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Fruit and seed morphology of six species previously placed in *Malcolmia* (Brassicaceae) in Turkey and their taxonomic value

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Abstract: The fruit and seed morphologies of 6 species previously placed in the genus *Malcolmia* W.T.Aiton were examined with stereomicroscopy and scanning electron microscopy (SEM) in order to determine the significance of fruit and seed features as taxonomic characters. This study presents macro- and micromorphological characters, including fruit and seed shape, size, colour, fruiting pedicel size, fruit hair and density, seed coat pattern, epidermal cell shape, and anticlinal and periclinal cell walls. The results showed that the morphological characteristics of the fruit and seed could be used as criteria to distinguish genera and tribes. The investigated features of specimens indicated that *Malcolmia chia* (L.) DC. and *M. flexuosa* (Sibth. & Sm.) Sibth. & Sm. should remain in the genus that belongs to the tribe *Anastaticeae*. *Malcolmia crenulata* (DC.) Boiss. and *M. exacoides* (DC.) Spreng. belong to the genus *Zuvanda* (Dvořák) Askerova of the tribe *Conringieae*. Finally, *Malcolmia africana* (L.) W.T.Aiton and *M. intermedia* C.A.Mey. belong to the genus *Strigosella* Boiss. of the tribe *Euclidieae*. A key is provided for the identification of the investigated species based on fruit and seed characters.

Key words: Cruciferae, fruit, *Malcolmia*, morphology, seed, SEM, taxonomy

Türkiye'de günümüze kadar *Malcolmia* (Brassicaceae) cinsinde yer alan 6 türün meyve ve tohum morfolojisinin taksonomik değeri

Özet: Önceleri *Malcolmia* W.T.Aiton. cinsine yerleştirilen 6 türün meyve ve tohum morfolojileri, stereomikroskobu ve taramalı elektron mikroskobu (SEM) ile incelenerek taksonomik karakter olarak önemleri belirlendi. Makro- ve mikromorfolojik karakterleri, meyve ve tohum şekilleri, boyu, renk, meyve sapı boyu, meyvede tüy ve yoğunluğu, tohumu örten desen, epidermal hücre şekli, antiklinal ve periklinal hücre duvarı incelendi. Sonuçlar gösterdi ki, türlerinin herbirinin meyve ve tohumunun morfolojik karakterlerinin, tribus ve cins ayrımında kullanılabileceğini gösterdi. *Malcolmia chia* (L.) DC. ve *M. flexuosa* (Sibth. & Sm.) Sibth. & Sm. tribus *Anastaticeae*'de ve *Malcolmia* cinsinde kaldı. *Malcolmia crenulata* (DC.) Boiss. ve *M. exacoides* (DC.) Spreng. *Zuvanda* (Dvořák) Askerova cinsi ve tribus *Conringieae*'de yer aldığı ve son olarak, *Malcolmia africana* (L.) W.T.Aiton ve *M. intermedia* C.A.Mey. *Strigosella* Boiss. cinsi ve tribus *Euclidieae*'de yer alması gerektiği çalışmamızın sonuçları ile desteklendi. İncelenen türlerin meyve ve tohum özellikleri temel alınarak, teşhis anahtarı oluşturuldu.

Anahtar sözcükler: Cruciferae, meyve, *Malcolmia*, morfolojisi, tohum, SEM, taksonomi

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Introduction

Most systematists agree that data concerning the macro- and microstructure of fruits and seeds are very significant for the classification of Angiosperm taxa. Heywood (1971) drew attention to the importance and impact of scanning electron microscopy (SEM) in his study of systematic problems, as very valuable information has been provided by using this technique. Recently, the application of SEM to the study of the fruit and seed coat has become widespread. Concerning the family Brassicaceae, the exo- and endomorphic characters of seeds have been studied by Murley (1951), El-Naggar (1996), Fayed and El-Naggar (1988, 1996), El-Naggar and El-Hadidi (1998), and Abdel Khalik and van der Maesen (2002). Vaughan and Whitehouse (1971) studied the macro- and micromorphological characters of approximately 90 genera and 200 species of Brassicaceae and paid special attention to the relationships between structure and existing taxonomy. Seed coat morphology is known to be an important character for taxonomic and evolutionary studies. Koul et al. (2000), Zeng et al. (2004), Moazzeni et al. (2007), Pınar et al. (2007, 2009), and Duran (2009) investigated the seed coat morphology and microsculpturing in certain genera of Brassicaceae and provided evidence for the close relationships among various genera. The present investigation determined seed coat pattern in 78 accessions encompassing 44 species representing 11 genera, namely *Brassica* L. (15 of 35 species), *Diplotaxis* DC. (12 of 25 species), *Sinapis* L. (4 of 5 species), *Eruca* Mill. (1 species), *Erucastrum* C.Presl (5 of 25 species), *Trachystoma* O.E.Schulz (1 of 3 species), *Hirschfeldia* Moench (1 species), and *Coincya* Rouy (1 of 6 species) of subtribe *Brassicinae*; and *Raphanus* L. (2 of 3 species), *Enarthrocarpus* Labill. (1 of 5 species), and *Moricandia* DC. (1 of 7 species) of subtribes *Raphaninae* and *Moricandiinae* (Koul et al., 2000).

At present, seed coat patterns are used for various purposes: to solve classification problems, to establish evolutionary relationships, to elucidate the adaptive significance of the seed coat, and to serve as genetic markers for the identification of genotypes in segregating hybrid progenies (Zeng et al., 2004; Bayrakdar et al., 2010; Poyraz et al., 2010).

The genus *Malcolmia* W.T.Aiton is a member of the family Brassicaceae and it comprises 10 species distributed throughout the world (Al-Shehbaz et al., 2006). *Malcolmia* was previously represented by 6 species in Turkey (Cullen, 1965). However, recent reports have indicated that the *Malcolmia* is represented by 4 species: *Malcolmia chia* (L.) DC., *M. flexuosa* (Sibth. & Sm.) Sibth. & Sm., *M. micrantha* Boiss. & Reut., and *M. graeca* Boiss. & Spruner, belonging to the tribe *Anastaticeae* (German et al., 2009; Warwick et al., 2010). *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. *Malcolmia crenulata* (DC.) Boiss. and *M. exacoides* (DC.) Spreng. (Özgökçe & Ünal, 2007) belong to the genus *Zuvanda* (Dvořák) Askerova (Askerova, 1985; Al-Shehbaz et al., 2007; Doğan et al, 2011) of the tribe *Conringieae* (German et al., 2009; Warwick et al., 2010), while *Malcolmia africana* (L.) W.T.Aiton and *M. intermedia* C.A.Mey. (Ünal & Özgökçe, 2008) belong to the genus *Strigosella* Boiss. (Botschantsev, 1972; Al- Shehbaz et al., 2007; Doğan et al., 2011) of the tribe *Euclidieae* (German et al., 2009; Warwick et al., 2010).

Malcolmia species are slender annual herbs. They have simple, forked, medifixed (bifid) or stellate hair. Sepals are saccate or not. Petals are reddish to pale lilac in colour. Fruit is a siliqua and seeds are located in one row in each loculus. Fruit morphology is of importance at the species level in *Malcolmia* according to *Flora of Turkey*.

Until now, no study has reported on the use of fruit and seed morphology for identification of *Malcolmia*, *Strigosella*, and *Zuvanda* taxonomy. The present investigations were carried out on the morphological characters of fruits and seeds in the 6 species from Turkey previously placed in *Malcolmia*. Stereomicroscopy and SEM observations were carried out to emphasise the taxonomic significance of fruits and seed morphological characters as criteria for the separation of species.

Materials and methods

Malcolmia, *Strigosella*, and *Zuvanda* species 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). Only mature fruits and seeds samples were taken for investigation and 15-20 dried fruits and seeds samples were examined for each species. Measurements and optical observations of fruit and seed colours were carried out under a Wild M5 stereomicroscope. For SEM, fruits and seeds were mounted directly onto stubs, using single-sided adhesive tape, and coated with gold. Photographs were taken with an EVO-50. The terms used for describing the seed surface patterns have been adopted according to the works of Murley (1951), Stearn (1992), and Koul et al. (2000).

Results

The morphological characters of the fruit and seeds from the studied species of *Malcolmia*, *Strigosella*, *and Zuvanda* are given below. Data obtained by stereomicroscope and SEM investigation are reviewed in Tables 2 and 3, as well as in Figures 1-3.

Fruit characters

The fruit types of *Malcolmia*, *Strigosella*, and *Zuvanda* species are siliqua. Style is absent. Stigma is small and 2-lobed or acute. Siliquae are linear, rigid, usually terete or rarely more or less 4-angled in *Malcolmia chia*, and ascending to spreading. However, the shape of siliqua is straight in *Strigosella africana* (L.) Botsch., *Malcolmia chia*, and *M. flexuosa*; slightly curved in *Zuvanda exacoides* (DC.) Askerova, *Z. crenulata* (DC.) Askerova, and *Strigosella intermedia* (L.) Botsch.; and prominently curved downwards and flexuous in *M. flexuosa* (Figure 1). Siliquae of *Z. crenulata* are tortulose.

Fruit length varies greatly among the examined species; the longest linear siliquae in *Z. crenulata* have a length of 50-107 × 1-2.1 mm and the smallest fruits measure 29-45 × 1-1.75 mm in *S. intermedia*, while the rest of the species demonstrate longer fruits of 32-75 × 0.8-2.5 mm. Fruiting pedicel lengths also have systematic significance. The pedicels of *Z. crenulata* are the longest, at 7-15 × 0.75-1.5 mm, while they are the smallest in *S. intermedia*, at 0.5-2.0 × 0.75-1.5 mm. However, fruiting pedicels in *M. flexuosa* are incrassate (to 2.5 mm). The colour of the fruit can also be of considerable diagnostic value. The colour of fruits varies from green in *Z. crenulata*, *M. chia*, and *Z. exacoides*, to yellowish-green in *S. africana* and *S. intermedia* and pale green to reddish-green in *M. flexuosa* (Table 2).

The surface of fruits varies from glabrous to hairy. In the genera *Malcolmia*, *Strigosella*, and *Zuvanda*, the surface was found to be glabrous in *Z. crenulata* and hairy in the rest of the species. The hair type and density on the fruit surface are significant characters used to differentiate between *Malcolmia*, *Strigosella*, and *Zuvanda* species. Trichomes consist of distinct, primary axis (stalk), and 2 (forked) branches. Stalkless trichomes consisting of 2 main branches are

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

Taxon	Locality	VANF
Malcolmia chia	Turkey, Antalya, 07.05.2009	10153
Malcolmia flexuosa	Turkey, İzmir, 05.05.2009	10145
Strigosella intermedia	Turkey, Iğdır, 06.04.2009	10019
Strigosella africana	Turkey, Siirt, 28.03.2009	10001
Zuvanda crenulata	Turkey, Şanlıurfa, 31.03.2009	10043
Zuvanda exacoides	Turkey, Siirt, 28.03.2009	10011

Table 2. The morphological characters of fruits the genera *Malcolmia*, *Strigosella*, and *Zuvanda*.

Taxon	Shape	Size (mm)	Colour (mm)	Pedicel	Hair type	Hair density *
M. chia	straight linear, more or less 4-angled	37-57 × 0.8-1.5	green	4-10 × 0.8-1.25	medifixed (2-4-fid)	++, +++
M. flexuosa	straight linear, curved downwards	64 × 1.5-2.5	pale green to reddish-green	5-14 × 1-2.5	medifixed (2-fid)	+, ++
S. africana	straight linear	39-75 × 1-1.5	green to yellowish-green	1.5-4 × 0.8-1.5	forked	+, ++
S. intermedia	curved linear	29-45 × 1-1.75	green to yellowish-green	0.5-2 × 0.75-1.5	setose	+, ++
Z. crenulata	linear or curved, ± tortulose	50-107 × 1-2.1	green	7-15 × 0.75-1.5	glabrous	-
Z. exacoides	linear, ± curved	44-58 × 1-2	green	5-6 × 0.8-1.1	scabrous	+

* Symbols indicate: -: not hairy; +: less hairy; ++: hairy; +++: densely hairy.

Table 3. The morphological characters of seeds of the genera *Malcolmia*, *Strigosella*, and *Zuvanda*.

Species	Shape	Size (mm)	Colour	Surface pattern	Epidermal cell shape	Anticlinal cell wall	Periclinal cell wall
M. chia	oblong	1.2 -1.4 × 0.4-0.6	dark brown	reticulate-papillate	isodiametric 5-6 gonal cells	raised, straight	flat to concave, smooth
M. flexuosa	oblong to narrowly oblong	1.5-1.7 × 0.6-0.7	brown	reticulate-striate	irregular narrowly polygonal cells	raised, straight to slightly sinuous	flat to concave, smooth-folded
S. africana	rectangular-oblong	0.9-1 × 0.5-0.6	yellowish-brown	reticulate-undulate	irregular 4-polygonal cells	raised-channelled, undulated to folded	flat to concave, coarse folds
S. intermedia	oblong to broadly oblong	1.2-1.3 × 0.7-0.8	yellowish with green line	reticulate	large isodiametric 5-6 gonal cells	raised, straight	flat to slightly concave, smooth
Z. crenulata	narrowly oblong to obovate	1.3-1.4 × 0.4-0.5	brown	foveolate	irregular 4-5 gonal cells	raised, straight to folded	flat to concave, fine folds
Z. exacoides	obovate	1.5-1.8 × 0.7-1	brown	rugose	irregular to elongate in one direction	channelled, undulate-sinuous	rugulate to concave folded

termed malpighiaceous (medifixed), and those with 4 branches are stellate. Scabrous trichomes consist of short, hardish points while setose trichomes consist of long and hardish or slightly curved hairs. The hair type varies and is seen to be forked in *S. africana*, setose in *S. intermedia*, medifixed in *M. chia* (2-4-fid) and *M. flexuosa* (2-fid), scabrous in *Z. exacoides*, and glabrous in *Z. crenulata*. Cuticular micropapillae are observed on medifixed hairs of *M. chia* and *M. flexuosa* (Table 2, Figure 2).

Seed characters

The shape of seeds among the investigated species showed a large variation. Seeds are rectangular-oblong in *S. africana*, narrowly oblong to obovate in *Z. crenulata*, oblong in *M. chia*, oblong to narrowly oblong in *M. flexuosa*, obovate in *Z. exacoides*, and oblong to broadly oblong in *S. intermedia*. Seed dimensions also vary greatly among the examined species; the largest obovate seeds in *Z. exacoides* have a diameter of 1.5-1.8 × 0.7-1 mm and the smallest

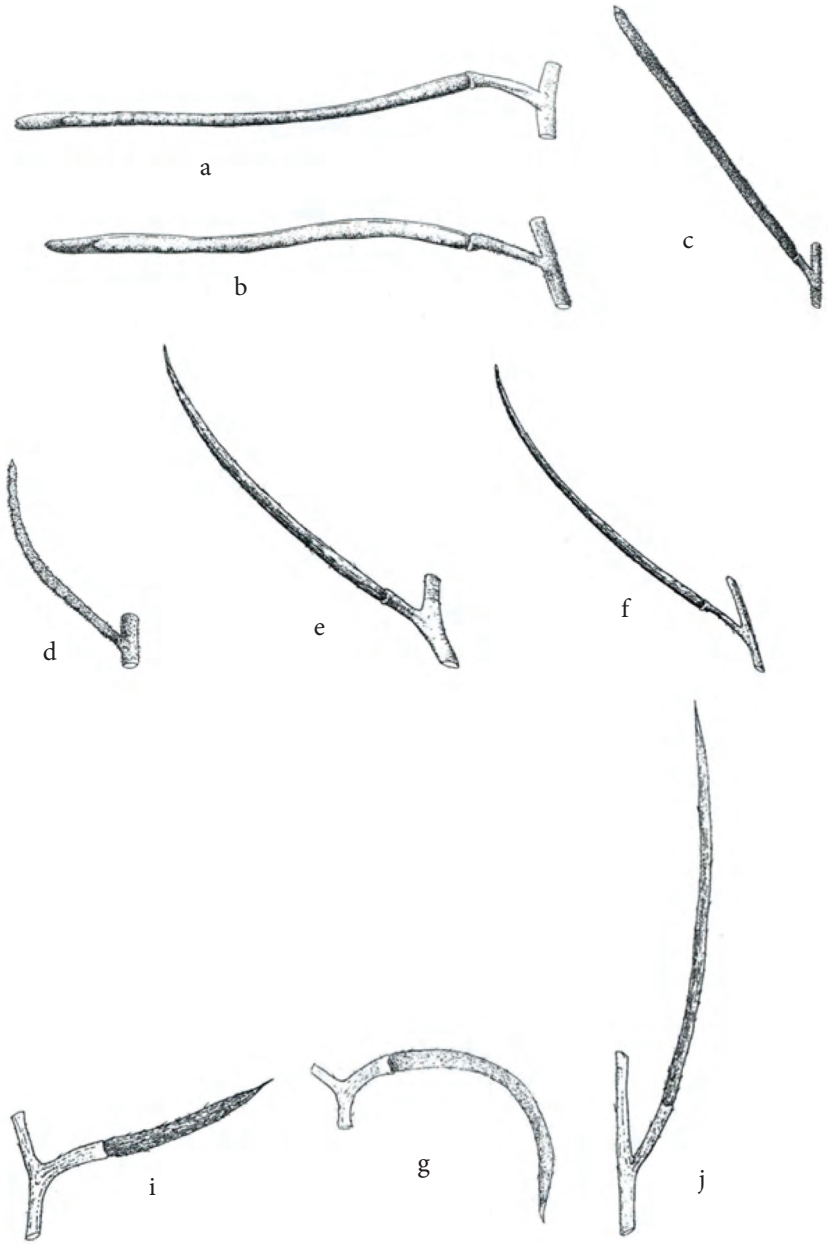

Figure 1. Siliquae shapes of *Malcolmia*, *Strigosella*, and *Zuvanda* species in natural size: a) *Z. crenulata*, b) *Z. exacoides*, c) *S. africana*, d) *S. intermedia*, e and f) *M. chia*, g and j) *M. flexuosa*.

seeds measure 0.9-1 × 0.5-0.6 mm in *S. africana*, while the rest of the species have slightly larger seeds. Seed colour was observed to be of high diagnostic value. The colour of seed varies from yellowish-brown in *S. africana* to brown in *Z. crenulata*, *M. flexuosa*, and *Z. exacoides*, while it is dark brown in *M. chia* and yellowish with green lines in *S. intermedia* (Table 3, Figures 2 and 3).

The surface patterns and epidermal cell shapes of seeds are of high diagnostic and systematic interest among the species. The surface pattern is reticulate-undulate in *S. africana*, foveolate in *Z. crenulata*, reticulate-papillate in *M. chia*, reticulate-striate in *M. flexuosa*, rugose in *Z. exacoides*, and reticulate in *S. intermedia*. The surface patterns of seed coats were found useful for distinguishing all *Malcolmia*,

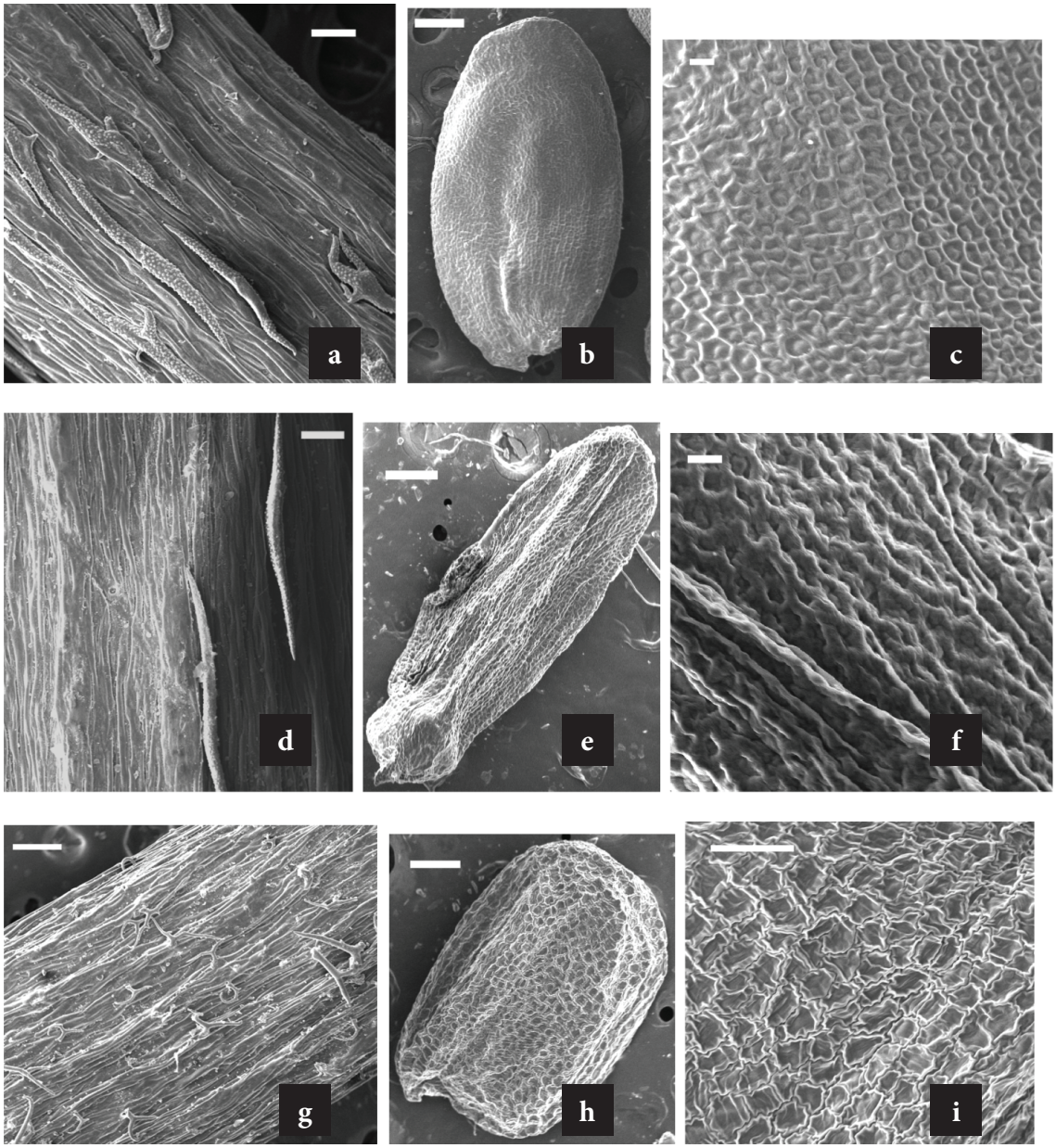

Figure 2. SEM photographs of *Malcolmia* and *Strigosella* species. *M. chia*: a) fruit, b) seed, c) seed coat pattern; *M. flexuosa*: d) fruit, e) seed, f) seed coat pattern; *S. africana*: g) fruit, h) seed, i) seed coat pattern. Scale bars: b, e, g, h = 200 mm; a, d, i = 100 mm; c, f = 20 mm.

Strigosella, and *Zuvanda* species. The shapes of epidermal cells show significant variations between species of these genera. They are irregular, 4-polygonal cells in *S. africana*; 4-5 gonal cells in *Z. crenulata*; large to narrow isodiametric, 5-6 gonal cells in *S. intermedia* and *M. chia*; irregular, narrowly polygonal cells in *M. flexuosa*; and irregular to elongate in 1 direction in *Z. exacoides*. Anticlinal and periclinal cell walls can also serve as a good diagnostic character for *Malcolmia*, *Strigosella*, and *Zuvanda* species. Anticlinal cell walls are generally well-developed in the genera *Malcolmia*, *Strigosella*, and *Zuvanda*. In this study, 3 types of cell boundaries were observed: 1) raised and straight in *M. chia* and *S. intermedia*, straight to slightly sinuous in *M. flexuosa*, straight to sinuous and folded in *Z. crenulata*; 2) channelled and undulate-sinuous in

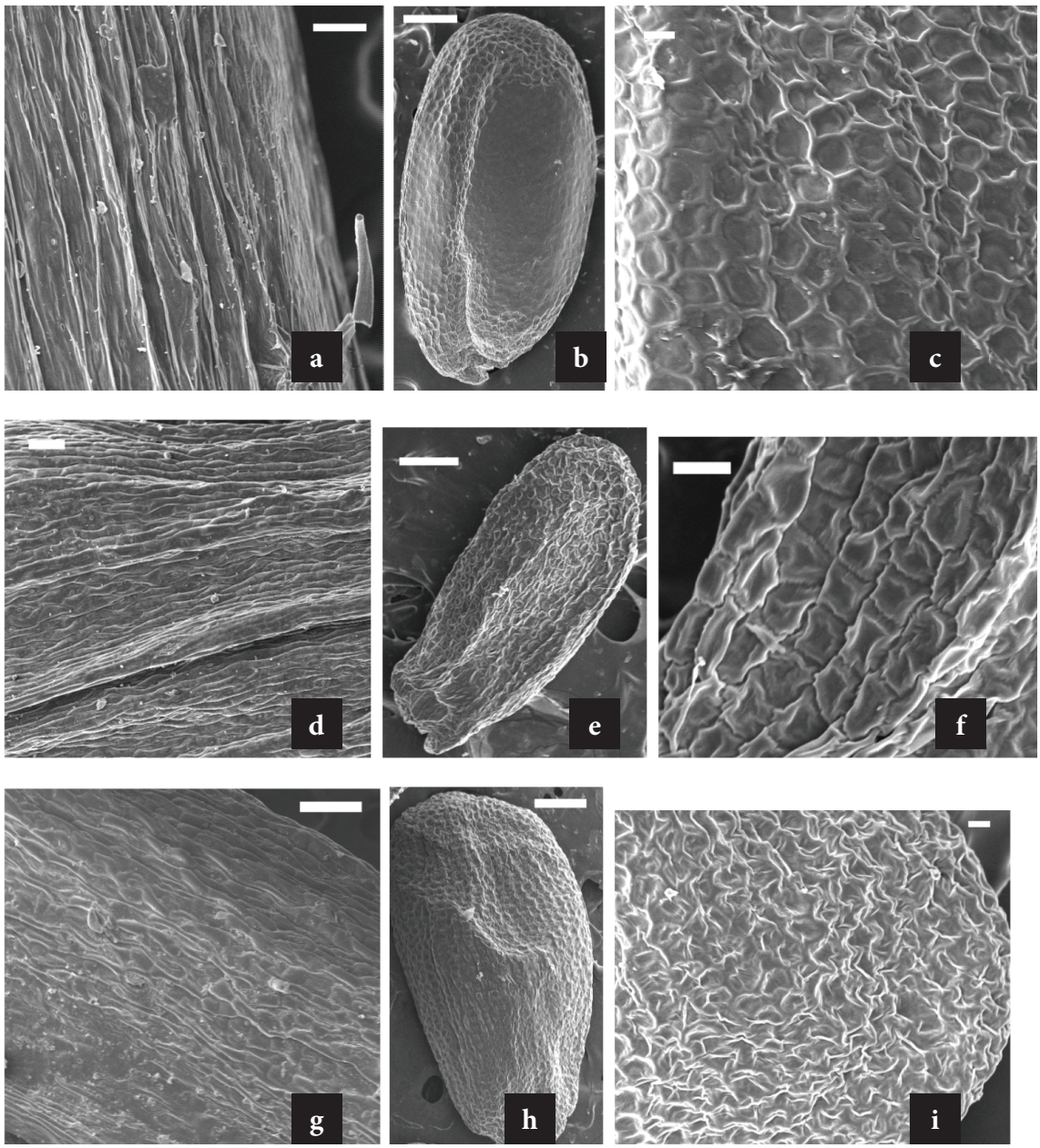

Figure 3. SEM photographs of *Strigosella* and *Zuvanda* species. *S. intermedia*: a) fruit, b) seed, c) seed coat pattern; *Z. crenulata*: d) fruit, e) seed, f) seed coat pattern; *Z. exacoides*: g) fruit, h) seed, i) seed coat pattern. Scale bars: b, e, h = 200 mm; a, d, g = 100 mm; c, f, i = 20 mm.

Z. exacoides; 3) raised-channelled and undulated to folded in *S. africana*. There are 2 different shapes for the periclinal cell wall: 1) flat to concave with coarse folds in *S. africana*, fine folds in *Z. crenulata*, smooth in *M. chia* and *S. intermedia*, smooth-folded in *M. flexuosa*; 2) rugulate to concave and folded in *Z. exacoides* (Table 3, Figures 2 and 3).

Discussion

The morphological characters of fruits and seeds provide valuable information about the evolutionary classification of flowering plants (Corner, 1976). In our observations, the fruit and seed characters of *Malcolmia*, *Strigosella*, and *Zuvanda* vary between different species, particularly with regard to fruit and

seed shapes, size, colour, fruit hair type and density, pedicel size, seed coat pattern, seed epidermal cell shape, and anticlinal and periclinal cell walls (Tables 2 and 3). Fruit and seed morphological characters are very important in separating species in *Malcolmia*, *Strigosella*, and *Zuvanda*. These features can be used in genera taxonomy.

Fruit morphology, especially hair types, was used for distinguishing *Malcolmia* species in *Flora of Turkey* (Cullen, 1965). While siliquae are spreading-pilose with mainly stalked forked hairs in *S. africana*, they are adpressed-pilose with bifid, stellate hairs or glabrous (*Z. crenulata*) in the rest of the species. Forked, stellate, and medifixed trichomes are also observed in different Brassicaceae species (Beilstein et al., 2006). Medifixed trichomes have cuticular micropapillae, which are quite characteristic for Brassicaceae (Barthlott, 1981). Fruiting pedicels are somewhat incrassate in *M. chia*, although they are identified as strongly incrassate in *M. flexuosa* according to *Flora of Turkey*. Our results are similar with those of *Flora of Turkey* (Cullen, 1965).

Seed surface patterns and seed coat anatomy have been shown to provide valuable characters for use in the delimitation of taxa in selected groups of Brassicaceae (Murley, 1951; Vaughan & Whitehouse, 1971; Barthlott, 1981; Koul et al., 2000), though such studies were lacking for *Malcolmia*, *Strigosella*, and *Zuvanda.* However, the micromorphological studies presented here have revealed that their surfaces feature reticulate, foveolate, and rugose patterns. The reticulate type of seed surface sculpturing is more common among the species studied (Barthlott, 1981; Koul et al., 2000; Zeng et al, 2004). A reticulate pattern is observed in *S. intermedia*. Moreover, some rare sculpturing types, such as reticulate-papillate, which is known in few taxa of Brassicaceae, e.g. *Capsella bursa-pastoris* (L.) Medik. (Tantawy et al., 2004) and *Diplotaxis tenuifolia* (L.) DC. (Koul et al., 2000), are also present in *M. chia* here. The reticulate-striate type is found in *M. flexuosa* while the reticulate-undulate type is observed in *S. africana*. *Z. exacoides* has a rugose surface pattern, which has also been determined in *Brassica cossoneana* Boiss. & Reut. (Koul et al., 2000). *Z. crenulata* has a foveolate surface pattern. Similarly, *Z. crenulata* resembled *Diplotaxis harra* (Forssk.) Boiss. in terms of seed surface pattern (Koul et al., 2000).

The genera *Malcolmia*, *Strigosella*, and *Zuvanda* have irregular 4-polygonal to isodiametric 5-6 gonal cells or irregular to elongate in 1 direction epidermal cell shapes. The present study observed 3 types of anticlinal cell walls and 2 types of periclinal cell wall boundaries, some types of which were occasionally represented by more than 1 species belonging to different genera (Barthlott, 1981; Koul et al., 2000; Abdel Khalik, 2006; Abdel Khalik & Osman, 2007).

The checklist of *Flora of Turkey* has recently added 2 species of *Zuvanda* and *Strigosella*, *Z. exacoides* (Özgökçe & Ünal, 2007; Al-Shehbaz et al., 2007) and *S. intermedia* (Ünal & Özgökçe, 2008; Al-Shehbaz et al., 2007), as new records. *S. intermedia* and *Z. exacoides* grow in a very limited area in Turkey and are under high grazing pressure. The recommended IUCN Red Category is Vulnerable (VU (B1a)) (IUCN, 2001). The closest species to *Z. exacoides* among Turkish *Zuvanda* is *Z. crenulata* (Özgökçe & Ünal, 2007). They can be easily differentiated, especially with regard to characters such as the lengths of the siliqua, fruiting pedicel, and seed; seed surface pattern; epidermal cell shape; and anticlinal-periclinal cell walls.

In conclusion, the present study supports the use of fruit and seed morphological characters as a parameter for species identification.

Key to *Malcolmia, Strigosella*, and *Zuvanda* species from Turkey, based on fruit and seed characters

1. Siliquae furcate, hairy; seeds rectangular-oblong, reticulate-undulate; epidermal cell shape irregular 4-polygonal cells, anticlinal cell wall raised-channelled.....…..*S. africana*

1. Siliquae setose, medifixed hairy, scabrous or glabrous; seeds oblong to broadly oblong, reticulate, epidermal cell shape large isodiametric 5-6 gonal cells, anticlinal cell wall raised, and straight ……2

2. Siliquae setose hairy, 29-45 mm long; seeds yellowish with green line, reticulate; epidermal cell shape isodiametric 5-6 gonal cells, anticlinal cell wall raised, periclinal cell wall flat to slightly concave…..…… *S. intermedia*

2. Siliquae glabrous, scabrous or medifixed hairy, longer than 45 mm, ± tortulose; seeds foveolate, epidermal cell shapes 4-5 gonal cells.....3

3. Siliquae glabrous, to 107 mm long, ± tortulose; seeds foveolate, epidermal cell shapes 4-5 gonal cells…….....…….*Z. crenulata*

3. Siliquae scabrous or medifixed hairy, to 75 mm long, not tortulose; seeds rugose, epidermal cell shapes irregular to elongate in 1 direction…….....……....4

4. Siliquae scabrous, 44-58 mm long, fruiting pedicels 5-6 mm long; seeds obovate, rugose...……....…..*Z. exacoides*

4. Siliquae medifixed hairy, fruiting pedicels 4-14 mm long; seeds oblong to narrowly oblong, reticulate-striate or reticulate-papillate ……5

5. Siliquae curved downwards, fruiting pedicels incrassate (to 2.5 mm wide); seeds 1.5-1.7 × 0.6-0.7 mm, brown, reticulate-striate ….....*M. flexuosa*

5. Siliquae not curved, fruiting pedicels not incrassate (to 1.25 mm wide); seeds 1.2-1.4 × 0.4-0.6 mm, dark brown, reticulate-papillate……...…….…….…… *M. chia*

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