

The importance of palynology in the taxonomy of genus *Silene*, based on pollen morphology

Received: 05.04.2014 / Accepted: 04.08.2014

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In this study, the palynology of 33 species of *Silene* belonging to 15 sections was investigated to examine the correspondence between palynology, morphological sections and phylogenetic trees. The pollen grains were prepared using the acetolysis method. Quantitative and qualitative micromorphological features of pollens were investigated using light microscopy and scanning electron microscopy. Differences in quantitative and qualitative pollen characters within sections were used to regroup species using a statistical hierarchical clustering method (average linkage). According to this classification, the species were divided into eight groups, some of which differed from morphological groupings, but had some similarities to previous phylogenetic studies.

Keywords: Average linkage, hierarchical clustering, Iran, phylogeny**اهمیت گرده‌شناسی در تاکسونومی جنس *Silene*، براساس ریخت‌شناسی گرده***

دریافت: ۱۳۹۳/۱۱/۱۶ / پذیرش: ۱۳۹۳/۵/۱۳

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خلاصه

Silene L. ایرانی متعلق به تیره *Caryophyllaceae* از روی صفات گرده‌شناسی به ۲۱ بخش تقسیم می‌شود. به علت اهمیت این جنس در فلور ایران و پیچیدگی‌های آن، ریخت‌شناسی گرده ۳۳ گونه *Silene* از ۱۵ بخش در مقاله حاضر مورد مطالعه قرار گرفته است تا تطابق میان گرده‌شناسی، بخش‌های ریخت‌شناسی و درختان تبارزایشی را مورد مطالعه قرار دهد. دانه‌های گرده با استفاده از روش استولیز آماده شدند. برای مطالعه صفات کمی و کیفی ریز ریخت‌شناسی گرده *Silene* میکروسکوپ نوری و الکترونی نگاره مورد استفاده قرار گرفتند. به منظور مطالعه تطابق میان گرده‌شناسی، بخش‌های ریخت‌شناسی و درختان تبارزایشی گونه‌ها با استفاده از یک روش آماری خوشه‌بندی سلسله مراتبی (روش پیوند میانگین) دوباره دسته‌بندی شدند. براساس این رده‌بندی، گونه‌ها به هشت دسته تقسیم شدند. با وجود برخی شباهت‌ها، بین گروه‌های گرده‌شناسی و بخش‌های ریخت‌شناسی مطابقت کامل وجود نداشت. به علاوه، بین مطالعه حاضر و مطالعات تبارزایشی *Silene* شباهت‌هایی مشاهده شد. رده‌بندی گونه‌ها در این دو مطالعه دارای تشابه اما با بخش‌های ریخت‌شناسی تطابق نداشت.

واژه‌های کلیدی: ایران، پیوند میانگین، تبار زایشی، خوشه بندی سلسله مراتبی

Introduction

Silene L. is the largest genus in the family *Caryophyllaceae* and includes many horticultural and medicinal plants (Swank 1932, Vestal 1952, Oxelman & Lidén 1995). There are approximately 700 species distributed mainly throughout the northern hemisphere, but also in North Africa and South America. Half of all species grow in the Middle East (Melzheimer 1988). The southern part of the Balkan Peninsula and southwestern Asia are major centers of biodiversity of *Silene* (Greuter 1995). The Iranian plateau has 140 species of *Silene*, which have been classified in 23 sections. Of these, nearly 100 species of *Silene*, classified in 21 sections, can be found in Iran (Edalatiyan *et al.* 2011). Approximately 30 species are endemic to the country (Melzheimer 1988), most of which are distributed in the western part of Iran (Edalatiyan *et al.* 2010).

Several important studies have been run on *Caryophyllaceae* and *Silene* palynology (Skvarla 1975, Skvarla & Nowicke 1976). The pollen of the *Caryophyllaceae* is periporate and the number of pores is a crucial factor in the genus classification (Faegri & Iversen 1975). The *Silene* taxa distributed in the Balkans have been revised biosystematically (Melzheimer 1977). In the sections *Siphonomorpha* Otth. and *Auriculatae* Boiss., ectexine sculpture of *S. italica*, *S. viridiflora* L. and *S. rhynchocarpa* Boiss. is punctate, and the sculpture of the sexine of *S. caryophylloides* subsp. *subulata* (Boiss.) Coode & Cullen is semireticulate (Ghazanfar 1984). The variation pattern in *S. latifolia* pollen morphology has been determined (Prentice 1987). Pollen morphology of some *Silene* species of the flora of Pakistan have been studied (Perveen 2000, Perveen & Qaiser 2006, Sahreen *et al.* 2008).

Pollen and seed morphology of some *Silene* species from several sections, for example: *Sclerocalycinae* (Boiss.) Schischk., *Chloranthae* Rohrb., *Otites* (Adans.) Otth., *Tataricae* Chowdhuri., *Siphonomorpha* Otth., and *Lasiostemones* Boiss. have been studied (Yildiz 1996, 2001a, 2001b, 2005, 2006, Yildiz *et al.* 2010, 2011, Arman & Gholipour 2013).

Molecular and cytological investigations on *Silene* species have been carried out (Oxelman & Lidén 1995, Oxelman *et al.* 1997, 2001, Popp & Oxelman 2001, 2004, Popp *et al.* 2005, Eggens *et al.* 2007, Sheidai *et al.* 2010, Gholipour & Sheidai 2010, Petri & Oxelman 2011, Ghahremaninejad *et al.* 2014).

Because of the importance of *Silene* in the flora of Iran and its complexities (Edalatiyan *et al.* 2010), the pollen morphology of 33 *Silene* species from 15 sections of Iran were studied in the present paper. This is the first study of its kind to examine whether the grouping of species corresponds to the grouping of pollen grains.

Materials and Methods

This study is mainly based on plant material deposited in some Iranian herbaria FAR, FUMH and TUH. Pollen grains were dehydrated with acetic acid and acetolysed (Erdtman 1969) to prepare the specimens for light microscopy (LM). Pollen grains were gathered from the bottom of the centrifuge tubes with the help of liquid glycerin jelly. Photographs were taken with a Canon Power Shot G10 digital camera and observed under a Zeiss research light microscope.

The measurements were carried out on these photographs using Microstructure measurement software. We recorded pollen diameter, the number of pores, interporal distance, pore diameter and exine thickness of 30 pollen grains of each species. The mean value and standard deviation using Microsoft Excel were calculated for each measurement separately. To investigate pollen shape, ornamentation, surface using scanning electron microscope (SEM), acetolysed pollen grains were mounted on the stubs attached with double sided adhesive tape and coated in a sputter coater using physical vapor deposition method (Hacking *et al.* 2007). We observed the morphology at 2000x and 10000x with a Phillips XL30 scanning electron microscope at Tarbiat Modares University (Tehran, Iran). The pollen terminology of Punt *et al.* (2007) has been used to describe the characters of the species.

To examine the correspondence between palynology and morphological sections and phylogenetic

trees, *Silene* species were grouped again using a statistical clustering method in the program R. This is defined as the task of assigning a set of objects into groups (called clusters) so that the objects in the same cluster are more similar to each other than to those in other clusters.

Hierarchical clustering produces a set of nested clusters organized as a dendrogram. We used average linkage, which classifies clusters based on the average distance from any member of one cluster to any member

of the other cluster, starting with each species in its own cluster and merging similar clusters (Rencher 2002).

Results

Iranian *Silene* species are about 100 species which are divided into 21 sections. In the present study, the pollen grains of 33 species from 15 sections were investigated and LM micrographs of all species were taken (Fig. 1).

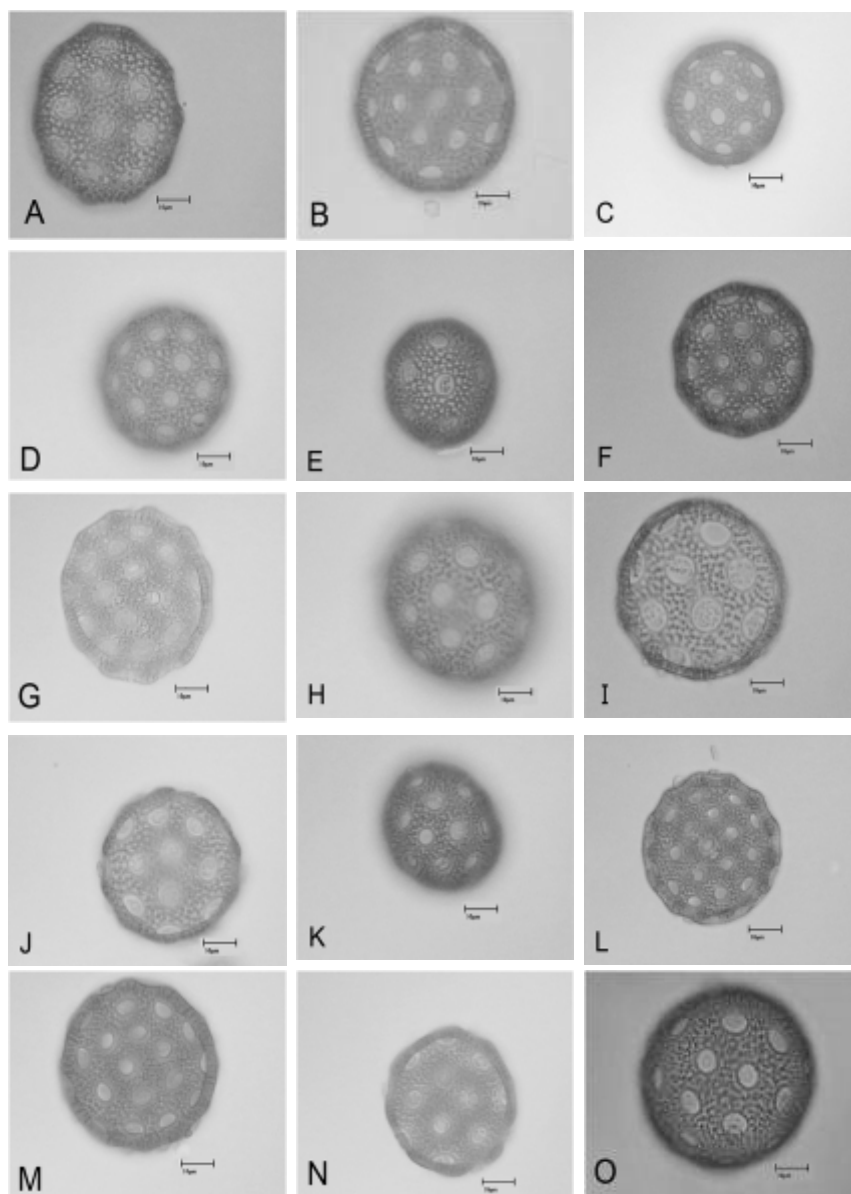


Fig. 1. (Legend in the next page.)

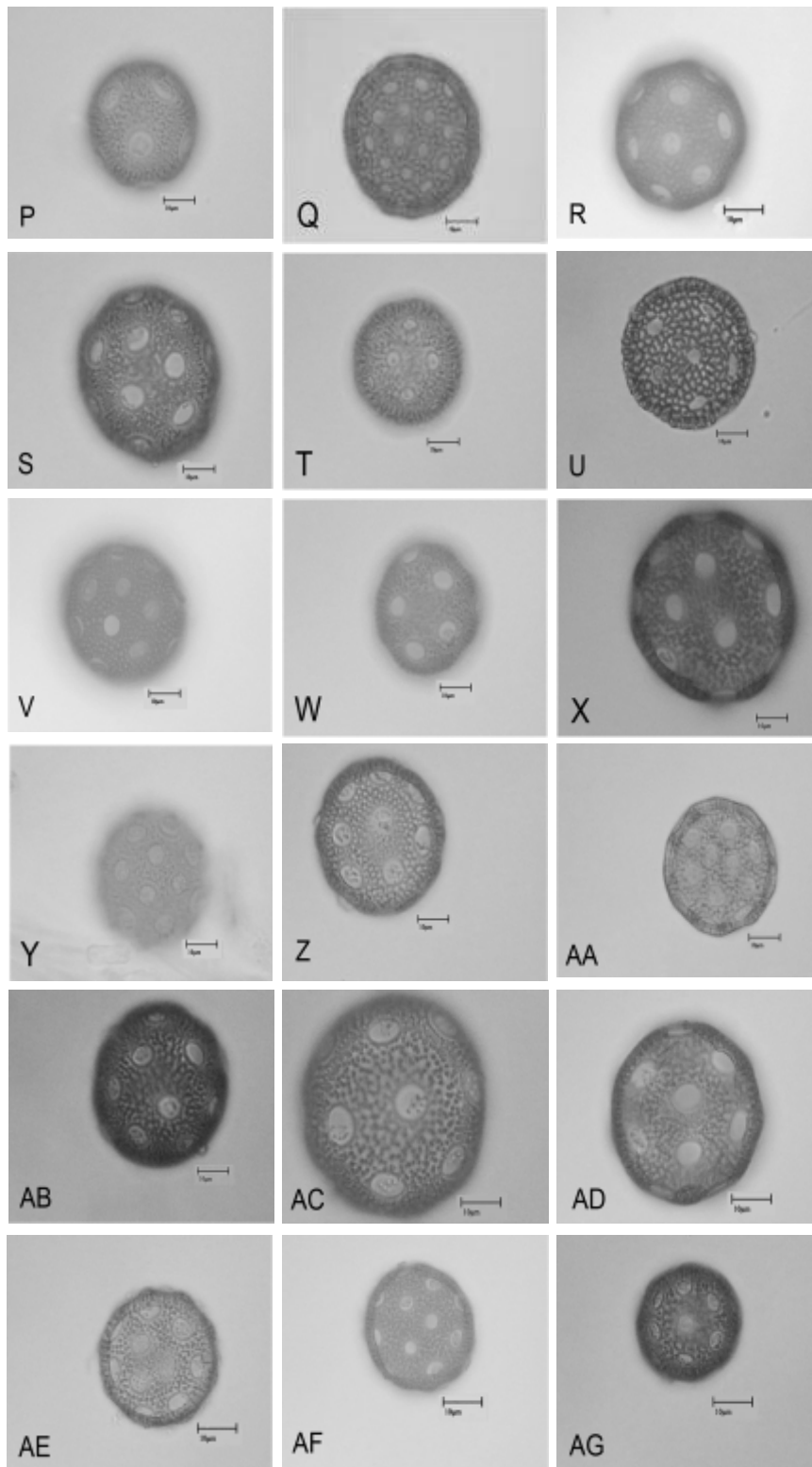


Fig. 1. A-AG. LM micrographs of *Silene* pollen grains. A. *S. aucheriana*, B. *S. crispans*, C. *S. ferdowsii*, D. *S. getraudiae*, E. *S. indepressa*, F. *S. meyeri*, G. *S. parjumanensis*, H. *S. palinotricha*, I. *S. persica*, J. *S. pseudaucheriana*, K. *S. renzii*, L. *S. villosa*, M. *S. arabica*, N. *S. compacta*, O. *S. conoidea*, P. *S. coniflora*, Q. *S. gallica*, R. *S. marschallii*, S. *S. claviformis*, T. *S. coronaria*, U. *S. latifolia*, V. *S. noctiflora*, W. *S. cyri*, X. *S. italica*, Y. *S. chaetodonta*, Z. *S. nana*, AA. *S. shafta*, AB. *S. bupleuroides*, AC. *S. chlorifolia*, AD. *S. swertiifolia*, AE. *S. brahuica*, AF. *S. cephalantha*, AG. *S. cappadocica* (Bar = 10 μ m).

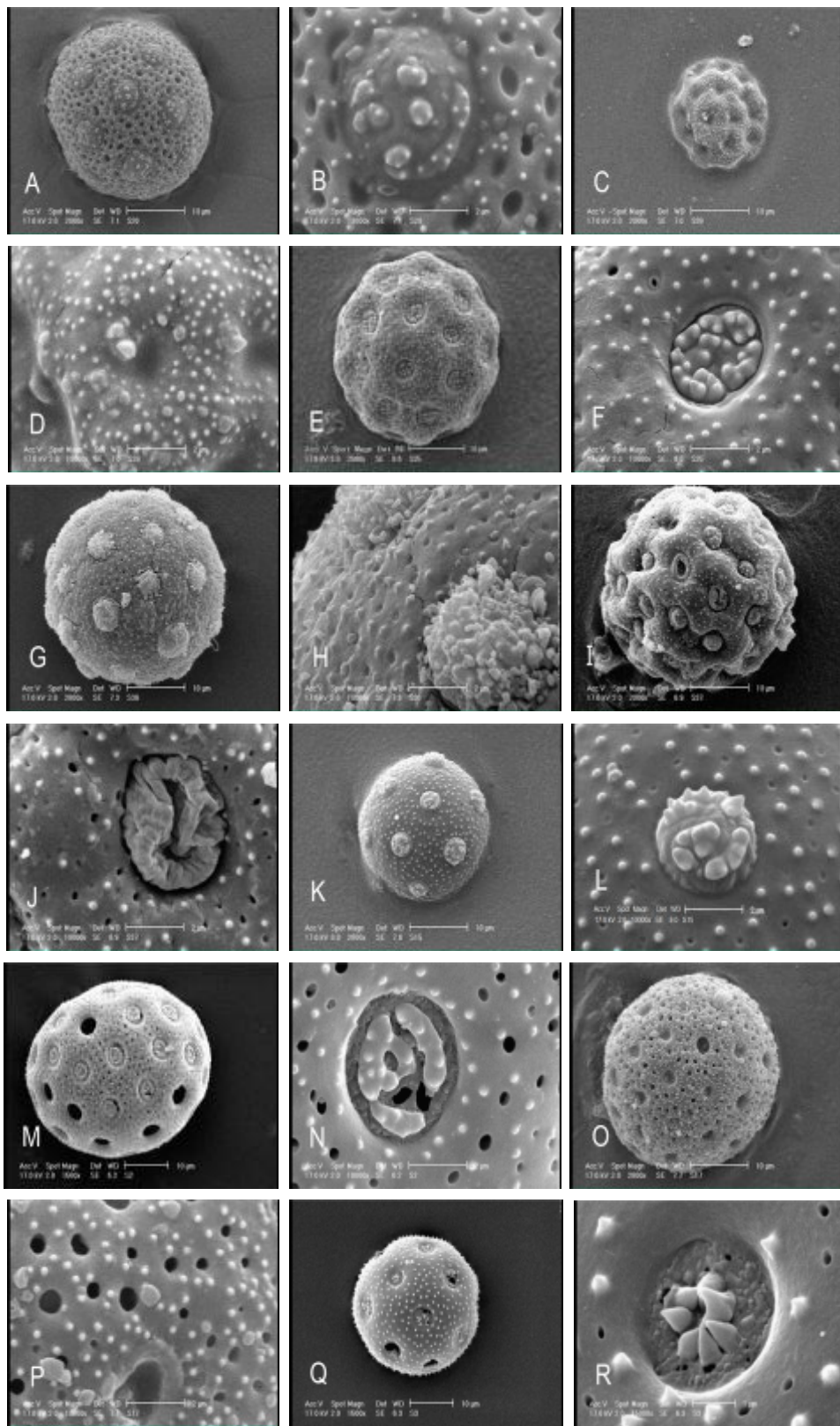


Fig. 2. (Legend in the next page.)

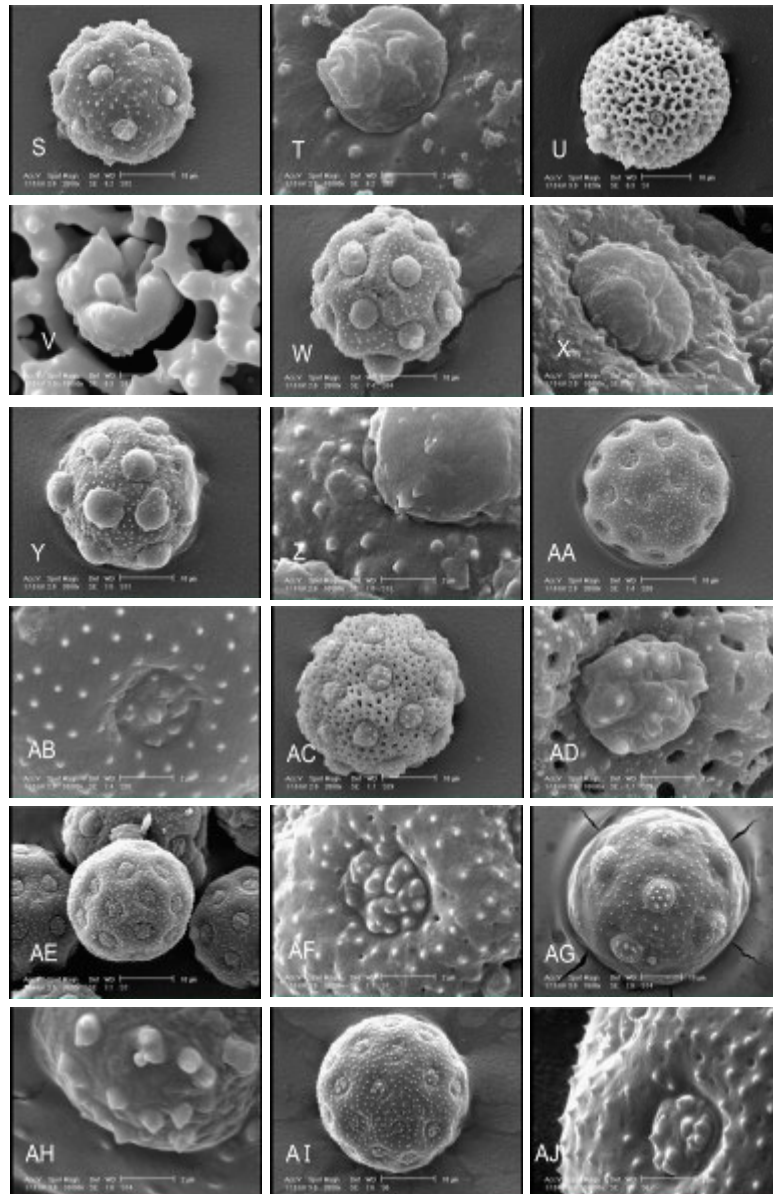


Fig. 2. A-AJ. SEM micrographs of *Silene* pollen grains. A. *S. aucheriana*, B. ornamentation of *S. aucheriana*, C. *S. ferdowsii*, D. ornamentation of *S. ferdowsii*, E. *S. getraudiae*, F. ornamentation of *S. getraudiae*, G. *S. parjumanensis*, H. ornamentation of *S. parjumanensis*, I. *S. arabica*, J. ornamentation of *S. arabica*, K. *S. compacta*, L. ornamentation of *S. compacta*, M. *S. conoidea*, N. ornamentation of *S. conoidea*, O. *S. gallica*, P. ornamentation of *S. gallica*, Q. *S. marschallii*, R. ornamentation of *S. marschallii*, S. *S. coronaria*, T. ornamentation of *S. coronaria*, U. *S. latifolia*, V. ornamentation of *S. latifolia*, W. *S. cyri*, X. ornamentation of *S. cyri*, Y. *S. italica*, Z. ornamentation of *S. italica*, AA. *S. chaetodonta*, AB. ornamentation of *S. chaetodonta*, AC. *S. nana*, AD. ornamentation of *S. nana*, AE. *S. schafta*, AF. ornamentation of *S. schafta*, AG. *S. chlorifolia*, AH. ornamentation of *S. chlorifolia*, AI. *S. cephalantha*, AJ. ornamentation of *S. cephalantha* (whole pollen grains bar = 10 μm , surface bar = 2 μm , figs 2.R & 2.V bar = 1 μm).

Moreover, in each section, SEM micrographs of at least one species were taken (Fig. 2). Each species has two micrographs. The first represents the general view of the pollen grain and the second, shows the details of its surface/ornamentation.

Using Microstructure measurement software and the taken micrographs, pollen characters of each species were prepared. The names of the species and the sections studied in this investigation together with the pollen characters of each species are demonstrated in Table 1.

Table 1. The names of the species and the sections studied of genus *Silene* together with pollen characters of each species

Taxon	Pollen diameter (μm)		Pore diameter (μm)		Interporal distance (μm)		Pore No.	Exine thickness (μm)		Pollen OR.	Structure
	M \pm SD	(R)	M \pm SD	(R)	M \pm SD	(R)		M(R)	M \pm SD		
Sect. <i>Auriculatae</i>											
<i>S. aucheriana</i>	48.06 \pm 4.1	41.9–58.4	7.18 \pm 1.1	4.68–9.7	6.35 \pm 1.4	2.47–11.0	18.8(15–26)	3.01 \pm 1.5	1.06–7.2	MR	SE
<i>S. crispans</i>	52.56 \pm 5.7	41.0–64.1	5.41 \pm 0.8	3.4–7.7	5.69 \pm 1.1	1.16–9.0	38.43(30–46)	4.07 \pm 1.5	1.82–7.2	MP	TE
<i>S. ferdowsii</i>	45.53 \pm 3.4	38.5–50.9	5.34 \pm 0.8	3.39–7.9	5.24 \pm 1.0	1.8–7.6	33.1(24–46)	3.47 \pm 1.5	1.28–7.4	MP	TE
<i>S. getraudiae</i>	43.41 \pm 2.5	36.8–46.9	5.53 \pm 0.8	3.3–7.5	5.35 \pm 1.2	3.74–10.2	26.3(21–38)	2.78 \pm 1.0	1.23–5.6	MP	TE
<i>S. indepressa</i>	38.39 \pm 1.4	34.9–41.3	5.92 \pm 0.6	4.24–8.0	7.57 \pm 1.5	4.87–12.5	15.47(12–21)	2.8 \pm 0.85	1.28–4.6	MR	SE
<i>S. meyeri</i>	45.39 \pm 2.6	40.3–52.2	5.81 \pm 0.9	3.62–8.2	5.92 \pm 1.3	2.91–10.1	27(22–32)	3.16 \pm 1.2	1.3–5.4	MP	TE
<i>S. parjumanensis</i>	50.3 \pm 3.4	41.7–56.8	6.25 \pm 1.2	3.91–9.6	5.39 \pm 1.2	2.03–9.1	32.03(27–38)	3.69 \pm 1.7	1.47–8.2	MP	TE
<i>S. palinotricha</i>	52.16 \pm 3.0	42.3–60.3	6.58 \pm 0.9	3.92–10.1	6.46 \pm 1.2	3.99–11.5	28.13(22–35)	3.76 \pm 1.4	1.27–6.7	MP	TE
<i>S. persica</i>	57.81 \pm 4.9	50.2–69.2	8.34 \pm 1.3	4.92–11.5	6.43 \pm 1.7	3.29–12.8	23.53(19–30)	3.87 \pm 2.0	1.09–9.3	MP	TE
<i>S. pseudaucheriana</i>	43.31 \pm 2.7	39.6–51.8	6.12 \pm 0.9	4.56–9.2	5.19 \pm 1.0	3.16–9.6	27.97(22–36)	3.17 \pm 1.6	1.09–6.6	MP	TE
<i>S. renzii</i>	40.63 \pm 2.5	35.7–46.3	5.11 \pm 0.7	3.79–7.9	5.98 \pm 1.1	3.36–11.3	22.2(17–28)	3.11 \pm 1.1	1.41–5.1	MP	TE
Sect. <i>Bipartitae</i>											
<i>S. villosa</i>	49.9 \pm 4.0	41.5–57.1	4.86 \pm 0.7	3.19–7.3	4.92 \pm 1.3	0.95–9.9	45.6(37–58)	3.63 \pm 1.5	1.14–6.5	MP	TE
<i>S. arabica</i>	49.99 \pm 4.3	40.0–58.2	5.36 \pm 0.8	3.3–7.3	5.8 \pm 1.1	1.95–8.4	35.6(25–44)	3.94 \pm 1.6	1.16–6.6	MP	TE
Sect. <i>Compactae</i>											
<i>S. compacta</i>	36.37 \pm 3.2	30.3–42.6	5.72 \pm 1.0	3.33–8.7	6.45 \pm 1.3	2.56–11.6	16.3(13–20)	2.71 \pm 1.0	1.08–4.7	MP	TE

Table 1. (contd)

Taxon	Pollen diameter (µm)		Pore diameter (µm)		Interporal distance (µm)		Pore No.	Exine thickness (µm)		Pollen OR.	Structure
	M±SD	(R)	M±SD	(R)	M±SD	(R)		M(R)	M±SD		
Sect. <i>Conoimorphae</i>											
<i>S. conoidea</i>	54.38±4.0	45.8–61.6	6.52±1.0	4.14–9.2	6.71±1.5	3.3–11.7	31.2(27–35)	3.58±1.4	1.04–8.2	MP	TE
<i>S. coniflora</i>	39.83±1.6	35.5–42.6	8.1±0.8	5.84–10.1	10.7±1.7	5.95–14.5	11.73(10–14)	2.59±1.3	0.75–5.2	MP	TE
Sect. <i>Lasiocalycinae</i>											
<i>S. gallica</i>	45.85±4.0	36.7–58.7	4.7±0.8	2.27–6.6	4.84±1.1	2.88–9.2	37.33(30–44)	3.76±1.4	1.54–6.5	P	TE
Sect. <i>Lasiostemones</i>											
<i>S. marschallii</i>	46.39±4	40.4–58.5	7.9±1.1	5.12–10.2	9.02±2.3	4.3–17.3	13.47(10–17)	3.37±1.7	1.38–7.8	MP	TE
<i>S. claviformis</i>	47.65±4.0	41.0–61.3	7.07±1.1	4.36–10.0	8.2±1.6	5.08–14.1	21.07(16–26)	3.68±1.5	1.09–6.6	MP	TE
Sect. <i>Lychnidiformes</i>											
<i>S. coronaria</i>	37.2±2.1	31.7–40.6	4.35±0.7	2.7–6.5	8.08±1.3	4.66–11.4	16.8(14–22)	3.2±1.1	1.49–5.3	MP	TE
Sect. <i>Melandrifformes</i>											
<i>S. latifolia</i>	41.14±2.4	36.2–46.4	4.59±0.7	3.14–6.7	8.42±2.1	1.08–13.9	12.63(9–17)	3.36±1.0	1.69–5.9	MR	SE
<i>S. noctiflora</i>	43.23±2.2	38.3–47.0	5.92±0.6	4.11–7.7	6.35±1.2	3.08–9.0	21.83(19–29)	3.1±1.0	1.61–4.8	MR	SE
Sect. <i>Oiites</i>											
<i>S. cyri</i>	37.47±2.2	32.4–41.3	5.83±1.0	33.83–7.8	7.48±1.2	4.46–10.0	17.23(15–21)	3.14±1.0	1.59–5.0	MP	TE
Sect. <i>Paniculatae</i>											
<i>S. italica</i>	59.55±5.0	50.5–70.2	8.25±1.7	5.08–13.6	10.2±2.9	1.5–19.9	17.47(12–25)	3.79±2.1	0.9–9.0	MP	TE

Table 1. (contd)

Taxon	Pollen diameter (µm)		Pore diameter (µm)		Interporal distance (µm)		Pore No.	Exine thickness (µm)		Pollen OR.	Structure
	M±SD	(R)	M±SD	(R)	M±SD	(R)		M(R)	M±SD		
Sect. <i>Rigidulae</i>											
<i>S. chaetodonta</i>	39.91±2.2	36.6–44.3	5.46±0.8	3.79–7.8	5.71±1.0	3.47–10.3	27.23(20–34)	2.53±1.0	1–4.9	MC	TE
Sect. <i>Saponariodeae</i>											
<i>S. nana</i>	46.83±3.3	38.4–53.9	6.25±0.9	4.07–8.7	5.76±1.3	1.85–9.7	23.73(20–30)	3.18±1.2	1.21–5.9	MR	SE
Sect. <i>Schaftae</i>											
<i>S. schafta</i>	36.17±3	30.4–44.0	5.41±0.9	2.89–9.0	5.02±1.6	2.47–11.4	22.5(15–38)	2.57±1.4	0.81–6.9	MP	TE
Sect. <i>Sclerocalycinae</i>											
<i>S. bupleuroides</i>	45.49±2.5	39.5–50.8	7.39±1.0	4.95–9.9	9.39±1.8	5.67–16.5	14(11–20)	3.78±1.4	1.19–8.0	MP	TE
<i>S. chlorifolia</i>	58.80±3.4	52.4–65.5	9.25±1.3	6.17–13.4	11.2±2.1	7.64–17.8	18.4(14–23)	3.30±1.4	1.3–5.78	MP	TE
<i>S. swertiifolia</i>	58.30±3.1	52.6–65.6	9.05±1.1	6.15–12.2	9.56±1.8	5.52–17.2	20.97(16–26)	3.63±2.0	1–7.36	MP	TE
<i>S. brahuica</i>	42.36±2.9	36.9–48.3	5.50±0.9	3.69–8.7	5.09±0.9	3.17–8.26	24.87(20–32)	3.50±1.5	1.08–6.4	MP	TE
<i>S. cephalantha</i>	36.97±2.7	31.5–42.2	4.85±0.7	3.22–6.8	5.65±1.2	2.91–10.5	20.13(15–30)	2.77±1.2	0.87–5.7	MP	TE
<i>S. cappadocica</i>	34.58±2.8	29.4–40.2	5.26±0.9	3.34–8.1	5.86±1.4	2.65–10.9	16.87(14–20)	2.60±1.0	1.17–5.2	MP	TE

M: mean value, SD: standard deviation, R: range, MP: microechinate- microperforate, MR: microechinate- reticulate, MC: microechinate, P: microechinate-perforate, OR: ornamentation, SE: Semitectate, TE: Tectate

There are several differences in quantitative pollen characters and sometimes in qualitative pollen characters of the species of the same section. On the other hand, when it comes under close scrutiny, there exist some similarities among the species which were classified into different sections.

In this study, the number of clusters was reduced from 33 to 32. By repeating the previous step between other clusters and the new cluster, old clusters were

merged together and new clusters, which have more species, are formed. Clustering of the data objects is obtained by cutting the dendrogram at the desired distance which is an experimental value and obtained by considering a sudden increase in divergence of characters in the clusters. In the present study, the suitable distance was 6.75 μm . The species studied here were divided into eight groups. The dendrogram and the groups of species with the distance of 6.75 μm are shown in figure 3.

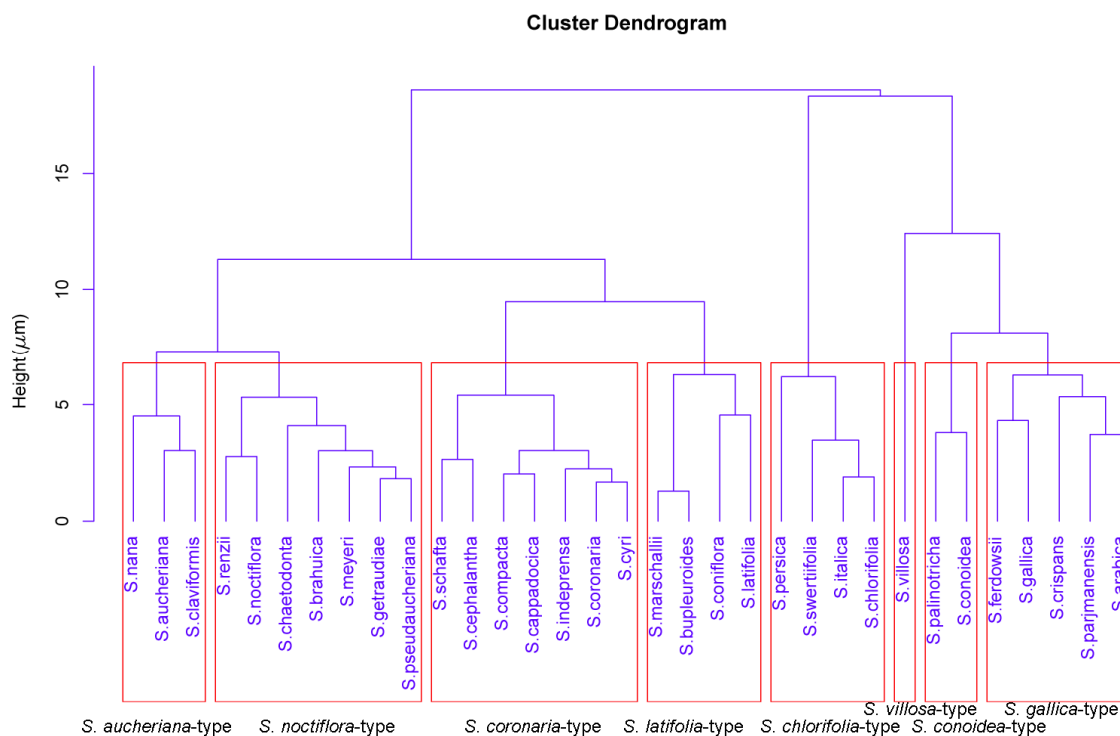


Fig. 3. The dendrogram and the eight groups of species obtained by the hierarchical clustering method (average linkage) with the distance of 6.75 μm .

In the traditional classification, these 33 species were divided into 15 sections. However, in the new clustering, which is based on quantitative pollen

characters, these 33 species were divided into eight types. The characters of these eight types of species were summarized in Table 2.

Table 2. Classifying the species into eight types according to quantitative pollen characters

Taxon	Average pollen diameter (µm)	Average pore diameter (µm)	Average interporal distance (µm)	Average pore No.	Average exine thickness (µm)
<i>S. aucheriana</i>-type					
<i>S. aucheriana</i>					
<i>S. claviformis</i>	46.83–48.1	6.25–7.18	5.76–8.2	18.8–23.73	3.01–3.68
<i>S. nana</i>					
<i>S. noctiflora</i>-type					
<i>S. renzii</i>					
<i>S. noctiflora</i>					
<i>S. chaetodonta</i>					
<i>S. brahuica</i>	39.91–45.4	5.11–6.12	5.09–6.35	21.83–28.0	2.53–3.5
<i>S. meyeri</i>					
<i>S. getraudiae</i>					
<i>S. pseudaucheriana</i>					
<i>S. coronaria</i>-type					
<i>S. schafta</i>					
<i>S. cephalantha</i>					
<i>S. compacta</i>					
<i>S. cappadocica</i>	34.58–38.4	4.35–5.92	5.02–8.08	15.47–22.5	2.57–3.2
<i>S. indepressa</i>					
<i>S. coronaria</i>					
<i>S. cyri</i>					
<i>S. latifolia</i>-type					
<i>S. marschallii</i>					
<i>S. bupleuroides</i>	39.83–46.4	4.59–8.1	8.42–10.72	11.73–14	2.59–3.78
<i>S. coniflora</i>					
<i>S. latifolia</i>					
<i>S. chlorifolia</i>-type					
<i>S. persica</i>					
<i>S. swertiifolia</i>	57.81–59.6	8.25–9.25	6.43–11.16	17.47–23.5	3.3–3.87
<i>S. italica</i>					
<i>S. chlorifolia</i>					
<i>S. villosa</i>-type					
<i>S. villosa</i>	49.9	4.86	4.92	45.6	3.63
<i>S. conoidea</i>-type					
<i>S. palinotricha</i>	52.16–54.4	6.52–6.58	6.46–6.71	28.13–31.2	3.58–3.76
<i>S. conoidea</i>					

Taxon	Average pollen diameter (µm)	Average pore diameter (µm)	Average interporal distance (µm)	Average pore No.	Average exine thickness (µm)
<i>S. gallica</i>-type					
<i>S. ferdowsii</i>					
<i>S. gallica</i>					
<i>S. crispans</i>	45.53–52.6	4.7–6.3	4.84–5.8	32.03–38.4	3.47–4.1
<i>S. parjumanensis</i>					
<i>S. arabica</i>					

Discussion

Species of the genus *Silene* are traditionally divided into several sections (Oth 1824, Candolle 1830, Boissier 1867, Rohrbach 1868, Shishkin 1936, Melzheimer 1988). The most significant characters separating sections are morphology and venation of the calyx, length and pubescence of the anthophore, shape and size of the corolla and coronal scales, and capsule and seed features (Edalatiyan *et al.* 2010). According to these characters, *S. aucheriana* and *S. crispans* have been assigned into the same section, named Sect. *Auriculatae*. The classification of all investigated species in this article corresponds to these authors' classification (mentioned above) which is demonstrated in the Table 1.

In the present study, we proposed a new classification based on quantitative palynological characters of studied *Silene* (Table 2). In this classification, *S. renzii*, *S. meyeri*, *S. getraudiae*, and *S. pseudaucheriana* from section *Auriculatae* are assigned into the *S. noctiflora*-type (Table 2).

Molecular studies using nuclear and plastid DNA have been used to resolve phylogenetic relationships within the genus *Silene* (Oxelman & Lidén 1995, Oxelman *et al.* 1997, 2001, Popp & Oxelman 2001, 2004, Popp *et al.* 2005, Eggens *et al.* 2007, Sheidai *et al.* 2010, Petri & Oxelman 2011, Ghahremaninejad *et al.* 2014). In some of these studies, molecular results have conflicted with morphological classification. For example, the circumscription of the sect. *Physolychnis*

has been expanded to include Siberian and Eurasian taxa (Petri & Oxelman 2011). Several taxa which have been previously classified into sections *Odontopetalae*, *Occidentales* and *Morrisonmontanae* have been presented in the sect. *Physolychnis* (Bocquet 1969). *S. noctiflora* and *S. latifolia* which were in one section, are distantly related in the studied cladogram using molecular markers such as MP-PCR AND AMP-PCR and SRAP (Ghahremaninejad *et al.* 2014). Trees obtained from morphological and RAPD studies on *Silene* species of sect. *Auriculatae* growing in Iran, differed in species groupings although agreed in some parts. For instance, NJ and Bayesian trees of RAPD data partly agree with morphological trees (Sheidai *et al.* 2010).

In the phylogenetic trees, using both nuclear and plastid genome sequencing, *S. schafta* and *S. cappadocica* and also *S. marschallii* and *S. bupleuroides* have been assigned into the same group, which were derived from common ancestors (Eggens *et al.* 2007). Similarly, in our study *S. schafta* and *S. cappadocica* were assigned into the same palynological group called *S. coronaria*-type; and *S. marschallii* and *S. bupleuroides* into *S. latifolia*-type (Table 2). However, *S. latifolia* and *S. noctiflora* have been assigned into the same phylogenetic group (Oxelman *et al.* 1997), but in our study these species were divided into different palynological groups.

Pollen morphology can be useful in identification and classification of taxa of the family *Caryophyllaceae*. Pollen morphological characters are considered

supplementary to the general plant morphology and play a significant role in taxonomic and evolutionary debate (Sahreem *et al.* 2008). Our palynological investigation and molecular studies indicate that the morphological sections were not classified naturally, but rather, artificially because they were only based on morphological characters due to their ease of use. However, the outright refusal of morphological sections is not feasible.

Acknowledgments

The authors wish to thank Soroush Safaie for his invaluable support in all stages of preparation of this paper.

Specimens examined:

Sect. *Auriculatae* Boiss. (1867)

S. aucheriana Boiss.: Iran: Lavestan, Noor Abad, Hatchameh to Chameh Karim, Pari strait, 1900 m, May 1988, Mozafarian 64398 (TUH).

S. crispans Litw.: Iran: south of Mashhad, Moghan, to Moghan cave, 2000 m, 24 May 2009, Joharchi & Zanguyi 42675 (FUMH).

S. ferdowsii Joharchi, Nejati & F. Ghahrem.: Iran: south-west of Sarakhs, Shorlagh, 700 m, 26 Apr. 1991, Faghih Nia & Zanguyi 21552 (FUMH).

S. getraudiae Melzh.: Iran: south-west of Sarakhs, between Pedli and Darzab, 2000 m, 31 May 1988, Joharchi & Zanguyi 17217 (FUMH).

S. indeprensa Schichk.: Iran: west of Bojnoord, Badranloo, 12 km to Dakal Mokhaberat road, 1920 m, 12 Jun. 1995, Rafeyi & Zanguyi 27303 (FUMH).

S. meyeri Fenzl ex Boiss. & Buhse: Iran: Marand, Mishodagh, 1700–2200 m, 6 Apr. 1990, Ghahreman & Mozafarian 9684 (TUH).

S. parjumanensis Podl.: Iran: west of Ghaen, Tajan, 2025 m, 3 May 2010, Joharchi & Nejati 43473 (FUMH).

S. palinotricha Fenzl ex Boiss.: Iran: north of Jajarm, top of Moshama village, 1680 m, 12 May 2009, Memariani & Zanguyi 42390 (FUMH).

S. persica Boiss.: Iran: Yasooj, Dena mountain, Bijan pass, 3000–3400 m, 16 Sept. 1995, Veizi & Hakimi 19409 (TUH).

S. pseudaucheriana Melzh.: Iran: Lavestan, Khoram Abad, Veisian, 10 Aug. 1999, Veis Karami 23964 (TUH).

S. renzii Melzh.: Iran: west of Ghaen, Tajan, 2025 m, 3 May 2010, Joharchi & Nejati 43471 (FUMH).

Sect. *Bipartitae* Boiss. (1867)

S. villosa Forssk.: Iran: Yazd, Ardekan to Nayin, 16 Apr. 1992, Ghahreman 14096 (TUH).

S. arabica Boiss.: Iran: north of Gonabad, Torbat-e Heidarieh to Goanabd road, 50 km to Gonabad, Loot Omrani, 1050 m, 9 Apr. 1989, Faghih Nia & Zanguyi 18052 (FUMH).

Sect. *Compactae* Boiss. (1867)

S. compacta Fisch.: Iran: Arasbaran, between Vaighan and Kharli Forked road, 1400 m, 6 Sept. 2005, Ghahreman, Hamzeh & Attar 35576 (TUH).

Sect. *Conoimorphae* Otth in DC. (1824)

S. conoidea L.: Iran: Neka, Hezar Jarib protected area, 5 Dec. 2009, Azadbakht 7256 (FAR).

S. coniflora Nees ex Otth.: Iran: part of southern slopes of Alborz mountains, around Vardije and Varish villages, 1843 m, 5 Aug. 2009, Gilani 8138 (FAR).

Sect. *Lasiocalycinae* Boiss. (1867)

S. gallica L.: Iran: Lahijan, Saharkhiz village, – 25 m, 27 May 2000, Naquinejad 27286 (TUH).

Sect. *Lasiostemones* Boiss. (1867)

S. marschallii C.A. Mey.: Iran: Marakan protected area, 1233 m, 26 May 2009, Nafisi 10535 (FAR).

S. claviformis: Litv.: Iran: south-west of Chenaran, 1–3 km of Frizi to Sorkheh, 1720–1810 m, 7 Jun. 2005, Emadzade, Memariani & Zanguyi 36551 (FUMH).

Sect. *Lychnidiformes* Melzh. (1988)

S. coronaria (L.) Clairv.: Iran: Golestan forest, south of Tangrah, Konkoo road, 12 Jul. 1986, Rezaei & Zanguyi 15539 (FUMH).

Sect. *Melandrifformes* Boiss. (1867)

S. latifolia Poir.: Iran: Siah Rood protected area, Roodbar, Alaei 3846 (FAR).

S. noctiflora L.: Iran: west of Mashhad, Binalood mountains, Kordineh, 1832 m, 6 Jun. 2005, Joharchi & Zanguyi 36536 (FUMH).

Sect. *Otites* (Adans.) Oth in DC. (1824)

S. cyri Schischk.: Iran: west of Bojnoord, Golestan forest, between Loooh and Dashte Shah, 13 km of east of Loooh, 1240 m, 8 Sept. 1986, Zanguyi & Alvani 5895 (FUMH).

Sect. *Paniculatae* Boiss. (1867)

S. italica (L.) Pers.: Iran: Neka, Hezar Jarib protected area, 1915 m, 27 Oct. 2009, Azadbakht 7569 (FAR).

Sect. *Rigidulae* Boiss. (1867)

S. chaetodonta Boiss.: Iran: Torbat-e Jam, Mashhad to Salehabad, between Baghbaghu and Natoo, 700 m, 17 May 1990, Joharchi & Zanguyi 20263 (FUMH).

Sect. *Saponariodeae* Boiss. (1867)

S. nana Kar & Kir.: Iran: north of Gonabad, Gonabad to Torbat-e Heidarieh road, 35 km, 850 m, 26 Apr. 1994, 25123 (FUMH).

Sect. *Schaftae* Boiss. (1867)

S. shafta Gmel.: Iran: Kiasar national park, 2068 m, Agheli 3453 (FAR).

Sect. *Sclerocalycinae* Boiss. (1867)

S. bupleuroides L.: Iran: Marakan protected area, 1239 m, 4 Jul. 2009, Nafisi 10745 (FAR).

S. chlorifolia Sm.: Iran: Ghoboor, Razi, 2040 m, 22 Jul. 1999, Ghahreman & Attar 22435 (TUH).

S. swertiifolia Boiss.: Iran: part of southern slopes of Central Albourz mountains, around Vardije and Varish villages, 1851 m, 22 May 2009, Gilani 8221 (FAR).

Sect. *Spergulifoliae* Boiss. (1867)

S. brahuica Boiss.: Iran: north-west of Torbat-e Jam, 16 km Salehabad to Doosti Dam, beside road, 550 m, 28 Apr. 2010, Joharchi & Memariani 42070 (FUMH).

S. cephalantha Boiss.: Iran: Marand, Zoonooz, between Zoonoozghah and Koohkamar, 2500 m, 26 Jun. 1994, Ghahreman & Mozafarian 17397 (TUH).

S. cappadocica Boiss & Heldr.: Iran: Marakan protected area, 1128 m, 3 May 2009, Nafisi 10360 (FAR).

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