Micro- and macromorphological study of *Stellaria* (*Caryophyllaceae*) and its closest relatives in Iran

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Abstract. Six species of *Stellaria* and two of its closest relatives were studied macro- and micromorphologically. Biometric study involved 26 quantitative and 31 qualitative characters. Seeds, pollen grains, leaf epidermis, and trichomes of the studied species were investigated by light microscope and SEM. Statistical analysis employed SPSS software. Principal Component Analysis defined the most variable characters. Some features, such as sepal number and hair presence, width, length and nerve number, different capsule features, etc. are of diagnostic importance. In micromorphological studies, shape of testa cells and their ornamentations are important seed features. In leaf and stem epidermis, cuticle shape and shape and structure of hairs are found to help in taxa separation. Species relationships are discussed in the article.

Keywords: **Iran,** micro- and macromorphology, phenetic analysis, *Stellaria*

Introduction

Stellaria L. (*Caryophyllaceae, Alsinoideae*) comprises *ca*. 150–200 species across the world (Bittrich 1993). This genus has nine species grouped in two sections. *S. blatteri* Mattf., *S. scaturiginella* Rech.f. and *S. sarcophylla* Rech.f. have an uncertain section (Rechinger 1988). According to *Flora Iranica, Stellaria* sections in Iran include: *Stellaria* L. with two annual species [*S. media* (L.) Vill. and *S. pallida* (Dumort.) Pire] and four perennial species which grow in the mountain areas, including *S. holostea* L., *S. persica* Boiss., *S. graminea* L., and *S. nemorum* L. Section *Pseudalsine* Boiss. consists of only one annual species (*S. alsinoides* Boiss. & Buhse) growing in the mountains of Iran. *Mesostemma kotschyanum* (Fenzl ex Boiss.) Vved. is a basionym for *Stellaria kotschyanum* Fenzl ex Boiss., but in 1988 Rechinger referred to this taxon as *Mesostemma* Vved. *Myosoton aquaticum* (L.) Moench, is occasionally treated as *Stellaria aquaticum* (L.) Scop. Recent molecular studies have shown that *M. aquaticum* nested within the *Stellaria* clades (Mahdavi 2012).

In genus *Stellaria,* seeds are small (0.4–3 mm long), numerous, seldom few or one, round to reniform, usually laterally compressed, and testa is variously sculpted by more or less papilliform cells, seldom completely smooth (Bittrich 1993). According to Yildiz (2002), the seed type of *S. holostea* is reniform to orbicular. Zareh (2005) pointed out that in the *Stellaria* species the seeds are tuberculate and compressed laterally. Chen & al. (2010) have studied micromorphological characters of the seed coat for 18 species of *Stellaria* from North China. They have found out that micromorphological features of the seed coat in *Stellaria* resembles a cogwheel. By these characters they divided *Stellaria* into two types: 1) granular or cobweb surface, without secondary sculpting, 2) granular or cobweb surface, with an outer surface with papillae. Miller & West (2012) have studied the *Stellaria* seed characters in Australia. Mahdavi & al. (2012) carried out the only study related to seed micromorphology of all *Stellaria* species in Iran.

Chanda (1962) determined some pollen features of *Stellaria aquatia, S.nemorum, S. media, S. apetala,*

S. holostea, S. palustris, S. graminea, and *S. longipes*. Basically, three pollen types were observed in the family: tricolpate with spinulose and tubuliferous/punctate ektexine, pantoporate with spinulose and tubuliferous/punctate ektexine; and pantoporate with reticulate ektexine (Nowicke 1975). Nilsson & al. (1977) reported a spinulose and perforate exine surface in the pantoporate pollen grains of *S. media*.

In Alsinoideae and Caryophylloideae, pantoporate pollens are frequent (Bittrich 1993). Taia (1994) found in subfamily Alsinoides that *Stellaria* was easily distinguished from *Spergularia* by their pollen features. He also determined the number of apertures in two of the studied *Stellaria* species. Yildiz (2001) determined some pollen features of *S. media* subsp. *pallida*, *S. nemorum*, *S. holostea,* and *Myosoton aquaticum*. He found that there were differences mainly in the pollen and pore diameter and number.

Chen & al. (2010) studied the leaf epidermis of 16 *Stellaria* species and four other related species from North China. They have found stomata apparatus on the abaxial epidermis. Most of the studied species also showed stomata apparatus on the adaxial epidermis. The types included anisocytic, anomocytic and anomotetracytic stomata apparatus, the shape of cells was irregular, with straight, arched, sinuate, or sinuolate anticlinal walls. The type of hairs on leaf epidermis included stellate, glandular pubescent, hair or lanate.

In this paper, micro- and macromorphology of the *Stellaria* species of Iran and their closest relatives were considered, in order to evaluate the species relationship and to provide an efficient identification key to the studied taxa.

Material and methods

Plant material

In the present study, 43 populations of six *Stellaria* L. species and two closest related genera in Iran were collected in the field: *Stellaria media, S. pallida, S. holostea, S. persica, S. graminea, S. alsinoides, Mesostemma kotschyanum,* and *Myosoton aquaticum*. The details of the voucher specimens and their localities are given in Table 1. All voucher specimens are deposited at the Herbarium of Alzahra University. In order to study the morphological characters for each location, at least 10 individuals were collected. A set of 26 quantitative and 31 qualitative characters were chosen (Table 2). The samples were studied by a Dino-Lite pro hand stereomicroscope. For SEM studies, the samples were stabilized on aluminum stocks and coated with a thin layer of gold. Then, the specimens were observed under a SEM electronic microscope, model S300. Stomatal index was calculated by division of the number of stomatal apparatuses by the number of stomatal apparatuses + the number of epidermal cells. To describe the seed coat, cell epidermis of the stomata and pollen morphology, we have followed the terminology of Punt & al. (1994), Yildiz & al. (2011) and Sahreen (2010).

Statistical analysis

To reveal the species relationship, cluster analysis and principal coordinate analysis (PCA) plotting were used. Multivariate analysis employed the average quantitative characters, while the qualitative characters were coded as binary/multistate characters. Standardized variables (mean = 0, variance = 1) were used in the statistical analysis. The average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficients in the cluster analysis of morphological data. SPSS ver. 9 (1998) was used for the statistical analysis.

Results

Seed morphology

Seed morphology characteristics of the eight studied taxa are listed in Table 3. The seed shape was reniform-round in *S. pallida* (Figs. 1-13) and *S. media* (Figs. 5-15); reniform in *S. holostea* (Figs. 3-11), *S. graminea* (Figs. 7-21), *S. persica* (Figs. 6-19), and *Myosoton aquaticum* (Figs. 4-25); pyriform in *S. alsinoides* (Figs. 2-17); and cubical-spherical in *M. kotschyanum* subsp. *kotschyanum* (Figs. 8-27) and *M. kotschyanum* subsp. *afghanicum* (Figs. 9-23). Seed colour is a diagnostic character in the *Stellaria* species. The seed colour was dark-brown in *S. media,* yellow in *S. alsinoides* and brown in the other species. The largest seeds occurred in *M. kotschyanum* subsp. *afghanicum* (3.28 mm long and 2.24 mm wide) and the smallest in *S. alsinoides* (0.72 mm long and 0.79 mm wide). Seed surface ornamentations have diagnostic value in the studied species. *S. pallida* (Fig. 12), *S. media* (Fig. 14), *S. holostea* (Fig. 10), and *Myosoton aquaticum* (Fig. 24) have shown broad polygonal

Table 1. Voucher details of the *Stellaria* species and related genera from Iran examined in this study.

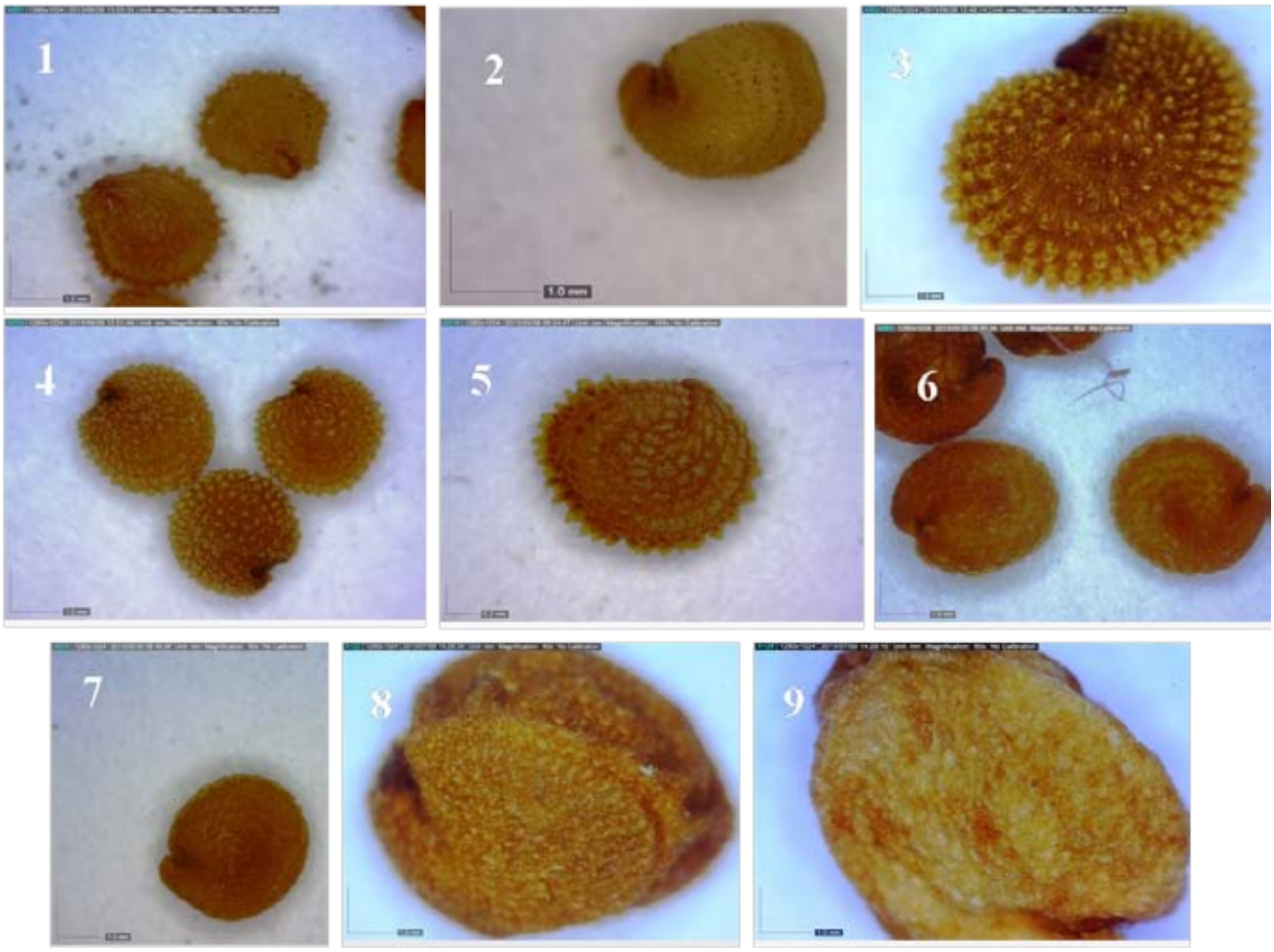
Species	Locality
Stellaria media (L.) Vill.	Tehran,Vanak, 1461 m, Esfandani 993 Tehran, Tehranpars, 1400 m , Esfandani 994 Tehran, Evin, 1649 m , Esfandani 995 Tehran, Lavizan, 1500 m, Esfandani 996 Tehran, Milad Tower, 1300 m ,Esfandani 997 Tehran, Enghelab sq., 1100 m , Esfandani 998 Alborz, Karaj, 1300 m, Esfandani 1000 Isfahan, City center, 1500 m, Esfandani 1010 Markazi, Arak, Senejan, 1800 m, Esfandani 1011 Tehran, Darband, 1600 m , Esfandani 1012 Azarbaijan, Tabriz, 1450 m, Habibi Tirtash 1013 Azarbaijan, Tabriz, University tabriz, 1400 m, Habibi Tirtash 1014 Golestan, Gorgan, 134 m, Esfandani 1015 Tehran, Shahriyar, 1600 m, Esfandani 1016 Golestan, Gorgan, Ramian, 200 m, Esfandani 1017 Tehran, Pasdaran street, 1500 m, Esfandani 1018 Mazandaran, Sari, 40 m, Esfandani 1019 Alborz, Karaj- Mardabad, 1280 m, Esfandani 1020 Tehran, Karaj, Chalus road, 1400 m, Esfandani 1021 Golestan, Gorgan, Gonbadkavous, 24 m, Habibi Tirtash 1022 Gilan, Rezvanshahr, 12 m, Esfandani 1023 Qom, Masoume Shrine, 950 m, Esfandani 1024 Mazandaran, Ramsar, 65 m , Esfandani 1025 Qazvin, Avaj, 1900 m, Esfandani 1027 Mazandaran, Tonekabon, 23 m, Esfandani 1029 Alborz, Karaj, Azimiyeh, 1320 m, Taghipour 992 Isfahan, Isfahan, Hezarjarib, 1600 m, Habibi Tirtash 991 Tehran, Resalat street, 1400 m, Esfandani 990 Tehran, Delavaran street, 1500 m, Esfandani 989 Tehran, Narmak, Hafthouz, 1380 m, Esfandani 988 Tehran, Kohsar, 1580 m, Esfandani 986 Khorassan, Sabzevar, 980 m, Esfandani 985
S. pallida (Dumort.) Pire	Golestan, Gorgan, Ramian, 200 m, Esfandani 1030 Golestan, Gorgan, Gonbadkavous, 24 m, Habibi Tirtash 1031
S. holostea L.	Tehran, Karaj, Chalus road, Siah bisheh, 2200 m, Esfandani 1032 Gilan, Jirandeh, 1300 m, Dadmehr1043
S. persica Boiss.	Tehran, Karaj, Chalus road, Kandovan Tunnel north slopes, 2750-2800 m, Esfandani 1035
S. graminea L.	Tehran, Karaj, Chalus road, Kandovan north slopes, 2750-2800 m, Esfandani 1036 Ardebil, Neour, 2400 m, Esfandani 1039
S. alsinoides Boiss. & Buhse	Mazandaran, Haraz road, Emam Zad-e-Hashem, 2700-2800 m, Esfandani 1038
Myosoton aquaticum (L.) Moench	Gilan, Rezvanshahr, Khoshabireh, 50 m, Esfandani 1040
Mesostemma kotschyanum (Fenzl ex Boiss.) Vved. subsp. *kotschyanum*	Tehran, Mountain of Tuchal, near fifth station of Tuchal Tele-cabin, 2900 m, Esfandani 1041
M. kotschyanum(Fenzl ex Boiss.) Vved. subsp. *afghanicum*	Azarbaijan, Tabriz, Sardasht, 1500 m, Azizi 1042

Table 2. List of selected characters and their codes in morphological studies.

No.	Characters	Numerical code
1	Plant height	mm
2	Length of basal leaves	mm
3	Width of basal leaves	mm
4	Length of stem leaves	mm
5	Width of stem leaves	mm
6	Bract length	mm
7	Bract width	mm
8	Pedicel length	mm
9	Number of seeds per capsule	
10	Number of flowers per inflorescence	
11	Number of calyx	
12	Calyx length	mm
13	Calyx width	mm
14	Number of petal	
15	petal length	mm
16	Petal width	mm
17	Cleft size of petals	mm
18	Internode length	mm
19	Number of stamen	
20	Number of stigma	
21	Capsule length	mm
22	Seed length	mm
23	Seed width	mm
24	Cleft size of capsule	mm
25	Number of suture capsules	
26	Vein number of sepals	
27	Growth period	0- annual 1- perennial
28	Bract apex	0- acute; 1- narrow; 2- absence
29	State of stem	0- unbranched; 1- branched
30	State of stem strength	0- thin; 1- strong
31	Stem hairs	1- unilateral hair; 2- multilateral hair; 3- bilateral hair
32	Cross-section of stem	0- round; 1- rectangular; 2- elliptical; 3-angular circular
33	Shape of basal leaves	0- linear; 1- linear-lanceolate; 2- lanceolate; 3- lanceolate–acuminate; 4- ovate; 5- ovate–broadly elliptical
34	Basal leaves apex	0- acute; 1- narrow
35	Basal leaves petiole	0- absence; 1- presence
36	Hair of basal leaves petiole	0- absence; 1- presence
37	Shape of cauline leaves	0- linear; 1- linear-lanceolate; 2- lanceolate; 3- lanceolate–acuminate; 4- ovate; 5- ovate–broadly elliptical
38	Cauline leaves apex	0- acute; 1- narrow
39	Cauline leaves petiole	0- absence; 1- presence
40	Hair of cauline leaves petiole	0- absence; 1- presence
41	Hair of cauline leaves margin	0- absence; 1- presence
42	Hair of cauline leaves lamina	0- absence; 1- presence
43	Shape of bract	0- linear; 1- linear-lanceolate; 2- lanceolate; 3- lanceolate–acuminate; 4- ovate; 5- less-ovate; 6- ovate– broadly elliptical
44	Seed shape	1- kidney-round; 2- kidney-shaped; 3- kidney-pear shaped; 4- globular cubic shaped;
45	Seed color	1- dark-brown; 2- brown; 3- yellow
46	Hair of bract	0- absence 1- presence
47	Shape of capsule	1- oblong; 2- oblong-ovate; 3- ovate; 4- spherical
48	Stamen color	1 gray or dark-purple; 2- yellow
49	Shape of petal	1- oblong; 2- ovate; 3- linear
50	State of petal	0- absence; 1- presence
51	Shape of petal lobes	1- linear- oblong; 2- linear
52	Texture of calyx	0- absence; 1- herbaceous, with a membranous margin
53	Hair of calyx	0- absence; 1- presence
54	Calyx apex	1- narrow; 2- acute
55	Shape of calyx	1- ovate-lanceolate; 2- lanceolate; 3- linear- lanceolate
56	Hair pedicle	0- absence; 1- presence
57	State bract texture	0- herbaceous; 1- herbaceous with a membranous margin; 2- membranous

Table 3. Seed characters of the *Stellaria* species and two of the closest related genera.

Taxa	Seed surface type	Width of testa cells (µm)	Length of testa cells (µm)	Seed granulation	Anticlinal wall	Epidermal cells shape	Seed color	Seed type	L/W seed	W (mm)	L (mm)
S. media	Convex	123	242	Fine	V-shape	Round	Dark Brown	Reni-R	0.96	1.30	1.26
S. pallida	Convex	107	117	Coarse	V-shape	Round	Brown	Reni-R	0.76	1.00	1.30
S. holostea	Convex and dome-shaped	150	305	Not	V-shape	Round	Brown	Reni	1.39	1.82	2.54
S. persica	Convex	115	193	Not	V-shape	Interlaced	Brown	Reni	1.25	0.89	1.12
S. graminea	Convex	249	141	Not	V-shape	Interlaced	Brown	Reni	1.19	1.04	1.24
S. alsinoides	Psilate – papilate	–	–	Not	Undulate	Elongate	Yellow	Pyri	0.91	0.79	0.72
Myosoton aquaticum	Convex	80	115	Not	V-shape	Round	Brown	Reni	1.20	0.75	0.90
Mesostemma kotschyanum. subsp. *kotschyanum*	Corrugate	95	180	Not	Irr, zip	Irregular	Brown	Cub-Sph	1.08	1.84	2.00
Mesostemma kotschyanum subsp. *afghanicum*	Corrugate	114	329	Not	Irr, zip	Irregular	Brown	Cub-Sph	1.46	2.24	3.28



Figs. 1-9. Seed shape of the *Stellaria* species and two of the closest related genera by stereomicroscope. Scale bar = 1 mm: 1) *S. pallida*; 2) *S. alsinoides*; 3) *S. holostea*; 4) *Myosoton aquaticum*; 5) *S. media*; 6) *S. persica*; 7) *S. graminea*; 8) *Mesostemma kotschyanum* subsp. *kotschyanum*; 9) *Mesostemma kotschyanum* subsp. *afghanicum*.

ornamentation, while in *S. graminea* (Fig. 20) and *S. persica* (Fig. 18) interlaced polygonal ornamentation was observed. Ornamentations are elongated polygonal in *S. alsinoides* (Fig. 16) and irregular polygonal in *Mesostemma* populations. Granulated rough surface was observed in *S. pallida* (Fig. 12) and fine granulated surface in *S. media* (Fig. 14), while the other studied species were not granulated. The seed surface type was convex in *S. pallida, S. media, S. persica,* and *S. graminea,* strongly convex in *S. holostea,* flat in *S. alsinoides,* and corrugate in the *Mesostemma* populations.

Palynological observations

LM investigations show that pollen grains in the *Stellaria* species and related genera are polyporate. Pollen shapes in polar and equatorial views are spheroid (Figs. 30-40). The comparative results of LM are given in Table 4. In the present study, the average size of pollen grains in the *Stellaria* species and related genera varied from 22.5 µm (*S. alsinoides*) to 42µm (*Myosoton aquaticum*). Pore diameters varied between 4.45 µm and 7.63 µm. The grain mesoporium ranged from 7.89 µm to 15.82 µm. The average exine thickness was between 1.37 µm (*S. alsinoides*) and 2.61 µm (*S. holostea*).

Leaf epidermis

The leaf epidermal characteristics of the eight species are listed in Tables 5 and 6. The LM characteristics of the adaxial (Ad) and abaxial (Ab) epidermis are illustrated in Figs. 55-66 and Figs. 67-78, respectively. SEM characteristics of Ab and Ad of the eight species are illustrated in Figs. 79–86 and 87–92, respectively. Apparently, stomatal and other epidermal features are constant within the species and, therefore, are good taxonomic characters.

Table 4. Pollen characters in the *Stellaria* species and two of the closest related genra under light microscopy.

Taxa	Exine protruding	Pollen shape	Pollen type	Exine thickness (µm)	Interpore distance (µm)	Pore diameter (µm)	Pollen diameter (µm)
S. media	Presence	Spheroidal	Polyporate	1.90	9.26	5.85	35.03
S. pallida	Presence	Spheroidal	Polyporate	1.43	9.25	4.47	32.12
S. holostea	Presence	Spheroidal	Polyporate	2.61	9.39	6.35	31.85
S. persica	Presence	Spheroidal	Polyporate	1.52	8.00	6.35	33.11
S. graminea	Presence	Spheroidal	Polyporate	2.59	11.12	6.40	31.07
S. alsinoides	Absence	Spheroidal	Polyporate	1.37	7.89	4.45	22.50
Myosoton aquaticum	Presence	Spheroidal	Polyporate	2.13	15.82	7.63	42.00
Mesostemma kotschyanum subsp. *kotschyanum*	Presence	Spheroidal	Polyporate	2.54	9.45	6.34	29.63

Table 5. Leaf epidermis characters in the *Stellaria* species and two of the closest related genera under light microscopy.

Character / Taxon	Adaxial epidermis				Abaxial epidermis						(Ab, Ad) epidermis		
	Shape of cells	Pattern of anticlinal walls	Stomatal apparatus cells	Shape of cells	Pattern of anticlinal walls	Stomatal apparatus cells	Size of Stomata	Stomatal index(%)	Stomato- type	Figure	Hair of epiderme	Shape of guard cell of stomata	Type of trichome
S. media	Irr	H-Sin	Absence	Irr	H-Sin	Presence	37.42×26	41.62	Ano-is	67	Absence	N-El	Multi cell
S. pallida	Irr	H-Sin	Presence	Irr	H-Sin	Presence	29×21.5	33.62	Ano-is	68	Absence	N-El	Multi cell
S. holostea (Fr)	Pol	Sm	Presence	Pol	Sm	Presence	29.75×18.37	34.37	Ano	73	Presence	N-El	Single cell
S. holostea (St)	Pol	Sm	Absence	Pol	Sm	Presence	32.14×22.42	31.22	Ano	74	Presence	N-El	Single cell
S.graminea (Fr)	Irr	H-Sin	Presence	Irr	H-Sin	Presence	28.57×18.85	18.54	Ano-is	75	Absence	N-El	Absence
S. graminea (St)	Irr	H-Sin	Presence	Irr	H-Sin	Presence	30×15	12	Ano-is	76	Absence	N-El	Absence
S. persica (Fr)	Irr	Sin	Presence	Irr	Sin	Presence	27×18	16	Ano-is	77	Absence	N-El	Absence
S. persica (St)	Irr	Sin	Presence	Irr	H-Sin	Presence	27×20	22	Ano-is	78	Absence	N-El	Absence
S. alsinoides	Pol	Sm	Presence	Pol	Sm	Presence	35×24	28	Ano-is	69	Absence	Ellip	Absence
Myosoton aquaticum	Irr	Sin	Presence	Irr	Sin	Presence	35×25	30.76	Ano-is	70	Presence	N-El	Multi cell
Mesostemma kotschyanum subsp. *kotschyanum*	Irr	Sin	Presence	Irr	Sin	Presence	30×25	69	Ano-is	72	Presence	Ellip	Multi cell
Mesostemma kotschyanum subsp. *afghanicum*	Irr	Sin	Presence	Irr	Sin	Presence	28×24	30	Ano-is	71	Absence	Ellip	Absence

Abbreviations: **Irr** – irregular , **Pol** – polygonal, **Ano** – anomocytic, **Ano-is** – anomo-anisocytic, **Sm** – smooth, **Sin** – sinuous, **H-sin** – highly sinuous, **Ellip** – elliptical, **N-El** – narrow elliptical, **Fr** – fertile, **St** – sterile.

Epidermal cells

Epidermal cells of the *Stellaria* species and relative genera examined under LM were usually irregular in form, with sinuous or highly sinuous anticlinal cell walls. In a few species they were polygonal, with smooth anticlinal cell walls. The ordinary cell shape in Ad and Ab of most species, including *S. media* (Figs. 55-67), *S. pallida* (Figs. 56-68), *S. graminea* (Figs. 63- 64 and 75-76), *S. persica* (Figs. 65-66 and 77-78), *Myosoton aquaticum* (Figs. 58-70), *M. kotschyanum* subsp. *kotschyanum* (Figs. 60-72), and *M. kotschyanum* subsp. *afghanicum* (Figs. 59-71), was irregular and the anticlinal cell walls were slightly or highly sinuate. Polygonal shapes and smooth walls occurred in Ad and Ab of *S. holostea* (Figs. 61-62 and 73-74) and *S. alsinoides* (Figs. 57-69).

Stomatal apparatus and guard cells

Stomata were of the anomocytic and anomo-anisocytic type. They were present on the surfaces in *S. pallida* (Figs. 56-68), *S. graminea* (Figs. 63-64 and 75-76), *S. persica* (Figs. 65-66 and 77-78), *Myosoton aquaticum* (Figs. 58-70), *M. kotschyanum* subsp. *kotschyanum* (Figs. 60-72), *M. kotschyanum* subsp. *afghanicum* (Figs. 59-71), *S. alsinoides* (Figs. 57-69), and *S. holostea* (Fertile) (Figs. 61-73). However, in *S. holostea* (sterile stems) (Figs. 62-74) and *S. media* (Figs. 55-67), stomata occurred only on the abaxial surface. The shapes of guard cells examined by LM were usually narrow elliptical in *S. media* (Figs. 55-67), *S. pallida* (Figs. 56-68), *S. graminea* (Figs. 63-64 and 75-76), *S. persica* (Figs. 65-66 and 77-78), *Myosoton aquaticum* (Figs. 58-70), and *S. holostea* (Figs. 61-62 and 73-74). The shapes of the guard cells were elliptical in *M. kotschyanum* subsp. *kotschyanum* (Figs. 60-72), *M. kotschyanum* subsp. *afghanicum* (Figs. 59-71) and *S. alsinoides* (Figs. 57-69). The outer stomatal rims were raised in *S. graminea* (Fig. 86), *S. persica* (Fig. 85), *S. holostea* (Fig. 79), *S. alsinoides* (Fig. 80), and *M. kotschyanum* subsp. *kotschyanum* (Fig. 84), and not raised in *S. media* (Fig. 82), *S. pallida* (Fig. 81) and *Myosoton aquaticum* (Fig. 83).

Cuticular and wax ornamentation

Hairs were absent on both Ad and Ab surfaces of *S. media* (Figs. 55-67), *S. pallida* (Figs. 56-68), *S. graminea* (Figs. 63-64 and 75-76), *S. persica* (Figs. 65-66 and 77-78), *M. kotschyanum* subsp. *afghanicum* (Figs. 59-71), and *S. alsinoides* (Figs. 57-69), but were present in *M. kotschyanum* subsp. *kotschyanum* (Figs. 60-72), *Myosoton aquaticum* (Figs. 58-70) and *S. holostea* (Figs. 61-62 and 73-74). Under SEM, the cuticular membrane of *S. alsinoides* (Fig. 87) and *S. holostea* (Fig. 88) was striated, while *in S. pallida* (Fig. 89) and *S. media* (Fig. 90) was wrinkled. In *S. graminea* (Fig. 91) and *S. persica* (Fig. 92), the cuticular membranes were wavy. No wax ornamentations were observed on *S. pallida* (Fig. 81), *S. media* (Fig. 82) and *Myosoton aquaticum* (Fig. 83). Granular wax ornamentation occurred in *M. kotschyanum* subsp. *kotschyanum* (Fig. 84) and *S. graminea* (Fig. 86). Wax ornamentation in *S. persica* (Fig. 85) was highly granular, in *S. holostea* (Fig. 79) lanate, and in *S. alsinoides* (Fig. 80) in the form of platelets.

Trichome

In the *Stellaria* species and related genera examined in this study, the following types of trichomes have been observed by stereomicroscope and by SEM on the stem surfaces. The comparative results are given in Table 7. There were three different trichome types or the plant was totally glabrous:

Type 1. Multicellular (2–12) simple trichomes, with a round apex, multilateral (Figs. 41-45).

Type 2. Multicellular (2–14) simple trichomes, with a round apex, unilateral (Figs. 46-48).

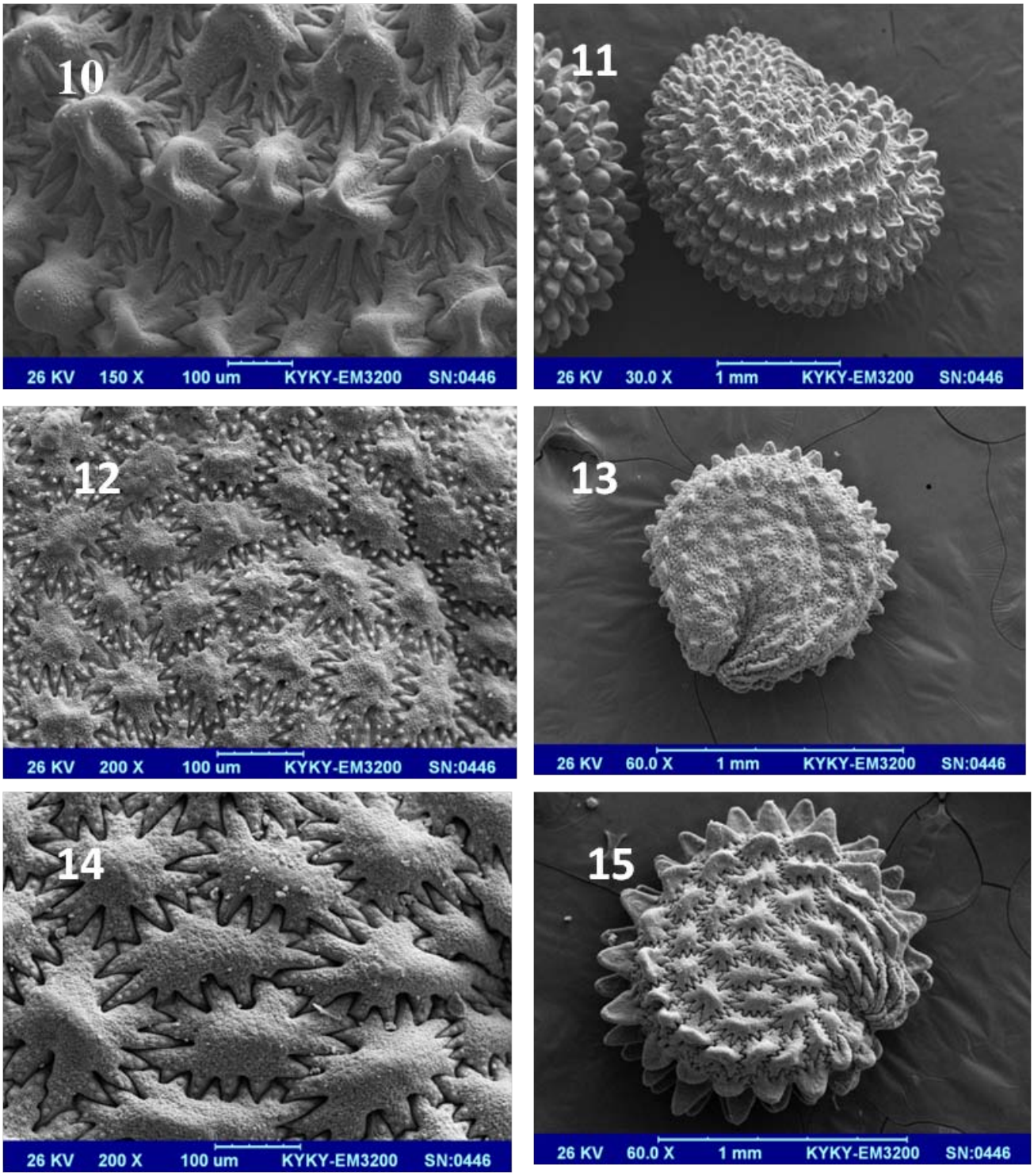
Type 3. Single-celled, simple, acicular trichomes, with an acute apex, multilateral (Figs. 49-51).

Table 6. Leaf abaxial epidermal characters in the *Stellaria* species and two of the closest related genera under SEM microscopy.

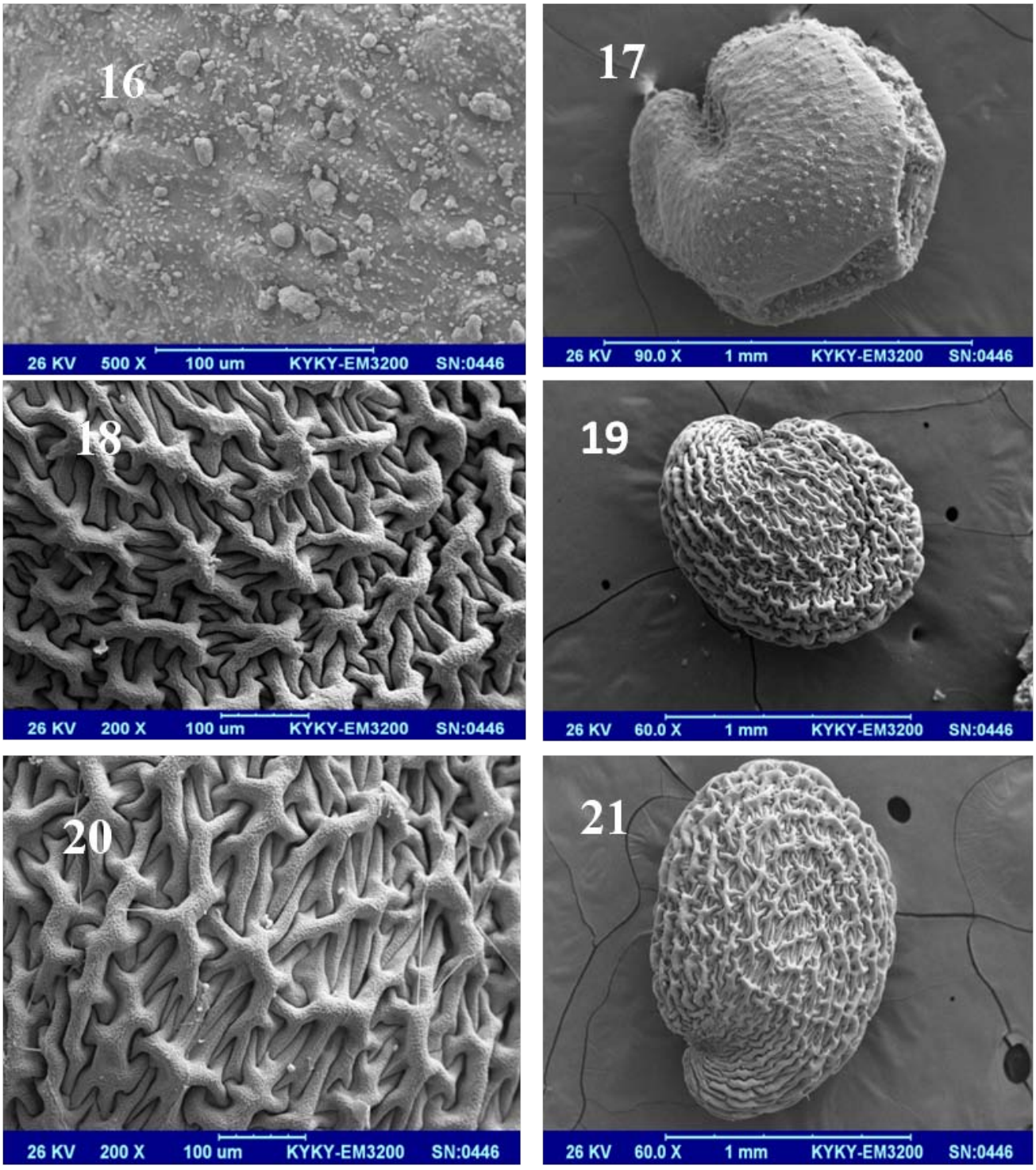
Taxa	Outer stomatal rim	Cuticular membrane	Wax ornamentation
S. media	Non-elevated	Wrinkled	Invisible
S. pallida	Non-elevated	Wrinkled	Invisible
S. holostea	Elevated	Straight	Lanate
S. graminea	Elevated	Wavy	Granular
S. persica	Elevated	Wavy	Highly granular
S. alsinoides	Elevated	Straight	Platelets
Myosoton aquaticum	Non-elevated	–	Invisible
Mesostemma kotschyanum subsp. *kotschyanum*	Elevated	–	Granular

Table 7. Stem trichome characteristics in the *Stellaria* species and two of the closest related genera by SEM and stereomicroscope.

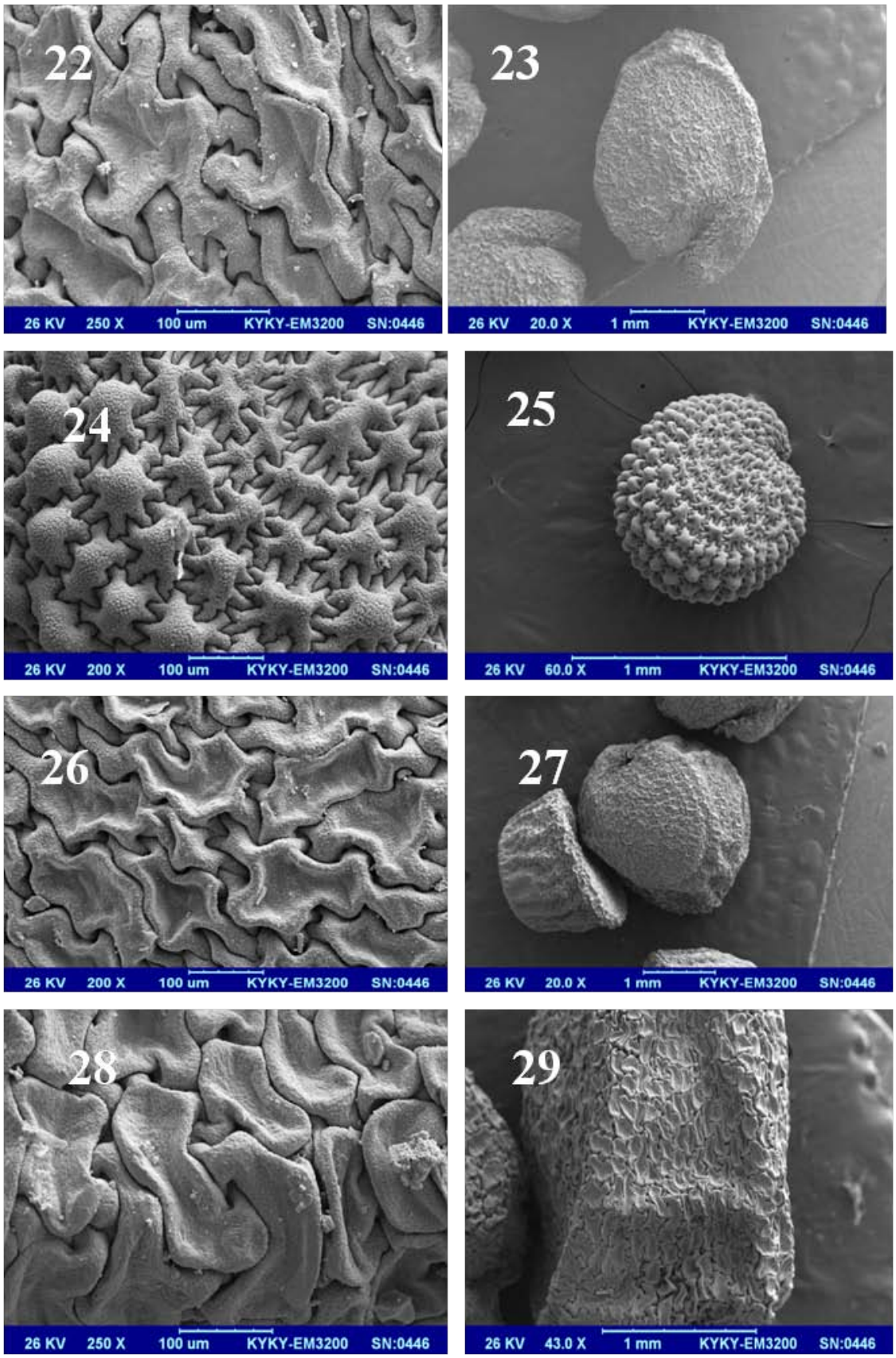
Taxon	Trichomes structure	Trichomes type	Stem trichomes
S. media	Multicellular	Simple	Unilateral
S. pallida	Multicellular	Simple	Unilateral
S. holostea (fertile)	Unicellular	Acicular	Multilateral
S. alsinoides	Multicellular	Simple	Multilateral
Myosoton aquaticum	Multicellular	Simple	Multilateral
Mesostemma kotschyanum subsp. *kotschyanum*	Multicellular	Simple	Multilateral



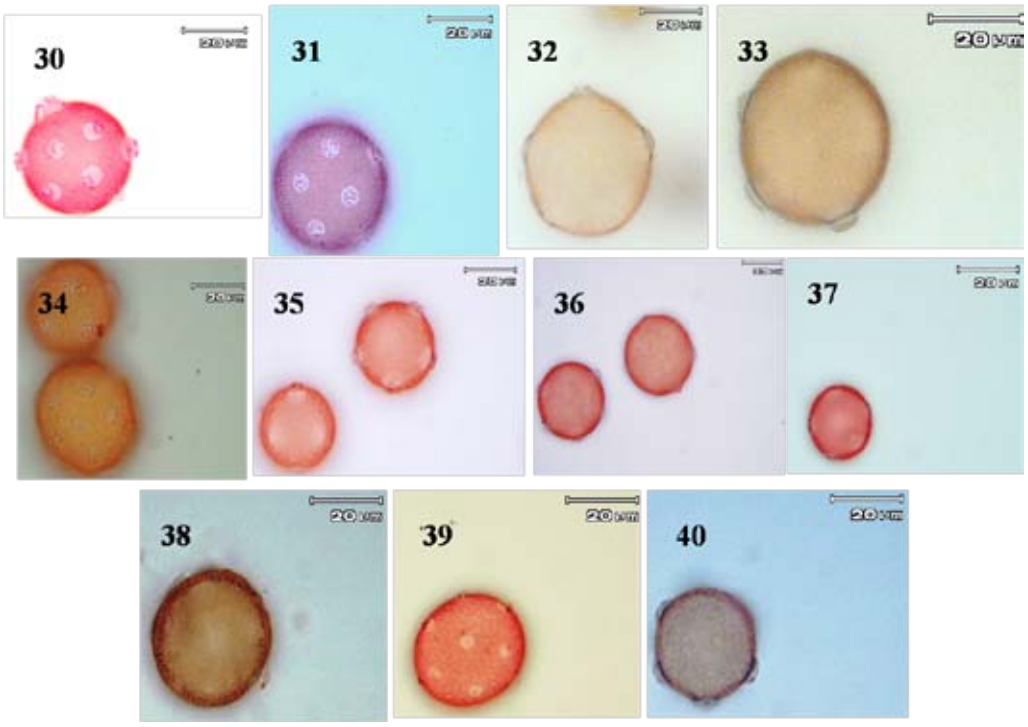
Figs. 10-15. SEM micrographs of seeds in the *Stellaria* species and two of the closest related genera: 10-11) *S. holostea*; 12-13) *S. pallida*; 14-15) *S. media*.



Figs. 16-21. SEM micrographs of seeds in the *Stellaria* species and two of the closest related genera: 16-17) *S. alsinoides*; 18-19) *S. persica*; 20-21) *S. graminea*.

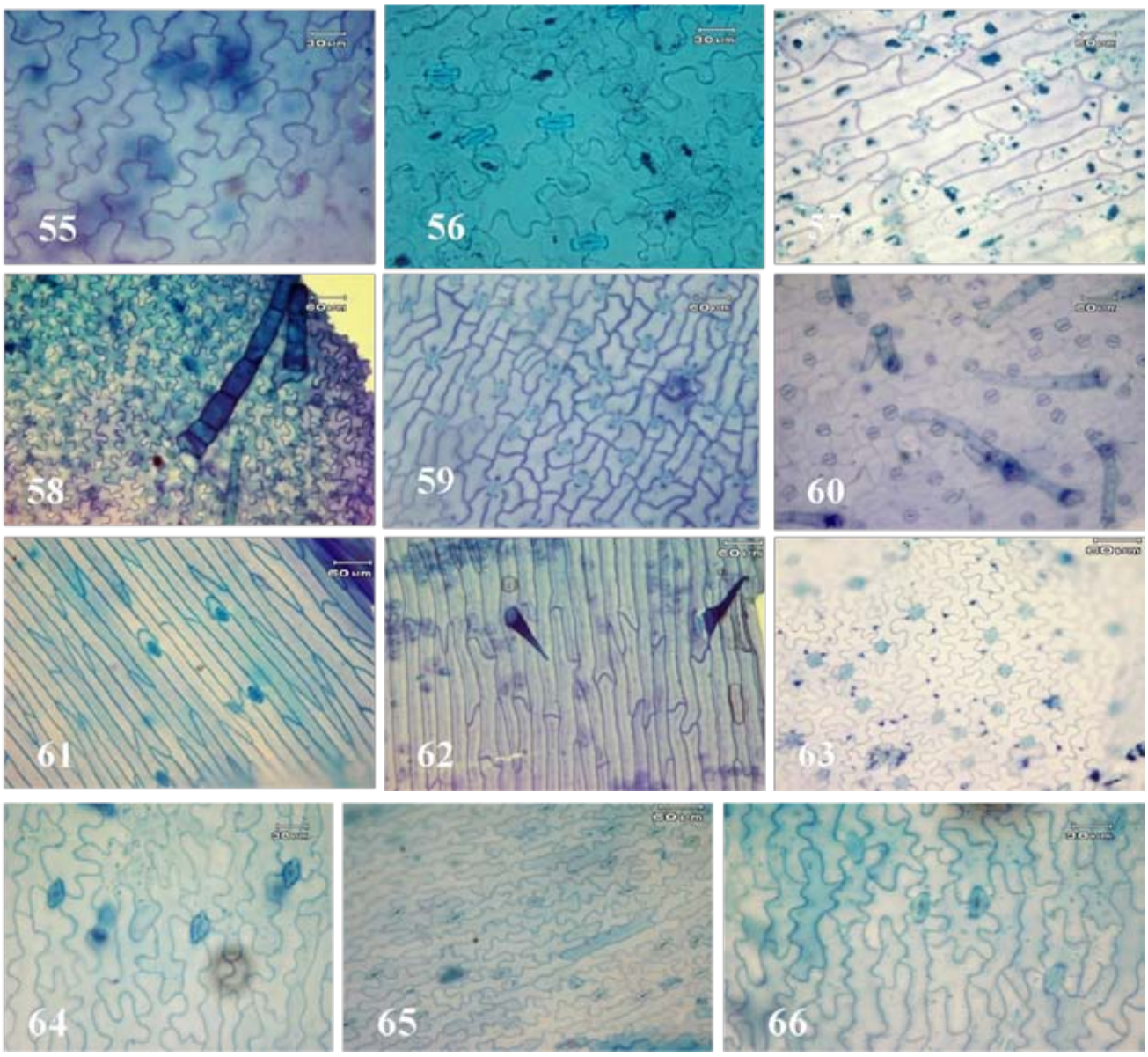


Figs. 22-29. SEM micrographs of seeds in the *Stellaria* species and two of the closest related genera: 22-23) *Mesostemma kotschyanum* subsp. *afghanicum*; 24-25) *Myosoton aquaticum*; 26-29) *Mesostemma kotschyanum* subsp. *kotschyanum;* 26-27) ventral surface; 28-29) lateral surface.

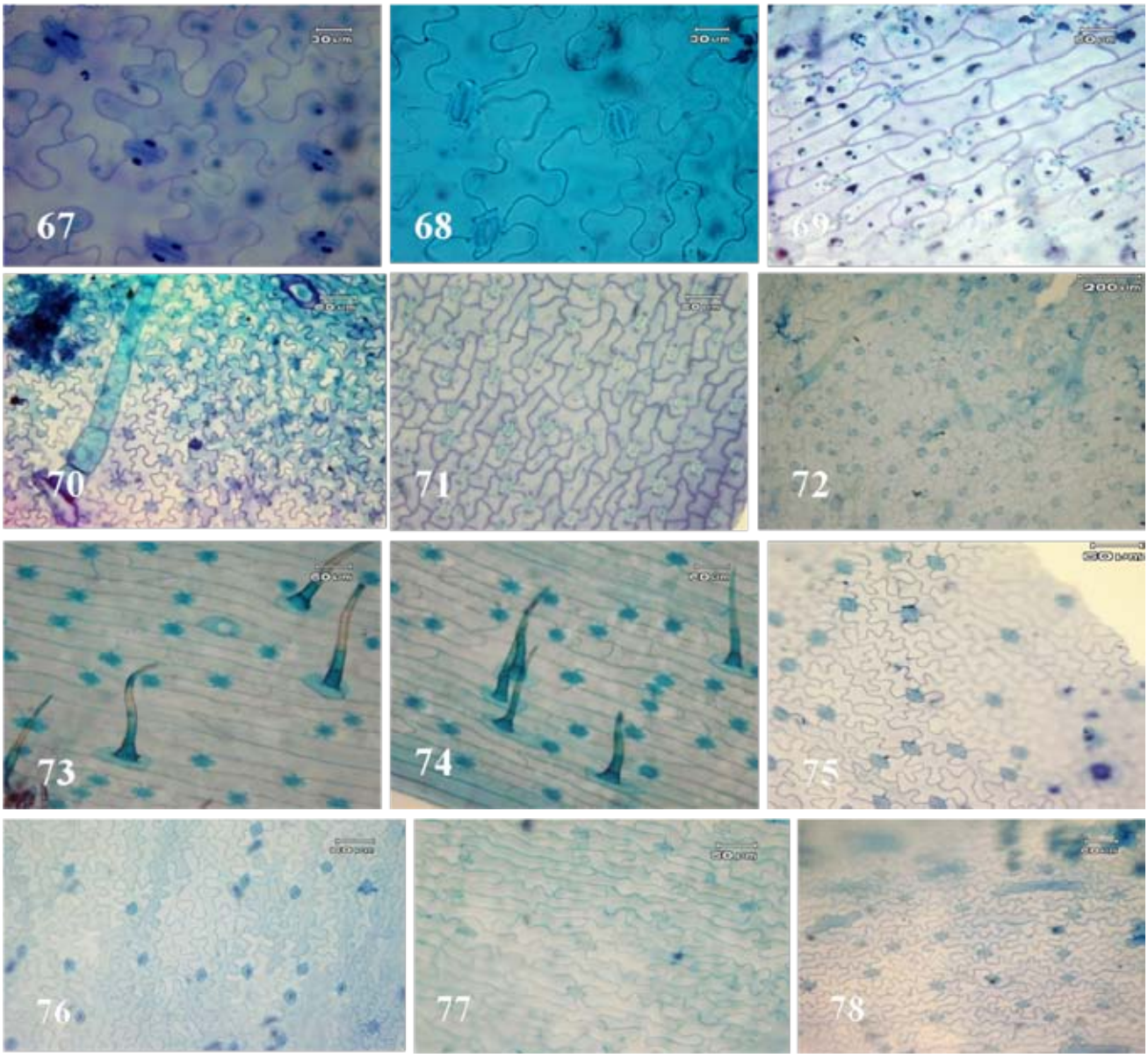


Figs. 30-40. Light micrographs of pollen grains in the *Stellaria* species and two of the closest related genera: 30) *S. media*; 31) *S. pallida*; 32-33) *Myosoton aquaticum*; 34) *S. holostea;* 35-36) *S. graminea*; 37) *S. alsinoides*; 38-39) *S. persica*; 40) *Mesostemma kotschyanum* subsp. *kotschyanum*.

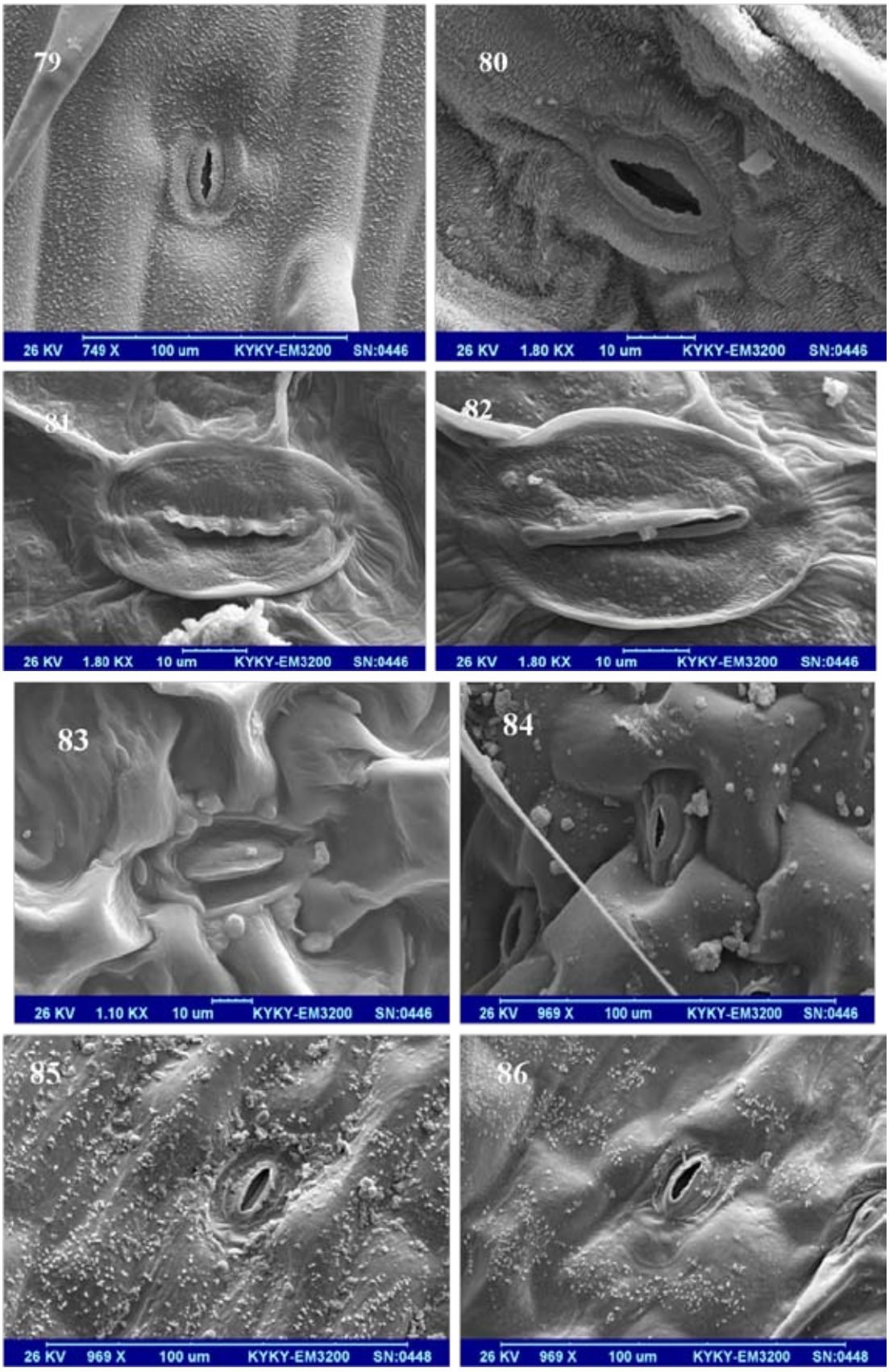
Figs. 41-54. Stereomicroscopic photographs and SEM micrographs of stem trichomes in the *Stellaria* species and two of the closest related gernera: 41) *Myosoton aquaticum*; 42-44) *Mesostemma kotschyanum* subsp. *kotschyanum*; 45) *S. alsinoides*; 46) *S. pallida*; 47-48) *S. media*; 49-51) *S. holostea*; 52) *Mesostemma kotschyanum* subsp. *afghanicum*; 53) *S. graminea*; 54) *S. persica*.



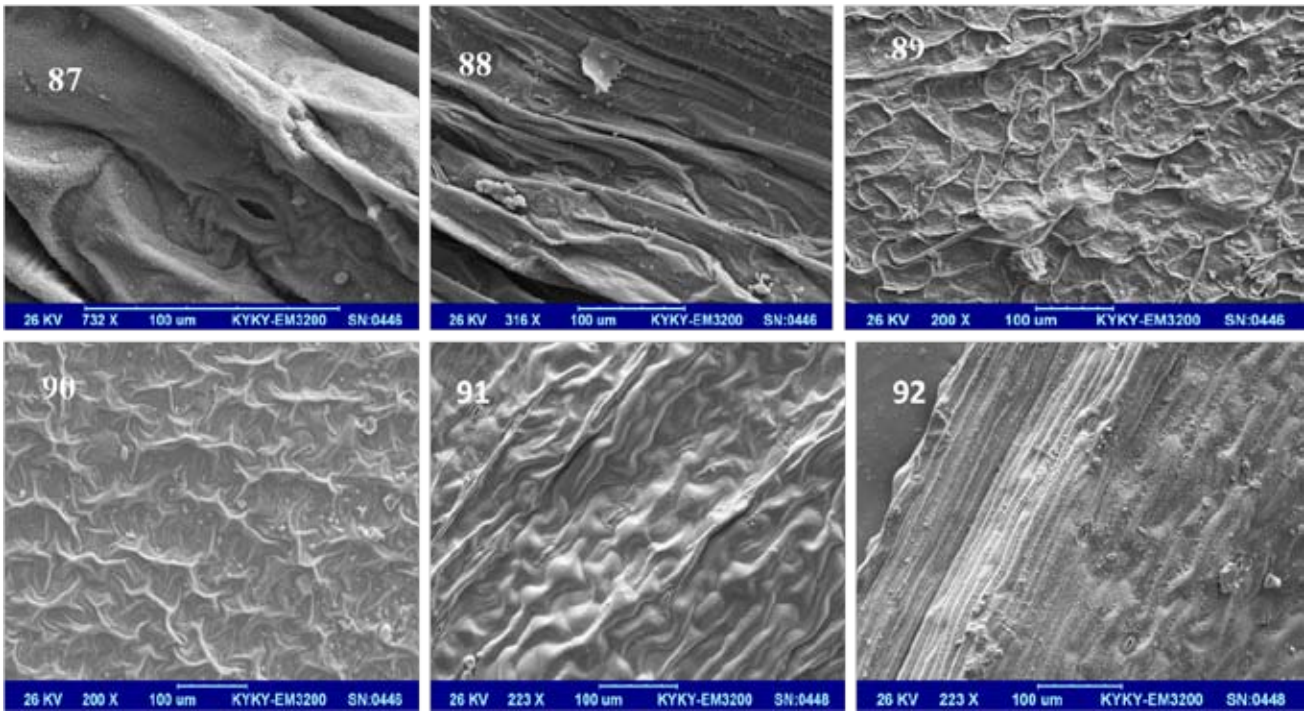
Figs. 55-66. Adaxial epidermis (by light microscopy) in the *Stellaria* species and two of the closest related genera: 55) *S. media*; 56) *S. pallida*; 57) *S. alsinoides*; 58) *Myosoton aquaticum*; 59) *Mesostemma kotschyanum* subsp. *afghanicum*; 60) *Mesostemma kotschyanum* subsp. *kotschyanum*; 61) *S. holostea* (fertile); 62) *S. holostea* (sterile); 63) *S. graminea* (fertile); 64) *S. graminea* (sterile); 65) *S. persica* (fertile); 66) *S. persica* (sterile).



Figs. 67-78. Abaxial epidermis (light microscopy) in the *Stellaria* species and two of the closest related genera under light microscopy: 67) *S. media*; 68) *S. pallida*; 69) *S. alsinoides*; 70) *Myosoton aquaticum*; 71) *Mesostemma kotschyanum* subsp. *afghanicum*; 72) *Mesostemma kotschyanum* subsp. *kotschyanum*; 73) *S. holostea* (fertile); 74) *S. holostea* (sterile); 75) *S. graminea* (fertile); 76) *S. graminea* (sterile); 77) *S. persica* (fertile); 78) *S. persica* (sterile).



Figs. 79-86. Electron micrographs of abaxial epidermis in the *Stellaria* species and two of the closest genera: 79) *S. holostea*; 80) *S. alsinoides*; 81) *S. pallida*; 82) *S. media*; 83) *Myosoton aquaticum*; 84) *Mesostemma kotschyanum* subsp. *kotschyanum*; 85) *S. persica*; 86) *S. graminea*.



Figs. 87-92. Electron micrographs of abaxial epidermis in the *Stellaria* species and two of the closest related genera: 87) *S. alsinoides*; 88) *S. holostea*; 89) *S. pallida*; 90) *S. media*; 91) *S. graminea*; 92) *S. persica*.

Results of the statistical analysis

According to the phenogram (Fig. 93) obtained by WARD method, two main clusters have been distinguished. In the first cluster, *S. media* and *S. pallida* from the *Stellaria* section and *Myosoton aquaticum* were located close to each other (in a subcluster1). *S. persica* and *S. graminea* (*Stellaria* section) were located in a subset and differed by hair absence, linear sessile leaves, 10 stamens, 3mer stigma, and deeply lobed petals. *S. alsinoides* (*Pseudalsine* section) and two of the above-mentioned taxa were also adjacent to each other (subcluster 2). However, *Mesostemma kotschyanum* subsp. *kotschyanum* and *M. kotschyanum* subsp. *afghanicum* nested also in this group. The *Mesostemma* taxa formed a separate set in the *Stellaria* by such features as seed shape, size, number per capsule, shape of cauline leaves and number of stigma. *S. holostea* (*Stellaria* section) formed a single cluster due to such features as lanceolate leaves, acicular hairs on the different plant parts, and capsule shape.

The second cluster consisted of sterile stems of *S. persica* and *S. graminea* and *S. holostea*. By means of factor analysis, it was shown that three factors were responsible for more than 76% of the variations in the studied *Stellaria* species and related genera in this study. The PCA analysis has revealed that the most variable characters were leaf texture, length, width, hairs and number of sepals, stigma number, number and size of capsule sutures and length, number of petals, petal presence, stem branching, pedicle hair, width of seed, capsule shape, sepal nerve number, shape of cauline and basal leaves; they all are of diagnostic value in separation of accessions in the eight studied species. The ordination of species based on PCA was also highly compatible with the related phenogram (Fig. 93). In order to study the relationship among the species on the basis of micromorphology, cluster analysis by WARD method based on evaluated characters was performed (Fig. 94). According to the phenogram, two main clusters were separated at *ca.* 15 level. The first cluster included two subclusters; in the first one, *S. persica* and *S. graminea* were virtually grouped, which was in concordance with the morphological phenogram. These two species showed difference only in the leaf cuticle. The second subcluster was composed of the *Mesostemma kotschyanum* subspecies. Micromorphological similarities in these two taxa were not unexpected. There were differences in the testa cell shape, vast peripheral surface, cubic seed shape, and seed size (*ca.* 3 mm) between *M. kotschyanum* subspecies and the other *Stellaria* taxa.

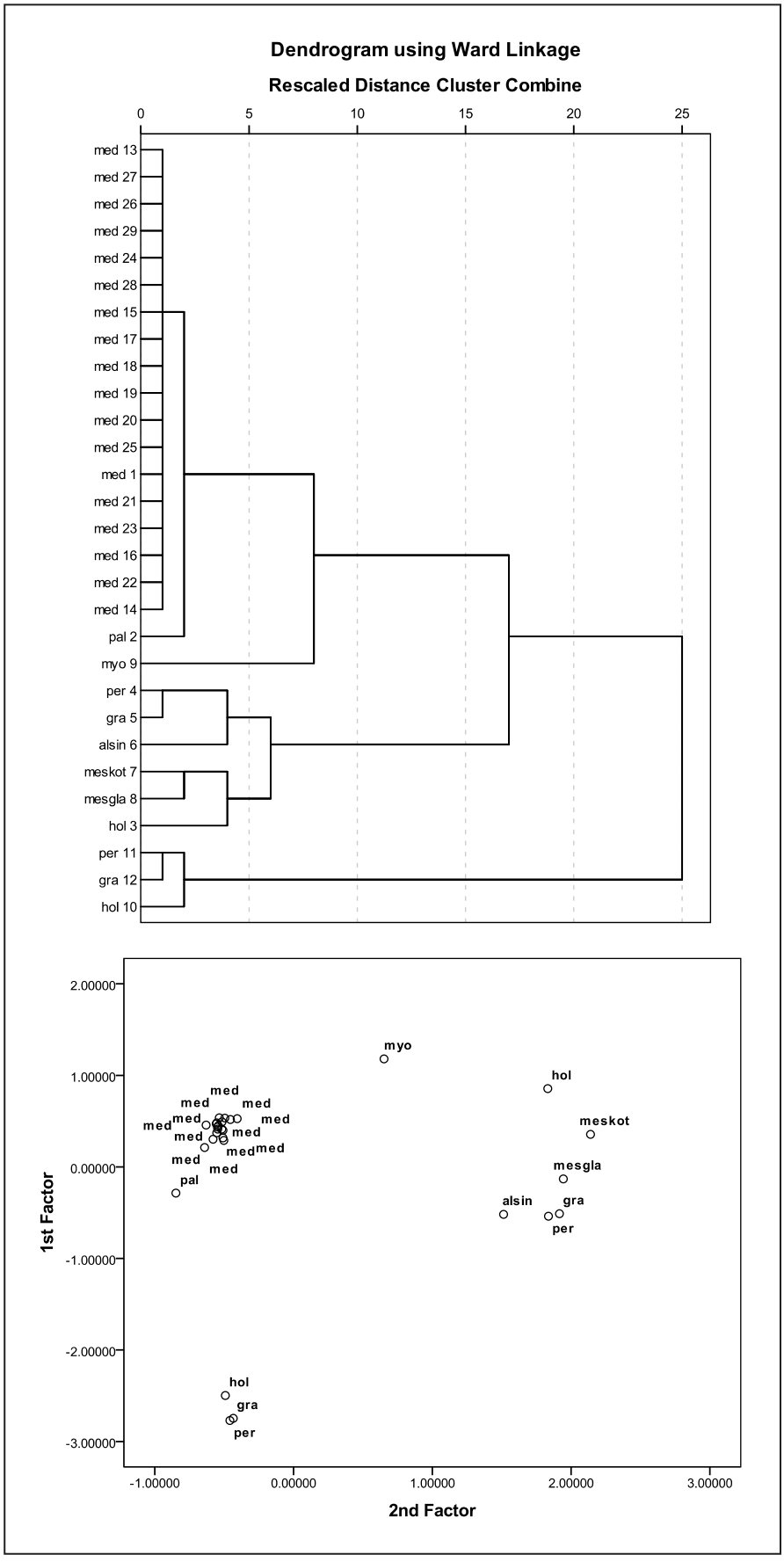


Fig. 93. Phenogram and ordination based on the morphological data of *Stellaria* and two of its closest related genera. Abbreviations: **med**= *S. media*; **pal**= *S. pallida*; **myo**= *Myosoton aquaticum*; **per4**= *S. persica* (Fertile); **gra5**= *S. graminea* (Fertile); **alsin**= *S. alsinoides*; **hol3**= *S. holostea* (Fertile); **per11**= *S. persica* (Sterile); **gra12**= *S. graminea* (Sterile); **hol10**= *S. holostea* (Sterile); **meskot**= *M. kotschyanum* subsp. *kotschyanum*; **mesgla**= *M. kotschyanum* subsp. *Afghanicum*.

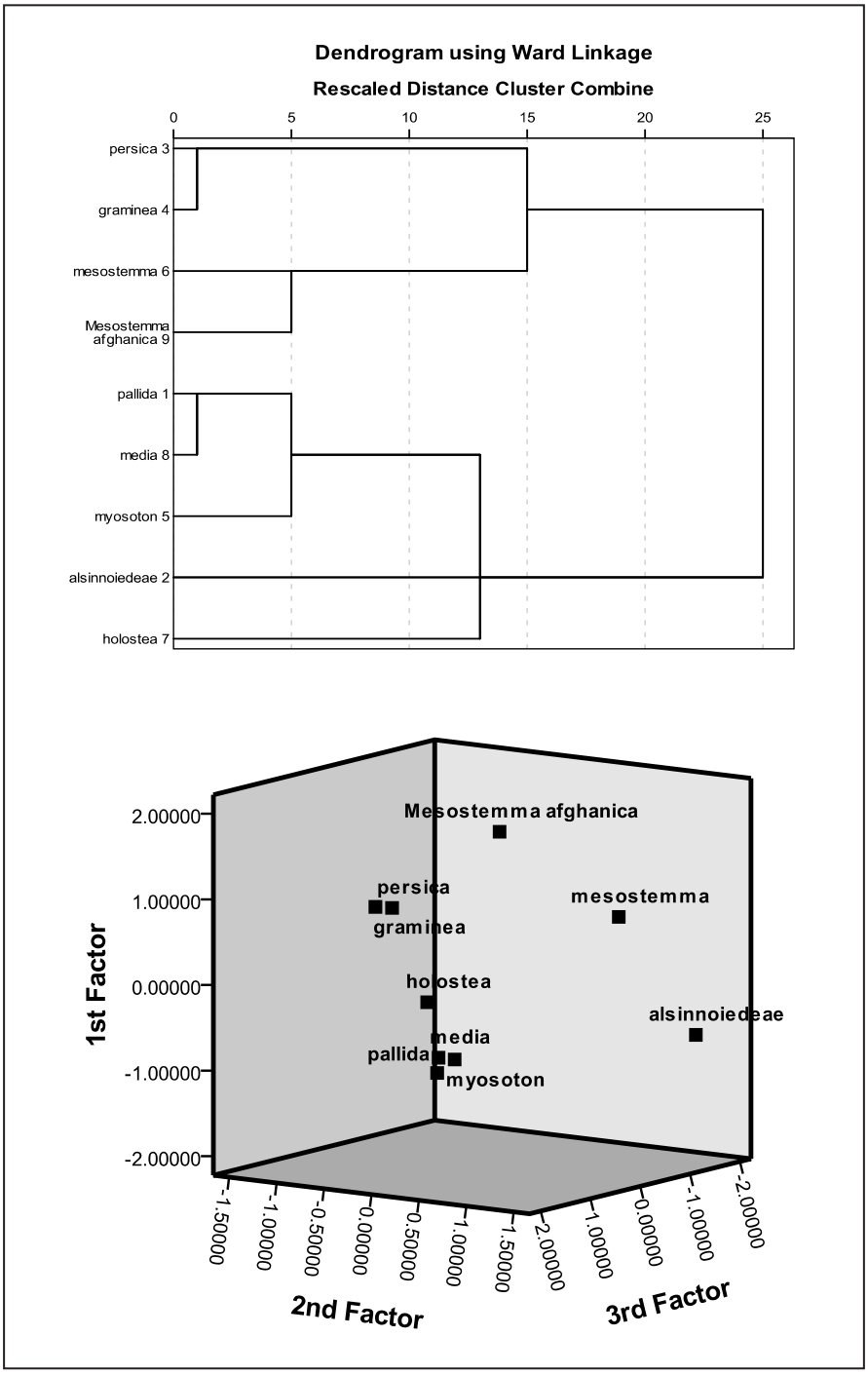


Fig. 94. Phenogram and ordination based on micomorphological data of *Stellaria* and two of its closest related genera. Abbreviations: **persica**= *S. persica*; **graminea**= *S. graminea*; **holostea**= *S. holostea*; **media**= *S. media*; **pallida**= *S. pallida*; **myosoton**= *myosoton aquaticum*; **alsinnoiedeae**= *S. alsonoides*; **mesostemma**= *Mesostemma kotschyanum* subsp. *kotschyanum*; **Mesostemma afghanic**a= *Mesostemma kotschyanum* subsp. *afghanicum*.

The second cluster consisted of five species. *Myosoton aquaticum* was located near *S. media* and *S. pallida,* as the morphological phenogram shows (Fig. 93). *S. alsinoides* and *S. holostea* were separated by such features as seed size, shape, colour, ornamentations of testa, epicuticular wax, and epidermal cell shape. Acicular hairs were also important in *S. holostea* separation.

By means of the factor analysis, it was shown that four factors were responsible for more than 87% of variation in the studied taxa. PCA analysis was carried out. Most variable characters were the shape of anticlinal epidermis wall, shape of testa cell, cuticular membrane, width of the seed peripheral surface, seed length, presence of extensive peripheral seed surface, seed length-to-width ratio, hair structure, and shape of the hair apex. Ordination of the species based on PCA was also highly compatible with the related phenogram (Fig. 94).

Discussion

An identification key based on the studied and evaluated features is provided:

1a. Styles 2 or 3, capsules dehiscence by 4 or 6 suture 2

1b. Styles 5(6), capsule dehiscence by 5 (6) suture .... *Myosoton aquaticum*

2a. Styles 2, capsule dehiscence by 4 suture, seed length *ca.* 3 mm .....3 *Mesostemma kotschyanum*

2b. Styles 2 or 3, capsule dehiscence by 4 or 6 suture, seed length *ca.* 1 mm 4

3a. Plant glabrous ..... subsp. *afghanicum*

3b. Plant hairy ..... subsp. *kotschyanum*

4a. Annual herb 5

4b. Prennial herb 7

5a. Sepals and petals 4, styles 2, leaves linear-lanceolate .... *S. alsinoides*

5b. Sepals and petals 5, styles 3, leaves ovate 6

6a. Petals are present, seeds 0.8–1.3 mm, dark-brown, stamens 3–7..... *S. media*

6b. Petals absent or seldom present, seeds 0.8 mm, light-brown, stamens 1-2..... *S. pallida*

7a. Leaves lanceolate-acuminate, hairy, petals bifid and 2x longer than sepals ..... *S. holostea*

7b. Leaves linear, glabrous, deeply bifid..... 8

8a. Infloresence with 1– 2 flowers, bracts leaf-like.... *S. persica*

8b. Infloresence with 3 flowers, bract membranous...*S. graminea*

The obtained results from macro- and micromorphological studies were in agreement. Results of seed micromorphology seem to be in agreement with those reported by Taia (1994), Chanda (1962), Punt & Hoen (1995), Yildiz (2001), Volponi (1987), and Perveen & Qaiser (2006). Seed micromorphology can separate the taxa into specific, sectional and generic ranks. Pollen grain features showed great similarity as Caryophyllaceae, which is a stenopalynous family, but there were some minor variations in size and ornamentations of the exine and operculum. In conclusion, morphological structure of the pollen grains seems to be useful for differentiating the taxa as an additional data source.

Chen & al. (2010) believed that epidermis studies alone had some limitations in the section classification and our findings are in concordance with their results. The observed different hair types have shown diagnostic value, besides the other data sources.

The main achievements are:

- *Stellaria alsinoides* is grouped inside genus *Stellaria* and should not be considered as *Tytthostemma alsinoides,* as was proposed by Mahdavi (2012).
- Despite its macro and micromorphological similarities to *S. media* and *S. pallida, Myosoton aquaticum* is clearly recognized as a separate taxon and should not be merged with the *Stellaria* genus. The main differences are multilateral hair distribution on the leaf and stem surface, stigma parts, capsule dehiscence, and shape and seed number per capsule (40 to 60). SDS_PAGE electrophoretic findings have contributed to the same conclusion (Esfandani Bozchaloyi 2013).
- The separate position of *Mesostemma kotschyanum* is confirmed under the present study. The results are in conformity with Mahdavi (2012) findings.

Perveen & Qaiser (2006)
Xubo & al. (2010)
Zareh (2005)

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