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Systematic Revision of *Erodium* species in Egypt as Reflected by Variation in Morphological Characters and Seed Protein Electrophoretic Profiles

[1]Sherif M. Sharawy and [2]Abdelfattah Badr
[1]Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt
[2]Department of Botany, Faculty of Science, Tanta University, Tanta 31529, Egypt

Abstract: The systematic relationships between the species of *Erodium* L'Hér. in the Egyptian flora have been reexamined, based on variation in vegetative and reproductive morphological characters as well as polymorphism in seed protein electrophoretic profile as revealed by SDS-PAGE. The relationships between the examined taxa have been expressed as UPGMA and NJ trees, based on the coefficient of similarity using the NTSYS-pc software program. In all trees, the species in section Plumosa have been delimited together as one group from another major group that comprises species of section Barbata. However, the delimitation of some species, did not agree with their current sub sectional delimitation. Similarities between *E. neuradifolium* of subsection Malacoidea and both *E. ciconium* (subsection Absinthioidea) and *E. gruinum* (subsection Gruina) as well as *E. cicutarium* and *E. moschatum* of subsection Cicutaria have been demonstrated in the trees bases on morphological and seed protein data. The remaining species of subsection Malacoidea i.e., *E. laciniatum*, *E. pulverulentum* and *E. malacoides* formed a cluster in which *E. malacoides* is clearly distinguished from the other two species. The clustering of *E. laciniatum* and *E. pulverulentum* reflects the morphological resemblances between them but does not warrant regarding the latter as a subspecies of the former.

Key words: *Erodium*, Geraniaceae, morphology, systematics, seed protein electrophoresis, Egyptian flora

INTRODUCTION

The genus *Erodium* L'Hér., of the family Geraniaceae, comprises about 60 annual or biennial, rosette forming herbs distributed in the temperate and subtropical regions of the World (Mabberley, 1997). The center of origin for the genus is assumed to be the East Mediterranean region but some species of Mediterranean origin have been naturalized in other parts of the World. The most famous example is *Erodium malacoides* (L.) Willd. that has been naturalized in Texas south of the USA (Lemke and Aplaca, 2006).

The genus differs from other genera in the Geraniaceae in the presence of five fertile and five sterile stamens compared to ten fertile stamens in the other genera; it also differs in the shedding of the ovary wall with the seeds at maturity. The genus was divided by Kunth (1912) into two sections: Plumosa, which included five species and Barbata, which included the remaining species of the genus delimited in ten subsections. Warburg (1938) viewed the delimitation of species as proposed by Kunth (1912) as satisfactory but transferred *Erodium ciconium* (L.) L'Hér. from subsection Absinthioidea, which comprises species with annual habit and colored hermaphrodite flowers, to subsection Gruina with which it agrees in all aspects except the rather pinnate leaves.

In the Egyptian flora, Forsskal (1775) reported the occurrence of *Erodium crassifolium* L'Hér. (*Geranium hirtum* Forrssk.) and Boissier (1867) described additional three species from Egypt. Ascherson and Schweinfurth (1887), in the first account on the Egyptian flora described another six species of the genus and in Taeckholm (1974) the genus was represented by 13 species. However, a systematic revision of the genus in Egypt by El-Hadidi *et al.* (1983) based on the differential characters of leaf, inflorescence, flowers and fruits, confirmed the presence of 14 species; four are delimited in section Plumosa Boiss. and ten in section Erodium (Barbata Boiss.), the species in the latter section were delimited in four subsections as recommended by Schönbeck-Temesy (1970), (Table 1). However, in the more recent Flora of Egypt written by Boulos (1999), the number of species was reduced to 13, he regarded *E. pulverulentum* (Boiss.) as subspecies of *E. laciniatum* (Cav.) Willd.

The chromosome number in the genus *Erodeum* is generally stable, the majority of the species are diploid

Corresponding Author: Abdelfattah Badr, Department of Botany, Faculty of Science, Tanta University, Tanta 31529, Egypt

Table 1: A list of the examined taxa of the Egyptian *Erodium* species, their sectional delimitation and the localities from, which they were collected

Serial No.	Taxa	Section	Subsection	Locality
1	*E. arborescens* (Desf.) Willd.	Plumosa	Plumosa	80 km east of Matruh
2	*E. ciconium* (L.) L'Hér.	Barbata	Absinthioidea	El-Arish, north Sinai
3	*E. cicutarium* (L.) L'Hér.	Barbata	Cicutaria	50 km west of Alexandria
4	*E. crassifolium* (L.) L'Hér.1	Plumosa	Plumosa	50 km west of Alexandria
5	*E. crassifolium* (L.) L'Hér. 2	Plumosa	Plumosa	80 km east of Matruh
6	*E. crassifolium* (L.) L'Hér. 3	Plumosa	Plumosa	El-Arish, north Sinai
7	*E. crassifolium* (L.) L'Hér. 4	Plumosa	Plumosa	Saint Catherine, south Sinai
8	*E. glaucophyllum* (L.) L'Hér.	Plumosa	Plumosa	El-Arish, Sinai
9	*E. gruinum* (L.) L'Hér. 1	Barbata	Gruina	80 km east of Matruh
10	*E. gruinum* (L.) L'Hér. 2	Barbata	Gruina	50 km South West Alexandria
11	*E. laciniatum* (Cav.) Willd.	Barbata	Malacoidea	50 km west of Alexandria
12	*E. malacoides* (L.) L'Hér.	Barbata	Malacoidea	Herbarial sheets (50 km West of Alexandria)
13	*E. moschatum* (L.) L'Hér.	Barbata	Cicutaria	80 km East Matruh
14	*E. neuradifolium* Delile ex Godr. 1	Barbata	Malacoidea	El-Arish, North Sinai
15	*E. neuradifolium* Delile ex Godr. 2	Barbata	Malacoidea	Saint Catherine, South Sinai
16	*E. oxyrrhynchum* M. Bieb.	Plumosa	Plumosa	Saint Catherine, South Sinai
17	*E. pulverulentum* (Cav.) Willd.	Barbata	Malacoidea	50 km West of Alexandria

with 2n = 20, but polyploidy, particularly tetraploidy (2n = 40), have been scored in a number of species (www.mobot.mobot.org). In the Egyptian flora, chromosome counts were reported in only five species (Badr and Hamoud, 1985), 2n = 20 was found in four species, while 2n = 18 was only encountered in *E. oxyrrhynchum* M.Bieb. subsp. *oxyrrhynchum*, the chromosomes in this species was longer than the other four. The chromosomes in the genus are short compared to other genera and further studies on the karyotype have been difficult to make (Badr and Hamoud, 1985). The homogeneity in chromosome number and size make the karyotype features of little use in the systematic of the genus.

The variation in the electrophoretic profile of storage seed proteins in polyacrylamide gel, in the presence of sodium dodecyle sulphate (SDS-PAGE), have been found useful in the study of systematics and evolution of plant species (Ladizinsky and Hymowitz, 1979; Vaughan, 1983). These proteins have provided valid source of evidence for addressing the systematic relationships, at the species level, in many genera of the family Fabaceae. For example, to differentiate between species in *Trifolium* (Badr, 1995), *Phaseolus* (Schmit *et al*., 1996), *Sesbania* (Badr *et al*., 1998), *Lathyrus* (Badr *et al*., 2000), *Astragalus* (Al-Nowaihi *et al*., 2002) and *Lupinus* (El-Shazly *et al*., 2006) and also in plants from other families such as *Mentha* from the Lammiaceae (Badr *et al*., 2003) and *Artemisia* from the Asteraceae (Mohamed, 2004). In the present research variation in vegetative and reproductive morphological characters as well as polymorphism in seed protein electrophoretic profile as revealed by SDS-PAGE are applied to reassess the taxonomic relationships of 17 samples of Egyptian *Erodium* representing 12 species, in the light of their previous taxonomic treatments.

MATERIALS AND METHODS

Seventeen accessions representing 12 species of *Erodium* were collected from their natural habitats in Egypt in 2004-2006. The sectional delimitation and localities of the examined material are given in Table 1. Herbarial material of *E. malacoides* was used for this study. The species were identified according to Taeckholm (1974) and Boulos (1999). Herbarium specimens of the examined taxa are deposited at the Herbarium of Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. A total of 26 vegetative and reproductive characters were considered, the examined characters include 22 two-state characters and four characters multi-state characters. A list of these characters and their states are given in Table 2.

For protein extraction, 2.0 g of mature seeds were mixed with an equal weight of pure, clean, sterile fine sand, powdered using a mortar and pestle and homogenized with 0.2 M Tris-HCl buffer, pH = 8 for 1 h in sterilized Eppendorf tubes. The extract was centrifuged at 12000 g for 10 min and the supernatant (protein extract) was transferred to new tubes and immediately used for electrophoresis. For electrophoresis, 40 μL of the extract were mixed with an equal volume of a sample buffer (0.125 M Tris-HCl, pH = 6.8, 2% SDS, 10% sucrose, 0.5% β-mercaptoethanol), denatured by boiling for 5 min in a water bath, cooled and 0.1% bromophenol blue as a tracking dye was added. For separation of protein components, 20 μL of this mixture were loaded in 12.5% gel slabs, which was prepared as described by Laemmli (1970). Electrophoresis was carried out in Tris-Glycine buffer (pH = 8.3) at 4°C and 125 volt for 2 h in a Consort Vertical Slab Gel Apparatus using a Pharmacia low-molecular weight protein mixture as standard marker. Gels were then stained in 0.1% Comassie Brilliant Blue

Table 2: List of examined morphological characters and their codes for numerical analysis

Serial No.	Character	State	Code
1	Habit	Annual	0
		Perennial	1
2	Stem	Erect	0
		Procumbent	1
3	Stem texture	Glabrous	0
		Hairy	1
		Hispid	2
		Bristles	3
4	Leaf composition	Simple	0
		Compound	1
5	Leaf blade	Undivided	0
		Divided	1
6	Shape of leaf blade	Cordate	0
		Ovate	1
7	Stipules texture	Membranous	0
		Hairy	1
		Scarious	2
8	Bract numbers	2-3	0
		> 3	1
9	Bracts	Free	0
		Connate at base	1
10	Flower color	Purple	0
		Violet	1
		Pink	2
11	No. of flowers in the inflorescence	< 5	0
		> 5	1
12	Flower symmetry	Actinomorphic	0
		Zygomorphic	1
13	Sepal length	< 5 mm	0
		> 5 mm	1
14	Sepal tip	Without bristles	0
		With bristles	1
15	Petal length	< 1 cm	0
		> 1 cm	1
16	Petal base spot	Absent	0
		Present	1
17	Stamens (filaments)	Glabrous	0
		Hairy	1
		Toothed	2
18	Stamen length	< 5 mm	0
		> 5 mm	1
19	Mericarp	Pitted below the beak	0
		Furrowed below the beak	1
20	Mericarp surface	Glandular	0
		Eglandular	1
21	Mericarp length	< 1 cm	0
		> 1 cm	1
22	Central column of fruit	Absent	0
		Present	1
23	Fruit beak	Plumose at base	0
		Plumose throughout its length	1
24	Beak bursting	From the top	0
		From the base	1
25	Beak surface	Glandular	0
		Eglandular	1
26	Beak length	< 5 cm	0
		> 5 cm	1

R-250 for 1 h destained and photographed while gels were wet and stored for subsequent examination. The bands produced in the electropherogram were scored and their molecular weights were calculated by comparison to the Pharmacia low molecular weight standard protein marker.

The 26 morphological characters and the 26 protein bands were coded and used to construct a number of trees that illustrate the relationships between the examined taxa of *Erodium*. For data analysis, the two-state morphological characters and the protein characters were coded as binary characters and coded as 0 or 1, whereas the multi-state characters were given codes ranging between 0 and 3 (Table 2). The relationships between the examined *Erodium* taxa have been expressed using the coefficient of similarity proposed by (Dice, 1945). The equation for this coefficient is included in the computer program NTSYS-pc (Rohlf, 2000), which has been used for data analysis. Construction of the trees illustrating the relationships between the studied samples was performed using the Unweighted Pair Group Method using Arithmetic Average (UPGMA) proposed by Sokal and Michener (1958) and the Neighbor Joining (NJ) method (Saitou and Nei, 1987) as implemented in the NTSYS-pc program (Rohlf, 2000).

RESULTS AND DISCUSSION

A total of 26 protein bands have been revealed in the electrophoretic profiles of the examined taxa of the Egyptian *Erodium* (Fig. 1). The molecular size of these bands ranges between over 100 kDa to about 10 kDa, the majority of the bands have been found polymorphic across the examined *Erodium* taxa. Protein bands were coded as 0 for absence and 1 for presence in the computer analysis. A glimpse on the electropherograms of the examined taxa shows abundance of polymorphic bands and only one monomorphic band with a MW of about 80 kDa. Some species are characterized by presence of a band that is absent from the profiles of other species. For example the four samples of *E. crassifolium* (14-17) exhibit a band with a MW of about 71 kDa that is only found in *E. oxyrrhynchum* (12); the latter species is also characterized by a small unique band of about 14 kDa. Other species are characterized by the absence of bands that are found in the remaining species, for example, a band with a MW of about 94 kDa is only absent from the profile of *E. moschatum* (13).

A UPGMA tree illustrating the relationship between the examined species of *Erodium*, based on morphological criteria, is shown in Fig. 2. In this tree the four species in section Plumosa are delimited together as a separate group from another major group that comprises eight species in section Barbata at a UPGMA distance coefficient of about 1.3. In the former group *E. arborescens*, *E. glaucophyllum* and *E. oxyrrhynchum* are clearly distinguished from the four samples of *E. crassifolium*, which are clustered at a low distance

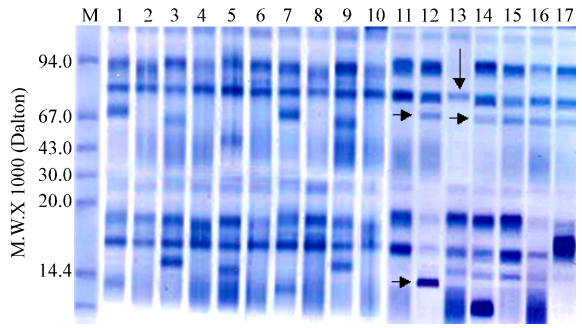

Fig. 1: SDS-PAGE profile of the seed storage protein for the examined taxa of the Egyptian *Erodium*, M refers to the Pharmacia low molecular weight marker and the No. 1-17 refer to the *Erodium* taxa as follows: 1 = *E. ciconium*, 2 = *E. arborescens*, 3 = *E. glaucophyllum*, 4 = *E. laciniatum*, 5 = *E. pulverulentum*, 6 = *E. gruinum*, 7 = *E. neuradifolium* 1, 8 = *E. neuradifolium* 2, 9 = *E. cicutarium*, 10 = *E. malacoides*, 11 = *E. gruinum*, 12 = *E. oxyrrhynchum*, 13 = *E. moschatum*, 14 = *E. crassifolium*1, 15 = *E. crassifolium* 2, 16 = *E. crassifolium* 3, 17 = *E. crassifolium* 4

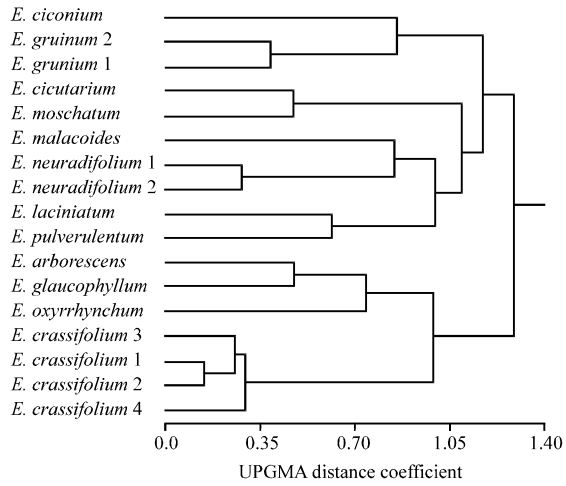

Fig. 2: UPGMA tree illustrating the relationships between the studied samples of *Erodium*, based on variation in morphological characters

coefficient (about 0.3) indicating close morphological resemblance between genotypes of *E. crassifolium* from different localities. The separation of *E. crassifolium* from the other species in section Plumosa is congruent with its distinction by leaf characters (El-Hadidi *et al*., 1983). Leaves in this species are compound bipinnatisect or bipinnatipartite and in the other three species simple undivided or 3-5 lobed.

The delimitation of the species placed in section Barbata, in the UPGMA tree, based on the variation in morphological criteria, generally agrees with their sectional delimitation as proposed by Schönbeck-Temesy (1970). However, *Erodium ciconium* of subsection Absinthioidea and *E. gruinum* of subsection Gruina are separated together from the other species at a distance coefficient of about 1.19. This may be congruent with an earlier view to delimit *E. ciconium* in subsection Gruina (Warburg, 1938). The two species of subsection Cicutaria (*E. cicutarium* and *E. moschatum*) are also recognized as a separate group, from the species of subsection Malacoidea, at a distance of 1.08. The species placed in section Malacoidea are divided into two clusters at a distance of about 1.0; one comprising *E. malacoides* and *E. neuradifolium* and the other *E. laciniatum* and *E. pulverulentum*. The clustering of the latter two species at a distance coefficient of about 0.63 reflects the morphological resemblances between them but does not warrant regarding the latter as a subspecies of the former as done by Boulos (1999). In fact the distance coefficient among *E. laciniatum* and *E. pulverulentum* is higher than other species pairs such as *E. cicutarium* and *E. moschatum* and *E. arborescens* and *E. glaucophyllum* (Fig. 2).

A NJ tree illustrating the relationship between the examined species of *Erodium*, based on the polymorphism in the seed protein electrophoretic profile is shown in Fig. 3. The topology of this tree generally resembles that of the tree based on morphological characters (Fig. 2) in that the examined species are delimited in two groups, one comprising the four species of section Plumosa and the other includes eight species of section Barbata. In the former group the three species *E. arborescens*, *E. glaucophyllum* and *E. oxyrrhynchum* are clearly distinguished from the four samples of *E. crassifolium* at a NJ distance coefficient of about 6.7. With regard to the four samples of the latter species, sample 4, that was collected from the mountains of Saint Catherine in South Sinai and sample 3, that was collected from Al-Arish in North Sinai are clearly distinguished from the other two samples, that were collected from Alexandria Matruh road West of Alexandria, indicating evident polymorphism in this species in the components of the seed storage protein.

In the second group, of the NJ tree based on polymorphism in seed protein electrophoretic profiles (Fig. 3), close relationship is expressed between *E. ciconium*, *E. gruinum* and *E. neuradifolium*. However, the topology of the tree and the relatively high distance between *E. ciconium* and *E. gruinum* do not support the grouping of *E. ciconium* in subsection Gruina as was proposed by Warburg (1938). These species together

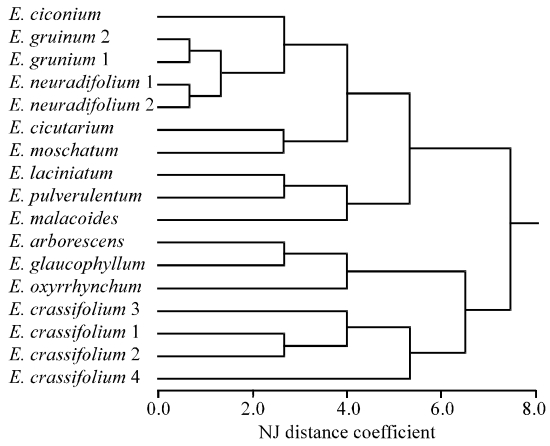

Fig. 3: NJ tree illustrating the relationships between the studied samples of *Erodium*, based on variation in seed protein electrophoretic data

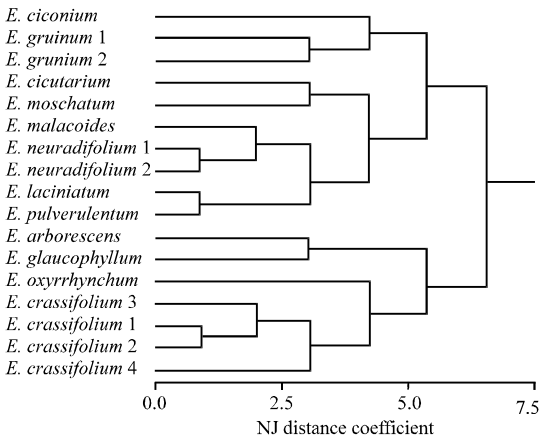

Fig. 4: NJ tree illustrating the relationships between the studied species of *Erodeum*, based on variation in morphological characters and polymorphism in seed protein electrophoretic profiles

form a cluster, at distance coefficient of about 2.7, in a subgroup that also comprises another cluster comprised of *E. cicutarium* and *E. moschatum* at a distance coefficient of 4.0. The delimitation of these species does not agree with their sub sectional delimitation proposed by Schönbeck-Temesy (1970) as given in Table 1. The remaining species of subsection Malacoidea i.e., *E. laciniatum*, *E. pulverulentum* and *E. malacoides* also form a second subgroup in which *E. malacoides* is clearly distinguished from the other two species. The clustering of *E. laciniatum* and *E. pulverulentum* reflects the morphological resemblances between them but does not warrant regarding the latter as a subspecies of the former as proposed by Boulos (1999). In fact the distance coefficient among *E. laciniatum* and *E. pulverulentum* is higher than other species pairs such as *E. cicutarium* and *E. moschatum* and *E. arborescens* and *E. glaucophyllum* (Fig. 3).

A NJ tree illustrating the relationship between the examined taxa of *Erodium*, based on variation in morphological criteria and polymorphism in seed protein electrophoretic profiles, is shown in Fig. 4. In this tree also the species in section Plumosa are clearly delimited as a separate group from species in section Barbata. However, in the group that comprises the species of section Plumosa, *E. arborescens* and *E. glaucophyllum* are distinguished from *E. oxyrrhynchum* that is clustered with, but clearly distinguished from, the four samples of *E. crassifolium*. Sample 4 and sample 3 of the latter species are distinguished from the other two samples reflecting some polymorphism in this species of the components of the seed storage protein (Fig. 1).

The delimitation of the species in section Barbata agrees with their sectional delimitation as proposed by Schönbeck-Temesy (1970). However, *Erodium ciconium* of section Absinthoides and *E. gruinum* of subsection Gruina are separated together from the other species at a NJ distance coefficient of about 4.4. The two species of subsection Cicutaria (*E. cicutarium* and *E. moschatum*) are also recognized as a separate group, from the species of subsection Malacoidea, at the same distance but show close similarity to each other as expressed by the small distance between them (about 3.0). The four species of subsection Malacoidea are divided into two clusters at a distance of about 3.0; one comprising *E. malacoides* and *E. neuradifolium* and the other *E. laciniatum* and *E. pulverulentum*. The small distance coefficient that separates the latter two species supports their consideration as one species as proposed by Boissier (1867). This view has been also recently supported by Boulos (1999), who regarded *E. laciniatum* in Egypt as two subspecies; ssp. *laciniatum* and ssp. *pulverulentum* (Boiss.).

The delimitation of some species in the NJ tree does not agree with their subsectional delimitation. This tree indicates similarities between *E. neuradifolium* of subsection Malacoidea and both *E. ciconium* (subsection Absinthioidea) and *E. gruinum* (subsection Gruina) as well as *E. cicutarium and E. moschatum* of subsection Cicutaria. The remaining species of subsection Malacoidea i.e., *E. laciniatum*, *E. pulverulentum* and *E. malacoides* formed a cluster in which *E. malacoides* is clearly distinguished from the other two species. Meanwhile, in this tree, the two species *E. laciniatum* and *E. pulverulentum* are grouped together, which is congruent with the morphological resemblances between them. The grouping of these two species

however, does not support the view of Boulos (1999) regarding the latter as a subspecies of the former.

In conclusion, the relationships between the Egyptian species of *Erodium*, based on variation in morphological characters and polymorphism in seed protein components, agree with their delimitation in the two sections Barbata and Plumosa. However, the delimitation of species in section Barbata differs from their delimitation in four subsections as proposed by Schönbeck-Temesy (1970) and El-Hadidi *et al.* (1983).

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