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Pollen and Seed Micromorphology of the some *Erodium* L'Herit (Geraniaceae) Species in Hatay Province

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ABSTRACT: In this study, pollen and seed micromorphology of 7 naturally distrubuted *Erodium* species (*E. acaule, E. amanum, E. botrys, E. cucitarium, E. gruinum, E. malacoides* and *E. moschatum*) in Hatay Province (Turkey) have been studied. Palynological analysis showed that pollen grains were generally medium, spheroidal and spheroidal subprolate, radially symmetrical, tricolporate and reticulate-clavate ornamentation. The seed characters were found ovate-eliptic and oblanceolate in shape, with rugose-foveate, reticulate-foveate and bireticulate-foveate ornamentation. The results demonstrated that characters such as pollen size, pollen shape, seed size, seed shape, seed surface ornamentation and seed cell pattern were found important and useful for the identification of *Erodium* species investigated.

Key Words: *Erodium*, pollen morphology, seed morphology, SEM, Hatay.

Hatay Yöresinde Yayılış Gösteren Bazı *Erodium* L'Herit (Geraniaceae) Türlerinin Polen ve Tohum Mikromorfolojileri

ÖZET: Bu çalışmada, Hatay'da doğal yayılış gösteren 7 *Erodium* türünün (*E. acaule* (L.) Becherer & Thell, *E. amanum* Boiss & Kotschy, *E. botrys* (Cav.) Bertol, *E. cucitarium* (L.) La'Herit, *E. gruinum* (L.) La'Herit, *E. malacoides* (L.) La'Herit ve *E. moschatum* (L.) La'Herit) polen ve tohum mikromorfolojileri incelenmiştir. Palinolojik analiz sonuçlarına göre polenlerin genellikle radial simetrik, medium büyüklükte, sferoidal ve sferoidal-subprolat şekillerde, trikolporat tipte ve retikulat-klavat yüzey ornamentasyonuna sahip olduğu belirlenmiştir. Tohum özellikleri incelendiğinde tohumların ovat-eliptik ve oblanseolat şekillerde ve rugose-foveat, retikulat-foveat ve biretikulat-foveat ornamentasyona sahip olduğu belirlenmiştir. Sonuçlar genel olarak değerlendirildiğinde polen şekli ve boyutu, tohum şekli ve boyutu, tohum yüzey süslemesi ve yüzey epidermal hücrelerin şeklinin incelenen *Erodium* türlerinin taksonomik ayrımında önemli ve kullanışlı olduğu belirlenmiştir.

Anahtar kelimeler: *Erodium*, polen morfolojisi, tohum morfolojisi, SEM, Hatay.

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INTRODUCTION

The genus *Erodium* L'Herit contains annual, biennial and perennial species and it is distributed on all continents except the Antarctica. Mediterranean region is a major center of diversity of the genus with 74 taxa (Fiz et al. 2006). The genus is divided into two sections (sect. *Plumosa* and sect. *Erodium*) and represented by 30 taxa with 25 species in Turkey, and 16 of these taxa are endemic (endemism rate 53%) (Davis 1967; Davis et al., 1988; Güner, 2000; Yıldırımlı and Doğru Koca, 2004; Günenç, 2015; Oskay et al., 2018). Some *Erodium* species have been used to bleeding and diarrhea (Baytop 1999), chronic and acute rheumatic (Hussein 1985), prostate and other urinary tract diseases (Mosaddegh et al., 2012), and as antiviral and antioxidant (Sroka et al., 1994) in traditional medicine.

The pollen morphology of the Geraniaceae has been studied by many previous studies such as Erdtman (1952), Moore and Webb (1978), Oltmann (1967), Kuprianova and Alyoshina (1972), Hutchinson (1969) and Bortenschlager (1967). There are many taxonomical studies about *Erodium* species such as Raina (1985) carried out monographic studies of Geraniaceae family from Kashmir (India) and studied pollen and seed characters of *E. cicutarium* and *E. tibetianura* species. Ou and Kao (1994) analyzed general taxonomic and morphological properties of *Erodium moschatum* in Taiwan. Özbucak (1995) studied taxonomic and morphological characters of some *Erodium* species in Central Black Sea Region of Turkey. Parmaksız (1997) analyzed general pollen characters and pollen type of *Erodium acaule, E. cucitarium* and *E. ciconium* species in Tokat (Turkey). Shehata (2008) analyzed nine *Erodium* species in Egypt to find out the evidence of possible taxonomic significance. Naggar (2008) investigated seed morphology and seed coat sculpture of 14 native species of *Erodium* in Egypt and he emphasised that size, shape, epidermal cell patterns of seeds were important for taxonomic of *Erodium* species. Recently, several studies about morphology, autecology, conservation biology, caryology, fruit, seed and pollen micromorphology of *E. somanum* and *E. pelargoniiflorum* carried out by Oskay (2010; 2017), Oskay et al. (2011; 2018). Ather et al. (2012) prepared palynology atlas of Pakistan and studied pollen morphology of *E. cucitarium* and *E. malacoides* species. Francis et al. (2012) studied general morphological, taxonomical, palinological properties of *E. cucitarium* in Canada.

Despite of many comprehensively studies which are carried out on morphological and taxonomical specifications of *Erodium* species, studies about palynological and seed micromorphological specifications of species are limited. Pollen and seed morphology are very important to characterize and classify any plant properly. The aim of the study is to expand morphological descriptions of studied species which includes their detailed pollen and seed specifications. These informations will be very useful for further studies on *Erodium* species. Also, these characters are also required for database preparation in this digital world by which further experiments or research will be done.

To the best our knowledge this is the first study about detailed micromorphology on pollen and seeds of *E. amanum* Boiss & Kotschy and *E. botrys* (Cav.) Bertol species and their systematic implications.

MATERIALS AND METHODS

The study is based on pollen and seed morphology of seven wild *Erodium* species from Hatay province (Table 1). The study area is located in the East Mediterranean region (35°48′-37°00′N latitude and 35°46′-36°41′ E longitude). It has a typical Mediterranean climate, with an annual average temperature of 18.1 °C and average annual rainfall of about

1.078 mm, most of which falls during winter months. The temperatures varies 7-32 °C experienced during January and July respectively (Altay et al., 2016).

The examined seed and pollen samples were collected from their natural habitats (Table 1). The plant specimens have identified by using the keys in Flora of Turkey and The East Aegean Islands (Davis, 1967). All pollen grains for light microscope examination were prepared according to the standard procedure of Wodehouse (1935). In the palynological study, suitable samples were taken from these materials for SEM and light microscope (Olympus CX21FS1). Polar length (P), equatorial width (E), P/E ratio, exhin and intin thickness, colpus and porus length (clg and plg), colpus and porus width (clt and plt) were measured on the average on 30 different pollen grains, and were assessed by the biometric method. Arithmetic means and standard deviations were also calculated. For SEM, pollen and seed samples were directly mounted on stubs. Samples were coated with gold in POLARON SC 7620 ionsputter and then observed by standard techniques using a scanning electron microscope (JEOL JSM-5500 LV). Erdtman (1952) and Punt et al. (2007) was used for pollen terninology and Barthlott (1981, 1984) was used for seed terminology.

Table 1. Localities of the studied *Erodium* taxa in Hatay province.

Taxa	Locations
E. acaule (L.) Becherer & Thell	Erzin district, İçmeler, road side 323 m. 15.03.2015
E. amanum Boiss & Kotschy	Belen district, Atik plateau, rocky slopes 980 m. 05.07.2015
E. botrys (Cav.) Bertol	Yayladağı district, Kışlak, dry woodland, 715 m. 12.04.2015
Erodium cicutarium (L.) La'Herit subsp. *cucitarium*	Antakya district, Antakya Castle, grassland, 440 m. 14.04.2015
E. gruinum (L.) La'Herit	Yayladağı district, Yayladağı Barrage, rocky slopes, 800 m. 12.04.2015
E. malacoides (L.) La'Herit	Kırıkhan district, Alan plateau, rocky slopes, 1123 m. 02.04.2015
E. moschatum (L.) La'Herit	Kırıkhan district, Çataltepe, grassland, 180 m. 02.04.2015

RESULTS AND DISCUSSION

Pollen morphology

All palynological analysis has shown that the pollen grains are monad, isopolar, radially symmetrical. Pollen size measured as medium in the all species, while pollen shape found spheroidal and spheroidal-subprolate (Figure 1). According to our results, the biggest pollen size was found in *E. gruinum* (99.98 × 92.91 µm) and the smallest in *E. moschatum* (55.75 × 52.81 µm). The pollen shapes were generally spheroidal, those of in *E. botrys*, *E. gruinum* and *E. moschatum* taxa were spheroidal-subprolate. Their apertures were found to be tricolporate and equatorial views were circular and exine ornamentations were striate-reticulate in the all species (Figure 1). P/E ratio varied from 1.00 (*E. acaule*) to 1.07 (*E. gruinum*). Exine thicknesses ranged from 3.53 µm (*E. amanum*) to 4.32 µm (*E. gruinum*); intine thicknesses ranged from 1.38 µm (*E. acaule*) to 2.20 µm (*E. cicutarium* subsp. *cicutarium*) respectively. The colpus lenght (clg) varied from 9.99 µm (*E. malacoides*) to 25.12 µm (*E. gruinum*); and colpus windth (clt) varied from 3.45 µm (*E. cicutarium* subsp. *cicutarium*) to 7.23 µm (*E. gruinum*). The porus lenght (plg) varied from 10.76 µm (*E. cicutarium* subsp. *cicutarium*) to 16.66 µm (*E. botrys*); and porus windth (plt) varied from 4.53 µm (*E. moschatum*) to 8.97 µm (*E. botrys*). Detailed results of palynological analyses were given in Table 2.

Raina (1985) determined that *E. cucitarium* had suboblate to oblate-spheroidalpollen grains, while we have found that this species had only spheroidal pollen grains. Exine ornamentation were medium size,

striate-reticulate surface. Similarly, Francis et al. (2012) reported that *E. cucitarium* had tricolporat and striate-reticulate surface and their results are similar to ours. Shehata (2008) studied pollen morphology of the Geraniaceae family from Egypt and similarly identified three types and three subtypes, based on the exine ornamentation. Although in that study it was reported that *E. cucitarium*, *E. gruinum, E. malacoides* and *E.moschatum* had prolate-spheroidal pollen grains, we have found that *E. cucitarium* and *E. moschatum* spheroidal pollen grains, while *E. gruinum* and *E. malacoides* spheroidal-subprolate pollen grains. The study emphasised that, pollen size (P×E) measured as 5236 µm in *E. cucitarium*, as 11766 µm in *E. gruinum*, as 5159 µm in *E. malacoides* and as 3360 µm in *E. moschatum*. However, in our study *E. cucitarium* as 2994 µm, *E. gruinum* as 9290 µm, *E. malacoides* as 3394 µm and *E. moschatum* as 2944 µm were found to be smaller size. Also, they reported that these species had striate-reticulate surface and it is in agreement with our results. Our results are consistent with previous study. These results show that there are several pollen characters of taxonomic significance in *Erodium.* The main palynological differences have been found especially in the pollen size and shape.

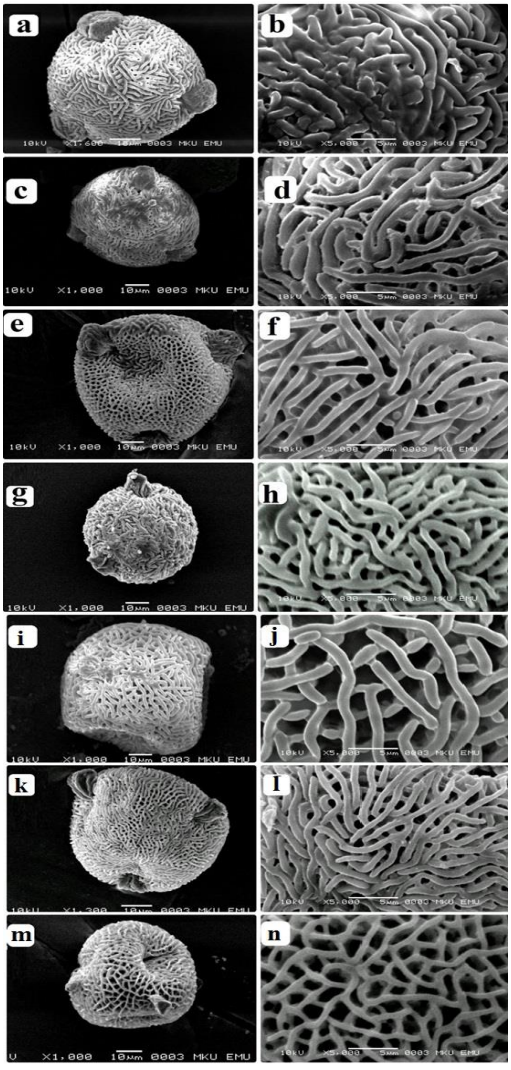

Figure 1. Pollen morphology of *Erodium* species (a-b: *E. acaule*, c-d: *E. amanum*, e-f: *E. botyrs*, g-h: *E. cicutarium*, i-j: *E. grunium*, k-l: *E. malacoides*, m-n: *E. moschatum*

Table 2. Pollen morphological characters of the *Erodium* species studied.

Taxa	*E. acaule*	*E. amanum*	*E. botrys*	*E. cicutarium*	*E. gruinum*	*E. malacoides*	*E. moschatum*
P (µm)	55.55±3.64	56.40±2.59	69.66±3.93	56.01 ±3.31	99.98±4.01	59.97 ±2.98	55.75± 3.16
E (µm)	55.18±3.09	55.02±2.57	65.91±3.60	53.47±3.42	92.91±4.62	56.58±3.32	52.81±3.15
P/E	1.00	1.02	1.05	1.04	1.07	1.06	1.05
Shape	Sp	Sp	Sp-Subpro	Sp	Sp-Subpro	Sp-Subpro	Sp
Clg (µm)	11.15±1.12	11.02±0.96	10.76±0.82	11.14±0.54	25.12±3.20	9.99±1.04	11.27±1.41
Clt (µm)	4.99±1.83	6.50±7.34	4.76±1.48	3.45±0.54	7.23±1.17	3.84±0.77	3.96±1.23
Plg (µm)	11.53±1.08	12.15±1.28	16.66±2.35	10.76±2.30	11.91±0.54	12.81±1.77	11.60±0.79
Plt (µm)	5.72±0,86	5.61±0.74	8.97±1.93	5.38±2.03	6.53±0.54	6.15±0.66	4.53±0.56
Exine (µm)	3.87±0.64	3.53±0.83	4.27±1.01	4.26±0.72	4.32±0.65	3.98±0.90	3.79±0.62
Intine (µm)	1.38±0.46	1.65±0.41	1.88±0.69	2.20±0.62	1.57±0.58	1.79±0.50	1.42±0.45

Abbreviations: P – polar axis; E – equatorial axis; Clg – length of colpi; Clt – width of colpi; Plg – length of porus; Plt – width of porus; Sp – Spheroidal, Sp-Subpro – Spheroidal-subprolate

Seed morphology

The seeds dehisceing along with mericarps and seed surface of the all species were glabrous. The seed features of possible taxonomic importance in the examined taxa are given in Table 3. The seeds were 2.69 to 5.32 mm in length and 0.75 to 1.72 mm in width. Seed sizes varied greatly among the all species, with the smallest seeds in *E. acaule* (2.95 × 0.75 mm), and the largest in *E. amanum* (5.32 × 1.69 mm). Seed shape were found ovate-elliptic or oblanceolate. The seed coat surfaces of the examined taxa were determined as reticulate-foveate, rugose-foveate or bireticulate-foveate (Figure 2). The hilum were usually distinct and have basal position in all species. The periclinal wall of the cells is variously concave or flattened. The shape of the epidermal cells showed significant variation among the examined species. Rectangular cells in *E. acaule*, *E. cucitarium* and *E. malacoides*, square-polygonal cells in *E. gruinum* and *E. amanum*, while polygonal cells observed in *E. moschatum* and *E. botrys* taxa (Figure 2).

In recent years, many workers studied seed morphology of *Erodium* species. For example, *E. cucitarium* described as conical seed shape, indistinct hilum, reticulate-areolate seed surface and elongate cell shape in Raina (1985); as narrow ovoid to oblanceolate seed shape, 3 × 1 mm, brown color, foveate seed surface and polygonal cell shape in Naggar (1992); as obovate seed shape, 2.60-3.05 × 0.85-1.10 mm, dark brown, distinct hilum and scalariform-reticulate seed surface in Parmaksız (1997); as narrow ovoid seed shape, 3 × 1 mm and light brown color in Francis et al. (2012); as oblanceolate seed shape, 2.6-3 × 0.6-1 mm, brown color, basal hilum and foveate seed surface in Ather et al. (2012). Similarly, we have found that this species had oblanceolate shape, 2.95 × 0.90 mm, brown color, basal hilum, reticulate foveate surface and rectangular cell shape.

Table 3. Seed morphological characters of the *Erodium* species studied.

Taxa	Seed shape	Seed length (mm)	Seed width (mm)	L/W seed	Seed surface	Cell shape
E. acaule	Ovate-eliptic	2.95±0.19	0.75±0.14	3.9	Rugose-foveate	Rectangular
E. amanum	Ovate-eliptic	5.32±0.42	1.69±0.20	3.1	Reticulate-foveate	Square-Polygonal
E. botrys	Oblanceolate	3.53±0.50	0.75±0.20	4.7	Rugose-foveate	Polygonal
E. cicutarium	Oblanceolate	2.95±0.10	0.90±0.12	3.3	Reticulate-foveate	Rectangular
E. gruinum	Oblanceolate	4.58±0.40	1.72±0.31	2.7	Bireticulate-foveate	Square-Polygonal
E. malacoides	Oblanceolate	2.69±0.23	0.83±0.12	3.2	Reticulate-foveate	Rectangular
E. moschatum	Oblanceolate	3.24±0.20	0.89±0.12	3.6	Rugose-foveate	Polygonal

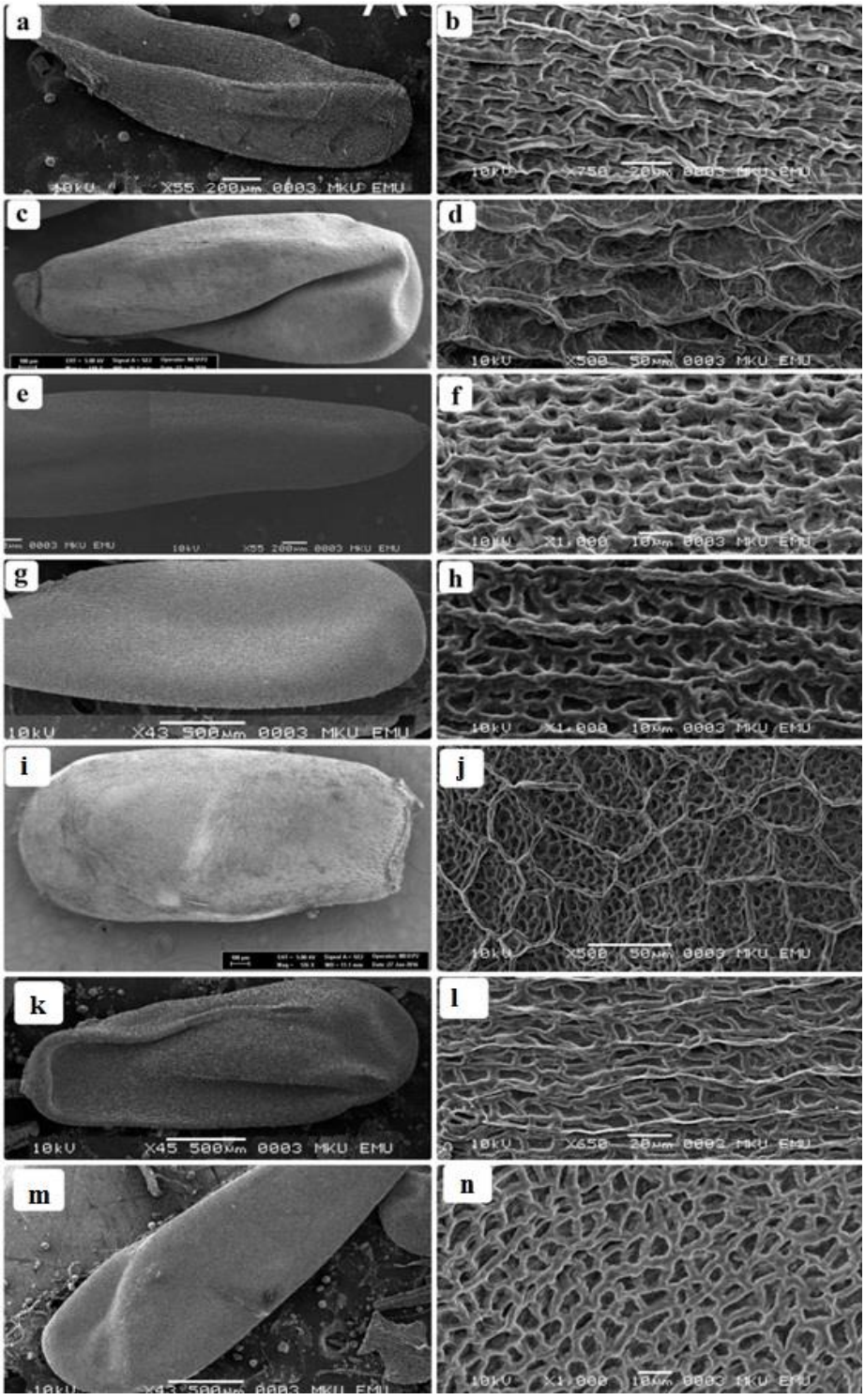

Figure 2. Seed micromorphology of *Erodium* species (a-b: *E. acaule*, c-d: *E. amanum*, e-f: *E. botyrs*, g-h: *E. cicutarium*, i-j: *E. grunium*, k-l: *E. malacoides*, m-n: *E. moschatum*)

Parmaksız (1997) reported that *E. acaule* had obovate shape, 2.3-2.7 × 0.7-0.9 mm, brown color, distinct hilum, scalariform-reticulate surface while we have found that ovate-eliptic shape, 2.95 × 0.75 mm, brown color, distinct hilum, rugose foveate surface and rectangular cell shape. Our results are similar to his results except shape and surface of seed. Although, Naggar (1992) emphasised that *E. grunium* had obclavate or cylindrical seed shape, 5.5 × 2 mm, brown, reticulate seed surface and polygonal cell shape, *E. moschatum* had narrow obovate seed

shape, 3 × 0.9 mm, brown, undulate seed surface and polygonal cell shape, and *E. malacoides* had obclavate or cylindrical seed shape, 5.5 × 2 mm, brown, reticulate seed surface and polygonal cell shape. Also, Ather et al. (2012) determined that *E. malacoides* had oblanceolate seed shape, 2.5-2.7 × 0.7-0.8 mm, dark brown, with basal hilum and scalariform seed surface. Similarly, the present study found that *E. grunium* had oblanceolate seed shape, 4.58 × 1.72 mm, brown, bireticulate-foveate seed surface and square-polygonal cell shape, *E. moschatum* had oblanceolate seed shape, 3.24 × 0.89 mm, brown, rugose-foveate seed surface and polygonal cell shape and *E. malacoides* had oblanceolate seed shape, 42.69 × 0.83 mm, brown, reticulate-foveate seed surface and rectangular cell shape.

These previous studies found data that could be useful in providing additional information for taxonomic delimitation at various levels in the genus. The findings of seed characteristics in this study are compared with the findings of seed characteristics by related previous studies, and it is seen that genus *Erodium* has its own distinct characters. These micromorphological characters like seed shape, size, surface ornamentation and epidermal cell patterns have revealed differences between *Erodium* species. Similarly, our results shown that seed size and surface ornamentation were significant characters of the investigated species. Our results are generally consistent with previous study.

This study is aimed to introduce pollen and seed micromorphological details of some *Erodium* species. The morphological characters of the investigated species were similar to those reported by various botanist in recent years. However, morphological findings including the pollen and seed features of *E. amanum* and *E. botrys* species was presented for the first time.

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