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Chapter 11

Morphological Studies on Seeds of Scrophulariaceae s.l. and Their Systematic Significance

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70572

Abstract

This study employed scanning electron microscopy and light microscopy to observe seed surface micromorphology and seed coat anatomy in the Scrophulariaceae s.l. to investigate seed characters of taxonomic importance. Seeds of 41 taxa corresponding to 13 genera of the family were carefully investigated. Seeds were minute and less than or slightly larger than 1 millimeter in length except for *Melampyrum* and *Pedicularis* species. The seed shape ranged from elliptical to broad elliptical and ovoid. In the studied species the surface sculpture was predominantly reticulate-striate, regular reticulate, sometimes colliculate, and rugose, or - rarely - ribbed, as in *Lindernia procumbens* and *Paulownia coreana*. Seed coats comprised the epidermis and the endothelium. Nevertheless, in all *Melampyrum* and some *Veronica* species the seed coat was very poorly represented and only formed by a papery layer of epidermis. According to correspondence analysis (CA) and unweighted pair group method with arithmetic mean (UPGMA) based cluster analysis the close affinities among the species of *Scrophularia* were well supported by their proximity to one another. Similarly, the proximity of *Melampyrum* species and *Pedicularis* species cannot be denied. In contrast, *Veronica* species were divided into two groups in CA plots and even three in the UPGMA tree. Regardless of the limited range taxa considered we found that similarities and differences in seed morphology between different genera could help us to understand the systematic relationships involved.

Keywords: Scrophulariaceae s.l., seed morphology, seed anatomy, scanning electron microscopy, light microscopy, surface sculpture, seed coat, systematics

1. Introduction

In traditional classification, Scrophulariaceae sensu lato (s.l.) are the largest family in the Lamiales. The members of the family can be distinguished from related families by bilaterally symmetrical and often tubular flowers, ovaries with axile placentation and numerous ovules, and many seeded capsular fruits. However, recent molecular studies have suggested that it is not the presence of a suite of uniquely derived characteristics that allows for the easy recognition of a member of Scrophulariaceae s.l. but, rather, the absence in Scrophulariaceae of the synapomorphies that characterize closely related families [1]. The taxonomic problem of the Scrophulariaceae s.l. is one aspect of the widespread difficulties that reflect the problems of describing natural groups within the order Lamiales. As with most large families, previous classification of the family includes many treatments that differ in their circumscription (for example, see [2]). The most influential classifications for the nineteenth century concept of Scrophulariaceae were those of in [3, 4], from which most contemporary classifications of the family are derived. A large-scale investigation of phylogenetic relationships in the Scrophulariaceae s.l. and related families using DNA sequence data has radically altered the circumscription of many families in Lamiales [1, 2, 5–7].

Before the revolution in molecular systematics, many studies proposed that Scrophulariaceae s.l. were monophyletic and several morphologically similar groups of taxa, which are now assigned to different families, and were placed together (for example, see [8]). For the first time in [2], the study identified two clearly separated clades consisting of members of the family and suggested that the Scrophulariaceae are polyphyletic. Subsequently, a third clade was identified consisting of parasitic members of the Scrophulariaceae and Orobanchaceae [9–11]. Later on five distinct phylogenetic lineages composed mainly of taxa previously assigned to Scrophulariaceae were recognized [5]. However, the emerging classification for the plants which are traditionally assigned to Scrophulariaceae consists of at least seven groups that bear the rank of family [12–14]. Most notable, following changes in the circumscription of families and the disintegration of Scrophulariaceae s.l., were the dramatic changes in the size of some families: the Scrophulariaceae itself was more than halved in size to just over 1800 species, and the Plantaginaceae increased to about 1900 species [15].

The morphological similarities and differences among the groups of taxa and their alignment in various families usually depend on the characters emphasized by different researchers. On the other hand, complications in discriminating between genera or groups of genera are usually the result of the available suite of usable characters, and thus, several comparative studies were carried out. In general, systematics uses morphological characteristics to carve diversity into its taxonomic subunits, and since the beginning of the discipline, plant systematics has frequently used morphological character ranges from roots, leaves, inflorescence, flowers, and fruit to seeds.

Due to its great uniformity, seed morphology has been recognized as an important source of useful phylogenetic information. A number of angiosperm taxa have already been investigated intensively in terms of their seed morphology, in combination with phenetic or phylogenetic analyses at the genus level. In the past, the variation in seed morphology has been used variously in plant systematics ranging from identification [16, 17] and taxonomic circumscription [18, 19] to phylogenetic inference [20, 21] and character-state evolution [22, 23]. Both macro- and micromorphological seed characters have been shown to be of essential systematic importance within and among the genera of traditional Scrophulariaceae, *Orobanchaceae*, and *Plantaginaceae* [24–35], in which seed morphological characters have been used widely to differentiate the different taxa or to find affinities between them.

Recent studies on seed morphology of Scrophulariaceae s.l. have focused mainly on common genera like *Veronica*, *Scrophularia*, and *Pedicularis*. Juan et al. [30] observed fruits and seeds of Scrophulariaceae from southwest Spain, and the systematic significance of seed morphology of *Veronica* and *Pedicularis* has been examined in some comprehensive studies [32–34]. Despite the aforementioned, no comparative studies on seed anatomy or seed coat characteristics together with surface structure have been conducted on any genera of Scrophulariaceae s.l. It is necessary to make extensive investigation of the seed morphology and anatomy of Scrophulariaceae s.l. to determine whether they can be used as additional support for disintegration of genera in the family. The objectives of this study were to (1) understand the utility of seed morphology and anatomy in Scrophulariaceae s.l. systematics, (2) discuss the proximity of studied genera based on these characters, and (3) highlight the characters that can be used to describe different genera and possible variation in infrageneric classification of *Veronica* on a similar basis.

2. Materials and methods

2.1. Specimens

More than 2500 seeds from 41 taxa and 56 accessions were investigated, corresponding to 13 genera of family Scrophulariaceae s.l. originating from seed bank and herbarium specimens at the Korea National Arboretum, Pocheon, Korea. Names of investigated species and accession numbers are presented in **Table 1**.

2.2. Scanning electron microscopy

Sampling seeds were directly taken from seed bank (stored in $-18^{\circ}C$) and thus no pretreatment was needed for scanning electron microscopy (SEM). Before SEM observation, the seed samples were rinsed with absolute ethyl alcohol and sputtered with gold coating in a KIC-IA COXEM ion-coater (COXEM Co., Ltd., Korea). SEM photographs were taken with the help of COXEM CX-100S scanning electron microscope at 20 kV at the Seed Test Laboratory of the Korea National Arboretum. Scale bars were added manually during image alignment.

2.3. Light microscopy

At least 10 to 12 seeds from each species were sectioned and investigated under light microscope. Microscopic slides were prepared using conventional microtome resin method. Mature seeds were dehydrated through alcohol series (50, 70, 80, 90, 95, and 100%). The complete dehydrated seeds were transferred in alcohol/Technovit combination (3:1, 1:1, 1:3, and 100% Technovit) and then embedded in Technovit 7100 resin. Histo-blocks were prepared from each

Family	Genus	Species	Voucher number
Linderniaceae	*Lindernia*	*Lindernia procumbens* (Krock.) Philcox	L10041
		L. crustacea (L.) F.Muell.	L3029
Phrymaceae	*Mazus*	*Mazus pumilus* (Burm.f.) Steenis	L10128
Orobanchaceae	*Lathraea*	*Lathraea japonica* Miq.	2014REC006
	Melampyrum	*Melampyrum koreanum* K. J. Kim & S. M. Yun	L9710
		M. roseum Maxim.	L2324
		M. roseum var. *japonicum* Franch. & Sav.	L1707
		M. roseum var. *ovalifolium* Nakai ex Beauverd	L10163
		M. setaceum (Maxim. ex Palib.) Nakai	L8578
		M. setaceum var. *nakaianum* (Tuyama) T.Yamaz.	L3449
	Pedicularis	*Pedicularis mandshurica* Maxim.	L10193
		P. resupinata f. *albiflora* (Nakai) W.T.Lee	L9042
		P. resupinata L.	2014KNA031
		P. resupinata var. *umbrosa* Kom. ex Nakai	L10246
		Pedicularis verticillata L.	L8699
		Phtheirospermum japonicum (Thunb.) Kanitz	L9691
	Siphonostegia	*Siphonostegia chinensis* Benth.	L10657
Paulowniaceae	*Paulownia*	*Paulownia coreana* Uyeki	2014 REC081
Plantaginaceae	*Limnophila*	*Limnophila indica* (L.) Druce	L10453
	Veronica	*Veronica arvensis* L.	L3304
		V. dahurica Steven	L10154
		V. didyma var. *lilacina* (*H. Hara*) T.Yamaz.	L2006
		V. incana L.	L10791
		V. kiusiana var. *diamantiaca* (Nakai) T.Yamaz.	L10532
		V. kiusiana var. *glabrifolia* (Kitag.) Kitag.	L9413
		V. linariifolia Pall. ex Link	L7983
		V. longifolia L.	L9903
		V. nakaiana Ohwi	2014GB024
		V. peregrina L.	L2695
		V. persica Poir.	L2702
		V. pusanensis Y. Lee	L11087
		V. pyrethrina Nakai	L10564
		V. rotunda var. *subintegra* (Nakai) T.Yamaz.	L10130
		V. undulata Wall.	2014 cc56
	Veronicastrum	*Veronicastrum sibiricum* (L.) Pennell	L10658

Family	Genus	Species	Voucher number
	Linaria	*Linaria japonica* Miq.	L8982
Scrophulariaceae s.s.	*Scrophularia*	*Scrophularia buergeriana* Miq.	L8488
		S. grayana Maxim. ex Kom.	L10574
		S. kakudensis Franch.	L9643
		S. koraiensis Nakai	L9617
		S. takesimensis Nakai	L12516

Table 1. List of the plant species with their voucher number included in this study.

embedded materials and then sectioned using a Leica RM2255 rotary microtome (Leica Microsystems GmbH, Germany). Serial sections of 4–6 μm thickness were cut with stainless blades, fixed onto a slide glass, and dried on electric slide warmer for about 12 h. In order to stain, dried slides were immersed in 0.1% Toluidine blue O for 60–90 s, rinsed with running water, and again dried with an electric slide warmer for more than 6 h to remove water. After complete removal of the water, slides were mounted with Entellan (Merck Co., Germany) and pressed with metal blocks for a couple days to remove air bubbles. After 2 days, the prepared slides were observed under an AXIO Imager A1 light microscope (Carl Zeiss, Germany). Photomicrographs were taken with an AxioCam MRc5 attached camera system, and seed coat measurement was carried out by using AxioVision software for Windows (release 4.7, 2008). Multiple image alignment was arranged by Photoshop CS4 for Windows 2007. None of the image-alteration facilities of Photoshop were used to modify the original images.

2.4. Morphometry and data analysis

A total of approximately 2500 seeds were used for morphometric measurement. Digital images of whole seeds were taken with a Leica DFC420 C multifocal camera attached to a Leica MZ16 FA microscope (Leica Microsystems). The length and width of a minimum of 20 seeds from each taxon were measured from digital images using Leica LAS V3.8 software for Windows. Seed length (SL) and width were measured, length/width ratios (LWRs) were calculated, and mean values are presented. Individual seed morphological parameters and their features are given in Appendix 1.

For the analysis, 13 seed characters were treated as non-ordered and coded as unweighted consecutive numbers (**Table 2** and Appendix 2). Correspondence analysis (CA) and cluster analysis (UPGMA) were performed to reveal whether the seed features allowed the grouping of taxa using PAST version 3.14 [36]. The eigenvectors and character scores of the first four axes in CA are presented in **Table 3**, together with the percentage of total variance. The results were presented in a two-dimensional biplot of axis 1 (in *X* axis) and axis 2 (*Y*-axis). To visualize the relationship between the different taxa based on seed features, a cluster analysis-based UPGMA tree using Euclidean distance measurement was constructed. Bootstrap support values were based on 100 replicates, and values above 50 were presented above tree branches.

1. Shape: 0, elliptical; 1 ovoid
2. Seed length: 0, <1 mm; 1, 1–2 mm, 2, >2 mm
3. Length/width ratio: 0, <2; 1, >2
4. Hilum position: 0, lateral; 1, basal.
5. Hilum character: 0, distinctly protuberant; 1, flat to indistinctly protuberant; 2, deep
6. Primary surface sculpture: 0, reticulate; 1, colliculate; 2, others
7. Epidermal cell shape: 0, rectangular; 1, polygonal; 2, irregular
8. Anticlinal wall: 0, shallow to indistinctly raised; 1, distinctly raised
9. Periclinal wall: 0, convex; 1, flat; 2, concave
10. Periclinal wall ornamentation: 0, striate; 1, papillate/granulate; 2, smooth/folded
11. Seed coat anatomy: 0, seed coat distinct; 1, indistinct
12. Epidermis: 0, well represent; 1, degenerate
13. Endothelium: 0, present; 1, absent

Table 2. Seed characters with their coding states used in analysis.

Character number	Character code	Axis 1	Axis 2	Axis 3	Axis 4
1	SH	−0.23118	−**0.76272**	−0.26612	0.263812
2	SL	0.007571	**0.467293**	0.281203	−0.31876
3	LWR	0.654796	**0.952965**	0.162676	−0.4085
4	HP	0.10317	0.025843	−0.09821	−**0.26307**
5	HC	0.065757	−**0.91809**	−0.06242	−0.80719
6	PSS	**0.817606**	0.046239	−0.7419	0.29745
7	ECS	−0.09684	−0.15219	−0.04569	**0.273533**
8	AW	−**0.8693**	0.080012	0.101048	0.143169
9	PW	−**0.88318**	0.176183	−0.03262	0.129772
10	PWO	**0.491091**	0.221064	−0.31801	−0.04532
11	SCA	**0.995628**	−0.19168	0.954453	0.561853
12	EP	0.382648	−0.61797	**0.775164**	−0.04003
13	EN	**0.91233**	−0.07943	0.531445	0.705178
	Eigenvalue	0.325	0.176	0.123	0.115
	% total variance	34.355%	18.675%	13.041%	12.213%

AW, anticlinal wall; EN, endothecium; EP, epidermis; HC, hilum character; HP, vn; LWR, length/width ratio; PSS, primary surface sculpture; PW, periclinal wall; PWO, primary wall ornamentation; SCA, seed coat anatomy; ECS, epidermal cell shape; SH, seed shape; and SL, seed length.

Table 3. Eigenvectors and character scores of the first four axes of a CA of the 13 seed characters.

http://dx.doi.org/10.5772/intechopen.70572

3. Results

A total of 41 taxa from Scrophulariaceae s.l. were studied, belonging to six families: Scrophulariaceae sensu stricto (s.s.; 1 genus, 5 species), Plantaginaceae (4 genera, 14 species, 4 varieties), Orobanchaceae (5 genera, 9 species, 4 varieties, 1 forma), Linderniaceae (1 genus, 2 species), Phrymaceae (1 species), and Paulowniaceae (1 species). Selected scanning electron micrographs and light micrographs of seeds are presented in **Figures 1–11**. A comprehensive description of seed features by family and genus is given here.

3.1. *Scrophularia* (Scrophulariaceae s.s)

Five species of *Scrophularia* were investigated in this study (**Figure 1A–O**). Seeds were minute with a small of variation in size within studied species; they were ovoid to broadly elliptical in shape and usually black or sometimes brown in color (Appendix 1, online supplementary resource; **Figure 1A, B, D, E, G, H, J, K, M, N**). The hilum was terminally positioned and slightly protruding. In all species, gross surface sculpture was typical reticulate-striate, and epidermal cells were polygonal or elongated in one direction (**Figure 1C, F, I, L, O**). The periclinal wall (PW) of the testal cell was slightly concave with parallel striation, and the anticlinal wall (AW) was highly raised, straight to sinuous, or wavy and unevenly thickened.

3.2. *Limnophila*, *Linaria*, *Veronica*, and *Veronicastrum* (Plantaginaceae)

Altogether, 18 taxa from 4 genera of Plantaginaceae, including 11 species and 4 varieties of the genus *Veronica*, were investigated. Seeds were minute: the largest seeds of *Veronica didyma* var. *lilacina* ranged from 1.01 × 0.81 mm to 1.49 × 1.25 mm, and the smallest seeds of *V. rotunda* var. *subintegra* ranged from 0.44 × 0.32 mm to 0.81 × 0.49 mm. Seed were pale yellow or dark brown to black in color, and seed shape (SS) ranged from ovoid, broad ovoid, to sub-spherical, and mostly they were flat or plano-convex and bifacial (**Figures 2A–O**, **3A–O**, **4A–O**, and **5A–F**). The surface sculpture was predominantly reticulate-striate, cristate, and sometimes reticulate-verrucate, as in *V. persica*, *V. arvensis*, and *V. didyma* var. *lilacina* (**Figures 2F, I** and **4O**), reticulate-corrugate as in *Veronica undulata* (**Figure 4L**), or typical reticulate as in *V. peregrina* (**Figure 2C**). Epidermal cell shape was polygonal, elongated, and isodiametric or rarely irregular as in *V. arvensis* (**Figure 2F**). The periclinal wall of the surface cell was flat to slightly concave and striate but rarely folded, whereas the anticlinal wall was slightly to highly raised, straight to wavy, folded, and sometimes of uneven thickness.

3.3. *Pedicularis*, *Melampyrum*, *Lathraea*, *Phtheirospermum*, and *Siphonostegia* (Orobanchaceae)

A total of 14 taxa belonging to 5 genera (6 taxa from *Melampyrum*, 5 taxa from *Pedicularis*, and 1 species from each three genera) of Orobanchaceae were investigated. The seeds were larger than the taxa of Scrophulariaceae s.s. and Plantaginaceae. *Melampyrum roseum* had the largest seeds (4.31 × 1.77 mm to 5.42 × 2.2 mm), whereas *Phtheirospermum japonica* had the smallest (1.04 × 0.49 mm to 1.89 × 0.84 mm; **Figures 6A–O**, **7A–O**, and **8A–L**). Mostly seeds were elliptical and cylindrical (*Melampyrum*), ellipsoidal, navicular (*Pedicularis*), ovoid to subglobose

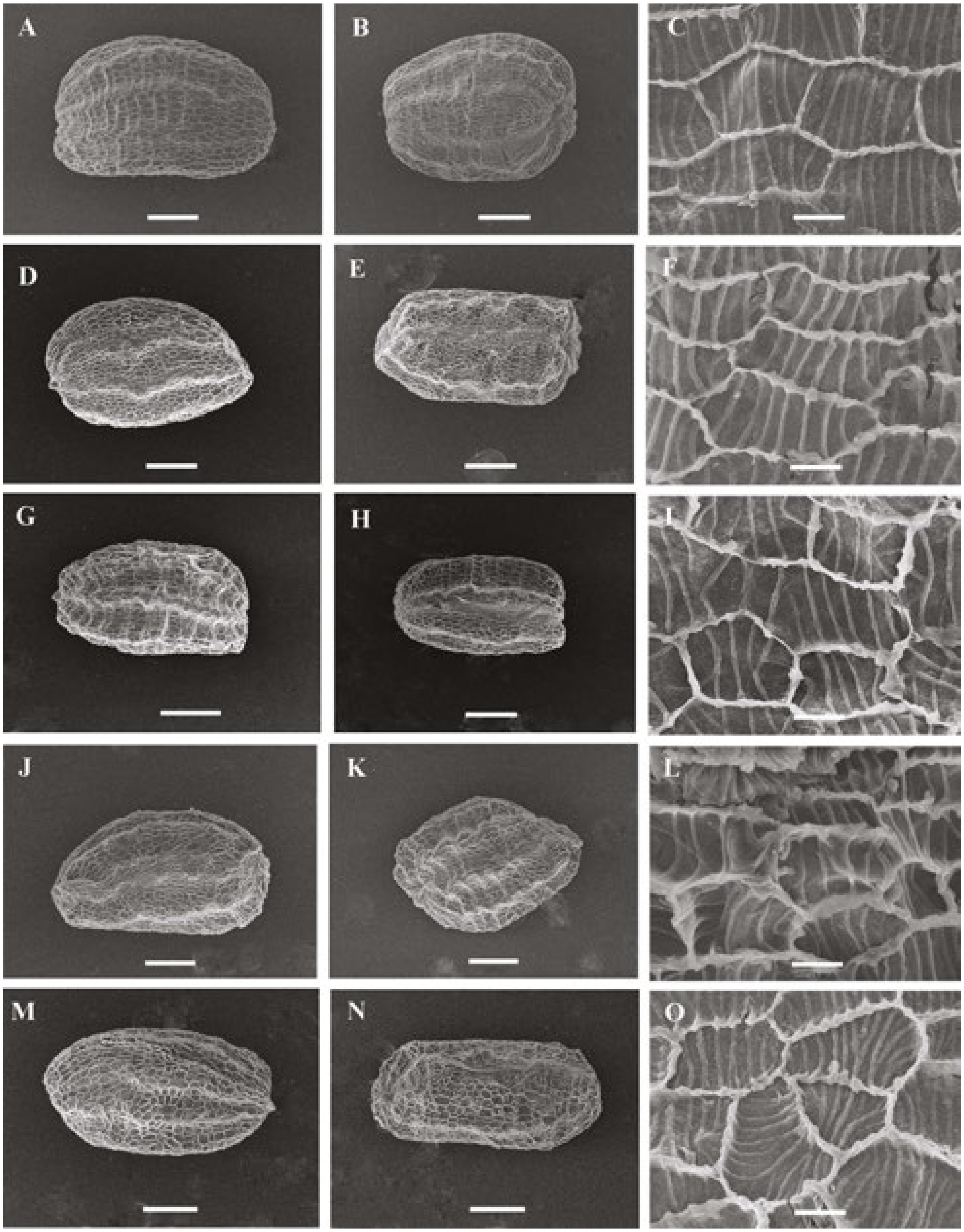

Figure 1. Scanning electron micrographs of seeds of Scrophulariaceae s.l. *Scrophularia kakudensis* (A, B, C), *S. koraiensis* (D, E, F), *S. takesimensis* (G, H, I), *S. buergeriana* (J, K, L), and *S. grayana* (M, N, O). Scale bars: 300 μm (A, B, D, E, G, H, J, K, M, N), 30 μm (C, O), and 20 μm (F, I, L).

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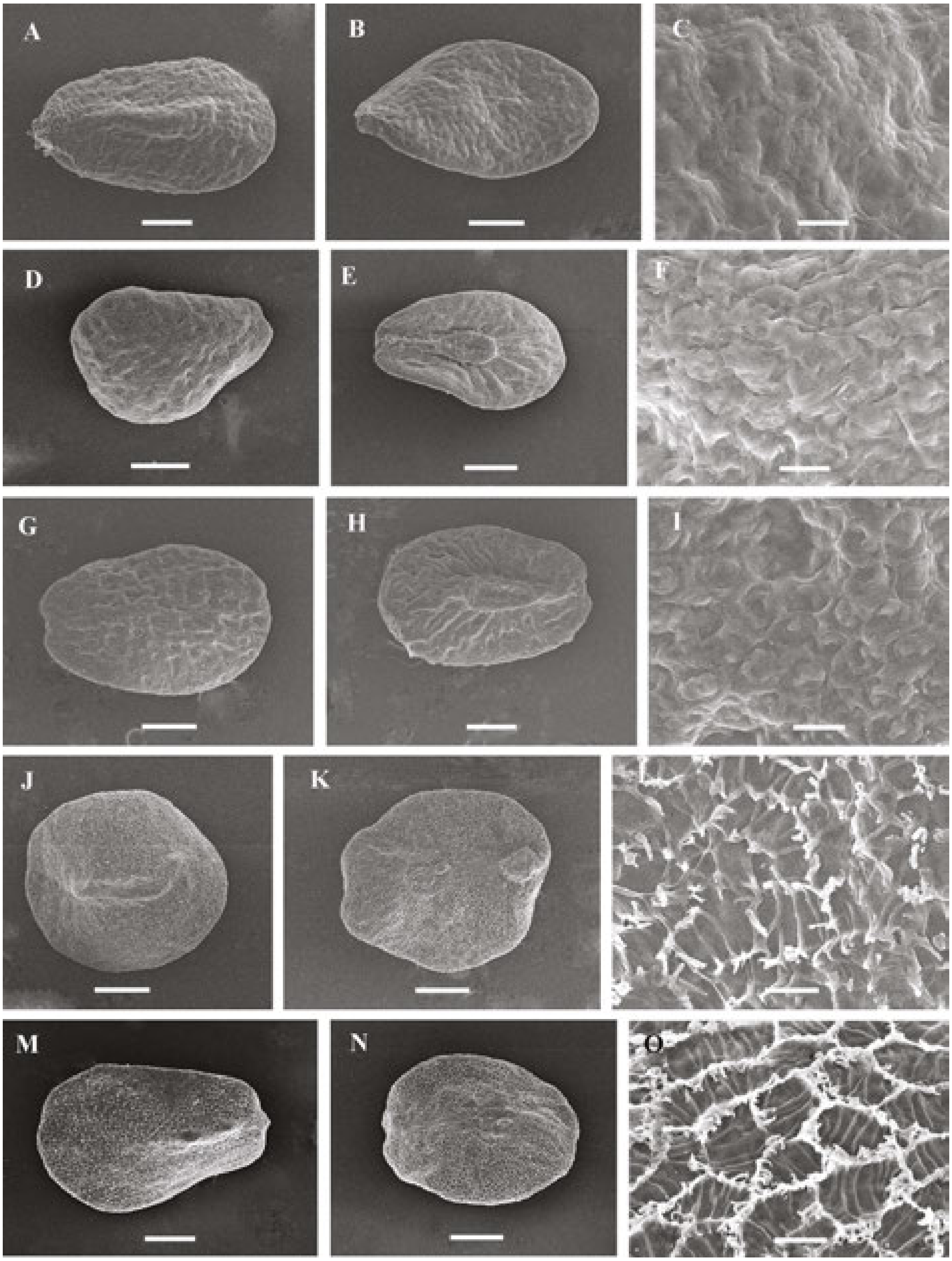

Figure 2. Scanning electron micrographs of seeds of Plantaginaceae. *Veronica peregrina* (A, B, C), *V. arvensis* (D, E, F), *V. persica* (G, H, I), *V. longifolia* (J, K, L), and *V. linariifolia* (M, N, O). Scale bars: 300 μm (D, E, G, H, J, K, M, N), 150 μm (A, B), and 20 μm (C, F, I, L, O).

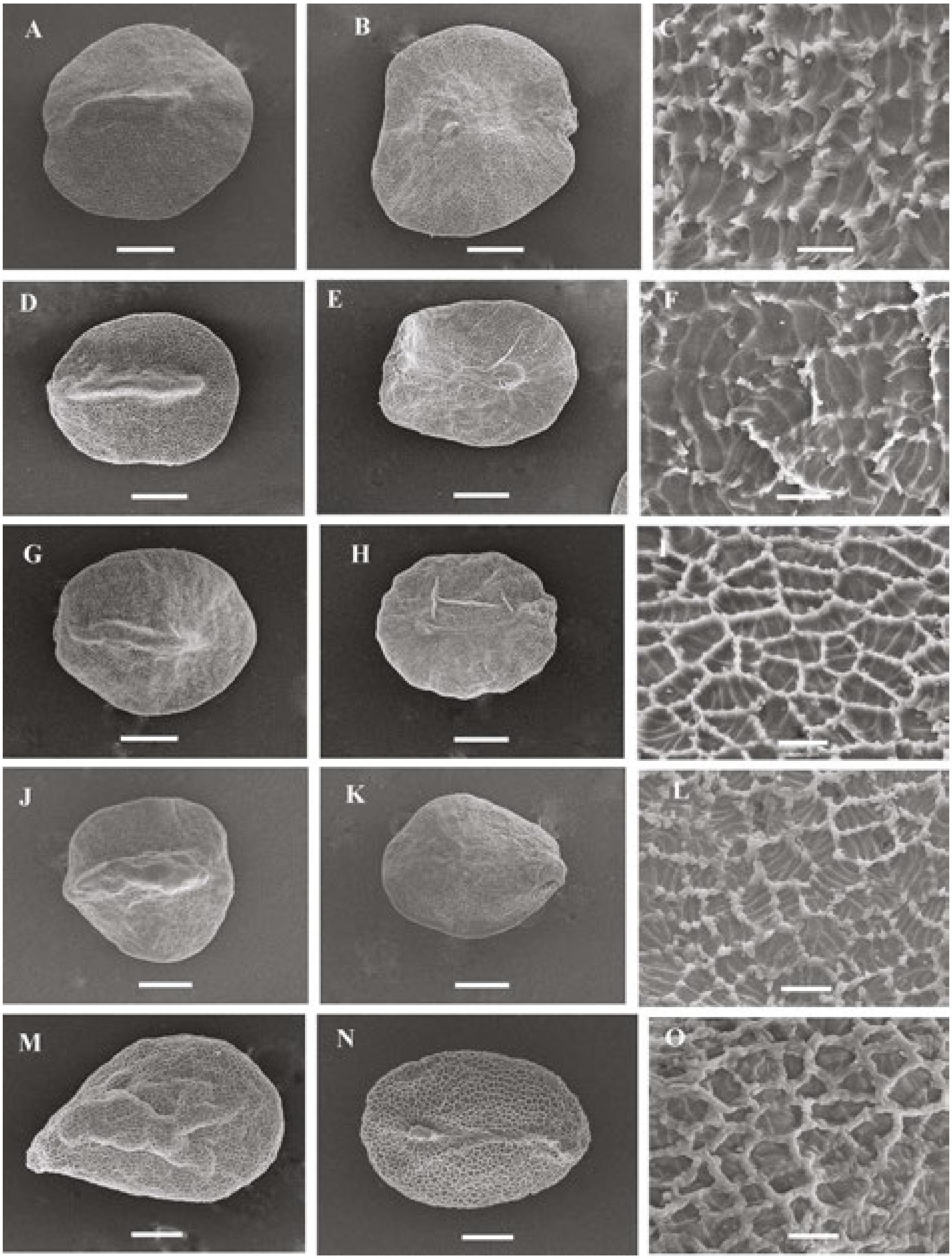

Figure 3. Scanning electron micrographs of seeds of Plantaginaceae. *Veronica dahurica* (A, B, C), *V. rotunda* var. *sabinteara* (D, E, F), *V. pusanensis* (G, H, I), *V. incana* (J, K, L), and *V. pyrethrina* (M, N, O). Scale bars: 300 μm (A, B, D, E, G, H, J, K), 150 μm (M, N), and 20 μm (C, F, I, L, O).

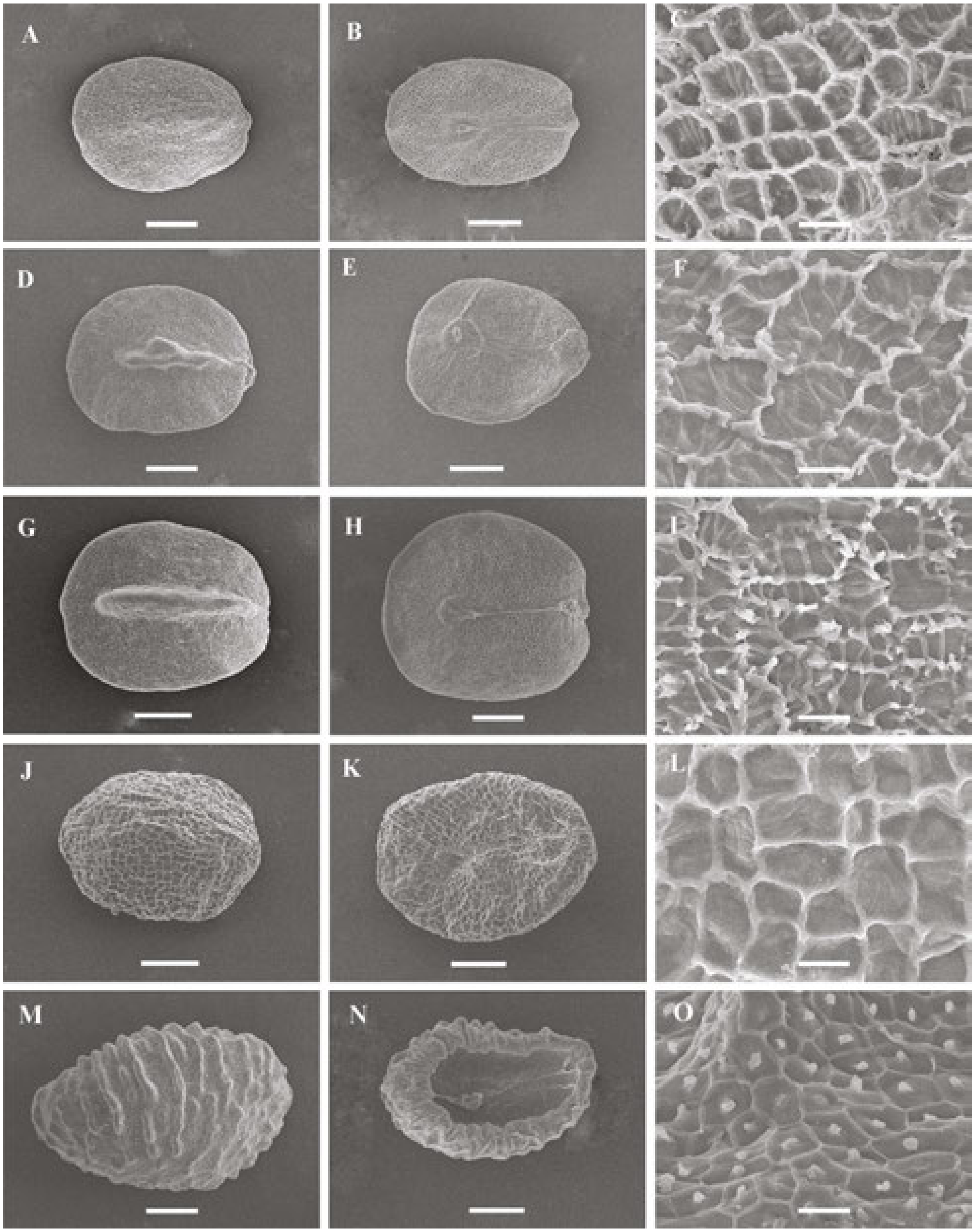

Figure 4. Scanning electron micrographs of seeds of Plantaginaceae. *Veronica nakiana* (A, B, C), *V. kiusiana* var. *diamentica* (D, E, F), *V. kiusiana* var. *glabrifolia* (G, H, I), *V. undulata* (J, K, L), and *V. didyma* var. *lilacina* (M, N, O). Scale bars: 300 μm (A, B, D, E, G, H, M, N), 150 μm (J, K), and 20 μm (C, F, I, L, O).

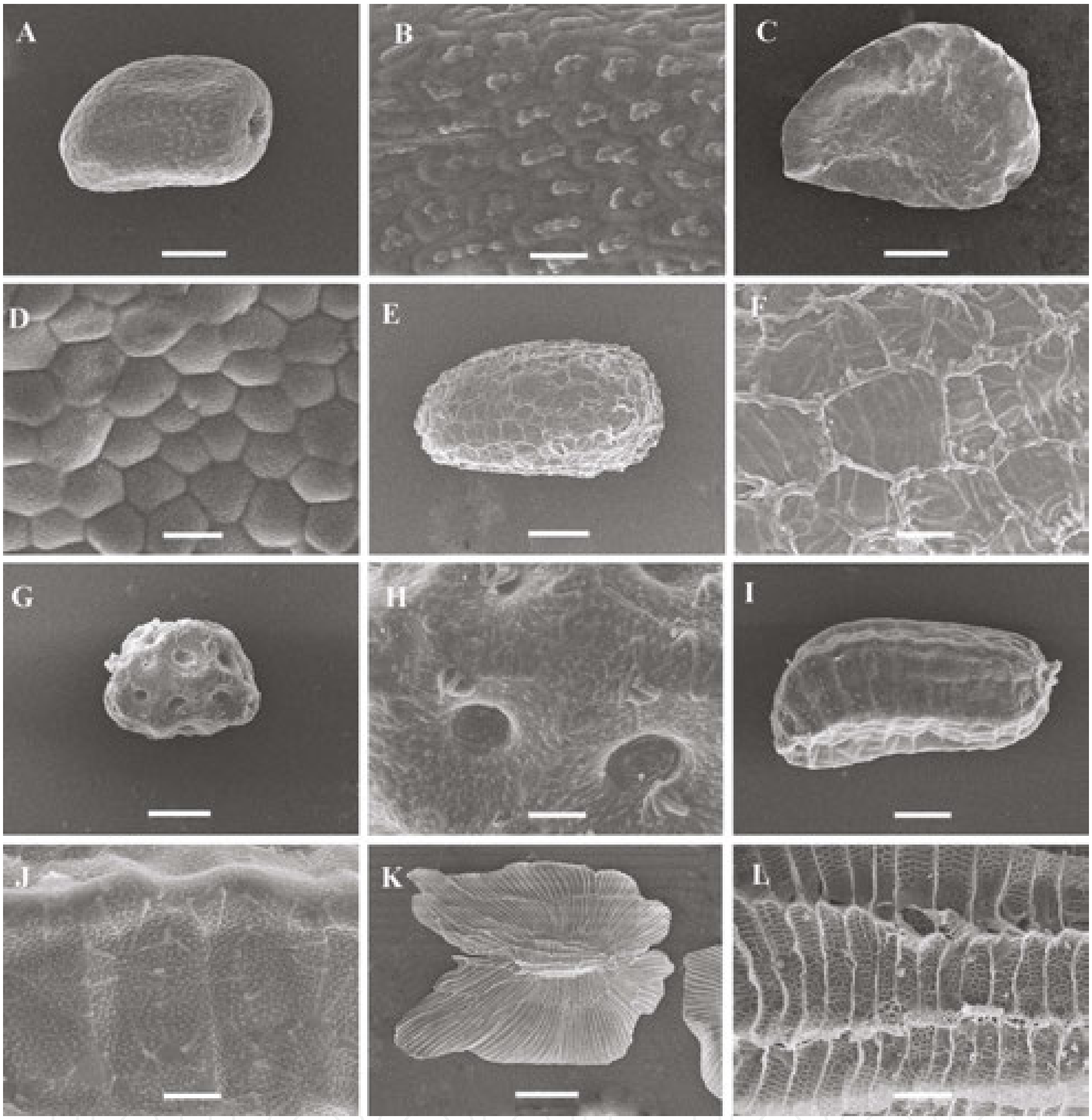

Figure 5. Scanning electron micrographs of seeds of Plantaginaceae, Linderniaceae, and Paulowniaceae. *Limnophila indica* (A, B), *Linaria japonica* (C, D), *Veronicastrum sibiricum* (E, F), *Lindernia crustacea* (G, H), *Lindernia procumbens* (I, J), *Paulownia coreana* (K, L). Scale bars: 1000 μm (K), 600 μm (C), 150 μm (A, G), 100 μm (L), 42 μm (F), 30 μm (B, D, H), and 15 μm (J).

(*Lathraea japonica*), ovoid (*Phtheirospermum japonica*), or elliptical beaked (*Siphonostegia chinensis*). The surface sculpture was highly variable, being rugose, colliculate, reticulate, scalariform, or papillate. The testa dermal cell shape was predominantly irregular; in some species, it was rectangular to elongated or polygonal-isodiametric. The testa periclinal wall was concave, flat concave, or slightly convex, with either smooth to finely folded or sessile papillae. The anticlinal walls were mostly raised but sometimes shallow and sometimes deep. The raised wall was smooth, finely papillate, or finely folded and unevenly thickened.

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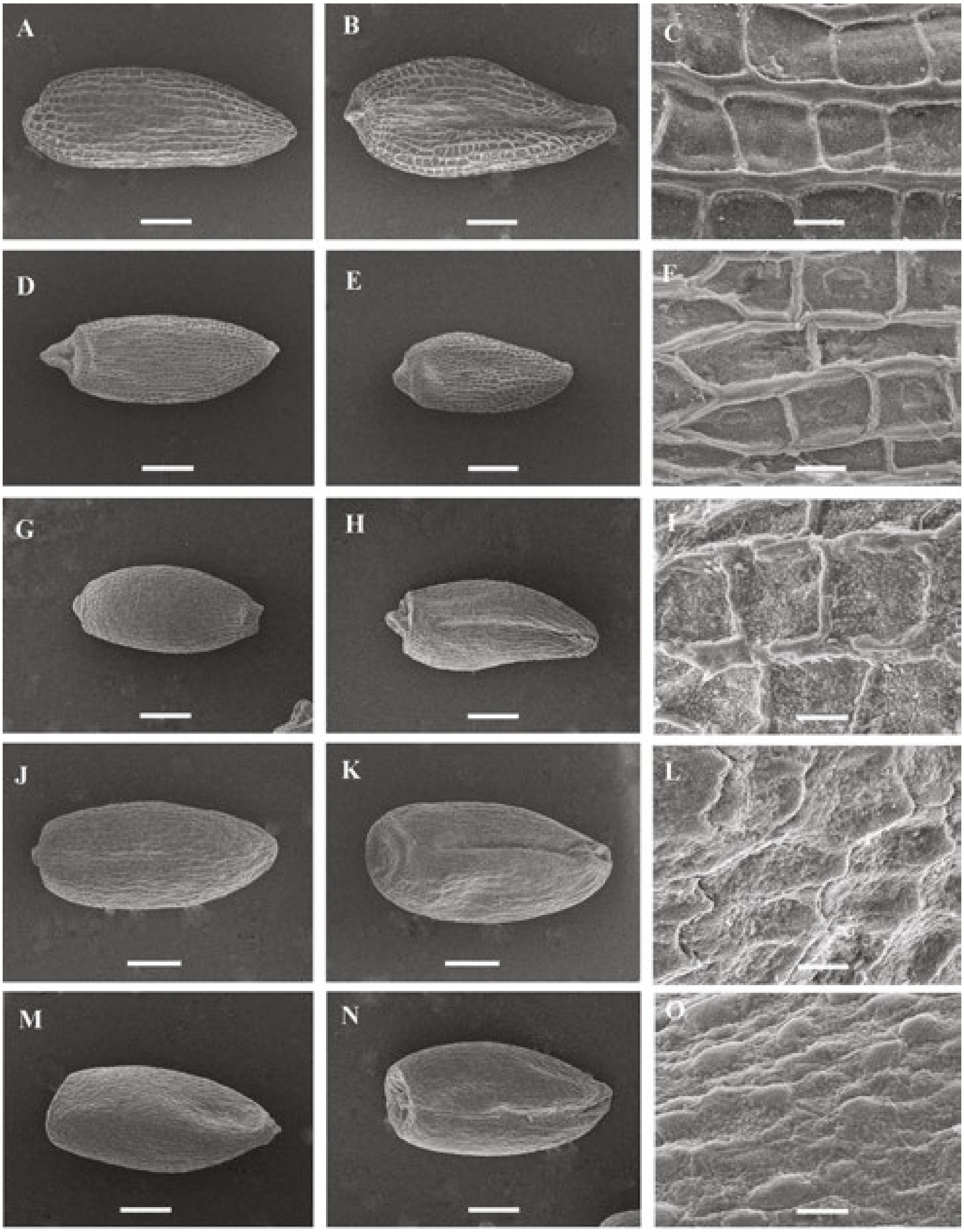

Figure 6. Scanning electron micrographs of seeds of Orobanchaceae. *Pedicularis mandshurica* (A, B, C), *P. resupinata* var. *umbrosa* (D, E, F), *P. verticillata* (G, H, I), *P. resupinata* (J, K, L), and *P. resupinata* for *albiflora* (M, N, O). Scale bars: 600 μm (A, B, D, E, G, H, J, K, M, N), 42 μm (C, F, I, L, O).

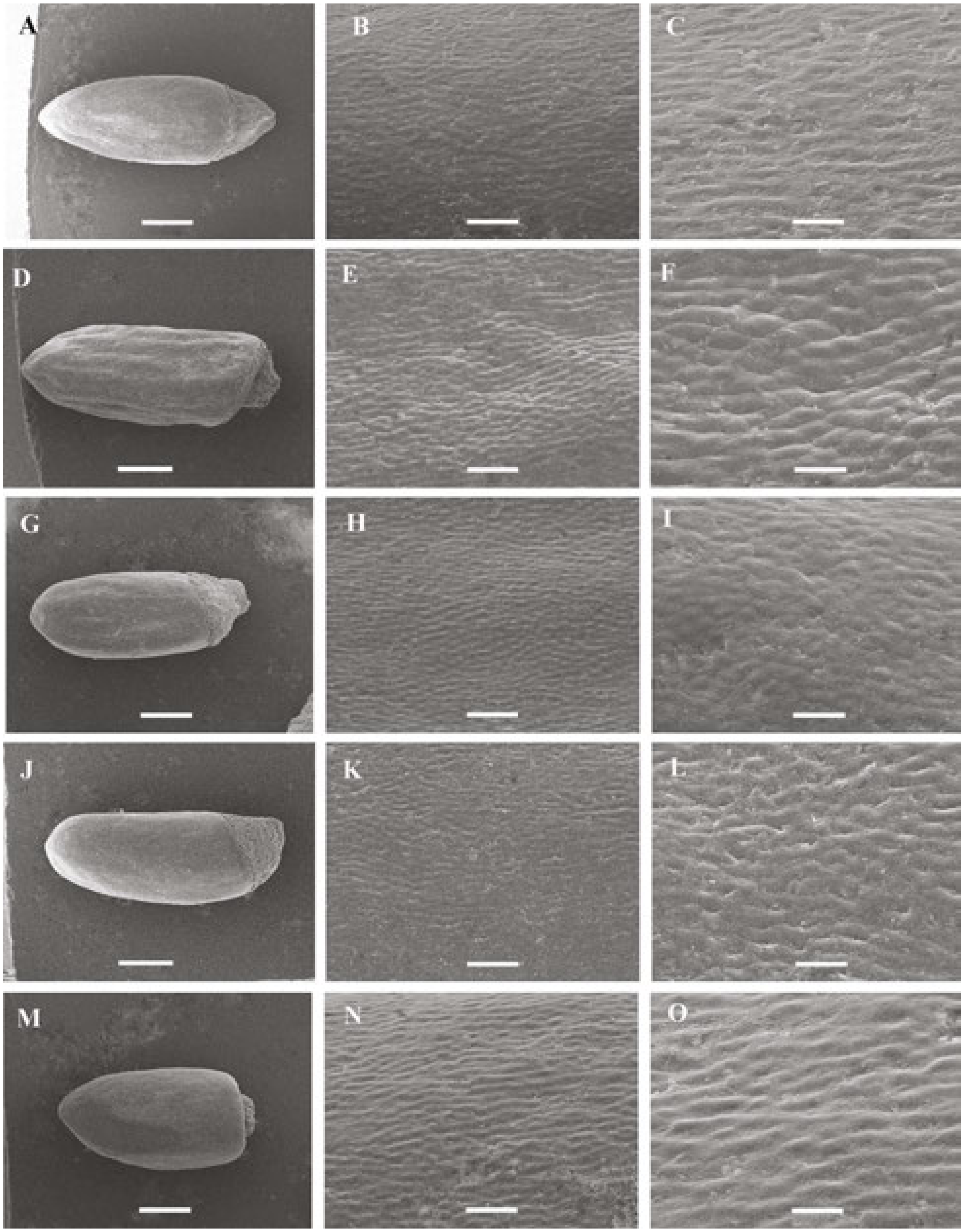

Figure 7. Scanning electron micrographs of seeds of Orobanchaceae. *Melampyrum koreanum* (A, B, C), *M. roseum* (D, E, F) *M. roseum* var. *japonicum* (G, H, I), *M. roseum* var. *ovalifolium* (J, K, L), and *M. setaceum* (M, N, O). Scale bars: 1000 μm (A, D, G, J, M), 100 μm (B, E, H, K, N), and 42 μm (C, F, I, L, O).

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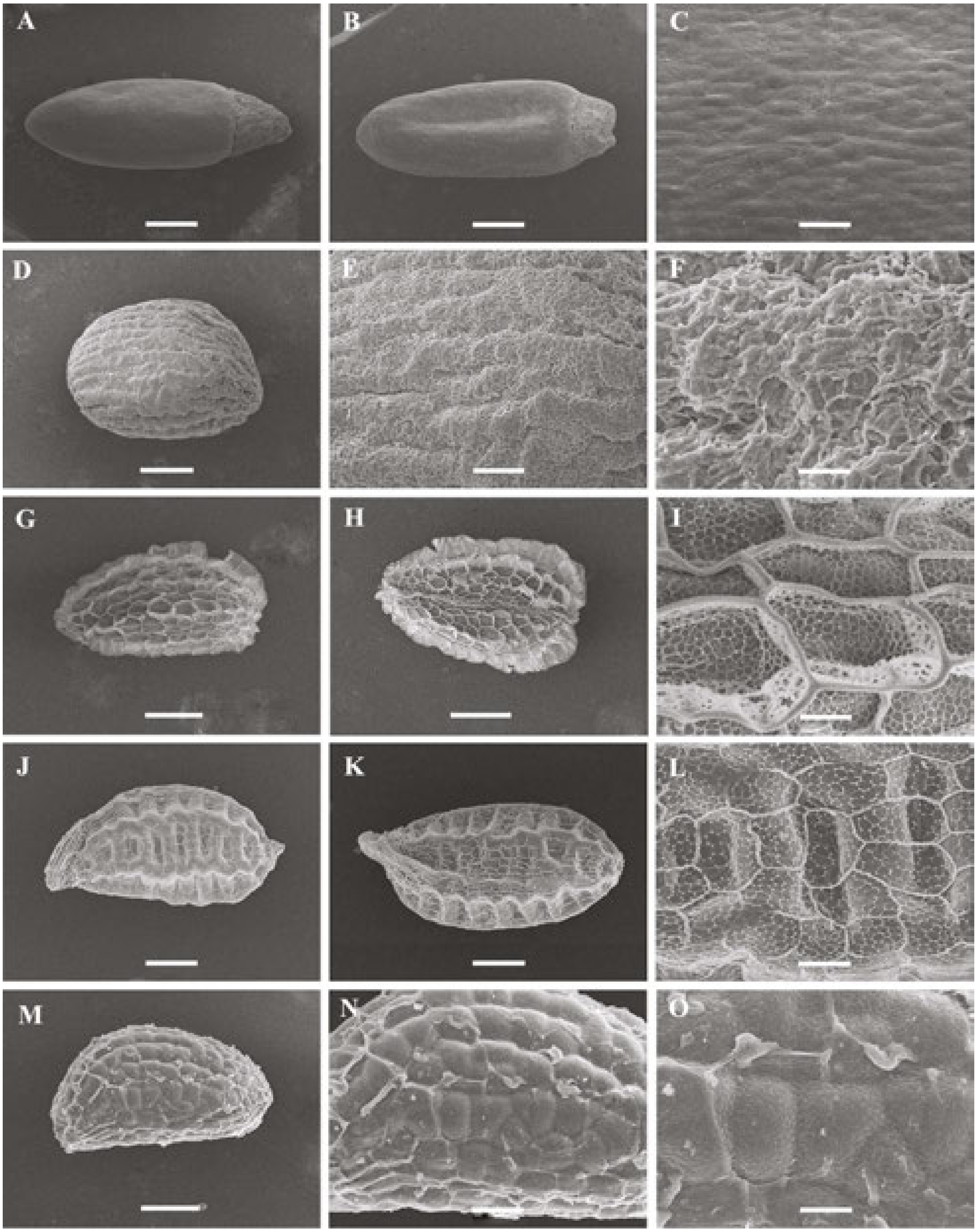

Figure 8. Scanning electron micrographs of seeds of Orobanchaceae and Phrymaceae. *Melampyrum setaceum* var. *nakaianum* (A, B, C), *Lathraea japonica* (D, E, F), *Phtheirospermum japonicum* (G, H, I), *Siphonostegia chinensis* (J, K, L), and *Mazus pumilus* (M, N, O). Scale bars: 1000 μm (A, B), 600 μm (D), 200 μm (E, J, K), 300 μm (G, H), 100 μm and 42 μm (C, F, I, L, M, NM), and 20 μm (O).

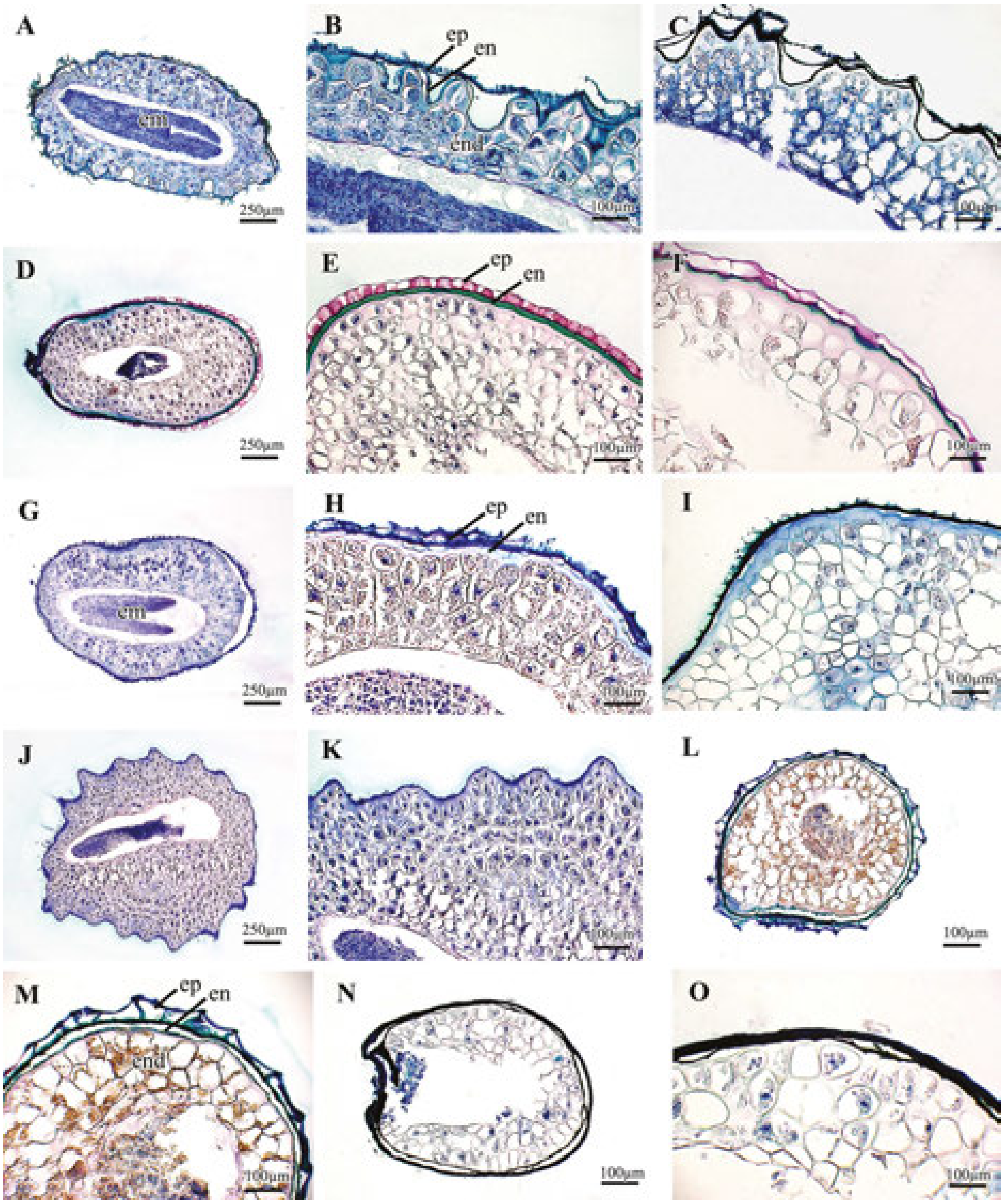

Figure 9. Seed anatomy of (A, B) *Scrophularia buergeriana*, (C) *S. koraiensis*, (D, E) *Veronica peregrina*, (F) *V. undulata*, (G, H) *V. incana*, (I) *V. pusanensis*, (J, K) *V. didyma* var. *lilacina*, (L, M) *Veronicastrum sibiricum*, and (N, O) *Limnophila indica*. em, embryo; en, endothelium; end, endosperm; and ep, epidermis.

3.4. *Lindernia* (Linderniaceae)

Two species were included and seeds were ovoid or ovoid to oblong (**Figure 5G–J**). The surface was ribbed, ridged, or rugose pitted. The epidermal cell shape was rectangular, elongated (*L. procumbens*), or irregular (*Lindernia crustacea*). The testal periclinal wall was flat,

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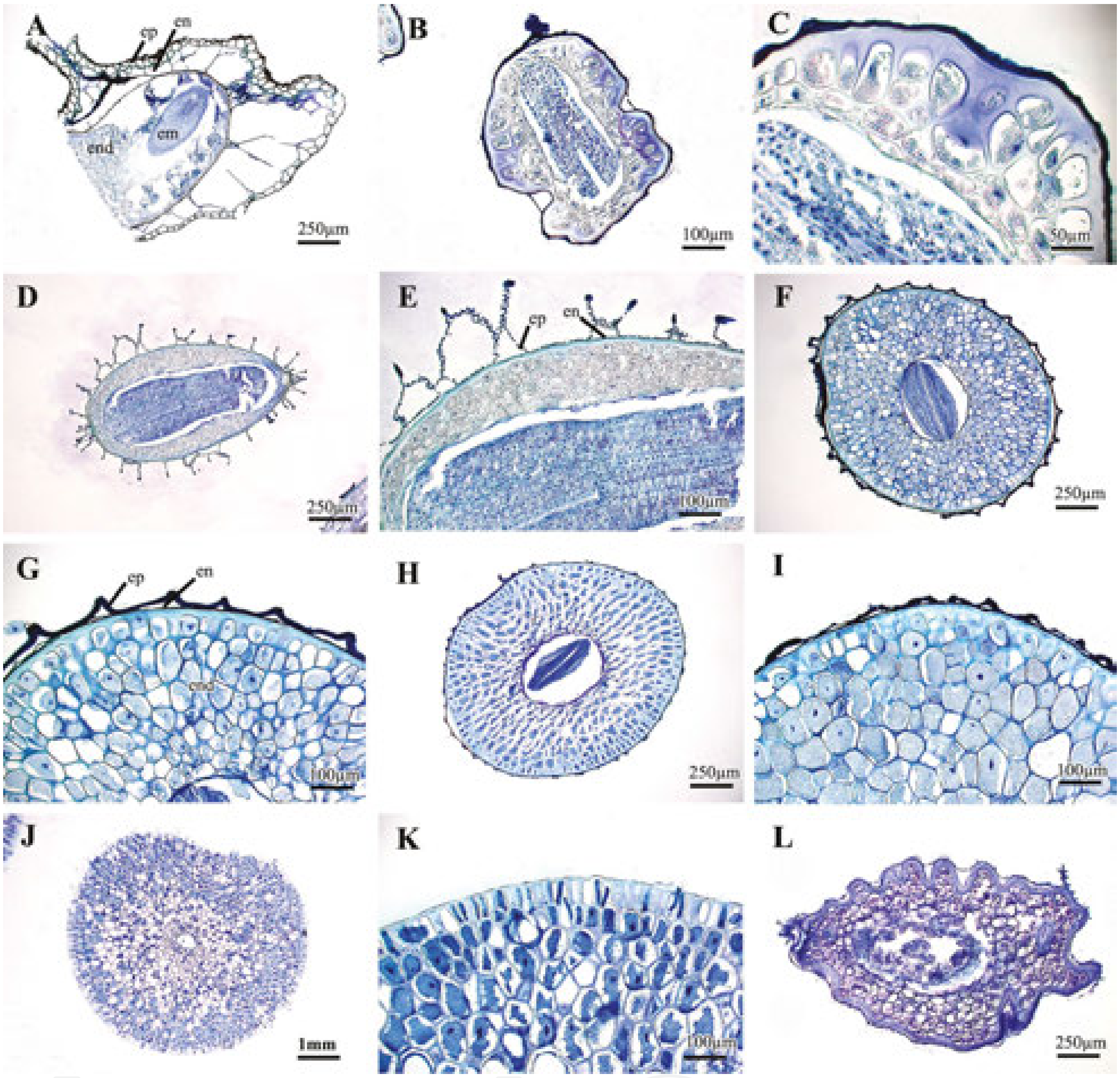

Figure 10. Seed anatomy of (A) *Linaria japonica*, (B, C) *Lindernia procumbens*, (D, E) *Phtheirospermum japonicum*, (F, G) *Pedicularis mandshurica*, (H, I) *P. resupinata*, (J, K) *Melampyrum roseum*, and (L) *Siphonostegia chinensis*. Abbreviations: em, embryo; en, endothelium; end, endosperm; ep, epidermis.

slightly concave, and finely granulate, whereas the anticlinal wall was slightly raised and finely granulate to folded.

3.5. *Paulownia* (Paulowniaceae)

An endemic species, *P. coreana*, was investigated. The seeds were small, fluffy winged, and pale yellow to white in color (**Figure 5K, L**). The testa surface was ribbed, and epidermal cells were polygonal-isodiametric in shape. The testa periclinal wall was flat to slightly concave, and its surface was smooth, whereas the anticlinal wall was raised, straight to sinuous, and unevenly thickened.

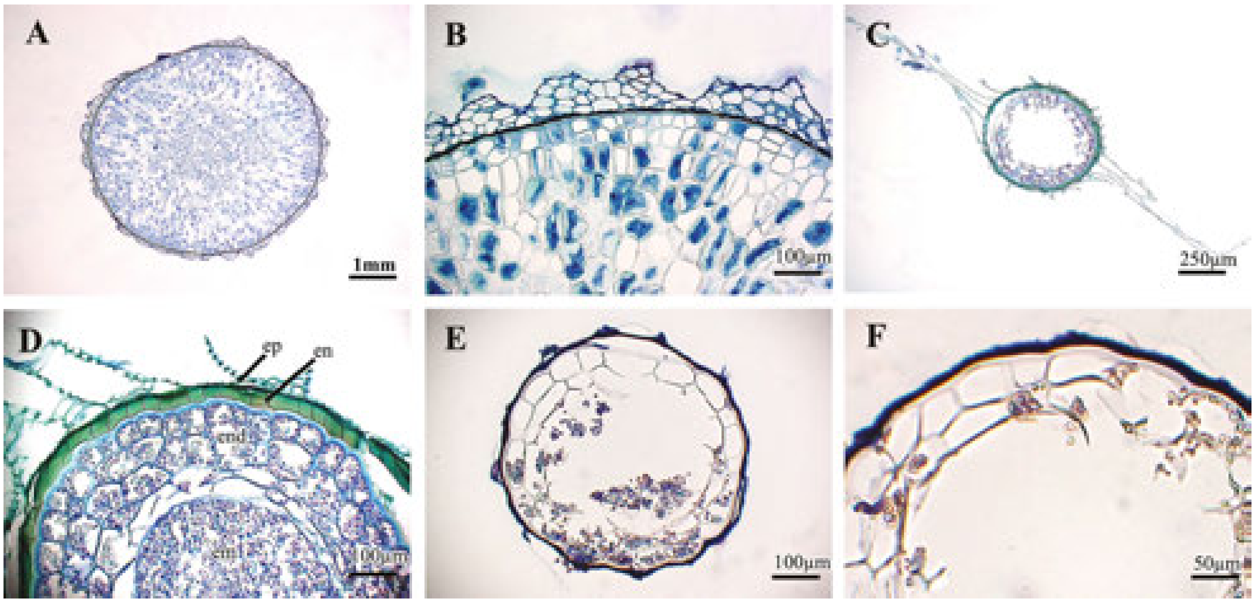

Figure 11. Seed anatomy of (A, B) *Lathraea japonica*, (C, D) *Paulownia coreana* and (E, F) *Mazus pumilus*. Abbreviations: em, embryo; en, endothelium; end, endosperm; ep, epidermis.

3.6. *Mazus* (Phrymaceae)

Only *Mazus pumilu*s was included in this study, and seeds were small ovoid or ellipsoidal in shape (**Figure 8M–O**). The surface sculpture was colliculate with rectangular to polygonal epidermal cells. The periclinal wall was convex with fine folds, whereas the anticlinal wall was shallow with fine folds.

3.7. Seed anatomy

The seed coat in Scrophulariaceae s.s. was somehow distinct, thin, and fairly comparable in all studied species. The epidermis was represented by a layer of degenerated cells followed by one or two endothelium layers (**Figure 9A–C**). In most places, endothelium cells were degenerated.

In 18 taxa of Plantaginaceae, the seed coat was distinct and more well characterized than in *Scrophularia* except in three species of *Veronica* (*V. arvensis, V. didyma* var. *lilacina*, and *V. persica*), in which the seed coat was unclear and degenerated (**Figures 9D–O** and **10A**). The epidermis was well represented in all species except in the aforementioned species, and it was noticeable in *V. peregrina* and *Veronicastrum sibericum* (**Figure 9L, M**). In all species, the epidermis was followed by one or two endothelium layers which were represented by either distinct cells in layers or degenerated layers.

The seeds of the six *Melampyrum* taxa were easily distinguished from rest of the Orobanchaceae species. They had largest seeds among the studied genera and a very thin seed coat in transverse section (**Figures 10D–L** and **11A, B**). The epidermis was well characterized, and endothelium was present in eight taxa of the family, excluding all *Melampyrum* species.

http://dx.doi.org/10.5772/intechopen.70572

The seed coat was well represented in *Lindernia* spp., *P. coreana*, and *M. pumilus*, although the epidermal cells were more clearly noticeable in the former rather than the latter two (**Figures 10B, C** and **11C–F**). In *M. pumilus*, the epidermis was characterized by a degenerated layer of cells. The endothelium was present but poorly developed in all species.

3.8. Data analyses

The relationships among the taxa for the 13 seed characteristics were analyzed using correspondence analysis (CA) and cluster analyses (**Figures 12** and **13**). In CA, the first four axes explained 78.869% of the total variance of the analyzed data (**Table 3**). Axis 1 described 34.355% of the variance based on the values of primary surface sculpture (PSS), anticlinal wall (AW), periclinal wall (PW), periclinal wall ornamentation (PWO), seed coat anatomy (SCA), and endothecium (EN) (**Table 3**). Axis 2 explained 18.675% of the data variability, of which seed shape (SH), seed length (SL), seed length/width ratio (LWR), and hilum character (HC) were the significant variables for the ordination of the species. Correspondingly, axis 3 and axis 4 were explained by 13.041 and 12.213% of the data variability, respectively. CA biplots revealed a cluster of taxa corresponding with primary surface sculpture, periclinal wall ornamentation, and seed coat anatomy (**Figure 12**). When the distribution of seed surface characters on the CA biplots was observed, most of the taxa with a reticulate surface were grouped on the negative side of axis 2, whereas the taxa with a colliculate and other types of surface

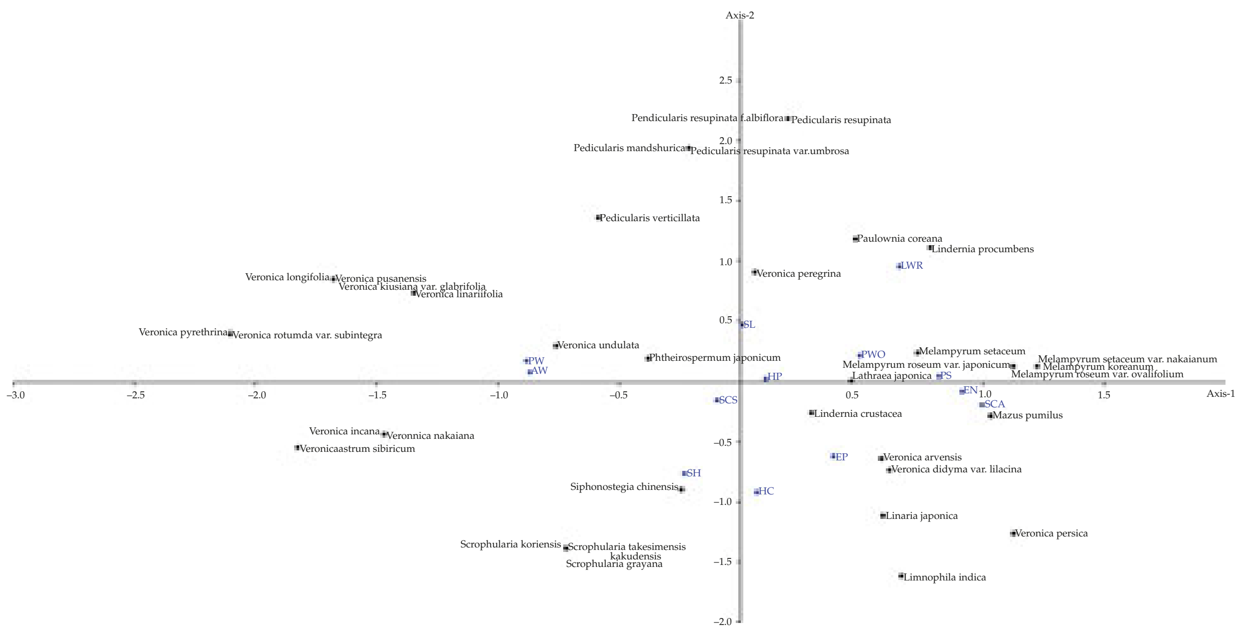

Figure 12. Correspondence analysis (CA) biplot of 13 seed characters sampled for 41 taxa of Scrophulariaceae s.l. Samples of different genera are represented by different symbols. AW, anticlinal wall; EN, endothecium; EP, epidermis; HC, hilum character; HP, hilum position; LWR, length/width ratio; PSS, primary surface sculpture; PW, periclinal wall; PWO, primary wall ornamentation; SCA, seed coat anatomy; ECS, epidermal cell shape; SH, seed shape; SL, seed length.

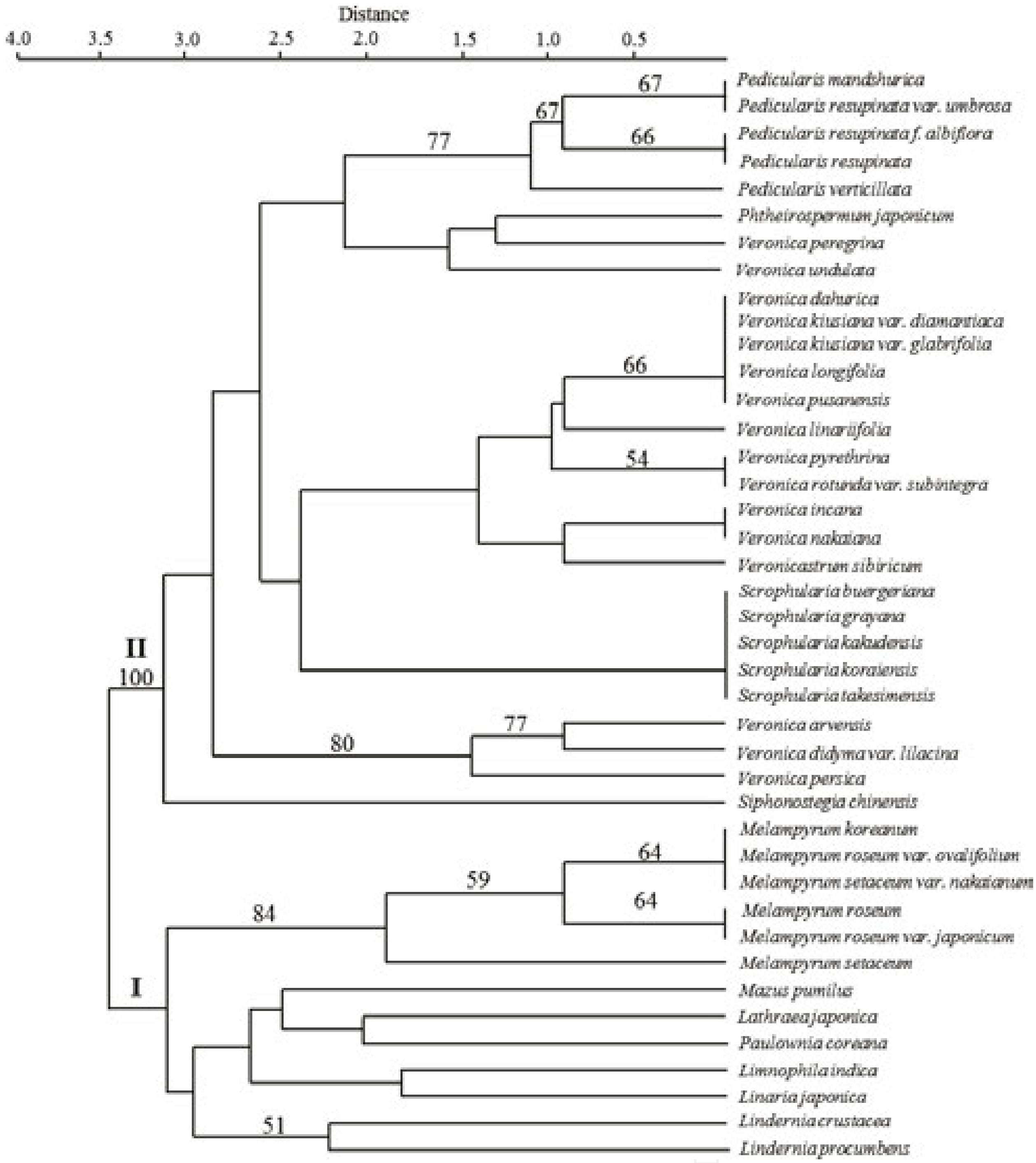

Figure 13. Seed morphological relationship among the taxa as displaced by UPGMA cluster diagram. Numbers above the branch represent bootstrap value.

were grouped on the opposite side. Likewise, all the taxa with striate periclinal walls were grouped on the negative side of axis 2, whereas taxa with papillate/granulate and smooth/folded walls, except *Veronica dahurica* and three *Pedicularis* species, were distributed on the positive side of axis 2. The cluster-based UPGMA tree revealed two main clusters [supported by a 100% bootstrap (BT) value]: cluster I included *Melampyrum*, *Mazus*, *Lathraea*, *Paulownia*, *Limnophila*, *Linaria*, and *Lindernia,* whereas cluster II comprised species of *Pedicularis*, *Veronica*, *Phtheirospermum*, *Veronicastrum*, *Scrophularia*, and *Siphonostegia* (**Figure 13**). In cluster I, there were two subclusters: the first one, which was supported by an 84% BT value, included *Melampyrum* species, and the second was formed by the remaining taxa of cluster I with bootstrap support of less than 50%. Correspondingly, in cluster II, there were three separate subclusters: *Pedicularis*/*Phtheirospermum*/*Veronica*, *Veronica*/*Veronicastrum*/*Scrophularia*, and three species of *Veronica*. *Siphonostegia chinensis* remained isolated and positioned at the bottom of the cluster. Interestingly, the subcluster formed by three *Veronica* species was supported by an 80% BT value, which was higher than for any other subclusters in cluster II which had bootstrap support <50%.

4. Discussion

4.1. Variations in seed morphology

This study demonstrated the high diversity of seed morphology in Scrophulariaceae s.l. in terms of seed shape, hilum character, primary ornamentation, epidermal cell characters, and seed coat anatomy. Variations are mainly found in seed primary sculpture, surface cell shape, and periclinal wall ornamentation. Seeds are minute, and most of the taxa are less or slightly larger than 1 millimeter in length except *Melampyrum* and *Pedicularis*. Within each taxon, seed size is variable; however, overall not much variation was found among the different species. That is why size is not very useful for the description of a particular taxon. Seed shape is also very heterogeneous, even with the same species. Mostly in studied species, the seed shape ranged from elliptical, broad elliptical, to ovoid. Elliptical seeds were cylindrical, navicular, flattened, or plano-convex. Seed of *Siphonostegia chinensis* is elliptical and beaked, that of *P. coreana* is winged, and seed of *V. didyma* var. *lilacina* is cupular. In general, however, in Scrophulariaceae s.l., seed shape related directly to its relative position in the fruit [30].

As far as surface sculpture and ornamentation are concerned, we found quite similar patterns in several species of same genera, particularly in *Veronica*, *Pedicularis*, *Scrophularia*, and *Melampyrum*. Our result agrees with previous studies regarding *Veronica* and *Pedicularis* [30, 32–34, 37, 38]. Out of 15 taxa of *Veronica* observed in this study, *V. arvensis, V. didyma* var. *lilacina*, and *V. persica* were reticulate-verrucate with a centrally located tubercle in epidermal cells, *V. undulata* had a reticulate-corrugate seed surface, and *V. peregrina* had a typical reticulate surface, whereas the rest of the species had a reticulate-striate surface with a cristate wall. In terms of the systematic significance of seed morphology in *Veronica*, Muñoz-Centeno et al. [32] described eight types of surface pattern in 123 species, and our samples represent four of them, although reticulate-striate is the most dominant pattern. Our results disagree with those of previous study [32] in terms of the surface pattern of *V. peregrina* as these previous authors mentioned a reticulate-corrugate surface pattern; in contrast, in our samples we observed a typical reticulate pattern. As regards seed anatomy data, *V. arvensis, V. didyma* var. *lilacina*, and *V. persica* had the most poorly differentiated seed coat, represented by a thin epidermal layer of almost degenerated cells, whereas *V. peregrina* and *V. undulata* had the most clearly defined epidermis and endothelium among the *Veronica* species. All the species with a reticulate-striate, cristate surface had well-characterized epidermis but indistinct endothelium.

Regarding *Pedicularis*, the results of our study agree with those of earlier results (for example, see [33]), that the regular-reticulate surface pattern is common among the studied taxa. Although gross primary sculpture looks like a reticulate pattern in all five *Pedicularis* taxa, there are substantial dissimilarities in secondary ornamentation and anticlinal wall formation. The anticlinal wall of *P. mandshuricais*, *P. verticillata*, and *P. resupinata* var. *umbrosa* was highly raised, whereas that of *P. resupinata* and *P. resupinata* f. *albiflora* was only slightly raised. The regular-reticulate, membranous-reticulate, cristate-reticulate, and the undulate primary ornamentations have been mentioned in previous studies [30, 33, 38–40], but the reticulate-tuberculate primary ornamentation found in *P. resupinata* f. *albiflora* is reported for the first time here. Nevertheless, the reticulate seed surface of *Pedicularis* is a common feature among genera of the families Orobanchaceae and Plantaginaceae [26, 30, 41–43]. The most usual and consistent feature observed among the five taxa of *Pedicularis* was a seed coat comprising a clearly defined epidermis and endothelium. On the other hand, most of the seed features were consistent within six *Melampyrum* taxa; variation was only observed in the gross surface sculpture with colliculate (*M. roseum* and *M. roseum* var. *japonicum*), rugose (*M. roseum* var. *ovalifolium* and *M. setaceum*), and rugose + colliculate (*M. koreanum* and *M. setaceum* var. *nakaianum*) surfaces.

In most cases, the seed coat comprised the epidermis and the endothelium. Nevertheless, in all *Melampyrum* and some *Veronica* species, the seed coat was very poorly represented and consisted only of a papery layer of epidermis. In this regard, our results agree with the findings of Juan et al. [30] who described the seed coat of Scrophulariaceae as being composed of the epidermis and endothelium; the latter is a useful character with which to distinguish the seeds of certain genera, particularly *Scrophularia* and *Verbascum*. Although they did not include any *Melampyrum* species, similar to our result, they indicated that some *Veronica* species consisted only of an epidermis, and no endothelium.

According to the CA analyses, the close affinities among the species of *Scrophularia* are well supported by their proximity to one another. Similarly, the proximity of *Melampyrum* species and of *Pedicularis* species is also apparent. Alternatively, in the CA plot, *Veronica* species are divided into two clusters. One is characterized by a concave periclinal wall, striate wall ornamentation, and a distinct seed coat, whereas the other, comprising only four species (*V. arvensis, V. persica, V. didyma* var. *lilacina*, and *V. peregrina*), has a convex periclinal wall and either papillate or smooth folded wall ornamentation. In addition, these four species differ in gross surface pattern as they have reticulate-verrucate or typical reticulate (*V. peregrina*) ornamentation instead of a reticulate-striate, cristate surface in the rest of the species (except *V. undulata*). Morphologically, *V. arvensis, V. peregrina*, and *V. persica* share an annual habit without rhizomes and flowers in terminal inflorescence; however, the latter species differs from the former two by having a longer pedicel than bract [44].

http://dx.doi.org/10.5772/intechopen.70572

4.2. Systematic implications of seed characters

In the UPGMA tree based on the seed morphological and anatomical characters, two major clusters were obtained, of them cluster II was supported by a 100% BT value (**Figure 13**). Cluster I, which included *Lindernia*, *Linaria*, *Limnophila*, *Paulownia*, *Lathraea*, *Mazus*, and *Melampyrum*, was a highly heterogeneous group of plants in life form and nature, ranging from annual or perennial herbs to trees, erect or prostrate, and creeping or submerged amphibious forms. Within cluster I, two subclusters were distinguished: the first one, supported by an 84% BT value, contained only the genus *Melampyrum*, whereas the remaining genera formed a separate subcluster. Six *Melampyrum* species can be differentiated from each other by primary sculpture and the nature of the periclinal wall; and UPGMA tree indicated that *M. setaceum* remains isolated from the rest of the *Melampyrum* species (59% BT). Morphologically, *M. setaceum* differed from other five species by linear to linear-lanceolate leaves and lanceolate bracts, whereas the rest of the species had lanceolate leaves and ovate to ovate lanceolate bracts. In another subcluster of cluster I, two other subclusters contained *Lindernia* (51% BT) and the remaining five genera (*Linaria*, *Limnophila*, *Paulownia*, *Lathraea*, and *Mazus*), forming a very heterogeneous group containing members from four families. Out of the five genera, *Paulownia* is a deciduous or evergreen tree, and *Limnophila*, an annual or perennial herb, usually grows in marshy areas with erect, prostrate, or creeping stems.

Despite being clustered in the same clade, seeds of two *Lindernia* species are very different from each other in terms of shape, primary sculpture, and epidermal cell shape. The primary surface sculpture of *L. procumbens* is ribbed with rectangular/elongated surface cells, whereas that of L. crustacea is rugose and pitted with irregular cells. These two species also differ in their leaf morphologies as the leaves of *L. procumbence* are sessile, elliptical to oblong, and somewhat rhomboid with entire or weakly toothed margins, whereas the leaves of *L. crustacea* are shortly petiolate, triangular-ovate to broadly ovate, and shallowly crenate or serrate. After observing the seeds of 14 *Lindernia* species, [45] classified the genus with ribbed and unribbed seeds and also indicated that this character shows good correspondence with subdivision of the genus explained in [46].

In cluster II, the *Siphonostagia* was separated first from rest of the genera despite belonging to the same family, Plantaginaceae, with *Pedicularis*. *Pedicularis*, which is known to be hemiparasitic like *Melampyrum*, formed the topmost subcluster in the tree (77% BT) and remained quite far from *Melampyrum*. Apart from their parasitic nature, these two genera share some morphological features including leafy bracts, campanulate calyces, didynamous stamens, and capitate stigmas. Yet again, three *Veronica* species (*V. arvensis, V. didyma* var. *lilacina*, and V. persica) constituted a remarkable case making a separate subcluster with 80% BT, whereas the rest of *Veronica* species grouped either with *Scrophularia*/*Veronicastrum* or with *Pedicularis*/*Phtheirospermum*. Many of the *Veronica* species combined with *Veronicastrum* and *Scrophularia,* although BT support for this subcluster was <50%. In our results, *Scrophularia* shared some similar seed features with *Veronica* and *Veronicastrum*; however, from a morphological point of view, the position of the *Scrophularia* in this subcluster is very confusing. As *Veronica* and *Veronicastrum* are characterized by perennial rhizomatous herbs (although some *Veronica* are annual and without rhizosomes), four corolla lobes, two stamens, and capitate stigmas.

Instead, *Scrophularia* are characterized by perennial herbs without rhizomes, five corolla lobes, four didynamous stamens, and bifid stigmas.

As predicted, three *Veronica* species (*V. arvensis*, *V. didyma* var. *lilacina*, and *V. persica*) form an isolated group. Based on the infrageneric classification as done in [47], V. arvensis (with haploid chromosome, n = 8) belongs to subgenus *Chamaedrys* and V. didyma var. *lilacina* (as *V. polita*) and *V. persica*, both having base chromosome n = 7, belong to *Pocilla*. *V. linariifolia*, *V. longifolia*, *V. dahurica*, and *V. incana*, all have the chromosome number n = 17, and the reticulate-striate, cristate group belongs to *Pseudolysimachium*, whereas *V. peregrina* (n = 9) belongs to subgenus *Beccabunga*. The present results confirmed what was reported in previous studies on *Veronica* (for example, [32, 34]), that seed morphological data can be employed to evaluate the relationships of taxa within the genus and that seed characters can also provide additional support to infrageneric discrimination in *Veronica*.

The SEM investigations reveal that the reticulate surface pattern as a primary sculpture is a confusing case. The pattern is found in the members of Orobanchaceae, Plantaginaceae, and Scrophulariaceae, although quite variations are observed in secondary striation and anticlinal wall pattern. The current study likewise concurs that there is no even single seed features representing all investigated species. Nonetheless, few seed characters somehow nicely characterized the group of particular taxa, for instance, seeds of Orobanchaceae are either elliptical cylindrical or ovoid in shape and of Plantaginaceae are elliptical flattened or ovoid flattened. Correspondingly, reticulate-striate surface sculpture of *Veronica*, *Veronicastrum,* and *Scrophularia* is comparable, although later one is without cristae. The seed coat anatomy indicates that the epidermis and endothelium are informative characters. All the *Scrophularia* species have indistinct epidermis but distinct endothelium, whereas most of the *Veronica* has distinct epidermis and endothelium. On the other hand, all the *Melampyrum* are without both layers, but other Plantaginaceae have well-distinct epidermis and endothelium.

The concept of Scrophulariaceae s.l. has changed considerably since the application of molecular approaches in plant systematics. Studies have shown that the traditional Scrophulariaceae are an unusual assemblage of plants distributed throughout the phylogenetic tree of Lamiales [1, 2, 5–7]. Despite the limited number of taxa investigated, the present seed morphological analysis highlights the high heterogeneity existing within the studied taxa of Scrophulariaceae s.l., which may be observed at a higher taxonomic rank than genus. In particular, primary surface sculpture, anticlinal and periclinal walls of epidermal cell, epidermal cell shape, and seed coat layers are the important seed features observed in this study, and these features can be used to distinguish different groups of taxa in the family. The results of this study therefore suggest that seed coat micromorphology and anatomy have significant taxonomic importance.

Acknowledgements

This study was supported financially by the project 'Studies on the Establishment of SeedBank base for the Asian network' at the Korea National Arboretum (KNA), Pocheon, Korea.

Appendix

Plant name	Shape	Average size (mm)	Length/width ratio	Hilum	Primary surface sculpture
Lindernia procumbens	Elliptical, oblong	0.35 × 0.16	2.11	Basal, distinctly protuberant	Ribbed
L. crustacea	Ovoid, sub globose	0.38 × 0.3	1.26	Basal, distinctly protuberant	Rugose, pitted
Mazus pumilus	Ovoid, elliptical	0.42 × 0.26	1.36	Basal, distinctly protuberant	Colliculate
Lathraea japonica	Ovoid	2.03 × 1.69	1.2	Lateral, distinctly protuberant	Rugose
Melampyrum koreanum	Elliptical, cylindrical	4.73 × 1.97	2.43	Lateral, flat to indistinctly protuberant	Rugose/poorly colliculate
M. roseum	Elliptical, cylindrical	4.96 × 1.98	2.51	Lateral, flat to indistinctly protuberant	Colliculate
M. roseum var. *japonicum*	Elliptical, cylindrical	3.79 × 1.65	2.32	Lateral, flat to indistinctly protuberant	Colliculate
M. roseum var. *ovalifolium*	Elliptical, cylindrical	4.35 × 1.74	2.51	Lateral, flat to indistinctly protuberant	Rugose
M. setaceum	Elliptical, cylindrical	4.15 × 1.55	2.7	Lateral, flat to indistinctly protuberant	Rugose
M. setaceum var. *nakaianum*	Elliptical, cylindrical	4.97 × 1.9	2.62	Lateral, flat to indistinctly protuberant	Rugose/poorly colliculate
Pedicularis mandshurica	Elliptical, navicular	2.99 × 1.27	2.38	Basal, distinctly protuberant	Reticulate
P. resupinata f. *albiflora*	Elliptical, navicular	2.83 × 1.27	2.25	Basal, distinctly protuberant	Reticulate, tuberculate
P. resupinata	Elliptical	2.69 × 1.32	2.05	Basal, distinctly protuberant	Reticulate
P. resupinata var. *umbrosa*	Elliptical	2.62 × 1.08	2.49	Basal, distinctly protuberant	Reticulate
P. verticillata	Elliptical, navicular	2.27 × 1.16	1.95	Basal, distinctly protuberant	Reticulate
Phtheirospermum japonicum	Ovoid	1.19 × 0.73	1.64	Basal, distinctly protuberant	Reticulate
Siphonostegia chinensis	Elliptical, beaked	0.92 × 0.46	2.05	Basal, deep	Reticulate
Paulownia coreana	Elliptical, winged	1.58 × 0.74	2.13	Basal, distinctly protuberant	Ribbed
Limnophila indica	Ovoid, elliptical	0.95 × 0.46	1.73	Basal, distinctly protuberant	Rugose
Veronica arvensis	Ovoid, flattened	0.96 × 0.64	1.51	Basal, distinctly protuberant	Reticulate-verrucate/Rugose
V. dahurica	Broad elliptical, flattened	1.02 × 0.89	1.16	Lateral, distinctly protuberant	Reticulate-striate, cristate
V. didyma var. *lilacina*	Ovoid, cupular	1.27 × 1	1.28	Lateral, distinctly protuberant	Reticulate-verrucate
V. incana	Ovoid, flattened	0.71 × 0.49	1.46	Basal, distinctly protuberant	Reticulate-striate, cristate
V. kiusiana var. *diamantiaca*	Broad elliptical, flattened	1.17 × 1.01	1.36	Lateral, distinctly protuberant	Reticulate-striate, cristate

Plant name	Shape	Average size (mm)	Length/width ratio	Hilum	Primary surface sculpture
V. kiusiana var. *glabrifolia*	Broad elliptical, flattened	1.17 × 1.01	1.16	Lateral, distinctly protuberant	Reticulate-striate, cristate
V. linariifolia	Broad elliptical, flattened	1.19 × 0.85	1.4	Basal, distinctly protuberant	Reticulate-striate, cristate
V. longifolia	Broad elliptical, flattened	1.19 × 0.99	1.54	Lateral, distinctly protuberant	Reticulate-striate, cristate
V. nakaiana	Broad ovoid, flattened	0.94 × 0.74	1.28	Basal, distinctly protuberant	Reticulate-striate, cristate
V. peregrina	Elliptical, flattened	0.71 × 0.41	1.73	Basal, distinctly protuberant	Faintly reticulate
V. persica	Ovoid, flattened	1.17 × 0.79	1.49	Basal, distinctly protuberant	Reticulate-verrucate
V. pusanensis	Broad elliptical, flattened	1.01 × 0.79	1.29	Lateral, distinctly protuberant	Reticulate-striate, cristate
V. pyrethrina	Broad elliptical, flattened	0.95 × 0.78	1.23	Lateral, distinctly protuberant	Reticulate-striate, cristate
V. rotunda var. *subintegra*	Broad elliptical, flattened	0.99 × 0.77	1.29	Lateral, distinctly protuberant	Reticulate-striate, cristate
V. undulata	Broad ovoid, plano-convex	0.62 × 0.40	1.56	Lateral, distinctly protuberant	Reticulate-corrugate
Veronicastrum sibiricum	Ovoid, plano-convex	0.73 × 0.46	1.61	Lateral, distinctly protuberant	Reticulate-striate, cristate
Linaria japonica	Ovoid, flattened	2.03 × 1.68	1.68	Basal, deep	Colliculate
Scrophularia buergeriana	Ovoid	10.3 × 0.71	1.47	Basal, deep	Reticulate-striate
S. grayana	Ovoid	1.08 × 0.76	1.45	Basal, deep	Reticulate-striate
S. kakudensis.	Ovoid	1.13 × 0.73	1.56	Basal, deep	Reticulate-striate
S. koraiensis	Ovoid	1.2 × 0.74	1.64	Basal, deep	Reticulate-striate
S. takesimensis	Ovoid	1.01 × 0.6	1.72	Basal, deep	Reticulate-striate

Epidermal cell shape	Periclinal wall	Anticlinal wall	Seed coat	Epidermis	Endothelium
Rectangular, elongated	Concave, finely granulate	Slightly raised, straight, finely granulated	Distinct	Well represented	Absent
Irregular	Concave, finely granulate	Slightly raised, folded	Distinct	Well represented	Absent
Rectangular, Polygonal	Convex, finely folded	Shallow, finely folded	Distinct	Well represented	Present
Irregular	Concave, smooth to folded	Distinctly raised, straight to wavy, finely folded, unevenly thickened	Distinct	Well represented	Present
Irregular	Convex, smooth to finely folded	Shallow or slightly raised	Indistinct	Degenerated	Absent
Irregular	Convex, smooth to finely folded	Shallow	Indistinct	Degenerated	Absent
Irregular	Convex or pitted, smooth to finely folded	Shallow, smooth, unevenly thickened	Indistinct	Degenerated	Absent

Epidermal cell shape	Periclinal wall	Anticlinal wall	Seed coat	Epidermis	Endothelium
Irregular	Convex or pitted, smooth to finely folded	Slightly raised, smooth, unevenly thickened	Indistinct	Degenerated	Absent
Irregular	Concave or pitted, smooth to finely folded	Slightly raised, smooth, unevenly thickened	Indistinct	Degenerated	Absent
Irregular	Convex, smooth to finely folded	Shallow	Indistinct	Degenerated	Absent
Rectangular, elongated	Flat, warty	Distinctly raised, straight, smooth to warty, unevenly thickened	Distinct	Well represented	Present
Rectangular, elongated	Flat with sessile tubercle	Slightly raised, straight	Distinct	Well represented	Present
Rectangular, elongated	Flat with sessile micro papillae	Slightly raised, straight,	Distinct	Well represented	Present
Rectangular, elongated	Flat with sessile micro papillae	Distinctly raised, straight, smooth or finely folded, unevenly thickened	Distinct	Well represented	Present
Rectangular, elongated	Flat, full of micro papillae	Distinctly raised, straight, smooth or finely folded, unevenly thickened	Distinct	Well represented	Present
Polygonal, isodiametric	Flat, smooth	Distinctly raised, straight to sinuous, unevenly thickened	Distinct	Well represented	Present
Polygonal, isodiametric	Flat, smooth to finely folded	Distinctly raised, straight to sinuous, unevenly thickened	Distinct	Degenerate	Present
Polygonal, isodiametric	Flat to slightly concave, smooth	Distinctly raised, straight to sinuous, unevenly thickened	Distinct	Well represented	Present
Irregular	Slightly convex, warty	Shallow	Distinct	Well represented	Present
Polygonal, elongated/isodiametric	Convex with central tubercle, smooth	Distinctly raised, straight to wavy, smooth	Indistinct	Degenerated	Present
Polygonal, elongated/isodiametric	Concave, striate	Distinctly raised, straight to wavy, smooth	Distinct	Well represented	Present
Polygonal, isodiametric	Convex with central tubercle, smooth	Distinctly raised, straight to wavy, smooth	Indistinct	Degenerated	Absent
Polygonal, elongated/isodiametric	Concave, striate	Distinctly raised, straight to wavy, folded	Distinct	Well represented	Present
Polygonal, elongated/isodiametric	Concave, striate	Distinctly raised, straight to wavy, folded	Distinct	Well represented	Present
Polygonal, elongated/isodiametric	Concave, striate	Distinctly raised, straight to wavy, folded	Distinct	Well represented	Present
Polygonal, elongated/isodiametric	Concave, striate	Distinctly raised, straight to wavy, folded	Distinct	Well represented	Present

Epidermal cell shape	Periclinal wall	Anticlinal wall	Seed coat	Epidermis	Endothelium
Polygonal, elongated/isodiametric	Concave, striate	Distinctly raised, straight to wavy, folded, unevenly thickened	Distinct	Well represented	Present
Polygonal, elongated	Concave, striate	Distinctly raised, straight to wavy, folded, unevenly thickened	Distinct	Well represented	Present
Polygonal, elongated/isodiametric	Flat, smooth/folded	Slightly raised, folded, unevenly thickened	Distinct	Well represented	Present
Polygonal, isodiametric	Convex with central tubercle, smooth	Slightly raised, folded, unevenly thickened	Indistinct	Degenerated	Absent
Polygonal, elongated/isodiametric	Concave, striate	Distinctly raised, straight to wavy, folded, unevenly thickened	Distinct	Well represented	Present
Polygonal, elongated	Concave, striate	Distinctly raised, straight to wavy, folded, unevenly thickened	Distinct	Well represented	Present
Polygonal, elongated	Concave, striate	Distinctly raised, straight to wavy, folded, unevenly thickened	Distinct	Well represented	Present
Polygonal, isodiametric	Concave, striate	Distinctly raised, folded, unevenly thickened	Distinct	Well represented	Present
Polygonal, elongated	Concave, striate	Distinctly raised, straight to wavy, folded, unevenly thickened	Distinct	Well represented	Present
Polygonal, isodiametric	Convex, finely folded	Shallow, finely folded, straight to wavy	Distinct	Well represented	Present
Polygonal, elongated	Concave, striate	Distinctly raised, straight to sinuous, unevenly thickened	Distinct	Degenerated	Present
Polygonal, elongated	Concave, striate	Distinctly raised, straight to sinuous, unevenly thickened	Distinct	Degenerated	Present
Polygonal, elongated	Concave, striate	Distinctly raised, straight to sinuous, unevenly thickened	Distinct	Degenerated	Present
Polygonal, elongated	Concave, striate	Distinctly raised, straight to sinuous, unevenly thickened	Distinct	Degenerated	Present
Polygonal, elongated	Concave, striate	Distinctly raised, straight to sinuous, unevenly thickened	Distinct	Degenerated	Present

Appendix 1. Seed characters studied for Scrophulariaceae s.l.

http://dx.doi.org/10.5772/intechopen.70572

Plant name	1	2	3	4	5	6	7	8	9	10	11	12	13
Lindernia procumbens	0	0	1	1	0	2	0	0	2	1	0	0	1
L. crustacea	1	0	0	1	0	2	2	0	2	1	0	0	1
Mazus pumilus	1	0	0	1	0	1	0, 1	0	0	2	0	0	0
Lathraea japonica	1	1	0	0	0	2	2	1	0	2	0	0	0
Melampyrum koreanum	0	2	1	1	1	2, 1	2	0	0	2	1	1	1
M. roseum	0	2	1	1	1	1	2	0	0	2	1	1	1
M. roseum var. *japonicum*	0	2	1	1	1	1	2	0	0	2	1	1	1
M. roseum var. *ovalifolium*	0	2	1	1	1	2	2	0	0	2	1	1	1
M. setaceum	0	2	1	1	1	2	2	0	2	2	1	1	1
M. setaceum var. *nakaianum*	0	2	1	1	1	2, 1	2	0	0	2	1	1	1
Pedicularis mandshurica	0	2	1	1	0	0	0	1	1	1	0	0	0
P. resupinata f. *albiflora*	0	2	1	1	0	0, 2	0	0	1	1	0	0	0
P. resupinata	0	2	1	1	0	0	0	0	1	1	0	0	0
P. resupinata var. *umbrosa*	0	2	1	1	0	0	0	1	1	1	0	0	0
P. verticillata	0	2	0	1	0	0	0	1	1	1	0	0	0
Phtheirospermum japonicum	1	1	0	1	0	0	1	1	1	2	0	0	0
Siphonostegia chinensis	0	0	0	1	2	0	1	1	1	2	0	0	0
Paulownia coreana	0	1	1	1	0	2	1	1	1	2	0	0	0
Limnophila indica	1	0	0	1	2	2	2	0	0	1	0	0	0
V. arvensis	1	1	0	1	0	0, 2	1	1	0	1	1	1	1
V. dahurica	0	1	0	0	0	0	1	1	2	0	0	0	0
V. didyma var. *lilacina*	1	1	0	0	0	0	1	1	0	1	1	1	1
V. incana	1	0	0	1	0	0	1	1	2	0	0	0	0
V. kiusiana var. *diamantiaca*	0	1	0	0	0	0	1	1	2	0	0	0	0
V. kiusiana var. *glabrifolia*	0	1	0	0	0	0	1	1	2	0	0	0	0
V. linariifolia	0	1	0	1	0	0	1	1	2	0	0	0	0
V. longifolia	0	1	0	0	0	0	1	1	2	0	0	0	0
V. nakaiana	1	0	0	1	0	0	1	1	2	0	0	0	0
V. peregrina	0	1	0	1	0	0	1	0	1	2	0	0	0
V. persica	1	0	0	1	0	0	1	0	0	1	1	1	1
V. pusanensis	0	1	0	0	0	0	1	1	2	0	0	0	0
V. pyrethrina	0	0	0	0	0	0	1	1	2	0	0	0	0
V. rotunda var. *subintegra*	0	0	0	0	0	0	1	1	2	0	0	0	0
V. undulata	1	1	0	0	0	0	1	1	2	2	0	0	0
Veronicastrum sibiricum	1	0	0	0	0	0	1	1	2	0	0	0	0
Linaria japonica	1	1	0	1	2	1	1	0	0	2	0	0	0

Plant name	1	2	3	4	5	6	7	8	9	10	11	12	13
Scrophularia buergeriana	1	1	0	1	2	0	1	1	2	0	0	1	0
S. grayana	1	1	0	1	2	0	1	1	2	0	0	1	0
S. kakudensis	1	1	0	1	2	0	1	1	2	0	0	1	0
S. koraiensis	1	1	0	1	2	0	1	1	2	0	0	1	0
S. takesimensis	1	1	0	1	2	0	1	1	2	0	0	1	0

Appendix 2. Data matrix of the character states used for analysis.

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