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Seed coat morphology of some species of the genus *Campanula* (Campanulaceae) in Iran

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Summary: Seed morphology of 13 taxa of *Campanula* distributed mainly in northern Iran was investigated by light microscopy (LM) and scanning electron microscopy (SEM). Two main types and two subtypes of surface ornamentation were observed. The seed surface may vary from striate, faintly striate or interrupted striate in *C. latifolia*, *C. lourica*, *C. rapunculoides*, *C. rapunculus* subsp. *lambertiana*, *C. ruprechtii*, *C. saxifraga* subsp. *aucheri* and *C. trachelium*, to striate-reticulate in *C. glomerata* subsp. *hispida*, *C. involucrata*, *C. lactiflora*, *C. patula*, *C. stevenii* subsp. *stevenii* and *C. stevenii* subsp. *beauverdiana*. Based on the shape of the testa cells and the arrangement of the anticlinal walls it is possible to distinguish the following two testa types: 1) cells narrowly oblong to subpolygonal in shape and with anticlinal walls being irregularly straight and 2) lanceolate cells with straight anticlinal walls. The results indicate that size and shape of the seeds are of little systematic significance, but the shape of testa cells and the surface ornamentation may be important for the taxonomy of the genus.

Keywords: *Campanula*, SEM, seed coat, Iran

Campanulaceae Juss. comprises about 84 genera (Lammers 2007) and is divided into five subfamilies: Campanuloideae Burnett, Lobelioideae Burnett, Nemacladoideae Lammers, Cyphioideae (A. DC) Walp and Cyphocarpoideae Miers (Lammers 1998). The largest and the most widespread are Campanuloideae and Lobelioideae, with 56 and 32 genera and about 1045 and 1192 species, respectively (Lammers 2007). The genus *Campanula* L. with about 420 species is one of the largest genera in the subfamily Campanuloideae (Lammers 2007). It is distributed in northern temperate and Mediterranean regions (Shetler & Morin 1986; Borsch et al. 2009). Recently, molecular phylogenetic analysis of the family was performed using DNA sequences of the chloroplast gene *petD* (Borsch et al. 2009). Borsch et al. (2009) have shown that core Campanuloideae comprise two major radiations of *Campanula* species: a *Musschia* clade (including *C. lactiflora*) and a *Jasione* clade. *Campanula* species might be considered as genetic resources able to persist under a wide range of ecological conditions (Vogler et al. 1999). In fact, these species vary in habitat from dwarf arctic and alpine species about five centimeters tall to large temperate grassland and woodland species growing up to two meters (Khansari et al. 2012). This genus is represented by ca. 44 species and 11 endemics classified into five subgenera and 10 sections in Iran (Schiman-Czeika 1965; Aghabeigi & Assadi 2008). Among them, 13 species and subspecies are from north of Iran and they are classified into two subgenera and five sections. These taxa are herbaceous with alternate leaves and 5-lobed calyx and corolla, trifid stigma and capsules containing numerous seeds. The delimitation of *Campanula* species within sections and subgenera can be problematic due to morphological variation and phenotypic plasticity in many regions of the world (Shetler & Morin 1986). In contrast to seed features, the seed coat is little affected by environmental conditions. However, the importance of seed morphology has been confirmed taxonomically (Barthlott 1981; Barthlott 1984; Shetler & Morin 1986; Takhtajan 1991).

Table 1. Collection data of *Campanula* species examined.

Taxa	Collection data
Campanula latifolia L.	Mazandaran: 50 km SW of Chalous, above the village Delir, *Assadi & Massoumi 51610* [TARI].
C. trachelium L.	Guilan: Asalem to Khalkhal, 190 m, *Wendelbo & Shirdelpour 14904* [TARI].
C. rapunculoides L.	Guilan: Harzevil, 700 m. *Gauba 3635* [IRAN].
C. involucrata Aucher ex A. DC.	Guilan: Manjil to Zanjan, Badamestan, 2000 m, *Iranshahr 35541* [IRAN].
C. glomerata subsp. *hispida* (Witasek) Hayek	Guilan: Amarlou, Damash, kuhe Angour-Chaleh 1750–1920 m, *Terme & Daryadel 3507* [IRAN].
C. saxifraga subsp. *aucheri* (A. DC.) Organ	Mazandaran: 40 km S of Ramsar, N slope of Khash-e Chal mountain, *Assadi & Massoumi 51140* [TARI].
C. ruprechtii Boiss.	Mazandaran: Ramsar, S of Javaherdeh, between Lapasar & Pish-chak, *Masoumi 56817* [TARI].
C. lourica Boiss.	Golestan: Gorgan, 14 km E of Chaman-e Bid, *Wendelbo & cobham, 14350* [TARI].
C. rapunculus subsp. *lambertiana* (A. DC.) Rech. f.	Mazandaran: Tonekabon, Khoramabad, Gireh-Sar, *Ershad & Ezadyar 3642* [IRAN].
C. lactiflora M. Bieb.	Guilan: Asalem-Khalkhal road, *Wendelbo & Assadi 18372* [TARI].
C. patula L.	Mazandaran: by Lavij river, 5 km S of Nosrat Abad, *Wendelbo & Assadi 14526* [TARI].
C. stevenii subsp. *stevenii* M. Bieb.	Mazandaran: Alasht, Emamzade Hashem (Savadkuh), *Termeh, Daneshpaguh & Zargani, 3714* [IRAN].
C. stevenii. subsp. *beauverdiana* (Fomin) Rech. f.	Mazandaran: Haraz road at Abgarm, *Assadi & Mozaffarian 33113* [TARI].

The most important findings of previous morphological studies of *Campanula* were the frequent occurrence of rugose, reticulate and striate ornamentations among different species of the genus (Shetler & Morin 1986; Akcin 2009; Alçitepe 2010; Cupido et al. 2011).

Data on seed coat morphology exist for only five species out of 13 taxa of *Campanula* currently known in northern Iran, i.e. *C. glomerata* subsp. *hispida*, *C. involucrata*, *C. lactiflora*, *C. rapunculoides* and *C. rapunculus* subsp. *lambertiana*. The aim of the present study was to examine seed morphology in the selected species of *Campanula* distributed in northern Iran in order to determine a possible application of these characters in the infrageneric classification of this genus and to obtain more data for further taxonomic studies.

Materials and methods

Dried mature seeds were removed from herbarium specimens deposited in the herbarium of the Iranian Research Institute of Plant Protection [IRAN] and the herbarium of Research Institute of Forests and Rangelands [TARI]. Examined species and their voucher numbers are shown in Table 1. Approximately 10 seeds from each taxon were analyzed. For SEM observation, the seeds were mounted on aluminum stubs using double-sided adhesive tape and then coated with

Table 2. Seed characters of the investigated species of *Campanula*. The subgenera and sections are arranged according to Damboldt (1976, 1978).

Subgenus / Section taxon	Seed shape	Seed length (mm)	Seed width (mm)	Seed surface	Testa cell outline	Seed type and subtype
Campanula / *Campanula* *C. latifolia*	oblong	2.3 (2.42 ± 0.06) 2.5	0.85 (1.04 ± 0.1) 1.2	striate	lanceolate	Ia
C. trachelium	ovoid to ellipsoid	1.2 (1.51 ± 0.1) 1.62	0.5 (0.6 ± 0.06) 0.67	+	+	Ia
C. rapunculoides	oblong	1.5 (1.64 ± 0.1) 1.7	0.62 (0.75 ± 0.06) 0.9	+	+	Ia
Campanula / *Scapiflorae* *C. saxifraga* subsp. *aucheri*	oblong	1.97 (2.08 ± 0.11) 2.22	0.75 (0.78 ± 0.02) 1.05	+	+	Ia
C. ruprechtii	ellipsoid	1.97 (1.99 ± 0.01) 2.02	0.75 (0.84 ± 0.03) 0.97	+	+	Ia
Campanula / *Involucratae* *C. involucrata*	ovoid to ellipsoid	2 (2.01 ± 0.01) 2.05	0.55 (0.75 ± 0.09) 0.95	striate-reticulate	narrowly oblong	II
C. glomerata subsp. *hispida*	ovoid	1.25 (1.26 ± 0.09) 1.37	0.35 (0.66 ± 0.04) 0.75	striate-reticulate	+	II
Campanula / *Saxicolae* *C. lourica*	ellipsoid	0.66 (0.67 ± 0.01) 0.69	0.2 (0.27 ± 0.06) 0.4	interrupted striate	lanceolate	Ib
Rapunculus / *Rapunculus* *C. rapunculus* subsp. *lambertiana*	ellipsoid	0.5 (0.53 ± 0.01) 0.55	0.23 (0.24 ± 0.01) 0.3	faintly striate	lanceolate	Ib
C. lactiflora	ovoid to broad ellipsoid	0.72 (0.82 ± 0.05) 0.88	0.35 (0.41 ± 0.03) 0.55	striate-reticulate	narrowly oblong	II
C. patula	ellipsoid	0.61 (0.68 ± 0.05) 0.76	0.27 (0.29 ± 0.01) 0.33	striate-reticulate	narrowly oblong to subpolygonal	II
C. stevenii subsp. *stevenii*	oblong	0.77 (0.86 ± 0.05) 0.92	0.23 (0.29 ± 0.04) 0.4	striate-reticulate	narrowly oblong	II
C. stevenii subsp. *beauverdiana*	ellipsoid	0.97 (0.97 ± 0.02) 1	0.2 (0.43 ± 0.01) 0.57	striate-reticulate	narrowly oblong	II

a thin layer of gold. SEM examination was carried out by means of a Vega Tescan SEM, at an accelerating voltage of 15 kV, in Razi Metallurgical Research Center (Figs. 1–3). Terminology of seed coat and surface sculpturing is according to Barthlott (1981, 1984) and Stearn (1996). Measurements were made with a light microscope (LM). Ten seeds from each species, if available, were measured (Table 2).

Results

The seed size ranges from 0.5–2.5 mm in length and from 0.2–1.2 mm in width. The smallest seed belongs to *C. rapunculus* subsp. *lambertiana* (0.5–0.55 × 0.23–0.3 mm) and the largest ones to *C. latifolia* (2.3–2.5 × 0.85–1.2 mm). The shape of seeds is oblong, ovoid to ellipsoid. Seeds are yellowish or brown. The seed surfaces of the studied taxa vary from striate, faintly striate or interrupted striate to striate-reticulate. There are two types of testa cells in respect of the arrangement of the anticlinal walls: 1) cells lanceolate with straight anticlinal walls and 2) cells narrowly oblong to subpolygonal in shape with anticlinal walls being irregularly straight or undulated. According to our micromorphological studies, two main types and two subtypes of seed coat have been recognized: Type I, which is generally characterized by a striate, interrupted striate or faintly striate testa, indicates the most heterogeneous group. Two subtypes under this main type were identified in *C. latifolia*, *C. lourica, C. rapunculoides*, *C. rapunculus* subsp. *lambertiana, C. ruprechtii, C. saxifraga* subsp. *aucheri* and *C. trachelium* which belong to four different sections (Table. 2).

Campanula latifolia, C. rapunculoides and *C. trachelium* of sect. *Campanula, C. saxifraga* subsp. *aucheri* and *C. ruprechtii* of sect. *Scapiflorae* (Boiss.) Kharadze are representative species of subgen. *Campanula.* They have similar micromorphological characteristics like striate sculpturing and lanceolate cells with straight anticlinal walls (Type I). Type II with striate-reticulate pattern was observed in *C. glomerata*, *C. involucrata*, *C. lactiflora* of sect. *Involucratae* (Fomin) Kharadze (subgen. *Campanula*) and *C. patula, C. stevenii* subsp. *stevenii* and *C. stevenii* subsp. *beauverdiana* of sect. *Rapunculus* (Boiss.) Kharadze (subgen. *Rapunculus* Boiss.).

Type I (Figs 1 A–I; 2A–E)

Striate, interrupted striate or faintly striate. Characterized by cells laterally compressed to indiscernible. Cells lanceolate with straight anticlinal walls and narrow or indistinct lumen. Two subtypes were identified within this type:

Subtype Ia

The surface pattern is striate with anticlinal walls extremely or relatively thick and rod-like in surface view. The striations are regular and prominent and completely stick to each other leading to a narrow lumen in *C. latifolia*, *C. rapunculoides* and *C. trachelium* L. (Fig. 1B, D, F) or to an indistinct lumen in *C. saxifraga* subsp. *aucheri* (Fig. 1H) and *C. ruprechtii* (Fig. 2A).

Subtype Ib

Seeds are smooth with interrupted striate or faintly striate surface. The cell outlines are indiscernible and characterized by longitudinal interrupted grooves or a striped appearance. This subtype was observed in *C. rapunculus* subsp. *lambertiana* (Fig. 2C) and *C. lourica* (Fig. 2E).

Type II (Figs 2F–I; 3A–H)

Striate-reticulate. Cells narrowly oblong to subpolygonal with anticlinal walls being irregularly straight or undulated and with slit-like to relatively wide lumen, observed in *C. glomerata* subsp. *hispida* (Fig. 2G), *C. involucratea* (Fig. 2I), *C. lactiflora* (Fig. 3B), *C. patula* (Fig. 3H), *C. stevenii* subsp. *beauverdiana* (Fig. 3D) and *C. stevenii* subsp. *stevenii* (Fig. 3F). The testa cells of *C. glomerata* subsp. *hispida*, *C. involucrata* and *C. lactiflora* have a relatively wider lumen than

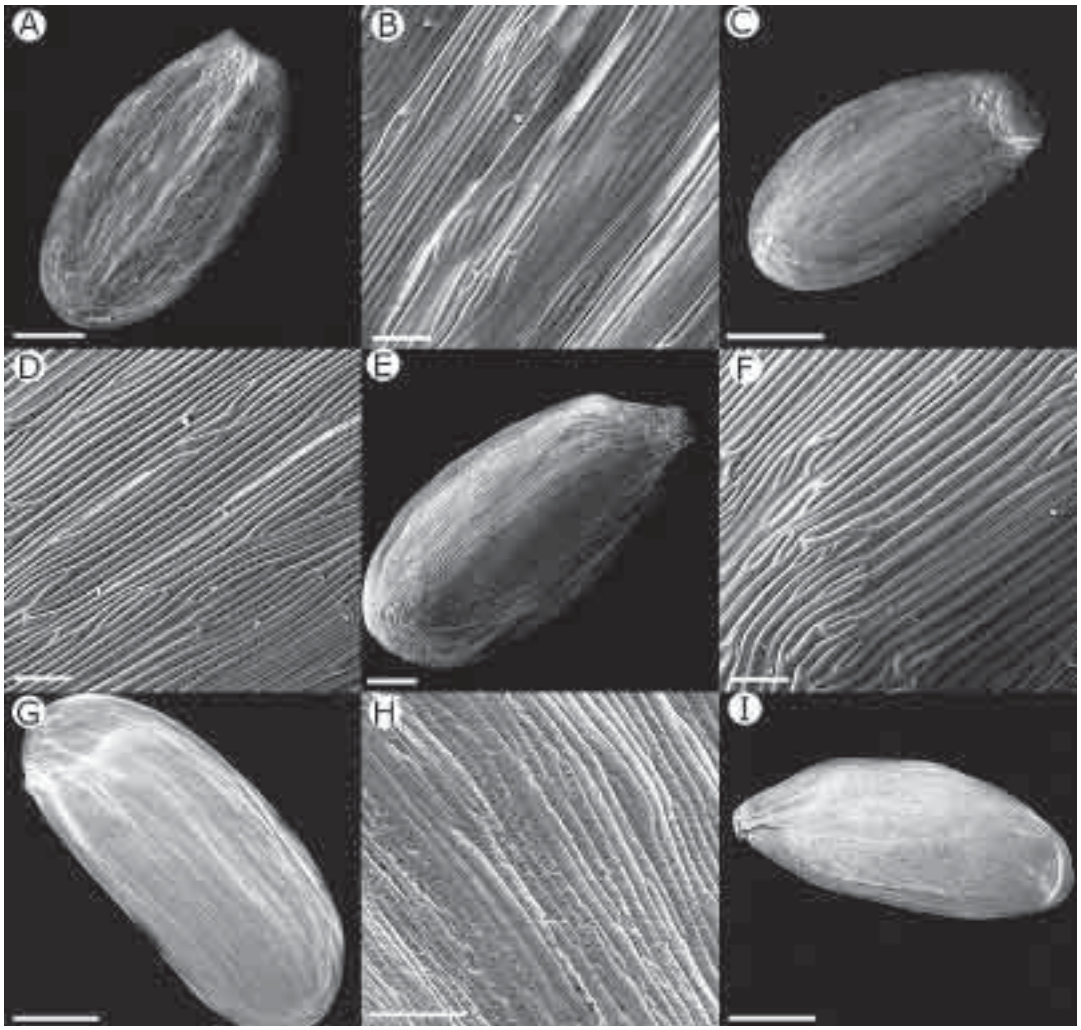

Figure 1. SEM micrographs of seed coat: A, B – *C. latifolia*; C, D – *C. rapunculoides*; E, F – *C. trachelium*; G, H – *C. saxifraga* subsp. *aucheri*; I – *C. ruprechtii*. Scale bars: A–D, F–G, I = 500 µm; E = 200 µm; H = 50 µm.

the other taxa. The cells are more elongated in *C. involucrata* (Fig. 2I). Seeds are more or less smooth in *C. stevenii* subsp. *stevenii* and *C. stevenii* subsp. *beauverdiana* (Fig. 3C, E). But among all species of this type, only testa cells of *C. patula* have irregularly depressed radial walls which create ridges that run more or less perpendicular to the long axis of the seed, giving the seed coat a rugose appearance (Fig. 3H).

Discussion

In the present study, we examined species that belong to two different subgenera of *Campanula*: subgenus *Campanula* and subgenus *Rapunculus*. Seed length in all species (with exception of *C. lourica*) of subgenus *Campanula* is more than 1.2 mm; it is 2.5 mm in *C. latifolia*, whilst seed length of the other subgenus is less than 1 mm. Significant differences were obtained in seed size for both length and width of all species studied (Table 2) according to the one-way Anova ($P < 0.05$). Based on our results, the seed shape and color are of only minor taxonomic value. The seed shape depends on the seed's relative position in the capsule. However, the

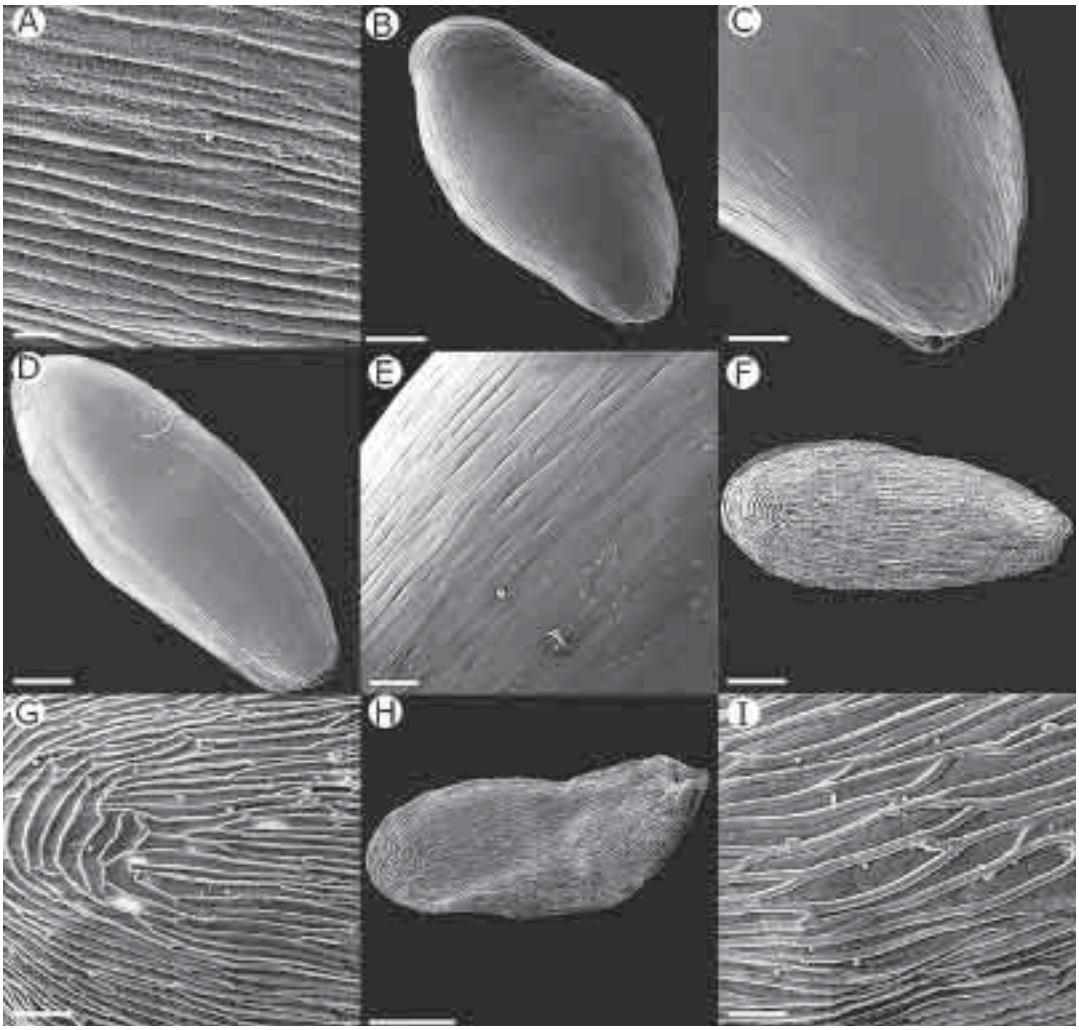

Figure 2. SEM micrographs of seed coat: A – *C. ruprechtii*; B, C – *C. rapunculus* subsp. *lambertiana*; D, E – *C. lourica*; F, G – *C. glomerata* subsp. *hispida*; H, I – *C. involucrata*. Scale bars: A, E = 20 µm; C, G, I = 50 µm; B, D = 100 µm; F = 200 µm; H = 500 µm.

micromorphological features of seeds seem to provide valuable evidence for classification of taxa and may reflect the phylogenetic position of taxa (Barthlott 1981, 1984). In addition to striate testa, other ornamentations, e.g. reticulate and rugose, have been reported for *Campanula* species (Alçitepe 2010; Cupido et al. 2011). The sculpture of seed coats offers a set of characters useful for the taxonomy of the genus.

Campanula latifolia, C. rapunculoides and *C. trachelium* of sect. *Campanula* differ from the latter species by having relatively thick anticlinal walls (vs. extremely thick and rod-like) and narrow lumen (vs. an indistinct lumen) (Figs 1B, D, F, H; 2A). The similar pattern, i.e. interrupted striate and faintly striate, was also observed in *C. lourica* (sect. *Saxicolae* (Boiss.) Kharadze) and *C. rapunculus* (sect. *Rapunculus*), respectively. Seed coat sculpture of *C. rapunculoides* and *C. rapunculus* studied here correspond completely to Akcin (2009) indicating the constancy of seed ornamental features among various populations of a certain species. However, the similarities between the seed surface of both species restricts the use of their micromorphological

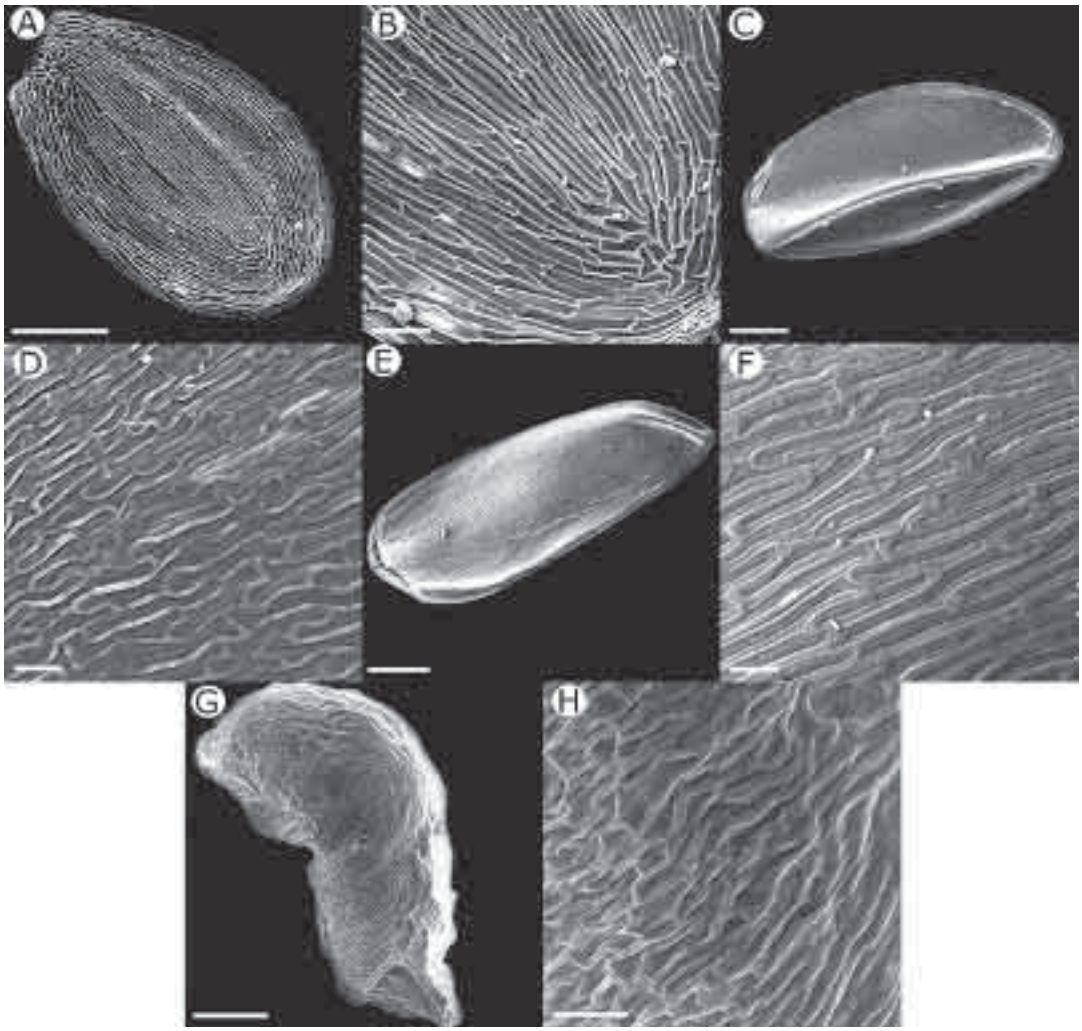

Figure 3. SEM micrographs of seed coat: A, B – *C. lactiflora*; C, D – *C. stevenii* subsp. *beauverdiana*; E, F – *C. stevenii* subsp. *stevenii*; G, H – *C. patula*. Scale bars: A, C, E = 200 µm; G = 100 µm; B = 50 µm; D, F, H = 20 µm.

characteristics for taxonomic purposes. But findings of differences of the vegetative and floral morphology of these species support their placement into separate sections.

Our study shows that *C. rapunculoides* and *C. trachelium* belonging to sect. *Campanula* have a close relationship based on seed coat ornamentation (subtype Ia). Furthermore, pollen surface of these two species is a characteristic feature of their relationship (Khansari et al. 2012). Both have an exine covered with echinae, although the density of echinae in *C. rapunculoides* is higher than in *C. trachelium*.

Campanula aucheri is treated as synonym of *C. saxifraga* subsp. *aucheri* (Lammers 2007), whereas these taxa have been considered as distinct species by Boissier (1875), Fedorov (1957), Schiman-Czeika (1965) and Damboldt (1978). These species seem to be distinct based on morphological characters such as pubescent or glabrous corolla and type of leaf margin (Aghabeigi & Assadi 2008). Both species show similarity in size (as mentioned before) and seed coat pattern, but from gross morphological aspects these two species appear to be unrelated. Moreover, the present

results show that sections *Campanula* and *Scapiflorae* are closely related on the basis of seed characters, e.g. striate ornamentation and lanceolate testa cells. This relationship was also observed between the sections *Involucratae* and *Rapunculus*. The seed surface and testa cells of examined taxa of these two sections were striate-reticulate and narrowly oblong to subpolygonal, except for *C. rapunculus*, in which they are faintly striate and lanceolate.

Striate ornamentation was reported for *C. glomerata*, *C. involucrata* and *C. lactiflora* (Akcin 2009) which disagrees with our results. This may be due to observations under low magnification (scale bar = 100 µm in the mentioned study) as opposed to our investigation using higher magnification (scale bar = 50 µm).

There are considerable differences in seed characters among *C. stevenii* subsp. *stevenii* and *C. stevenii* subsp. *beauverdiana*. For example, the average seed size of *C. stevenii* subsp. *stevenii* (0.77–0.92 × 0.23–0.4 mm) is smaller than of *C. stevenii* subsp. *beauverdiana* (0.97–1 × 0.2–0.57 mm); seed shape in the former taxon is oblong, whereas in the latter taxon it is ellipsoid. Furthermore, seed coat of these two subspecies can be recognized by differences in thickening of the inner face of anticlinal walls: uneven thickening in *C. stevenii* subsp. *stevenii* and even thickening in *C. stevenii* subsp. *beauverdiana* (Fig. 3C–F). From a morphological point of view, *C. stevenii* subsp. *stevenii* can be distinguished by a glabrous calyx tube vs. papillose calyx tube in *C. stevenii* subsp. *beauverdiana*.

There are only few studies about adaptive significance of seed coat sculpturing and ornamentation in Campanulaceae. Water is the main dispersal factor for seeds of Campanulaceae, because the seeds are generally smooth and rounded (Shetler & Morin 1986). In the present study, *C. glomerata*, *C. involucrata* and *C. lactiflora* have epidermal cells with thin anticlinal walls and relatively wide lumina that give the seeds greater buoyancy in moist habitat (Shetler & Morin 1986). Although the micromorphological characteristics of the seed surfaces did not permit the distinction of all examined species, the observed patterns allow the identification of infrageneric groups. Therefore, extended investigations including the seeds of other *Campanula* taxa are needed for a better diagnostic determination.

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