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SEM-variations in style and stigma of some *Erodium* L'Hér. Ex aiton. and *Geranium* L. Species and importance in their classification

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Abstract
This investigation dealt with the study of the style and stigmatic characters of 13 Egyptian species, 9 belonging to genus *Erodium* and 4 to genus *Geranium* using both the stereo- and scanning electron microscopes. The studied characters are the hair density and type on the style, length, width, shape and epidermal characters of the stigma. The obtained results revealed that the most important character in distinguishing between the two genera is the length and shape of stigma, while the hair density, type, length can be of use in the identification of the species under these two genera. *G. dissectum* and *G. rotundifolium* are the only two species with multicelluler capitates glandular hairs, while *E. moschatum* is the only species with short unicellular hairs. From this study, we can conclude that the taxa in family Geraniaceae have great similarity in their floral characters and need more detailed studies to unveil the diagnostic features between them.

Keywords: *Erodium, geranium*, stigma, style, taxonomy

Introduction

Floral characters considered the most valuable tool in understanding taxa relation, pollination and evolution. The essential organs in the flowers; gynoecium and androecium; play a key role in the process of plant evolution. Stigma considered from the neglected organ in taxonomical decisions. Heslop-Harrison (1981) [9] pointed to the importance of the stigma in understanding certain morphological and physiological attitude within angiosperm. He mentioned that there is close relation between the stigma, reproductive physiology and pollination type within the species. Edlund *et al*. (2004) [8] pointed to the importance of the stigmatic surface and its chemical compositions to know the pollination type and evaluate the degree of evolution within the taxa. The flower in both *Erodium* and *Geranium* is hypogenous, usually actinomorphic, perfect, pentamerous and arranged in definite inflorescences. The androecium consists of ten to fifteen free stamens, with sterile ones and arranged in two whorls. The gynoecium consists of one ovary with five united carpels which developed into schizocarpic fruits. The style and stigma differs between the species, but usually there is long style ended with free stigmas. In both genera; *Erodium* and *Geranium*; the outer anthers develop first and discharge their pollen or turn into sterile scales in case of the genus *Erodium*, and then the inner anthers discharge their pollen. This two-step process expands the period of pollen production and affects the pollination type. Philipp (1985) [16] pointed to the variability in maturation between the androecium and gynoecium within *Geranium* species and varies from protandry to homogamy to protogyny. As the result, the type of pollination differs between the species with gradual transition between self-pollinated to insect pollinated. Kozuharova (2002) [14] reported five pollination trends within the *Geranium* species based on flower symmetry and position as well as the evolutionary state in each taxa. *Erodium* species are usually pollinated by bees.

The Geraniaceae includes five genera, *Erodium* L'Herit., *Geranium* L., *Monsonia* L., *Sarcocaulon* (D.C.) Sweet and *Pelargonium* L'Herit. (Hutchinson 1969, Zomlefer 1994, ITIS 2007-2010) [10, 24, 11] which shared a common schizocarpic fruits. The family is divided into two tribes, the Geranieae, which includes *Erodium, Geranium, Monsonia* and *Sarcocaulon*, with actinomorphic flowers, while *Pelargonium*, with zygomorphic flowers, present alone in the second tribe Pelargonieae. In spite of that many *Erodium* species recorded with zygomorphic flowers and some *Pelargonium* species with actinomorphic flowers. *Geranium* is distinguished from the other genera by the ten fertile stamens. The number of fertile stamens in other genera differs from 15 in *Monsonia* and *Sarcocaulon*, 5 in *Erodium* and 2 - 7 in *Pelargonium* (Verhoeven and Venter 1992) [21]. In fact the similarity between the three genera, *Erodium, Geranium* and *Pelargonium* is great and many taxa transferred from one genus to the other. Taylor (1993) [19] indicated to the great similarities between taxa of both genera and they can be identified by their fruits. The fruits consist of the mericarp and carpel beak which is the style and stigmas. To understand the variations between both genera; *Erodium* and *Geranium;* the style and stigma of thirteen Egyptian taxa belonging to the genera *Erodium* (9) and *Geranium* (4) investigated, measured, photographed and scanned. Micro-morphological characters of the stigma epidermis have been studied in order to found distinctive variations between the two genera and the different species within the two genera. In the Egyptian flora the recorded species of the genus *Erodium* are fourteen, while those belonging to the genus *Geranium* are six

(Tackholm, 1974; Boulos, 1999, 2009) [6, 7] but the available species from both genera with mature ovaries was 13 species which investigated in this work.

Materials and Methods

This work is focused on the characters of the style and stigma of 13 species; *Erodium* (9 species) and *Geranium* (4 species). The work depends on both fresh and herbarium materials (table 1). The collected specimens were identified using Täckholm, (1974) and Boulos, (1999, 2009) [6, 7]. Taxonomic authorities for Latin names and synonymy of the species investigated in this study were based on either the Australian plant name index (APNI) or Tropicos. Voucher specimens were kept in the Faculty of Science, Alexandria University Herbarium (ALEX). Fresh flowers of the collected taxa gathered from Alexandria roads during March and April 2020, dried and preserved as dry specimens for examination under both the stereo-and scanning microscopes. The upper part of the dry style mounted onto SEM stubs with double sided cello tape, coated with 30 nm gold using Fine Coat Sputter JFC 1100 then examined and photographed using JEOL-JSM.I T200 Series Scanning Electron Microscope allocated in the electron microscope unit, Faculty of Science, Alexandria University, Egypt. Photographs of the matured mericarps with the style and stigmas are taken by mobile camera and some of them from different net cite to clarify the different species stigmas.

Measurements of the length and width of the stigma in mm and taken by the aid of the stereomicroscope, ten flowers from each taxa subjected to the measurements and investigations. The width of the clavate stigmas is taken from the widest part of it.

Table 1: Studied species, information of the herbarium sheets, source of materials, confirmation of nomenclature and synonyms

No	Taxa	Collector and date	Locality	Source	Confirmation of nomenclature	Synonyms
1	<u>*Erodium*</u> *E. bryoniaefolium* Boiss., 1842	Taia,W.K. 8/3/2020	Alex. Abu-qir Rd.	Alex. Sporting	Tropicos.org	*E. oxyrhinchum* subsp. *bryoniifolium* (Boiss.) Schoenb.-Tem., 1970
2	*E. cicutarium (*L.) L'Her. Ex Aiton	Ayyad *et al*. 22/2/1996	Borg El-Arab	ALEX	Tropicos.org	*G. cicutarium* L.
3	*E. deserti* (Eig) Eig	Taia,W.K. 16/4/2020	Near Marina	Alex. Marina Rd.	The Plant List, record kew-2798217	*E. touchyanum* Delile ex Godr. *E.moschatum* (L.) L´Her
4	*E. glaucophyllum* (L.) Aiton	Heneidy *et al*. 12/2/2002	Borg El-Arab	ALEX	Database in: USDA, ARS	No synonym
5	*E. gruinum* (L.) L'Her.	Heneidy *et al*. 12/2/2002	Borg El-Arab	ALEX	Tropicos.org	No synonym
6	*E. hirtum* Wild	Ayyad *et al*. 6/4/1996	El-Omayed	ALEX	APNI	*Geranium hirtum* Forssk
7	*E. laciniatum* var. *pulverulentum* (Cav.) Willd.	Ayyad *et al*. 6/4/1996	El-Omayed	ALEX	APNI	No synonym
8	*E. malacoides* (L.) L'Her. ex Aiton	Ayyad *et al*. 3/5/1998	Matruh	ALEX	Database in: USDA, AR	No synonym
9	*E. moschatum* (L.) L'Her. ex Aiton	Taia, W.K. 2/4/2020	Alex. Sporting	Alex. Sporting	APNI	*Geranium cicutarium v*ar. *moschatum* L.
10	<u>*Geranium*</u> *G. dissectum* L.	Rizk *et al*. 4/5/1996	Rashid	ALEX	APNI	*Geranium laxum Hank*
11	*G. molle* L.	Rizk *et al*. 12/3/1992	Borg El-Arab	ALEX	APNI	No synonym
12	*G. trilophum* Boiss	Ayyad *et al*. 7/4/1992	Sinai, Saint Katrine	ALEX	APNI	No synonym
13	*G. rotundifolium* L.	Ayyad *et al*. 7/4/1992	Sinai, Saint Katrine	ALEX	APNI	No synonym

Table 2: Ultrastructural characteristics of the stigma of the studied taxa Abbreviation used: St W= style wall: DH=densely hairy, DW=densely wooly, H=hairy, SH=sparsely hairy, W=wooly; HT=hair type: MMP=multicelluler multiserriate pointed, MUP=multicellular uniserriate pointed, MUCG=multicelluler uniserriate capitates glandular, UUP=unicellular uniserriate pointed;

No	Taxa	St W	HT	Stigma				
				Length mm	Width mm	Shape	Periclinal wall	Relief of cell boundaries
1	*E. bryoniaefolium*	D.W	MMP	2.9-3.3 (3.0±1.23)	1.9-2.1 (2.0±058)	Finger	Convex rounded	Sunken
2	*E. cicutarium*	W	MMP	4.5-4.9 (4.2±1.08)	1.1-1.2 (1.1±0.08)	Finger	Convex rectangular	Sunken

3	*E. deserti*	D.W	MMP	3.8-4.2 (4.1±0.62)	1.0-1.3 (1.1±0.18)	Finger	Convex Oval	Sunken
4	*E. glaucophyllum*	D.W	MUP	2.2-2.8 (2.3±1.22)	1.4-1.6 (1.3±0.78)	Finger	Convex rectangular	Sunken
5	*E. gruinum*	D.W	MMP	10.9-11.8 (11.1±2.22)	3.0-3.2 (3.1±1.18)	Clavate flag	Convex rectangular	Elevated
6	*E. hirtum*	D.W	MMP	4.5-4.8 (4.6±0.98)	1.0-1.2 (1.1±0.38)	Finger	Convex rectangular	Sunken
7	*E. laciniatum*	D.W	MMP	4.0-4.4 (4.2±0.99)	1.0-1.2 (1.1±0.29)	Clavate flag	Convex oval	Sunken
8	*E. malacoides*	D.W	MMP	13.0-14.2(13.6±2.78)	1.2-1.4 (1.3±0.46)	Clavate flag	Convex elongated	Sunken
9	*E. moschatum*	SH	UUP	2.6-2.8 (2.7±0.78)	1.0-1.2 (1.1±0.33)	Finger	Concave rectangular	Sunken
10	*G. dissectum*	DH	MUCG	3.2-3.6 (3.5±1.23)	1.0-1.8 (1.4±1.07)	Clavate flag	Concave rectangular	Elevated
11	*G. molle*	DH	MUP	6.0-6.6 (6.2±1.58)	1.2-1.4 (1.3±0.78)	Clavate flag	Convex rectangular	Elevated
12	*G. trilophum*	H	MUP	5.0-5.4 (5.1±2.88)	1.9-2.1 (2.0±0.48)	Clavate flag	Flat elongated	Superficial
13	*G. rotundifolium*	H	MUCG	4.8-5.6 (5.1±1.68)	1.0-1.2 (1.1±0.28)	Clavate flag	Concave elongated	Elevated

Key to the studied taxa according to their style and stigma characters

1-Finger-shaped stigma
2-Stigma length from 2-2.8 mm
3-The style is densely hairy……*E. glaucophyllum*
3-The style is sparsely hairy…….*E. moschatum*
2-Stigma length from 3.0-3.5 mm……..*E. bryoniaefolium*
2-Stigma length from 3.8-4.2 mm……*E. deserti*
2-Stigma length from 4.5-5.0 mm……..*E. cicutarium* & *E. hirtum*
1-Clavate shape stigma
2-Stigma length from 3.0-3.6 mm……*G. dissectum*
2-Stigma length from 4.0-4.5 mm……*E. laciniatum*
2-Stigma length from 4.8-5.6 mm
3- Style hairy
4-The hairs multicellular multiserriate pointed…..*G. trilophum*
4-The hairs multicellular uniserriate capitated glandular.. *G.rotundifolium*
2-Stigma length from 6.0-6.6 mm……..*G. molle*
2-Stgma length from 10.9-11.8 mm……*E. gruinum*
2-Stigma length over 13.0 mm……..*E. malacoides*

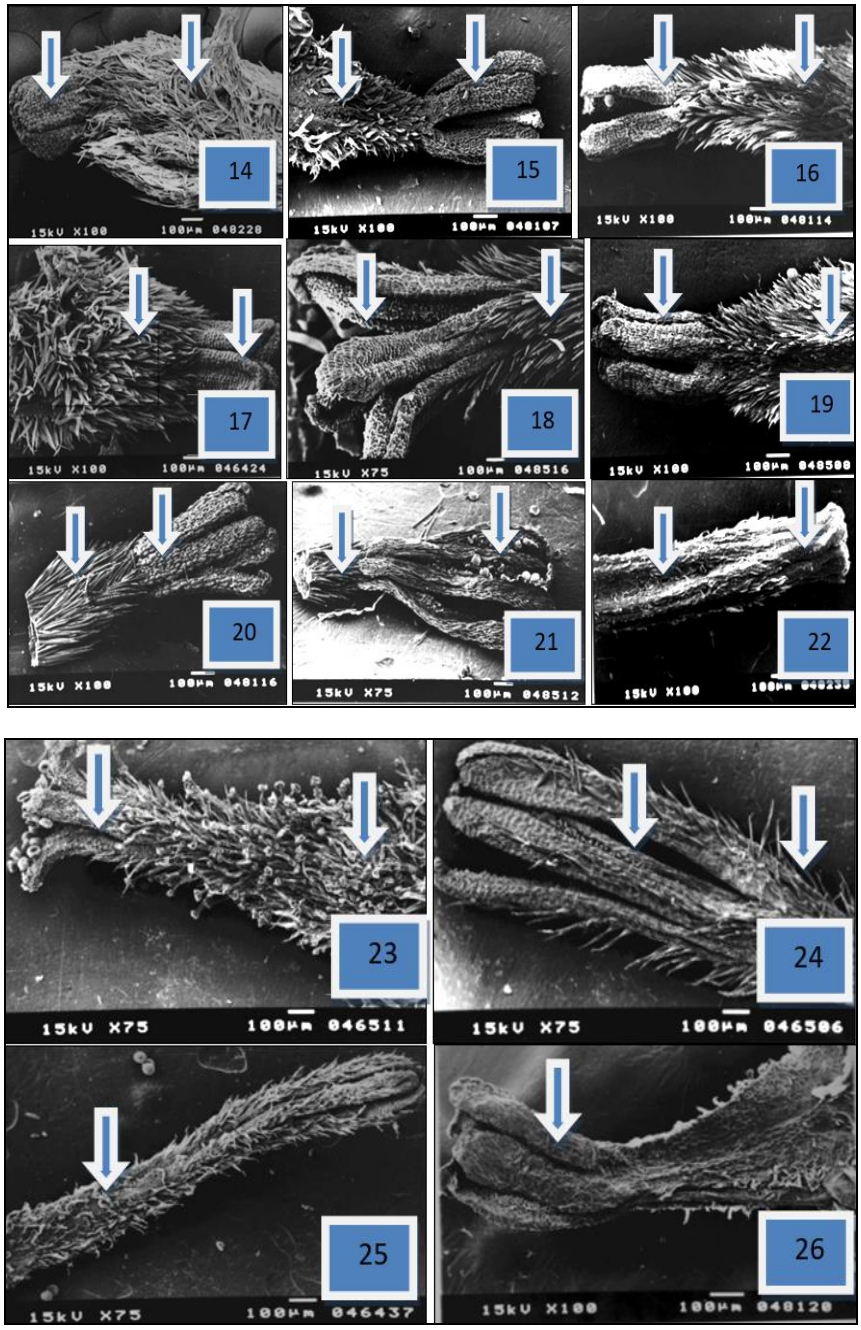

Plate 1: Photographs 1-13, Light photographs showing the style and stigma in the different studied species. 14-26 SEM photographs showing the hairs on the style and shape, length and epidermal ornamentation of the stigma. Arrows indicate to the stigma and trichomes.

Results and Discussion

The taxonomic ranking of any taxon is based on special feature that characterized this taxon. Most taxonomic works pre-molecular studies depend on the floral morphological characters. Androecium, fruit and seed characters gained more attention than the style and stigmatic variations. Thus the stigma in flowering plants has been little studied in most taxonomic works although it plays an important role in pollen capture, type of pollination and can help in the taxonomic differentiations. Family Geraniaceae, is a Mediterranean and cold region family, it has five genera; *Geranium* Tourn. ex L. (430 species), *Pelargonium* L'Hér. ex Aiton (280 species), *Erodium* L'Hér. ex Aiton (80 species), *Monsonia* L.(40 species) and Hyseocharis (1-3 species)(steven 2001 onwards). *Erodium* and *Geranium* are phylogenetically closest related. The two genera are considered from the taxonomically difficult taxa in the absence of the fruits. The mericarp gained more attention in the taxonomical divisions of the *Geranium* species more than the other morphological characters. Yeo (1984) [23] classified the *Geranium* species into three subgenera; *Geranium* with ten sections, *Robertium* with eight sections and *Erodioideae* with four sections; on the basis of seed-ejection type. The former discharge the seeds in ballistic explosion, the middle with forcible discharge of the mericarp containing the seeds without the awn while the later discharge the mericarp containing the seeds with the attached awn which called *Erodium-type*. Each of these subgenera divided into sections according to other characters. In fact the diagnostic features of the species

under these two genera based on fruit margin and awn, seed discharge and leaf morphology. Aedo (1996, 2000, 2001, 2003, 2017) [1-5] revised the different sections belonging to genus Geranium in series of research works, and he found that the hair type and density beside the some floral morphological features can help in the discrimination of the taxa under the genus. Troshkina (2021) found the mericarp size and its epidermal cell shrinkage are useful characters in the taxonomy of the taxa under genus Geranium section Recurvata. Meanwhile Thomas and Andrea (2017) found that the classification of the species belonging to genus Geranium based on fruit morphology is an artificial classification and need more taxonomic investigations. In fact the two genera, Erodium and Geranium, are closely related and come in near clades in recent phylogenetic works (Keshavarzi 2015, Jeiter et al. 2017) [13, 12]. Accordingly this work has been done to investigate how much the morphological character of the stigma in 9 Erodium and 4 Geranium species can help in their taxonomy and identification.

The results of this investigation are summarized in table 2 and illustrated in the photographs (1- 26). All the studied species have long and broad style ending with five free stigmas. The photographs (1-13) clarify the shapes of the styles and stigmas as well as the density of the hairs within the studied species by mobile camera. From these photographs we can see the density of hairs covering the styles within the different species. The studied Erodium species have densely hairy styles (Photos. 1-8), except E. moschatum has sparsely hairy style (Photo.9).The studied Geranium species, both G.dissectum and G.molle (Photos. 10, 11) are densely hairy, while G.trilophum and G.rotundifolium are hairy with lesser density (Photos 12, 13).

From the stereomicroscope examination the type of hairs on the style wall can be identified. The style wall is richly covered by long multicellular, multiserriate pointed hairs with different density within the Erodium species. In the studied Geranium species multicellular, capitates, glandular hairs are found in both G.dissectum and G.rotundifolium (Photos. 23, 26). Unicellular uniserriate hairs are found in E. moschatum only (Photo. 22). The hairs are dense and covered the style completely of the Erodium species (Photos. 14-21). In E.gruinum, E.hirtum and E.laciniatum the hairs look like stout bristles which made it difficult to examine their cellular structures (Photos. 18-20). The rest of the studied Erodium species the hairs are long and soft (Photos. 14,15,16,21 and 22). In E. glaucophyllum the style covered by dense hairs, but they are shorter than the other species (Photo. 17). The investigated Geranium species the density of the hairs are considerably less than those on the Erodium taxa. This observation support Aedo observation, as the hair type and density can be of use in the division of the two genera.

The stigma lobes, considerably long and look like fingers, or clavate flags. The length and width of the stigma measured in mm and varied from 2.3 mm in E. glaucophyllum and reach 14.2 mm in E. malacoides. The width of the finger-shaped stigmas is uniform throughout the whole length of the five parts, while of the clavate-shaped ones it widen toward the tips. Measurements of the clavate-like stigmas are at the widest part. The width of the stigma branches are from 1.0 to 2.0 mm in all the studied taxa, except E.grunium in which the stigma width reach 3.2 mm. The surface of the stigma branches has convex rounded, rectangular or even oval shaped periclinal walls with sunken, superficial or elevated cell boundaries. In E. grunium, E. hirtum and E. laciniatum the periclinal walls arranged in a way resemble the scales (Photos. 5, 6 & 7).

From the obtained results, the style and stigma within the studied taxa can be used in the discrimination between the species under the two genera. The shape of the stigma and its length and width can be used to distinguish the studied species as shown in the constructed Key.

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