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Seed macro- and micromorphology in *Allium* (Amaryllidaceae) and its phylogenetic significance

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• **Background and Aims** Macro- and micromorphology of seeds are diagnostic characteristics of importance in delimiting taxa in *Allium* (Amaryllidaceae). However, there is no consensus on the phylogenetic significance of testa cell characteristics and whether they reflect the different evolutionary levels recognized in *Allium*.
• **Methods** Seeds of 95 species (98 samples) representing 14 subgenera and 58 sections of *Allium* were examined using scanning electron microscopy (SEM) for such traits as periclinal wall surface area of ten testa cells, distance between testa cells (macromorphology), testa cell shapes, and arrangement and structure of anticlinal and periclinal walls (micromorphology). The data matrix was subjected to cladistic analysis. The produced phylogenetic tree was examined against the molecular tree obtained from publically available ITS sequences.
• **Key Results** The periclinal wall surface area of ten testa cells and the distance between them, examined for the first time, were found useful for delimitation of species in *Allium*. Based on seed macro- and micromorphology, we present a taxonomic key and a hypothetical reconstruction of the migration routes during the early stages of evolution of *Allium*.
• **Conclusions** The ancestors of *Allium* originated in an area bounded by the Caucasus, Central Asia and Iran. The seed testa morphology-based evolutionary state of a species is determined by two parameters: the shape of the periclinal walls and curvature of the anticlinal walls.

Key words: Seed macromorphology, seed micromorphology, phylogenetic analysis, SEM, testa cell, *Allium* taxonomy.

INTRODUCTION

Allium (Amaryllidaceae), one of the largest genera in the family (Friesen *et al.*, 2006; Li *et al.*, 2010), comprises more than 1000 species classified into 15 subgenera and 85 sections (Friesen *et al.*, 2006; Fritsch *et al.*, 2010). The genus ranges throughout the northern hemisphere with the main centre of diversity in southwest and central Asia (Khassanov, 2018). Species of *Allium* are easily recognized by their membranous or fibrous bulb tunic, free or nearly free tepals, subgynobasic style and distinct odour and taste (Friesen *et al.*, 2006). The genus is economically significant since it contains many essential vegetable crops and ornamental and medicinal plants (Fritsch and Friesen, 2002).

Studies of the seed morphology of *Allium* have shown that not only the shape and size of the seeds but also the sculpturing of the testa are diverse among but not within species and are taxonomically important characteristics (von Bothmer, 1974; Pastor, 1981; Kruse, 1984, 198, 1988, 1994; Ilarslan and Koyuncu, 1997; Fritsch *et al.*, 2006; Neshati and Fritsch, 2009; Choi and Cota-Sanchez, 2010; Bednorz *et al.*, 2011; Celep *et al.*, 2012; Lin and Tan, 2017; Veiskarami *et al.*, 2018; Baasanmunkh *et al.*, 2021). Kruse (1984, 1986, 1988, 1994) showed that many seed testa character combinations in *Allium* are section- and species-specific and Fritsch *et al.* (2006) suggested that verrucae and anticlinal curvature type of the epidermal part of seeds may indicate a stage of evolutionary development. Later, Celep *et al.* (2012) in their study of micromorphological seed characteristics, such as the shape of the cells of the testa and the sculpturing of the periclinal walls, concluded that the seed coat patterns in *Allium* in general reflect phylogenetic trends, but cannot tell apart the basal and advanced evolutionary levels within the genus. In contrast, Lin and Tan (2017) distinguished three evolutionary developmental stages of testa cell characteristics (primitive, intermediate and advanced) and accordingly divided *Allium* into six distinct groups (*Tuberosum*, *Mongolicum*, *Strictum*, *Atrosanguineum*, *Platyspathum* and *Delicatulum*). Both the above studies (Celep *et al.*, 2012; Lin and Tan, 2017), however, had a very limited geographic coverage (Turkey and Xinjiang Provinces of China, respectively), and limited coverage of taxa within *Allium* (9 and

19 sections, respectively). The limited geographic and taxonomic scope of the above studies seriously affected the generality of their findings. This narrow geographic and taxonomic focus is common for all the *Allium* seed micromorphology studies conducted to date (Table 1). A study based on a much more comprehensive coverage of the genus taxonomy and geography is needed to resolve the question of the phylogenetic significance of testa cell characteristics and whether they reflect the different evolutionary levels recognized in *Allium*. In an attempt to fill the existing gaps, we conducted the widest to date coverage of *Allium*, comprising 95 species (98 samples; the choice of species was made to ensure that all taxa within the genus were represented) representing 58 sections and 14 subgenera.

For the past two decades, DNA markers (plastid DNA and nuclear ribosomal DNA) have been utilized to reveal evolutionary processes and taxonomic relationships within the entire genus *Allium* (Dubouzet and Shinoda, 1999; Fritsch and Friesen, 2002; Friesen *et al.*, 2006; Li *et al.*, 2010) or groups within it, such as subgenus *Amerallium* (Samoylov *et al*., 1995, 1999), subgenus *Melanocrommyum* (Dubouzet and Shinoda, 1998; Gurushidze *et al.*, 2008; Fritsch *et al.*, 2010) and section *Cepa* (Miller) Prokhanov (Yusupov *et al*., 2019, 2021; Liu *et al.*, 2020). In our study, we used publicly available (deposited in NCBI) sequences of the ITS region to construct a phylogenetic dendrogram of 72 species, 56 sections and 14 subgenera of *Allium* (the species were chosen from a list of 95 species available from the NCBI) representing three evolutionary lineages (EL1, EL2, EL3) (Friesen *et al.*, 2006; Li *et al.*, 2010; Xie *et al.*, 2020) to determine how closely the molecular- and seed morphology-based *Allium* phylogenies correspond.

Our goal was to describe seed macro- and micromorphology and adequately evaluate the diagnostic value of testa morphological characteristics in the entire genus *Allium* and to compare the results with existing hypotheses on the evolution and biogeography of the genus (Li *et al.*, 2010, 2016; Xie *et al*., 2020; Hauenschild *et al.*, 2017). More specific questions included the following: (1) Are various types of testa cell ornamentations indicative of the stage of evolutionary process in *Allium*? (2) Which factors resulted in different testa cell characteristics? (3) Do any previously uninvestigated testa cell characteristics have taxonomic value in *Allium*? To answer the last question, we studied two seed surface ultrastructure traits for the first time: periclinal wall surface area of ten testa cells and the distance between two adjacent testa cells.

MATERIALS AND METHODS

Taxon sampling and specimen examination

Ninety-five species representing 14 subgenera and 58 sections of *Allium* L. were investigated in this study. Information on taxonomic affiliation, collection data and deposition of voucher specimens are provided in Table 2. Specimens of *Allium* were examined from the National Herbarium of Uzbekistan, the Institute of Botany in Uzbekistan Academy of Sciences (TASH), the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN), the herbarium of the Institute of Botany, Chinese Academy of Sciences (PE) and the herbarium of the Botanical Institute of the National Herbarium of Georgia (TBI).

The seeds were examined under a Zeiss Sigma 300 (Zeiss, Oberkochen, Germany) scanning electron microscope (SEM) at 7 kV at Kunming Institute of Botany, Chinese Academy of Sciences, to determine that they were typical in size and maturity. Dried seed samples were then affixed to specimen tabs and then coated with platinum in a sputter coater. The seeds were then examined for morphometric measurement and observation using the SEM. For the average morphometric measurements, three to five seeds for each sample, depending on availability, were measured. The terminology of seed micromorphology follows Barthlott and Ehler (1977) and Celep *et al.* (2012) for explaining the seed surface elements. The seed measurements employed ImageJ software (Schneider *et al.*, 2012). The distance between testa cells and ten testa cells of the periclinal surface area was measured using the special plugin 3D viewer of the software ImageJ. There were ten repetitions of each measurement type per species.

The taxa investigated in this study were classified in accordance with Friesen *et al.* (2006), Li *et al.* (2010), Choi *et al.* (2012); Fritsch and Abbasi (2013) (subgenus *Melanocrommyum*),

Table 1. *List of the seed coat studies with the number of investigated taxa and the geographic range covered*

No.	Author	Subgenus	Section	Species	Locality
1	Kruse (1984)	7	10	35	Worldwide
2	Kruse (1986)	10	19	70	Worldwide
3	Kruse (1988)	8	15	53	Worldwide
4	Kruse (1994)	8	23	105	Worldwide
5	Fritsch *et al.* (2006)	1	15	88	Worldwide
6	Neshati and Fritsch (2009)	4	11	20	Iran
7	Choi and Cota-Sanchez (2010)	2	3	5	Canada
8	Bednorz *et al.* (2011)	5	5	8	Poland
9	Choi *et al.* (2012)	9	16	35	Asia and North America
10	Celep *et al.* (2012)	4	9	62	Turkey
11	Lin and Tan (2017)	7	19	38	China
12	Duman *et al.* (2017)	1	1	6	Turkey
13	Veiskarami *et al.* (2018)	2	6	23	Worldwide
14	Khorasani *et al.* (2020)	1	5	13	Central Asia
15	Baasanmunkh *et al.* (2020)	7	24	48	Central Asia
16	Baasanmunkh *et al.* (2021)	5	13	24	Worldwide
17	**Present study**	**14**	**58**	**95**	**Worldwide**

TABLE 2. *Seed testa micromorphology of 95 species (98 samples) of* Allium *(Amaryllidaceae)*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
1	**Subg. *Nectaroscordum* (Lindl.) Asch. & Graebn** Sect. *Nectaroscordum* (Lindl.) Gren. & Godr., *A. tripedale* Trautv.	3.22–1.8	1.79	(Broadly) ovoid (shrivelled)	0.012	0.007–0.01	4–7 edged, close.	5 edged	Straight to arched	Gradually concave from edge to centre, many intermediate verrucae on edge	Azerbaijan, Karabakh, TBI1034308	1A; 8A
2	**Subg. *Amerallium* Traub** Sect. *Arctoprasum* Kirschl. (*Ophioscorodon* (Wallr.) Endl.) *A. ursinum* L.	2.46–2.54	0.97	Broadly ovoid	0.012	0.003–0.005	4–7 edged, loose with reticulate tissue	5 edged	Straight to arched	Gradually concave from edge to centre, one large verruca in centre	Niederosterreich, Austria (PE 00156365)	1B; 8B
3	Sect. *Bromatorrhiza* Ekberg. *A. wallichii* Kunth	3.04–1.78	1.71	Ovoid (shrivelled)	0.022	0.001–0.002	4–8 edged, loose with reticulate tissue.	6 edged	Straight to arched	Flat to slightly concave from edge to centre, many small verrucae	Yunnan, China (KUN 0359135)	1C; 8C
4	Sect. *Caulorhizideum* Traub *A. validum* S. Watson	4.73–1.81	2.61	Narrowly ovoid	0.019	0.01–0.013	4–8 edged, close	6 edged	Straight to arched	Flat, many small verrucae	Washington, USA (PE00156367)	1D; 8D
5	Sect. *Amerallium* Traub *A. geyeri* S. Watson	2.13–1.45	1.47	Ovoid	0.011	0.002–0.007	5–7 edged, loose with clear meshes of reticulate tissue and with narrow connecting thread.	6 edged	Straight to arched	Flat to slightly convex, many small verrucae	Riverton, USA (PE 00114968)	1E; 8E
6	Sect. *Molium* G. Don ex Koch *A. moly* L.	2.86–2.32	1.22	Broadly ovoid (shrivelled)	0.022	0.007–0.017	4 to many edged, loose with reticulate tissue.	6 edged	Arched to S-type	Gradually convex, 1–4 intermediate verrucae on central area of epidermis	Tubingen, Germany (KUN 0358576)	1F; 8F
7	**Subg. *Caloscordum* (Herb.) R.M. Fritsch** Sect. *Caloscordum* (Herb.) Baker. *A. neriniflorum* (Herb.) Baker.	1.71–1.63	1.05	Broadly ovoid shrivelled)	0.012	0.0009–0.004	4–7 edged, loose with reticulate tissue.	6 edged	Straight to arched	Slightly convex, many small verrucae	Heilongjiang Province, China (PE 00115792)	1G; 8G
8	**Subg. *Anguinum* (G. Don ex Koch) N. Friesen** Sect. *Anguinum* G. Don ex Koch *A. prattii* C.H. Wright	2.20–1.66	1.32	Broadly ovoid	0.007	0.005–0.01	77	6 edged	Straight to Arched	Gradually concave from edge to centre, intermediate to large verrucae	Yunnan, China (KUN 0358650)	1H; 8H

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
9	**Subg. *Porphyroprason* (Ekberg) R.M. Fritsch** Sect. *Porphyroprasum* Ekberg. *A. oreophilum* C. A. Mey.	2.39–1.95	1.23	Broadly ovoid	0.015	0.0009–0.004	Many edged to oblong, loose with inserted pattern	Oblong	S-type	Convex, many small verrucae	Osh, Kyrgyzstan (TASH 65)	1I; 8I
10	**Subg. *Vvedenskya* (Kamelin) R.M. Fritsch** Sect. *Vvedenskya* Kamelin *A. kujukense* Vved.	2.05–1.56	1.31	Broadly ovoid	0.01	0.002–0.005	Many edged to oblong, loose with inserted pattern.	Oblong	Arched to S-type	Flat to convex, plane	Shymkent, Kazakhstan (TASH 2)	1J; 8J
11	**Subg. *Melanocrommyum* (Webb et Berth.) Rouy.** Sect. *Acanthoprason* Wendelbo *A. akaka* S.G. Gmel. ex Schult. & Schult.f.	2.69–1.92	1.40	(Broadly) ovoid (shrivelled)	0.016	0.0002–0.00068	Orbicular to oblong, loose with inserted pattern	Oblong	U- to Ω-type	Gradually convex, many intermediate on central area	Azerbaijan, TBI1032951	1K; 8K
12	Sect. *Megaloprason* Webb et Berth. *A. insufficiens* Vved.	1.66–1.16	1.43	(Broadly) ovoid (shrivelled)	0.02	0.001–0.002	Variably ovoid to elliptic, close with inserted pattern	Oblong	S- to U-type	Slightly convex, many small and intermediate verrucae	Kurgan Tyube, Tajikistan (TASH 1)	1L; 8L
13	*A. sarawschanicum* Regel	2.42–1.93	1.25	(Broadly) ovoid (shrivelled)	0.016	0.0005–0.001	Orbicular to elliptic, close with inserted pattern	Orbicular	U-type	Globular convex, 3–8 intermediate verrucae	Kashkadarya, Uzbekistan (TASH 110)	1M; 8M
14	Sect. *Miniprason* R.M. Fritsch *A. karataviense* Regel	3.78–2.68	1.41	(Broadly) ovoid (shrivelled)	0.014	0.001–0.004	Orbicular to elliptic, close with inserted pattern	Orbicular	U- to Ω-type	Globular convex, 1–5 intermediate verrucae on central area	Pskem, Tashkent, Uzbekistan (TASH) 10820151) TASH	1N; 8N
15	Sect. *Popovia* F.O. Khassanov et R.M. Fritsch *A. gypsaceum* Popov et Vved.	2.26–1.65	1.37	(Broadly) ovoid (shrivelled)	0.011	0.004–0.009	Oblong, close with inserted pattern	Oblong	Ω-type	Flat to convex, many intermediate and large verrucae, granulose	Surkhandarya, Uzbekistan (TASH 33 (1))	1O; 8O

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
16	Sect. *Verticillata* Kamelin *A. verticillatum* Regel	1.93–1.42	1.36	Flattened ovoid (shrivelled)	0.012	0	Oblong, close with inserted pattern	Oblong	Arched to S-type	Convex or concave, many small verrucae	Chatlkal Range, Tashkent, Uzbekistan (TASH 146)	2A; 9A
17	Sect. *Acmopetala* R.M. Fritsch *A. costatovaginatum* Kamelin et Levichev	2.45–1.46	1.68	(Broadly) ovoid (shrivelled)	0.009	0.00064–0.00097	Ovoid to oblong, close with inserted pattern	Oblong	Ω-type	Gradually convex, many intermediate verrucae	Bashkysylsai, Tashkent, Uzbekistan (TASH 21 Isotypus)	2B; 9B
18	*A. tschimganicum* B. Fedtsch.	2.76–1.77	1.56	(Broadly) ovoid (shrivelled)	0.015	0.003–0.005	Elliptic to oblong, close with inserted pattern	Elliptic	S- to U-type	Convex with granules, one large verruca on central area, intermediate verrucae on edge	Parkent, Tashkent, Uzbekistan (TASH 40)	2C; 9C
19	*A. tashkenticum* F.O. Khass. & R.M. Fritsch ex F.O. Khass	3.01–1.56	1.93	Flattened ovoid	0.01	0	Elliptic to triangular, close with inserted pattern	Elliptic	Ω-type	Gradually convex, 1–4 intermediate verrucae on central area and many small verrucae on edge	Nurekatasay, Tashkent, Uzbekistan (TASH 972)	2D; 9D
20	*A. zergericum* F. Khass. et R.M. Fritsch	2.81–2.00	1.405	Broadly ovoid (shrivelled)	0.02	0	Suborbicular to elliptic, close with inserted pattern	Elliptic	Ω-type	Convex or concave, many small verrucae	Fergana mountain, Uzbekistan (TASH Typus)	2E; 9E
21	Sect. *Aroidea* F.O. Khass. et R.M. Fritsch *A. aroides* Vved. et Popov	3.21–1.63	1.97	Flattened ovoid	0.014	0–0.00048	4 to many edged, close	6 edged	Arched to S-type	Slightly concave or convex from edge to centre, many small verrucae	Samarkand, Uzbekistan (TASH)	2F; 9F
22	Sect. *Asteroprason* R.M. Fritsch *A. cristophii* Trautv.	3.08–2.45	1.26	Broadly ovoid (shrivelled)	0.02	0–0.002	Oblong to suborbicular, close with inserted pattern	Oblong	U- to Ω-type	Convex, many intermediate verrucae, granulose	Babadurmaz, Turkmenistan (TASH 24)	2G; 9G
23	Sect. *Compactoprason* R.M. Fritsch *A. giganteum* Regel	2.48–1.77	1.4	(Broadly) ovoid (shrivelled)	0.016	0.002–0.005	Elliptic to oblong, loose with inserted pattern	Oblong	U- to Ω-type	Convex, many intermediate verrucae, granulose	Tashkent, Uzbekistan (TASH)	2H; 9H
24	*A. komarowii* Lipsky	3.80–2.31	1.65	Broadly ovoid	0.019	0–0.002	Ovoid to irregular, loose with inserted pattern	Oblong	U- to Ω-type	Convex, many intermediate verrucae	Jizakh, Uzbekistan (TASH)	2I; 9I

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
25	Sect. *Tulipifolia* R.M. Fritsch & N. Friesen *A. robustum* Kar. & Kir.	2.54–1.52	1.67	Ovoid	0.012	0.002–0.003	4–5 edged to oblong, close with inserted pattern	Rectangular	S-type	Convex with granulate, one large verruca in centre, intermediate verrucae on edge	Dzungarian Alatau , Kazakhstan (TASH)	2J; 9J
26	Sect. *Kaloprason* C. Koch *A. alexeianum* Regel	2.14–1.57	1.36	Broadly ovoid (shrivelled)	0.015	0.0006–0.001	Orbicular to oblong, loose with inserted pattern	Oblong	U- to Ω-type	Gradually convex, many intermediate verrucae on central area, many small verrucae on edge, granulose	Jizakh, Uzbekistan (TASH 956)	2K; 9K
27	*A. baissunense* Lipsky	3.13–1.83	1.71	(Broadly) ovoid (shrivelled)	0.009	0–0.002	Orbicular to oblong, loose with inserted pattern	Oblong	U- to Ω-type	Globular convex, many intermediate verrucae on central area, granulose, many small verrucae on edge	Surkhandarya, Uzbekistan (TASH 538)	2L; 9L
28	*A. protensum* Wendelbo.	2.4–1.4	1.71	Flattened ovoid	0.012	0–0.002	Orbicular to oblong, loose with inserted pattern	Oblong	U- to Ω-type	Gradually convex, many intermediate verrucae on central area, many small verrucae on edge, granulose	Tashkent, Uzbekistan (TASH)	2M; 9M
29	*A. rhodanthum* Vved.	3.1–2.1	1.48	(Broadly) flat ovoid	0.017	0–0.002	Suborbicular to elliptic, close with inserted pattern	Elliptic	U- to Ω-type	Slightly convex, many intermediate verrucae on central area, many small verrucae on edge, granulose	Surkhandarya Uzbekistan (TASH 21)	2N; 9N
30	Sect. *Procerallium* R.M. Fritsch *A. stipitatum* Regel	3.43–2.57	1.33	Broadly ovoid	0.03	0.00086–0.004	Elliptic to triangular, loose with inserted pattern	Elliptic	U- to Ω-type	Convex with granules, 1–5 intermediate verrucae on central area and many small verrucae on edge	Jizzakh, Uzbekistan (TASH 308)	2O; 9O
31	Sect. *Regeloprason* C. Koch *A. regelii* Trautv.	4.31–2.85	1.51	(Broadly) ovoid	0.027	0	Orbicular to elliptic, close with inserted pattern	Orbicular	U- to Ω-type	Slightly concave, many small verrucae	Mary, Turkmenistan (TASH)	3A; 10A

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
32	*A. cupuliferum* Regel	2.71–2.25	1.2	(Broadly) ovoid (shrivelled)	0.013	0–0.001	Orbicular to elliptic, close with inserted pattern	Orbicular	U- to Ω-type	Gradually convex, many intermediate verrucae on central area of shrivelled epidermis	Forish, Jizzakh, Uzbekistan (TASH)	3B; 10B
33	*A. isakulii* R.M. Fritsch & F.O. Khass.	2.69–1.95	1.38	(Broadly) ovoid (shrivelled)	0.013	0–0.003	Orbicular to elliptic, loose with inserted pattern	Orbicular	U- to Ω-type	Gradually convex, 1–4 intermediate verrucae on central area, many small verrucae	Forish, Jizzakh, Uzbekistan (TASH)	3C; 10C
34	Sect. *Stellata* (F.O. Khass. & R.M. Fritsch) R.M. Fritsch *A. taeniopetalum* Popov & Vved.	2.29–1.71	1.34	(Broadly) ovoid	0.02	0–0.001	Orbicular to elliptic, loose with inserted pattern	Orbicular	Ω-type	Gradually convex, one large verruca central area, many intermediate and many small verrucae on edge	Forish, Jizzakh, Uzbekistan (TASH)	3D; 10D
35	Sect. *Melanocrommyum* Webb & Berth. *A. cardiostemon* Fisch. & C.A. Mey.	2.52–1.37	1.84	Flattened ovoid (shrivelled)	0.008	0–0.003	Orbicular to oblong, loose with inserted pattern	Oblong	Ω-type	Gradually convex, many intermediate verrucae on central area, many small verrucae	Armenia, TBI1033229	3E; 10E
36	*A. woronowii* Miscz. ex Grossh.	2.02–1.52	1.33	Broadly ovoid	0.014	0.002–0.008	Orbicular to elliptic, close with inserted pattern	Orbicular	S- to U-type	Gradually convex, many intermediate and small verrucae	Azerbaijan, Nachitshevan, Shachbuz TBI1034296	3F; 10F
37	**Subg. *Butomissa* (Salisb.) N. Friesen** Sect. *Austromontana* N. Friesen *A. oreoprasum* Schrenk.	3.03–1.87	1.62	Ovoid	0.02	0.003–0.009	Elliptic to triangular, loose with reticulate tissue and inserted pattern	Elliptic	S- to U-type	Concave or convex, many intermediate and small verrucae, granulose	Naryn, Kyrgyzstan (TASH 453)	3G; 10G
38	Sect.*Butomissa* Webb et Berth. *A. ramosum* L.	3.93–2.34	1.68	Flattened ovoid	0.018	0.002–0.008	Long to narrowly 4–7 edged to elliptic, loose with reticulate tissue	Elliptic	Arched to S-type	Plane to slightly concave, many small verrucae	Gorno-Altaisk, Russia (PE 00138973)	3H; 10H
39	*A. ramosum L.*	3.97–2.33	1.7	(Broadly) ovoid (shrivelled)	0.02 m	0.001–0.003	4–7 edged, close with irregularly inserted pattern	6 edged	Arched to S-type	Flat to slightly concave or convex, many small verrucae	Kibray, Tashkent, Uzbekistan (TASH)	3I; 10I

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
40	*A. tuberosum* Rottler ex Spreng.	3.45–2.37	1.45	(Broadly) ovoid (shrivelled)	0.04	0.002–0.005	Long to narrowly 4–7 edged to elliptic, loose with reticulate tissue	Elliptic	Arched to S-type	Plane to slightly concave or convex, many small verrucae	Guizhou, China (KUN 0358965)	3J; 10J
41	*A.* aff. *tuberosum* Rottler ex Spreng.	2.79–1.60	1.74	Flattened ovoid	0.021	0.003–0.006	5–7 edged, loose with unclear meshes of reticulate tissue	6 edged	Straight to arched	Gradually concave or convex from edge to centre, small to intermediate verrucae, granulose	Jalal–Abad, Kyrgyzstan (TASH 284)	3K; 10K
42	**Subg. *Cyathophora* (R.M. Fritsch) R.M. Fritsch** Sect. *Coleoblastus* Ekberg. *A. mairei* Levl.	2.10–1.55	1.35	Broadly ovoid	0.013	0.005–0.008	5–7 edged, loose with clear meshes of reticulate tissue	6 edged	Straight to arched	Gradually concave from thickened edge to centre, many small verrucae in central area of epidermis	Yunnan, China (KUN 0358567)	3L; 10L
43	Sect. *Cyathophora* R.M. Fritsch *A. cyathophorum* Bureau & Franch.	2.64–1.32	2	Flattened ovoid	0.015	0.004–0.010	5–6 edged, loose with reticulate tissue and inserted pattern	6 edged	Straight to arched	Gradually convex from edge to centre, small to intermediate verrucae, granulose	Yunnan, China (KUN 0358252)	3M; 10M
44	**Subg. *Rhizirideum* (G. Don ex Koch) Wendelbo** Sect. *Caespitosoprason* N. Friesen *A. subangulatum* Regel	2.10–1.30	1.61	Flattened ovoid	0.007	0.0008–0.002	5–6 edged, loose with reticulate tissue and inserted pattern	6 edged	S-type	Slightly convex, many small verrucae	Qinghai, China (PE 01570803)	3N; 10N
45	Sect. *Eduardia* N. Friesen *A. przewalskianum* Regel	2.70–1.50	1.8	Ovoid	0.014	0.005–0.008	Long to narrowly 4–7 edged to elliptic, loose with clear meshes of reticulate tissue with indented connecting thread	Elliptic	Straight to arched	Gradually concave from edge to centre, marginal bulge, many small verrucae	Tibet, China (KUN 0358856)	3O; 10O
46	Sect. *Tenuissima* (Tzagolova) Hanelt. *A. tenuissimum* L.	2.22–1.40	1.58	(Broadly) ovoid (shrivelled)	0.016	0–0.003	5–7 edged, loose with reticulate tissue and inserted pattern	6 edged	S-type	Slightly concave or convex, many small verrucae	Jilin, China (PE 00139786)	4A; 11A
47	Sect. *Rhizirideum* G. Don ex Koch s.s. *A. denudatum* Redouté	1.77–1.31	1.29	Broadly ovoid	0.007	0.002–0.011	5–7 edged, loose with inserted pattern and with clear meshes of reticulate tissue and indented connecting thread	6 edged	Straight to arched	Gradually concave from edge to centre, marginal bulge, many small verrucae	Russia, Dagestan, TBI1033015	4B; 11B

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
48	**Subg. *Allium*** Sect. *Brevispatha* Valsecchi *A. margaritae* B. Fedtsch.	2.50–1.30	1.92	Ovoid	0.007	0.001–0.003	4–6 edged, loose with meshes of reticulate tissue	5 edged	S-type	Slightly convex, many small verrucae	Taraz, Jambyl, Kazakhstan (TASH 544 (16))	4C; 11C
49	Sect. *Codonoprassum* Rchb. *A. lenkoranicum* Miscz. ex Grossh.	3.60–1.90	1.89	Ovoid	0.01	0.003–0.005	Triangular to 5 edged, loose with unclear meshes of reticulate tissue and inserted pattern	5 edged	S-type	Convex, many intermediate verrucae, granulose	Akhal, Turkmenistan (TASH 2)	4D; 11D
50	Sect. *Crystallina* F.O. Khass. et Yengalycheva *A. crystallinum* Vved.	3.31–1.87	1.77	Flattened ovoid	0.022	0.002–0.005	Orbicular to elliptic, loose with inserted pattern	Elliptic	S- to U-type	Globular convex, 1–5 intermediate and many small verrucae	Kashkadarya, Uzbekistan (TASH 143)	4E; 11E
51	Sect. *Eremoprasum* (Kamelin) F.O. Khassanov, R.M. Fritsch et N. Friesen *A. popovii* Vved.	2.03–1.48	1.37	(Broadly) ovoid	0.006	0.001–0.004	Orbicular to oblong, loose with inserted pattern	Oblong	S-type	Convex, many small verrucae	Navoi, Uzbekistan (TASH 45)	4F; 11F
52	*A. sabulosum* Stev.	2.72–1.66	1.62	Flattened ovoid	0.019	0.002–0.004	Orbicular to elliptic, loose with inserted pattern	Elliptic	U- to Ω-type	Slightly concave or convex, many small verrucae	Tomdi, Navoi, Uzbekistan (TASH 177)	4G; 11G
53	Sect. *Kopetdagia* F.O. Khass. *A. kopetdagense* Vved.	2.75–2.16	1.27	(Broadly) ovoid	0.01	0.001–0.005	5–7 edged, loose with inserted unclear pattern	5 edged	Arched to S-type	Gradually convex from edge to centre, one large verruca on central area and many small verrucae on edge, granulose	Karakala, Turkmenistan (TASH)	4H; 11H
54	Sect. *Minuta* F.O. Khass. *A. anisotepalum* Vved.	2.41–1.01	2.39	Narrowly ovoid, flattened ovoid	0.006	0.001–0.002	Orbicular to elliptic, loose with inserted pattern	Elliptic	U- to Ω-type	Slightly convex, many small verrucae	Uzbek-Gava, Jalal-Abad, Kyrgyzstan (TASH)	4I; 11I
55	*A. minutum* Vved.	2.35–1.02	2.3	(Narrowly) ovoid	0.012	0.002.–0.005	Orbicular to elliptic, loose with clear meshes of reticulate tissue with indented connecting thread	Elliptic	U- to Ω-type	Slightly convex, many small and granulose intermediate verrucae	Zaalaisky Range, Kyrgyzstan (TASH 65)	4J; 11J
56	Sect. *Allium* *A. affine* Ledeb.	2.54–1.17	2.017	Flattened ovoid	0.018	0.004–0.06	Orbicular to oblong, loose with inserted pattern and with indented connecting thread	Oblong	U-type	Convex, many intermediate verrucae	Georgia, TBI1032955	4K; 11K

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
57	*A. atroviolaceum* Boiss.	3.26–1.58	2.06	(Narrowly) flattened ovoid	0.02	0.001–0.003	Orbicular to elliptic, loose with inserted pattern	Elliptic	U-type	Convex, one large verruca in centre, many intermediate verrucae on edge, granulose	Zangiata, Tashkent, Uzbekistan (TASH)	4L; 11L
58	*A. aucheri* Boiss.	3.11–1.42	2.19	Narrowly ovoid	0.022	0.002–0.006	Oblong to elliptic, loose with reticulate tissue and inserted pattern	Elliptic	S- to U-type	Concave, many intermediate and small verrucae, granulose	Armenia, Semenovskii Pass, TBI10332208	4M; 11M
59	*A. dictyoscordum* Vved.	3.15–2.20	1.43	Broadly ovoid	0.024	0.003–0.008	Orbicular to oblong, loose with inserted pattern and with indented connecting thread	Oblong	S- to U-type	Convex, many intermediate verrucae	Akhal, Turkmenistan (TASH 53)	4N; 11N
60	*A. filidens* Regel	3.23–2.14	1.51	(Broadly) ovoid	0.012	0.002–0.004	Orbicular to elliptic, loose with inserted pattern	Elliptic	U- to Ω-type	Convex, 1–3 large verrucae in centre, many intermediate verrucae on edge, granulose	Tashkent, Uzbekistan (TASH 35546)	4O; 11O
61	*A. guttatum* Steven	2.46–1.15	2.14	(Narrowly) flattened ovoid (shrivelled)	0.011	0.002–0.006	Orbicular to elliptic, loose with inserted pattern and with unclear meshes of reticulate tissue with indented connecting thread	Elliptic	U- to Ω-type	Convex, 5–7 intermediate verrucae, granulose	Bessarabia, Eastern Europe (PE 00114993)	5A; 12A
62	*A. ugami* (Vved.) R.M. Fritsch & F.O. Khass.	3.02–1.88	1.6	(Broadly) ovoid	0.016	0.001–0.005	Orbicular to elliptic and triangular, loose with inserted pattern	Elliptic	U- to Ω-type	Convex, one or two large granulose verrucae in centre, many intermediate granulose verrucae on edge	Tashkent, Uzbekistan (TASH 173 Syntypus)	5B; 12B
63	*A. vineale* L.	2.77–1.21	2.29	(Narrowly) ovoid (shrivelled)	0.016	0.002–0.011	Orbicular to oblong, loose with inserted pattern	Oblong	U- to Ω-type	Gradually convex from edge to centre, marginal bulge or not, many intermediate and small verrucae	Armenia, TBI1034279	5C;12C
64	Sect. *Avulsea* F.O. Khass. *A. fibrosum* Regel	2.55–1.27	2	Flattened ovoid	0.013	0.002–0.004	Orbicular to elliptic, loose with inserted pattern	Elliptic	S- to U-type	Convex, one or two large verrucae in centre, many intermediate verrucae on edge, granulose	Ashgabat, Turkmenistan (TASH25)	5D; 12D

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
65	*A. griffithianum* Boiss.	2.14–1.15	1.86	Ovoid	0.008	0.0009–0.004	Orbicular to elliptic and triangular, loose with inserted pattern	Elliptic	U-type	Gradually convex, large verrucae in centre, many small and intermediate verrucae on edge, granulose	Zaaminsu, Jizzakh, Uzbekistan (TASH)	5E; 12E
66	*A. pamiricum* Wendelbo	3.31–1.48	2.24	Flattened ovoid	0.009	0–0.003	Orbicular to elliptic, close with inserted pattern	Elliptic	U-type	Concave or convex, 1–4 intermediate verrucae in centre, many small verrucae on edge, granulose	Khorog, Tajikistan (TASH)	5F; 12F
67	Sect. *Brevidentia* F.O. Khass. et Yengalycheva *A. brevidens* Vved.	2.40–1.33	1.8	Ovoid	0.01	0.002–0.005	Orbicular to elliptic and triangular, loose with inserted pattern and with unclear meshes of reticulate tissue with indented connecting thread	Elliptic	U-type	Convex, 1–3 intermediate granulose verrucae on central area and many small verrucae	Babatag range, Tajikistan (TASH 546)	5G; 12G
68	*A. ophiophyllum* Vved.	2.12–1.21	1.75	Ovoid (shrivelled)	0.012	0.002–0.007	Orbicular to oblong, loose with inserted pattern	Oblong	S- to U-type	Globular convex, many intermediate granulose verrucae	Kashkadarya, Uzbekistan (TASH 482)	5H; 12H
69	Sect. *Coerulea* (Omelczuk) F.O. Khass. *A. caesioides* Drobow ex Vved.	1.70–0.91	1.87	Ovoid	0.01	0.003–0.008	Orbicular to oblong, loose with inserted pattern	Oblong	S- to U-type	Gradually concave or convex from edge to centre, marginal bulge or not, many small verrucae	Jalal–Abad, Kyrgyzstan (TASH)	5I; 12I
70	*A. elegans* Drobow.	2.29–1.53	1.5	(Broadly) ovoid (shrivelled)	0.01	0.003–0.01	Orbicular to elliptic, loose with inserted pattern and with unclear meshes of reticulate tissue with indented connecting thread	Elliptic	S-type	Convex, 1–5 intermediate granulose verrucae and many small verrucae	Sugd, Tajikistan (TASH 87)	5J; 12J
71	*A. caesium* Schrenk.	2.16–1.15	1.88	Ovoid (shrivelled)	0.012	0.002–0.007	Orbicular to oblong and elliptic, loose with inserted pattern and with unclear meshes of reticulate tissue with indented connecting thread	Oblong	S- to U-type	Convex, one or two large granulose verrucae and many small verrucae	Namangan, Uzbekistan (TASH)	5K; 12K

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
72	*A. delicatulum* Siev. ex Schult. & Schult. f.	2.32–1.08	2.15	Ovoid	0.008	0.001–0.004	Orbicular to oblong and elliptic, loose with inserted pattern	Oblong	U- to Ω-type	Gradually concave from edge to centre, marginal bulge or not, small verrucae	Tarangul lake, Severo-Kazakhstan, Kazakhstan (TASH 1111)	5L; 12L
73	*A. caeruleum* Pall.	2.13–1.40	1.52	(Broadly) ovoid	0.012	0.001–0.003	Triangular to elliptic, loose with inserted pattern	Elliptic	Ω-type	Gradually concave from edge to centre, marginal bulge or not, small verrucae	Suusamyr, Kyrgyzstan (TASH 1115)	5M; 12M
74	Sect. *Mediasia* F.O. Khass., S.C. Yengalycheva et N. Friesen *A. turkestanicum* Regel	2.93–1.89	1.55	(Broadly) ovoid	0.011	0.001–0.007	5–7 edged, loose with inserted pattern and with large clear meshes of reticulate tissue with broadly connecting thread	6 edged	Arched to S-type	Convex, many small verrucae	Jizzakh, Uzbekistan (TASH)	5N; 12N
75	Sect. *Multicaulia* F.O. Khass. et Yengalycheva. *A. ferganicum* Vved.	2.42–1.19	2.03	Ovoid	0.015	0.001–0.005	Orbicular to elliptic and oblong with inserted pattern and with large clear meshes of reticulate tissue with broadly connecting thread	Oblong	U-type	Convex, 1–3 intermediate verrucae and many small verrucae	Namangan, Uzbekistan (TASH 1099)	5O; 12O
76	*A. borszczowii* Regel	3.58–2.51	1.43	(Broadly) ovoid	0.023	0.0008–0.003	Triangular to elliptic, loose with inserted pattern	Elliptic	U-type	Slightly convex, many small verrucae	Karakalpakstan, Uzbekistan (TASH 18)	6A; 13A
77	*A. borszczowii* Regel	3.06–1.37	2.23	Flattened ovoid	0.018	0.002–0.004	Triangular to elliptic and oblong, loose with inserted pattern	Oblong	U- to Ω-type	Convex, many small verrucae	Badhyz, Turkmenistan (TASH 1172)	6B; 13B
78	Sect. *Haneltia* F.O. Khass. *A. haneltii* F.O. Khass. & R.M. Fritsch	2.41–1.22	1.97	Flattened ovoid	0.019	0–0.003	Triangular to elliptic and oblong, loose with inserted pattern	Elliptic	U-type	Slightly convex, 1–4 intermediate granulose verrucae and many small verrucae	Pop, Namangan, Uzbekistan (TASH 107)	6C; 13C
79	Sect. *Pallasia* (Tzagolova) F.O. Khass., R.M. Fritsch et N. Friesen *A. pallasii* Murr.	2.72–1.39	1.96	Ovoid (shrivelled)	0.011	0.002–0.009	Oblong to elliptic, loose with inserted pattern and with small clear meshes of reticulate tissue with narrowly connecting thread	Oblong	Arched to S-type	Flat to concave, many small verrucae	Xinjiang, China (KUN 0358637)	6D; 13D
80	*A. tanguticum* Regel	2.45–1.39	1.76	Flattened ovoid	0.008	0.007–0.021	4–7 edged, loose with clear meshes of reticulate tissue and indented connecting thread	6 edged	Straight to arched	Gradually concave from edge to centre, marginal bulge, small verrucae	Gansu, China (PE 00139762)	6E; 13E

TABLE 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
81	**Subg. *Reticulatobulbosa* (Kamelin) N. Friesen** Sect. *Nigrimontana* N. Friesen *A. drobovii* Vved.	3.97–2.31	1.72	Flattened ovoid	0.025	0.008–0.012	4–7 edged, loose with inserted pattern and with reticulate tissue	6 edged	Straight to arched	Gradually concave from thickened edge to centre, one large granulose verruca in centre, many small verrucae on edge	Pskem, Tashkent, Uzbekistan (TASH 10820151)	6F; 13F
82	Sect. *Reticulatobulbosa* Kamelin *A. lineare* L.	3.00–1.54	1.95	Flattened ovoid	0.02	0.006–0.15	4–7 edged, loose with inserted pattern and with clear meshes of reticulate tissue and indented connecting thread	6 edged	Straight to arched	Gradually concave from thickened edge to centre, one large granulose verruca in centre, many small verrucae on edge	Kazakhstan (TASH 805)	6G; 13G
83	Sect. *Campanulata* Kamelin *A. barsczewskii* Lipsky	3.83–2.05	1.87	Ovoid	0.01	0.005–0.009	5–7 edged, loose with clear meshes of reticulate tissue and indented connecting thread	6 edged	Straight to arched	Gradually convex from thickened edge to centre, many granulose verrucae in centre, many small verrucae on edge	Parkent, Tashkent, Uzbekistan (TASH)	6H; 13H
84	Sect. *Scabriscapa* (Tscholok.) N. Friesen *A. eriocoleum* Vved.	2.85–2.07	1.37	(Broadly) ovoid (shrivelled)	0.01	0.006–0.008	4–7 edged, loose with clear meshes of reticulate tissue and broadly connecting thread	6 edged	Straight to arched	Gradually convex from thickened edge to centre, one large granulose verruca in centre, many small verrucae on edge	Tashkent, Uzbekistan (TASH)	6I; 13I
85	*A. sulphureum* Vved.	2.84–2.07	1.37	Broadly ovoid	0.012	0.003–0.006	Orbicular to elliptic, loose with inserted pattern and with unclear meshes of reticulate tissue with indented connecting thread	Elliptic	U- to Ω-type	Convex, one or two large granulose verrucae in centre, many intermediate granulose verrucae on edge	Surkhandarya, Uzbekistan (TASH 736)	6J; 13J
86	***Subg. Polyprason* Radic** Sect. *Daghestanica* (Tscholok.) N. Friesen *A. albovianum* C.H. Wright	3.13–1.48	2.11	Ovoid (shrivelled)	0.005	0.003–0.011	4–many edged, loose with inserted pattern and with clear meshes of reticulate tissue and indented connecting thread	6 edged	Arched to S-type	Gradually concave from edge to centre, intermediate to large verrucae	Georgia, TBI1038711	6K; 13K

Table 2. *Continued*

No.	Taxon	Seed length and width (mm average measurement)	L/W ratio (mm)	Seed shape	Area of 10 periclinal wall testa surface (mm^2 average measurement)	Distance between testa cells (mm)	Testa cells: shape, arrangement	Dominant testa cell shapes	Anticlinal wall: curvature type	Periclinal wall: shape and micromorphology	Herbarium data	Figure: seed; cell
87	Sect. *Falcatifolia* *A. korolkowii* Regel	2.17–1.16	1.87	Flattened ovoid	0.008	0.003–0.005	5–6 edged to oblong, loose with clear meshes of reticulate tissue and with indented connecting thread	Oblong	S-type	Convex, many small verrucae in centre, intermediate verrucae on edge	Almaty, Kazakhstan (TASH 5739)	6L; 13L
88	*A. carolinianum* DC.	3.24–1.80	1.3	Ovoid (shrivelled)	0.01	0.005–0.009	4–7 edged, loose with reticulate tissue	6 edged	Straight to arched	Slightly concave, many small verrucae	Surkhandarya, Uzbekistan (TASH)	6M; 13M
89	Sect. *Oreiprason* F. Herm. *A. talassicum* Regel	2.77–1.62	1.71	Flattened ovoid	0.006	0.002–0.006	5–7 edged, loose with inserted pattern and with clear meshes of reticulate tissue	6 edged	Straight to arched	Convex, many small verrucae	Aflatun river, Jalal–Abad, Kyrgyzstan (TASH)	6N; 13N
90	**Subg. *Cepa*(Mill.) Radic** Sect. *Cepa* (Mill.) Prokh.	3.52–2.60	1.35	Broadly ovoid	0.008	0.002–0.007	4–7 edged, loose with unclear meshes of reticulate tissue	6 edged	Straight to arched	Convex, many small verrucae	Khujand, Tajikistan (TASH 264)	6O; 13O
91	*A. praemixtum* Vved.	3.30–2.42	1.36	(Broadly) ovoid (shrivelled)	0.009	0.002–0.006	5–7 edged, loose with reticulate tissue	6 edged	Straight to arched	Globular convex, many small verrucae	Khujand, Tajikistan (TASH 265)	7A; 14A
92	*A. altaicum* Pall.	3.58–1.98	1.8	(Broadly) ovoid (shrivelled)	0.01	0.005–0.014	4–7 edged, loose with reticulate tissue and with narrow connecting thread	6 edged	Straight to arched	Slightly convex, many small verrucae	Almaty, Kazakhstan (TASH 1174)	7B; 14B
93	*A. galanthum* Kar. & Kir.	3.39–2.18	1.55	(Broadly) ovoid (shrivelled)	0.008	0.003–0.009	4–7 edged, loose with reticulate tissue and with narrow connecting thread	6 edged	Straight to arched	Convex, many small verrucae	Naryn, Kyrgyzstan (TASH 1280)	7C; 14C
94	*A. pskemense* B. Fedtsch.	3.86–2.85	1.35	(Broadly) ovoid (shrivelled)	0.012	0.006–0.014	4–7 edged, loose with reticulate tissue and with narrow connecting thread	6 edged	Straight to arched	Convex, many small verrucae	Pskem, Tashkent, Uzbekistan (TASH)	7D; 14D
95	*A. oschaninii* O. Fedtsch.	3.47–2.32	1.5	(Broadly) ovoid	0.01	0.004–0.008	5–7 edged, loose with reticulate tissue and with narrow connecting thread	6 edged	Straight to arched	Gradually convex, many small to intermediate verrucae	Malguzar, Jizzakh, Uzbekistan (TASH 214)	7E; 14E
96	Sect. *Schoenoprasum* Dumort. *A. karelinii* Poljak.	2.73–1.42	1.92	Flattened ovoid	0.004	0.002–0.006	4–7 edged, loose with clear meshes of reticulate tissue and with narrow connecting thread	6 edged	Straight to arched	Flat to slightly convex, many small verrucae	Almaty, Kazakhstan (TASH3916)	7F; 14F
97	Sect. *Annulopraso*n Egorova *A. fedtschenkoanum* Regel	3.57–1.74	2.05	Flattened ovoid	0.009	0.002–0.006	4–7 edged to oblong, close	6 edged	Straight to arched	Globular convex, many small verrucae on surface	Surkhandarya, Uzbekistan (TASH 580)	7G; 14G
98	Sect. *Condensatum* N. Friesen *A. condensatum* Turcz.	3.05–1.64	1.86	Flattened ovoid	0.011	0.003–0.011	Long 4–7 edged, close with unclear meshes	6 edged	Straight to arched	Flat to slightly convex, many small verrucae	Hebei, China (KUN 0358234)	7H; 14H

(Lin and Tan, 2017).

Fritsch (2016) (subgenus *Melanocrommyum*), Fritsch *et al.* (2010) (subgenus *Melanocrommyum*), Sinitsyna *et al.*, (2016), Khassanov (2018) (subgenus *Allium*), Baasanmunkh *et al.* (2021) and Friesen *et al.* (2021) (Supplementary Data Table S1). The orthography of taxonomic names was adopted from the World Checklist of Selected Plant Families (WCSP, 2022).

Phylogenetic analyses

To investigate the phylogenetic significance of seed testa cell micromorphology, DNA sequences of 72 species, 56 sections and 14 subgenera representing three major lineages of *Allium* were downloaded from NCBI (see Appendix) and the phylogenetic tree based on ITS sequences of *Allium* was constructed. Furthermore, the correlation of testa cell characteristics with evolutionary trends was examined and discussed. The species were compared through five seed morphological parameters [cell arrangement, anticlinal wall undulation, periclinal wall (shape), periclinal wall (verrucae), seed shape]. The sequences were aligned using MEGA 7.0 (Hall, 1999). The best-fitting substitution models for Bayesian inference were selected using MrModeltest 2.3 (Nylander *et al.*, 2004). Bayesian inference employed MrBayes v.3.2.6 with a Metropolis-coupled Markov chain Monte Carlo approach and maximum likelihood employed RAxML v.8.2.10 in the GTRGAMMA substitution model at the Cipres Portal (https://www.phylo.org/portal2). Phylogenetic analyses were also performed with the maximum parsimony method using PAUP* 4.0a169 (current). The maximum parsimony bootstrap analysis was performed with heuristic search, TBR (tree bisection–reconnection) branch-swapping, 1000 bootstrap replicates, random addition sequence with ten replicates, and a maximum of 1000 trees saved per round.

RESULTS AND DISCUSSION

Information on taxonomic affiliation, vouchers, measurements of seed macro- and micromorphology of 95 species (98 samples) of *Allium* are provided in Table 2. SEM photographs of seeds of all taxa studied are presented in Figs 1–14.

We recognized the following categories of seed shape: ovoid, broadly ovoid, narrowly ovoid, ovoid shrivelled and flattened ovoid (Table 2). Figures 1–7 show the seed shapes of the species of *Allium* organized according to their taxonomic position. In our morphometric measurements, *A. caesioides* Wendelbo [subgenus *Allium*, section *Coerulea* (Omelczuk) F.O. Khass (Fig. 5I)] had the shortest (1.70 mm) and narrowest (0.91 mm) seeds; *A. validum* S. Watson (subgenus *Amerallium*, section *Caulorhizideum* Traub, Fig. 1D) had the longest (4.73 mm). The widest seeds (2.85 mm) were observed in *A. regelii* Trautv. [subgenus *Melanocrommyum* (Webb et Berth.) Rouy, section *Regeloprason* C. Koch (Fig. 3A)]. *Allium ursinum* L. (subgenus *Amerallium*, section *Arctoprasum* Kirschl., Fig. 1B) had the smallest length/width (L/W) ratio (0.97); *A. validum* (subgenus *Amerallium*, section *Caulorhizideum*, Fig. 1D) had the highest L/W ratio (2.61). The greatest periclinal wall surface area (0.04 mm^2) of ten testa cells was in *A. tuberosum* Rottler ex Spreng. [subgenus *Butomissa* (Salisb.) N. Friesen, section *Butomissa* Webb et Berth. Fig. 10J)]. The least periclinal wall surface area (0.004 mm^2) of 10 testa cells was in *A. karelinii* Poljak. (subgenus *Cepa*, section *Schoenoprasum* Dumort., Fig. 14F). The greatest distance between two testa cells was in *A. validum* (subgenus *Amerallium*, section *Caulorhizideum*, Fig. 8D); the shortest distance between two testa cells was in several species of subgenus *Melanocrommyum* (*A. verticillatum* Regel, *A. zergericum* F.O. Khass. & R.M. Fritsch, *A. tashkenticum* F.O. Khass. & R.M. Fritsch, *A. regelii* Trautv.) (Figs 9A, D, N and 10A)

Among 95 species (98 samples), the most important differences were in the shape and arrangement of epidermal cells of the seed testa, particularly in the shape and micromorphology of the anticlinal and periclinal walls. We used the following categories of shape of the epidermal cells: oblong, four- to eight-edged, many edged, orbicular, elliptic, rectangular, triangular, and suborbicular. The types of cellular arrangement were loose or close and with reticulate tissue or with an inserted pattern (Table 2). Nearly all the seeds of all species were verrucate, except in one species, *A. kujukense* Vved. (subgenus *Vvedenskya*, section *Vvedenskya*, Fig. 8J). The types of representative features and dominant shapes of the seed testa cells are provided in Table 3 to convey the features of the testa cells more effectively.

Morphological characteristics of five selected traits in the phylogenetic tree

The five morphological characters mapped onto the parsimony strict consensus tree can be seen in Fig. 15. The first character (1, Fig. 15), cell arrangement, has two states: with inserted pattern and without inserted pattern. Mostly, the second evolutionary lineage and the middle parts of the phylogenetic tree represent cell arrangement with inserted pattern. The anticlinal wall undulation type (2, Fig. 15) had three states: (1) straight to arched; (2) arched to S or S-type; and (3) U- to Ω- type and Ω-type. The members of the first evolutionary lineages and upper part of the third evolutionary lineages show mostly straight to arched anticlinal walls. Periclinal wall shape (3, Fig. 15) includes three different types (convex, flat, concave). The second characteristic of periclinal walls (verrucae on the periclinal wall) (4, Fig. 15) was grouped according to the height of verrucae on the periclinal wall (without, small, intermediate and large). The last studied characteristic of seed morphology (5, Fig. 15) is its shape. Although seed shape includes various types, the traits were assigned to three categories (broadly ovoid, narrowly ovoid, and ovoid or flattened ovoid).

Previous studies suggested that undulating anticlinal walls with a close surface and distinctly convex periclinal walls with prominent verrucae were the advanced characteristics, while straight or arched anticlinal walls with flat, smooth or evenly granulose periclinal walls were ancient characteristics (von Bothmer, 1974; Pastor, 1981; Kruse, 1984, 1986, 1988, 1994; Ilarslan and Koyuncu, 1997; Fritsch *et al.*, 2006; Neshati and Fritsch, 2009; Lin and Tan, 2017). Our study suggested that such characteristics as loose cellular arrangement, small verrucae or not verrucate, concave periclinal wall, straight to arched anticlinal wall and broadly ovoid seed reflected a basal evolutionary

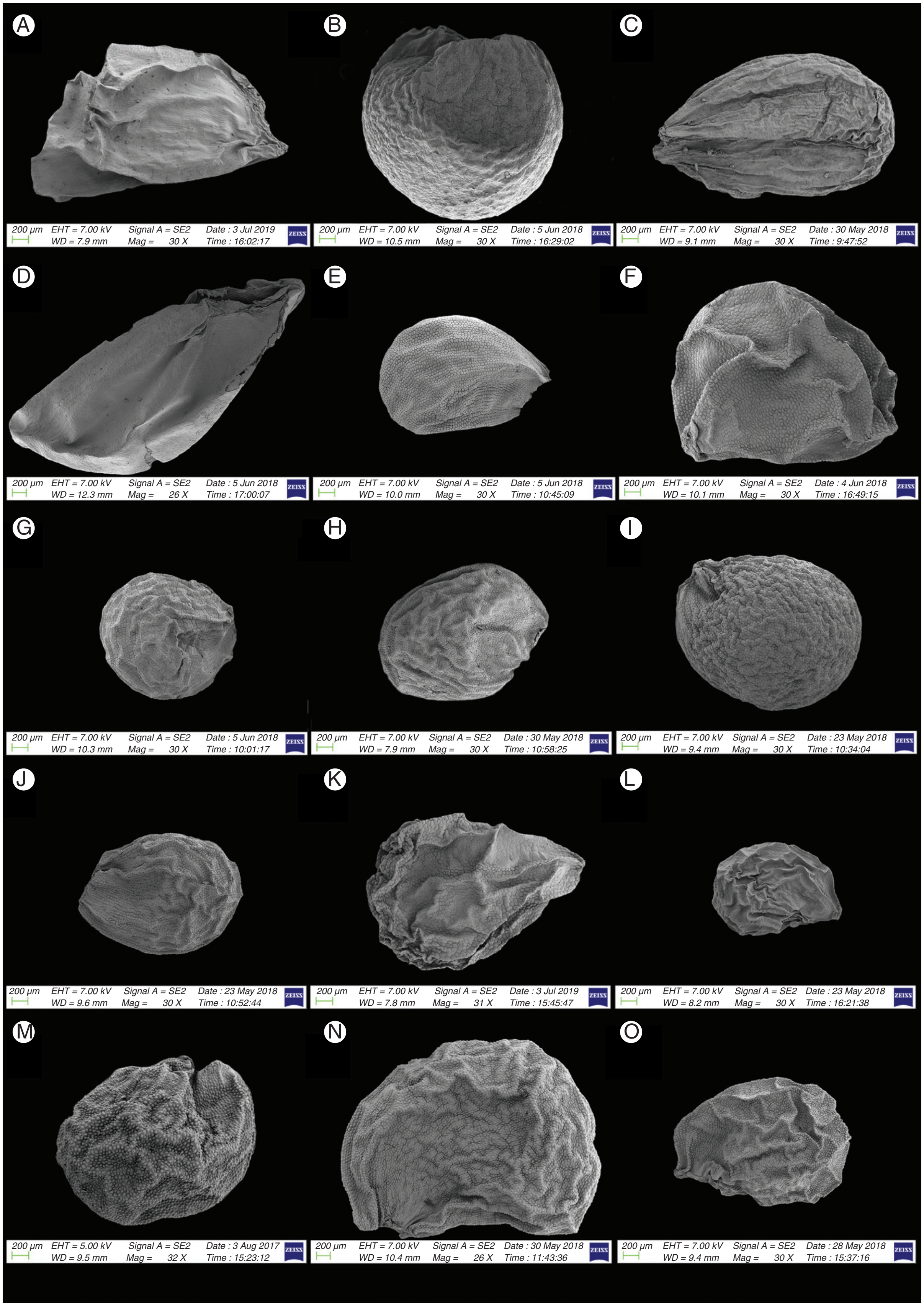


FIG. 1. SEM micrographs of seeds of 15 *Allium* species. (A) Subg. *Nectaroscordum*: *A. tripedale* (sect. *Nectaroscordum*). (B–F) Subg. *Amerallium*: (B) *A. ursinum* (sect. *Arctoprasum*); (C) *A. wallichii* (sect. *Bromatorrhiza*); (D) *A. validum* (sect. *Caulorhizideum*); (E) *A. geyeri* (sect. *Amerallium*); (F) *A. moly* (sect. *Molium*). (G) Subg. *Caloscordum*: *A. neriniflorum* (sect. *Caloscordum*). (H) Subg. *Anguinum*: *A. prattii* (sect. *Anguinum*). (I) Subg. *Porphyroprason*: *A. oreophilum* (sect. *Porphyroprasum*). (J) Subg. *Vvedenskya*: *A. kujukense* (sect. *Vvedenskya*). (K–O) Subg. *Melanocrommyum*: (K) *A. akaka* (sect. *Acanthoprason*); (L) *A. insufficiens* (sect. *Megaloprason*); (M) *A. sarawschanicum* (sect. *Megaloprason*); (N) *A. karataviense* (sect. *Miniprason*); (O) *A. gypsaceum* (sect. *Popovia*).

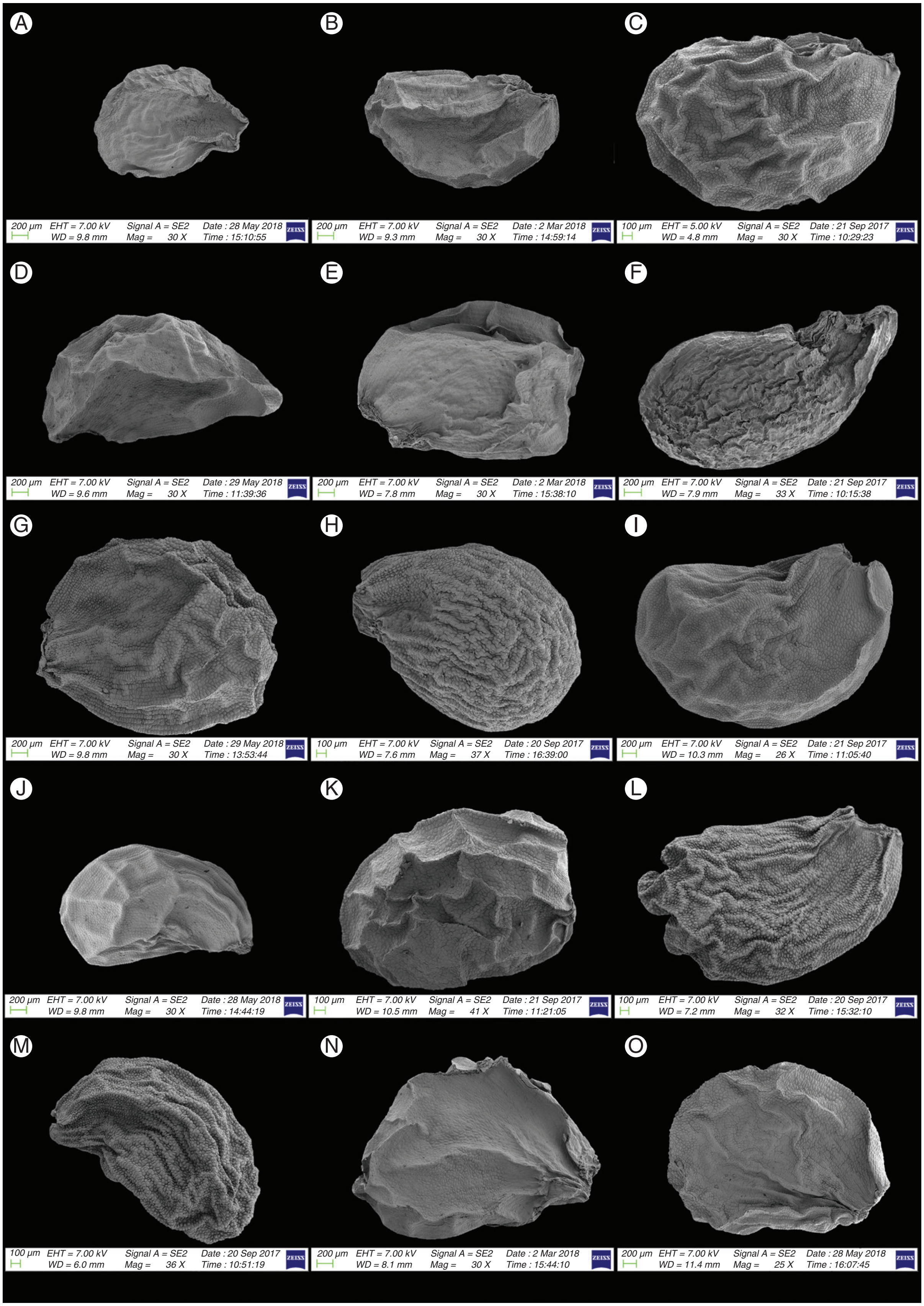


FIG. 2. SEM micrographs of seeds of 15 *Allium* species in subg. *Melanocrommyum*: (A) *A. verticillatum* (sect. *Verticillata*); (B) *A. costatovaginatum* (sect. *Acmopetala*); (C) *A. tschimganicum* (sect. *Acmopetala*); (D) *A. tashkenticum* (sect. *Acmopetala*); (E) *A. zergericum* (sect. *Acmopetala*); (F) *A. aroides* (sect. *Aroidea*); (G) *A. cristophii* (sect. *Asteroprason*); (H) *A. giganteum* (sect. *Compactoprason*); (I) *A. komarowii* (sect. *Compactoprason*); (J) *A. robustum* (sect. *Tulipifolia*); (K) *A. alexeianum* (sect. *Kaloprason*); (L) *A. baissunense* (sect. *Kaloprason*); (M) *A. protensum* (sect. *Kaloprason*); (N) *A. rhodanthum* (sect. *Kaloprason*); (O) *A. stipitatum* (sect. *Procerallium*).

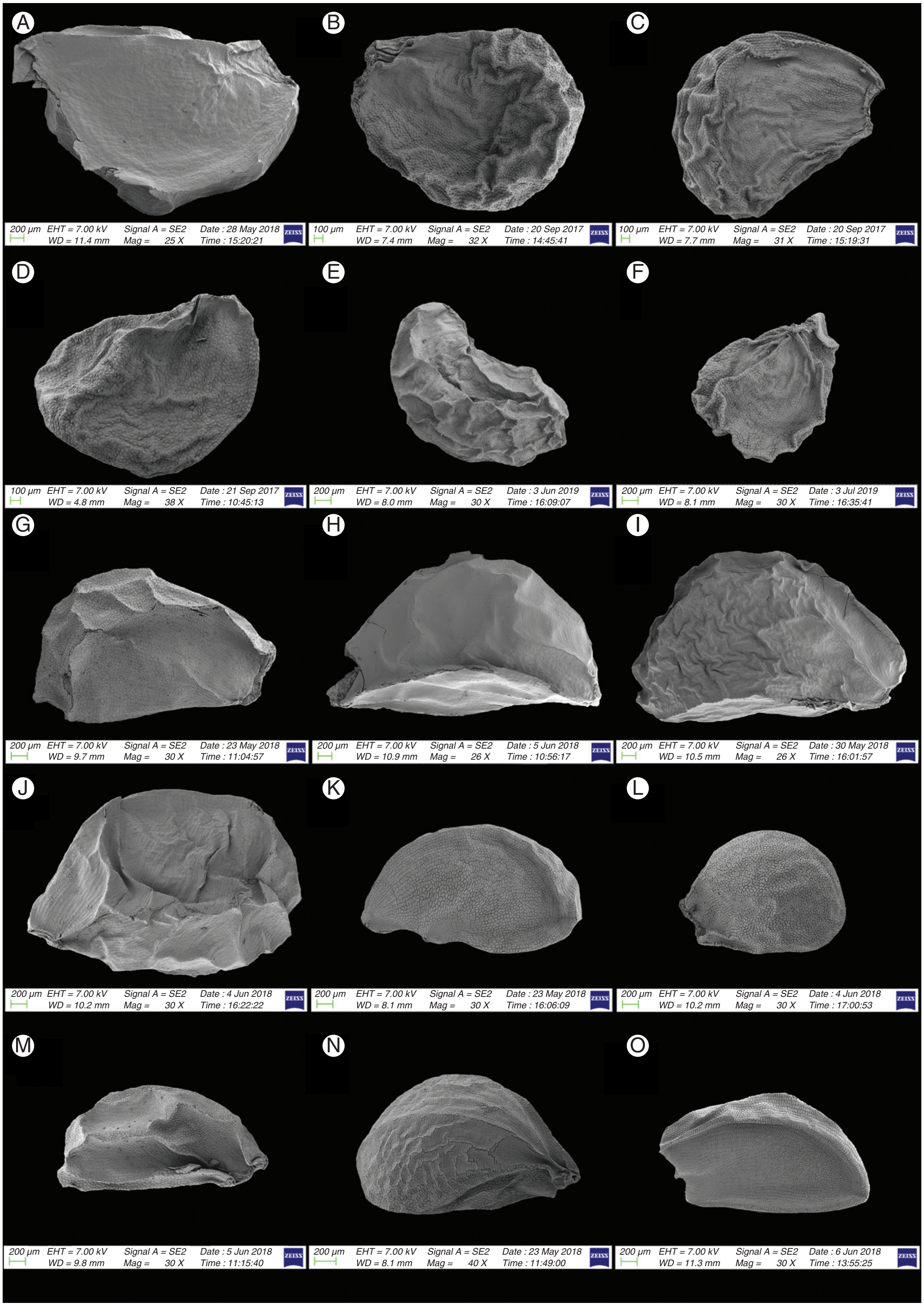


FIG. 3. SEM micrographs of seeds of 15 *Allium* species. (A–F) Subg. *Melanocrommyum*: (A) *A. regelii* (sect. *Regeloprason*); (B) *A. cupuliferum* (sect. *Regeloprason*); (C) *A. isakulii* (sect. *Regeloprason*); (D) *A. taeniopetalum* (sect. *Stellata*); (E) *A. cardiostemon* (sect. *Melanocrommyum*); (F) *A. woronowii* (sect. *Melanocrommyum*). (G–K) Subg. *Butomissa*: (G) *A. oreoprasum* (sect. *Austromontana*); (H) *A. ramosum* (sect. *Butomissa*); (I) *A. ramosum* (sect. *Butomissa*); (J) *A. tuberosum* (sect. *Butomissa*); (K) *A.* aff. *tuberosum*. (L, M) Subg. *Cyathophora*: (L) *A. mairei* (sect. *Coleoblastus*); (M) *A. cyathophorum* (sect. *Cyathophora*). (N, O) Subg. *Rhizirideum*: (N) *A. subangulatum* (sect. *Rhizomatosa*); (O) *A. przewalskianum* (sect. *Eduardia*).

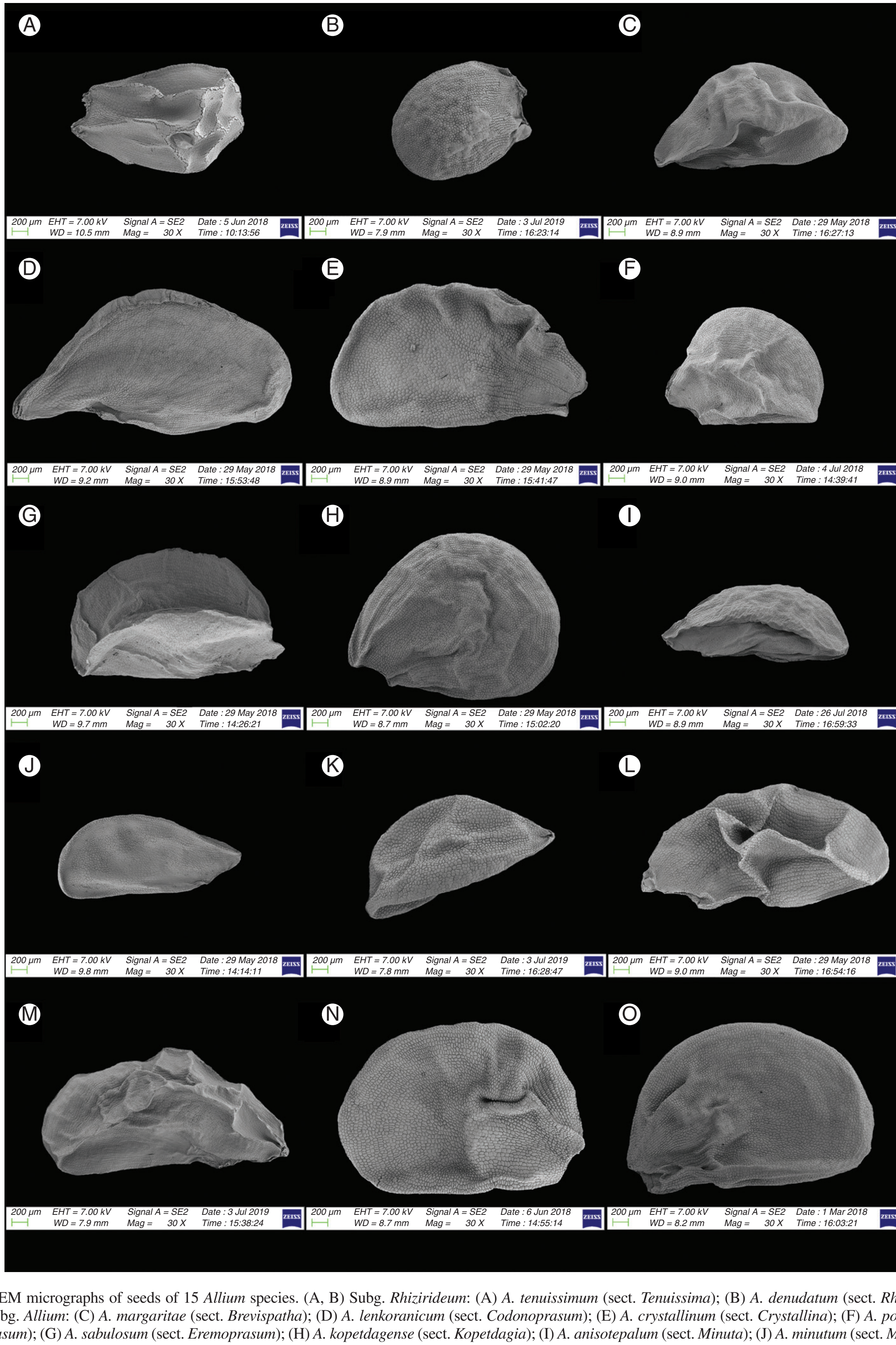


FIG. 4. SEM micrographs of seeds of 15 *Allium* species. (A, B) Subg. *Rhizirideum*: (A) *A. tenuissimum* (sect. *Tenuissima*); (B) *A. denudatum* (sect. *Rhizirideum*). (C–O) Subg. *Allium*: (C) *A. margaritae* (sect. *Brevispatha*); (D) *A. lenkoranicum* (sect. *Codonoprasum*); (E) *A. crystallinum* (sect. *Crystallina*); (F) *A. popovii* (sect. *Eremoprasum*); (G) *A. sabulosum* (sect. *Eremoprasum*); (H) *A. kopetdagense* (sect. *Kopetdagia*); (I) *A. anisotepalum* (sect. *Minuta*); (J) *A. minutum* (sect. *Minuta*); (K) *A. affine* (sect. *Allium*); (L) *A. atroviolaceum* (sect. *Allium*); (M) *A. aucheri* (sect. *Allium*); (N) *A. dictyoscordum* (sect. *Allium*); (O) *A. filidens* (sect. *Allium*).

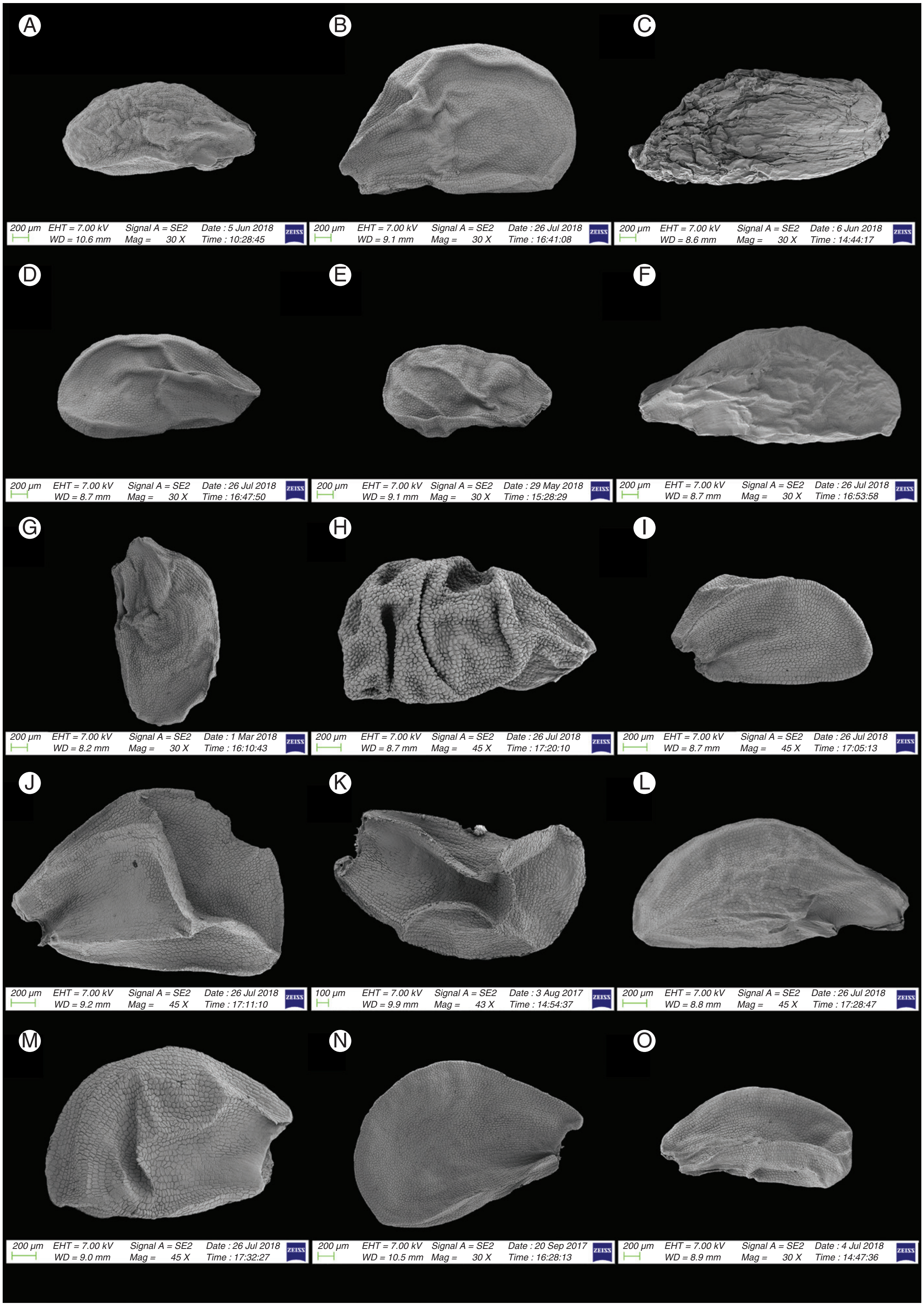


FIG. 5. SEM micrographs of seeds of 15 *Allium* species in subg. *Allium*: (A) *A. guttatum* (sect. *Allium*); (B) *A. ugami* (sect. *Allium*); (C) *A. vineale* (sect. *Allium*); (D) *A. fibrosum* (sect. *Avulsea*); (E) *A. griffithianum* (sect. *Avulsea*); (F) *A. pamiricum* (sect. *Avulsea*); (G) *A. brevidens* (sect. *Brevidentia*); (H) *A. ophiophyllum* (sect. *Brevidentia*); (I) *A. caesioides* (sect. *Coerulea*); (J) *A. elegans* (sect. *Coerulea*); (K) *A. caesium* (sect. *Coerulea*) (L) *A. delicatulum* (sect. *Coerulea*); (M) *A. caeruleum* (sect. *Coerulea*); (N) *A. turkestanicum* (sect. *Mediasia*); (O) *A. ferganicum* (sect. *Multicaulia*).

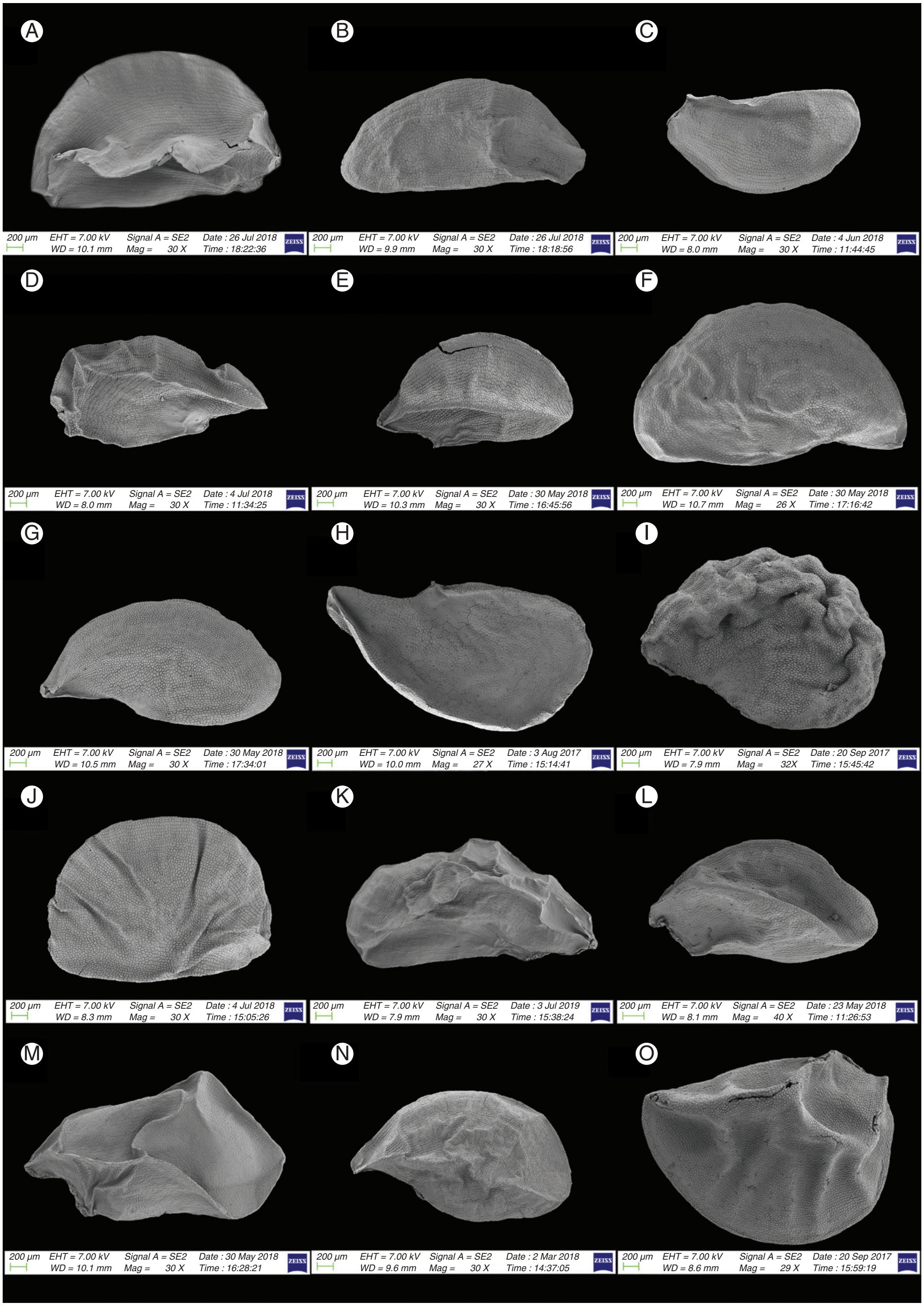


Fig. 6. SEM micrographs of seeds of 15 *Allium* species. (A–E) Subg. *Allium*: (A) *A. borszczowii* (sect. *Multicaulia*); (B) *A. borszczowii* (sect. *Multicaulia*); (C) *A. haneltii* (sect. *Haneltia*); (D) *A. pallasii* (sect. *Pallasia*); (E) *A. tanguticum* (sect. *Pallasia*). (F–J) Subg. *Reticulatobulbosa*: (F) *A. drobovii* (sect. *Nigrimontana*); (G) *A. lineare* (sect. *Reticulatobulbosa*); (H) *A. barsczewskii* (sect. *Campanulata*); (I) *A. eriocoleum* (sect. *Scabriscapa*); (J) *A. sulphureum* (sect. *Scabriscapa*). (K–N) Subg. *Polyprason*: (K) *A. albovianum* (sect. *Oreoprason*); (L) *A. korolkowii* (sect. *Falcatifolia*); (M) *A. carolinianum* (sect. *Falcatifolia*); (N) *A. talassicum* (sect. *Oreiprason*). (O) Subg. *Cepa*: *A. praemixtum* (sect. *Cepa*).

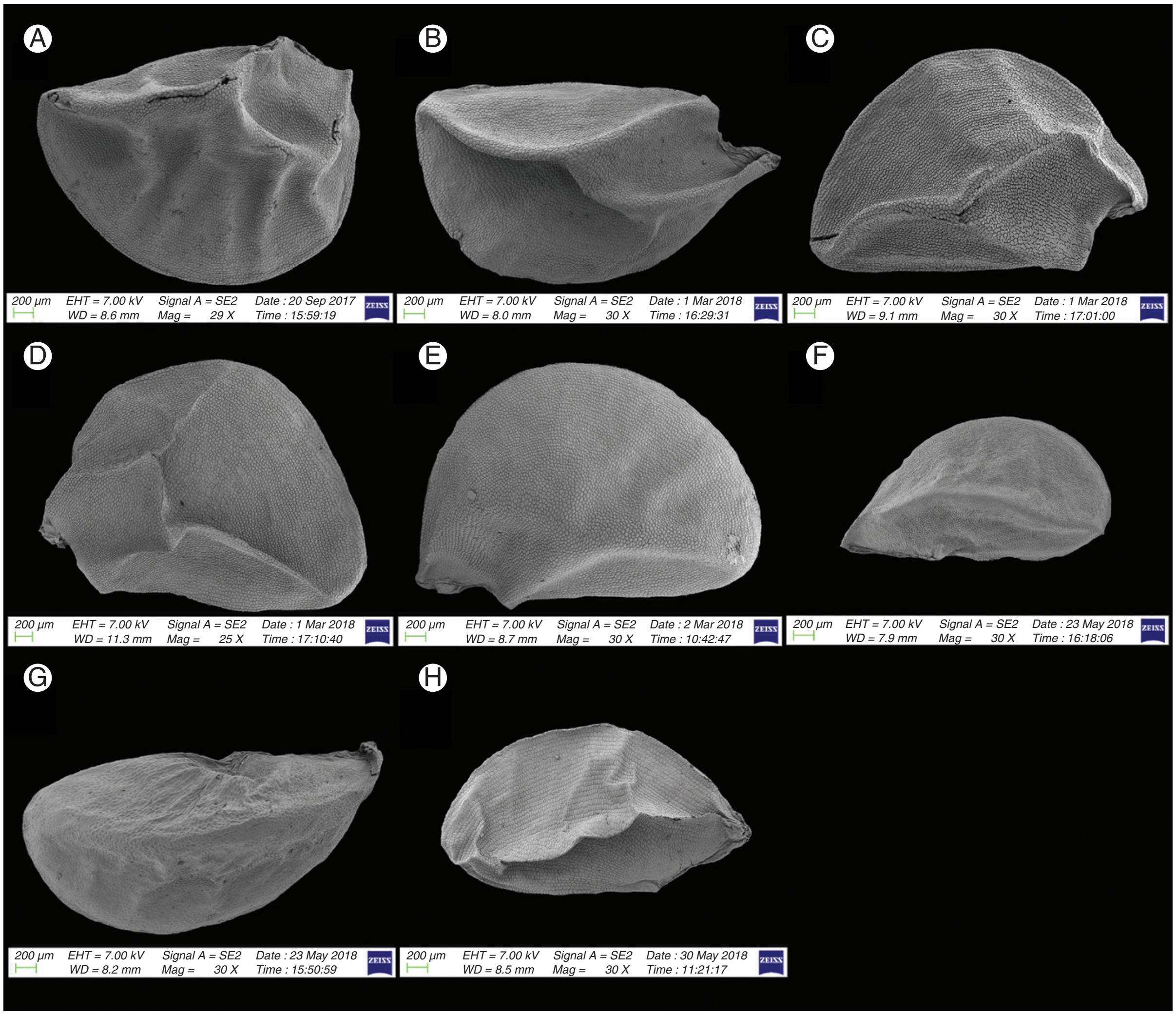


Fig. 7. SEM micrographs of seeds of eight *Allium* species in subg. *Cepa*: (A) *A. praemixtum* (sect. *Cepa*); (B) *A. altaicum* (sect. *Cepa*); (C) *A. galanthum* (sect. *Cepa*); (D) *A. pskemense* (sect. *Cepa*); (E) *A. oschaninii* (sect. *Cepa*); (F) *A. karelinii* (sect. *Schoenoprasum*); (G) *A. fedschenkoanum* (sect. *Annuloprason*); (H) *A. condensatum* (sect. *Condensatum*).

trend. At the same time, reticulate tissue or a mesh-like cellular arrangement, intermediate verrucae, a flat periclinal wall, arched to S or S-type and narrowly ovoid seeds were intermediate characteristics. Closely arranged cells, large verrucae, convex periclinal walls, U- to Ω-type and Ω-type and ovoid or flattened ovoid seeds reflected an advanced evolutionary trend. Members of the first evolutionary lineage along with the representatives of the outgroup [testa characteristics of outgroup members were derived from Kruse (1986)] in the phylogenetic tree showed more primitive characteristics. However, most traits possessed by the members of the second evolutionary lineage were advanced, but they mostly had broadly ovoid seeds (ancient character). Members of the third evolutionary lineage showed mostly primitive testa characteristics (except in subgenus *Allium*, which exhibited advanced characteristics), but with mostly convex periclinal walls, which is an advanced characteristic. The detailed characteristics of the subgenera and sections examined in this study are provided below.

Subgenus *Nectaroscordum* (Lindl.) Asch. & Graebn.

Subgenus *Nectaroscordum* is represented by *A. tripedale* Trautv. from section *Nectaroscordum* (Lindl.) Gren. & Godr. (Table 2). The seed morphology of this species is described here for the first time. The seeds were ovoid (shrivelled, somewhat broadly), seed size was 3.22–1.8 mm, and the L/W ratio was 1.79 (Fig. 1A). The distance between testa cells was 0.007–0.01 mm, close arrangement, periclinal wall surface area of ten cells was 0.012 mm^2, cells of anticlinal wall undulation type were straight to arched, cells of periclinal wall type were gradually concave from edge to centre, there were many intermediate verrucae on edge, and the dominant testa shape was five-edged (Fig. 8A).

In 1986, Kruse (1986) studied the testa cell sculpture of *A. siculum* Ucria as a representative of the subgenus *Nectaroscordum*, and the testa cell structure of this species was very similar to that of *A. ursinum* of the subgenus *Amerallium*

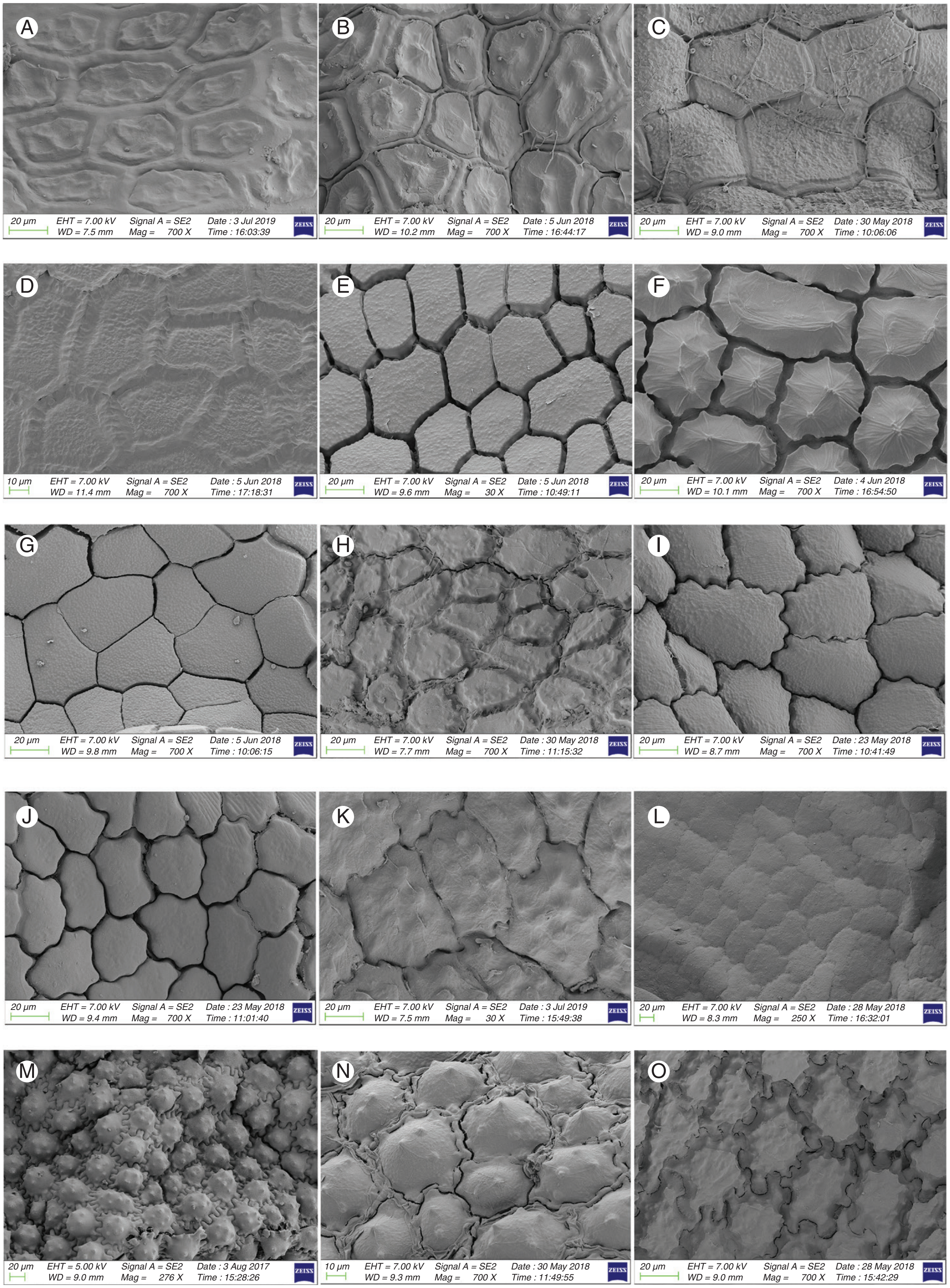


FIG. 8. SEM micrographs of seeds of 15 *Allium* species. (A) Subg. *Nectaroscordum*: *A. tripedale* (sect. *Nectaroscordum*). (B–F) Subg. *Amerallium*: (B) *A. ursinum* (sect. *Arctoprasum*); (C) *A. wallichii* (sect. *Bromatorrhiza*); (D) *A. validum* (sect. *Caulorhizideum*); (E) *A. geyeri* (sect. *Amerallium*); (F) *A. moly* (sect. *Molium*). (G) Subg. *Caloscordum*: *A. neriniflorum* (sect. *Caloscordum*). (H) Subg. *Anguinum*: *A. prattii* (sect. *Anguinum*). (I) Subg. *Porphyroprason*: *A. oreophilum* (sect. *Porphyroprasum*). (J) Subg. *Vvedenskya A. kujukense* (sect. *Vvedenskya*). (K–O) Subg. *Melanocrommyum*: (K) *A. akaka* (sect. *Acanthoprason*); (L) *A. insufficiens* (sect. *Megaloprason*); (M) *A. sarawschanicum* (sect. *Megaloprason*); (N) *A. karataviense* (sect. *Miniprason*); (O) *A. gypsaceum* (sect. *Popovia*).

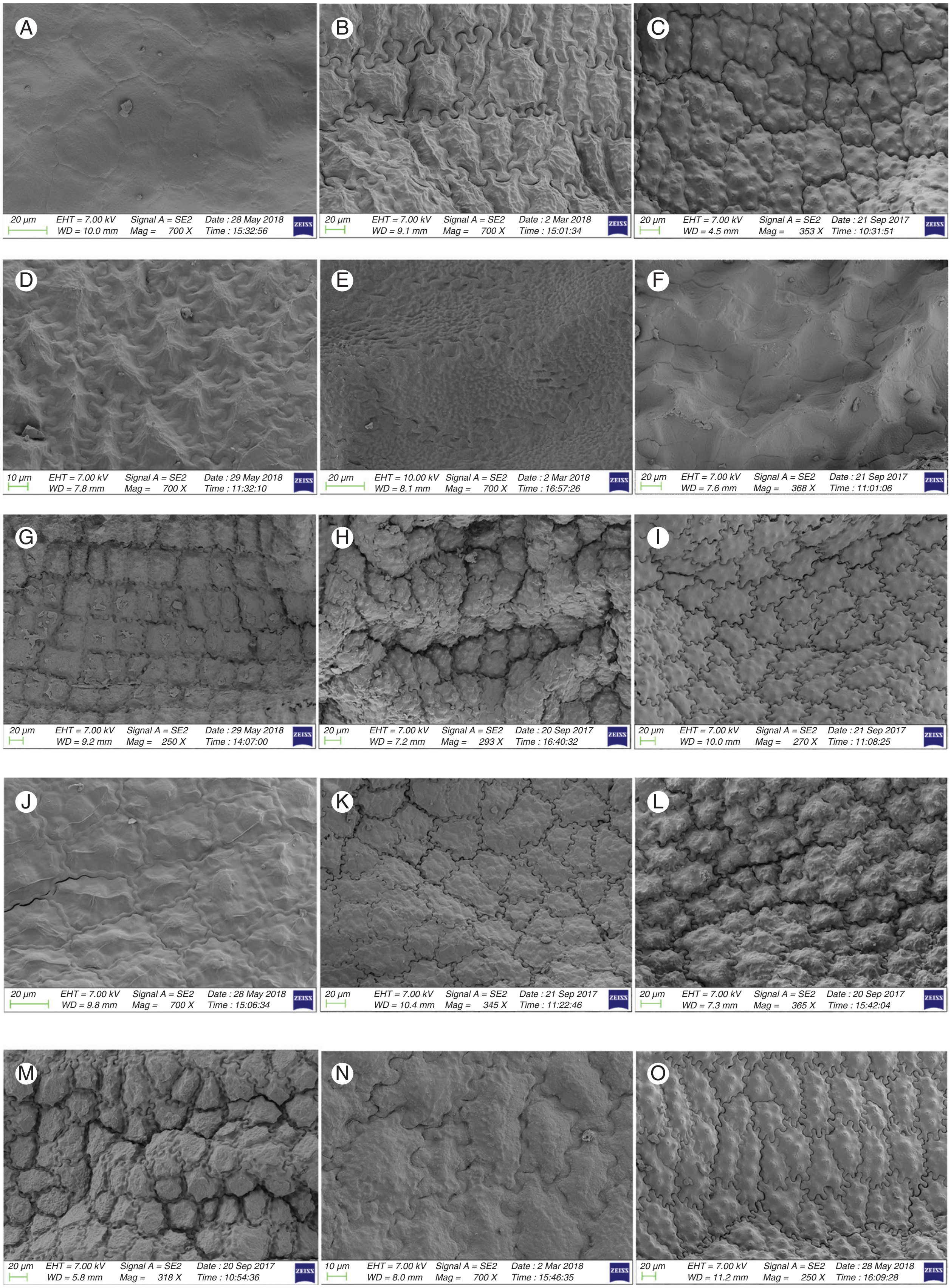


FIG. 9. SEM micrographs of seeds of 15 *Allium* species in subg. *Melanocrommyum*: (A) *A. verticillatum* (sect. *Verticillata*); (B) *A. costatovaginatum* (sect. *Acmopetala*); (C) *A. tschimganicum* (sect. *Acmopetala*); (D) *A. tashkenticum* (sect. *Acmopetala*); (E) *A. zergericum* (sect. *Acmopetala*); (F) *A. aroides* (sect. *Aroidea*); (G) *A. cristophii* (sect. *Asteroprason*); (H) *A. giganteum* (sect. *Compactoprason*); (I) *A. komarowii* (sect. *Compactoprason*); (J) *A. robustum* (sect. *Tulipifolia*); (K) *A. alexeianum* (sect. *Kaloprason*); (L) *A. baissunense* (sect. *Kaloprason*); (M) *A. protensum* (sect. *Kaloprason*); (N) *A. rhodanthum* (sect. *Kaloprason*); (O) *A. stipitatum* (sect. *Procerallium*).

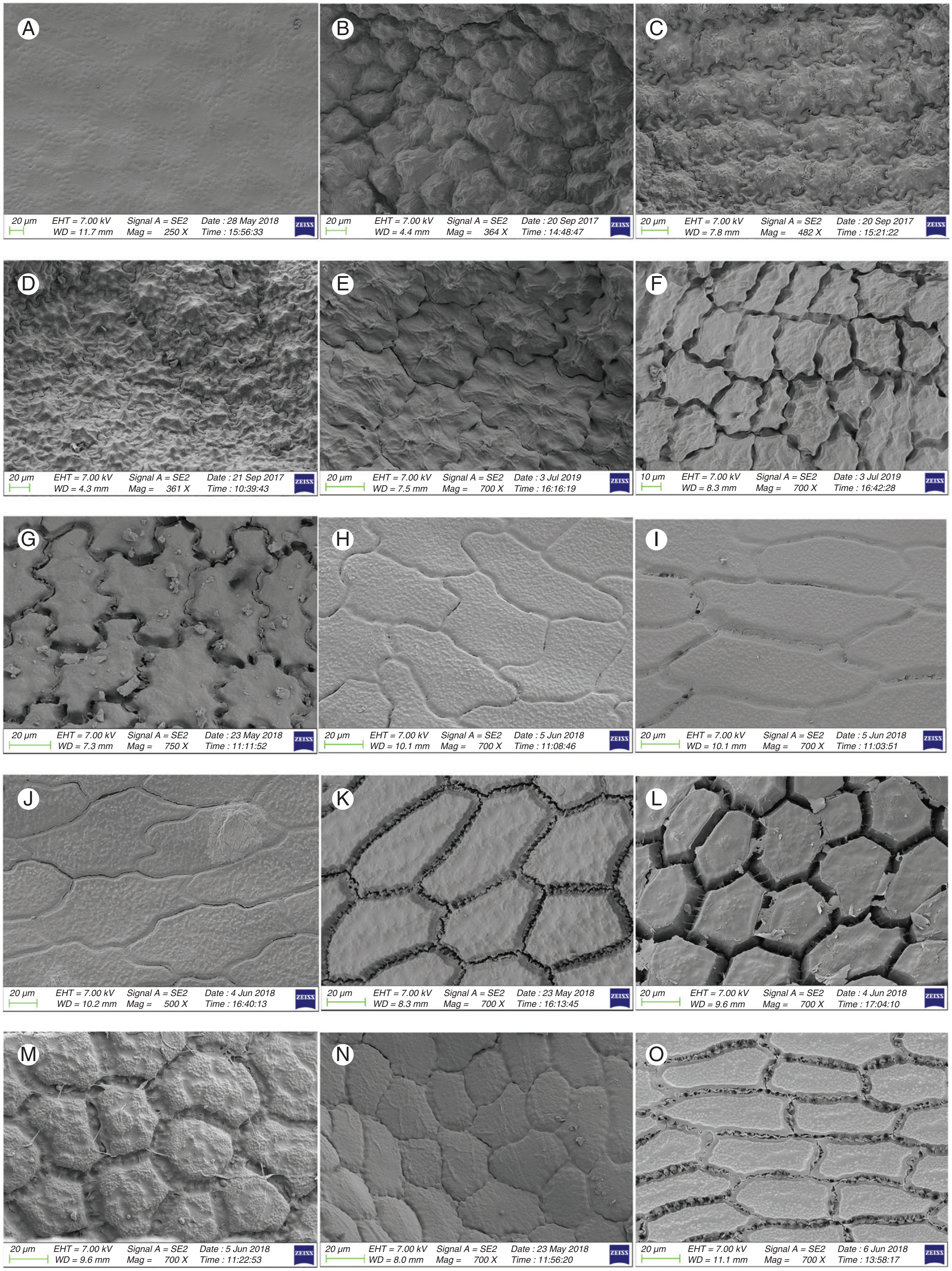


Fig. 10. SEM micrographs of seeds of 15 *Allium* species. (A–F) Subg. *Melanocrommyum*: (A) *A. regelii* (sect. *Regeloprason*); (B) *A. cupuliferum* (sect. *Regeloprason*); (C) *A. isakulii* (sect. *Regeloprason*); (D) *A. taeniopetalum* (sect. *Stellata*); (E) *A. cardiostemon* (sect. *Melanocrommyum*); (F) *A. woronowii* (sect. *Melanocrommyum*). (G–K) Subg. *Butomissa*: (G) *A. oreoprasum* (sect. *Austromontana*); (H) *A. ramosum* (sect. *Butomissa*); (I) *A. ramosum* (sect. *Butomissa*); (J) *A. tuberosum* (sect. *Butomissa*); (K) *A.* aff. *tuberosum*. (L, M) Subg. *Cyathophora*: (L) *A. mairei* (sect. *Coleoblastus*); (M) *A. cyathophorum* (sect. *Cyathophora*). (N, O) Subg. *Rhizirideum*: (N) *A. subangulatum* (sect. *Rhizomatosa*); (O) *A. przewalskianum* (sect. *Eduardia*).

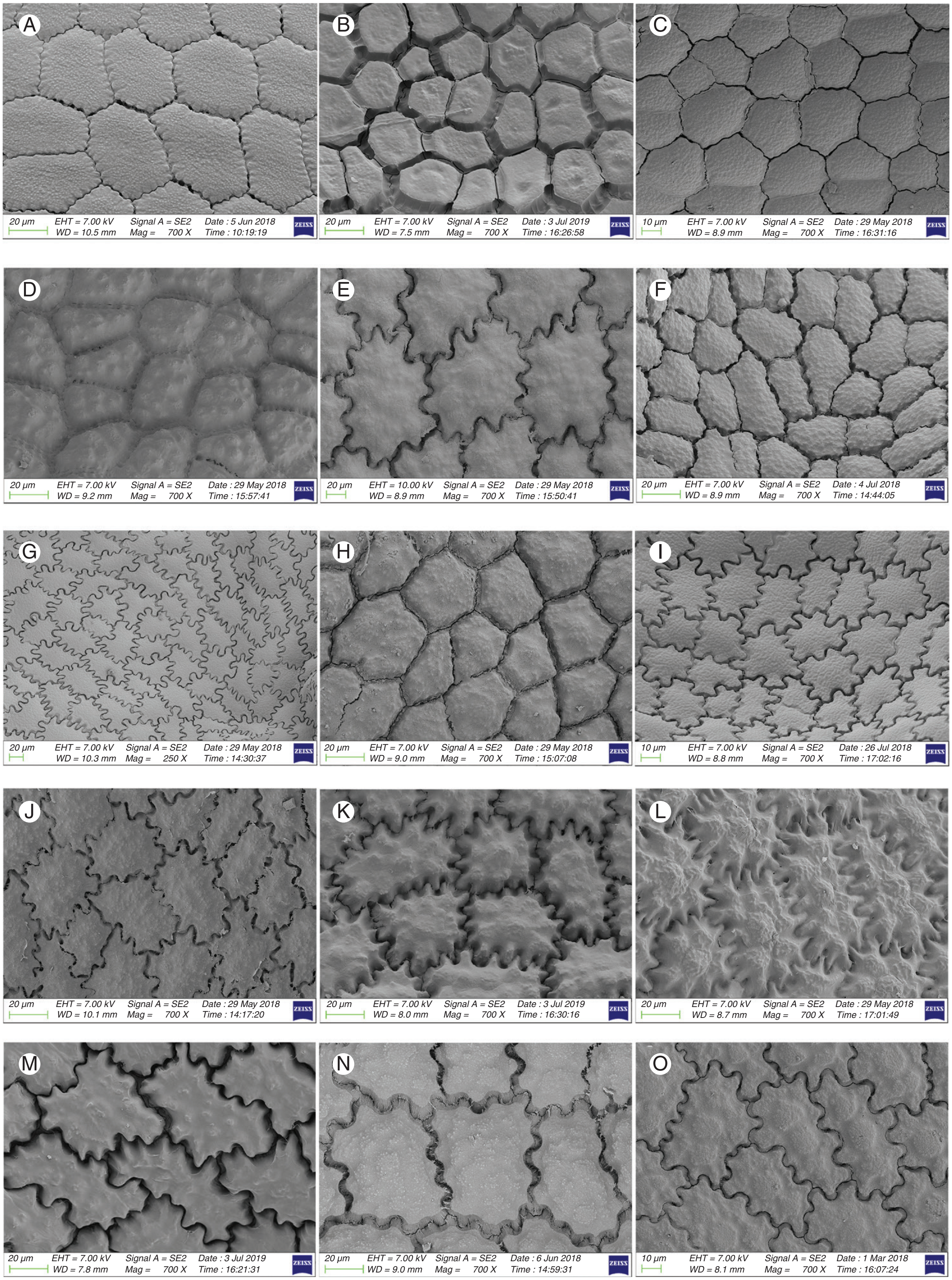


FIG. 11. SEM micrographs of seeds of 15 *Allium* species. (A, B) Subg. *Rhizirideum*: (A) *A. tenuissimum* (sect. *Tenuissima*); (B) *A. denudatum* (sect. *Rhizirideum*). (C–O) Subg. *Allium*: (C) *A. margaritae* (sect. *Brevispatha*); (D) *A. lenkoranicum* (sect. *Codonoprasum*); (E) *A. crystallinum* (sect. *Crystallina*); (F) *A. popovii* (sect. *Eremoprasum*); (G) *A. sabulosum* (sect. *Eremoprasum*); (H) *A. kopetdagense* (sect. *Kopetdagia*); (I) *A. anisotepalum* (sect. *Minuta*); (J) *A. minutum* (sect. *Minuta*); (K) *A. affine* (sect. *Allium*); (L) *A. atroviolaceum* (sect. *Allium*); (M) *A. aucheri* (sect. *Allium*); (N) *A. dictyoscordum* (sect. *Allium*); (O) *A. filidens* (sect. *Allium*).

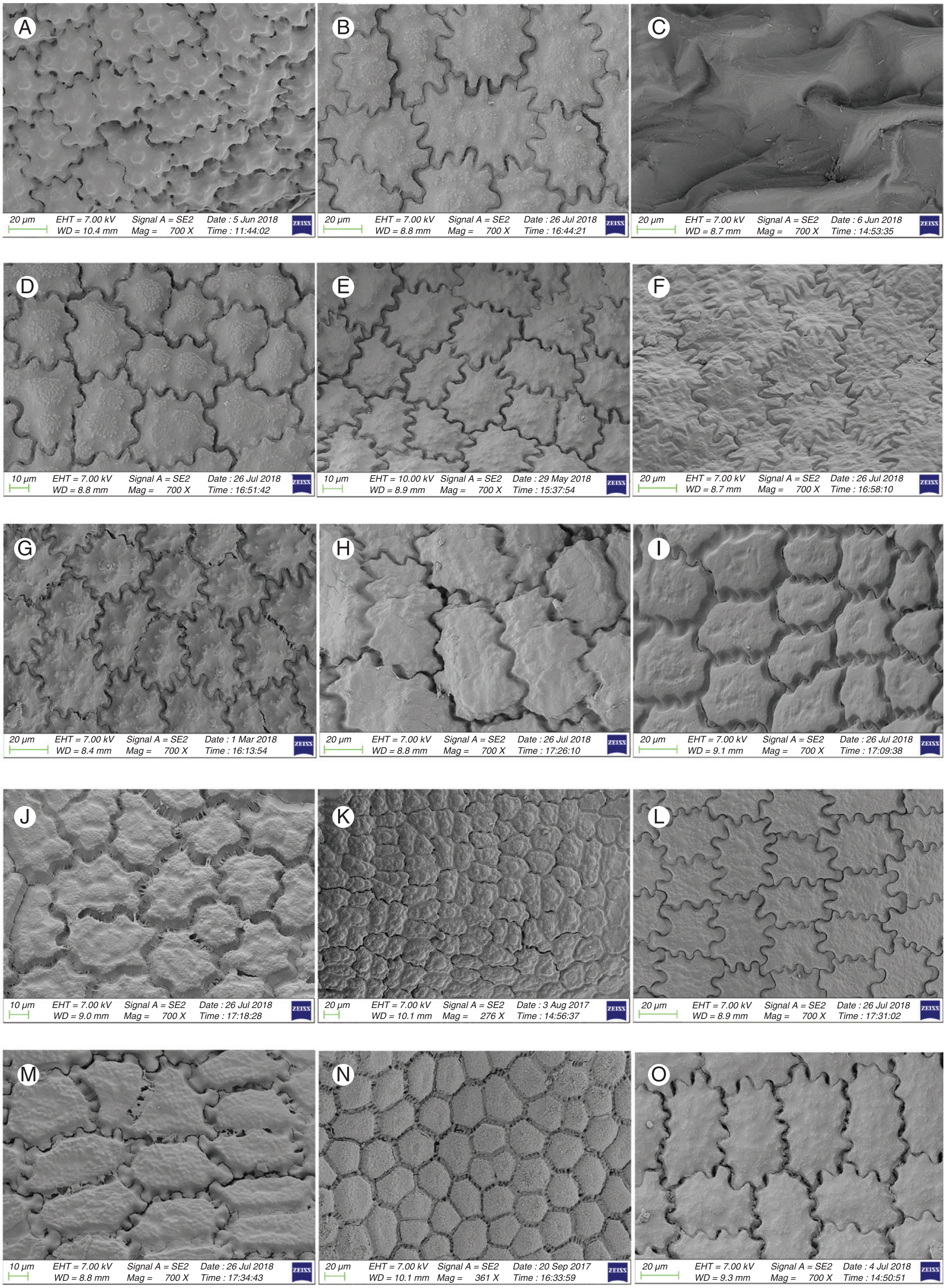


FIG. 12. SEM micrographs of seeds of 15 *Allium* species in subg. *Allium*: (A) *A. guttatum* (sect. *Allium*); (B) *A. ugami* (sect. *Allium*); (C) *A. vineale* (sect. *Allium*); (D) *A. fibrosum* (sect. *Avulsea*); (E) *A. griffithianum* (sect. *Avulsea*); (F) *A. pamiricum* (sect. *Avulsea*); (G) *A. brevidens* (sect. *Brevidentia*); (H) *A. ophiophyllum* (sect. *Brevidentia*); (I) *A. caesioides* (sect. *Coerulea*); (J) *A. elegans* (sect. *Coerulea*); (K) *A. caesium* (sect. *Coerulea*) (L) *A. delicatulum* (sect. *Coerulea*); (M) *A. caeruleum* (sect. *Coerulea*); (N) *A. turkestanicum* (sect. *Mediasia*); (O) *A. ferganicum* (sect. *Multicaulia*).

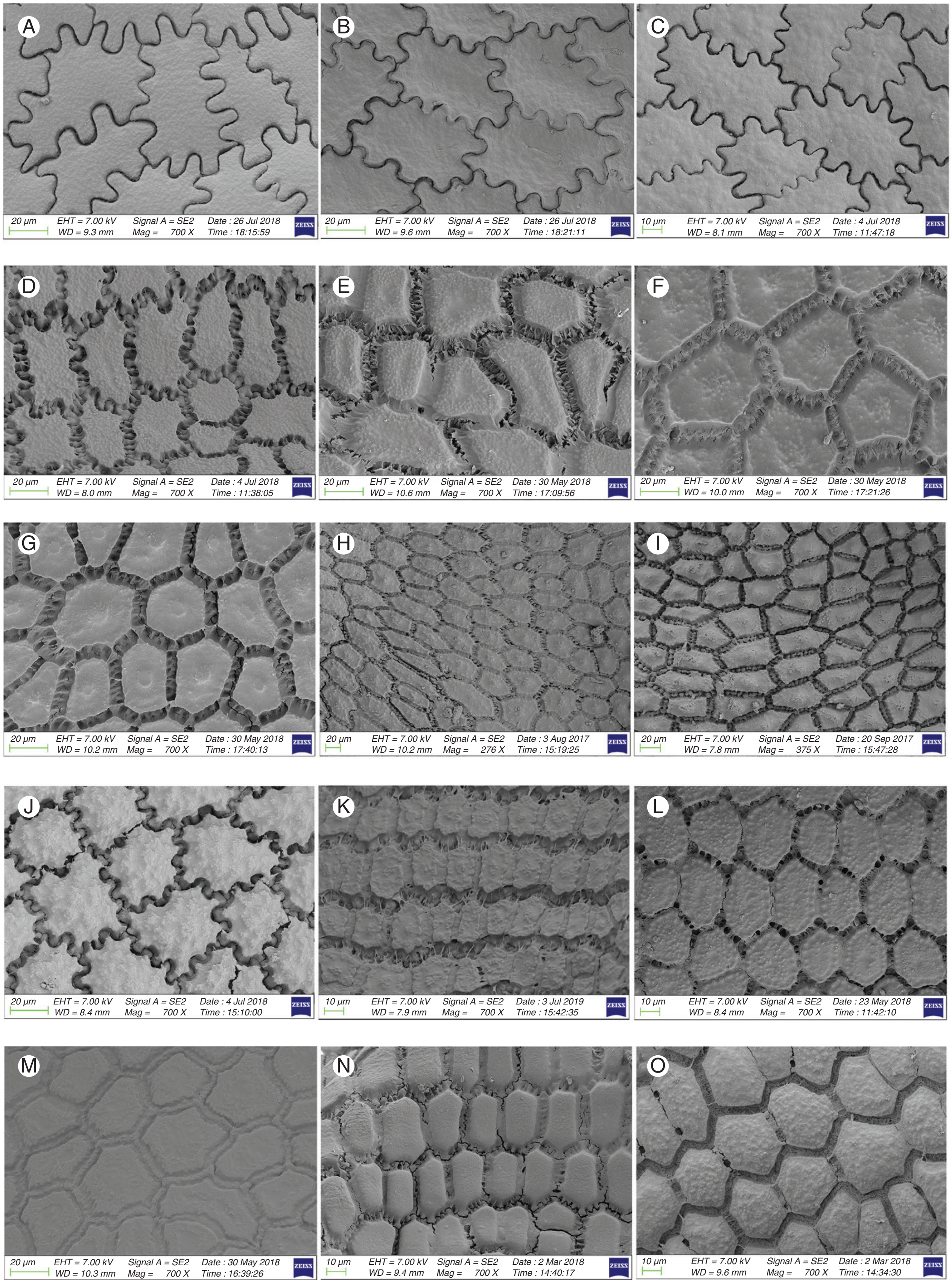


Fig. 13. SEM micrographs of seeds of 15 *Allium* species. (A–E) Subg. *Allium*: (A) *A. borszczowii* (sect. *Multicaulia*); (B) *A. borszczowii* (sect. *Multicaulia*); (C) *A. haneltii* (sect. *Haneltia*); (D) *A. pallasii* (sect. *Pallasia*); (E) *A. tanguticum* (sect. *Pallasia*). (F–J) Subg. *Reticulatobulbosa*: (F) *A. drobovii* (sect. *Nigrimontana*); (G) *A. lineare* (sect. *Reticulatobulbosa*); (H) *A. barsczewskii* (sect. *Campanulata*); (I) *A. eriocoleum* (sect. *Scabriscapa*); (J) *A. sulphureum* (sect. *Scabriscapa*). (K–N) Subg. *Polyprason*: (K) *A. albovianum* (*sect. Oreoprason*); (L) *A. korolkowii* (sect. *Falcatifolia*); (M) *A. carolinianum* (sect. *Falcatifolia*); (N) *A. talassicum* (sect. *Oreiprason*). (O) Subg. *Cepa*: *A. praemixtum* (sect. *Cepa*).

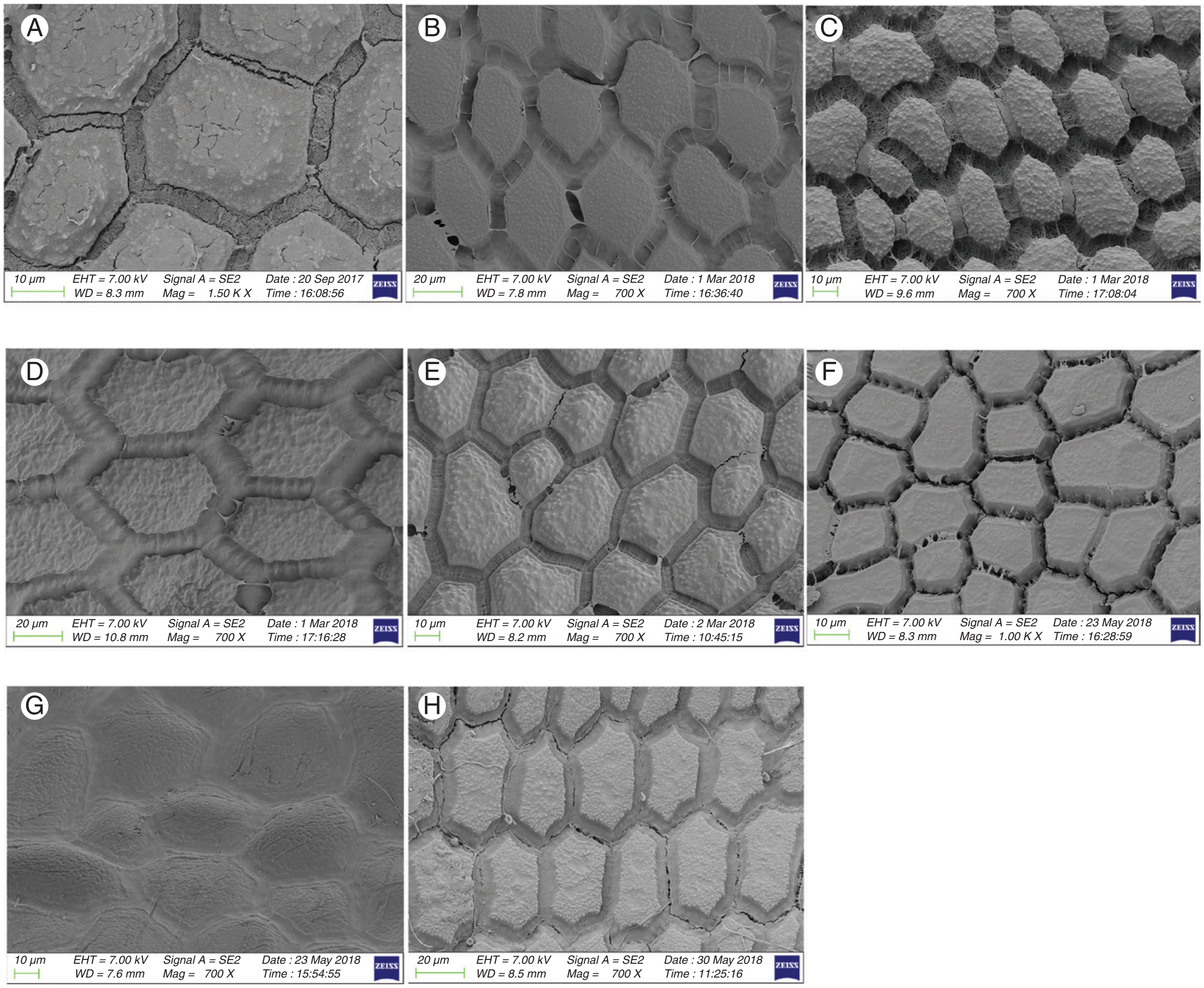


FIG. 14. SEM micrographs of seeds of eight *Allium* species in subg. *Cepa*: (A) *A. praemixtum* (sect. *Cepa*); (B) *A. altaicum* (sect. *Cepa*); (C) *A. galanthum* (sect. *Cepa*); (D) *A. pskemense* (sect. *Cepa*); (E) *A. oschaninii* (sect. *Cepa*); (F) *A. karelinii* (sect. *Schoenoprasum*); (G) *A. fedschenkoanum* (sect. *Annuloprason*); (H) *A. condensatum* (sect. *Condensatum*).

(section *Arctoprasum*). In comparing testa cell sculpturing of *A. tripedale* (Fig. 8A) and *A. ursinum* (Fig. 8B), we saw that they were to some extent similar. Our observations support Kruse's findings (1986). For example, undulation type (straight to arched), periclinal wall shape (gradually concave from edge to centre) and periclinal wall surface area of ten testa cells (0.012 mm^2) were the same, but these two species differed from each other in seed size, shape, cell arrangement and verrucae. This connection, to a certain extent, indicates taxonomic relationship. According to the molecular-based phylogenetic tree of Li *et al.* (2010), both of them belong to the first evolutionary lineage of *Allium*.

Allium tripedale (section *Nectaroscordum*) belongs to the first evolutionary lineage in the phylogenetic tree (Fig. 15) and shows mostly group-specific traits for the first evolutionary lineage (cell arrangement without inserted pattern, straight to arched anticlinal wall, concave periclinal wall, broadly ovoid seeds). However, the verrucae on the periclinal wall were intermediate in size rather than being small in this species.

Subgenus *Amerallium* Traub

The five species examined varied in seed shape from broadly ovoid (*A. moly* L., *A. ursinum*) to ovoid (*A. wallichii* Kunth, *A. geyeri* S. Watson) and narrowly ovoid (*A. validum* S. Watson). The L/W ratio ranged from 0.97 to 2.61 (Fig. 1B–F). The distance between testa cells was 0.001–0.013 mm, close, loose or loose with reticulate tissue arrangement, and the periclinal wall surface area of ten cells was 0.012–0.022 mm^2 (Table 2). The cells of anticlinal wall undulation type were mostly straight to arched, except for *A. moly* (section *Molium*) having arched to S-type (Fig. 8F). In most sections the periclinal wall type cells were flat with many small verrucae (sections *Caulorhizideum*, *Amerallium* Traub, *Bromatorrhiza* Ekberg) and gradually convex with several intermediate verrucae (section *Molium*) or gradually concave with large verrucae in the centre (section *Arctoprasum*). The dominant testa shape was five- to six-edged (Fig. 8B–F).

The species of section *Molium* were described by Kruse (1988) as having wide depressed channel-like anticlinal walls and specific verrucate testa patterns with a distinctly raised

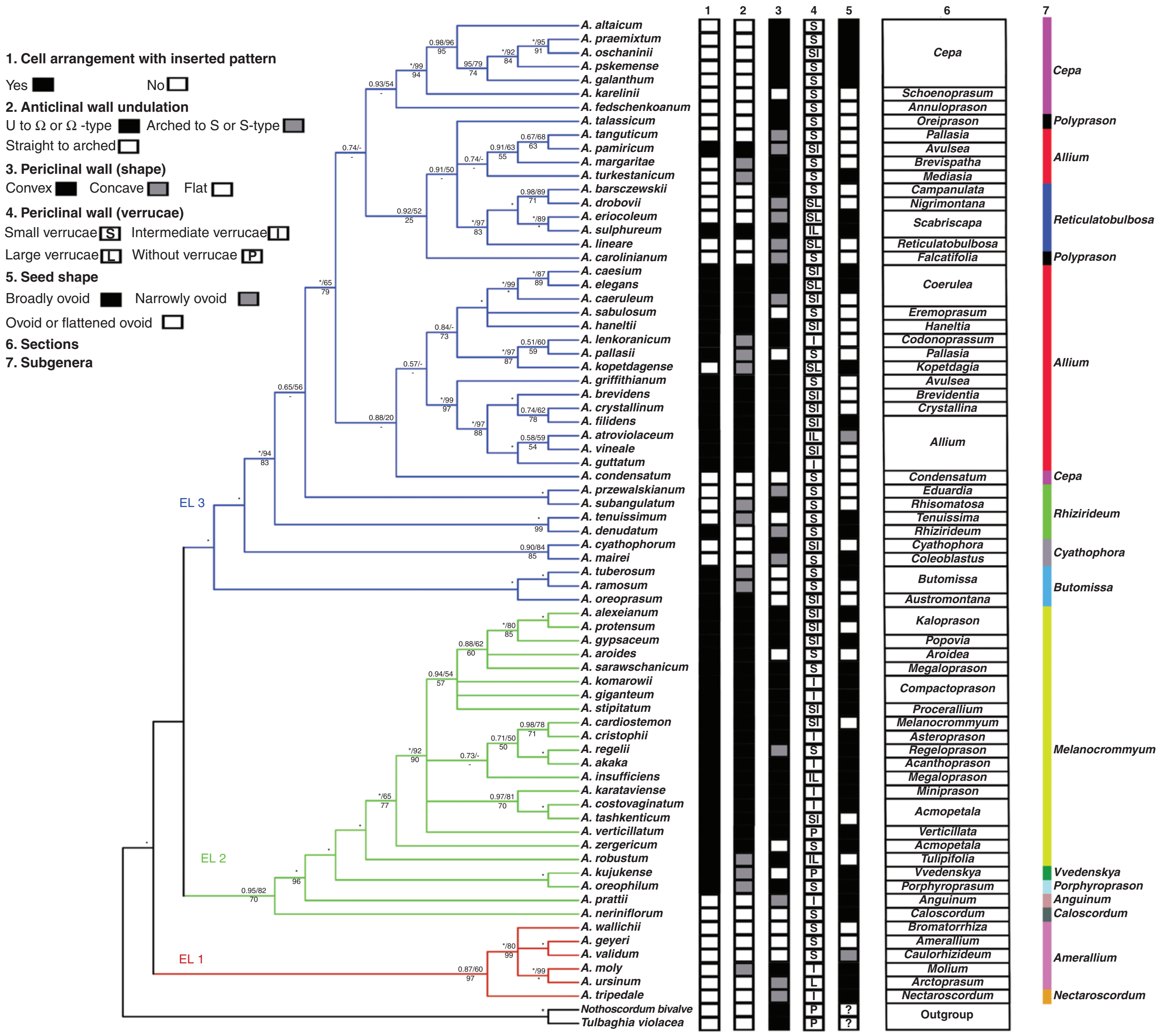
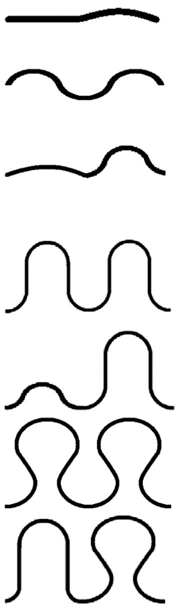


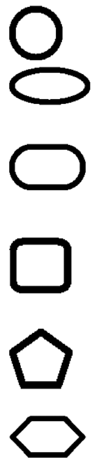
FIG. 15. The trees were constructed with Bayesian inference posterior probability/maximum parsimony, which are given on each branch; maximum likelihood is below branches. *Maximum support in all three analyses or maximum support value for only one or two methods. Morphological characteristics of five selected features were used to evaluate the three evolutionary lineages. The testa characteristics of species selected as outgroup [*Nothoscordum bivalve*, *Tulbaghia violacea* were taken from Kruse (1986)].

central verruca surrounded by small granules. However, Bednorz *et al.* (2011) found that *A. moly* had anticlinal walls typical for section *Molium* but periclinal walls were flatter and non-granulate although having similar verrucate sculpturing and S-like undulation. Our observations confirmed the findings of Bednorz *et al.* (2011) for the same species (*A. moly*).

All species of subgenus *Amerallium* analysed in the phylogenetic analysis were representatives of the first evolutionary lineage (Fig. 15). The majority showed traits of the first evolutionary lineage. Most characteristics of *A. ursinum* (section *Arctoprasum*) are primitive traits. However, *A. ursinum* shows advancement in the verrucae on the periclinal wall (large). *Allium moly* (section *Molium*) shows primitive characteristics (cell arrangement without inserted pattern, broadly ovoid seeds), intermediate characteristics (arched to S-type anticlinal walls, intermediate verrucae) and advanced characteristics in the convex periclinal wall. Most characteristics in *A. wallichii* (section *Bromatorrhiza*) are primitive and match the first evolutionary linage (except for ovoid or flattened ovoid seeds, which are an advanced characteristic). *Allium validum* (section *Caulorhizideum*) mostly reflects primitive characteristics. However, in terms of seed shape and periclinal wall it was intermediate (flat periclinal wall, narrowly ovoid). Most characteristics shown by *A. geyeri* (section *Amerallium*) are primitive with the exception of the flat periclinal wall (intermediate trait) and ovoid or flattened ovoid seeds (advanced character) (Fig. 15).

TABLE 3. *Dominant curvature types of anticlinal wall and dominant testa cell shapes*





A. Anticlinal wall: dominant curvature type		B. Dominant testa cell shape	
Straight to arched	7	Orbicular	1
S-type	6	Elliptic	2
Arched to S-type	5	Oblong	3
U-type	4	Rectangular	4
S- to U-type	.3	5 edged	5
Ω-type	2	6 edged	6
U- to Ω-type	1		

Subgenus *Caloscordum* (Herb.) R.M. Fritsch

Only *A. neriniflorum* (Herb.) Baker was investigated in section *Caloscordum* (Herb.) R.M. Fritsch (Table 2). The seeds of *A. neriniflorum* were broadly ovoid and shrivelled, were 1.71–1.63 mm long and had an L/W ratio of 1.05 (Fig. 1G). The distance between testa cells was 0.0009–0.004 mm and the reticulate tissue was loosely arranged. The surface area of ten testa cells was 0.012 mm^2, seed testa cell anticlinal wall undulation type was straight to arched, the periclinal wall was slightly convex with many small verrucae, and the dominant testa shape was six-edged (Fig. 8G).

According to Choi *et al.* (2012) four types of moderately flat periclinal walls can be distinguished in *Allium* (smooth, minutely roughened, granulate and verrucate). *Allium neriniflorum* exhibits the smooth type and lacks micro relief. Our observations confirmed this finding. In addition, we observed verrucae on slightly convex periclinal walls in this species.

Allium neriniflorum (section *Caloscordum*) shows all characteristics of the first evolutionary line (cell arrangement without inserted pattern, straight to arched anticlinal walls, flat periclinal wall, broadly ovoid seeds). Therefore, its position is in the basal part of the second evolutionary line (Fig. 15).

Subgenus *Anguinum* (G. Don ex Koch) N. Friesen

Only *A. prattii* C.H. Wright was investigated in section *Anguinum* (Table 2). It is native to Eastern Asia. The seeds of *A. prattii* were broadly ovoid and 2.20–1.66 mm long, and L/W ratio was 1.32 (Fig. 1G). The distance between testa cells was 0.005–0.01 mm and close with an inserted pattern, surface area of ten testa cells was 0.007 mm^2, seed testa cell anticlinal wall undulation was straight to arched, the periclinal wall was gradually concave from edge to centre, there were intermediate to large verrucae, and the dominant testa shape was six-edged (Fig. 8H).

Choi *et al.* (2012) examined three species (*A. microdictyon* Prokh., *A. ochotense* Prokh., *A. tricoccum* Aiton) representing subgenus *Anguinum*. The micromorphology of the seeds of these species were similar and showed nearly smooth periclinal walls and straight anticlinal walls with a convex channel. Our observations were partly congruent with these reports as our results showed intermediate to large verrucae on gradually concave periclinal walls and straight to arched anticlinal walls for *A. prattii*.

Allium prattii (section *Anguinum*) shows characteristics of the first evolutionary lineage (cell arrangement without inserted pattern, straight to arched anticlinal wall, flat periclinal wall, broadly ovoid seeds). Therefore, its position in the phylogenetic tree is in the basal part of the second evolutionary lineage (Fig. 15). However, in terms of verrucae on the periclinal walls, *A. prattii* has intermediate type verrucae.

Subgenus *Porphyroprason* (Ekberg) R.M. Fritsch

Allium oreophilum was investigated from section *Porphyroprason* (Ekberg) R.M. Fritsch (Table 2). *Allium oreophilum* is native to Asia. The seeds of *A. oreophilum* were broadly ovoid, the seeds were 2.39–1.95 mm long, and the L/W ratio was 1.23 (Fig. 1I). The distance between testa cells was 0.0009–0.004 mm and loose with inserted pattern, surface area of ten testa cells was 0.015 mm^2, seed testa cell anticlinal wall undulation type was S-type, the periclinal wall was convex with many small verrucae, and the dominant testa shape was oblong (Fig. 8I).

Lin and Tan (2017) examined two sections (*Oreiprason* F. Herm., section *Falcatifolia* N. Friesen) of subgenus *Porphyroprason* and found them to be characterized by having straight to arched anticlinal walls and concave periclinal walls with different sized verrucae. Baasanmunkh *et al.* (2021) also investigated these sections and reported that all the species examined possessed almost the same characteristics: straight anticlinal walls and densely granulate periclinal walls. However, *A. hymenorrhizum* Ledeb. showed one central verruca and small marginal verrucae. Our observation is partly congruent with the above-mentioned studies as *A. oreophilum* showed many small verrucae on convex periclinal walls and S-type anticlinal walls.

Allium oreophilum (section *Porphyroprasum*) shows the characteristics that belong to the second and third evolutionary lineages in the phylogenetic tree (Fig. 15). However, in terms of periclinal wall verrucae type and seed shape the species shows group-specific characters for the first evolutionary lineage (small verruca type, broadly ovoid seeds).

Subgenus *Vvedenskya* (Kamelin) R.M. Fritsch

Only *A. kujukense* was investigated from section *Vvedenskya* (Table 2), which is native to Kazakhstan. The seeds of *A. kujukense* were broadly ovoid and 2.05–1.56 mm long, with an L/W ratio of 1.31 (Fig. 1J). The distance between testa cells was 0.002–0.005 mm and loose with inserted pattern, surface area of ten testa cells was 0.01 mm^2, seed testa cell anticlinal wall undulation type was arched to S-type, the periclinal wall was flat to convex without verrucae, and the dominant testa shape was oblong (Fig. 8J).

Our study was the first to investigate *A. kujukense*. The testa cell structure was similar to that of *A. oreophilum* in our study, with a loose inserted pattern, oblong testa cells dominating. However, there were differences in the periclinal walls with many small verrucae (plane for *A. kujukense*) and undulation type of anticlinal wall (S-type) in *A. oreophilum.*

Allium kujukense differs from *A. oreophilum* by having a flat periclinal wall without verrcuae (Fig. 15).

Subgenus *Melanocrommyum* (Webb & Berth.) Rouy

Twenty-six species from 15 sections of subgenus *Melanocrommyum* were investigated (Table 2). The seeds were broadly ovoid or ovoid shrivelled (*A. akaka* S. G. Gmelin ex Schult. & Schult. f., *A. insufficiens* Vved., *A. sarawschanicum* Regel, *A. karataviense* Regel, *A. gypsaceum* Popov & Vved., *A. costatovaginatum* Kamelin & Levichev, *A. tschimganicum* B. Fedtsch., *A. zergericum* F.O. Khass. & R.M. Fritsch, *A. cristophii* Trautv., *A. giganteum* Regel, *A. komarowii* Lipsky, *A. alexeianum* Regel, *A. baissunense* (Lipsky) F.O. Khass. & R.M. Fritsch, *A. stipitatum* Regel, *A. regelii* Trautv., *A. cupuliferum* Regel, *A. isakulii* R.M. Fritsch & F.O. Khass., *A. taeniopetalum* Popov & Vved., *A. woronowii* Miscz. ex Grossh.), flattened ovoid (shrivelled) (*A. verticillatum* Regel, *A. aroides* Popov & Vved., *A. protensum* Wendelbo, *A. tashkenticum*, *A. cardiostemon* Fisch. & C.A. Mey.) and ovoid (*A. robustum* Kar. & Kir.) (Figs 1K–3F) with an L/W ratio of 1.2–1.97 (Table 1). The distance between testa cells was 0–0.009 mm and close or loose with inserted pattern, and the area of ten testa cells was 0.008–0.03 mm^2. The seed testa cell anticlinal wall undulation type was mostly U- to Ω-type (*A. akaka*, *A. karataviense*, *A. cristophii*, *A. giganteum*, *A. alexeianum*, *A. rhodanthum*, *A. protensum*, *A. stipitatum*, *A. regelii*, *A. cupuliferum*, *A. isakulii*) and Ω type (*A. gypsaceum*, *A. costatovaginatum*, *A. zergericum*, *A. tashkenticum*, *A. cardiostemon*), rarely S- to U-type (*A. insufficiens*, *A. tschimganicum*, *A. woronowii*) and S-type (*A. robustum*). The periclinal walls were mostly convex with one to eight intermediate verrucae. The dominant testa shape was elliptic to oblong (Figs 8K–10F).

According to previous studies (Fritsch *et al.*, 2006; Neshati and Fritsch, 2009; Choi and Cota-Sanchez, 2010; Bednorz *et al.*, 2011; Celep *et al.*, 2012; Lin and Tan, 2017) convex periclinal walls with several large verrucate sculptures and combined S- to Ω-type undulate anticlinal walls are the most characteristic of this subgenus. We examined 15 sections in this subgenus. Our observations confirmed previous findings. Earlier papers (Kruse, 1994; Fritsch *et al.*, 2006) suggested that among species of subgenus *Melanocrommyum*, *A. aroides* (section *Aroidea*) and *A. verticillatum* (section *Verticillata*) had more or less flat and granulose periclinal cell walls without verrucae. Our findings matched these characteristics for the same species.

Nineteen species of subgenus *Melanocrommyum* were used in the phylogenetic analysis. Most species showed characteristics of the third evolutionary lineages in the phylogenetic tree (Fig. 15). *Allium robustum* (section *Tulipifolia* R.M. Fritsch & N. Friesen) mostly showed advanced characteristics (cellular arrangement with inserted pattern, convex periclinal wall with large and small verrucae, ovoid or flattened ovoid seeds). However, in terms of anticlinal wall type *A. robustum* exhibited the arched to S or S undulation type. Characteristics of *A. zergericum* and *A. costatovaginatum* (section *Acmopetala* R.M. Fritsch) are primitive (small verrucae, broadly ovoid seeds for *A. zergericum*; broadly ovoid seeds for *A. costatovaginatum*) the remaining traits as advanced characteristics. Two species of section *Compactoprason* R.M. Fritsch (*A. komarowii A. giganteum*) were used in the phylogenetic analysis. Those two species shared the same advanced seed characteristics (cell arrangement with inserted pattern, U- to Ω-type or Ω-type anticlinal wall, convex periclinal wall), intermediate characteristics (intermediate verrucae) and primitive characteristics (broadly ovoid seeds). Two species of section *Megaloprason* Webb et Berth. (*A. insufficiens*, *A. sarawschanicum*) were used in the phylogenetic analysis. These two species show the same seed characteristics of a primitive character (broadly ovoid seeds) and other advanced characteristics. The seeds of *A. gypsaceum* (section *Popovia* F.O. Khass. & R.M. Fritsch) show primitive characteristics (broadly ovoid seeds), intermediate characteristics (small and intermediate verrucae) and other characteristics as advanced traits. *Allium aroides* (section *Aroidea*) showed primitive characteristics (small verrucae) and intermediate characteristics (flat periclinal wall), with the remaining characteristics advanced. Most characteristics shown by *A. stipitatum* (section *Procerallium* R.M. Fritsch) were advanced. However, in the type of verrucae (small and intermediate) and seed shape (broadly ovoid) the species reflects primitive characteristics. Two species of section *Kaloprason* C. Koch (*A. alexeianum*, *A. protensum*) were used in the phylogenetic analysis. These two species have almost the same primitive seed characteristics (small and intermediate verrucae), and other advanced characteristics. However, *A. alexeianum* reflected primitive seed characteristics (broadly ovoid). *Allium karataviense* (section *Minipraso*n R.M. Fritsch) showed mostly advanced and primitive characteristics (broadly ovoid seeds), intermediate (intermediate and small verrucae). Almost all the characteristics of *Allium tashkenticum* and *A. costatovaginatum* (section *Acmopetala* R.M. Fritsch) are advanced. Most characteristics reflected by *A. cristophii* (section *Asteroprason*) are advanced characteristics. However, in terms of verruca type this species shows advanced traits (small and intermediate verrucae). *Allium cardiostemon* (section *Melanocrommyum*) shows the characteristics that belong to the third evolutionary lineage (advanced). However, regarding verrucae type the species shows primitive traits (small and intermediate verrucae). *Allium akaka* (section *Acanthoprason*) shows characteristics that are primitive (broadly ovoid seeds), intermediate (intermediate verrucae type) and advanced (remaining traits). Most characteristics shown by *A. verticillatum* (section *Verticillata*) are advanced characteristics. But in terms of seed shape and verrucae type the taxon has primitive characteristics (broadly ovoid, without verrucae) (Fig. 15).

Subgenus *Butomissa* (Salisb.) N. Friesen

In this subgenus three species (five samples) from two sections were investigated (Table 2). The seeds of this subgenus were ovoid and L/W ratio was 1.45–1.74 (Fig. 3G–K).

The distance between testa cells was 0.002–0.009 mm and loose with reticulate tissue, and the area of ten testa cells was 0.018–0.04 mm^2. The seed testa cell anticlinal wall undulation type was mostly arched to S-type (section *Butomissa*) or S- to U-type (section *Austromontana* N. Friesen). The periclinal walls of this subgenus were mostly flat (somewhat slightly convex or concave) with many small verrucae. The dominant testa shape was elliptic (Fig. 10G–K).

According to previous papers (Kruse, 1984, 1986, 1988, 1994; Ilarslan and Koyuncu, 1997; Fritsch *et al.*, 2006), dominant sculpture patterns in the subgenus *Butomissa* were straight anticlinal walls and granulous sculptures of the periclinal walls. After a few years Choi *et al.* (2012) also found irregularly curved anticlinal wall boundaries covered with granulate periclinal walls in two species: *A. ramosum* L. and *A. tuberosum* (section *Butomissa*). But afterwards Lin and Tan (2017) examined three species (*A. tuberosum*, *A. ramosum*, *A. oreoprasum* Schrenk) and described more diverse sculpture patterns in terms of periclinal and anticlinal walls. In addition to sculptural characteristics described previously, we found convex and concave periclinal walls, a close cellular arrangement dominating an arched to S-type anticlinal wall in *A. oreoprasum*. Baasanmunkh *et al.* (2021) distinguished the two sections (*Butomissa* and *Austromontana*) on the basis of seed shape. In our research the seed shapes of the two studied sections were also easily distinguished. We examined the two samples of *A. ramosum* from Russia and Uzbekistan. Although the samples were from different places the testa cell ornamentation of samples has nearly the same pattern.

Three species of this subgenus were used in the phylogenetic analysis (Fig. 15). Two species, *A. ramosum* and *A. tuberosum* (section *Butomissa*) show nearly the same characters of small verrucae (broadly ovoid for *A. tuberosum*) as primitive characteristics, a flat periclinal wall, arched to S or S-type anticlinal wall as intermediate characteristics, and remaining traits as advanced. *Allium oreoprasum* (section *Austromontana*) shows both primitive (cell arrangement with inserted pattern, small and intermediate verrucae) and advanced traits (convex periclinal wall, U- to Ω-type or Ω-type, ovoid or flattened ovoid seed shape).

Subgenus *Cyathophora* (R.M. Fritsch) R.M. Fritsch

In this subgenus two species from two sections were investigated (Table 2). The seeds were flattened ovoid or broadly ovoid, and the L/W ratio was 1.35–2 (Fig. 3L, M). The distance between testa cells was 0.004–0.01 mm and loose with reticulate tissue, and the area of ten testa cells was 0.013–0.015 mm^2. The seed testa cell anticlinal wall undulation type was strongly straight to arched. The periclinal walls were gradually convex or gradually concave with one large verruca in the centre or small to intermediate verrucae, but always granulose verrucae. The dominant testa shape was six-edged (Fig. 10L, M).

Two species, *A. mairei* H. Lév. and *A. cyathophorum* Bur. et Franch., belonging to sections *Coleoblastus* Ekberg. and *Cyathophora* R.M. Fritsch, were examined in this research. The seed coat patterns of the two sections are rather similar. The differences between the two sections were only in the shapes of periclinal walls. When compared with other related subgenera the testa cell characteristics of this subgenus were rather similar to those of subgenus *Reticulatobulbosa* as the subgenus had straight to arched anticlinal walls and gradually concave or convex periclinal walls from edge to centre. However, each species of subgenus *Reticulatobulbosa* showed one large granulose verruca in centre.

All examined species of this subgenus were used in the phylogenetic analysis (Fig. 15). Most species show the characteristics belonging to the first and second evolutionary lineages despite their place in the third evolutionary lineage. *Allkum mairei* (section *Coleoblastus*) has only primitive characteristics (cell arrangement without inserted pattern, straight to arched anticlinal wall, small verrucae, broadly ovoid seeds, concave periclinal wall), whereas *A. cyathophorum* (section *Cyathophora*) shows primitive characteristics (cell arrangement without inserted pattern, straight to arched anticlinal wall, small and intermediate verrucae) and advanced characteristics (convex periclinal wall, ovoid or flattened ovoid seed shape).

Subgenus *Rhizirideum* G. Don f. ex Koch

In this subgenus four species from four sections were investigated (Table 2). The seeds were flattened ovoid or (broadly) ovoid, and the L/W ratio was 1.58–1.87 (Figs 3N–O and 4A, B). The distance between testa cells was 0–0.008 mm and loose with reticulate tissue, and the area of ten testa cells was 0.007–0.016 mm^2. The seed testa cell anticlinal wall undulation type was variable, showing transitions from straight to arched and to S-type forms, always with a marginal bulge. The periclinal walls were flat to convex (except in *A. przewalskianum* Regel, where they were concave at the centre) with many intermediate verrucae, which gave rise to a marginal bulge on the edge. The dominant testa shape was six-edged (Figs 10N–O and 11A, B).

The species *A. subangulatum* Regel, *A. przewalskianum*, *A. tenuissimum* L. and *A. denudatum* Redouté, belonging to sects *Rhizirideum* Traub, *Rhizomatosa* Egorova, *Eduardia* N. Friesen and *Tenuissima* (Tzagolova) Hanelt., were examined. Bednorz *et al.* (2011) examined *A. nutans* L. (section *Rhizirideum*) and found that the species had hollowly depressed, straight, strip-like anticlinal walls and periclinal walls that were convex and verrucate with central verruca periclinal walls. Lin and Tan (2017) examined two species of section *Rhizirideum* (*A. nutans*, *A. senescens* L.) and found that the species possesses small to intermediate verrucae on flat or gradually concave periclinal walls and straight to arched anticlinal walls. Our results support these previous authors' findings. Based on phylogenetic molecular research (Friesen *et al.*, 2020), sections *Caespitosoprason* and *Rhizomatosa* are the same sections or synonyms. According to previous reports (Kruse, 1988; Choi *et al.*, 2012; Lin and Tan, 2017; Baasanmunkh *et al.*, 2021), most species in section *Rhizirideum* were characterized by having straight anticlinal walls and convex periclinal walls with intermediate verrucae or granules. Our observations support these findings, but *A. subangulatum* possesses an S-type anticlinal wall. Lin and Tan (2017) examined *A. przewalskianum* (section *Eduardia*) and found straight to arched anticlinal walls and gradually concave periclinal walls, which is similar to our findings.

S-type anticlinal walls were reported for *A. anisopodium* Ledeb. and *A. tenuissimum* (Kruse, 1988; Choi *et al.*, 2012), and for *A. anisopodium* Ledeb., *A. tenuissimum* and *A. vodopjanovae* N. Friesen (Baasanmunkh *et al.*, 2021). We examined the type species of section *Tenuissima* (*A. tenuissimum*) and our findings were congruent with all of the above-mentioned findings.

The four species of subgenus *Rhizirideum* were used in the phylogenetic analysis (Fig. 15). Most species showed characteristics of the first and second evolutionary lineages despite their place in the third evolutionary lineage. *Allium tenuissimum* (section *Tenuissima*) showed primitive (cell arrangement without inserted pattern, small verrucae, broadly ovoid seeds) and intermediate characteristics (arched to S or S-type anticlinal wall, flat periclinal wall). Almost all of the characteristics of *A. przewalskianum* (section *Eduardia*) were primitive except for the seed shape (ovoid or flattened ovoid). *Allium subangulatum* (section *Rhizomatosa* Egorova) showed primitive (cell arrangement without inserted pattern, small verrucae), intermediate (arched to S or S-type anticlinal wall) and advanced characteristics.

Subgenus *Allium*

Thirty-two species from 14 sections of subgenus *Allium* were investigated (Table 2). The seeds were (broadly) ovoid, except for species of section *Minuta* F.O. Khass. and some species of section *Allium*, which were narrowly ovoid. The L/W ratio was 1.27–2.39 (Figs 4C–6E). The distance between testa cells was 0–0.06 mm and loose with inserted pattern and with reticulate tissue, and the area of ten testa cells was 0.006–0.024 mm^2. The seed testa cell anticlinal wall undulation in subgenus *Allium* was known to vary from arched to S-, U- and Ω-type, but in this study the species never exhibited the straight to arched type. The periclinal walls were convex to concave with many small, intermediate and large verrucae. The dominant testa shape was oblong to elliptic (Figs 11C–13E).

Due to the diversity and richness of subgenus *Allium* among other subgenera, a perfect phylogenetic classification of this complex subgenus has not been perfectly developed. According to previous papers (Fritsch *et al.*, 2006; Neshati and Fritsch, 2009; Choi and Cota-Sanchez, 2010; Bednorz *et al.*, 2011; Celep *et al.*, 2012; Lin and Tan, 2017; Veiskarami *et al.*, 2018; Baasanmunkh *et al.*, 2021) macromorphologies and testa cell sculpture were highly variable in subgenus *Allium*. In addition, Celep *et al.* (2012), Lin and Tan (2017), Veiskarami *et al.* (2018) and Baasanmunkh *et al.* (2021) reported that the testa cells of the seeds of most species of the subgenera *Allium* and *Melanocrommyum* were rather similar. They both showed convex periclinal walls with several large verrucae and S- to Ω-type anticlinal walls. We examined sections *Brevispatha* Valsecchi, *Codonoprasum* Rchb., *Crystallina* F.O. Khassanov et S.C. Yengalycheva, *Eremoprasum* (Kamelin) F.O. Khass., R.M. Fritsch et N. Friesen, *Kopetdagia* F.O. Khassanov, *Minuta*, *Allium* Wendelbo, *Avulsea* F.O. Khassanov, *Brevidentia* F.O. Khass. et Yengalycheva, *Coerulea*, *Mediasia* F.O. Khass., S.C. Yengalycheva et N. Friesen, *Multicaulia* F.O. Khass. et S.C. Yengalycheva, *Haneltia* and *Pallasia* (Tzagolova) F.O. Khass., R.M. Fritsch et N. Friesen of subgenus *Allium*.

Cesmedziev and Terzijski (1997) found convex periclinal walls that were concave in the centre in section *Brevispatha*, but Celep *et al.* (2012) examined three members of section *Brevispatha* and found that they had flat, granulous periclinal walls with flat verrucae and straight to curved anticlinal walls. Our observations of *A. margaritae* B. Fedtsch. confirmed their findings, but the species we examined did not have a concavity in the centre. Kruse (1994) reported that section *Brevispatha* showed a general similarity to the seed surface pattern of section *Codonoprasum*. In our study we examined *A. lenkoranicum* Miscz. ex Grossh., representing section *Codonoprasum* and found that this species was similar to *A. margaritae*; these two sections share the same group (the second clade) of subgenus *Allium* according to a previous molecular phylogenetic study (Friesen *et al.*, 2006). Veiskarami *et al.* (2018) also examined representatives of the first and the second groups of this subgenus and suggested that potential applicability of anticlinal wall traits present synapomorphy for certain clades. In our research the sections belonging to the second group of subgenus *Allium* showed more ancient characteristics and at the same time the first group showed the advanced character state. In our research, a majority of the sections of subgenus *Allium* supported the evolutionary clades within subgenus *Allium* suggested in the molecular phylogenetic study by Friesen *et al.* (2006). Two samples of *A. borszczowii* from different places were examined. Although the samples were collected from different places (Uzbekistan and Turkmenistan) the testa cell pattern has nearly the same ornamentation.

Nineteen species of subgenus *Allium* were used in the phylogenetic analysis. Most species showed characteristics of the third evolutionary lineages in the phylogenetic tree (Fig. 15). Characteristics of *A. margaritae* (section *Brevispatha*) belonged to three evolutionary lines (cell arrangement without inserted pattern and small verrucae as primitive characteristics, arched to S or S-type as intermediate character, convex periclinal wall and ovoid or flattened ovoid seeds as advanced characteristics). Seed testa characteristics of *A. turkestanicum* Regel (section *Mediasia*) were similar to those of *A. margaritae* (section *Brevispatha*). However, the only difference was in seed shape (broadly ovoid for *A. turkestanicum*). All characteristics shown by *A. crystallinum* Vved. (section *Crystallina*) are advanced (except for small and intermediate verrucae). *Allium filidens* Regel, *A. atroviolaceum* Boiss., *A. guttatum* Steven and *A. vineale* L. are displayed in the phylogenetic tree as representatives of section *Allium*. Those species show the same characteristics in cell arrangement (with inserted pattern), anticlinal wall (U- to Ω-type or Ω-type) and periclinal wall (convex) as advanced characteristics. Seed testa characteristics of *A. brevidens* Vved. (section *Brevidentia*) were the same as those of *A. filidens* (section *Allium*) except for seed shape characteristics (ovoid or flattened ovoid). *Allium caeruleum* Pall., *A. elegans* Drobow and *A. caesium* Schrenk are displayed in the phylogenetic tree as representatives of section *Coerulea*. The three species show the same characteristics regarding cell arrangement (with inserted pattern) and anticlinal wall (U- to Ω-type or Ω-type) as advanced characteristics. However, while *A. caeruleum* has concave periclinal walls *A. elegans* and *A. caesium* have convex periclinal walls. There is also a difference in seed shape between these species. Testa characteristics of *A. haneltii* F.O. Khass. & R.M. Fritsch (section *Haneltia*) are the same as those of *A. brevidens* (section *Brevidentia*). *Allium pallasii* Murr. (section *Pallasia*) had a cell arrangement with inserted pattern, ovoid or flattened ovoid seed shape as advanced characteristics and arched to S or S-type, flat periclinal wall as intermediate characteristics. *Allium kopetdagense* Vved. (section *Kopetdagia*) showed three different characteristics (cell arrangement without inserted pattern, broadly ovoid seeds as primitive characteristic; arched to S or S-type anticlinal wall as intermediate characteristics; and convex periclinal wall as an advanced characteristic (Fig. 15).

Subgenus *Reticulatobulbos* (Kamelin) N. Friesen

Five species from four sections of subgenus *Reticulatobulbosa* were investigated (Table 2). The seeds were flattened ovoid or (broadly) ovoid and the L/W ratio was 1.37–1.95 (Fig. 6F–J). The distance between testa cells was 0.005–0.015 mm and loose with a clear reticulate mesh and indented connecting thread, and the area of ten testa cells was 0.01–0.025 mm^2. The seed testa cell anticlinal wall undulation type was straight to arched. The periclinal walls were convex with one large verruca at the centre (mostly granulose) and many small verrucae on the edge. The dominant testa shape was six-edged (Fig. 13F–J).

Allium drobovii Vved., *A. lineare* L., *A. barsczewskii* Lipsky, *A. eriocoleum* Vved. and *A. sulphureum* Vved. in subgenus *Reticulatobulbosa* were analysed. According to previous findings (Kruse, 1984, 1986, 1988, 1994; Ilarslan and Koyuncu, 1997; Fritsch *et al.*, 2006), straight anticlinal walls and granulous sculptures of the periclinal walls dominate in the subgenus. Lin and Tan (2017) examined two species, *A. strictum* Schrad. and *A. flavidum* Ledeb., of section *Reticulatobulbosa.* In our research *A. lineare* represented this section. Many previous studies (Choi *et al.*, 2012; Lin and Tan, 2017; Baasanmunkh *et al.*, 2021) reported concave periclinal walls with one large central verruca and straight to arched anticlinal walls for section *Reticulatobulbosa*. Our observations confirmed these findings for *A. lineare.* Kruse (1988) found that *A. barsczewskii* (section *Campanulata* Kamelin) had U-type undulate anticlinal walls with verrucate periclinal walls. However, in our research *A. barsczewskii* (section *Campanulata*) had convex periclinal walls with many granulose verrucae in the centre and straight to arched anticlinal walls. The seed surface ultrastructure of section *Scabriscapa* (Tscholok.) N. Friesen was analysed by Neshati and Fritsch (2009) and Baasanmunkh *et al.* (2021). According to the former authors *A. scabriscapum* Boiss. had convex periclinal walls with central verrucae and strip-like anticlinal walls. The latter researchers reported periclinal walls with one central verruca, marginal small verrucae and straight anticlinal walls for *A. trachyscordum* Vved. Our observations were similar to those of both authors, but *A. eriocoleum* had straight to arched anticlinal walls. *Allium drobovii* (section *Nigrimontana* N. Friesen) was analysed here for the first time. Its testa sculpture was similar to other representatives of the genus.

All species of subgenus *Reticulatobulbosa* examined in our study were used in the phylogenetic analysis as representative of the third evolutionary lineage (Fig. 15). *Allium drobovii* (section *Nigrimontana*) and *A. lineare* (section *Reticulatobulbosa*) showed the same seed testa characteristics: cell arrangement without inserted pattern, straight to arched anticlinal wall type, concave periclinal wall as a primitive character state and small and large verrucae on the periclinal wall, and ovoid or flattened ovoid seeds as an advanced character. The testa cells of *A. barsczewskii* (section *Campanulata*) were similar to the above-mentioned sections. However, the periclinal walls of *A. barsczewskii* were convex. Most characteristics shown by these species, such as cell arrangement without inserted pattern, straight to arched anticlinal walls and intermediate and small verrucae, and concave periclinal wall, belong to the first evolutionary lineages. However, *A. barsczewskii* showed convex periclinal walls. *Allium eriocoleum* (section *Scabriscapa*) had primitive testa characteristics such as those of sections *Nigrimontana* and *Reticulatobulbosa*. However, in the broadly ovoid seeds the species is primitive (Fig. 15).

Subgenus *Polyprason* Radic

We investigated four species from three sections of subgenus *Polyprason* (*A. albovianum* C.H. Wright, *A. korolkowii* Regel *A. carolinianum*, *A. talassicum* Regel; Table 2). The seeds were flattened ovoid or (broadly) ovoid with an L/W ratio of 1.3–2.11 (Fig. 6K–N). The distance between testa cells was 0.001–0.021 mm and loose with a clear reticulate mesh and indented connecting thread, and the area of ten testa cells was 0.005–0.02 mm^2. The anticlinal walls of seed testa cells of most species were straight to arched. The periclinal walls were gradually concave or gradually convex with many small verrucae on the edge. The dominant testa shape was six-edged and oblong (Fig. 13K–N).

In subgenus *Polyprason*, several species of sections *Falcatifolia* and *Oreiprason* were studied by Lin and Tan (2017) and Baasanmunkh *et al.* (2021). The reports in both studies showed that the two sections have similar seed testa patterns. However, we found some differences between the sections in anticlinal and periclinal wall traits. The former section (section *Falcatifolia*: *A. carolinianum*) matched with the results presented by the above-mentioned authors regarding anticlinal wall traits. The testa characteristics of section *Oreiprason* (*A. albovianum*) did not support the findings reported by Lin and Tan (2017) and Baasanmunkh *et al.* (2021) since the species had arched to S-type anticlinal walls. According to Baasanmunkh *et al.* (2021) subgenera *Polyprason* and *Reticulatobulbosa* were similar in seed shape and seed testa features in the species they examined. Our observations were congruent with their findings.

Two species (*A. talassicum*, *A. carolinianum*) representing sections *Oreiprason* and *Falcatifolia* were used in the phylogenetic analysis as representatives of subgenus *Polyprason* (Fig. 15). Most characteristics of these species belong to the first evolutionary lineage (cell arrangement without inserted pattern, straight to arched anticlinal wall type, small verrucae on the periclinal wall). However, in terms of periclinal wall shape and seed shape the species showed advanced characteristics (convex periclinal wall, ovoid or flattened ovoid seed shape) (Fig. 15).

Subgenus *Cepa* (Mill.) Radic

Eight species from four sections of subgenus *Cepa* were investigated (Table 2). The seeds of subgenus *Cepa* were flattened ovoid, except in section *Cepa* (broadly ovoid) and the L/W ratio was 1.86–2.05 (Figs 6O and 7). The distance between testa cells was 0.002–0.014 mm and loose with clear reticulate mesh tissue and indented connecting threads, and the area of ten testa cells was 0.004–0.011 mm^2. The seed testa cell anticlinal wall undulation type was straight to arched. The periclinal walls were convex with many small verrucae. The dominant testa shape was six-edged (Figs 13O and 14).

We studied two samples of each of *A. praemixtum* Vved. and *A. altaicum* Vved. species and one sample of each of *A. galanthum* Kar. & Kir., *A. pskemense* B. Fedtsch., *A. praemixtum*, *A. oschaninii* O. Fedtsch., *A. karelinii* Poljal., *A. fedschenkoanum* Regel and *A. condensatum* Turcz. species in subgenus *Cepa*. Kruse (1988, 1994) and Bednorz *et al.* (2011) observed species of *Cepa* and found that the seed testa sculpturing of the periclinal walls of most species was densely granulate. Lin and Tan (2017) also examined seven members of subgenus *Cepa*; the testa characteristics of these species were very similar to that of the species Kruse (1988, 1994) and Bednorz *et al.* (2011) examined. Our research also showed the same results as those of Lin and Tan (2017). We found that all of the species we examined in subgenus *Cepa* had straight to arched anticlinal walls. According to Lin and Tan (2017) *A. galanthum* is characterized by having straight to arched anticlinal walls and periclinal walls with intermediate verrucae. Our observations were congruent with their findings. Baasanmunkh *et al.* (2021) examined *A. oschaninii* (section *Cepa*) and supported the reports of Veiskarami *et al.* (2018) that *A. oschaninii* showed straight anticlinal walls and periclinal walls with large verrucae. In contrast, we found straight to arched anticlinal walls and gradually convex periclinal walls with small to intermediate verrucae for the same species. Species in section *Cepa* share the same testa sculpture pattern except that one species, *A. praemixtum*, had globular convex periclinal walls with many small verrucae. Lin and Tan (2017) examined *A. atrosanguineum* Schrenk from section *Annuloprason* Egorova. It was found that this species had S-type anticlinal walls and periclinal walls with tuberculate or many intermediate verrucae. Baasanmunkh *et al.* (2021) examined *A. fedschenkoanum* of section *Annuloprason* and found similarity between the testa sculpturing of *A. atrosanguineum* and *A. fedschenkoanum*. They found that only the inflorescence colour and habitat differed between them. We also examined *A. fedtschenkoanum* and agree with their findings. From previous findings of the above-mentioned authors, a group-specific characteristic for section *Annuloprason* is many intermediate or small verrucae on globular periclinal walls. However, according to Lin and Tan (2017), *A. weschniakowii* Regel (section *Annuloprason*) did not reflect these characteristics. Further study is needed to study testa characteristics of section *Annuloprason.* The two samples of *A. praemixtum* were examined and turned out to have nearly the same seed testa pattern.

Allium altaicum, *A. galanthum*, *A. pskemense*, *A. fedschenkoanum*, *A. praemixtum*, *A. oschaninii*, *A. karelinii* and *A. condensatum*, representing sects *Cepa*, *Annuloprason*, *Schoenoprasum* and *Condensatum* of subgenus *Cepa*, were used in the phylogenetic analysis (Fig. 15). *Allium altaicum*, *A. pskemense* and *A. galanthum* (sect. *Cepa*) show the same character states of the testa cells, such as cell arrangement (without inserted pattern), anticlinal wall undulation (straight to arched), verrucae on the periclinal wall (small) and seed shape (broadly ovoid) in the phylogenetic tree. All of these characteristics are primitive (except for the convex periclinal wall) despite their location in the third evolutionary lineage. Testa cell characteristics of *A. fedschenkoanum* (section *Annuloprason*) are similar to those of members of section *Cepa*. However, in seed shape *A. fedschenkoanum* has ovoid or flattened ovoid seeds.

Taxonomic, evolutionary and biogeographical significance of seed macro- and micromorphology in the genus *Allium*

The results of our study are in line with the common view that seed macromorphology (size and shape) and micromorphology (testa cell shape, shape and sculpturing of periclinal walls, curvature of anticlinal walls) in *Allium* are the key taxonomic characteristics at species rank (von Bothmer, 1974; Pastor, 1981; Kruse, 1984, 1986, 1988, 1994; Ilarslan and Koyuncu, 1997; Fritsch *et al.*, 2006; Neshati and Fritsch, 2009; Choi and Cota-Sanchez, 2010; Bednorz *et al.*, 2011; Celep *et al.*, 2012; Lin and Tan, 2017; Veiskarami *et al.*, 2018; Baasanmunkh *et al.*, 2021). In addition, our seed coat micromorphology-based classification supported the molecular phylogenetic trees by Friesen *et al.* (2006) and Li *et al.* (2010), confirming the findings of previous investigators (Celep *et al.*, 2012; Lin and Tan, 2017).

Fritsch *et al.* (2006) recognized two seed testa characters, verrucae types on periclinal walls and the shape of anticlinal walls, as directly reflecting the primitive versus advanced evolutionary developmental stages in *Allium*. Celep *et al.* (2012) disagreed with this view, stating that 'Seed coat patterns appear to mark different evolutionary levels inside of many taxonomic groups and variation of the testa characters is sufficient to distinguish taxa at sectional level. However, seed coat patterns do not directly indicate basal or advance evolutionary levels.' The latter view was challenged by Lin and Tan (2017), who claimed that the seed testa characteristics directly correspond to the primitive, intermediate and advanced developmental stages. Our results support this claim and also suggest that the micromorphology (as well as macromorphology) of seeds of species of *Allium* reflect evolutionary levels. Such traits as loose cellular arrangement, verrucae small or absent, concave periclinal walls, straight to arched anticlinal wall, and broadly ovoid seeds appear to be ancient traits in *Allium*. The reticulate mesh-like cellular arrangement, intermediate verrucae, flat periclinal wall, arched to S or S-type, and narrowly ovoid seeds reflect the intermediate developmental stage. Close cellular arrangement, large verrucae, convex periclinal wall, U- to Ω- type and Ω-type, ovoid or flattened ovoid seeds appear to be the most advanced traits. Members of the first evolutionary lineage along with the species selected as the outgroup species, *Nothoscordum bivalve*, *Tulbaghia violacea* [testa characteristics of outgroup species were derived from Kruse (1986)] show more primitive characteristics (Fig. 15). Members of the second evolutionary lineage possess intermediate characteristics, except for members of subgenus *Melanocrommyum*, which show advanced characteristics (Table 2). In the third evolutionary lineage, the taxa retained mostly primitive testa characteristics (except in subgenus *Allium*, in which the species have mostly convex periclinal wall and undulated anticlinal walls, which are advanced characteristics) (Fig. 15). All members of the subgenera *Allium* and *Melanocrommyum* had advanced testa cell features, confirming previous findings (Fritsch *et al.*, 2006; Neshati and Fritsch, 2009; Choi and Cota-Sanchez, 2010; Bednorz *et al.*, 2011). According to Hanelt *et al.* (1992) bulb-forming species evolved from rhizomatous species and subgenera *Allium* and *Melanocrommyum* are derived from subgenus *Rhizirideum*. Adaptive evolution of morphological characteristics or complex hybridization are two possible reasons for the advanced testa characteristics reflected in *Melanocrommyum* and also for less advanced characters of the subgenera in the third evolutionary lineage.

If the seed macro- and micromorphology is phylogenetically informative, we can try to infer, using the testa cell characteristics, the possible migration routes of *Allium* during its early evolution. Most probably, *Allium* originated in the early Eocene (Xie *et al.*, 2020). The place of origin is difficult to infer from the currently available data, but most probably it was an area confined to the Caucasus, Central Asia and Iran. From there, the taxa comprising the first evolutionary lineage migrated either to the west (towards southern Europe and the eastern Mediterranean) or to the east (towards eastern Asia and the Far East). For example, subgenus *Nectaroscordum* apparently took the western route. In our study, *A. tripedale*, representing subgenus *Nectaroscordum* shows the most primitive testa cell characteristics (straight to arched anticlinal walls, concave periclinal walls). The sister to *Nectaroscordum*, subgenus *Amerallium*, apparently took both routes, and as a result its representatives are now in North America, the Mediterranean and eastern Asia. Some researchers (Hanelt *et al.*, 1992; Li *et al.*, 2010) suggested that *Amerallium* originated in Asia and migrated to North America via the Bering Land Bridge. Biogeographers have long studied the relationships between eastern Asian and western North American biotas (Li, 1952; Thorne, 1972; Zhengyi, 1983; Hong, 1993; Wen, 1999; Nie *et al.*, 2005; Zhou *et al.*, 2012). The migrations of Asian and American biota have been hypothesized to be via the Bering Land Bridge across the northern Pacific (Hopkins, 1967) during most of the Tertiary. This bridge remained accessible for floristic exchanges until the Pliocene (Hamilton, 1983). For example, Nie *et al.* (2005) examined two closely related species of *Kelloggia*, *K. chinensis* and *K. galioides* (Rubiaceae), distributed in East Asia and North America, respectively, and found Asia to be the place of origin. Sanmartin *et al.* (2001) recognized three distinct phases of trans-Beringian migrations: Bering Land Bridges I, II and III. The first migration between the two continents (Bering Land Bridge I) took place from the late Cretaceous to the Palaeogene around 70–20 million years ago. This period embraces the time when species of *Allium* arose and the three evolutionary lineages emerged (Xie *et al.*, 2020). Our seed testa morphological study of sections *Arctoprasum*, *Molium* and *Bromatorrhiza* from the Old World and sections *Amerallium* and *Caulorhizideum* from the New World support this hypothesized migration. European species of *Amerallium* (*A. ursinum*) have concave periclinal walls but North American *Amerallium* (*A. validum*, *A. geyeri*) and the Chinese species (*A. wallichii*) have flat or flat to slightly convex periclinal walls and straight to arched anticlinal walls (Table 2). We hypothesize from this that the ancestral species of *Allium* had concave periclinal and straight to arched anticlinal walls. The ancestral species apparently was similar to *A. tripedale* of section *Nectaroscorum* (Fig. 16). Further evolution of the testa cell structure was towards flat periclinal and arched to S, U anticlinal walls (second evolutionary lineage), and then to convex periclinal and U, U- to Ω-type, Ω-type anticlinal walls (second and third evolutionary lineages). Primitive testa cell characteristics in the sections from the third evolutionary lineage, such as the eastern Asian section *Butomissa* (*A. tuberosum*), suggest that these characteristics were apparently inherited from ancient ancestors of the first evolutionary line, while some primitive characteristics could have also been acquired through hybridization (Fig. 16). European section *Molium* (*A. moly*), with less primitive seed testa traits (convex periclinal wall and arched to S-type anticlinal wall), could be one of the ancestors of the subgenera in the second and third evolutionary lineages that have more advanced testa cell characteristics. Whether species bear primitive or advanced traits mostly determines two seed characteristics: the shape of the periclinal wall and the type of undulation of the anticlinal wall. The proposed evolution of the seed testa in the biogeographical context is presented in Figure 16.

Conclusions

This study provided detailed information on the seed micro- and macromorphological characteristics of the genus *Allium* (Amaryllidaceae). Our results showed that testa cell characteristics are useful for identifying species of *Allium*: the anticlinal versus periclinal walls at the sectional level and the verrucae on the periclinal walls within sections. Two new characteristics of the testa cells, surface area of periclinal walls and distance between two adjacent cells, examined for the first time in this study, were found to be taxonomically important. We hypothesize, based on the observed testa cell diversity patterns, that the ancestors of *Allium* originated in a region bounded by the Caucasus, Central Asia and Iran. The seed testa morphology-based evolutionary state of a species is determined by two parameters: the shape of the periclinal walls and curvature of the anticlinal walls.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: list of examined species used in the analyses with their correct taxonomic positions based on existing reliable publications.

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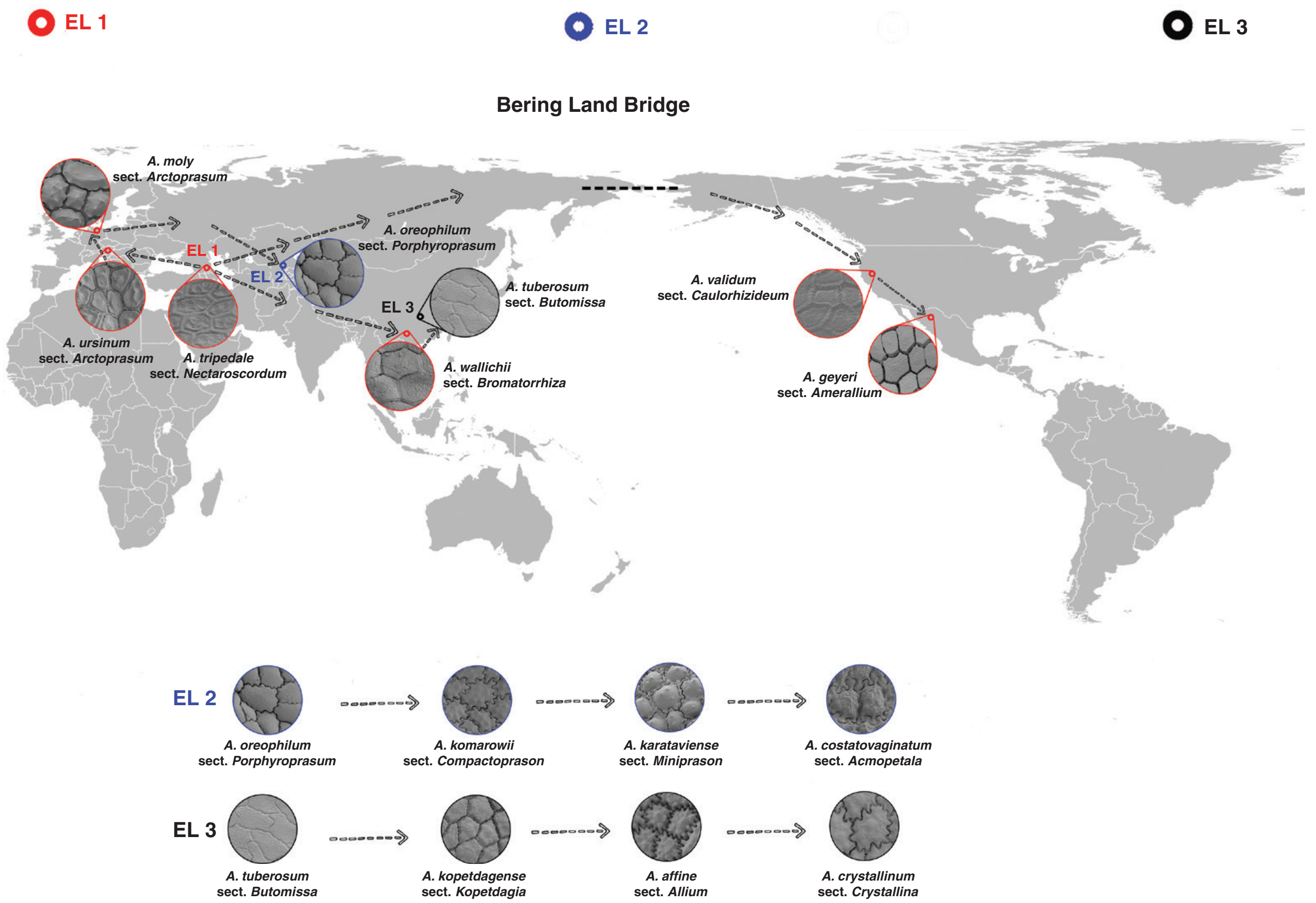

FIG. 16. Distribution of *Allium* throughout the world based on testa cell characteristics of seeds. Locating and branching of species on the map was carried out according to herbarium data (Table 2) and position in the ITS-based phylogenetic tree (Fig. 15), respectively.

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APPENDIX

Previously published ITS accessions obtained from GenBank. ITS accessions of *Allium* and outgroups obtained from GenBank. [1]Friesen *et al.* (2000); [2]Friesen *et al.* (2006); [3]Gurushidze *et al.* (2008); [4]Nguyen *et al.* (2008); [5]Li *et al.* (2010); [6]Abugalieva *et al.* (2017); [7]Friesen *et al.* (2001, Botanical Garden of the University of Osnabrueck, Germany, unpubl. res.); [8]Wheeler *et al.* (2013); [9]Khedim *et al.* (2017, Université des Sciences et de la Technologie Houari Boumediene, Algeria, unpubl. res); [10]Bagheri *et al.* (2020); [11]Friesen *et al.* (2003, Botanical Garden of the University of Osnabrueck, Germany, unpubl. res.); [12]Veiskarami *et al.* (2019, University of Tehran, Iran, unpubl. res.); [13] Stafford *et al.* (2016).

Allium**:** *A. akaka* S.G. Gmel. ex Schult. & Schult.f. FM177242[3]; *A. albidum* Fisch. ex M. Bieb. AJ411841[2]; *A. alexeianum* Regel FM177247[3]; *A. altaicum* Pall. AJ411928[2]; *A. aroides* Popov & Vved. AJ411915[2]; *A. atroviolaceum* Boiss. AJ411884[2]; *A. barsczewskii* Lipsky MG282011[6]; *A. brevidens* Vved. AJ412721[7]; *A. caeruleum* Pall. AJ411903[2]; *A. caesium* Schrenk AJ412731[7]; *A. cardiostemon* Fisch. & C.A.Mey. AJ411971[2]; *A. carolinianum* DC. AJ250290[1]; *A. condensatum* Turcz. AJ412752[7]; *A. costatovaginatum* Kamelin & Levichev FM177286[3]; *A. christophii* Trautv. AJ411966[2]; *A. crystallinum* Vved. AJ412724[7]; *A. cyathophorum* Bureau & Franch. GQ181093[5]; *A. drobovii* Vved. AJ411895[2]; *A. elegans* Drobow AJ412730[7]; *A. eriocoleum* Vved. MG282015[6]; *A. fedschenkoanum* Regel AJ411844[2]; *A. filidens* Regel AJ412723[7]; *A. galanthum* Kar. & Kir. AJ411905[2]; *A. geyeri* S.Watson KC119659[8]; *A. giganteum* Regel FM177320[3]; *A. griffithianum* Boiss. AJ411862[2]; *A. guttatum* Steven MG546819[9]; *A. gypsaceum* Popov & Vved. AJ411969[2]; *A. haneltii* F.O.Khass. & R.M.Fritsch AJ412725[7]; *A. insufficiens* Vved. FM177334[3]; *A. karataviense* Regel AJ411922[2]; *A. karelinii* Poljak AJ411876[2]; *A. komarowii* Lipsky AJ411967[2]; *A. kopetdagense* Vved. AJ411950[2]; *A. kujukense* Vved. AJ411947[2]; *A. lenkoranicum* Miscz. ex Grossh. MT303162[10]; *A. lineare* L. AJ411951[2]; *A. mairei* H.Lév. AJ250298[1]; *A. margaritae* B.Fedtsch. AJ412732[7]; *A. moly* L. AJ412743[7]; *A. neriniflorum* (Herb.) G.Don AJ411913[2]; *A. oreophilum* C.A.Mey. AJ411931[2]; *A. oreoprasum* Schrenk AJ411867[2]; *A. oschaninii* O.Fedtsch. AJ411940[2]; *A. pallasii* Murray GQ181077[5]; *A. pamiricum* Wendelbo AJ412736[7]; *A. praemixtum* Vved. AJ411873[2]; *A. prattii* C.H.Wright GQ181087[5]; *A. protensum* Wendelbo FM177380[3]; *A. przewalskianum* Regel AJ411852[2]; *A. pskemense* B.Fedtsch. AJ411907[2]; *A. ramosum* L. AJ250295[1]; *A. regelii* Trautv. AJ411972[2]; *A. robustum* Kar. & Kir. FM177391[3]; *A. sabulosum* Steven ex Bunge MG282024[6]; *A. sarawschanicum* Regel AJ411935[2]; *A. stipitatum* Regel AJ411911[2]; *A. subangulatum* Regel AJ411870[2]; *A. sulphureum* Vved. AJ412759[7]; *A. talassicum* Regel AJ411865[2]; *A. tanguticum* Regel AJ411893[2]; *A. tashkenticum* F.O.Khass. & R.M.Fritsch; FM177434[3]; *A. tenuissimum* L. AJ411846[2]; *A. tripedale* Trautv. HF934350[11]; *A. tuberosum* Rottler ex Spreng. AJ411914[2]; *A. turkestanicum* Regel AJ411968[2]; *A. ursinum* L. AJ412744[7]; *A. validum* S.Watson EU096188[4]; *A. verticillatum* Regel AJ411910[2]; *A. vineale* L. MK776887[12]; *A wallichii* Kunth AJ250294[1]; *A. zergericum* F.O.Khass. & R.M.Fritsch FM177456[3].

Outgroup: *Nothoscordum bivalve* (L.) Britton AJ250301[1]; *Tulbaghia violacea* Harv. KU692190[13].

KEY TO THE 95 SPECIES OF *ALLIUM* USED IN THIS STUDY BASED ON SEED MACRO- AND MICROMORPHOLOGY

1a. Anticlinal walls straight to arched, arched to S-type, S-type.
2a. Seed shape (broadly) ovoid (shrivelled).
3a. Periclinal wall shapes concave.
4a. Cell arrangement close.
5a. Seed length and width 3.22–1.8 mm, dominant testa cell shape 5-edged, periclinal walls with many intermediate verrucae on edge, testa shapes 4- to 7-edged…… *A. tripedale*
5b. Seed length and width 2.20–1.66 mm, dominant testa cell shape 6-edged, periclinal walls with intermediate to large verrucae, testa shapes 4- to many-edged …… *A. prattii*
4b. Cell arrangement loose.
6a. Periclinal walls with one large verruca in centre or intermediate to large verrucae.
7a. Anticlinal wall straight to arched, seed length and width 2.46–2.54 mm, area of 10 periclinal wall testa surface cells 0.012 mm^2, seed shape broadly ovoid, periclinal wall with one large verruca in centre …… *A. ursinum*
7b. Anticlinal wall arched to S-type, seed length and width 3.13–1.48 mm, area of 10 periclinal wall testa surface cells 0.005 mm^2, seed shape ovoid (shrivelled), periclinal wall with intermediate to large verrucae …… *A. albovianum*
6b. Periclinal walls with many small verrucae (in central area of epidermis).
8a. Dominant testa cell shape elliptic or oblong.
9a. Periclinal wall arched to S-type, plane to slightly concave.
10a. Seed length and width 3.45–2.37 mm, area of 10 periclinal wall testa surface cells 0.04 mm^2, dominant testa cell shape elliptic. …… *A. tuberosum*
10b. Seed length and width 2.72–1.39 mm, area of 10 periclinal wall testa surface cells 0.011 mm^2, dominant testa cell shape oblong. ……*A. pallasii*

9b. Periclinal wall straight to arched, with marginal bulge, gradually concave from edge to centre .... *A. przewalskianum*
8b. Dominant testa cell shape 6-edged.
11a. Seed shape broadly ovoid, periclinal wall gradually concave, cell arrangement with clear meshes of reticulate tissue.
12a. Seed length and width 2.10–1.55 mm, area of 10 periclinal wall testa surface cells 0.013 mm^2, cell arrangement without inserted pattern and indented connecting thread, periclinal wall with many small verrucae in central area of epidermis. .... *A. mairei*
12b. Seed length and width 1.77–1.31 mm, area of 10 periclinal wall testa surface cells 0.007 mm^2, cell arrangement with inserted pattern and indented connecting thread, periclinal wall with marginal bulge, many small verrucae. .... *A. denudatum*
11b. Seed shape ovoid (shrivelled), periclinal wall (flat to) slightly concave, cell arrangement with clear meshes of reticulate tissue.
13a. Periclinal wall with inserted pattern, anticlinal wall S-type. .... *A. tenuissimum*
13b. Periclinal wall without inserted pattern, anticlinal wall straight to arched.
14a. Area of 10 periclinal wall testa surface 0.022 mm^2, distance between testa cells 0.001–0.002 mm, periclinal wall flat to slightly concave from edge to centre. .... *A. wallichii*
14b. Area of 10 periclinal wall testa surface 0.01 mm^2, distance between testa cells 0.005–0.009 mm, periclinal wall slightly concave. .... *A. carolinianum*
3b. Periclinal wall shape convex.
15a. Anticlinal wall straight to arched, arched to S-type.
16a. Cell arrangement with connecting thread.
17a. Cell arrangement with narrow connecting thread.
18a. Periclinal wall convex.
19a. Seed length and width 3.39–2.18 mm, area of 10 periclinal wall testa surface cells 0.008 mm^2, distance between testa cells 0.003–0.009 mm. .....*A. galanthum*
19b. Seed length and width 3.86–2.85 mm, area of 10 periclinal wall testa surface cells 0.012 mm^2, distance between testa cells 0.006–0.014 mm. .... *A. pskemense*
18b. Periclinal wall gradually, flat to slightly or slightly convex.
20a. Periclinal wall with many small verrucae, flat to (slightly) convex periclinal wall.
21a. Seed length and width 2.13–1.45 mm, cell arrangement with clear meshes of reticulate tissue, distance between testa cells 0.002–0.007 mm. .... *A. geyeri*
21b. Seed length and width 3.58–1.98 mm, cell arrangement with reticulate tissue, distance between testa cells 0.005–0.014 mm. .... *A. altaicum*
20b. Periclinal wall with many small to intermediate verrucae, gradually convex periclinal wall. .... *A. oschaninii*
17b. Cell arrangement with (broadly) indented connecting thread.
22a. Anticlinal wall straight to arched, periclinal wall gradually convex from thickened edge to centre, without inserted pattern.
23a. Seed length and width 3.83–2.05 mm, periclinal wall with many granulose verrucae in centre, many small verrucae on edge. .... *A. barsczewskii*
23b. Seed length and width 2.85–2.07 mm, periclinal wall with one large granulose verruca in centre, many small verrucae on edge. .... *A. eriocoleum*
22b. Anticlinal wall straight to arched, periclinal wall convex, with inserted pattern. .... *A. turkestanicum*
16b. Cell arrangement without connecting thread.
24a. Periclinal walls straight to arched.
25a. Seed length and width 1.71–1.63 mm, distance between testa cells 0.0009–0.004 mm, periclinal wall slightly convex. .... *A. neriniflorum*
25b. Seed length and width 1.71–1.63 mm, distance between testa cells 0.002–0.007 mm, periclinal globular convex. .... *A. praemixtum*
24b. Periclinal walls arched to S-type.
26a. Cell arrangement without inserted pattern, dominant testa cell shape 6-edged. .... *A. moly*
26b. Cell arrangement with inserted pattern, dominant testa cell shape 5-edged, oblong.
27a. Seed length and width 2.05–1.56 mm, periclinal wall flat to convex, plane. .... *A. kujukense*
27b. Seed length and width 2.75–2.16 mm, periclinal wall gradually convex from edge to centre, one large verruca on central area and many small verrucae on edge, granulose. .... *A. kopetdagense*
15b. Anticlinal wall S-type.
28a. Dominant testa cell shape 5-edged, rectangular.
29a. Periclinal wall shape convex with large and/or intermediate verrucae.
30a. Seed length and width 2.54–1.52 mm, cell arrangement close, periclinal wall with one large verruca in centre, intermediate verrucae on edge. ...... *A. robustum*
30b. Seed length and width 3.60–1.90 mm, cell arrangement loose, periclinal wall with many intermediate verrucae. .... *A. lenkoranicum*
29b. Periclinal wall shape slightly convex with many small verrucae. .... *A. margaritae*
28b. Dominant testa cell shape oblong, elliptic.
31a. Cell arrangement with indented connecting thread. .... *A. elegans*
31b. Cell arrangement without indented connecting thread.
32a. Seed length and width 2.39–1.95 mm, area of 10 periclinal wall testa surface cells 0.015 mm^2, testa cell shape many-edged to oblong. .... *A. oreophilum*
32b. Seed length and width 2.03–1.48 mm, area of 10 periclinal wall testa surface cells 0.006 mm^2, testa cell shape orbicular to oblong. .... *A. popovii*
2b. Seed shape narrowly (flattened) ovoid.
33a. Cell arrangement close.
34a. Anticlinal wall straight to arched.
35a. Periclinal wall globular convex. .... *A. fedtschenkoanum*
35b. Periclinal wall flat, flat to slightly convex.
36a. Seed length and width 3.05–1.64 mm, cell arrangement with unclear meshes, periclinal wall flat to slightly convex. .... *A. condensatum*
36b. Seed length and width 4.73–1.81 mm, cell arrangement without unclear meshes, periclinal wall flat. ....*A. validum*
34b. Anticlinal wall arched to S-type.
37a. Seed length and width 1.93–1.42 mm, cell arrangement with inserted pattern, testa cell shape oblong. .... *A. verticillatum*
37b. Seed length and width 3.21–1.63 mm, cell arrangement without inserted pattern, testa cell shape 4- to many-edged. .... *A. aroides*
33b. Cell arrangement loose.
38a. Cell arrangement with connecting thread.
39a. Periclinal wall gradually concave.
40a. Area of 10 periclinal wall testa surface cells 0.008 mm^2, periclinal wall with marginal bulge, small verrucae. ....*A. tanguticum*
40b. Area of 10 periclinal wall testa surface cells 0.02 mm^2, periclinal wall with one large granulose verruca in centre, many small verrucae on edge. ...... *A. lineare*
39b. Periclinal wall flat to slightly convex or convex.
41a. Seed length and width 2.17–1.16 mm, dominant testa cell shape oblong, anticlinal wall S-type, periclinal wall with many small verrucae in centre, intermediate verrucae on edge. .... *A. korolkowii*
41b. Seed length and width 2.73–1.42 mm, dominant testa cell shape 6-edged, anticlinal wall straight to arched, periclinal wall with many small verrucae. .... .*A. karelinii*
38b. Cell arrangement without connecting thread.
42a. Periclinal wall plane to slightly, gradually concave.

43a. Periclinal wall straight to arched.
44a. Seed length and width 2.79–1.60 mm, distance between testa cells 0.003–0.006 mm, periclinal walls with small to intermediate verrucae. ... *A.* aff. *tuberosum*
44b. Seed length and width 3.97–2.31 mm, distance between testa cells 0.008–0.012 mm, periclinal walls with one large granulose verruca in centre, many small verrucae on edge. .... *A. drobovii*
43b. Periclinal wall arched to S-type. .... *A. ramosum*
42b. Periclinal wall (gradually) (slightly) convex.
45a. Periclinal wall straight to arched.
46a. Area of 10 periclinal wall testa surface cells 0.015 mm^2, gradually convex periclinal walls with small to intermediate verrucae. .... *A. cyathophorum*
46b. Area of 10 periclinal wall testa surface cells 0.006 mm^2, convex periclinal walls with many small verrucae. .... *A. talassicum*
45b. Periclinal wall S-type. .... *A. subangulatum*
1b. Anticlinal walls S- to U-type, U-type, U- to Ω-type, Ω-type.
47a. Loose cell arrangement.
48a. Seed shape (broadly) ovoid (shrivelled).
49a. Periclinal wall shape concave.
50a. Dominant testa cell shape elliptic.
51a. Seed length and width 3.03–1.87 mm, anticlinal wall S- to U-type, periclinal wall with many intermediate and small verrucae. .... *A. oreoprasum*
51b. Seed length and width 2.13–1.40 mm, anticlinal wall Ω-type, periclinal wall with marginal bulge or not, small verrucae. .... *A. caeruleum*
50b. Dominant testa cell shape oblong.
52a. Distance between testa cells 0.003–0.008 mm, anticlinal wall S- to U-type. .... *A. caesioides*
52b. Distance between testa cells 0.001–0.004 mm, anticlinal wall U- to Ω-type. .... *A. delicatulum*
49b. Periclinal wall shape convex.
53a. Cell arrangement with connecting thread.
54a. Dominant testa cell shape oblong.
55a. Anticlinal wall S- to U-type.
56a. Convex periclinal wall.
57a. Seed length and width 3.15–2.20 mm, area of 10 periclinal wall testa surface cells 0.024 mm^2, periclinal wall with many intermediate verrucae. .... *A. dictyoscordum*
57b. Seed length and width 2.16–1.15 mm, area of 10 periclinal wall testa surface cells 0.012 mm^2, periclinal wall with 1 or 2 large granulose verrucae and many small verrucae. .... *A. caesium*
56b. Globular convex periclinal wall. .... *A. ophiophyllum*
55b. Anticlinal wall U-type. .... *A. ferganicum*
54b. Dominant testa cell shape elliptic.
58a. Anticlinal wall U-type, periclinal wall with 1–3 intermediate granulose verrucae on central area and many small verrucae. .... *A. brevidens*
58b. Anticlinal wall U- to Ω-type, periclinal wall with 1 or 2 large granulose verrucae in centre, many intermediate granulose verrucae on edge. ... *A. sulphureum*
53b. Cell arrangement without connecting thread.
59a. Globular, gradually convex periclinal walls.
60a. Dominant testa cell shape oblong.
61a. Periclinal wall many intermediate verrucae.
62a. Distance between testa cells 0.0002–0.00068 mm, anticlinal wall U- to Ω-type. .... *A. akaka*
62b. Distance between testa cells 0.002–0.007 mm, anticlinal wall S- to U-type. .... *A. ophiophyllum*
61b. Periclinal wall many intermediate verrucae and many small verrucae on edge.
63a. Seed length and width 2.14–1.57 mm, area of 10 periclinal wall testa surface cells 0.015 mm^2, periclinal wall gradually convex........ *A. alexeianum*
63b. Seed length and width 3.13–1.83 mm, area of 10 periclinal wall testa surface cells 0.009 mm^2, periclinal wall globular convex. .... *A. baissunense*
60b. Dominant testa cell orbicular, elliptic.
64a. Large verruca or verrucae on central area of periclinal wall.
65a. Area of 10 periclinal wall testa surface cells 0.02 mm^2, anticlinal wall Ω-type. .... *A. taeniopetalum*
65b. Area of 10 periclinal wall testa surface cells 0.008 mm^2, anticlinal wall U-type. .... *A. griffithianum*
64b. One to four intermediate verrucae on central area of periclinal wall. .... *A. isakulii*
59b. Convex periclinal wall.
66a. Dominant testa cell shape oblong.
67a. Seed length and width 2.48–1.77 mm, testa cell shapes elliptic to oblong. .... *A. giganteum*
67b. Seed length and width 3.80–2.31 mm, testa cell shapes ovoid to irregular. .... *A. komarowii*
66b. Dominant testa cell shape elliptic.
68a. Intermediate verrucae on central area of periclinal wall. .... *A. stipitatum*
68b. Large verrucae on central area of periclinal wall.
69a. Seed length and width 3.23–2.14 mm, area of 10 periclinal wall testa surface cells 0.012 mm^2. .... *A. filidens*
69b. Seed length and width 3.02–1.88 mm, area of 10 periclinal wall testa surface cells 0.016 mm^2. .... *A. ugami*
48b. Seed shape flattened ovoid, narrowly ovoid.
70a. Dominant testa cell shape oblong.
71a. Anticlinal wall U- to Ω-type.
72a. Seed length and width 2.4–1.4 mm, distance between testa cells 0–0.002 mm. .... *A. protensum*
72b. Seed length and width 2.77–1.21 mm, distance between testa cells 0.002–0.011 mm. .... *A. vineale*
71b. Anticlinal wall Ω-type, U-type.
73a. Area of 10 periclinal wall testa surface cells 0.008 mm^2, distance between testa cells 0–0.003 mm, anticlinal wall Ω-type, cell arrangement without indented connecting thread. .... *A. cardiostemon*
73b. Area of 10 periclinal wall testa surface 0.018 mm^2, distance between testa cells 0.004–0.06 mm, anticlinal wall Ω-type, cell arrangement with indented connecting thread. .... *A. affine*
70b. Dominant testa cell shape elliptic.
74a. Cell arrangement with connecting thread.
75a. Periclinal walls slightly convex, many small and granulose intermediate verrucae. .... *A. minutum*
75b. Periclinal walls convex, five to seven intermediate verrucae, granulose. .... *A. guttatum*
74b. Cell arrangement without connecting thread.
76a. Periclinal wall globular, slightly convex.
77a. Testa cell shapes orbicular to elliptic.

78a. Globular, slightly convex periclinal wall.
79a. Seed length and width 3.31–1.87 mm, area of 10 periclinal wall testa surface cells 0.022 mm^2, anticlinal wall S- to U-type periclinal wall with 1–5 intermediate and many small verrucae. .... *A. crystallinum*
79b. Seed length and width 2.41–1.01 mm, area of 10 periclinal wall testa surface cells 0.022 mm^2, anticlinal wall U- to Ω-type, periclinal wall with many small verrucae. .... *A. anisotepalum*
78b. Convex periclinal wall.
80a. Seed length and width 3.26–1.58 mm, anticlinal wall U-type. .... *A. atroviolaceum*
80b. Seed length and width 2.55–1.27 mm, anticlinal wall S- to U-type. .... *A. fibrosum*
77b. Testa cell shapes triangular to elliptic and oblong.
81a. Periclinal wall with many small verrucae. .... *A. borszczowii*
81b. Periclinal wall with 1–4 intermediate granulose verrucae and many small verrucae. .... *A. haneltii*
76b. Periclinal wall globular, slightly concave.
82a. Seed shape flattened ovoid, anticlinal wall U- to Ω-type, periclinal wall with many small verrucae. .... *A. sabulosum*
82b. Seed shape narrowly ovoid, anticlinal wall S- to U-type, periclinal wall with many intermediate and small verrucae. ....*A. aucheri*
47b. Close cell arrangement.
83a. Dominant testa cell shape orbicular.
84a. Anticlinal wall U- to Ω-type or U-type.
85a. Globular convex periclinal wall.
86a. Seed length and width 2.42–1.93 mm, distance between testa cells 0.0005–0.001 mm, anticlinal wall U-type. .... *A. sarawschanicum*
86b. Seed length and width 3.78–2.68 mm, distance between testa cells 0.001–0.004 mm, anticlinal wall U- to Ω-type. .... *A. karataviense*
85b. Slightly concave, gradually convex periclinal wall.
87a. Periclinal wall slightly concave with many small verrucae. .... *A. regelii*
87b. Periclinal wall gradually convex with many intermediate verrucae on central area of shrivelled epidermis. .... *A. cupuliferum*
84b. Anticlinal wall S- to U-type. .... *A. woronowii*
83b. Dominant testa cell shape elliptic, oblong.
88a. Seed shape broadly ovoid (shrivelled).
89a. Dominant testa cell shape elliptic.
90a. Anticlinal wall S- to U-type, periclinal wall convex and granulate, one large verruca on central area, intermediate verrucae on edge. .... *A. tschimganicum*
90b. Anticlinal wall Ω-type, periclinal wall convex or concave, many small verrucae. .... *A. zergericum*
89b. Dominant testa cell shape oblong.
91a. Anticlinal wall Ω-type.
92a. Distance between testa cells 0.004–0.009 mm, periclinal wall flat to convex with many intermediate and large verrucae. .... *A. gypsaceum*
92b. Distance between testa cells 0.00064–0.00097 mm, periclinal wall gradually convex with many intermediate verrucae. .... *A. costatovaginatum*
91b. Anticlinal wall S- to U-type, U- to Ω-type.
93a. Seed length and width 1.66–1.16 mm, anticlinal wall S- to U-type, periclinal wall slightly convex, many small and intermediate verrucae. .... *A. insufficiens*
93b. Seed length and width 3.08–2.45 mm, anticlinal wall U- to Ω-type, periclinal wall convex, many intermediate verrucae. .... *A. cristophii*
88b. Seed shape flattened ovoid.
94a. Test cell shapes orbicular or suborbicular to elliptic.
95a. Area of 10 periclinal wall testa surface 0.017 mm^2, anticlinal wall U- to Ω-type. .... *A. rhodanthum*
95b. Area of 10 periclinal wall testa surface 0.009 mm^2, anticlinal wall U-type. .... *A. pamiricum*
94b. Test cell shapes elliptic to triangular. .... *A. tashkenticum*