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Using Morphology and Micromorphology Characters for Identification of *Silene* L. Species in North-East of Iran

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Abstract: *Silene* L. from Caryophyllaceae, comprises about 92 species in Iran which 16 species have been recorded from North-East of Iran. In present research, seven *Silene* species and four subspecies from four sections from Khorassan Razavi Province in North-East of Iran identified using floral segments morphology e.g. shape of calyx's denth, margin of calyx's denth, paracorolla shape and micromorphological characters like seed coat and pollen ornamentation. The results demonstrated using above mentioned characters are very useful for exact identification of *Silene* species.

Key words: *Silene*, micromorphology, pollen, palynology, Silenoideae, floral segments

INTRODUCTION

With about 700 species, the genus *Silene* L. is one of the largest and perhaps the best known of the family Caryophyllaceae. *Silene* from Caryophyllaceae family. Silenoideae subfamily (Melzheimer, 1988). This genus is distributed in the east of Meditranea, central Asia, Italy, Turkey, Iran, Iraq, Russia, England, Spain (Boissier, 1884; Komarov, 1936; Tutin, 1964; Zohary, 1966; Davis, 1967; Anzalone, 1982; Melzheimer, 1988). The most recent revision of Iranian *Silene* L. is that of Melzheimer (1988). The 92 species and 14 subspecies from 20 sections for Iran have been recorded. 16 of those from eight sections distributed in North-East of Iran. This genus is important for having ecdysteroids, phyto- ecdysteroids, Saponin, tritrepens (Meng, 2001; Jurgens, 2002, 2004). In earlier taxonomical researches, *Silene* L. species have been identified on the basis of calyx shape, calyx's nerves, basal and cauline leaves shape (Ghazanfar, 1984; Yildiz, 2006a, b). To identify on the basis of these characters is very difficult because the species had transitional or all of above mentioned characters. In *Silene* species, the identification basis of floral segments morphological characters like paracorolla shape, calyx's denth shape, the margin of calyx's denth, shape of petals lobes and micromorphology of seed coat and pollen ornamentation is still need to investigate. So, studies carried out seven species and four subspecies from four sections in Khorassan Razavi Province in North-East of Iran. This research furthermore aims to identify exactly and to construct a useable key for studied species on the basis of seed coat and pollen ornamentation. Seed coat characterization has been firstly reported. There are some articles to mentioned on pollen ornamentation in sections *Siphonomorpha* and *Auriculatae* (Ghazanfar, 1984). Also, Moore *et al.* (1991) and Punt and Hoen (1995) studied the pollen of *S. latifolia* subsp. *alba* and *S. noctiflora*, respectively.

MATERIALS AND METHODS

In May to June each year between 2005 and 2006, the herbarium materials at FUM H were intensively studied. Also, specimens of *Silene* were collected from Khorassan Razavi in North-East

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Table 1: The localities of studied *Silene* species

Section	Species	Localities
<i>Sclerocalycinae</i> Boiss.	<i>S. bupleuroides</i> L. subsp. <i>bupleuroides</i>	Mashhad, Moghan to Maj- Gusouleh, 2000 m, Zangooi and Faghihnia, 23637.
	<i>S. bupleuroides</i> L. subsp. <i>ramosa</i>	Mashhad, north-west of Kardeh, Balghour, 1750 m, Zangooi and Fagihian, 27544.
	<i>S. swertifolia</i> Boiss.	Mashhad, Moghan village, 1750 m. Jafari and Fathi, 34; West of Kang, 1900 m, Faghihnia and Zangooi, 17877; Seiedy to Haft Hous, 1270 m. Fathi, 41; Moghan mountains, 1750 m, Jafari and Fathi, 34; Piveh jen mountains, 2100 m, Rashed and Zangooi, 10248.
<i>Auriculatae</i> Boiss.	<i>S. indepressa</i> Schischk.	Between Mashhad and Kalat. Sandough Shekan, 1700 m, Zangooi and Faghihian, 20870; Kalat road, Sandough shekan mountains, 1550 m, Joharchi and Zangooi, 16847.
	<i>S. gertraudiae</i> Melzh.	Mashhad to Torbat-Heidarieh, Robat Sephid, 2000 m, Fathi, Azizzadeh, 25; Robat Sefid mountains, 1800 m, Fathi and Aziz zadeh, 1800 m, 22.
<i>Melandriformes</i> Boiss.	<i>S. latifolia</i> Poir. subsp. <i>eriocalycinae</i> (Boiss) Greuter and Burdetand	70 km Mashhad to Kalat, 1600 m, Zangooi Faghihnia, 24683.
	<i>S. latifolia</i> Poir. subsp. <i>alba</i> (Mill.) Greuter and Burdet	Mashhad, Zoshk, 1500 m, Safavi and Joharchi, 12575; North of Mashhad, Talghour, 1550 m, Joharchi, 13012.
	<i>S. latifolia</i> Poir. subsp. <i>persica</i> (Boiss and Buhse) Melzh	Mashhad, Moghan. Mountain, 1900 m, Faghihnia and Zangooi 20382; Moghan mountains, 1900 m, Faghihnia and Zangooi, 20385.
	<i>S. noctiflora</i> L.	South-west of Mashhad, Azghad, 1600 m, Hosseinzade and Zangooi, 24262.
<i>Conoimorpha</i> Otth.	<i>S. conoidae</i> L.	Mashhad, north-west of Kardeh- Balghour. 1750 m, Faghihnia and Zangooi, 27532; Mashhad to Kalat, after Ghotijghi, 1400 m, Fathi, 5.

of Iran (Table 1). The photos of type specimens from G, W and LINN herbariums observed and studied. Floral segments morphology were studied for identification of the species by stereo microscope and micromorphological study of seed coat and pollen ornamentation were studied by scanning electromicroscope. In the palynological study, the pollen were extracted from the anther and dehydrated by glacial acetic acid, then acetolised, finally studied by light microscope and scanning electromicroscope LEO1450VP. Erdtman (1952), Moore *et al.* (1991). The pollen terminology was adapted from Punt (Punt and Hoen, 1995; Punt *et al.*, 2007). Finally two keys were prepared for *Silene* on the basis of seed coat and pollen ornamentation.

RESULTS

The Floral Segments Morphology and Micromorphology

The results showed *Melandriformes*, all of subspecies of *S. latifolia* had subulate to triangular calyx's denth with ciliolate margin, obovate petals lobes and fimbriate paracorolla but in *S. noctiflora* just were observed differences in ellipsodic paracorolla and broad rectangular lobes of petals. Also, one difference was observed in seed coat ornamentation. In *Sclerocalycinae*, both of species had triangular calyx's denth with narrow margin. In *S. swertifolia* paracorolla shape in all of species was ellipsodic. The petals shape were obovate in *S. swertifolia* while oblong obovate in *S. bupleuroides*. Seed coat ornamentation was observed circular in *S. swertifolia* and oblong dentate in *S. bupleuroides*. In *Auriculatae*, calyx's denth shape and margin of them, paracorolla shape and seed coat ornamentation were different. It sounds this was very variable. In *S. conoidae* from sect. *Conoimorpha*, special

characters were different from another sections like; calyx's denth shape and margin, paracorolla shape and petals lobes.

Palynology

The pollen ornamentation were reticulate. *Auriculatae* and *Melandriiformes* were scabrate-granulate in another sections. Tectum *Melandriiformes* and *Sclerocalycinae* were semi-ectate but *Conoimorpha* and *Auriculatae* were tectate-imperforate. The results of floral segments morphology and seed coat, pollen micro morphology studies on *Silene* species have been shown in Table. 2, Fig. 1a-j, 2a-g, 3a-g and 4a-j.

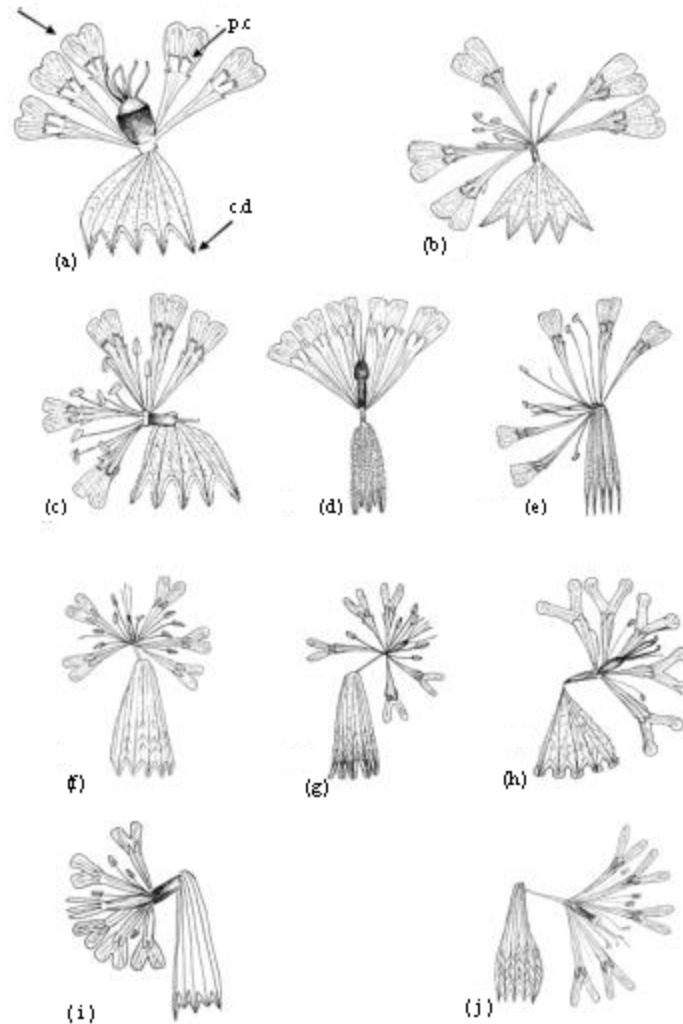


Fig. 1: Drawing of floral segments. c. d: calyx denth, p: petal, p. c: paracorolla. (a) *S. latifolia* subsp. *alba*, (b) *S. latifolia* subsp. *eriocalycinae*, (c) *S. latifolia* subsp. *persica*, (d) *S. noctiflora*, (e) *S. conoidae*, (f) *S. swertifolia*, (g) *S. gertraudae*, (h) *S. indepressa*, (i) *S. bupleuroides* subsp. *bupleuroides*, (j) *S. bupleuroides* subsp. *ramosa*

Table 2: The floral morphology and micromorphology characters of the studied *Slene* species

Species	Calyx's denth shape	The margin of calyx's denth	Paracoroll shape	The lobes of petals shape	Seed coat ornamentation	Pollen ornamentation	Tectum	Microechinate on the muri
<i>S. latifolia</i> subsp. <i>alba</i>	Subulate, acuminate	Narrow at the base acuminate or acute, ciliolate	Fimbriate	Obovate, auriculate with shallow cleft	Irregular broad polygonal	Reticulate	Tectate-perforate (semi-tectate)	+
<i>S. latifolia</i> subsp. <i>ericalycinae</i>	Triangular, acute	Narrow, ciliolate	Fimbriate	Obovate, auriculate with entire apex	Irregular broad polygonal	Reticulate	Tectate-perforate (semi-tectate)	+
<i>S. latifolia</i> subsp. <i>persica</i>	Triangular, subulate, mucronulate	Almost narrow, ciliolate	Fimbriate	Obovate, auriculate with entire apex	irregular broad polygonal	reticulate	Tectate- perforate (semi-tectate)	+
<i>S. noctiflora</i>	Triangular, acute, alternately short and long	Almost narrow, ciliolate at the base	Ellipsodic	Broad rectangular with shallow cleft and entire apex	Broad dentate polygonal	Reticulate	Tectate-perforate (semi- tectate)	-
<i>S. conoidae</i>	Subulate, mucronulate	Very narrow, ciliolate at the base	Fimbriate	Obovate with shallow cleft, auriculate	Oblong. crenate polygonal	Scabrate-granulate	Tectate-imperforate	-
<i>S. swertifolia</i>	Triangular, acute	Narrow, ciliolate	Ellipsodic, subulate, acute	Obovate	Circular	Scabrate-granulate	Tectate-imperforate	-
<i>S. gertauidae</i>	Lanceolate, acute	Broad, obtuse auriculate	Oblong-obovate	Oblong with deep cleft,	Oblong- dentate polygonal, non prominent	Reticulate	tectate- perforate (semi- tectate)	-
<i>S. indepressa</i>	Triangular, obtuse	Circular, obtuse, ciliolate at the apex	Circular-obovate	Oblong with deep cleft, auriculate	Irregular reticulate	Rectangular	tectate- perforate (semi-tectate)	+
<i>S. bupleuroides</i> subsp. <i>bupleuroides</i>	Triangular, alternately acute and obtuse	Broad at the base	Ellipsodic or subulate, acute	Oblong-obovate	Oblong- dentate, prominent	Scabrate-Granulate	Tectate-imperforate	-
<i>S. bupleuroides</i> subsp. <i>ramosa</i>	Triangular, mucronulate	Broad	Ellipsodic or subulate, acute	Oblong- obovate with deep cleft	Oblong- dentate, prominent	Scabrate-granulate	tectate-imperforate	-

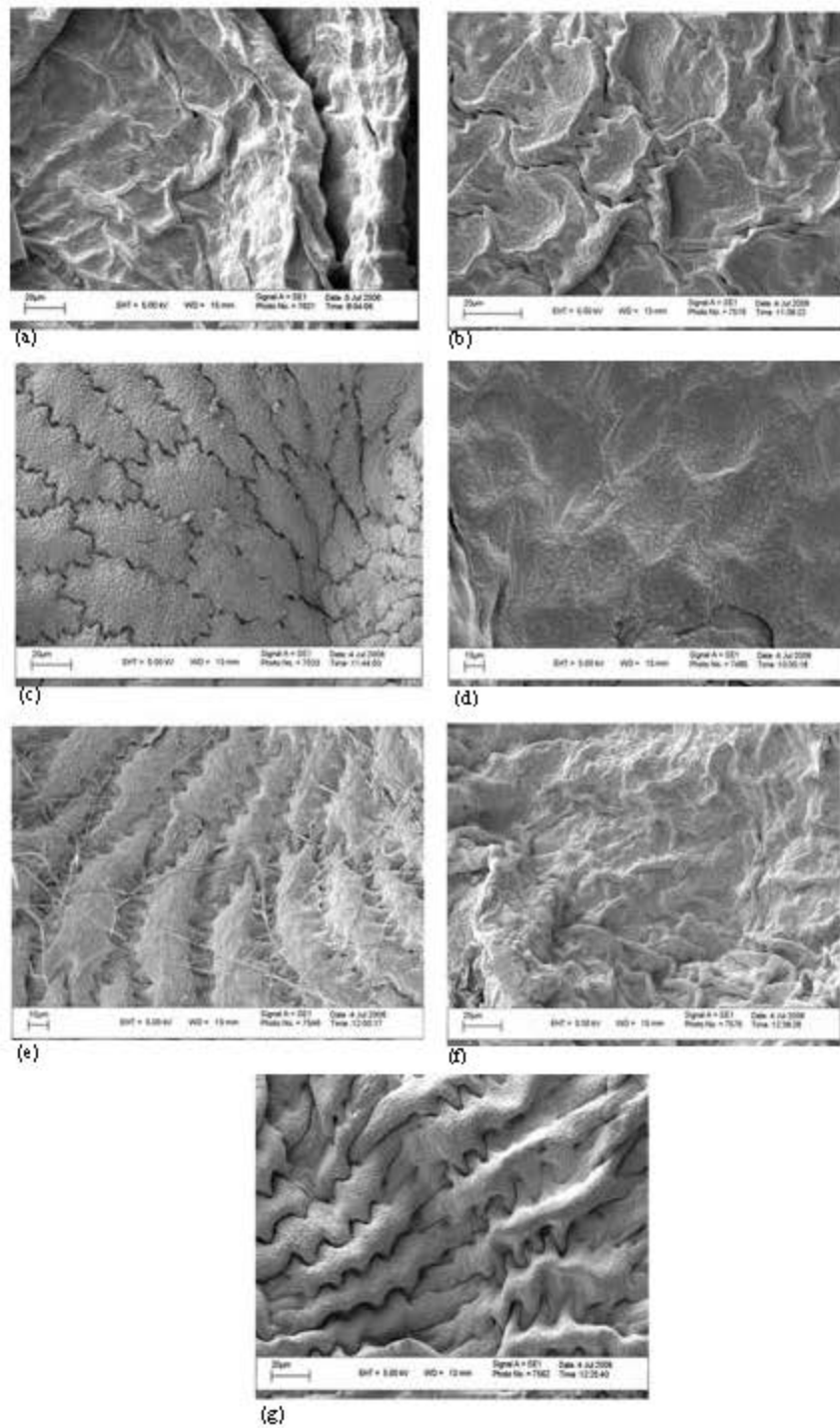


Fig. 2: Electromicrograph of seed coat (1000x) (a) *S. latifolia*, (b) *S. noctiflora*, (c) *S. conoidea*, (d) *S. swertifolia*, (e) *S. gertrudiae*, (f) *S. indeprusa*, (g) *S. bupleuroides*

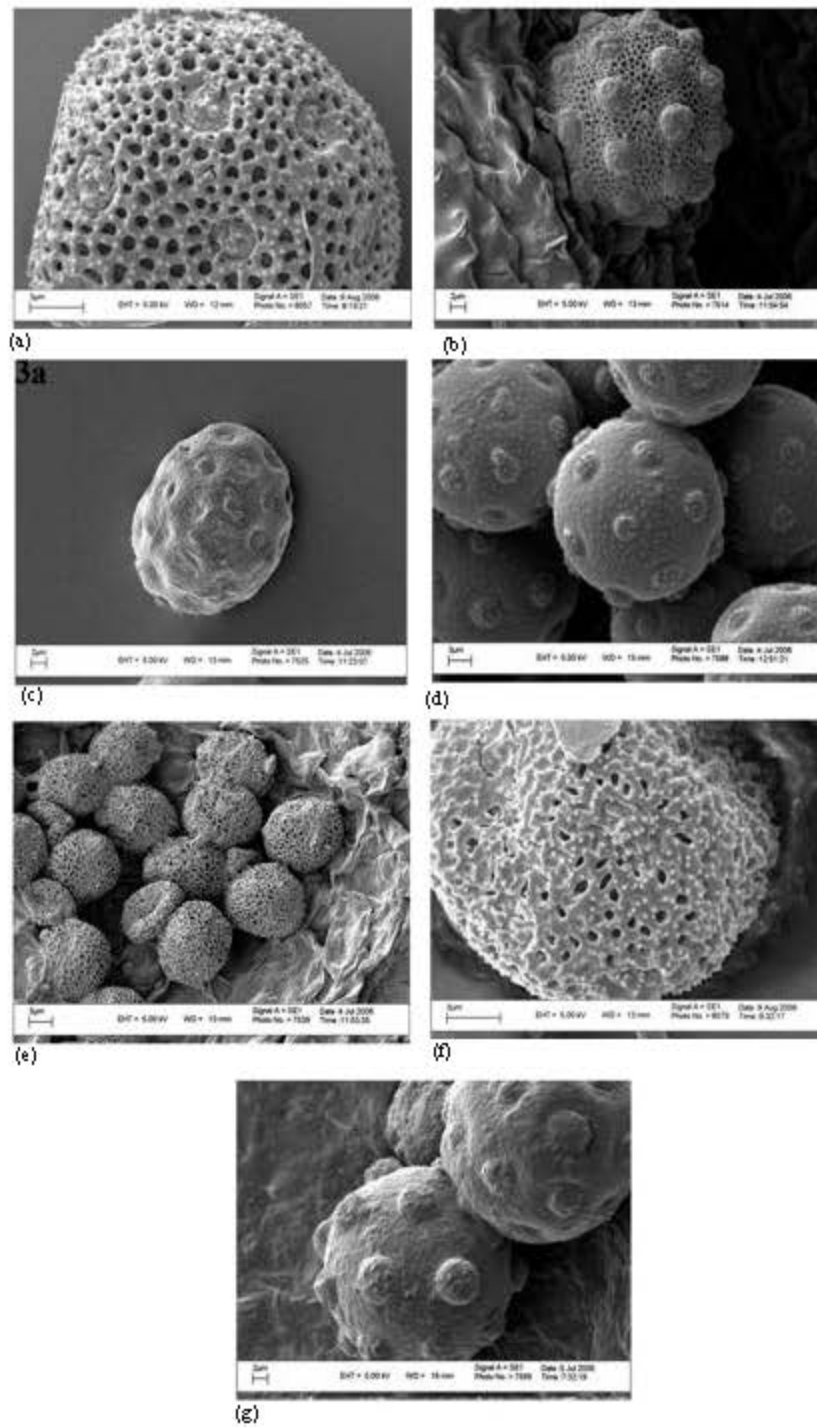


Fig. 3: Electromicrograph of pollen ornamentation (a) *S. latifolia* (10000x), (b) *S. noctiflora* (4000x), (c) *S. conoides* (4000x), (d) *S. swertifolia* (4000x), (e) *S. germanica* (4000x), (f) *S. indepressa* (10000x) and (g) *S. bupleuroides* (10000x)

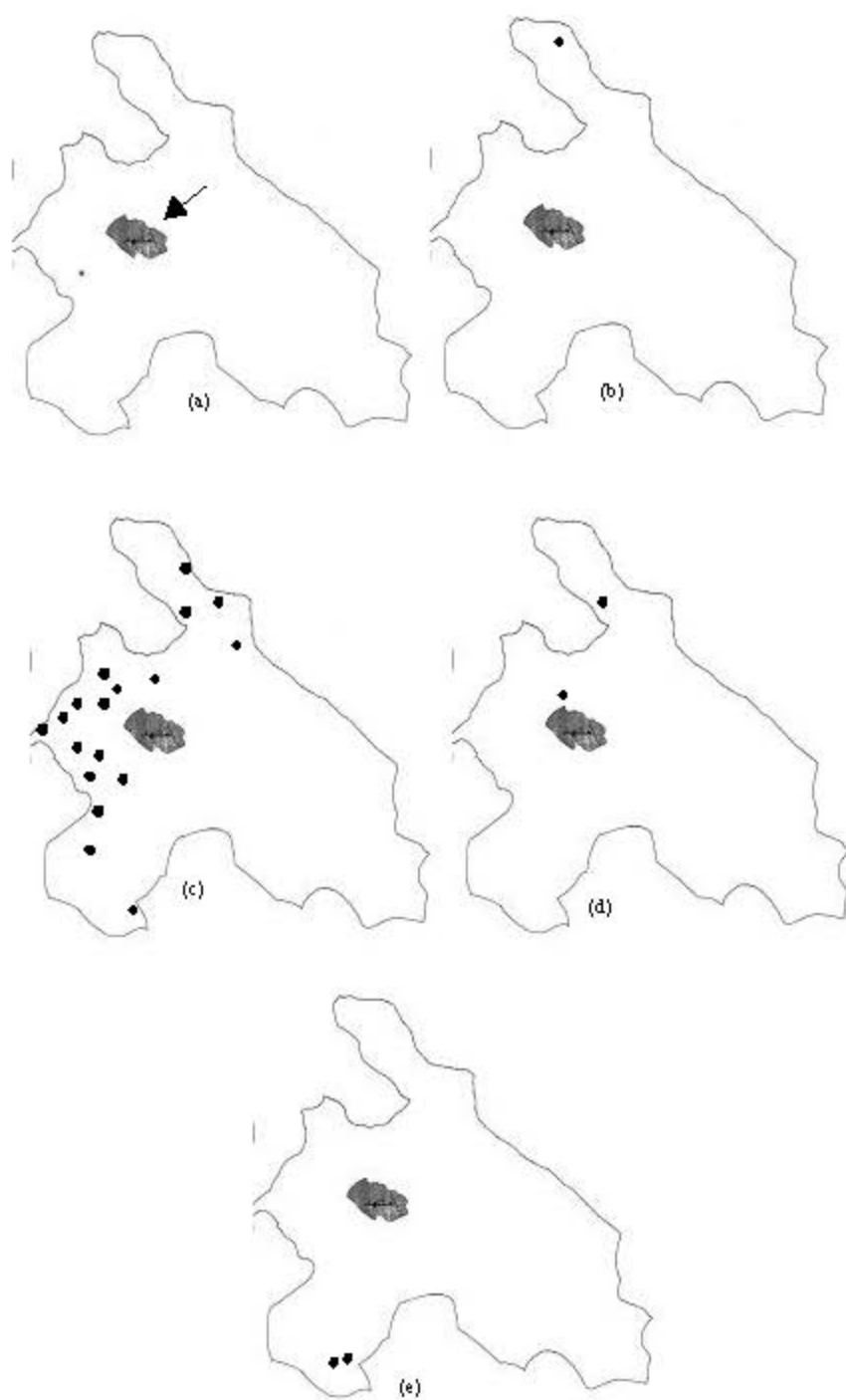


Fig. 4: Continued

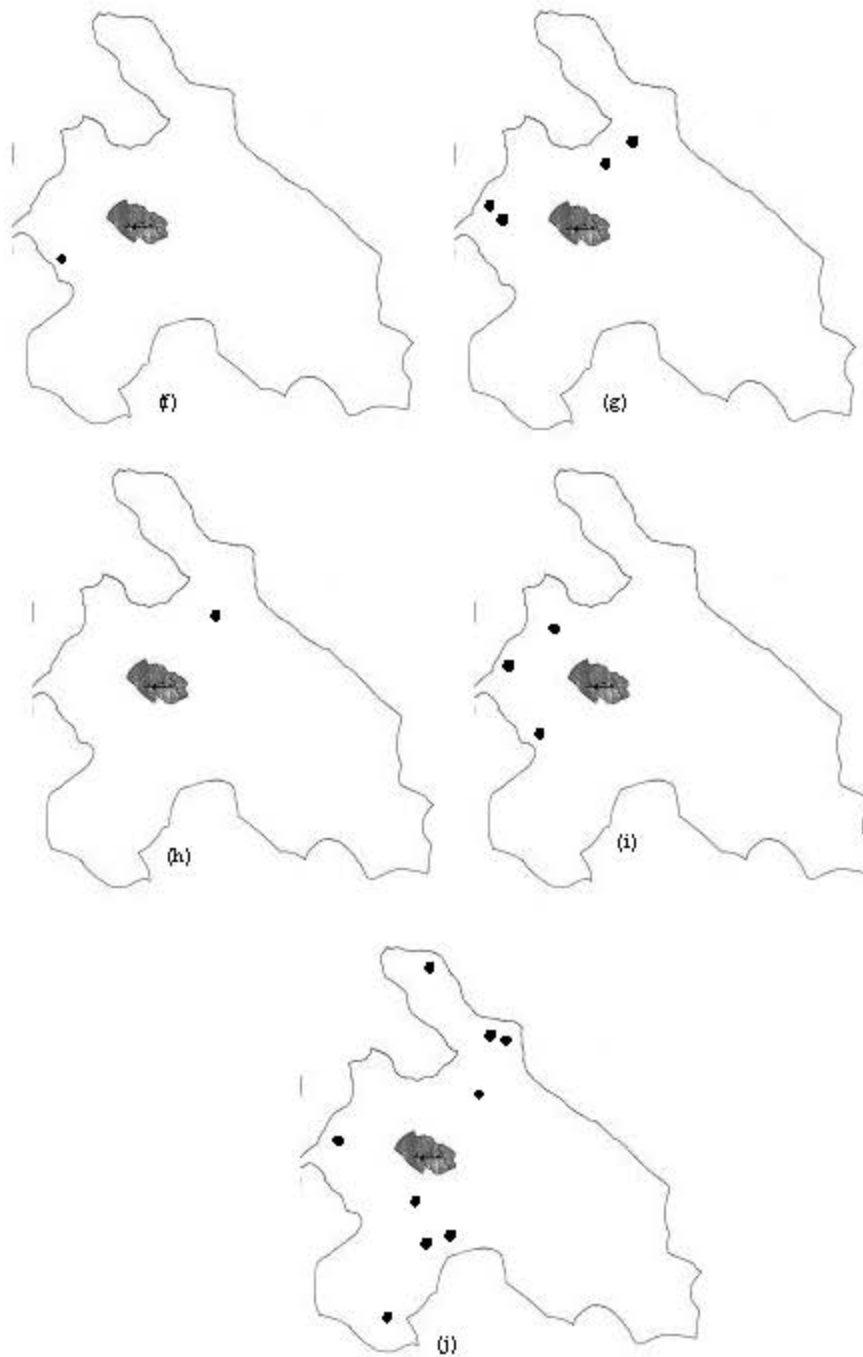


Fig. 4: The maps of distribution. The arrow showing Mashhad city. (a) *S. bupleuroides* subsp. *bupleuroides*, (b) *S. bupleuroides* subsp. *ramosa*, (c) *S. swertifolia*, (d) *S. indepressa*, (e) *S. gertracidae*, (f) *S. noctiflora*, (g) *S. latifolia* subsp. *alba*, (h) *S. latifolia* subsp. *ericalcynae*, (i) *S. latifolia* subsp. *persica*, (j). *S. conoides*

DISCUSSION

Results of morphological and micromorphological studies of floral segments and seed coat usually showed the characters of each species different from each other which it is difficult to justify including within a single section on the basis of these characters. But using these characters help to identify exactly species. In the earlier pollen ornamentation studies on *Auriculatae* and *Siphonomorpha*, Ghazanfar explained ectexine is punctate, tubulifera/spinulosa in sect. *Siphonomorpha* is but *Auriculatae* is punctate, reticulate, semi-tectate (Ghazanfar, 1984). Punt and Hoen also explained pollen ornamentation of *S. noctiflora* (Punt and Hoen, 1995; Punt *et al.*, 2007). Another reports of pollen is related to *S. behen* L. *S. gigantea* L. var. *gigantea* by Yildiz (1996, 2001, 2006a, b). The present research showed, *Auriculatae* and *Melandrifformes* are similar as semi- tectate, reticulate occasionally with microechinate above the muri. *Sclerocalycinae* and *Conoimorpha* are similar because having tectate and granulate pollen. So, species of each sections have similar pollen characters. Finally, we recommend using floral segments morphology and micromorphology study on seed coat and pollen ornamentation help to identify *Silene* species exactly.

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