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POLLEN MORPHOLOGY OF THE GENUS *ARENARIA* L. (SUBGENUS *ARENARIA*) (CARYOPHYLLACEAE) IN TURKEY

CAHİT DOĞAN AND NİHAN ÇAKIR

[1]*Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey*
[*]*Corresponding author's email: cdogan@hacettepe.edu.tr*

Abstract

In this study, the palynological characters of 16 perennial taxa belonging to subgenus *Arenaria* L. (Caryophyllaceae) from Turkey were examined. We studied the morphological features of the pollen grains by using light microscopy (LM) and scanning electron microscopy (SEM), and microphotographs of the pollen grains were taken. As a result of the morphological observation of pollen grains using LM, that pollen size and numbers of pores on the pollen grain differ between taxa. In conclusion, the pollen type of the taxa belonging to subgen. *Arenaria* are pantoporate (periporate), the pollen shape is prolate spheroidal, the exine structure is subtectate, and the ornamentation is microechinate perforate. The pores are covered with an operculum and the surface of the operculum is microechinate. The pollen properties of each taxon in the genus are given in detail.

Key words: *Arenaria*, Caryophyllaceae, Micromorphology, Palynology, Taxonomy.

Introduction

Arenaria is a genus in the Alsinoideae subfamily of Caryophyllaceae family (Aktaş *et al.*, 2010). It has 306 species that are found globally, but it is mainly found in Eurasia, America, and North Africa (Williams, 1897; Li-hua, 1996; Fatemeh *et al.*, 2010).

The taxa in the genus *Arenaria* are annual or perennial herbs or rarely spiny subshrubs, and are often caespitose or matforming. The leaves are exstipulate, suborbicular to linear or setaceous. The inflorescences are terminal or occasionally also axillary, and are composed of 3-50-flowered cymes (though sometimes as few as one), cymose panicles, or clusters. They have five sepals that are free, herbaceous, scarious, or coriaceous, with a single midrib or with the midrib more prominent than the lateral veins, though it will sometimes have an equally three-veined midrib. They have five petals that are white, entire or rarely subemarginate. They have ten stamens, of which the outer five have obsolete, single, or bipartite basal glands, and three styles. The capsule opening has six teeth or occasionally six valves. The seeds are exstrophiolate and black, but are sometimes reddish. It is very difficult to distinguish the species of *Arenaria* from each other on the basis of their external morphological characteristics (McNeill, 1967). It has also been reported that the taxa in *Arenaria* appear to have similar morphological features at first glance, and their distinctive characteristics can only be observed through detailed analysis (Weaver, 1970).

A revision of *Arenaria* in Turkey was carried out by McNeill (1967), and published in the *Flora of Turkey and the East Aegean Islands* vol. 2 (Davis, 1967). This reports that there are 54 taxa in the flora of Turkey that belong to genus *Arenaria*. Further studies conducted by several researchers have described eight new taxa in this genus (Celebioglu & Favarger, 1989; Tan & Vural, 2000; Aytaç & Duman, 2004; Parolly & Eren, 2006). In another study, *A. yunus-emrei* Aytaç and Duman, which was one of the eight newly described new taxa, was proved to be the synonym of *A. angustifolia* McNeill (Parolly & Eren, 2006). As a result, the total number of the taxa belonging to *Arenaria* reached 65 taxa.

McNeill (1967) stated that there were 16 perennial taxa in Turkey that belong to subgenus *Arenaria*. Tan and Sorger (1987) described four new perennial species, *A. eliasiana* Kit Tan and Sorger, *A. sivasica* Kit Tan and Sorger, *A. mons-cragus* Kit Tan and Sorger, and *A. angustifolioides* Kit Tan and Sorger, all of which belong to this group. As a result, perennial taxa in subgen. *Arenaria* reached 20. Of these, 14 were endemic with an endemism rate of 70% (Davis *et al.*, 1988; Güner *et al.*, 2000).

Classifications that rely exclusively on external morphological characters are not always sufficient to distinguish taxa. In some cases, other characters of the taxa are also needed for an accurate identification. Palynology is an important field of study that is frequently used to solve problems in systematic classification. In this study, the pollen morphology of 16 perennial taxa belonging to the subgen. *Arenaria* growing in Turkey were examined. This study aimed to contribute palynologically to the taxonomic problems of this group.

Materials and Methods

Specimen collection: In order to obtain herbarium specimens (with flowers) of the 20 perennial taxa belonging to subgen. *Arenaria*, we contacted experts studying these taxa. We obtained herbarium specimens (with flowers) of the 16 taxa belonging to subgen. *Arenaria* from the herbaria of HUB (Department of Biology, Hacettepe University, Ankara, Turkey), GAZI (Faculty of Science, Gazi University, Ankara, Turkey), ISTE (Faculty of Pharmacy, Istanbul University, Istanbul, Turkey), ISTF (Faculty of Science, Istanbul University, Istanbul, Turkey), ISTO (Faculty of Forestry, Istanbul University, Istanbul, Turkey), and from the collection of Department of Biology, Bozok University (Yozgat, Turkey). We also contacted the directors/curators of herbaria outside Turkey in order to obtain herbarium specimens (with flowers) of the remaining five taxa, namely *A. eliasiana* Kit Tan & Sorger, *A. sivasica* Kit Tan & Sorger, *A. mons-cragus* Kit Tan & Sorger, *A. sipylea* Boiss., and *A. angustifolioides* Kit Tan & Sorger. However, these taxa could not be supplied, and thus we were unable to study their pollen morphology.

The pollen morphology of *A. yunus-emrei*, which was described as a new species by Aytaç & Duman (2004), but was considered to be a synonym of *A. angustifolia* by Parolly & Eren (2006), was also included in the present study. However, although *A. yunus-emrei* is no longer considered to be a separate species, we decided to include it in our analysis to test the validity of the current classification. Consequently, we examined the pollen morphology of 16 taxa, including *A. yunus-emrei*, in the present work.

List of voucher specimens is given in Table 1.

Palynological analyses: Pollen slides were prepared using the methods of Erdtman (1960). The pollen were measured minimum of 100 times until the Gaussian curves of their A and B axes; pore lengths (Plg) and pore widths (Plt); exine, sexine, and nexine thicknesses; pore numbers; and pore-to-pore distances were obtained. The mean, standard deviation, minimum and maximum values of these palynological characteristics using SPSS Statistics 23 software were evaluated, following the methods of Sokal & Rohlf (1969). Additionally, we evaluated data obtained from the study, such as A and B axes; Plg; Plt; exine, sexine, and nexine thicknesses; pore numbers; and pore-to-pore distances, using PAST 3 software for cluster analysis on the basis of the Euclidean distance.

Microphotographs of the pollen grains were taken with an Olympus E330 imaging system connected to CX41 light microscopy (LM).

Polleniferous material of the examined taxa was placed into centrifuge tubes for scanning electron microscopy (SEM). Distilled water was added to the tubes to soften the anthers and release of pollen, and they were then kept in a hot water-bath for 10 minutes. Each tube was stirred with a clean glass rod in order to ensure pollen release. Then, the mixture in the tube was strained using a wire mesh filter with 250 µm holes, and transferred to another centrifuge tube. These tubes were centrifuged for 20 minutes, and the distilled water was removed. Both 3% glutaraldehyde and 0.1 M 7.2 pH phosphate tampon solutions were used for pollen fixation. Then, 5 mL of this mixture was placed in each tube, and they were stored at room temperature overnight. After the fixation process, the tubes were washed three times with phosphate tampon, and centrifuged each time for 20 minutes. These tubes were left for 5 minutes each in a 25%, 50%, 75%, 90%, and 100% ethanol series, and were centrifuged for 20 minutes after each of these series. In this way, the water in the specimens was removed. After the ethanol from the final series was removed, the tubes were left upside down on a filter paper for 5 minutes so that any remaining ethanol would drain out of the tubes. The pollen-ethanol mixture precipitated on the bottom of the tubes was extracted with a glass Pasteur pipette and layered on stubs that had previously been coated with carbon tapes (Karcz, 2013). The stubs were left to dry overnight in a 25°C drying oven and then coated with gold-palladium mixture for 2 minutes by the central laboratory at METU (Middle East Technical University, Ankara, Turkey). The pollen grains were studied using QUANTA 400F field emission electron microscope. The exine ornamentation and structure were noted. In addition, microphotographs of the pollen grains were also taken.

The terminology of Erdtman (1969), Faegri & Iversen (1975), and Punt *et al.*, (2007) was used to describe the pollen grain morphology. We also used the evolutionary sequence specified in the *Flora of Turkey and the East Aegean Islands* (Davis, 1967) for the pollen descriptions of subgen. *Arenaria* taxa (Davis, 1967; Davis *et al.*, 1988; Güner *et al.*, 2000).

Table 1. Voucher specimens of subgen. *Arenaria* taxa examined in the present study.

Taxa	Collection site	Collection date	Collectors	Herbarium
A. antitaurica	B6 Kayseri, Sarız Kırkısrak Köyü, Salıngaç mevkisi, Binboğa dağları, kayalık alanlar, 2100 m, 382506 K-363946 D	16.07.2009	M. Koç-747, E. Hamzaoğlu & Budak	Bozok University
A. uninervia	C3 Antalya, Akseki, Çukurkay Yaylası, Eğerbeli kuzey yamaçları, kaya üzeri, 2200-2400 m	17.07.1997	A. Duran-2843	GAZI
A. kotschyana subsp. *kotschyana*	C5 Konya, Ereğli, Aydos Dağı, Delimahmutlu Otlak Tepe, nemli çayır, 1700 m	19.08.1978	S. Erik-3044	HUB
A. kotschyana subsp. *stenophylla*	A5 Amasya, Akdağ, Suluova, Seyfe Köyü, Ovacık Yaylası, Taşlıdağ mevkii, taşlı yamaç, 1400 m	27.07.2005	E. Hamzaoğlu-3792	Bozok University
A. tmolea	C3 Konya, Seydişehir-Derebucak arası, Rezebeli Geçidi sola zirve, kalker kayalık, 2180 m, 372655 K-0314202 D	14.07.2011	E. Hamzaoğlu-6141	Bozok University
A. balansae	C4 Karaman, Ermenek, Kazancı Kasabası, Kocaş mevkii, nemli taşlı alan, 1500 m	23.06.1988	H. Sümbül-3046	HUB
A. rotundifolia subsp. *rotundifolia*	A8 Artvin, Kozlu köyünden Avana Yaylası, alpin çayır, 2500 m, 413312 K-0413938 D	27.07.2010	E. Hamzaoğlu-6011	Bozok University
A. speluncarum	C4 Karaman, Ermenek Tekeçatı Yellibel, Kamışlı Dere, kalker mağara ağzı, 1530 m	29.09.1994	M. Vural-7263, M. Koyuncu & M. Ekici	GAZI
A. angustifolia	C4 Ermenek-Karaman, Tekeçatı, Kamışdere, tarla kenarı, 1400 m	26.08.1992	M. Vural-8151, M. Koyuncu, M. Ekici	GAZI
A. yunus-emrei	C4 Karaman, Ermenek-Hadim, Başyayla, kayalık, 1500 m	30.07.1998	A. Güner-12652 *et al.*	GAZI
A. filicaulis subsp. *filicaulis*	A5 Çorum, Ortaköy, İncesu, İncesu Kanyonu girişi, kalker kayalık, 620 m, 401582 K-352061 D	18.06.2005	E. Hamzaoğlu-3685	Bozok University
A. filicaulis. subsp. *graeca*	B2 Kütahya, Kütahya-Gediz, 21. Km., Göksuyu sağ kıyısı, kireç kayaları, 960 m	14.06.1954	H. Demiriz-1808	ISTF
A. deflexa. subsp. *deflexa*	C2 Muğla, Sandras Dağı, Beşparmak Tepesi, 1975 m	26.07.1977	E. Özhatay-19425	ISTO
A. deflexa subsp. *pubescens*	C3 Antalya, Kemer, Tahtalıdağ, Çukuryayla Ağla yaylası arası, kalkerli kuzey yamaç, *Cedrus libani* ormanı	23.08.1978	H. Peşmen-4101	HUB
A. deflexa subsp. *pseudofragillima*	C3 Antalya, Kemer, TV kulesi tepesi, *Cedrus libani* ormanı ve açıklıkları, kalkerli arazi, 1200-1700 m	14.06.1976	H. Peşmen-3919	HUB
A. deflexa subsp. *microsepala*	B1 İzmir, Ödemiş, Bozdağ kayak mevkii üstü, kayalık yamaçlar, 381923 K-0280713 D	17.06.2009	M. Koç-252, Hamzaoğlu & Ü. Budak	Bozok University

Results

Pollen morphology: Pollen morphology of subgen. *Arenaria* taxa is conformed to Caryophyllaceae family. Pollen grains of Subgen. *Arenaria* taxa are small (20.20-25.77 µm) to medium (26.10-29.10 µm) sized.

The main palynological features of the subgen. *Arenaria* taxa are summarized in Table 2, and Figs. 1-5 A-P.

Size, symmetry, and shape: The mean value of A axis length of the analyzed pollen grains was 20.36-29.10 µm, B axis length was 19.68-28.11 µm, and A/B ratio was between 1.02-1.04. The shape of the pollen grains was found to be radial symmetrical or prolate spheroidal. The results obtained from morphological measurements showed that *A. balansae* Boiss. had the smallest pollen grains, while *A. filicaulis* subsp. *filicaulis* Fenzl had the largest pollen grains (Table 2).

Apertures: The pollen grains were pantoporate (periporate), and their pore numbers varied between five and twenty-three. The pore-to-pore distance was 4.99-9.95 µm. The Plg were 2.42-4.31 µm, Plt were 2.16-3.79 µm, and the Plg/Plt ratios varied between 1.10-1.29 (Table 2). The pores were circle-shaped with their outer lines mostly apparent, and were clean-cut and orderly arrayed. They were operculate that showed microechinate ornamentation (Figs. 1-5 A-P).

Exine: The exine structure was subtectate, and the average exine thickness was 1.93-2.77 µm (Table 2). The sexine was found to be thicker than the nexine. Exine ornamentation was microechinate perforated, and the microspinules on the exine surface were generally unevenly distributed (Figs. 1-5 A-P).

Numerical analysis of the palynological character states: Cluster analysis based on the results of the pollen measurements of 16 taxa of subgen. *Arenaria* using the Euclidean distance was done (Fig. 6). Dendrogram obtained showed that the 16 taxa included in this study clustered in to four main groups. The discrete clusters formed by these taxa found in these groups due to their morphological similarities proved that data from pollen were very useful for revealing the systematic relationships of the taxa.

In the first group, *A. antitaurica* McNeill, *A. uninervia* McNeill, *A. kotschyana* subsp. *kotschyana* Fenzl, *A. kotschyana* subsp. *stenophylla* (Bornm.) McNeill, and *A. tmolea* Boiss. clustered together. These taxa were also listed as similar species or subspecies to each other in the *Flora of Turkey and the East Aegean Islands* (Davis, 1967). In these taxa, we found that the A/B ratio was 1.03-1.04, the mean a axis lengths were 24.58-27.80 µm, the mean B axis lengths were 23.85-26.85 µm, and their pore numbers varied between nine and twelve (Table 2).

In the second group, *A. balansae* and *A. rotundifolia* subsp. *rotundifolia* Bieb. clustered together. These taxa had an A/B ratio of 1.03, mean A axis lengths of 20.36-21.87 µm, mean B axis lengths of 19.68-21.16 µm, and their pore numbers varied between 14 and 15 (Table 2). These two taxa were again considered to be similar species or subspecies in the *Flora of Turkey and the East Aegean Islands* (Davis, 1967).

In the third group, *A. speluncarum* McNeill, *A. angustifolia* McNeill, *A. yunus-emrei*, *A. filicaulis* subsp. *filicaulis*, and *A. filicaulis* subsp. *graeca* (Boiss.) McNeill formed a cluster. These had an A/B ratio of 1.02-1.04, mean A axis lengths of 26.33-29.10 µm, mean B axis lengths of 25.32-28.11 µm, and their pore number varied between 16 and 20 (Table 2). Furthermore, in this group, the cluster analysis of *A. yunus-emrei*, identified as a synonym of *A. angustifolia*, allowed the comparison of these taxa side by side, which supported the currently accepted taxonomy.

In the fourth group, *A. deflexa* subsp. *deflexa* Dec., *A. deflexa* subsp. *pubescens* McNeill, *A. deflexa* subsp. *pseudofragillima* McNeill, and *A. deflexa* subsp. *microsepala* McNeill clustered together. They had an A/B ratio of 1.02-1.04, mean A axis lengths of 20.97-24.41 µm, mean B axis lengths of 20.20-23.94 µm, and their pore numbers varied between 15 and 18 (Table 2). Since these four taxa were subspecies of *A. deflexa*, it was expected that they would be clustered in the same group.

Discussion

In the present study, we examined the pollen morphology of the taxa belonging to subgen. *Arenaria* in Turkey in detail, and determined the palynological characteristics of this group.

It has been reported in the literature that pollen characteristics of the Caryophyllaceae family are distinctive enough not only at the family but also at the generic and the specific level (Erdtman, 1969). A number of researchers have found that pollen axes within the family vary between 20.00-65.00 µm (Vishnu-Mittre & Gupta, 1964; Melzheimer, 1975; Iwarsson, 1977; Volponi, 1987; Al-Eisawi, 1989; Bittrich, 1993; Parent & Richard, 1993; Yıldız, 1996; Yıldız, 2001; Yıldız, 2005; Yıldız, 2006; Yıldız *et al.*, 2008; Ataşlar *et al.*, 2009; Aktaş *et al.*, 2010; Poyraz & Ataşlar, 2010). According to these studies, the exine of Caryophyllaceae family taxa is tectate/pertectate, and its tectum is mostly perforated. In addition, the ornamentation types of the pollen of this family are echinate, but are sometimes reticulate. The pollen type is pantoporate in general, but is tricolpate in some genera, or tricolporate in the genus *Polycarpaes*.

The PalDat palynology database, which is run by AutPal–the Society for the Promotion of Palynological Research (University of Vienna, Vienna, Austria), lists the palynological features of 25 taxa belonging to Caryophyllaceae family (Buchner *et al.*, 2017). These researchers found that the pollen grains of taxa belonging to the Caryophyllaceae family are radially symmetrical, isopolar, pantoporate, and spheroid, and they all have opercula. In addition, it was reported that Caryophyllaceae pollen grains generally have microechinate perforate ornamentations. The results of the present study are in agreement with the Buchner *et al.*, (2017) regarding the palynological characteristics of taxa in the Caryophyllaceae family.

Table 2. Pollen morphological data of subgen. *Arenaria* taxa.

Taxa	Pollen shape	A/B	A axis (µm)		B axis (µm)		Exine (µm)		Sexine (µm)		Nexine (µm)		Plg (µm)		Plt (µm)		Plg/Plt	Distance between pores (µm)		Pore number	
			M (S)	Min-Max	M (S)	Min-Max	M (S)	Min-Max	M (S)	Min-Max	M (S)	Min-Max	M (S)	Min-Max	M (S)	Min-Max		M (S)	Min-Max	M (S)	Min-Max
A. antitaurica	prolate spheroidal	1.04	26.92 (±1.39)	19.00-30.00	25.77 (±1.46)	23.00-29.00	2.19 (±0.40)	2.00-3.00	1.10 (±0.29)	1.00-2.00	1.09 (±0.30)	1.00-2.00	4.31 (±0.84)	2.00-6.00	3.34 (±0.89)	2.00-5.00	1.29	9.95 (±0.82)	5.00-15.00	9 (±1.44)	5-12
A. uninervia	prolate spheroidal	1.03	24.58 (±1.17)	22.00-27.00	23.87 (±1.21)	21.00-27.00	2.70 (±0.46)	2.00-3.00	1.41 (±0.46)	1.00-2.00	1.29 (±0.49)	1.00-2.00	3.94 (±0.69)	2.00-5.00	3.46 (±0.64)	2.00-5.00	1.14	7.73 (±1.30)	5.00-10.00	11 (±1.26)	8-14
A. kotschyana subsp. *kotschyana*	prolate spheroidal	1.03	25.30 (±1.49)	21.00-28.00	24.58 (±1.48)	21.00-28.00	2.22 (±0.42)	2.00-3.00	1.22 (±0.42)	1.00-2.00	1.00 (±0.00)	1.00-1.00	3.33 (±0.62)	2.00-5.00	2.77 (±0.60)	2.00-5.00	1.20	8.09 (±1.30)	5.00-11.00	12 (±1.07)	10-15
A. kotschyana subsp. *stenophylla*	prolate spheroidal	1.04	24.84 (±1.54)	21.00-30.00	23.85 (±1.45)	19.00-28.00	2.45 (±0.41)	2.00-3.00	1.42 (±0.40)	0.50-2.00	1.03 (±0.19)	0.50-2.00	2.98 (±0.83)	2.00-6.00	2.58 (±0.65)	2.00-5.00	1.16	7.81 (±1.26)	5.00-11.00	11 (±1.28)	9-14
A. tmolea	prolate spheroidal	1.04	27.80 (±1.56)	24.00-31.00	26.85 (±1.54)	22.00-30.00	2.42 (±0.49)	2.00-3.00	1.28 (±0.45)	1.00-2.00	1.14 (±0.35)	1.00-2.00	4.01 (±0.76)	3.00-6.00	3.41 (±0.74)	2.00-5.00	1.18	9.09 (±1.07)	7.00-11.00	11 (±0.86)	9-13
A. balansae	prolate spheroidal	1.03	20.36 (±1.20)	17.00-23.00	19.68 (±1.18)	16.00-22.00	1.93 (±0.20)	1.50-3.00	1.05 (±0.22)	0.50-1.00	0.88 (±0.17)	1.00-2.00	3.39 (±0.68)	2.00-5.00	2.86 (±0.65)	2.00-4.00	1.19	5.21 (±0.69)	4.00-7.00	14 (±1.04)	12-16
A. rotundifolia subsp. *rotundifolia*	prolate spheroidal	1.03	21.87 (±1.41)	19.00-26.00	21.16 (±1.33)	18.00-25.00	2.17 (±0.35)	1.50-3.00	1.20 (±0.30)	0.50-2.00	0.97 (±0.35)	0.50-2.00	2.42 (±0.55)	2.00-4.00	2.16 (±0.37)	2.00-3.00	1.12	5.84 (±0.07)	4.00-8.00	15 (±1.59)	10-19
A. speluncarum	prolate spheroidal	1.04	26.44 (±1.53)	23.00-31.00	25.32 (±1.48)	22.00-29.00	2.77 (±0.51)	2.00-4.00	1.73 (±0.45)	1.00-2.00	1.04 (±0.20)	1.00-2.00	2.84 (±0.56)	2.00-4.00	2.58 (±0.55)	2.00-4.00	1.10	5.65 (±0.88)	4.00-8.00	16 (±1.40)	14-19
A. angustifolia	prolate spheroidal	1.02	26.33 (±1.50)	23.00-30.00	25.79 (±1.62)	21.00-30.00	2.64 (±0.53)	2.00-4.00	1.62 (±0.53)	1.00-3.00	1.02 (±0.14)	1.00-2.00	3.07 (±0.75)	2.00-5.00	2.75 (±0.70)	2.00-4.00	1.10	4.99 (±0.69)	3.00-7.00	20 (±1.53)	15-23
A. yunus-emrei	prolate spheroidal	1.04	26.40 (±1.32)	23.00-30.00	25.42 (±1.34)	22.00-29.00	2.62 (±0.49)	2.00-3.00	1.60 (±0.49)	1.00-2.00	1.02 (±0.14)	1.00-2.00	4.25 (±0.63)	3.00-5.00	3.79 (±0.61)	2.00-5.00	1.12	5.03 (±0.67)	4.00-7.00	20 (±1.40)	17-23
A. filicaulis subsp. *filicaulis*	prolate spheroidal	1.04	29.10 (±1.78)	25.00-33.00	28.11 (±1.66)	25.00-32.00	2.44 (±0.50)	2.00-3.00	1.41 (±0.49)	1.00-2.00	1.03 (±0.17)	1.00-2.00	3.52 (±0.81)	2.00-5.00	3.08 (±0.77)	2.00-5.00	1.14	7.51 (±1.11)	5.00-10.00	17 (±1.16)	14-20
A. filicaulis subsp. *graeca*	prolate spheroidal	1.02	26.57 (±1.42)	24.00-31.00	26.10 (±1.49)	22.00-31.00	2.33 (±0.41)	2.00-3.00	1.19 (±0.32)	0.50-2.00	1.14 (±0.37)	1.00-2.00	4.09 (±0.65)	2.00-5.00	3.72 (±0.70)	2.00-5.00	1.10	5.74 (±0.82)	4.00-9.00	16 (±1.31)	13-19
A. deflexa subsp. *deflexa*	prolate spheroidal	1.04	21.02 (±1.15)	19.00-24.00	20.20 (±1.09)	18.00-22.00	2.04 (±0.15)	1.50-2.50	1.05 (±0.18)	0.50-1.50	0.99 (±0.19)	0.50-1.50	3.36 (±0.76)	2.00-5.00	2.88 (±0.73)	2.00-5.00	1.17	5.59 (±0.83)	4.00-8.00	15 (±1.14)	12-17
A. deflexa subsp. *pubescens*	prolate spheroidal	1.03	20.97 (±1.27)	16.00-23.00	20.38 (±1.17)	14.00-23.00	2.10 (±0.23)	2.00-3.00	1.07 (±0.21)	1.00-2.00	1.03 (±0.15)	0.50-1.50	3.52 (±0.64)	2.00-5.00	2.94 (±0.68)	2.00-4.00	1.20	5.22 (±0.92)	3.00-8.00	15 (±1.23)	12-18
A. deflexa subsp. *pseudofragillima*	prolate spheroidal	1.03	22.18 (±1.04)	20.00-25.00	21.46 (±1.05)	19.00-25.00	2.07 (±0.26)	2.00-3.00	1.07 (±0.26)	1.00-2.00	1.00 (±0.00)	1.00-1.00	3.40 (±0.78)	2.00-5.00	3.04 (±0.68)	2.00-5.00	1.12	5.99 (±0.80)	4.00-8.00	15 (±1.17)	12-17
A. deflexa subsp. *microsepala*	prolate spheroidal	1.02	24.41 (±0.92)	22.00-26.00	23.94 (±1.00)	21.00-26.00	2.02 (±0.14)	2.00-3.00	1.01 (±0.10)	1.00-2.00	1.01 (±0.10)	1.00-2.00	2.79 (±0.61)	2.00-4.00	2.47 (±0.54)	2.00-4.00	1.13	5.27 (±0.76)	4.00-7.00	18 (±1.12)	15-21

M: Mean values, S: Standard deviation, Min-Max, Plg.: Pore length, Plt.: Pore width.

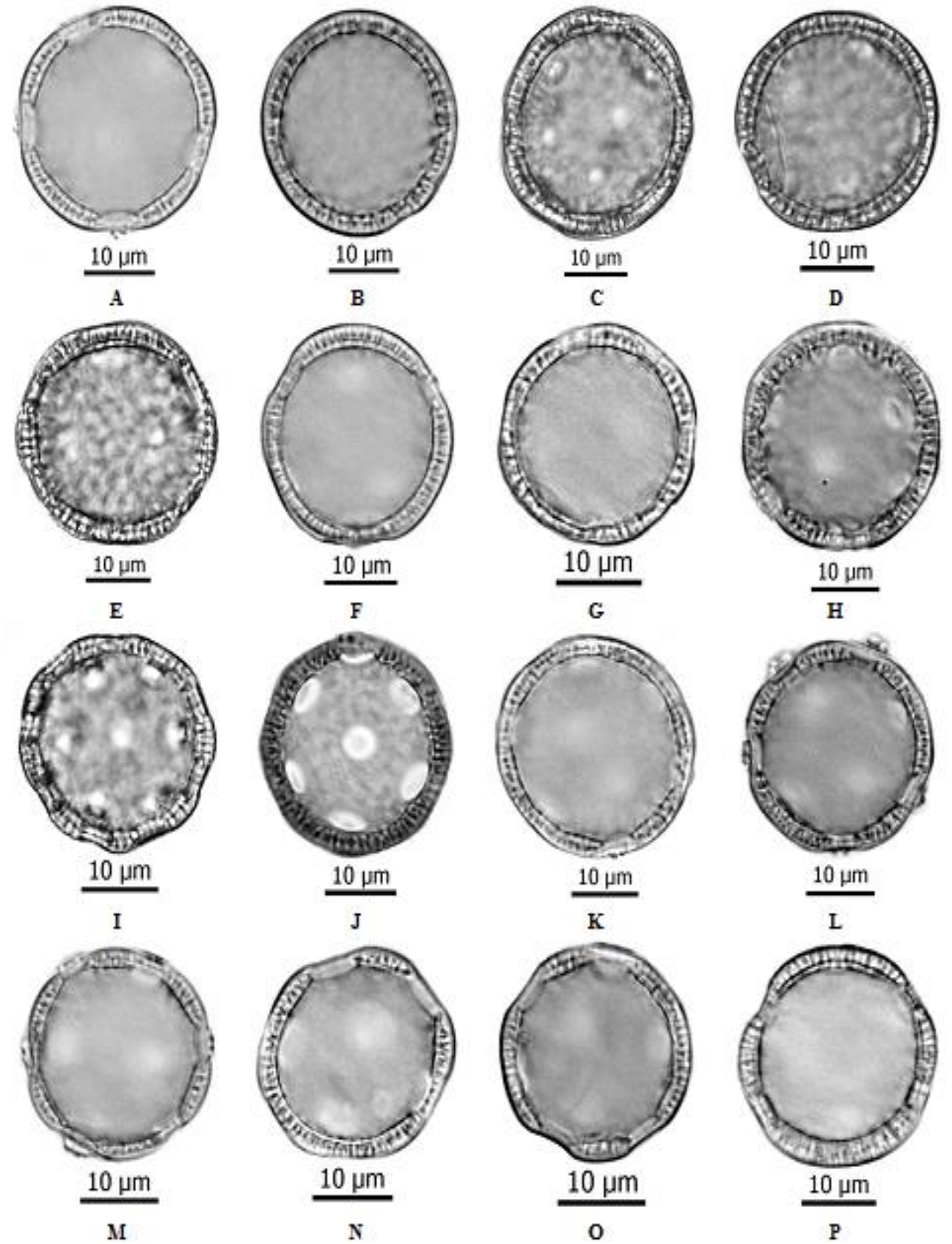

Fig. 1. LM photos of representative subgen. *Arenaria* taxa (optical section: LO analysis): (A) *A. antitaurica*, (B) *A. uninervia*, (C) *A. kotschyana* subsp. *kotschyana*, (D) *A. kotschyana* subsp. *stenophylla*, (E) *A. tmolea*, (F) *A. balansae*, (G) *A. rotundifolia* subsp. *rotundifolia*, (H) *A. speluncarum*, (I) *A. angustifolia*, (J) *A. yunus-emrei*, (K) *A. filicaulis* subsp. *filicaulis*, (L) *A. filicaulis* subsp. *graeca*, (M) *A. deflexa* subsp. *deflexa*, (N) *A. deflexa* subsp. *pubescens*, (O) *A. deflexa* subsp. *pseudofragillima*, (P) *A. deflexa* subsp. *microsepala*.

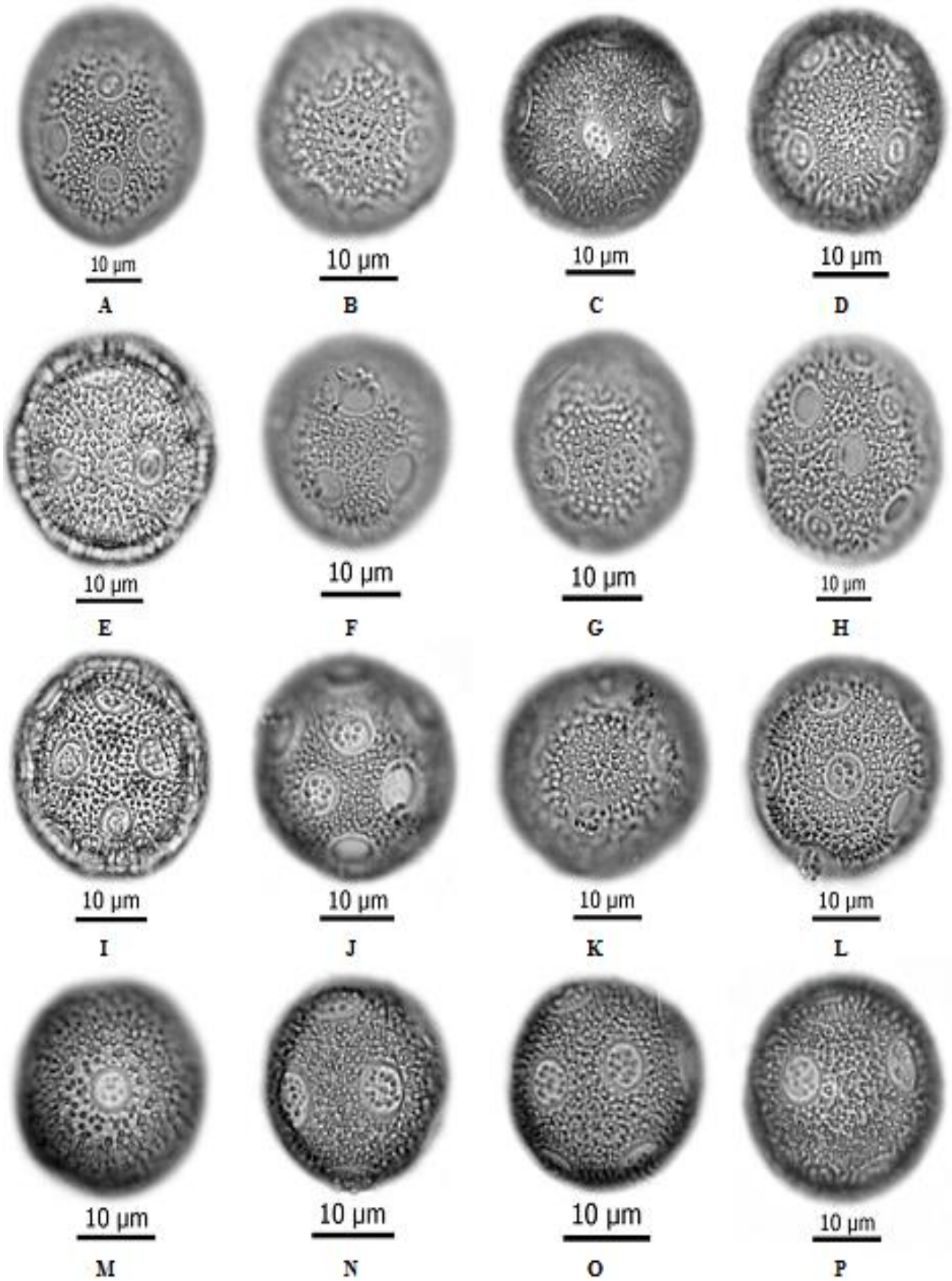

Fig. 2. LM photos of representative subgen. *Arenaria* taxa (ornamentation): (A) *A. antitaurica*, (B) *A. uninervia*, (C) *A. kotschyana* subsp. *kotschyana*, (D) *A. kotschyana* subsp. *stenophylla*, (E) *A. tmolea*, (F) *A. balansae*, (G) *A. rotundifolia* subsp. *rotundifolia*, (H) *A. speluncarum*, (I) *A. angustifolia*, (J) *A. yunus-emrei*, (K) *A. filicaulis* subsp. *filicaulis*, (L) *A. filicaulis* subsp. *graeca*, (M) *A. deflexa* subsp. *deflexa*, (N) *A. deflexa* subsp. *pubescens*, (O) *A. deflexa* subsp. *pseudofragillima*, (P) *A. deflexa* subsp. *microsepala*.

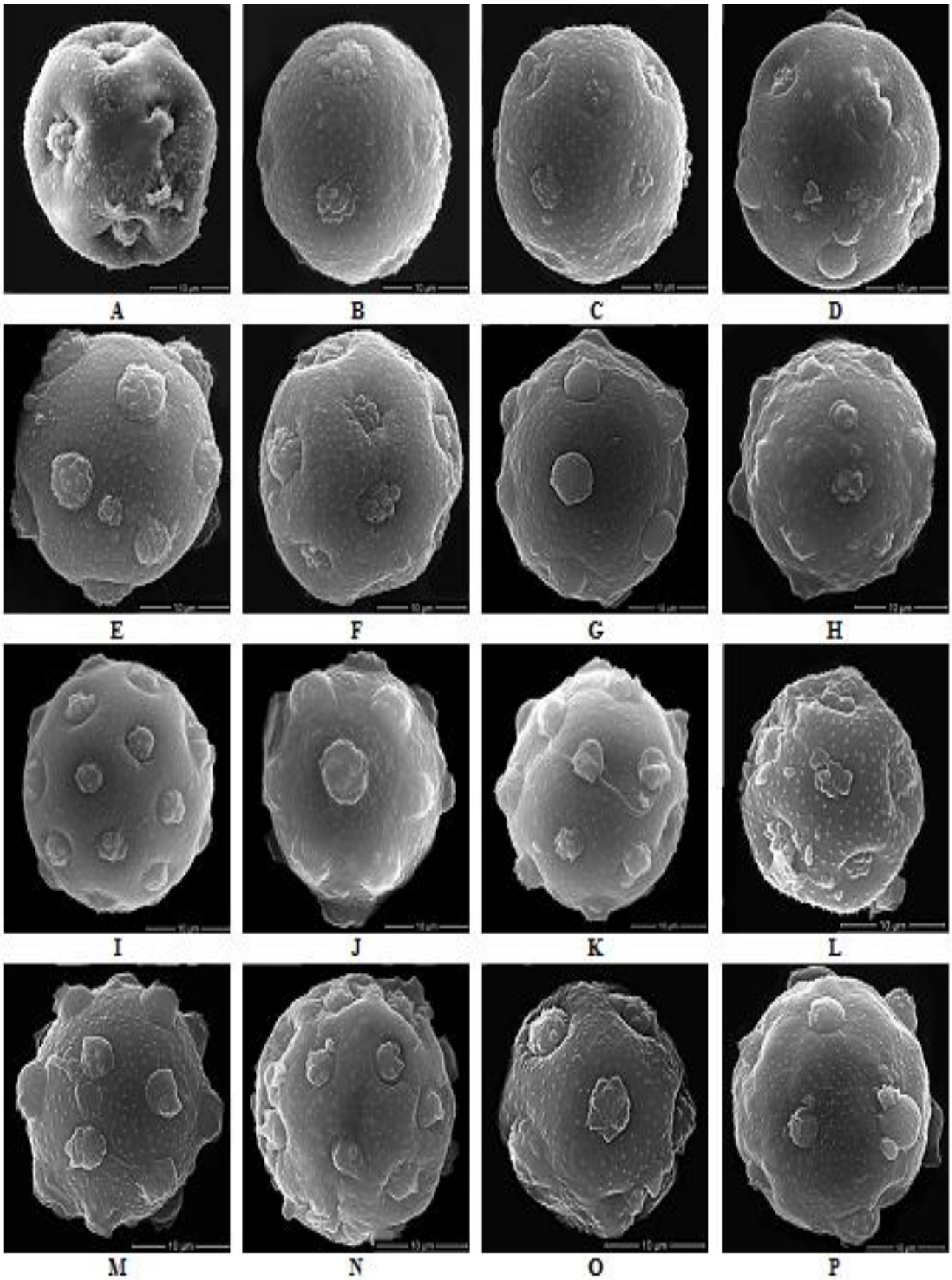

Fig. 3. SEM micrographs of pollen grains in the subgen. *Arenaria* taxa examined (general views): (A) *A. antitaurica,* (B) *A. uninervia,* (C) *A. kotschyana* subsp. *kotschyana,* (D) *A. kotschyana* subsp. *stenophylla,* (E) *A. tmolea,* (F) *A. balansae,* (G) *A. rotundifolia* subsp. *rotundifolia,* (H) *A. speluncarum,* (I) *A. angustifolia,* (J) *A. yunus-emrei,* (K) *A. filicaulis* subsp. *filicaulis,* (L) *A. filicaulis* subsp. *graeca,* (M) *A. deflexa* subsp. *deflexa,* (N) *A. deflexa* subsp. *pubescens,* (O) *A. deflexa* subsp. *pseudofragillima,* (P) *A. deflexa* subsp. *microsepala.*

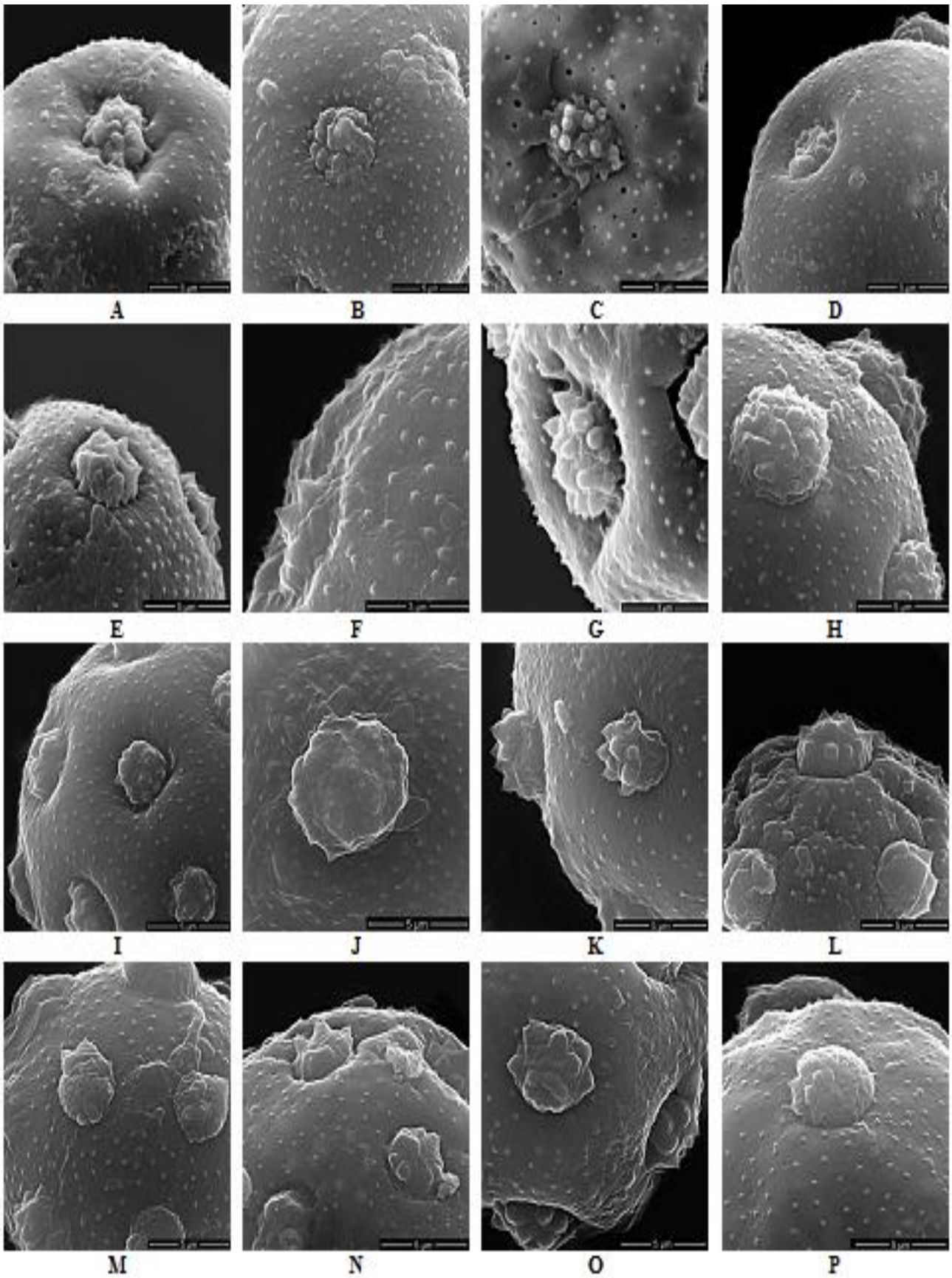

Fig. 4. SEM micrographs of pollen grains in the subgen. *Arenaria* taxa examined (pore and operculum): (A) *A. antitaurica*, (B) *A. uninervia*, (C) *A. kotschyana* subsp. *kotschyana*, (D) *A. kotschyana* subsp. *stenophylla*, (E) *A. tmolea*, (F) *A. balansae*, (G) *A. rotundifolia* subsp. *rotundifolia*, (H) *A. speluncarum*, (I) *A. angustifolia*, (J) *A. yunus-emrei*, (K) *A. filicaulis* subsp. *filicaulis*, (L) *A. filicaulis* subsp. *graeca*, (M) *A. deflexa* subsp. *deflexa*, (N) *A. deflexa* subsp. *pubescens*, (O) *A. deflexa* subsp. *pseudofragillima*, (P) *A. deflexa* subsp. *microsepala.*

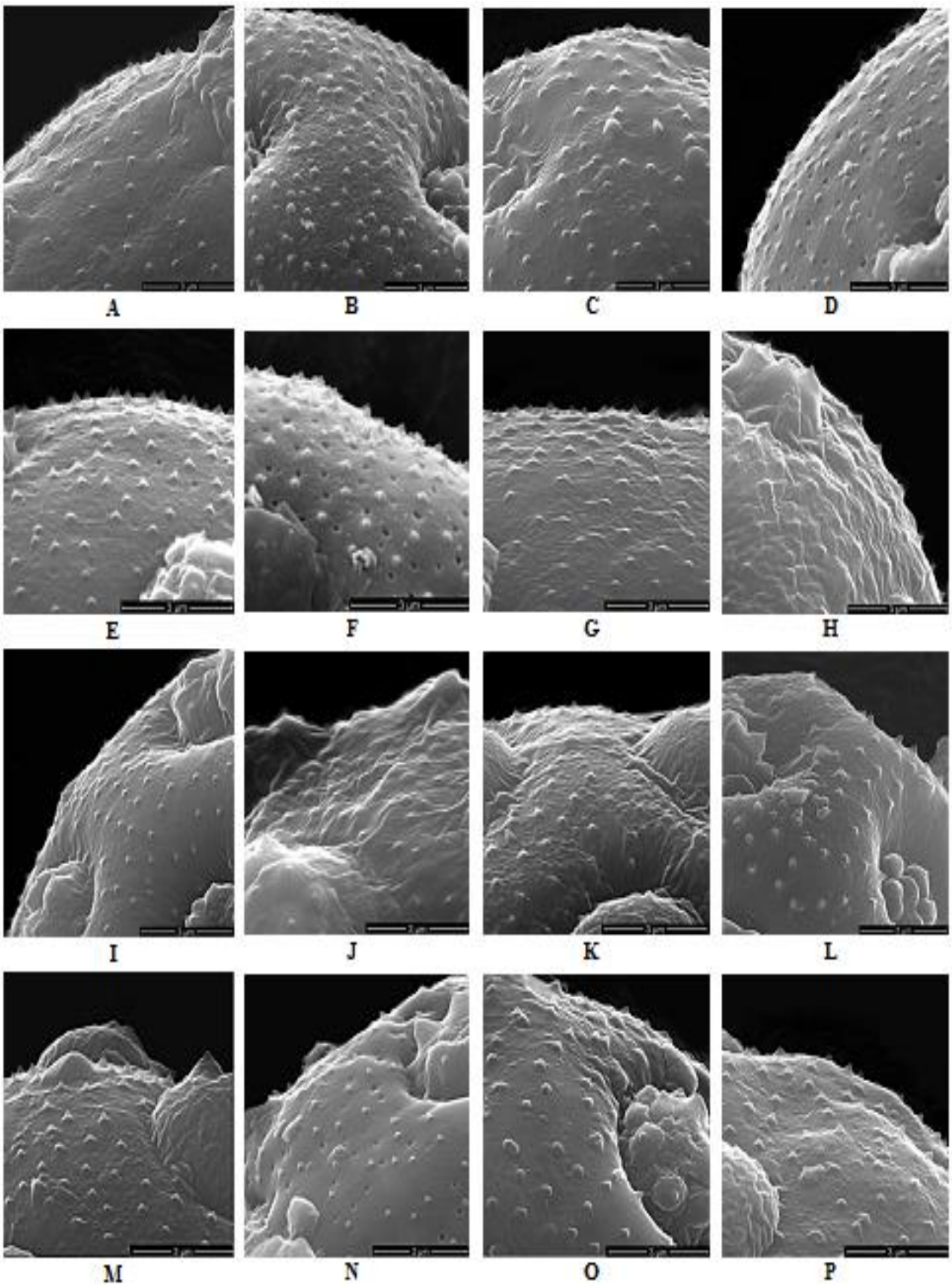

Fig. 5. SEM micrographs of pollen grains in the subgen. *Arenaria* taxa examined (surface details): (A) *A. antitaurica*, (B) *A. uninervia*, (C) *A. kotschyana* subsp. *kotschyana*, (D) *A. kotschyana* subsp. *stenophylla*, (E) *A. tmolea*, (F) *A. balansae*, (G) *A. rotundifolia* subsp. *rotundifolia*, (H) *A. speluncarum*, (I) *A. angustifolia*, (J) *A. yunus-emrei*, (K) *A. filicaulis* subsp. *filicaulis*, (L) *A. filicaulis* subsp. *graeca*, (M) *A. deflexa* subsp. *deflexa*, (N) *A. deflexa* subsp. *pubescens*, (O) *A. deflexa* subsp. *pseudofragillima*, (P) *A. deflexa* subsp. *microsepala.*

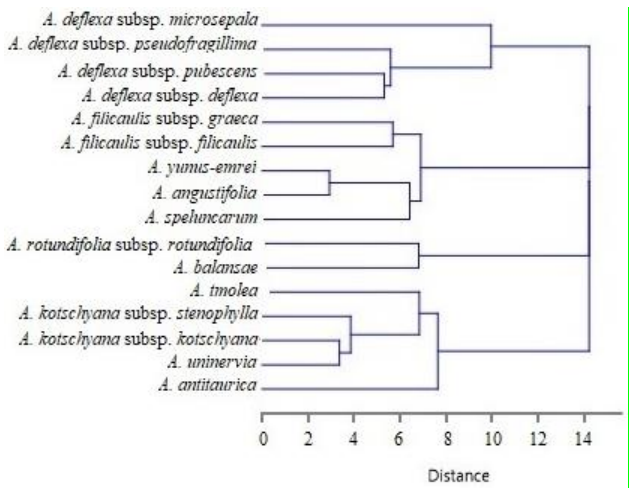

Fig. 6. Dendrogram of subgen. *Arenaria* taxa, constructed on the basis of the shortest Euclidean distances.

In her study on the Caryophyllaceae genera present in Argentina, Volponi (1987) analyzed the pollen morphologies of seven *Arenaria* species, *A. achalensis* Griseb, *A. bisulca* (Bartl.) Fenzl ex Rohrb., *A. catamarcensis* Pax, *A. lanuginosa* (Michx.) Rohrb., *A. rivularis* Phil, *A. serpens* H.B.K., and *A. serpyllifolia* L., using LM and SEM. Of these taxa, only *A. serpyllifolia* is found in Turkey. In that study, all of the pollen belonging to *Arenaria* taxa were described as spheroid-polyhedral. It was also reported that the exine structure was subtectate, its ornamentation was perforated and spinuled, the pollen grains were pantoporate (with 13-24 pores), the pores were operculate, and that the operculum surface was covered with conical bumps. Volponi (1987) found that pollen axes were 26.40-44.80 µm, and exine thickness varied between 1.60-3.20 µm.

In another study, Yıldız (2001) investigated the pollen morphology of 45 taxa belonging to Caryophyllaceae family using LM and SEM. The pollen grains of four of these taxa (*A. serpyllifolia*, *A. ledebouriana* var. *ledebouriana* Fenzl, *Minuartia juniperina* (L.) Marie et Petitm, and *M. verna* (L.) Hiern) were described as similar to *Arenaria* pollen grains. Yıldız (2001) reported that the pollen grains were tectate and microperforate, with a spheroid or pantoporate pollen type, and a pore number ranged between nine and twenty-one. He reported that the pores were operculate with spinules on their surfaces. The mean value of pollen axes was 27.83-29.72 µm, and their exine thickness was 2.14-2.89 µm.

The findings of our study are generally in agreement with the findings of the studies carried out by Volponi (1987) and Yıldız (2001). Yıldız (2001) asserted that the exine structure was tectate, and the exine ornamentation was microperforate. However, since the term microperforate refers to holes that are smaller than 1.00 µm, is not an appropriate term to be used in this case. The spinules on the surface of the pollen grains were also ignored while describing the exine ornamentation. Volponi (1987) described the shape of *Arenaria* pollen grains as spheroid-polyhedral. It is possible that the pollen grains might have become deformed during SEM analyses, and acquired this shape as a result. We speculate that Volponi might also have overlooked this potential deformation.

In the present study, the pollen of *A. kotschyana* subsp. *kotschyana* and *A. kotschyana* subsp. *stenophylla* were analyzed in detail, and it was found that they had very similar palynological characteristics (Table 2). A and B axes lengths were found to be within the same value range. The results showed that the observed morphological similarities in both subspecies could also be seen in their pollen.

A. yunus-emrei, which was described by Aytaç & Duman (2004) as a new species, was determined to be as a synonym of *A. angustifolia* by Parolly & Eren (2006). In our work, we studied the pollen of *A. yunus-emrei* alongside the pollen of *A. angustifolia.* We found that pollen of both specimens had similar pollen grains (Fig. 6). Hence, the taxonomic decision that Parolly & Eren (2006) made has been supported palynogically.

Another significant finding in the present study is that the palynological characteristics of *A. filicaulis* subsp. *filicaulis*, and *A. filicaulis* subsp. *graeca* differed from each other (Table 2). Therefore, we suggest that these subspecies should be re-evaluated with regard to their taxonomy and chromosome numbers.

Carlström (1986) compared the morphology, seed micromorphology, and chromosome numbers of four subspecies of *A. deflexa*, *A. deflexa* subsp. *deflexa*, *A. deflexa* subsp. *pubescens*, *A. deflexa* subsp. *pseudofragillima*, and *A. deflexa* subsp. *microsepala*, all of which grow in the Aegean Region and the Southeast of Turkey. She reported that the taxa showed morphological differences, even they grow in the same habitat and that they had both diploid (2n=22) and tetraploid (4n=44) chromosome numbers. In our study, we evaluated the pollen of these four *A. deflexa* subspecies independently of each other. We found that the pollen grains of *A. deflexa* subsp. *deflexa*, *A. deflexa* subsp. *pubescens*, and *A. deflexa* subsp. *pseudofragillima* are very similar with regard to their characteristics, such as A and B axes length and pore number, while *A. deflexa* subsp. *microsepala* differs from the other subspecies in several characteristics including its A and B axes length and pore number (Table 2). The differences between these taxa are attributed to the diversity in chromosome numbers; however, further analysis of their taxonomical status is required in order to obtain more precise results.

The present work is the first study to analyze the pollen of the subgen. *Arenaria* in detail. This study was based on a different approach to the classification of the genus using palynological characters, to solve its taxonomical problems. The results of this study showed that all the taxa had monomorphic pollen grains, when general palynological characteristics were taken into consideration. We found that the pollen grains were stenopalynous in terms of their shape, aperture type, and ornamentation. However, there were remarkable differences between the taxa with regard to pollen axis lengths and pore numbers. When we assessed these results as a whole, we found that pollen grains of subgen. *Arenaria* taxa had sufficiently distinct properties to support the current taxonomy. The present study provides pollen morphological characteristics that hopefully will be useful for future systematic and phylogenetic analyses.

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References

Aktaş, K., Y. Altan, C. Özdemir, P. Baran and T. Garnatje. 2010. Comparative pollen morphology of Turkish species of *Petrorhagia* (Caryophyllaceae) and its systematic implications. *Biologia*, 65(3): 444-450.

Al-Eisawi, D. 1989. Pollen morphology of Caryophyllaceae in Jordan. *Mitt. Bot. Staatssamml. Münch.*, 28: 599-614.

Ataşlar, E., I. Potoğlu Erkara and S. Tokur. 2009. Pollen morphology of some *Gypsophila* L. (Caryophyllaceae) species and its taxonomic value. *Turk. J. Bot.*, 33: 335-351.

Aytaç, Z. and H. Duman. 2004. Six new taxa (Caryophyllaceae) from Turkey. *Ann. Bot. Fennici*, 41: 213-221.

Bittrich, V. 1993. Caryophyllaceae. In: (Eds.): Kubitzki, K., J.G. Rohwer and V. Bittrich. *The families and genera of vascular plants, flowering plants, Dicotyledons, Magnoliid, Hamamelid and Caryophyllid families*. Springer, Berlin.

Buchner, R., A. Frosch-Radivo, H. Halbritter, M. Hesse, U. Schachner, S. Ulrich, M. Weber and R. Zetter. 2017. Palynological database. Society for the Promotion of Palynological Research in Austria. URL: http://www.paldat.org (May 2017).

Carlström, A. 1986. A revision of *Arenaria* sect. Orientales ser. Orientales incl. ser. *Deflexae* (Caryophyllaceae) in the Aegean and SW Turkey. *Willdenowia*, 15: 359-374.

Celebioglu, T. and C. Favarger. 1989. Two new species of Caryophyllaceae of Turkey. *Candollea*, 44: 329-336.

Davis, P.H. 1967. *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, Edinburgh, vol. 2: pp. 17-38.

Davis, P.H., R.R. Mill and K. Tan. 1988. *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, Edinburgh, vol. 10: pp. 65-67.

Erdtman, G. 1960. The acetolysis method. *A Revised Description Svensk Bo. Tidskr.*, 54: 561-564.

Erdtman, G. 1969. *Handbook of palynology, morphology, taxonomy, ecology, an introduction to the study of pollen grains and spores.* Hafner Pub. New York.

Faegri, K. and J. Iversen 1975. *Textbook of modern pollen analysis*. Hafner Press, Munksgaard, Copenhagen.

Fatemeh, F., M. Sheidai and M. Asadi. 2010. Cytological study the genus *Arenaria* L. (Caryophyllaceae). *Caryologia*, 63(2): 149-156.

Güner, A., N. Özhatay, T. Ekim and K.H.C. Başer. 2000. *Flora of Turkey and the East Aegean Islands.* Edinburgh University Press, Edinburgh, vol. 11: pp. 44-45.

Iwarsson, M. 1977. Pollen morphology of east African Caryophyllaceae. *Grana*, 16: 15-22.

Karcz, J. 2013. *Scanning electron microscopy in biology. University of Silesia, Faculty of Biology and Environmental Protection.* URL: www.semlab.us.edu.pl (May, 2013).

Li-hua, Z. 1996. On the geographical distribution of *Arenaria* L., Northwest Plateau Institute of Biology. *Academia Sinica*, 34: 229-241.

McNeill, J. 1967. *Arenaria* L. In: (Ed.): Davis, P.H. *Flora of Turkey and the East Aegean Islands.* Edinburgh University Press, Edinburgh, vol. 2: pp. 17-38.

Melzheimer, V. 1975. Pollen systematische untersuchungen in der Gattung *Silene* L. (Caryophyllaceae). *Can. J. Bot.*, 52: 1225-1231.

Parent, J. and P.J.H. Richard. 1993. Morphologie pollinuque des Caryophyllaceae du Que'bec nordique, der territoires adjacents et de l'archipel archique Canadien. *Can. J. Bot.*, 7(71): 887-905.

Parolly, G. and Ö. Eren. 2006. Contributions to the Flora of Turkey, 1. *Willdenowia*, 36: 823-844.

Poyraz, E.İ. and E. Ataşlar. 2010. Pollen and seed morphology of *Velezia* L. (Caryophyllaceae) genus in Turkey. *Turk. J. Bot.*, 34: 179-190.

Punt, W., P.P. Hoen, S. Blackmore, S. Nilsson and A. Le Thomas. 2007. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palynol.*, 143: 1-81.

Sokal, R.R. and F.J. Rohlf. 1969. *Biometry: the principles and practice of statistics in biological research*. Freeman & Company, San Francisco.

Tan, K. and F. Sorger. 1987. Even more new taxa from south and east Anatolia II. *Pl. Syst. Evol.*,155: 93-103.

Tan, K. and M. Vural. 2000. *Arenaria* L., *In*: Güner, A., N. Özhatay, T. Ekim and K.H.C. Başer (Eds.) *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, Edinburgh, vol. 11: pp. 44-45.

Vishnu-Mittre, S. and H.P. Gupta. 1964. Studies of Indian pollen grains. *Pollen et Spores*, 6: 99-111.

Volponi, C.R. 1987. Palynological study of Argentine species of *Arenaria* L. and *Stellaria* L. (Caryophyllaceae). *Candollea*, 42(2): 545-551.

Weaver, R.E.J. 1970. The Arenarias of the southeastern granitic flat-rocks. *Bulletin of the Torrey Botanical Club*, 97(1): 40-52.

Williams, F.N. 1897. A revision of the genus *Arenaria* Linn. J. Linn. Journal of the Linnean Society of London. *Botany*, 33: 326-437.

Yıldız, K. 1996. Pollen morphology of some *Silene* L. (Caryophyllaceae) taxa distributed in northwest Anatolia. *Turk. J. Bot.*, 20: 231-241.

Yıldız, K. 2001. Pollen morphology of Caryophyllaceae species from Turkey. *Pak. J. Bot.*, 33(4): 329-355.

Yıldız, K. 2005. A palynological investigation on *Silene* L. (Caryophyllaceae) species distributed in North Cyprus and West Anatolia. *CBÜ Fen Bil. Der.*, 1(2): 61-71.

Yıldız, K. 2006. Morphological and palynological investigation on *Silene gigantea* L. var. *gigantea* and *Silene behen* L. (Caryophyllaceae) distributed in western Anatolia and northern Cyprus. *Turk. J. Bot.*, 30: 105-119.

Yıldız, K., E. Minareci, A. Çırpıcı and M.Y. Dadandı. 2008. A karyotypic study on *Silene*, section *Siphonomorpha* species of Turkey. *Nordic J. Botany*, 26 (5-6): 368-374.

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