

ARTICLE

## Seed morphology diversity in some Iranian endemic *Silene* (Caryophyllaceae) species and their taxonomic significance

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**ABSTRACT** Seed morphology diversity of 16 endemic *Silene* species was studied using Scanning Electron Microscope (SEM) to describe the seed micromorphology features and evaluate their taxonomic significance. Fifteen qualitative and quantitative characters were measured using SEM micrographs and stereomicroscopy. The seeds shape of the most species is reniform. The size of seeds ranges from 0.85 × 0.62 mm in case of *S. elymaitica* to 2.28 × 1.4 mm in *S. nizvana*. The ornamentation of seed coat is papillate in *S. elymaitica* and *S. oligophylla*, tuberculate in *S. daenensis* and the seed surface of the rest is smooth. Three types of the edge of testa cells; V form, U form and entire were observed. This study indicates that seed characters, such as shape, size, dorsal surface, the shape and size of testa cells, the testa cells edge and testa cells surface ornamentation are useful in identification and classification of the species studied. An identification key is also presented based on seed morphology characters.

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**KEY WORDS**

micromorphology  
*Silene*  
seed coat ornamentation  
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*Silene* L. with over 700 annual, perennial herbaceous and rarely subshrub species is the largest genus of the family Caryophyllaceae, mainly growing in various habitats of temperate regions of the Northern hemisphere (Chowdhuri 1957; Greuter 1995; Oxelman 1995). Due to the large number of species, as well as the wide range of habitats and geographical distribution, the circumscription, classification and nomenclature of the genus have long been controversial (Chowdhuri 1957; Greuter 1995; Oxelman 1995; Eggens et al. 2007; Gholipour et al. 2010). In spite of some taxonomic studies on the various aspects of the genus *Silene*, our knowledge about the genus is still incomplete (Ghazanfar 1983 and 1989; Greuter 1995; Oxelman 1995; Oxelman 1996; Oxelman et al. 1997; Yildiz et al. 1998; Oxelman et al. 2001; Popp et al. 2005; Popp et al. 2007; Gholipour and Sheidai 2009; Gholipour et al. 2010).

The genus *Silene* is represented by ca. 105 species in the flora of Iran, mainly growing in the mountainous areas, out of which 36 species are endemic with very limited geographical distribution (Melzheimer 1988; Gholipour and Sheidai 2010). Endemic species are important on both global and local levels, as they provide unique genetic diversity for further studies and provide local people with priceless services (Newmark 2002). They increase the amount of genetic diversity and offer insight into biogeographical questions, such as where certain species originated and how distribution patterns have changed over time (Sharbek 2008). Some local endemic *Silene* species such

as *S. daenensis* Melzh., *S. dschuparensis* Boiss., *S. hirticalyx* Boiss. & Hausskn., *S. oligophylla* Melzh., *S. psudaucheriana* Melzh., *S. nizvana* Melzh. and *S. pseudonurensis* Melzh. have so far been recorded only from locus classicus, are some important parts of gene pool of the flora of Iran (Melzheimer 1985; Gholipour and Sheidai 2009).

Seed morphology studies of some species of the genus *Silene* have been revealed the taxonomic significance of those characters (El-oqla et al. 1990; Hosny et al. 1993; Hong et al. 1999; Minuto et al. 2006; Yildiz 1998; Perveen 2009; Fawzi et al. 2010). The seed morphology of seven Iranian *Silene* species including *S. bupleuroides* L., *S. swertifolia* Boiss., *S. indeprensa* Schischk., *S. gertraudiae* Melzh., *S. noctiflora* L., *S. latifolia* Poir. and *S. conoidea* L. have preliminary been described (Jafari et al. 2009) but there are no similar data on the other species. The present study aims to describe the seed micro morphology of 16 Iranian endemic *Silene* species and evaluate their taxonomic significance for the first time.

### Materials and methods

The seeds of 16 *Silene* species belonging to three sections (*Auriculatae* Boiss., *Lasiostemones* Boiss. and *Sclerocalycinae* Boiss.) were collected from natural habitats during the fruiting season of 2007-2010 years (Table 1). Plant specimens were identified using Flora Iranica (Melzheimer 1988) and compared with the type specimens deposited in G, W and IRAN herbaria. The vouchers are deposited in Shahid Beheshti and Sari Payame Noor Universities herbaria. Five fully developed seeds per species were selected using binocular stereoscope at 15x and 30x magnifications. Seeds attached

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**Table 1.** *Silene* species studied and voucher information. Abbreviation of the Section: *Auriculatae*: Au, *Sclerocalycinae* Scl and *Lasiostemones* Las.

Taxa	Code	Section	Voucher
<i>S. daenensis</i> Melzh.	dae	Au	Kuhgyloieh va Boyer Ahmad, Yasuj, Sisakht, Dena, 4000 m, 28 July 2008, A. Gholipour 8779.
<i>S. dschuparensis</i> Boiss.	dsc	Au	Kerman, Mahan, Tigran, Kuh-e Jupar, 2811 m, 26 June 2007, A. Gholipour 8673.
<i>S. elymaitica</i> Bornm.	ely	Au	Chaharmahal-o Bakhtiari, Farsan, Kuhrang, Zard kuh, 3350 m, 29 July 2008, A. Gholipour 8781.
<i>S. gynodioica</i> Ghazanfar	gyn	Au	Zanjan, Dandi, Kuh- e Anguran, 2469 m, 21 July 2007, A. Gholipour 8694-1.
<i>S. gertraudiae</i> Melzh.	ger	Au	Semnan, Sorkheh to Firouzkuh, 1284 m, 2 June 2007, A. Gholipour 8620.
<i>S. hirticalyx</i> Boiss. & Hauskn.	hir	Au	Kermanshah, Ravansar to Paveh, km 10, Kuh- e Shaho, 2550 m., 24 July 2007, A. Gholipour 86101.
<i>S. nizvana</i> Melzh.	niz	Au	Semnan to Firouzkuh, km 25, Aftar, 1825 m, 2 June 2007, A. Gholipour 8621.
<i>S. oligophylla</i> Melzh.	oli	Au	Zanjan to Manjil, before Abbar, 2160 m, 19 June 2008, A. Gholipour 87066
<i>S. palinotricha</i> Fenzl ex Boiss.	pal	Au	Semnan, Ahvan pass, km 30 semnan, 2056 m, 1 June 2007, A. Gholipour 8618.
<i>S. parrowiana</i> Boiss. & Hauskn.	par	Las	Kermanshah, Bisetoun Mountain, 1295 m, 11 Nov. 1996, T. Khanezad.
<i>S. persica</i> Boiss.	pic	Au	Kuhgyloieh va Boyer Ahmad, Yasuj, Sisakht, Dena, 3200 m, 28 July 2008, A. Gholipour 8777.
<i>S. persepolitana</i> Melzh.	pep	Au	Fars, Shiraz, Bamo protected area, 12 May 2010, A. Gholipour 89016.
<i>S. pseudaucheriana</i> Melzh.	pau	Au	Lurestan, Azna, Drreh tacht, Oshoran kuh, 2535 m, 30 July 2008, A. Gholipour 87092.
<i>S. pseudonurensis</i> Boiss.	pnu	Au	Kerman, Chatrod, Horjund- e Soud Kuh, 2850 m, 27 June 2007, A. Gholipour 8675.
<i>S. sojakii</i> Melzh.	soj	Au	Semnan to Firouzkuh, km 30, Gandab, 2140 m, 2 June 2007, A. Gholipour 8625
<i>S. stapfii</i> Melzh.	sta	Scl	Yazd, Taft, Dehbala, 2400 m, 2 May. 2007, A. Gholipour 8606

on stubs, were coated with thin layer of gold-palladium in a sputter-coater. The prepared samples were observed and photographed by Scanning Electron Microscope (SEM) model Cam Scan MV 2300 at an acceleration voltage of 15 kV at Tehran University. Four micrographs were taken per taxon in lateral, dorsal and ventral views. The data were measured based on micrographs by image tool software and stereomicroscope observations. Totally 15 qualitative and quantitative characters were studied (Table 2). The scientific literatures on the seed morphology of *Silene* were followed for terminology (Hong et al. 1999; Minuto et al. 2006; Fawzi et al. 2010).

In order to group the species studied based on the seed morphology features, different clustering methods were performed using SPSS ver. 9 software. All characters were coded as multistate characters. Standardized seed morphology data (mean = 0, variance = 1) were used to determine taxonomic and Euclidean distances among the species were used in clustering analysis (Sheidai et al. 2010).

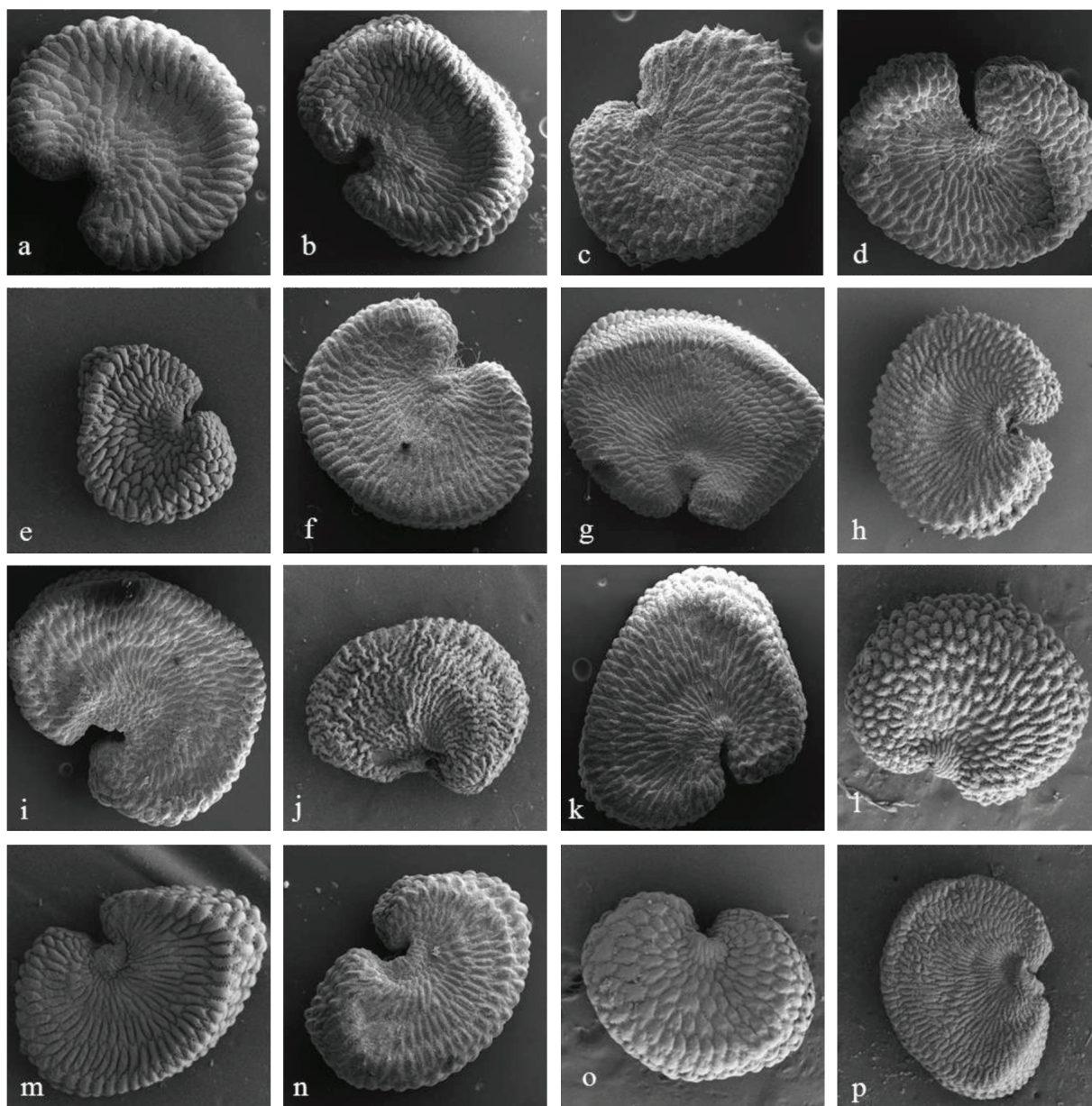
## Results

Seed morphology features of 16 Iranian endemic *Silene* species were described in detail for the first time (Table 2). The seed shape of 50 percent of the studied species is reniform and the rest have asymmetrical reniform and orbicular reniform seeds in outline (Fig. 1a-p). The size of the seeds range from  $0.85 \times 0.62$  mm in case of *S. elymaitica* to  $2.28 \times 1.4$  mm of *S. nizvana* (Table 2). The hilum of the seeds in all studied species locates in the median hole on the ventral face, but the ratio of hilum region length to width varies from 1.33 in case of *S. pseudaucheriana* to 4.4 in *S. nizvana* (Fig. 1g and m and

Table 2). The lateral surface of the seeds of the most studied species are concave, but convex in *S. parrowiana* (Fig. 1j) and flat in *S. stapfii* (Fig. 1p and Table 2). Three types of the seed dorsal surface are observed; convex in *S. daenensis*, *S. persepolitana*, *S. pseudaucheriana*, *S. oligophylla* and *S. sojakii*, flat in *S. hirticalyx* and *S. stapfii* and concave in the rest (Fig. 2 a-p). Seed coat ornamentation is papillate in *S. elymaitica* and *S. oligophylla* (Fig. 1 c and h), tuberculate in *S. daenensis* (Fig. 1 a), but in the rest is smooth. The shape of testa cell is fusiform-elliptic in *S. daenensis* and *S. nizvana* (Fig. 3 a and g), elliptic in *S. persepolitana* and *S. sojakii* (Fig. 3 l and o) and fusiform in the other species. Testa cell edge is sub entire in *S. palinotricha* (Fig. 3 i) and U form in *S. parrowiana* (Fig. 3 j) whereas it is V form in the other species (Fig. 3 a, b, c, e, f, g, h, k, l, m, n, o and p).

Based on the seed morphology data the different clustering methods produced similar results. The dendrogram of Ward method is presented here. The studied species arranges in three major clusters, A, B and C (Fig. 4). The first major cluster divides into two sub clusters, A1 and A2. *Silene stapfii* belongs to the section *Sclerocalycinae* along with *S. hirticalyx*, *S. oligophylla* and *S. daenensis* from the section *Auriculatae* constitute the first subcluster. A2 subcluster contains *S. gynodioica*, *S. elymaitica*, *S. gertraudiae*, *S. pseudonurensis* and *S. dschuparensis* all belong to the section *Auriculatae*. The second major cluster (B) comprises of closely related species of section *Auriculatae*, *S. sojakii* and *S. persepolitana*. *S. parrowiana* from the section *Lasiostemones* and remainders of the section *Auriculatae* place in the third major cluster (C).

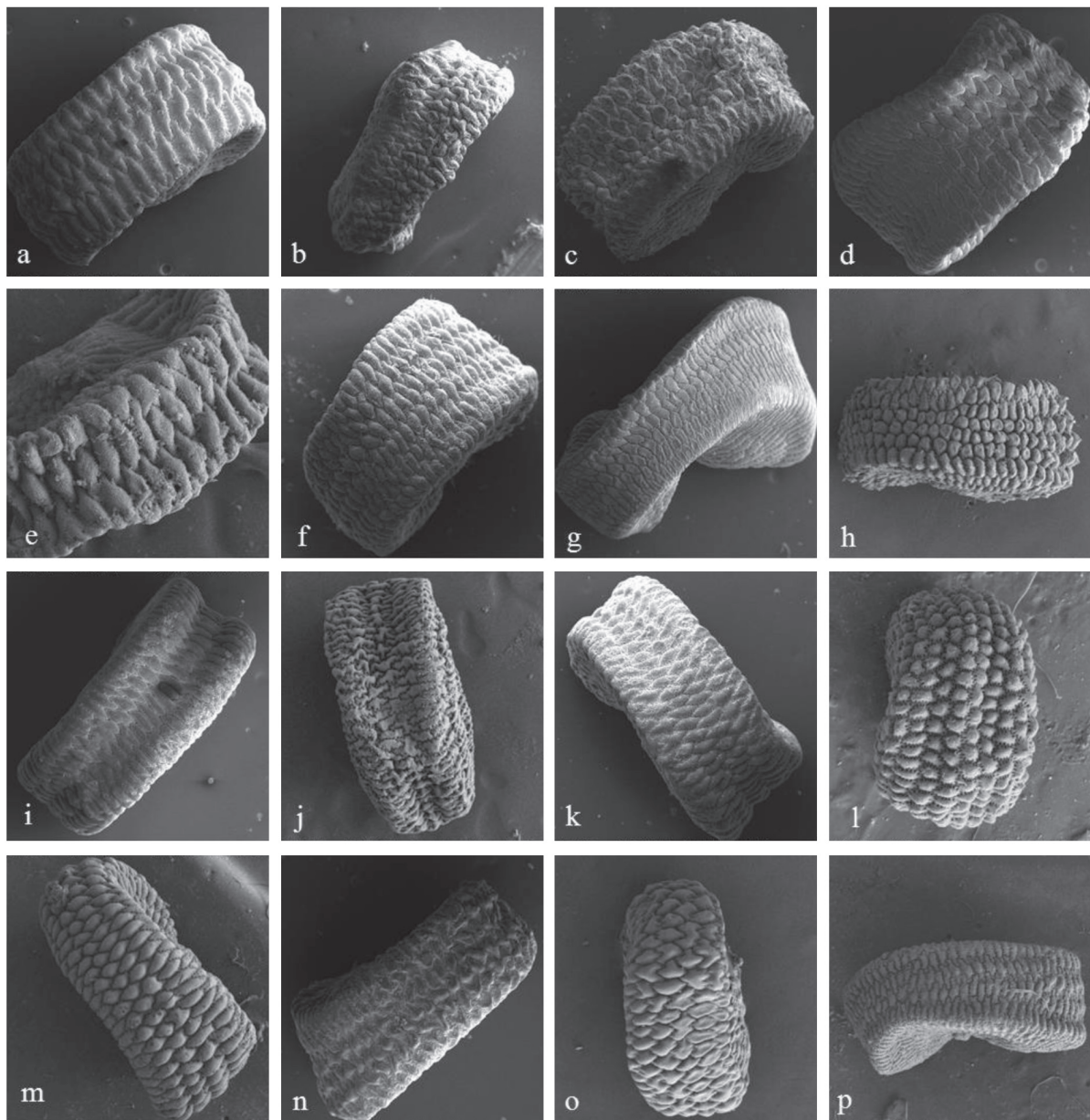




**Figure 1.** SEM micrographs of the seed shape of *Silene* species studied. Reniform: a, b, c, d, f, l, n and o. Orbicular reniform: e, h and p. Asymmetrical reniform: g, i, j, k and m. a. *S. daenensis*; b. *S. dschuparensis*; c. *S. elymaitica*; d. *S. gertraudiae*; e. *S. gynodioica*; f. *S. hirticalyx*; g. *S. nizvana*; h. *S. oligophylla*; i. *S. palinotricha*; j. *S. parrowiana*; k. *S. persica*; l. *S. persepolitana*; m. *S. psudaucheriana*; n. *S. psudonurensis*; o. *S. sojakii*; p. *S. stapfii* (Scale bar: g, m, p 1 mm and in the other cases 500  $\mu$ m).

- 10. Seeds orbicular reniform..... 11
  - 10. Seeds asymmetrical reniform..... 12
  - 11. Lateral and dorsal surface concave... *S. gynodioica*
  - 11. Lateral and dorsal surface flat..... *S. stapfii*
  - 12. Testa cell edge U form or entire..... 13
  - 12. Testa cell edge V form..... 14
  - 13. Testa cell edge U form, lateral surface convex.....
- S. parrowiana*
- 13. Testa cell edge entire, lateral surface concave.....

- S. palinotricha*
- 14. Seeds size 2.28  $\times$  1.44mm, testa cell fusiform-elliptic... *S. nizvana*
- 14. Seeds size shorter, testa cell fusiform..... 16
- 15. Seeds size 1.7  $\times$  1.2 mm, hilum region size 436.3  $\times$  322.8  $\mu$ m ..... *S. psudaucheriana*
- 15. Seeds size 1.4  $\times$  1.1mm, hilum region size 448.4  $\times$  170.9  $\mu$ m ..... *S. persica*



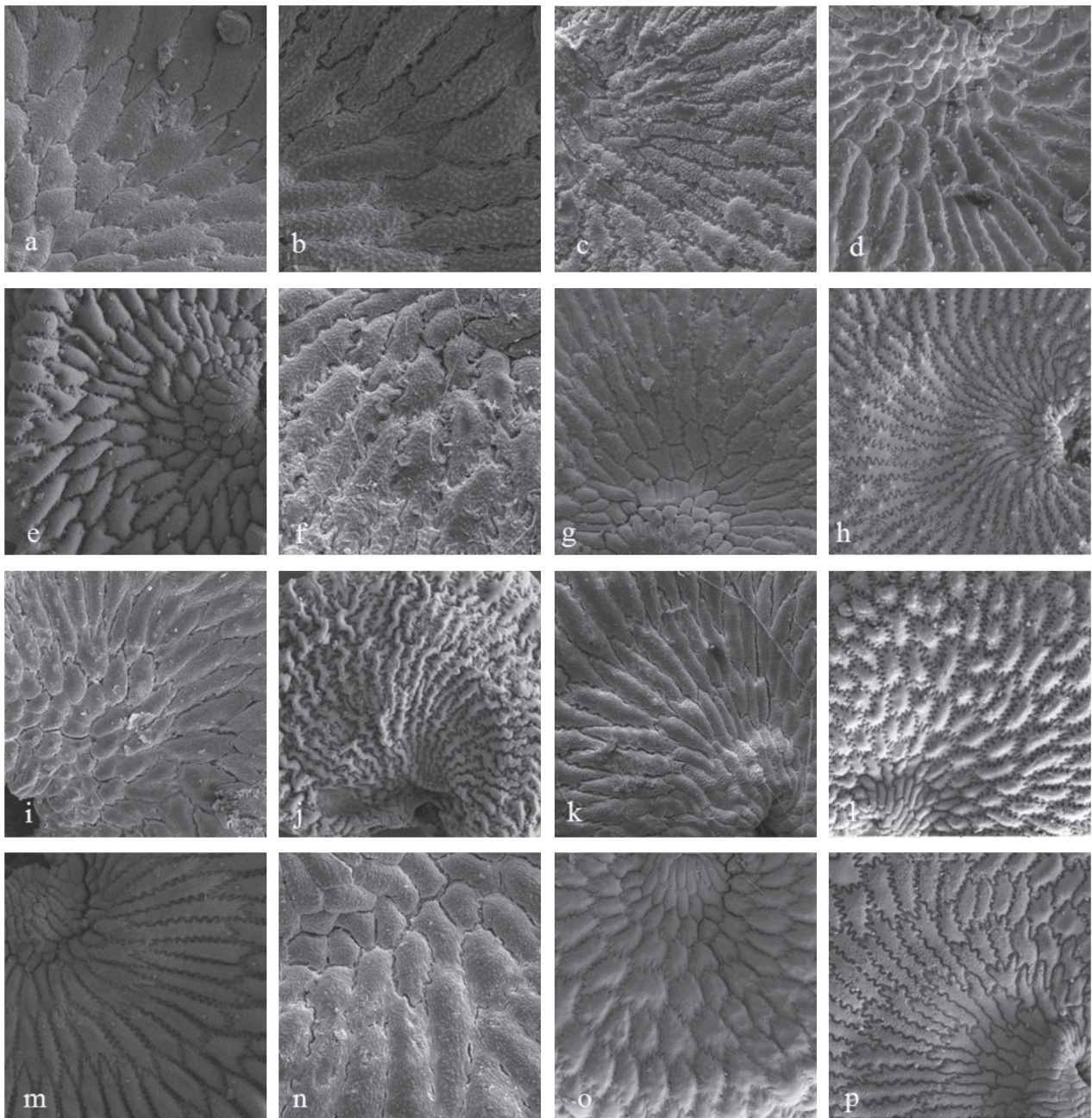
**Figure 2.** SEM micrographs of seed dorsal surface of *Silene* species studied. Concave: b, c, d, e, i, j, k, n. Convex: a, h, l, m, o. Flat: f, p. a. *S. daenensis*; b. *S. dschuparensis*; c. *S. elymaitica*; d. *S. gertraudiae*; e. *S. gynodioica*; f. *S. hirticalyx*; g. *S. nizvana*; h. *S. oligophylla*; i. *S. palinotricha*; j. *S. parrowiana*; k. *S. persica*; l. *S. persepolitana*; m. *S. psudaucheriana*; n. *S. psudonurensis*; o. *S. sojakii*; p. *S. stapfi* (Scale bar: g, m, p 1mm; a, b, d, f, h, i, j, k, l, n, o 500  $\mu$ m; c, e 200  $\mu$ m).

## Discussion

It has been reported that, the seed shape of the most species of the genus *Silene* is generally reniform in outline and the size of seeds is small (Yildiz and Cirpici 1998; Zareh 2005; Perveen 2009; Fawzi et al. 2010). The results of present study are in accordance with that study (Table 2 and fig. 1a-p). The range of variation in dorsal surface of the studied species is in accordance with the available data (Hong et al. 1999;

Fawzi et al. 2010).

Sheidai et al. (2010) indicated that *S. dschuparensis* and *S. psudonurensis* as well as *S. sojakii* and *S. persepolitana* are morphologically closely related species. These pairs of species show the most similarity based on seed morphology features (Fig. 4). *S. hirticalyx* (section *Auriculatae*) and *S. stapfi* (section *Sclerocalycinae*) are closely related species according to seed morphology analysis. Therefore the result of the present study is in accordance with the suggestion

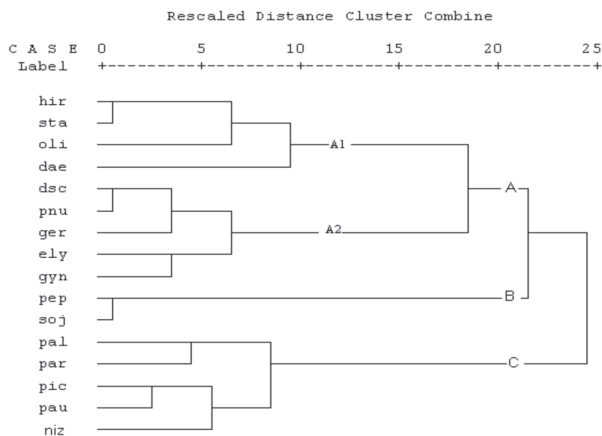


**Figure 3.** SEM micrographs of seed coat surface features of *Silene* species studied. Testa cell: Fusiform; a, b, c, d, f, h, l, j, k, m, n, p. Fusiform – elliptic; e, g. Elliptic, l, o. a. *S. daenensis*; b. *S. dschuparensis*; c. *S. elymaitica*; d. *S. gertraudiae*; e. *S. gynodioica*; f. *S. hirticalyx*; g. *S. nizvana*; h. *S. oligophylla*; i. *S. palinotricha*; j. *S. parrowiana*; k. *S. persica*; l. *S. persepolitana*; m. *S. psudaucheriana*; n. *S. psudonurensis*; o. *S. sojakii*; p. *S. stapfii* (Scale bar: a, b, c, f, n 100  $\mu$ m and in the other cases 200  $\mu$ m).

of Melzheimer (1988) about the affinity of *S. hirticalyx* to the section *Sclerocalycinae* species (Fig. 4). *Silene nizvana* morphologically showed the most dissimilarity to the other species of the section *Auriculatae* (Sheidai et al. 2010). *S. nizvana* placed far away from the other species in the dendrogram of clustering analysis of the species based on seed

morphology characters (Fig. 4).

*S. parrowiana* was clearly separated from the section *Auriculatae* species based on RAPD markers analysis (Sheidai et al. 2010) but is placed in the third major cluster (C) together with the section *Auriculatae* species based on seed morphology analysis as well as *S. stapfii* from the section *Scleroca-*



**Figure 4.** Ward dendrogram of *Silene* species studied based on seed morphology features. Acronyms refer to Table 1.

*lycinae* (Fig. 4). Therefore in accordance with the previous study (Fawzi et al. 2010) the seed morphology characters are not useful in the species distinction at the section level.

In conclusion the taxonomic significance of some seed characters such as seed shape, dorsal surface shape, the shape and size of testa cell, the edge of testa cells and testa cell surface ornamentation have been indicated at the species level. However further studies contributing broader species sampling is needed for a more comprehensive conclusion.

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