

**POLLEN MORPHOLOGY OF SIX SPECIES PREVIOUSLY PLACED
IN MALCOLMIA (BRASSICACEAE) IN TURKEY**

**AYLA KAYA^{*}, MURAT ÜNAL¹, FEVZİ ÖZGÖKÇE², BEKİR DOĞAN³
AND ESRA MARTİN⁴**

*Department of Pharmaceutical Botany, Faculty of Pharmacy,
Anadolu University, TR-26470 Eskisehir, Turkey.*

Keywords: Pollen morphology, *Malcolmia*, *Strigosella*, *Zuvanda*, Turkey

Abstract

The genus *Malcolmia* R. Br. (Brassicaceae) is taxonomically problematic and some of its species have recently been transferred to the genera *Strigosella* Boiss. and *Zuvanda* Dvorak. In this study, pollen morphology of some species of *Malcolmia*, *Strigosella* and *Zuvanda*, previously placed in *Malcolmia* genus, were examined under scanning electron microscope (SEM), in order to determine the significance of pollen features as taxonomic characters. The results reveal rather uniform morphological features, however fine details are characteristic to distinguishing pollen grains in the species of the genus. The pollen grains in three genera are tricolpate and the surface sculpturing type is reticulate. The basic shape of the pollen grains in species studied is euprolate, subprolate-euprolate and euprolate-perprolate. While pollen grains of *S. africana* is the smallest in all examined species, *M. chia* is the biggest. However, three genera can be easily separated by the length of the polar and equatorial axes themselves.

Introduction

The genus *Malcolmia* R.Br. is a member of Brassicaceae and it comprises ten species distributed throughout the world (Al-Shehbaz *et al.* 2006). *Malcolmia* was previously represented by six species in Turkey (Cullen 1965). In recent studies, two more species belonging to *Malcolmia* genus have added to the flora of Turkey as new records. These are: *M. exacoides* (DC.) Spreng. and *M. intermedia* C. A. Mey.. Thus, the number of *Malcolmia* species distributed in Turkey has increased to 8. The genus *Malcolmia* is taxonomically problematic (Doğan *et al.* 2011) and some of its species have recently been transferred to the genera *Strigosella* Boiss. and *Zuvanda* Dvorak. However, recent reports have indicated that the *Malcolmia* is represented by four species: *M. chia* (L.) DC., *M. flexuosa* (Sibth. & Sm.) Sibth. & Sm., *M. micrantha* Boiss. & Reut. and *M. graeca* Boiss. & Sprun. belonging to the tribe *Anastaticae* (German *et al.* 2009). *M. micrantha* is a synonym of *M. chia* (Meikle 1977). Cullen (1965) stated that *M. graeca*'s presence in Turkey is in need of confirmation; this confirmation has not been found despite many floristic investigations. *M. crenulata* (DC.) Boiss. and *M. exacoides* (DC.) Spreng. (Özgökçe and Ünal 2007) belong to the genus *Zuvanda* (Dvořak) Askerova (Askerova 1985, Al-Shehbaz *et al.* 2007, Doğan *et al.* 2011) of the tribe *Conringieae* (German *et al.* 2009), while *M. africana* (L.) R. Br. and *M. intermedia* C. A. Mey. (Ünal and Özgökçe 2008) belong to the genus *Strigosella* Boiss. (Al-Shehbaz *et al.* 2007, Doğan *et al.* 2011) of the tribe *Euclidieae* (German *et al.* 2009).

Pollen morphology has provided an approach to the systematic relationships among the genera of the Brassicaceae (Doğan and İnceoğlu 1990, Brochmann 1992, Khan 2004, Pınar *et al.* 2009, Mutlu and Erik 2012). Pollen morphology of *Malcolmia* is poorly known; only a few

*Author for correspondence: <aykaya@anadolu.edu.tr>. ¹Department of Biology, Faculty of Education, Yüzüncü Yıl University, TR-65080 Van, Turkey. ²Department of Biology, Faculty of Science, Yüzüncü Yıl University, TR-65080 Van, Turkey. ³Department of Science Educations, Faculty of A. K. Education, Necmettin Erbakan University, TR 42060-Konya, Turkey. ⁴Department of Biotechnology, Faculty of Science, Necmettin Erbakan University, TR 42060-Konya, Turkey.

occurrences of its pollen are available in published work. Khalik *et al.* (2002) and Perveen *et al.* (2004) investigated pollen morphology of some species of the genus *Malcolmia*. Kizilpinar *et al.* (2012) reported pollen morphology of some species of the genus *Malcolmia* prepared according to the acetolysis method from Turkey. This study has been based only on light microscopy (LM) studies.

The present investigations were carried out on the morphology characters of pollen grains in *Malcolmia*, *Strigosella* and *Zuvanda* species from Turkey previously placed in the genus *Malcolmia*. Scanning electron microscope (SEM) observations are carried out to emphasise the taxonomic significance of pollen morphological characters. This study is therefore the first comprehensive survey of pollen in the genera to utilize SEM.

Material and Methods

The species, namely *Malcolmia*, *Strigosella* and *Zuvanda* were collected from different localities in Turkey (Table 1). Voucher specimens were deposited in the Herbarium of the faculty of Science and Arts of Yüzüncü Yıl University (VANF). For scanning electron microscopy (SEM), pollens were mounted directly on stubs, using single-side adhesive tape and coated with gold. Photographs were taken with EVO-50. Twenty pollen grains for each species were examined.

Table 1. A list of the examined specimens of the genera *Malcolmia*, *Strigosella* and *Zuvanda*.

Taxon	Locality	VANF
<i>Malcolmia chia</i>	Turkey, Antalya, 07.05.2009	10153
<i>M. flexuosa</i>	Turkey, İzmir, 05.05.2009	10145
<i>Strigosella intermedia</i>	Turkey, Iğdır, 06.04.2009	10019
<i>S. africana</i>	Turkey, Siirt, 28.03.2009	10001
<i>Zuvanda crenulata</i>	Turkey, Şanlıurfa, 31.03.2009	10043
<i>Z. exacoides</i>	Turkey, Siirt, 28.03.2009	10011

Results and Discussion

Representative pollen grains are illustrated in Fig. 1 and the main palynological features of the studied species of *Malcolmia*, *Strigosella* and *Zuvanda* are summarized in Table 2. The pollen grains of the studied species of *Malcolmia*, *Strigosella* and *Zuvanda* are single, isopolar, radially symmetric and tricolpate. Exine ornamentation is reticulate (Fig. 1). The mean of polar axis and equatorial axis varies from 23.4 and 14.1 μm , in *S. africana*, to 36.8 and 22.7 μm , in *M. chia*. The shape of pollen grains ranges from subprolate-euprolate (P/E =1.33-1.80, Fig. 1-d₁,g₁), euprolate (P/E =1.40-1.95, Fig. 1-c₁,f₁,h₁) to euprolate-perprolate (P/E =1.83-2.0, Fig. 1-e₁). The outline in polar view is elliptic while the outline in equatorial view is triangular (Fig. 1 a,b). Regarding sculpturing of exine only one basic type can be distinguished: reticulate with irregular polygonal lumina shape (Fig. 1). The mean of colpi length and colpus width varies from 17.58 and 0.79 μm , (in *S. africana*) to 27.35 and 1.60 μm , (in *M. chia*). The colpi converge close to the polar ends. The lumina width, punctum number in the lumina and muri surface show variation among the species studied. Therefore, they provide useful criteria for separating the species. For example, the lumina width in one species, *S. intermedia*, is < 1.8 μm , but it is until to 4 μm in the remaining species. The range of muri width varies from 0.3 - 1.1 μm , in *S. intermedia*, to 0.5 - 2.8 μm , in *M. chia*. The punctum number in the lumina is 0 - 1 in *Z. crenulata*, *M. chia*, *M. flexuosa* and 0 - 4 in the remaining species. The surface of muri can be smooth-undulate in all the species.

M. chia, pollen grains euprolate in shape and $36.8 \times 22.7 \mu\text{m}$ in size, colpi $27.35 \times 1.60 \mu\text{m}$, sculpture reticulate, lumina width $0.8 - 4 \mu\text{m}$, shape of lumina irregular polygonal, punctum number in the lumina 0 - 1, muri width $0.5 - 2.8 \mu\text{m}$ and surface of muri smooth-undulate (Fig. 1- c_1, c_2). *M. flexuosa*, pollen grains subprolate-euprolate in shape and $29.4 \times 19 \mu\text{m}$ in size, colpi $22.55 \times 1.55 \mu\text{m}$, sculpture reticulate, lumina width $0.7 - 3 \mu\text{m}$, shape of lumina irregular polygonal, punctum number in the lumina 0-1, muri width $0.5 - 2.5 \mu\text{m}$ and surface of muri smooth-undulate (Fig. 1- d_1, d_2).

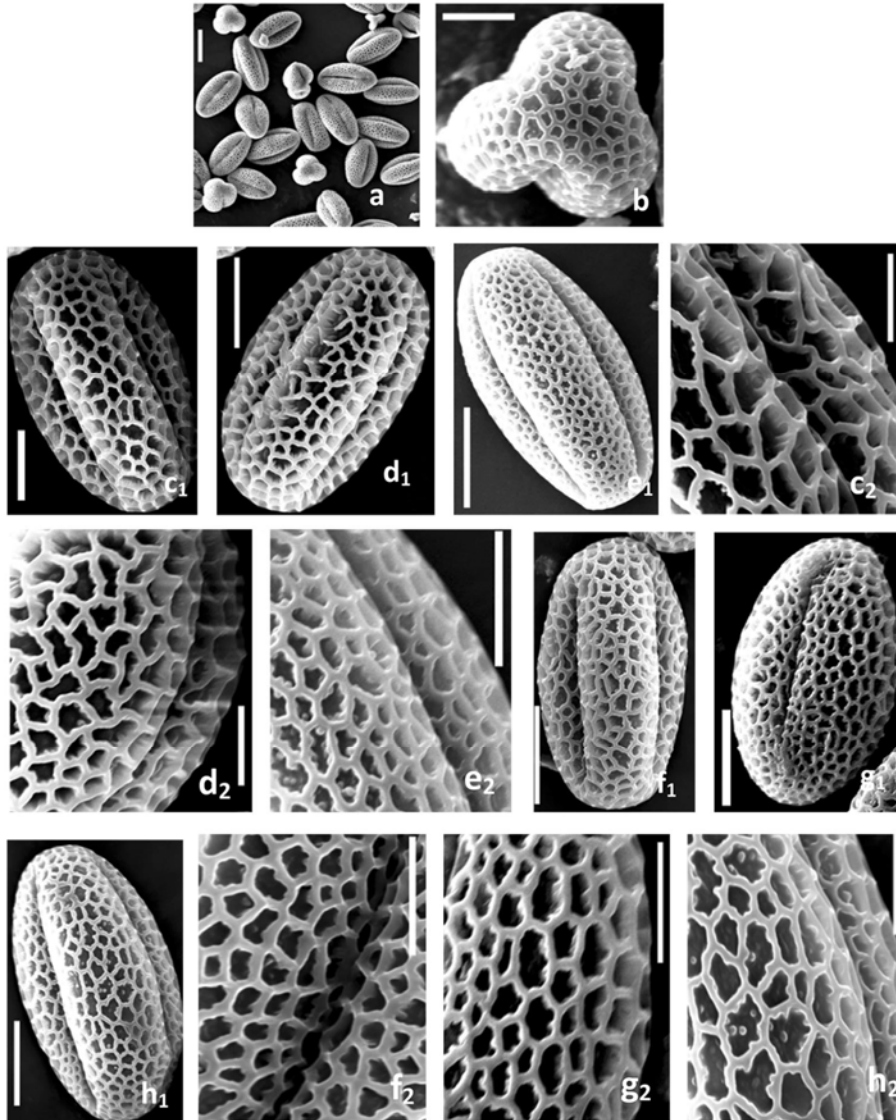


Fig. 1. Pollen grains of the genera in *Malcolmia*, *Strigosella* and *Zuvanda*. a- Equatorial and polar view, b- Polar view, $c_{1,2}$ - *M. chia*, $d_{1,2}$ - *M. flexuosa*, $e_{1,2}$ - *S. intermedia*, $f_{1,2}$ - *S. africana*, $g_{1,2}$ - *Z. crenulata*, $h_{1,2}$ - *Z. exacoides*. Scale bars: a = $20 \mu\text{m}$; c_1 - h_1 = $10 \mu\text{m}$; b, c_2 - h_2 = $5 \mu\text{m}$.

Table 2. Pollen morphology characters of the genera *Malcolmia*, *Strigosella* and *Zuvanda*.

Species	Polar axis mean/range (μm)	Equatorial axis mean/range (μm)	P/E ratio mean/range	Shape (μm)	Sculpturing (μm)	Colpus length (μm)	Colpus width (μm)	Lumina shape (μm)	Lumina width	Punctum number	Muri width	Muri surface
<i>M. chia</i>	36.8 (31.6-42)	22.7 (20-25.4)	1.62 (1.58-1.65)	Euprolate	Reticulate	27.35	1.60	Irregular polygonal	0.8-4	0-1	0.5-2.8	Smooth or undulate
<i>M. flexuosa</i>	29.4 (22.2-36.4)	19 (16.6-21.4)	1.54 (1.33-1.70)	Subprolate-euprolate	Reticulate	22.55	1.55	Irregular polygonal	0.7-3	0-1	0.5-2.5	Smooth or undulate
<i>S. africana</i>	23.4 (18.2-28.6)	14.1 (13-15.3)	1.65 (1.40-1.86)	Euprolate	Reticulate	17.58	0.79	Irregular polygonal	0.2-2.8	0-4	0.3-1.8	Smooth or undulate
<i>S. intermedia</i>	29.8 (25-34.6)	15.4 (13.6-17.3)	1.93 (1.83-2.0)	Euprolate-perprolate	Reticulate	25.0	0.90	Irregular polygonal	0.1-1.8	0-4	0.3-1.1	Smooth or undulate
<i>Z. crenulata</i>	25.2 (18.4-32)	15.7 (13.7-17.7)	1.60 (1.33-1.80)	Subprolate-euprolate	Reticulate	20.43	0.89	Irregular polygonal	0.3-2.8	0-1	0.3-1.8	Smooth or undulate
<i>Z. exacoides</i>	32.8 (26.6-39)	18.3 (16.6-20)	1.79 (1.62-1.95)	Euprolate	Reticulate	25.90	1.08	Irregular polygonal	0.5-4	0-4	0.3-2.0	Smooth or undulate

S. intermedia; pollen grains euprolate-perprolate in shape and $29.8 \times 15.4 \mu\text{m}$ in size, colpi $25 \times 0.90 \mu\text{m}$, sculpture reticulate, lumina width $0.1 - 1.8 \mu\text{m}$, shape of lumina irregular polygonal, punctum number in the lumina 0 - 4, muri width $0.3 - 1.1 \mu\text{m}$ and surface of muri smooth-undulate (Fig. 1-e₁,e₂). *S. africana*; pollen grains euprolate in shape and $23.4 \times 14.1 \mu\text{m}$ in size, colpi $17.58 \times 0.79 \mu\text{m}$, sculpture reticulate, lumina width $0.2-2.8 \mu\text{m}$, shape of lumina irregular polygonal, punctum number in the lumina 0 - 4, muri width $0.3 - 1.8 \mu\text{m}$ and surface of muri smooth-undulate (Fig. 1-f₁,f₂).

Z. crenulata; pollen grains subprolate-euprolate in shape and $25.2 \times 15.7 \mu\text{m}$ in size, colpi $20.43 \times 0.89 \mu\text{m}$, sculpture reticulate, lumina width $0.3-2.8 \mu\text{m}$, shape of lumina irregular polygonal, punctum number in the lumina 0 - 1, muri width $0.3 - 1.8 \mu\text{m}$ and surface of muri smooth-undulate (Fig. 1-g₁,g₂). *Z. exacoides*; pollen grains euprolate in shape and $32.8 \times 18.3 \mu\text{m}$ in size, colpi $25.90 \times 1.08 \mu\text{m}$, sculpture reticulate, lumina width $0.5 - 4 \mu\text{m}$, shape of lumina irregular polygonal, punctum number in the lumina 0-4, muri width $0.3 - 2.0 \mu\text{m}$ and surface of muri smooth-undulate (Fig. 1-h₁,h₂).

Brassicaceae is a stenopalynous family, pollen grains are usually reticulate and tricolpate (Erdtman 1952). The most common shape is prolate and this type is present in genera of Brassicaceae (Khalik *et al.* 2002, Khan 2004). The main characteristic features of pollen in the *Malcolmia*, *Strigosella* and *Zuvanda* species studied here are shed as monad, radially symmetrical, isopolar and they possess tricolpate aperture type and a reticulate exine sculpturing. *S. intermedia* has characteristic pollen grains with euprolate-perprolate shape and narrow lumina ($0.1 - 1.8 \mu\text{m}$) width (Fig. 1-e₁,e₂) and easily distinguished from the remaining species (Table 2). Although *S. africana*, *M. chia* and *Z. exacoides* have pollen grains with euprolate shape, pollen grains of *M. chia* are the biggest and without punctum or with only one punctum in the lumina (Fig. 1-c₁,c₂) and pollen grains of *S. africana* are the smallest (Fig. 1-f₁,f₂) and it has narrower lumina ($0.2-2.8 \mu\text{m}$) width. *Z. crenulata* and *M. flexuosa* have similar pollen grains with subprolate-euprolate and lumina and muri width ($0.3 - 2.8$ and $0.7 - 3 \mu\text{m}$, $0.3 - 1.8$ and $0.5 - 2.5 \mu\text{m}$, respectively). The muri surface of the pollen grains in all species is smooth-undulate. Average colpus lengths range from 17.58 to $27.35 \mu\text{m}$ and width from 0.79 to $1.60 \mu\text{m}$ among all species examined. Pollen grains of *S. africana* can be distinguished from the remaining species examined by their colpus length. The colpus length is smaller than $20 \mu\text{m}$ in *S. africana* while it is equal or longer than $20 \mu\text{m}$ in the others. Pollen grains of *M. chia* and *M. flexuosa* can also be distinguished by their wider ($1.55 - 1.60 \mu\text{m}$) colpus than the remaining species ($0.79 - 1.08 \mu\text{m}$).

The most comprehensive studies of Brassicaceae pollen is that of Rollins and Banerjee (1979). They examined the pollen grains of 227 species in 132 genera representing of Brassicaceae and found that the most pollen type is tricolpate. Doğan and İnceoğlu (1990) examined pollen grains of the taxa of the genus *Isatis* L. in Turkey and they have divided the *Isatis* pollen type into four groups by palynological features. The first group includes only tricolpate pollen grains and our examined all pollen grains have been located in to the first group. According to Brochmann's study (1992) Nordic *Draba* L. species show a characteristic reticulate sculpturing which is also resemble to our pollen sculpturing. Anchev and Deneva (1997) investigated 17 Brassicaceae species and they classified the pollen into two types. The first type is reticulate with lumina equal and our examined pollen grains have been located on the first group. Appel and Al-Shehbaz (2002) also reported tricolpate, reticulate pollen in the family Brassicaceae. Khalik *et al.* (2002) reported pollen morphology of some tribes of Brassicaceae from Egypt. They explained that *M. africana*'s pollen shape is prolate and claimed that ornemantation of pollen grains belonging to *M. africana*, *M. nana* and *M. pygmaea* are reticulate, reticulate and coarsely reticulate, respectively. *M. africana*'s pollen shape and pollen ornemantation of this three species

is similar to our examined pollen grains. Perveen *et al.* (2004) recognized four distinct pollen types on the basis of tectal surface in Brassicaceae: *Arabis bijuga*-type was characterized by finely reticulate tectum which included *Malcolmia scorpioides* (Bunge) Boiss.; *Erysimum melicentae*-type was characterized by medium reticulate tectum which included *Malcolmia strigosa* Boiss.; *Draba lanceolata*-type was characterized by coarsely reticulate tectum which included *Malcolmia cabulica* (Boiss.) Hook. F. Thoms and *Malcolmia africana*; and *Farsetia ramosissima*-type was characterized by reticulate-rugulate tectum which included *Malcolmia behboudiana* Rech. f.. Our examined all pollen grains would be included in the *Draba lanceolata*-type. Mutlu and Erik (2012) investigated pollen morphology of the genus *Arabis* in Turkey and they reported three pollen type to the results of cluster analysis and these types were named as *Alpina* (type I), *Nova* (type II), and *Hirsuta* (type III). And, they determined tricolpate pollen and reticulate ornamentation in the genus *Arabis*. In the study of Kızılpınar *et al.* (2012), five species of the genus *Malcolmia* were reported from Turkey. These taxa are tricolpate and they have reticulate ornamentation and it is compatible with our findings. Kızılpınar *et al.* are indicated two different group of pollen shape in their study: prolate-spheroidal and oblate-spheroidal. While *M. flexuosa* and *M. crenulata* are placed in the first group, *M. africana*, *M. chia* and *M. excooides* are placed in the second group. However, we determined the pollen shape is euprolate for *M. chia*, *S. africana* and *Z. excooides*, subprolate-euprolate for *M. flexuosa*, *Z. crenulata* and euprolate-perprolate for *S. inretmedia*. This difference in pollen shape may be caused by various natural conditions and working method. Because their study has been based only on light microscopy (LM) with acetolysis method.

Present results reveal rather uniform morphological features. And pollen features have little variations. However, fine details as polar-equatorial axis, colpus length and width, lumina and muri width are characteristic to distinguish *Malcolmia*, *Strigosella* and *Zuvanda* species.

Acknowledgements

The authors would like to thank Yüzüncü Yıl University (BAP Project no: 2008-FED-123) for financial support during this study.

References

- Al-Shehbaz IA Beilstein MA and Kellogg EA 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): An overview. *Pl. Syst. Evol.* **259**: 89-120.
- Al-Shehbaz IA Mutlu B and Dönmez AA 2007. The Brassicaceae (Cruciferae) of Turkey, updated. *Turk. J. Bot.* **31**: 327-336.
- Appel O and Al-Shehbaz IA 2002. Cruciferae. In: Kubitzki, K. (ed.), The families and genera of vascular plants, V. Flowering plants Dicotyledons, Malvales, Capparales and Non-Betalain Caryophyllaceae. C. Bayer. Springer-Verlag, Berlin Heidelberg, New York. pp. 75-174.
- Anchev M and Deneva B 1997. Pollen morphology of seventeen species from family Brassicaceae (Cruciferae). *Phytol Balcanica* **3**: 75-82.
- Askerova RK 1985. *Zuvanda* - A new genus of the family Brassicaceae, *Bot. Zhurn, Moscow, Leningrad.* pp. 522-524.
- Brochmann C 1992. Pollen and seed morphology of Nordic *Draba* (Brassicaceae): Phylogenetic and ecological implications. *Nord. J. Bot.* **12**: 657-673.
- Cullen J 1965. *Malcolmia*. In: Davis, P.H. (ed.), *Flora of Turkey and the East Aegean Islands*, University Press, Edinburgh. Vol. **1**, pp. 460-462.
- Doğan C and İnceoğlu O 1990. Pollen morphology of some *Isatis* L. taxa in Turkey. *Turk. J. Bot.* **14**: 12-31.

- Doğan B Ünal M Özgökçe F Martin E and Kaya A 2011. Phylogenetic relationships between *Malcolmia*, *Strigosella*, *Zuvanda*, and some closely related genera (Brassicaceae) from Turkey revealed by inter-simple sequence repeat amplification. *Turk. J. Bot.* **35**: 17-23.
- Erdtman G 1952. Pollen morphology and plant taxonomy: Angiosperms. Chronica Botanica Co., Waltham, Massachusetts.
- German D Friesen N Neuffer B Al-Shehbaz IA and Hurka H 2009. Contribution to ITS phylogeny of the Brassicaceae, with special reference to some Asian taxa. *Pl. Syst. Evol.* **283**: 33-56.
- Khalik KA Van Den Berg RG Van Der Maesen LJG and El Hadidi MN 2002. Pollen morphology of some tribes of Brassicaceae from Egypt and its systematic implications. *Feddes Repertorium* **113**: 3-4.
- Khan R 2004. Studies on the pollen morphology of the genus *Arabidopsis* (Brassicaceae) from Pakistan. *Pak. J. Bot.* **36**: 229-234.
- Kızılpınar İ Altınözlü H Doğan C 2012. Pollen morphology of the some species of the genus *Malcolmia* (Brassicaceae). *Mellifera* **12-23**: 24-29.
- Meikle RD 1977. Flora of Cyprus. Published by The Bentham-Moxon Trust Royal Botanic Garden, Kew.
- Mutlu B and Erik S 2012. Pollen morphology and its taxonomic significance of the genus *Arabis* (Brassicaceae) in Turkey. *Pl. Syst. Evol.* **298**: 1931-1946.
- Özgökçe F and Ünal M 2007. A new record for Turkey: *Malcolmia exacoides* (DC.) Spreng. (Brassicaceae). *Turk. J. Bot.* **31**: 345-347.
- Perveen A Qaiser M and Khan R 2004. Pollen flora of Pakistan –XLII Brassicaceae. *Pak. J. Bot.* **36**: 683-700.
- Pınar NM Duran A Çeter T and Tuğ GN 2009. Pollen and seed morphology of the Genus *Hesperis* L. (Brassicaceae) in Turkey. *Turk. J. Bot.* **33**: 83-96.
- Rollins RC and Banerjee M 1979. Pollen of Cruciferae. 33-64, Cambridge, M.A. Bussey. Inst. Harvard University.
- Ünal M and Özgökçe F 2008. A new record for Turkey: *Malcolmia intermedia* C.A. Mey. (Brassicaceae). *Turk. J. Bot.* **32**: 415-417.

(Manuscript received on 3 March, 2016; revised on 15 May, 2017)