaceae are characterized by the presence of annulus which is absent in the rest of the studied taxa. These findings corroborate with Yildiz (2005); Krachai *et al.* (2009); Pinar *et al.* (2009); Osman (2012); Mostafavi and Mehregan (2014); El-Ghamery *et al*. (2018) and Shiha (2020).

The examined seeds of the studied taxa showed significant variation in color, shape, epidermal cell shape, external surface sculpture pattern and periclinal wall sculpture. The seed color was brown (light, yellowish or dark) in most studied taxa except in *H. boveanus* and *P. boveana* where the seed color was black. Moreover, the seed was black and covered with long, unicellular, shiny hairs in *P. sinaica* which distinguished it from other taxa. Weitz *et al.* (1993) and Aydin (2019) reported the presence of such trichomes in their studies. The smallest seeds were measured in *M. serbaliana* (0.4- 0.8 × 0.1- 0.3 mm), while the largest in *S. leucophylla* (2.9- 3.5 × 1.6- 1.9 mm). The seeds exhibited various shapes: the most obvious was the cuboid shape in *P. boveana*, reniform in the three studied *Silene* species. Elliptic shape, oblong ovate, obovate and subglobose also were recorded. According to Hosny and Zareh (1992/1993) and Ibrahim (2015), the forgoing data reported the reniform seed shape in *Silene* species. Rabei *et al*. (2016) studied the seed of *S. oreosinaica* and also described it as a reniform shape. *B. multiceps* a member of Caryophyllaceae, have been studied as an orbicular shape in agreement with El-Ghamery *et al.* (2018).

The fascinating character is the surface sculpture pattern where numerous types were recorded. These were cerebelloid in *H. boveanus*, scalariforn in *B. multiceps*, tuberculate- ruminate in *A. pubescens*, rugose in *M. serbaliana*, ruminate in *B. kaiseri* and *O. syriacum* subsp. *sinaicum*, reticulate in *A. fresenii* and *P. sinaica*, reticulate-verrucate in *P. boveana*, reticulate- scalariform in *R. arabica*, acileate- verrucate in *S. oreosinaica* and colliculate in *S. leucophylla* and *S. schimperiana*. The epidermal cell is arranged in three shape *viz*, isodiametric, polygonal and irregular. The anticlinal walls were undulate in *O. syriacum* subsp. *sinaicum* and *S. oreosinaica*, sinuate in *B. kaiseri*, *H. boveanus*, *S. leucophylla* and *S. schimperiana* or straight in the rest of the studied taxa. Another diagnostic character in addition to the sculpture pattern is the periclinal wall surface sculpture as it was glebulate in *H. boveanus*, striate-microreticulate in *B. kaiseri*, verrucate in *P. boveana*, granulate in the three studied *Silene* species, obscure in *A. fresenii* or smooth in the rest of the studied taxa. The present study considered the first description of seeds in *A. fresenii*, *B. kaiseri* and *M. serbaliana*. However, their characteristics followed the general characters of their genera. Similar types of seed surface sculpture patterns in other species of *Astragalus* were reported by Ekici *et al.* (2005); Vural *et al.* (2008) and Shemetova *et al.* (2018). The reticulate, rugose, ruminate and smooth slightly tuberculate nutlet surface ornamentation were recorded by Kamel (2014) within the studied taxa of Lamiaceae.

The present study indicated that pollen grains and seeds of endemic taxa in SKP had high percentages of C, O, P, Mg, N and Ca. Boughediri (1991) found that phosphorus and potassium had the highest values for most date palm cultivars. K also appears to dominate in the studied taxa belonging to Plumerioideae and Cerberoideae, while Ca was equal or dominant in the studied taxa of Apocynoideae (Wolter and Nilsson 1990). The consistent appearance of P at the aperture area of pollen of *Hordeum vulgare* and *Sesamus indicum* was attributed as one factor regulating the regulate the uptake of water and rapid growth of pollen (Rehman *et al.* 2002, 2004). Bacha *et al.* (1997) found that pollen grains of date palm contained the highest concentrations of N. Stanley and Linskens (1974) reported that high mineral contents of pollen might be due to the high mineral content in the soil horizons in dry areas where this species grows. The highest percentages of C and O may due to they represented as organic matter in these taxa, since they have been classified as essential elements for the plants (Mengel and Kirkby 1982). Variations in the composition of taxa pollens and seeds reflect the differences in the floral origin of pollen and the plant growth conditions such as soil and geographic origin (Stanley 1971; Hassan 2011). The capacity of the parent plant to accumulate salts in the pollen and seeds is also related to the species. Moreover, Ca plays a vital role in plant growth and development cycles such as pollen tube growth and fertilization (Aly 2018). Ca also plays a role in determining the direction of pollen tube growth and has an essential signaling, physiological, and regulatory role during sexual reproduction in flowering plants (Ge *et al.* 2007; Prajapati and Jain 2010).

Conclusion

Results of this study were helpful in the authentication and identification of the endemic taxa in SKP. Also, the examined characteristics were useful in the identification and discrimination between the studied taxa. However, some of these taxa need further investigation to verify their exclusion from endemism (*B. multiceps, H. boveanus*, *S. shimperiana* and *P. sinaica* var. *sinaica*). These taxa are recorded in the countries other than Egypt but without coordinates, photos or herbarium sheets. Therefore, these taxa are not excluded from the endemism in the present study.

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Author Contributions

Mohamed M. El-Khalafy designed the colored plates, acquisition, analysis and interpretation of results; drafted, revised and approved the final version of the manuscript for submission; Dalia A. Ahmed, Kamal H. Shaltout and Yassin M. Al-Sodany conceived and interpreted the results; revised the article and approved the final version for submission; Soliman A. Haroun planned the study revised the article and approved the final version to be submitted; Mohamed A. Salem prepared initial draft, revised the script, designed the colored plates, analysed and interpreted results and approved the final version to be submitted.

Conflict of Interests

All of the authors confirm that there are no conflicts of interest.

Data Availability

Please contact the authors for data requests.

Ethics Approval

Not applicable in this paper

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