Turk J Bot
31 (2007) 171-182

Research Article

Pollen Morphology of the Turkish *Romulea* Maratti (Iridaceae)

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Received: 01.12.2006
Accepted: 28.02.2007

Abstract: Pollen morphology of the Turkish *Romulea* Maratti taxa (Iridaceae) was examined using light and scanning electron microscopy. The taxa are similar in some aspects, such as pollen size and spinulate-microperforate exine sculpturing. Most taxa are monosulcate; however, in 2 taxa, *R. bulbocodium* (L.) Seb. & Mauri var. *crocea* (Boiss & Heldr.) Baker and var. *leichtliniana* (Heldr. ex Hal.) Bég., growing sporadically in south-western Turkey, the existence of pollen type variability (monosulcate, disulcate, trisulcate, trisynsulcate, tetrasulcate, penta-aperturate (with longer and shorter sulci), and monoporate) from single pollen sacs was recorded for the first time. In addition, aperture morphology was found to be variable within most taxa; operculate with 2 bands (bands are free or joined), operculate with 1 band (band is straight or curved), operculate with a circular band, or occasionally insulate.

Key Words: Iridaceae, *Romulea*, pollen morphology, Turkey

Türkiye'deki *Romulea* Maratti Taksonlarının Polen Morfolojisi

Özet: Bu çalışmada Türkiye'de yetişen *Romulea* (Iridaceae) taksonlarının polen morfolojileri ışık ve elektron mikroskobu ile incelenmiştir. Taksonların polenleri polen boyutu ve spinulat-mikroperforat ekzin süslenmesi gibi bazı özellikler bakımından benzerlik göstermektedir. Taksonların çoğunun polenleri monosulkattır. Bununla birlikte, cinsin güneybatı Anadolu'da seyrek olarak yayılış gösteren iki taksonunda (*R. bulbocodium* (L.) Seb. & Mauri var. *crocea* (Boiss & Heldr.) Baker ve var. *leichtliniana* (Heldr. ex Hal.) Bég.) aynı polen kesesinden alınan polenlerin monosulkat, disulkat, trisulkat, trisinsulkat, tetrasulkat, pentaaperturat (kısa ve uzun sulkuslara sahip) yada monoporat olarak değişkenlik gösterdiği ilk kez belirlenmiştir. Ayrıca, apertür morfolojisinin çoğu taksonda değişken olduğu ortaya konmuştur. Apertür iki bantlı operkulat (bandlar serbest yada birbirine bağlı), tek bantlı operkulat (band düz ya da kıvrılmış), halka şeklinde bantlı operkulat, ya da nadiren insulat özellikler göstermektedir.

Anahtar Sözcükler: Iridaceae, *Romulea*, polen morfolojisi, Türkiye

Introduction

Romulea Maratti is a member of the subfamily Crocoideae (syn. Ixioideae) in the family Iridaceae. The genus comprises approximately 90 species, most of which are found in southern Africa (Manning & Goldblatt, 2001). Some 12 to 15 species occur in the Mediterranean Basin, Near East, Canary Islands, the Azores, and southern Europe. The southern African and the few tropical African species are uniformly hermaphroditic and self-compatible, but, in the Mediterranean region, reproductive systems include hermaphroditism and gynodioecy (Moret et al., 1992). *Romulea bulbocodium* and its variants have been cultivated for their showy flowers in Europe (Dahlgren et al., 1985), but have never been cultivated in Turkey.

In the Turkish flora 7 *Romulea* taxa (*R. bulbocodium* (L.) Seb. & Mauri var. *bulbocodium*, var. *crocea* (Boiss & Heldr.) Baker, var. *leichtliniana* (Heldr. ex Hal.) Bég., *R. tempskyana* Freyn, *R. linaresii* Parl. subsp. *graeca* Bég., *R. ramiflora* Ten. subsp. *ramiflora*, and *R. columnae* Seb. & Mauri subsp. *columnae*) have been recognised by Marais (1984). He stressed that the taxonomic treatment is made difficult in the eastern Mediterranean due to the occurrence of gynodioecism. Yet, this case has not been

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observed in Turkish species to date. Nevertheless, the reproductive systems of some *Romulea* species living in other parts of the Mediterranean region have been investigated. Moret et al. (1992, 1993) studied the reproductive systems of several *Romulea* species growing in Morocco and proposed an evolutionary interpretation based on the degree of herkogamy and gynodioecy in different species. Moussel et al. (1996) reported the stigma and style morphohistological features and various pollination modes of 6 species of *Romulea* found in France.

Pollen morphology of *Romulea* species that occur in Spain was examined by Martin Cacao & Fernández (1990), while that of the North African species was studied by A. Bari, but is as yet unpublished. Goldblatt et al. (1991) carried out a pollen morphological investigation of some selected African *Romulea* species.

Previous studies of the Turkish *Romulea* taxa focused only on morphological observations by Marais (1984) and anatomical observations by Erol & Küçüker (2003). The primary aim of the present study was to examine the pollen grains of the Turkish taxa of the genus in detail in order to provide palynological information. It was also aimed to make contributions to the knowledge of pollen morphology of the Mediterranean taxa of the genus.

Materials and Methods

The pollen material was taken from herbaria (the Herbarium of Gazi University-GAZI, of Hacettepe University-HUB, and İstanbul University-ISTF). The voucher specimens are listed below.

In all, 13 specimens from 7 taxa were examined. More than one specimen per taxon, with the exception of *R. bulbocodium* var. *bulbocodium* and var. *leichtliniana*, were investigated to determine possible infraspecific variation. Unfortunately, availability of the taxa mentioned above is limited.

For light microscopy (LM) study, the pollen from single pollen sacs of each sample was first treated with 70% alcohol to remove oily substances and was then embedded in glycerine-jelly stained with safranin. The following parameters were measured: pollen size (long axis (LA) and short axis (SA), sulcus width, pore width, operculum length and width (if straight), and interopercular distance when the operculum was 2-banded, number of spinules per 100 μm^2, exine (sexine and nexine) thickness, and intine (exintine and endintine) thickness. Reported pollen size represents the mean of about 50 pollen grains. About 10 measurements for the other parameters were obtained. To determine pollen type and operculum type, 2 pollen slides for each sample were examined from traverse covering the whole slide. The spinule number per 100 μm^2 was counted using the Leica Q-win programme. Photomicrographs were made with a Nikon E 600 microscope.

Scanning electron microscopy (SEM) was also used to investigate exine patterning in detail. The pollen was mounted on stubs with gold after treatment with 70% alcohol and air-drying, and photomicrographs were taken with a Jeol JSM-5600 electron microscope.

The palynological terminology mainly follows Punt et al. (1994).

Specimens investigated

The taxa are in taxonomic order as given by Marais (1984).

R. bulbocodium (L.) Seb. & Mauri var. *bulbocodium*: B1 İzmir: O. Erol 1 (ISTF).

R. bulbocodium (L.) Seb. & Mauri var. *crocea* (Boiss & Heldr.) Baker: C3 Antalya: A.A. Dönmez 4369 (HUB), C3 Antalya: A. Duran 2062 (GAZI), C3 Antalya: O. Erol 2 - A. Kılıç (ISTF).

R. bulbocodium (L.) Seb. & Mauri var. *leichtliniana* (Heldr. ex Hal.) Bég.: C6 Hatay: O. Erol 3 (ISTF).

R. tempskyana Freyn: C2 Antalya: A.A. Dönmez 4298 (HUB), C2 Muğla: A. Güner 8100-M. Vural, H. Duman, A.A. Dönmez (HUB).

R. linaresii Parl. subsp. *gracea* Bég: A1 Çanakkale: A.A. Dönmez 10432 (HUB), A1 Edirne: A. Akyol 2 (HUB).

R. ramiflora Ten. subsp. *ramiflora*: B1 Balıkesir: O. Erol 4 (ISTF), B1 Balıkesir: O. Erol 5 (ISTF).

R. columnae Seb. & Mauri subsp. *columnae*: A1 Çanakkale: A.A. Dönmez 10433 (HUB), B1 İzmir: Ş. Yıldırımlı 1258 (HUB).

Results

General pollen morphological features

Some pollen morphological features of the Turkish *Romulea* taxa examined in this study under LM are summarised in Table 1. A general description is given below.

Pollen grains are typically ellipsoid. Long axis ranges from 40.5 to 77.5 µm, while short axis ranges from 36 to 70 µm. Pollen type is predominantly monosulcate (Figures 1a-b, 4a). In *R. bulbocodium* var. *crocea* and var. *leichtliniana* pollen types are variable: monosulcate, disulcate (Figure 1c), trisulcate (Figure 1d), trisynsulcate (Figure 1e), tetrasulcate (Figure 1f), and monoporate (Figure 1h-i). In addition to these pollen types, the penta-aperturate (with longer and shorter sulci) type was also recorded in the latter (Figure 1g).

In sulcate pollen grains the sulcus is longer than the long axis of the pollen grain because it extends to the proximal face. It is usually very wide (8-40 µm). In porate pollen grains pore width ranges from 5 to 38 µm.

The aperture is mostly operculate, which is surrounded by small exine islands (Figure 2a-b), or occasionally insulate (Figure 2c). In sulcate-operculate pollen grains, operculum length ranges from 25 to 77 µm, while operculum width ranges from 2 to 8 µm. Interopercular distance of 2-banded opercula is between 0.5 and 12 µm.

Exine is semi-tectate. Sexine (1.5-2.25 µm) is thicker than nexine (< 0.5 µm). Exine sculpturing is spinulate-microperforate under SEM (Figure 4b). Spinule number per 100 μm^2 ranges from 15 to 58. The exine plate irregularly protrudes towards the aperture(s) (Figure 2d).

The intine is thick. The exintine is 0.5-3.5 µm and endintine is 0.5 µm thick. The former thickens at the aperture(s) up to 7 µm.

Operculate aperture morphology

Operculate aperture morphology is remarkably variable in most taxa, even within taxa, while the opercular exine has the same exine sculpturing as the rest of the grains in all the taxa.

Table 1. Some pollen morphological features of the Turkish *Romulea* taxa examined under LM.

Taxa	Specimen	LA µm	SA µm
Romulea bulbocodium var. *bulbocodium*	B1 İzmir: O. Erol 1	(57.5)-63.6-(70)	(41)-58.45-(67.5)
var. *crocea*	C3 Antalya: A. A. Dönmez 4369	(51)-58.44-(70)	(51)-57.74-(59)
	C3 Antalya: O. Erol 2 - A. Kılınç	(51)-58.66-(73)	(46)-57.2-(68)
	C3 Antalya: A. Duran 2062	(57)-64.16-(73)	(51)-58.08-(70)
var. *leichtliniana*	C6 Hatay: O. Erol 3	(51)-56.34-(66)	(47)-59.90-(70)
R. tempskyana	C2 Antalya: A. A. Dönmez 4298	(57.5)-64.15-(72.5)	(50)-56.07-(63.7)
	C2 Muğla: A. Güner 8100 et al.	(58.5)-62.28-(65.25)	(45)-54.09-(58.5)
R. linaresii subsp. *graeca*	A1 Çanakkale: A. A. Dönmez 10432	(47.5)-54.42-(77.5)	(43.75)-50.17-(67.5)
	A1 Edirne: A. Akyol 2	(45)-51.12-(63)	(38.25)-47.25-(54)
R. ramiflora subsp. *ramiflora*	B1 Balıkesir: O. Erol 4	(47)-51.90-(66)	(44)-47.16-(53)
	B1 Balıkesir: O. Erol 5	(47)-52.60-(61)	(39)-44.08-(58)
R. columnae subsp. *columnae*	A1 Çanakkale: A. A. Dönmez 10433	(45)-60.8-(72.5)	(43.7)-56.45-(65)
	B1 İzmir: Ş. Yıldırımlı 1258	(40.5)-44.82-(51.75)	(36)-41.22-(47.25)

Abbreviations:
LA: Long axis
SA: Short axis

Table 1. continued

Taxa	Specimen	Pollen type	Slg
Romulea bulbocodium var. *bulbocodium*	B1 İzmir: O. Erol 1	ms	> LA
var. *crocea*	C3 Antalya: A. A. Dönmez 4369	ms (13%), ds (18%), ts (53%), tss (16%)	> LA
	C3 Antalya: O. Erol 2 - A. Kılınç	ms (16%), ds (26%), ts (16%), tss (26%), trs (6%), mp (10%)	> LA
	C3 Antalya: A. Duran 2062	ms (20%), ds (40%), ts (13%), tss (7%), trs (13%), mp (7%)	> LA
var. *leichtliniana*	C6 Hatay: O. Erol 3	ms (17%), ds (24%), ts (26%), tss (9%), trs (20%), pta (2%), mp (2%)	> LA
R. tempskyana	C2 Antalya: A. A. Dönmez 4298	ms	> LA
	C2 Muğla: A. Güner 8100 et al.	ms	> LA
R. linaresii subsp. *graeca*	A1 Çanakkale: A. A. Dönmez 10432	ms	> LA
	A1 Edirne: A. Akyol 2	ms	> LA
R. ramiflora subsp. *ramiflora*	B1 Balıkesir: O. Erol 4	ms	> LA
	B1 Balıkesir: O. Erol 5	ms	> LA
R. columnae subsp. *columnae*	A1 Çanakkale: A. A. Dönmez 10433	ms	> LA
	B1 İzmir: Ş. Yıldırımlı 1258	ms	> LA

ds: Disulcate
mp: Monoporate
ms: Monosulcate
pta: Penta-aperturate (with longer and shorter sulci)
Slg: Sulcus length
trs: Tetrasulcate
ts: Trisulcate
tss: Trisynsulcate

Table 1. continued

Taxa	Specimen	Slt µm	Plt µm	Operculum type
R. bulbocodium var. *bulbocodium*	B1 İzmir: O. Erol 1	(20)-29.90-(38)	-	2-b/ insulate
var. *crocea*	C3 Antalya: A. A. Dönmez 4369	(13)-17.85-(25)	-	2-b/1-b
	C3 Antalya: O. Erol 2 - A. Kılınç	(8)-10.8-(16)	(5)-18.05-(35)	2-b/1-b
	C3 Antalya: A. Duran 2062	(8)-10.4-(13)	(11)-18.3-(28)	2-b/1-b
var. *leichtliniana*	C6 Hatay: O. Erol 3	(13)-17.40-(26)	(9)-18.13-(38)	2-b/1-b
R. tempskyana	C2 Antalya: A. A. Dönmez 4298	(29)-34.75-(40)	-	2-b/ insulate
	C2 Muğla: A. Güner 8100 et al.	(19)-23.40-(33)	-	2-b/ insulate
R. linaresii subsp. *graeca*	A1 Çanakkale: A. A. Dönmez 10432	(12)-17.65-(24)	-	2-b/1-b
	A1 Edirne: A. Akyol 2	(14)-21.85-(31)	-	2-b/1-b
R. ramiflora subsp. *ramiflora*	B1 Balıkesir: O. Erol 4	(20)-25.60-(34)	-	2-b/1-b
	B1 Balıkesir: O. Erol 5	(12)-21.30-(39)	-	2-b/1-b
R. columnae subsp. *columnae*	A1 Çanakkale: A. A. Dönmez 10433	(23)-29.60-(38)	-	2-b/1-b/ insulate
	B1 İzmir: Ş. Yıldırımlı 1258	(15)-23.15-(32)	-	2-b/1-b/ insulate

Plt: Pore width
Slt: Sulcus width
2-b: two-banded
1-b: one-banded

Table 1. continued

Taxa	Specimen	Operculum band (s)		
		Oplg µm	Oplt µm	IOD µm
R. bulbocodium var. *bulbocodium*	B1 İzmir: O. Erol 1	(48)-56.58-(68)	(4)-5.05-(8)	(3)-7.95-(12)
var. *crocea*	C3 Antalya: A. A. Dönmez 4369	(30)-37.10-(54)	(3)-4.07-(6)	(2)-4.35-(8)
	C3 Antalya: O. Erol 2 - A. Kılınç	(40)-46.16-(54)	(2)-3.12-(4)	(1)-1.60-(3)
	C3 Antalya: A. Duran 2062	(59)-60-(61)	(3)-3.75-(5)	(0.5)-1.32-(2)
var. *leichtliniana*	C6 Hatay: O. Erol 3	(31)-40.50-(58)	(2)-3.02-(4)	(2)-2.70-(4)
R. tempskyana	C2 Antalya: A. A. Dönmez 4298	(48)-56.15-(64)	(4)-4.47-(5)	(6)-8.65-(12)
	C2 Muğla: A. Güner 8100 et al.	(50)-59.77-(77)	(3)-4.7-(6)	(3)-4.4-(7)
R. linaresii subsp. *graeca*	A1 Çanakkale: A. A. Dönmez 10432	(28)-42.32-(54)	(2)-2.45-(3)	(0.5)-1.25-(3)
	A1 Edirne: A. Akyol 2	(32)-41.5-(52)	(2)-2.47-(4)	(3)-4.4-(9)
R. ramiflora subsp. *ramiflora*	B1 Balıkesir: O. Erol 4	(39)-44.80-(51)	(3)-4.05-(6)	(3)-4.40-(6)
	B1 Balıkesir: O. Erol 5	(36)-41.91-(54)	(2)-3.45-(5)	(2)-4.1-(8)
R. columnae subsp. *columnae*	A1 Çanakkale: A. A. Dönmez 10433	(37)-47.07-(53)	(2)-3.12-(4)	(2)-4.85-(8)
	B1 İzmir: Ş. Yıldırımlı 1258	(25)-38-(64)	(3)-3.9-(5)	(3)-5.55-(11)

IOD: Interopercular distance Oplg: Operculum band length Oplt: Operculum band width

Table 1. continued

Taxa	Specimen	SPN per 100 µm^2	Exine µm	
			S	N
R. bulbocodium var. *bulbocodium*	B1 İzmir: O. Erol 1	(15)-18-(22)	(1.75)-1.85-(2.25)	<0.5
var. *crocea*	C3 Antalya: A. A. Dönmez 4369	(16)-19-(24)	(1.5)-1.72-(1.75)	<0.5
	C3 Antalya: O. Erol 2 - A. Kılınç	(27)-31-(36)	(1.5)-1.57-(1.75)	<0.5
	C3 Antalya: A. Duran 2062	(32)-36-(40)	(1.5)-1.65-(1.75)	<0.5
var. *leichtliniana*	C6 Hatay: O. Erol 3	(23)-26-(30)	(1.5)-1.85-(2.25)	<0.5
R. tempskyana	C2 Antalya: A. A. Dönmez 4298	(22)-26-(34)	(1.55)-1.77-(2)	<0.5
	C2 Muğla: A. Güner 8100 et al.	(19)-21-(25)	(1.75)-1.8-(2)	<0.5
R. linaresii subsp. *graeca*	A1 Çanakkale: A. A. Dönmez 10432	(30)-44-(51)	(1.5)-1.62-(2)	<0.5
	A1 Edirne: A. Akyol 2	(43)-51-(58)	(1.5)-1.82-(2)	<0.5
R. ramiflora subsp. *ramiflora*	B1 Balıkesir: O. Erol 4	(45)-50-(54)	(1.5)-1.75-(2)	<0.5
	B1 Balıkesir: O. Erol 5	(48)-54-(57)	(1.5)-1.7-(1.75)	<0.5
R. columnae subsp. *columnae*	A1 Çanakkale: A. A. Dönmez 10433	(42)-49-(54)	(1.5)-1.75-(2)	<0.5
	B1 İzmir: Ş. Yıldırımlı 1258	(39)-43-(47)	(1.5)-1.75-(2)	<0.5

N: Nexine S: Sexine SPN: Spinule number

Table 1. continued

Taxa	Specimen	Intine µm		Exi at the aperture
		Exi	Endi	
R. bulbocodium var. *bulbocodium*	B1 İzmir: O. Erol 1	(1)-1.75-(2.75)	0.5	(1.5)-2.9-(4)
var. *crocea*	C3 Antalya: A. A. Dönmez 4369	(0.5)-0.95-(2)	0.5	(3)-4.2-(6)
	C3 Antalya: O. Erol 2 - A. Kılınç	(0.5)-0.9-(1.5)	0.5	(3)-5.1-(7)
	C3 Antalya: A. Duran 2062	(0.5)-0.92-(1.5)	0.5	(3)-4.8-(7)
var. *leichtliniana*	C6 Hatay: O. Erol 3	(0.5)-1.40-(2.5)	0.5	(2.5)-5.35-(7)
R. tempskyana	C2 Antalya: A. A. Dönmez 4298	(1)-1.92-(3.5)	0.5	(2)-4.05-(7)
	C2 Muğla: A. Güner 8100 et al.	(1)-1.75-(3)	0.5	(2.5)-3.75-(6)
R. linaresii subsp. *graeca*	A1 Çanakkale: A. A. Dönmez 10432	(1)-1.14-(2)	0.5	(2)-2.87-(3.5)
	A1 Edirne: A. Akyol 2	(1)-1.37-(2)	0.5	(1.5)-2.8-(3.5)
R. ramiflora subsp. *ramiflora*	B1 Balıkesir: O. Erol 4	(0.5)-1.4-(2)	0.5	(1.5)-2.15-(3)
	B1 Balıkesir: O. Erol 5	(0.5)-0.82-(1)	0.5	(1)-1.60-(3)
R. columnae subsp. *columnae*	A1 Çanakkale: A. A. Dönmez 10433	(0.5)-0.9-(2.5)	0.5	(1.5)-2.65-(3)
	B1 İzmir: Ş. Yıldırımlı 1258	(0.5)-0.9-(1.5)	0.5	(1.5)- 2.6-(4)

Endi: Endintine Exi: Exintine

In pollen grains with a 2-banded operculum the bands are usually almost equal in length. The bands are free (Figure 1a) or joined (Figures 1e, 3a-e). They are connected to the main exine plate via the exine protrusion(s) (Figure 3f).

In pollen grains with a 1-banded operculum the band is straight (Figure 2b) or curved (Figure 3g). Circular-banded opercula are also present (Figure 3h). The band is free (Figure 2b) or connected to the main exine plate via the exine protrusion(s) (Figure 3g-h). In some pollen grains the operculum is connected to an exine fragment (Figure 3g-h).

Discussion

The results of this study reveal that the pollen of Turkish *Romulea* taxa share some morphological features, such as pollen size, pollen wall thickness (Table 1), and exine sculpturing (Figure 4b); however, the 2 taxa *R. bulbocodium* var. *crocea* and var. *leichtliniana* are heterogeneous in pollen type (Figure 1a-i). Moreover, most of the taxa show various operculum features, even within taxa (Figures 2a-b, 3a-h).

Aperture type is predominantly monosulcate in the genus. In previous accounts by Goldblatt et al. (1991, 2002), Manning & Goldblatt (2001), and Martin Cacao & Fernández (1990), *Romulea* pollen was described as monosulcate with spinulate exine sculpturing. We, however, observed that in *R. bulbocodium* var. *crocea* and var. *leichtliniana* growing sporadically in south-western Turkey, other pollen types, such as monosulcate, disulcate, trisulcate, trisynsulcate, tetrasulcate, penta-aperturate (with longer and shorter sulci), and monoporate, from single pollen sacs are present. This condition appears to distinguish the 2 taxa from the closely related taxon, *R. bulbocodium* var. *bulbocodium*, as well as the other *Romulea* taxa, which are characterised by invariably monosulcate pollen. The study by Erol & Küçüker (2003) revealed that the 2 taxa also differ from *R. bulbocodium* var. *bulbocodium* in the morphology of micro-papillae of the basal leaves. They have horn-like papillae, whereas var. *bulbocodium* has finger-like papillae.

Monosulcate pollen is the most common type in Iridaceae (Goldblatt et al., 1991). It is considered primitive in Monocotyledons and, indeed, among

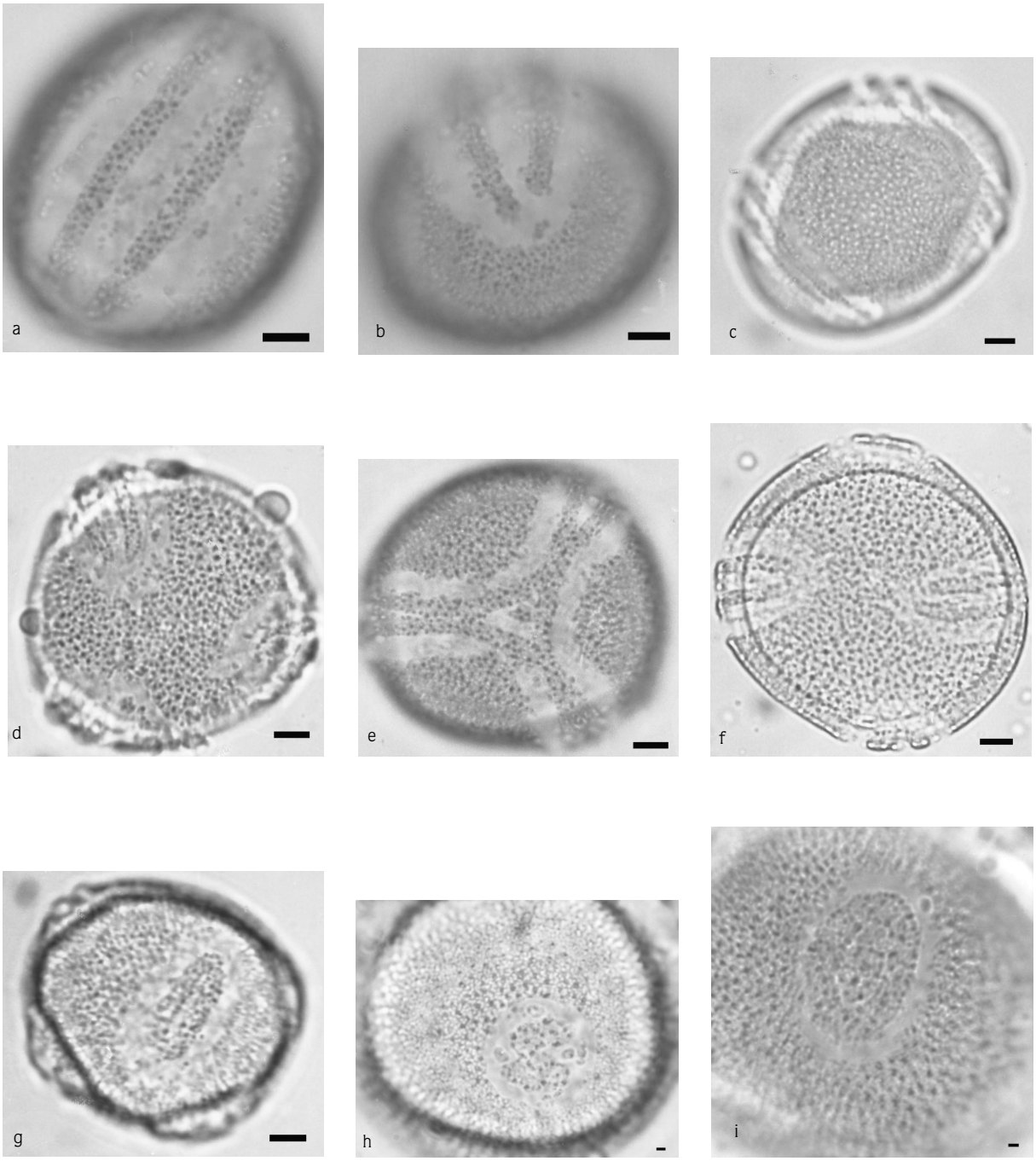

Figure 1. Pollen types under LM. *Romulea tempskyana* (a-b), *R. bulbocodium* var. *leichtliniana* (c-d, f-i), *R. bulbocodium* var. *crocea* (e). a: Monosulcate pollen in distal view (with 2-banded operculum); b: Monosulcate pollen in lateral view (with 2-banded operculum); c: Disulcate pollen (with 2-banded operculum); d: Trisulcate pollen (with 2-banded operculum); e: Trisynsulcate pollen (with 2-banded operculum); f: Tetrasulcate pollen (with 2-banded operculum); g: Penta-aperturate pollen (with circular-banded operculum); h-i: Monoporate (operculate) pollen. Scale bar: a-e, g: 8 µm; f: 6 µm; h-i: 2 µm.

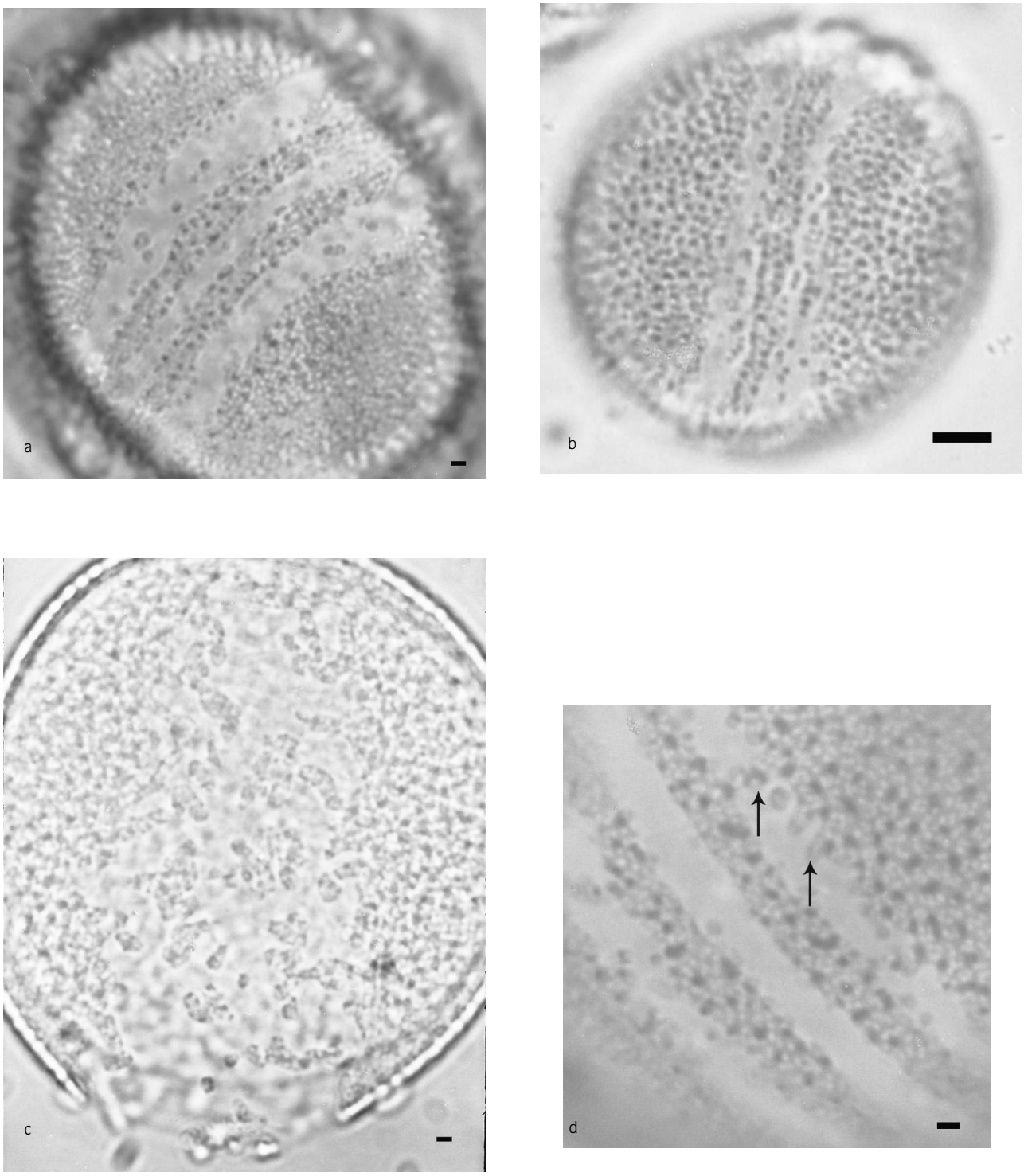

Figure 2. Aperture morphology under LM. *R. bulbocodium* var. *leichtliniana* (a-b), *R. columnae* subsp. *columnae* (c), *R. bulbocodium* var. *crocea* (d). a: Sulcus with 2-banded operculum (bands free) surrounded by small exine islands; b: Sulcus with 1-banded operculum surrounded by small exine islands; c: Insulate sulcus; d: Exine protrusions towards the aperture (arrows). Scale bar: b: 8 µm; a, c-d: 2 µm.

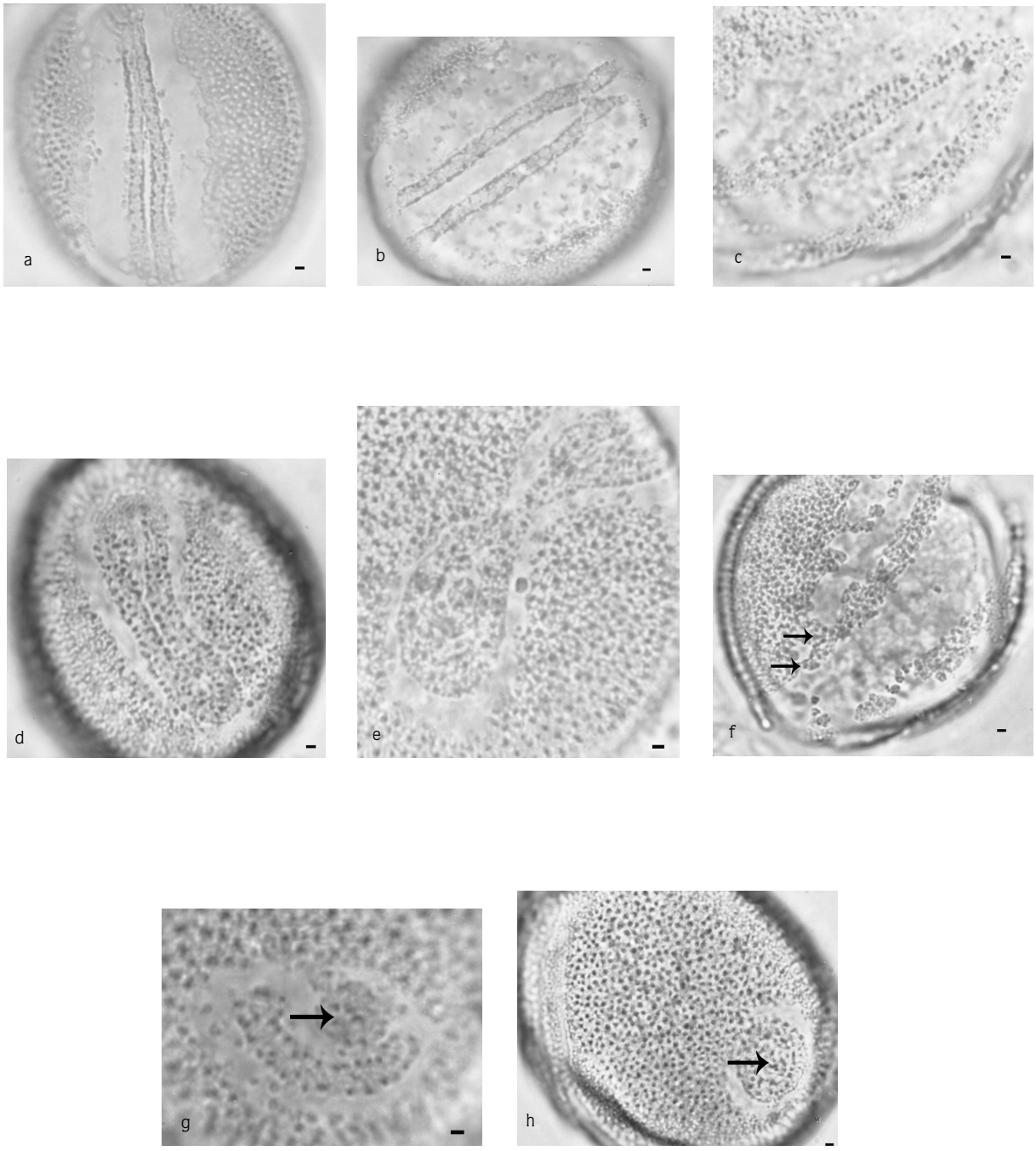

Figure 3. Operculum morphology under LM. *R. linaresii* subsp. *graeca* (a), *R. columnae* subsp. *columnae* (b, f), *R. tempskyana* (c), *R. bulbocodium* var. *crocea* (d-e, h), *R. bulbocodium* var. *leichtliniana* (g). a-f: 2-banded operculum, a: Bands joined nearly in the middle; b: Bands joined near one end; c: Bands joined at one end; d: Bands joined at both ends e: Bands joined at both ends and in the middle f: A band connected to the main exine plate via exine protrusions (arrows); g: Curved 1-banded operculum connected to an exine fragment (arrow); h: Circular-banded operculum connected to an exine fragment in the middle (arrow). Scale bar: 2 µm.

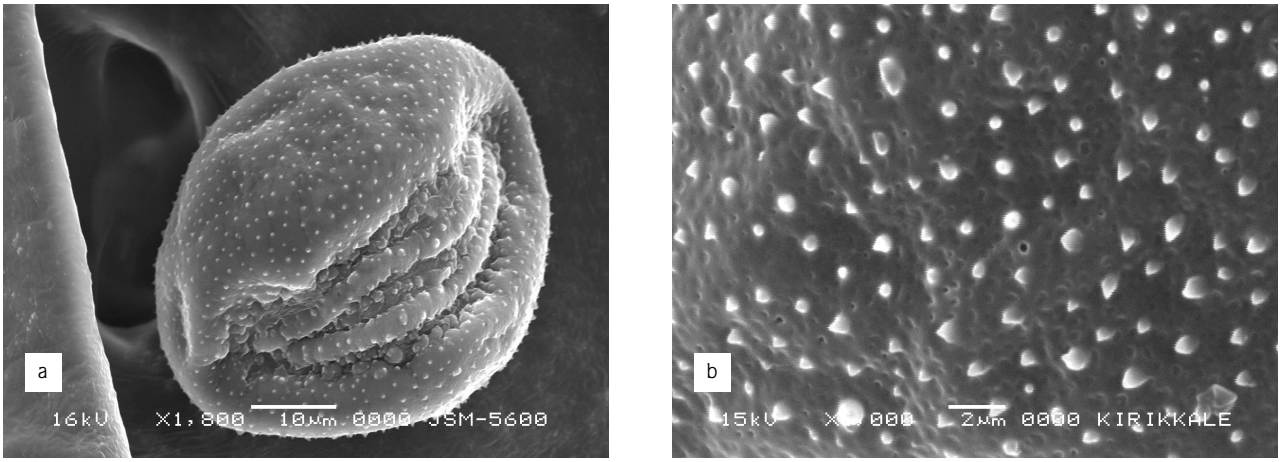

Figure 4. The pollen grains of selected *Romulea* taxa under SEM. *R. tempskyana* (a), *R. bulbocodium* var. *leichtliniana* (b) a: Monosulcate pollen with 2-banded operculum; b: Exine sculpturing with spinules and micro-perforations.

Angiosperms in general (Zavada, 1983). However, other aperture (pollen) types have been previously recorded in Iridaceae and in other Monocotyledons; for example in *Crocus* L. (spiral furrows, more or less extensive colpi and pores) (Chichiriccò, 1999) and *Aristea* Sol. Ex Aiton (trisulcate and dizonasulculate) (Le Thomas et al., 1996; Goldblatt et al., 2004), in the family Iridaceae in *Tulipa* L. (tri-aperturate and inaperturate) and *Lilium* L. (triporate), in the family Liliaceae (Kosenko, 1999), and in Palmae (trichotomosulcate) (Harley, 1990). According to Goldblatt et al. (1991), other aperture types are clearly derived states in Iridaceae. Moret et al. (1992) showed that *R. bulbocodium* var. *dioica* Batt. growing in Morocco has a complex reproductive system that combines gynodioecy and herkogamy, accompanied by sexual dimorphism in the flower. Moret et al. (1993) proposed that such a complex reproductive system represents an advanced condition. There is unfortunately no further information about the biology of the taxa of *Romulea* found in Turkey, with the exception of morphological and anatomical observations. Considering the occurrence of aperture polymorphism in the pollen grains of *R. bulbocodium* var. *crocea* and var. *leichtliniana*, we suggest that among the Mediterranean members of the genus these 2 taxa may have advanced positions and are isolated in the genus by their specialised pollen grains.

Pollen variability within a single species is, in fact, not uncommon. For example, Borsch & Wilde (2000) found an extreme variability in pollen aperture condition as well as in exine sculpturing in *Nelumbo* Adams. (Nelumbonaceae). In the Arecales (Arecaceae) and Asparagales there are examples of trichotomosulcy and monosulcy occurring in the same anthers, and these examples are linked to simultaneous cytokinesis (Harley, 2004). Borsch & Wilde (2000) described possible phenomena of pollen variability below the species level, such as ploidy and the differentiation of populations. In discussing possible causes of aperture transformations Blackmore & Crane (1998) consider that variations in meiosis play a major role in determining aperture position and symmetry. Harley (2004) further concludes that the greatest influence on aperture number, polarity, and morphology appears to be ontogenetic in origin, rather than slow evolutionary adaptation. Therefore, in order to assess the factor(s) responsible for aperture diversity in *R. bulbocodium* var. *crocea* and var. *leichtliniana*, further biological studies (chromosome cytology, investigation of ontogeny and of reproductive systems, etc.) of different populations and individuals are certainly warranted.

Concerning operculum morphology, the pollen grains of most *Romulea* taxa found in Turkey are rather variable (Figures 1a, e, 2b, 3a-h). The 2-banded operculum predominates, but 1-banded opercula are also present in most of the taxa (*R. bulbocodium* var. *crocea* and var. *leichtliniana*, *R. linaresii* subsp. *graeca*, *R. ramiflora* subsp. *ramiflora*, and *R. columnae* subsp. *columnae*). In addition, insulate pollen grains are occasionally recorded in some of the taxa (*R. bulbocodium* var. *bulbocodium*, *R. tempskyana*, and *R. columnae* subsp. *columnae*). Goldblatt et al. (1991) also predominantly recorded the

2-banded operculum type (sometimes joined in *R. bulbocodium*, *R. engleri* Bég. and *R. major* Schousboe) and occasionally 1-banded (in *R. engleri*) or disorganised (in *R. bifrons* Pau, *R. columnae* and *R. rollii* Parl.) operculum types in the African *Romulea* species. Martin Cacao & Fernández (1990) reported only the 2-banded operculum type in the *Romulea* taxa (*R. bulbocodium*, *R. clusiana* (Lange) Nyman, *R. ramiflora* subsp. *ramiflora* and subsp. *gaditana* (G.Kunze) Marais) from West Andalucia in Spain. With respect to variable aperture opercula, the Turkish *Romulea* taxa resemble some of the African *Romulea* species mentioned above. In terms of pollen size (LA), the Turkish *Romulea* taxa are similar to the African species as well as the western Mediterranean members of the genus. In the Turkish taxa the mean value for LA ranges from 52.25 to 63.6 µm, while in the African species it ranges from 60 to 63 µm (Goldblatt et al., 1991), and in those from Spain it ranges from 47.62 to 55.84 µm (Martin Cacao & Fernández, 1990).

The developmental controls giving rise to different operculum types are unknown; however, Furness & Rudall (2003) suggested that the position of the operculum may be related to that of the microtubule-organising centres during meiosis.

It is suggested that the operculum may have a variety of functions, such as protection of the delicate apertural area from pathogens and/or dehydration, especially in wind-pollinated plants or in plants from dry habitats, as well as a role in harmomegathy (Furness & Rudall, 2003). According to Goldblatt et al. (2002), the genus *Romulea* depends on insects for pollen transport. We observed that in the Turkish *Romulea* taxa, the exine is relatively thin and the aperture(s) is (are) usually large (Table 1). Additionally, the species grow in open forests or grassy places in southern and south-western Turkey. They flower in the early spring when their habitats become very humid due to heavy rainfall. Taking the pollination mechanism and habitat conditions into consideration, it is supposed that the opercula in the Turkish *Romulea* taxa may provide protection against entry of pathogens, such as bacteria and fungi, into the aperture(s). Considering the relatively thin exine, they may also possibly have a harmomegathic function and strengthen the pollen grains.

Based on exine sculpturing, the *Romulea* pollen grains are characterised by a spinulate-microperforate exine (Figure 4d). Pollen ornamentation (exine sculpturing) is usually related to pollination mechanisms. Grayum (1986) suggested that spinose pollen is closely associated with fly pollination and it more effectively guarantees the attachment of pollen among the hairs or bristles of rapidly flying insects. However, Grayum also states the fact that exine sculpturing is not the only pollen character that may be affected by pollinator selections. As stated above, the *Romulea* species are pollinated by insects (Goldblatt et al., 2002); therefore, the spinulate exine of the *Romulea* pollen could be, in part, correlated with insect pollination.

Acknowledgements

This is a revised section of the PhD thesis submitted by the first author, which was supported by the Scientific Research Unit of Hacettepe University (Project no 00 02 601 001). We thank the curators of GAZI, HUB, and ISTF herbaria for providing us with sampling facilities. We are indebted to Osman Erol and Sırrı Yüzbaşıoğlu of İstanbul University for their help during our studies at ISTF. We also thank the Electron Microscope Unit of Kırıkkale University for access to the SEM.

References

Blackmore S & Crane PR (1998). The evolution of apertures in the spores and pollen grains of embryophytes In: Owens SJ, Rudall PJ (ed.), *Reproductive Biology*, pp. 159-182. Kew: Royal Botanic Gardens.

Borsch T & Wilde V (2000). Pollen variability within species, populations, and individuals, with particular reference to *Nelumbo* In: Harley MM, Moron CM, Blackmore S (ed.), *Pollen and Spores: Morphology and Biology*, pp. 285-299. Kew: Royal Botanic Gardens.

Chichiriccò G (1999). Development stages of the pollen wall and tapetum in some *Crocus* species. *Grana* 38: 31-41.

Dahlgren RMT, Clifford HT & Yeo PF (1985). *The Families of the Monocotyledons: Structure, Evolution and Taxonomy*. Berlin: Springer Verlag.

Erol O & Küçüker O (2003). Morpho-anatomical observations on three *Romulea* (Iridaceae) taxa of Turkey. *Bocconea* 16: 607-613.

Furness CA & Rudall PJ (2003). Aperture with lids: distribution and significance of operculate pollen in Monocotyledons. *Int J Plant Sci* 164: 835-854.

Goldblatt P, Manning JC & Bari A (1991). Sulcus and operculum structure in the pollen grains of Iridaceae subfamily Ixioideae. *Ann Missouri Bot Gard* 78: 950-961.

Goldblatt P, Bernhardt P & Manning JC (2002). Floral biology of *Romulea* (Iridaceae: Crocoideae): a progression from a generalist to a specialist pollination system. *Adansonia* 24: 243-262.

Goldblatt P, Le Thomas A & Suárez-Cervera M (2004). Phylogeny of the Afro-Madagascan *Aristea* (Iridaceae) revisited in the light of new data on pollen morphology. *Botan J Linn Soc* 144: 41-68.

Grayum MH (1986). Correlations between pollination biology and pollen morphology in the Araceae, with some implications for angiosperm evolution In: Blackmore S, Ferguson IK (ed.), *Pollen and Spores: Form and Function*, pp. 313-327. London: Academic Press.

Harley MM (1990). Occurrence of simple, tectate, monosulcate or trichotomosulcate pollen grains within the Palmae. *Rev Palaeobot Palyno* 64:137-147.

Harley MM (2004). Triaperturate pollen in monocotyledons: configurations and conjectures. *Plant Syst Evol* 247: 75-122.

Kosenko VN (1999). Contributions to the pollen morphology and taxonomy of the Liliaceae. *Grana* 38: 20-30.

Le Thomas A, Suarez-Cervera M & Goldblatt P (1996). Deux types polliniques originaux le genre *Aristea* (Iridaceae – Nivenioideae): implications phylogéniques. *Grana* 35: 87-96.

Manning JC & Goldblatt P (2001). A synoptic review of *Romulea* (Iridaceae: Crocoideae) in sub-Saharan Africa, The Arabian Peninsula and Socotra including new species, biological notes, and a new infrageneric classification. *Adansonia* 23: 59-108.

Marais W (1984). *Romulea* Maratti In: Davis PH (ed.), Flora *of Turkey and the East Aegean Islands*, vol. 8 , pp. 438-441. Edinburgh: Edinburgh University Press.

Martin Cacao M & Fernández I (1990). Contribucion al estudio palinologico de la familia Iridaceae en Andalucia Occidental (excepto el genero *Iris* L.). *Lagascalia* 15: 189-198.

Moret J, Bari A, Le Thomas A & Goldblatt P (1992). Gynodioecy, herkogamy and sex-ratio in *Romulea bulbocodium* var. *dioica* (Iridaceae). *Evol Trend Plant* 6: 99-109.

Moret J, Bari A & Le Thomas A (1993). Evolution of herkogamy and gynodioecy in Moroccan species of *Romulea* (Iridaceae). *Pl Syst Evol* 184: 241-257.

Moussel B, Moussel C & Moret J (1996). Les systémes de reproduction des especes françaises du genre *Romulea* (Iridaceae): données morphohistologiques. *Can J Bot* 74: 140-149.

Punt W, Blackmore S, Nilsson S & Le Thomas A (1994). *Glossary of Pollen and Spore Terminology*. Utrecht: LPP Foundation.

Zavada M (1983). Comparative morphology of monocot pollen and evolutionary trends of apertures and wall structure. *Bot Rev* 49: 331-379.