

## Phylogeny of seed dormancy in Convolvulaceae, subfamily Convolvuloideae (Solanales)

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• **Background and Aims** The water gap is an important morphoanatomical structure in seeds with physical dormancy (PY). It is an environmental signal detector for dormancy break and the route of water into the non-dormant seed. The Convolvulaceae, which consists of subfamilies Convolvuloideae (11 tribes) and Humbertoideae (one tribe, monotypic Humberteae), is the only family in the asterid clade known to produce seeds with PY. The primary aim of this study was to compare the morphoanatomical characteristics of the water gap in seeds of species in the 11 tribes of the Convolvuloideae and to use this information, and that on seed dormancy and storage behaviour, to construct a phylogenetic tree of seed dormancy for the subfamily.

• **Methods** Scanning electron microscopy (SEM) was used to define morphological changes in the hilum area during dormancy break; hand and vibratome sections were taken to describe the anatomy of the water gap, hilum and seed coat; and dye tracking was used to identify the initial route of water entry into the non-dormant seed. Results were compared with a recent cladogram of the family.

• **Key Results** Species in nine tribes have (a) layer(s) of palisade cells in the seed coat, a water gap and orthodox storage behaviour. *Erycibe* (Erycibeae) and *Maripa* (Maripeae) do not have a palisade layer in the seed coat or a water gap, and are recalcitrant. The hilar fissure is the water gap in relatively basal Cuscuteae, and bulges adjacent to the micropyle serve as the water gap in the Convolvuloideae, Dicranostyloideae (except Maripeae) and the Cardiochlamyaeae clades. Seeds from the Convolvuloideae have morphologically prominent bulges demarcated by cell shape in the sclereid layer, whereas the Dicranostyloideae and Cardiochlamyaeae have non-prominent bulges demarcated by the number of sub-cell layers. The anatomy and morphology of the hilar pad follow the same pattern.

• **Conclusions** PY in the subfamily Convolvuloideae probably evolved in the aseasonal tropics from an ancestor with recalcitrant non-dormant seeds, and it may have arisen as Convolvulaceae radiated to occupy the seasonal tropics. Combinational dormancy may have developed in seeds of some *Cuscuta* spp. as this genus moved into temperate habitats.

**Key words:** Convolvulaceae, evolution, hilar fissure, physical dormancy, water gap.

### INTRODUCTION

Physical dormancy (PY) is caused by a water-impermeable seed or fruit coat that develops during the maturation drying stage of seed (Van Staden *et al.*, 1989; Baskin and Baskin, 1998) or fruit (Li *et al.*, 1999) development. A specialized morphoanatomical area termed the 'water gap' is present in the seed (or fruit) coat of physically dormant seeds. The water gap plays an important role as an environmental signal detector that synchronizes dormancy break in seeds to the favourable season for germination and seedling establishment, thus allowing subsequent completion of the plant's life cycle (Van Staden *et al.*, 1989; Baskin *et al.*, 2000; Baskin, 2003). During dormancy break, an opening or slit is formed in the water gap (i.e. the water gap opens) allowing water to enter into the seed (Baskin *et al.*, 2000). Thus, the water gap and the water-impermeable seed (or fruit) coat are important components of PY.

The Convolvulaceae is the only known family in the evolutionarily highly advanced asterid clade (Angiosperm Phylogeny

Group, 2003) that produces seeds with PY (Baskin *et al.*, 2000). This family consists of two subfamilies and 12 tribes: subfamily Convolvuloideae, with 11 tribes, and subfamily Humbertoideae, with one tribe, Humberteae, which is monotypic (*Humbertia madagascariensis*; Stefanovic *et al.*, 2002). Jayasuriya *et al.* (2008c) studied dormancy breaking and germination requirements of seeds in species representing each of the 11 tribes of subfamily Convolvuloideae and discussed the results from a phylogenetic perspective. The results showed that there are differences in type of dormancy and in storage behaviour in seeds of Convolvuloideae. Patterns of germination and dormancy seem to follow rather closely the phylogenetic and taxonomic demarcations proposed by Stefanovic *et al.* (2003). Seeds of advanced tribes have only PY, with the exception of *Maripa panamensis* (tribe Maripeae) with non-dormant recalcitrant seeds and *Bonamia menziesii* with non-dormant orthodox seeds. Species that belong to the more basal tribes produce non-dormant recalcitrant (Erycibeae) or physically dormant orthodox (Cardiochlamyaeae) seeds. Seeds of the Cuscuteae, a tribe derived directly from basal tribes, have PY or combinational dormancy (PY + PD, where PD = physiological dormancy, i.e. dormancy caused by low growth potential of the embryo; see Baskin and

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TABLE 1. Tribes and species of Convolvulaceae in which seed morphology and anatomy were studied

Tribe	Species	Anatomy	Morphology	
			SEM	Photomicrographs
Ipomoeae	<i>Ipomoea hederacea</i>	✓	✓	
	<i>Ipomoea indica</i>	✓		✓
	<i>Ipomoea lacunosa</i>	✓	✓	
	<i>Ipomoea triloba</i>	✓		✓
Merremieae	<i>Merremia dissecta</i>	✓	✓	
	<i>Merremia similis</i>	✓		✓
	<i>Merremia tuberosa</i>	✓		✓
Convolvuleae	<i>Calystegia sepium</i>	✓		✓
	<i>Calystegia soldanella</i>	✓	✓	
	<i>Convolvulus arvensis</i>	✓	✓	
Aniseieae	<i>Aniseia martinicensis</i>	✓		✓
Dichondreae	<i>Dichondra micrantha</i>	✓	✓	
	<i>Rapona tiliifolia</i>	✓		✓
Cresseae	<i>Bonamia grandiflora</i>	✓		✓
	<i>Bonamia menziesii</i>	✓	✓	
	<i>Evolvulus nuttallianus</i>	✓	✓	
Jacquemontieae	<i>Jacquemontia ovalifolia</i>	✓	✓	
	<i>Jacquemontia reclinata</i>	✓		✓
	<i>Cardiochlamys madagascariensis</i>	✓	✓	
Cardiochlamyaeae	<i>Cardiochlamys madagascariensis</i>	✓	✓	
Maripeae	<i>Maripa panamensis</i>	✓		✓
Cuscutaeae	<i>Cuscuta australis</i>	✓	✓	
	<i>Cuscuta gronovii</i>	✓	✓	
Erycibeae	<i>Erycibe henryi</i>	✓		

Baskin, 2004 for descriptions of classes of seed dormancy) (Gaertner, 1956; Tingey and Allred, 1961; Meulebrouck *et al.*, 2008).

The primary objective of research reported in this paper was 2-fold: (1) to identify the water gap and describe it anatomically and morphologically in seeds of species representing the 11 tribes in the Convolvulaceae, subfamily Convolvuloideae; and (2) to use the information on morphology and anatomy of the water gap, along with that on dormancy and germination (Jayasuriya *et al.*, 2008c), to construct a phylogenetic tree of seed dormancy for the Convolvuloideae.

## MATERIALS AND METHODS

### Seed collection

Seeds were collected from plants of at least one species representing each of the 11 tribes (*sensu* Stefanovic *et al.*, 2003) in the Convolvulaceae, subfamily Convolvuloideae in various states of the USA (Florida, Tennessee, Kentucky and Hawaii), Panama, Taiwan and Sri Lanka. *Convolvulus arvensis*, *Calystegia soldanella*, *Cardiochlamys madagascariensis*, *Cuscuta europea* and *Rapona tiliifolia* seeds were obtained from the Millennium Seed Bank collection, Kew Botanic Gardens, Kew, UK. Seeds were mailed to the University of Kentucky, Lexington, Kentucky, USA, and studies were carried out within 1–2 months after receipt. The tribes and species included in the study are listed in Table 1.

### Dormancy break

Artificial treatments or treatments simulating natural treatments (Jayasuriya *et al.*, 2008c) were used to break dormancy in seeds with PY. No dormancy-breaking treatments were

applied to *Bonamia menziesii* (collected on four different occasions), *Maripa panamensis* or *Erycibe henryi* since they were non-dormant (Jayasuriya *et al.*, 2008c).

### Morphological changes after dormancy break

Dormant (non-treated) and non-dormant (seeds made non-dormant using artificial treatment or simulated natural treatment) seeds of *Ipomoea lacunosa*, *I. hederacea*, *Merremia dissecta*, *Convolvulus arvensis*, *Cuscuta australis*, *C. gronovii*, *Jacquemontia ovalifolia*, *Dichondra micrantha*, *Evolvulus nuttallianus*, *Bonamia menziesii* (innately non-dormant seeds only) and *Cardiochlamys madagascariensis* were coated with gold–palladium in a Technics Hummer VI sputter coater and scanned with a Hitachi S-800 FE scanning electron microscope. Micrographs were compared to identify the water gap and changes on the seed surface after treatment. For *Aniseia martinicensis*, photomicrographs were taken using a light stereomicroscope (Leica S6D) with a Canon powershot S40 digital camera. Dormant and non-dormant seeds were also observed under a dissecting microscope. Twelve non-dormant seeds of each of the above-mentioned species were kept on wet filter paper and observed under a dissecting microscope at time 0 and every 30 min for 6 h. Morphology of the hilar area of dormant and non-dormant seeds of *Ipomoea lacunosa*, *I. hederacea*, *I. triloba*, *I. indica* (Ipomoeae); *Merremia dissecta*, *M. similis*, *M. tuberosa* (Merremieae); *Convolvulus arvensis*, *Calystegia soldanella*, *C. sepium* (Convolvuleae); *Aniseia martinicensis* (Aniseieae); *Jacquemontia ovalifolia*, *J. reclinata* (Jacquemontieae); *Evolvulus nuttallianus*, *Bonamia grandiflora*, *B. menziesii* (Cresseae); *Dichondra micrantha*, *Rapona tiliifolia* (Dichondreae); and *Cuscuta australis* and *C. gronovii* (Cuscutaeae) were studied under the dissecting microscope.

TABLE 2. Characters and character states used in construction of the dendrogram

Character	Tribes										
	IP	ME	CO	AN	CR	DI	JA	CU	MA	ER	CA
1	Orthodox	1	1	1	1	1	1	1	0	0	1
2	Physical dormancy	1	1	1	1	1	1	1	0	0	1
3	Presence of dormancy	1	1	1	1	1	1	1	0	0	1
4	No physiological dormancy	1	1	1	1	1	1	0	1	1	1
5	Presence of bulge	1	1	1	1	1	1	0	1	0	1
6	Formation of slits around bulges	1	1	1	1	1	1	1	0	0	1
7	Slits smooth	1	1	1	0	0	0	0	0	0	0
8	Palisade in seed coat away from hilum	1	1	1	1	1	1	1	0	0	1
9	Palisade in hilum	1	1	1	1	1	1	1	1	0	1
10	Seeds per fruit >1	1	1	1	1	1	0	1	1	0	0
11	Water gap	1	1	1	1	1	1	1	0	0	1
12	Absence of bulging cell layer	1	1	1	0	0	0	0	0	0	0
13	Fruit dry	1	1	1	1	1	1	1	1	0	1
14	Bulging cell layer not fleshy	1	1	1	1	1	1	1	0	0	1
15	Size of hilum >10% of seed	1	1	1	1	1	0	0	1	0	0
16	Absence of third protrusion	1	1	1	1	0	0	0	1	1	0
17	Bulge oval-shaped	1	1	1	1	0	0	0	0	0	0
18	Seed MC <15%	1	1	1	1	1	1	1	0	0	1
19	Location of bulge on side of MP only	1	1	1	1	0	0	1	0	0	0
20	Hilum fissure not point-like	1	1	1	1	1	1	1	1	0	1
21	Hilum fissure not linear	1	1	1	1	0	0	0	0	0	0
22	Hilum fissure horseshoe-shaped	1	1	1	1	0	0	0	0	0	0
23	Location of hilum not central	1	1	1	1	0	0	0	0	0	0
24	Location of hilum peripheral	1	1	1	1	0	0	0	0	0	0
25	Change in cell shape in bulge anatomy	1	1	1	1	0	0	0	0	0	0
26	Shape of hilar pad heart-shaped	1	1	1	1	0	0	1	0	0	0
27	Hilar ring	1	1	1	1	1	1	1	1	0	0

0 = No, 1 = Yes. AN, Aniseieae; CA, Cardiochlamyae; CO, Convolvuleae; CR, Cresseae; CU, Cuscuteae; DI, Dichondreae; IP, Ipomoeae; JA, Jaquemontiae; MA, Maripae; MC, moisture content; ME, Merremiae; MP, micropyle.

#### Dye tracking of putative water gap

Six dormant (non-treated) and non-dormant (made non-dormant using artificial treatment or simulated natural treatment) seeds each of *I. lacunosa*, *M. dissecta*, *C. arvensis*, *C. australis*, *J. ovalifolia*, *D. micrantha*, *E. nuttallianus*, *B. menziesii*, *M. panamensis* and *E. henryi* were immersed in saturated aniline blue solution. Seeds were retrieved at 30 min intervals for 3 h, surface blotted with filter paper and transverse cuts made through the upper and lower parts of the hilum. These cuts were photographed using a Canon EOS 30D camera with a Canon 95 mm macro lens.

#### Anatomy of water gap, hilum and seed coat away from hilum

Hand and Vibratome (Vibratome 1500, St Louis, MO) sections (25 µm) were made in the water gap, hilum and seed coat away from the hilum of seeds of *I. lacunosa*, *I. hederacea*, *I. triloba*, *I. indica*, *M. dissecta*, *M. similis*, *M. tuberosa*, *C. soldanella*, *C. sepium*, *C. arvensis*, *A. martinicensis*, *J. ovalifolia*, *J. reclinata*, *E. nuttallianus*, *B. grandiflora*, *B. menziesii*, *D. micrantha*, *R. tiliifolia*, *C. australis*, *C. gronovii*, *M. panamensis*, *C. madagascariensis* and *E. henryi*. These sections were observed under an Olympus light microscope (Model BX50), and photomicrographs were taken using a Canon EOS 30D camera. Photomicrographs were used to describe and compare the anatomy of the species.

#### Phenetic cluster analysis

External morphological features, anatomical characters, seed germination and seed storage behaviour (from Jayasuriya *et al.*, 2008c) were used to analyse the similarities of the 11 tribes in Convolvulaceae, subfamily Convolvuloideae (Table 2). The nearest neighbour linkage method of the Sorensen index was used to analyse similarities between tribes. PCord04 software (Jongman *et al.*, 1995) was used for the similarity analysis and for construction of the dendrogram.

## RESULTS

#### Dormancy break

Different dormancy-breaking treatments were effective in breaking the PY of different species. Dipping in boiling water for 4, 6, 10, 10, 10, 15 and 15 s broke the dormancy of seeds of *I. lacunosa*, *M. dissecta*, *C. arvensis*, *C. australis*, *J. ovalifolia*, *C. sepium* and *A. martinicensis*, respectively. Alternately dipping seeds in boiling water and ice water (10 s/10 s) 10 times broke the PY of seeds of *D. micrantha*, *C. soldanella* and *E. nuttallianus*. Dry storage at 35/20 °C for 2 and 5 months broke dormancy in seeds of *I. hederacea* and *I. indica*, respectively, and dry storage at ambient laboratory temperatures broke dormancy in *I. triloba* seeds. Sensitive seeds (*sensu* Jayasuriya *et al.*, 2008a) of *J. ovalifolia*

(Jayasuriya et al., 2008c) and *C. australis* (Jayasuriya et al., 2008b) became non-dormant by incubating them at 35/20 °C on wet sand for 2 weeks and 1 month, respectively, whereas sensitive seeds of *I. lacunosa* became non-dormant when they were incubated at 35 °C for 3 h (Jayasuriya et al., 2008a).

#### Morphological changes during dormancy break

Two prominent bulges adjacent to the micropyle were observed on dormant seeds of *I. lacunosa*, *M. dissecta* and *C. arvensis* (Fig. 1). No bulges were observed on dormant seeds of *C. australis* (Fig. 1U). Two bulges adjacent to the micropyle were non-prominent in dormant seeds of *J. ovalifolia*, *D. micrantha*, *E. nuttallianus*, *C. madagascariensis* (Fig. 1), *A. martinicensis* (Fig. 2A, C) and *B. menziesii* (non-dormant seeds; Fig. 3). However, bulge demarcation was faintly present on seeds of these species. No opening was seen on dormant seeds, with the following exceptions: cracks were observed in the hilar pad of dormant seeds of *M. dissecta*, and the hilar fissure of dormant seeds of *J. ovalifolia* appeared to be open. No micropyle was observed in seeds of any species except the non-dormant seeds of *B. menziesii* and *Cardiochlamys madagascariensis*.

The only openings observed in non-dormant seeds (made non-dormant using artificial dormancy-breaking treatments) of *C. arvensis* and *A. martinicensis* were slits around one or both bulges adjacent to the micropyle. In addition to the slits around the bulges in *I. lacunosa* and *M. dissecta*, cracks were seen on the hilar pad. In *J. ovalifolia*, *D. micrantha*, *E. nuttallianus* and *C. madagascariensis*, the hilar fissure seemed to be open in non-dormant seeds. In non-dormant seeds of *I. lacunosa*, *M. dissecta*, *C. arvensis*, *J. ovalifolia* and *A. martinicensis* (Fig. 2B, D), one or both bulges fell from the seed after 30 min of imbibition, and also during coating for scanning electron microscopy (SEM). In four of these species (*I. lacunosa*, *M. dissecta*, *C. arvensis* and *J. ovalifolia*), bulges were oval-shaped, with a distinct margin; therefore, a smooth margin is formed during separation of the bulges (Fig. 1E, F, K). In seeds of *A. martinicensis*, the bulges were also oval-shaped, but they did not form a smooth margin during separation; instead, the margin was rough dentate (Fig. 2B, D). Bulges on seeds of *E. nuttallianus*, *D. micrantha* and *C. madagascariensis* were not distinct, and the slit occurred only at the bulge margin on the micropylar and hilar pad side (Fig. 1N, P, R). Thus, no slit was formed in the chalazal end of the bulge. Therefore, bulges were not separated entirely from the seeds. However, the part of the bulge closest to the upper bulge margin was detached from seeds of *C. madagascariensis* after 3 h of imbibition. In non-dormant seeds of *C. australis*, the only opening observed was the hilar fissure (Fig. 1V).

Open fissures were observed in the micropyle and hilum (Fig. 3) in (innately) non-dormant seeds of *B. menziesii*.

After 2–3 h of imbibition, cracks were observed throughout the seed coats of non-dormant seeds of all the species (images not shown). Slits around the bulges similar to those seen in scanning electron micrographs were observed in seeds of *I. hederacea*, *I. triloba*, *I. indica*, *M. similis*, *M. tuberosa*, *C. soldanella*, *C. sepium*, *J. reclinata*, *B. grandiflora*, *R. tiliifolia* and *C. gronovii* under the dissecting microscope (images not shown).

#### Dye tracking of putative water gap

No stain was observed in non-treated seeds immersed in aniline blue except for the (innately) non-dormant seeds of *B. menziesii*, *E. henryi* and *M. panamensis* (images not shown). In *B. menziesii* seeds, blue staining was observed in tissues under the hilar pad and micropylar region after 30 min of soaking in the dye (images not shown). After 2 h, staining was observed throughout the seed. In seeds of *E. henryi* and *M. panamensis*, staining was observed immediately under the seed coat throughout the seed, but deep staining was observed in the hilar area, especially under the hilar fissure.

In treated (non-dormant) seeds of *I. lacunosa*, *M. dissecta*, *C. arvensis*, *A. martinicensis*, *J. ovalifolia*, *E. nuttallianus* and *D. micrantha*, staining was observed only in the tissues below the bulge after 30 min of imbibition (Fig. 4). No staining was observed in tissues under the hilar pad. However, after 2 h of imbibition, tissues below the seed coat were stained throughout the seed (data not shown).

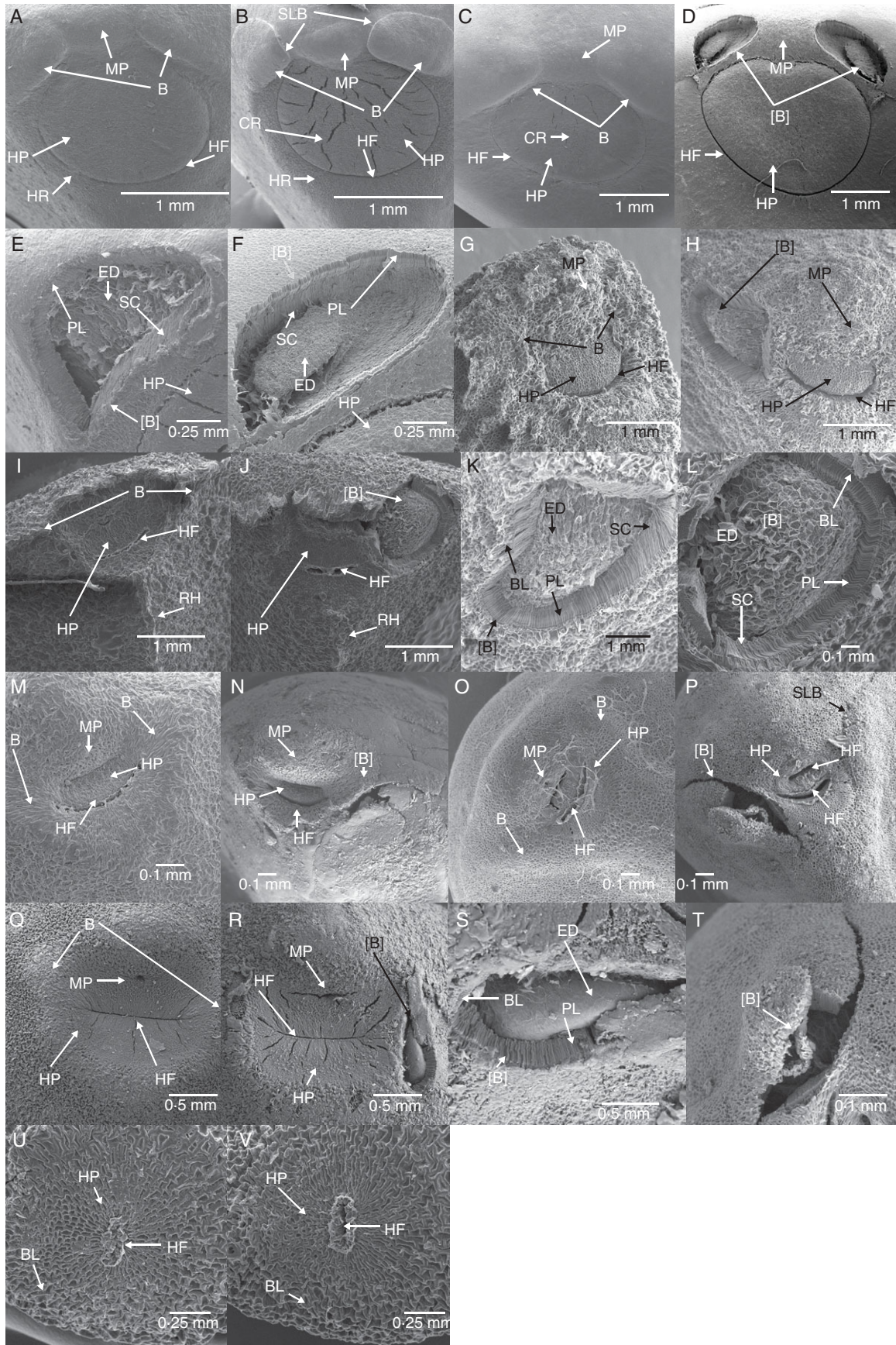
Staining was observed only in tissues under the hilar fissure in treated (non-dormant) seeds of *C. australis* after 15 min of soaking in dye (Fig. 4). After 1 h of soaking, tissues were stained throughout the seed (data not shown).

#### Anatomy of water gap, hilum and seed coat away from hilum

*Seed coat away from hilum.* Seed coat anatomy did not vary much among species, except for *M. panamensis* and *E. henryi* (Fig. 5). Seed coats of the other species mainly consist of three layers. The outermost layer is the epidermis, which is one cell thick. In *J. ovalifolia*, *E. nuttallianus*, *D. micrantha*, *C. madagascariensis* and *C. australis*, cells in this epidermal layer enlarge to form a distinct layer of bulging cells throughout the seed coat (Fig. 5E–G, I, K). Some epidermal cells of the seed coat of *I. lacunosa*, *M. dissecta*, *C. arvensis* and *A. martinicensis* also form bulging cells (Fig. 5A–D), but in seeds of these species the bulging cell layer is not a prominent layer. The epidermal bulging cell layer of *E. henryi* and of *M. panamensis* has collapsed and formed sticky substances on the seed coat (Fig. 5H, J).

FIG. 1. Electron micrographs of *Ipomoea lacunosa* dormant seed (A) and non-dormant seed (B); *Merremia dissecta* dormant seed (C) and non-dormant seed (D); *I. lacunosa* detached bulge (E); *M. dissecta* detached bulge (F); *Convolvulus arvensis* dormant seed (G) and non-dormant seed (H); *Jaquemontia ovalifolia* dormant seed (I) and non-dormant seed (J); *C. arvensis* detached bulge (K); *J. ovalifolia* detached bulge (L); *Evolvulus nuttallianus* dormant seed (M) and non-dormant seed (N); *Dichondra micrantha* dormant seed (O) and non-dormant seed (P); *Cardiochlamys madagascariensis* dormant seed (Q), non-dormant seed (R) and detached bulge (S); *Dichondra micrantha* detached bulge (T); and *Cuscuta australis* dormant seed (U) and non-dormant seed (V). Abbreviations: B, bulge; [B], detached bulge; BL, bulging cell layer; CR, cracks in hilar pad; ED, endodermal cells; HF, hilar fissure; HP, hilar pad; HR, hilar ring; MP, remnants of micropyle; PL, palisade cell layer; RH, raphe; SC, sclereid cell layer; SLB, slits around bulges.







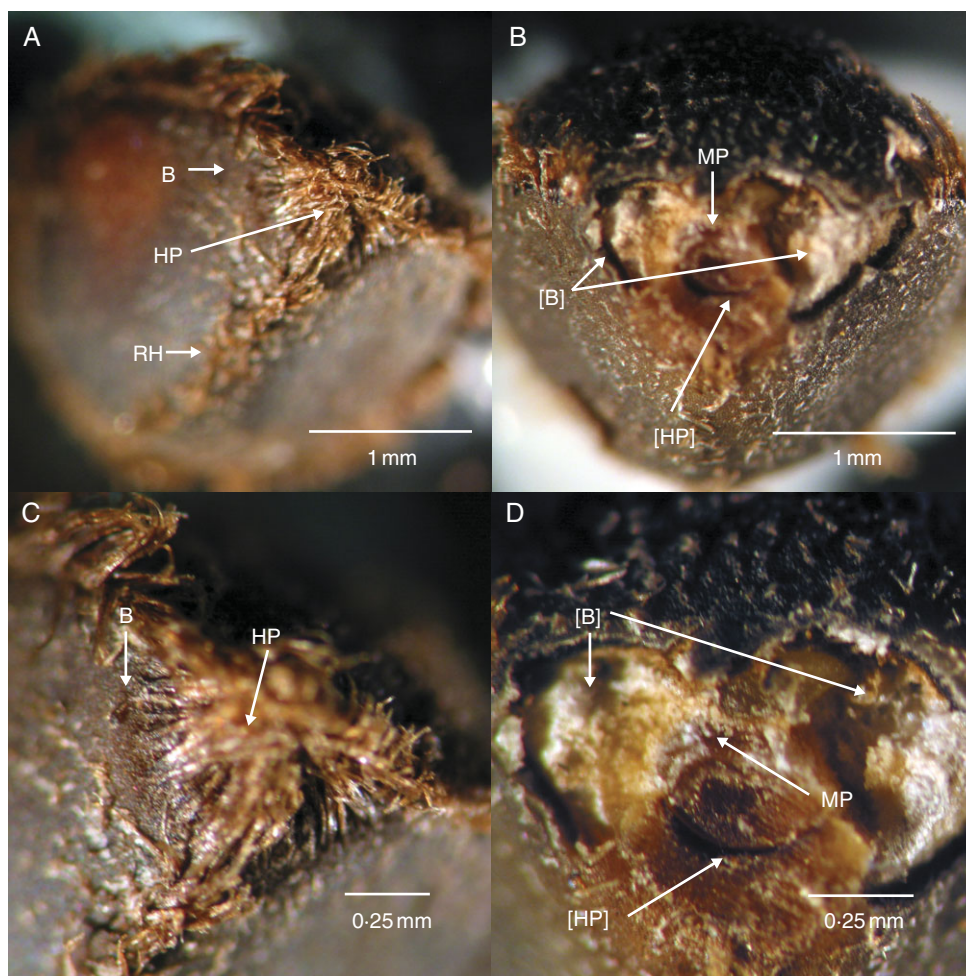


FIG. 2. Photomicrographs of *Aniseia martinicensis* dormant seed (A), non-dormant seed (B), hilar area of dormant seed (C) and detached bulge (D). Abbreviations: B, bulge; [B], detached bulge; HP, hilar pad; [HP], hilar pad, hair removed; MP, micropyle remnants; RH, raphe.

The second layer or middle layer of the seed coat is the palisade layer, which is not present in the seed coat of *E. henryi* and *M. panamensis*. The palisade layer of seeds of all the other species studied consists of a single cell layer with a light line (*linea lucida*) in the upper quarter of the cell. In *M. dissecta*, two light lines were observed (Fig. 5B); one is located in the upper quarter of the palisade layer and the other in the lower quarter. The innermost layer consists of 4–8 sub-cell layers with square or elongated sclereid cells. In *M. dissecta*, cells of the sclereid layer in the seed coat were pigmented, whereas in all other species pigmentation was observed in the epidermal or bulging cell layer.

Seed coats of *E. henryi* and *M. panamensis* differ from those of the other species studied (Fig. 5H, J). Except for the epidermal layer, there were no similarities between the seed coats of *E. henryi* and *M. panamensis* and those of the other species. The seed coat of *E. henryi* consists of two layers of cells, one of which is the epidermis. The other (innermost) layer consists of square sclereid cells, which are larger than the sclereid cells of other species. Below the sclereid layer is the crushed cell layer, which seems to consist of crushed remnants of the inner layers of the integument and nucellus. The seed coat of *M. panamensis* has a similar arrangement to that of

*E. henryi*. However, two or more sub-cell layers were observed in the sclereid layer. Sclereid cells were square and thus similar to those of *E. henryi*. The zone of crushed cells below the sclereid layer in *M. panamensis* is thicker than that in *E. henryi*.

*Hilum*. The anatomy of the hilar pad was basically similar in seeds of *I. lacunosa*, *M. dissecta*, *C. arvensis*, *A. martinicensis*, *J. ovalifolia*, *E. nuttallianus*, *D. micrantha*, *C. australis*, *C. madagascariensis* and *M. panamensis* (Fig. 6). However, there were differences in the location and shape of the hilar fissure. In seeds of *I. lacunosa*, *M. dissecta*, *C. arvensis* and *A. martinicensis*, the hilar fissure is located on the margin of the hilar pad between the hilar pad and the hilar ring and is horseshoe-shaped. In contrast, in *J. ovalifolia*, *E. nuttallianus*, *D. micrantha*, *C. australis*, *C. madagascariensis* and *M. panamensis* the hilar fissure is linear-shaped and is located in the middle of the hilar pad.

Apart from these differences, the basic anatomy of the hilar pad is similar for all the species studied, except *E. henryi* (Fig. 6J, K), and consists of five or six layers. The outermost layer is a square-cell epidermal layer. However, in *C. australis* (Fig. 6I), the outermost layer also consists of

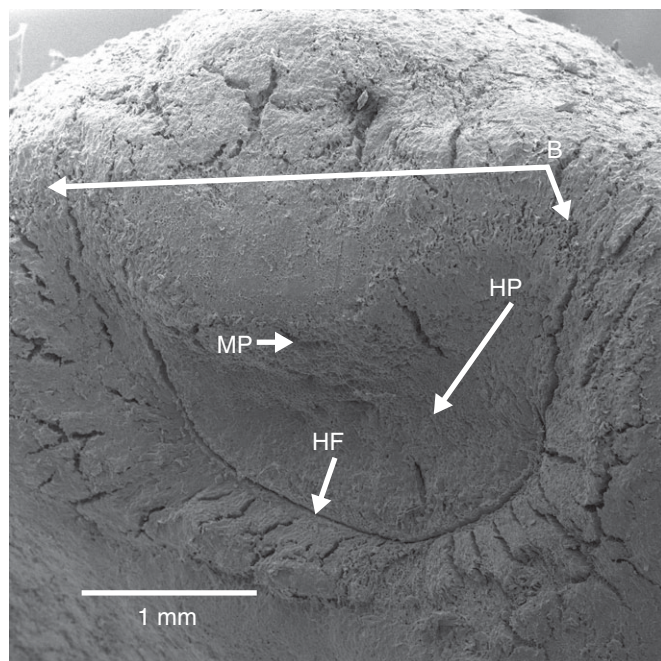


FIG. 3. Electron micrograph of *Bonamia menziesii* non-dormant seed. B, bulge; HP, hilar pad; HF, hilar fissure; MP, micropyle remnants.

palisade-like epidermal cells, which do not have a light line. The palisade-like epidermal cells consist of one sub-cell layer. The counter palisade layer is located below the palisade-like epidermal layer in *C. australis* and below the epidermis in all other species except *Erycibe* (no hilar pad). The counter palisade layer does not have a light line. A sclereid layer (outer sclereid layer), two or three cells thick, of square to elongate cells occurs below the counter palisade. The above-mentioned layers in all the species are pigmented in the hilar pad. The palisade layer with a light line is located below the outer sclereid layer. Another sclereid layer (inner sclereid layer) 4–6 cells thick is located below the palisade layer. These sclereid cells are also square to elongate. In *M. panamensis*, the hilar area (counter palisade + palisade) and bulge (palisade) are the only places where a palisade layer was observed.

*Erycibe henryi* does not have a hilar pad. Instead, the hilum in this species is a small opening in the micropylar end. The hilar fissure is just a point-like depression in the seed coat. However, the seed coat around the hilar fissure is thicker than it is in the rest of the seed coat. This area consists of 6–9 cell layers. All these cells are polygonal-shaped sclereid cells with thick walls.

**Water gap.** The water gap of *I. lacunosa*, *M. dissecta*, *C. arvensis*, *A. martinicensis*, *J. ovalifolia*, *E. nuttallianus*, *D. micrantha* and *C. madagascariensis* is a slit around the bulges adjacent to the micropyle. There is a clear transition between bulge–hilar pad and bulge–seed coat away from the hilum. In seeds of *I. lacunosa*, *M. dissecta*, *C. arvensis* and *A. martinicensis*, this transition is due to a change in the shape of cells in the sclereid layer (Fig. 7A–H). Cell shape changes from polygonal to elongated at the hilar pad–bulge margin and from elongated to square at the bulge–seed coat

away from hilum margin. Other than this difference in cell shape, the palisade layer undergoes an orientational change precisely at the transition.

In seeds of *J. ovalifolia*, *E. nuttallianus*, *D. micrantha* and *C. madagascariensis*, the number of sub-cell layers, as well as cell shape, changes in the hilar–bulge margin (Fig. 7I–N, R, S). In *J. ovalifolia* and *E. nuttallianus*, the number of sclereid subcell layers increased from four to eight from hilar pad to bulge. In *D. micrantha*, the number of sclereid sub-cell layers decreased from four to two from hilar pad to bulge.

Although the bulge is not functional in *M. panamensis*, its presence was observed in seeds of this species (Fig. 7O, P). The palisade layer in the hilar pad continues into the bulge. However, at the bulge–seed coat away from hilum margin, the palisade layer merges with the sclereid layer. The sclereid layer decreases from six to eight sub-layers at the hilar pad to three or four sub-layers in the bulge. The number of sclereid sub-layers is further reduced to two or three at the bulge–seed coat away from hilum margin. No anatomical demarcation was observed in *E. henryi* in the area that corresponds to the bulge in other seeds.

The hilar fissure is the water gap in *C. australis*. Formation of the hilar fissure in seeds of *C. australis* is primarily due to discontinuation of the counter palisade layer and of the bulging epidermal cells (Fig. 7Q). However, there also is a suture-type discontinuity in the inner palisade layer. The inner palisade cells in the fissure region are much shorter than those in the rest of the seed coat. The size of sclereid cells under the hilar fissure is also shorter than it is in other parts of the hilar pad. There is only one layer of sclereid cells in the hilar fissure. The inner part of the fissure is filled with remnants of cells, which may be those of the nucellus and/or of the innermost integument layers. No remnants of the micropyle were observed in mature seeds.

**The case of *Bonamia menziesii*.** The seed coat away from the hilum of *B. menziesii* seeds does not differ from that of the other seeds with PY in Convolvulaceae (Fig. 8A). However, the hilar pad anatomy differs in several ways from that of physically dormant seeds of other species. In *B. menziesii* seeds, the hilar pad has four layers, not six (Fig. 8B). The outermost layer is the counter palisade without a light line, and below the counter palisade layer is a single layer of square-shaped sclereids. Another sclereid layer is the innermost layer in the hilar pad. The palisade layer with a light line occurs between these two sclereid layers.

Furthermore, the micropylar opening in seeds of *B. menziesii*, which is located immediately above the hilar pad, seems to be open (Fig. 8C). The channel through the micropylar opening was filled with crushed cell materials. This channel is lined with palisade cells, except for two openings: one to the outside and one to the endosperm. The hilar fissure also seemed to be open, and the opening also was filled with these sticky crushed cell materials (images not shown).

#### Phenetic cluster analysis

Phenetic cluster analysis revealed two clusters of tribes (Fig. 9): (1) Ipomoeae, Merremieae, Convolvuleae and Aniseieae; and (2) Cresseae, Dichondreae, Jaquemontieae



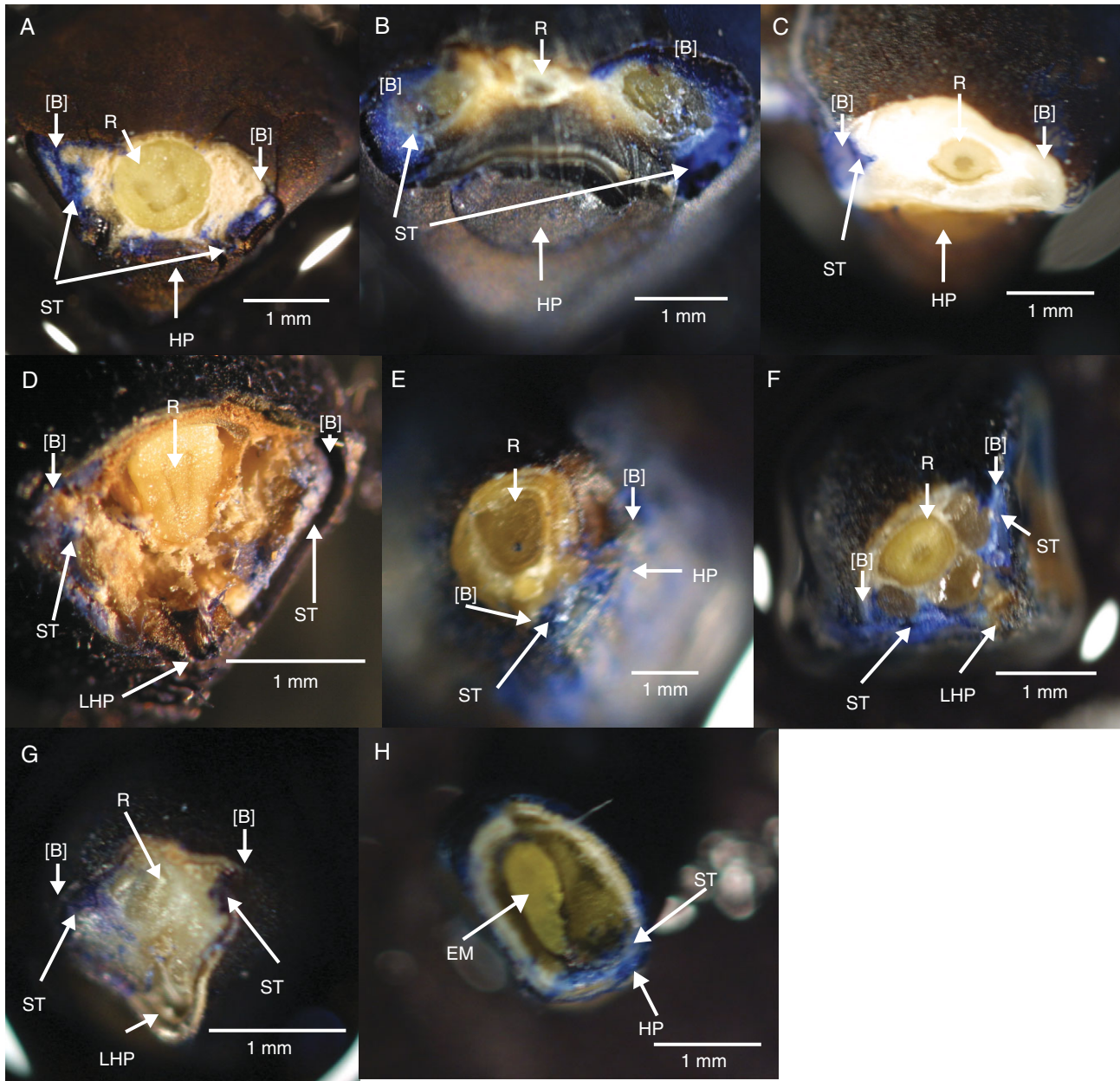


FIG. 4. Photomicrographs of non-dormant seeds of *Ipomoea lacunosa* (A), *Merremia dissecta* (B), *Convolvulus arvensis* (C), *Aniseia martinicensis* (D), *Jaquemontia ovalifolia* (E), *Evolvulus nuttallianus* (F), *Dichondra micrantha* (G) and *Cuscuta australis* (H) immersed in aniline blue for 30 min. Abbreviations: [B], tissues below the bulge; EM, embryo; HP, hilar pad; LHP, lower hilar pad margin; R, radicle; ST, stain.

and Cardiochlamyaeae. Cuscutaeae, Maripeae and Erycibeae are isolated from each other and from the two clusters at a distance of 97.5.

## DISCUSSION

The two bulges adjacent to the micropyle in seeds of *I. lacunosa* are the initial route (water gap) for entry of water into non-dormant seeds (Jayasuriya *et al.*, 2007). In the present study, it is shown that the water gaps of physically dormant seeds of other Convolvulaceae, except *Cuscuta*, are also the bulges adjacent to the micropyle. Koller and Cohen (1959) claimed

that a plug-like structure acts as the water gap in seeds of *Convolvulus lanatus*, *C. negevensis* and *C. secundus*, but they did not describe the structure anatomically. Callihan (1933) observed opening and closing of the hilar fissure of ethanol-treated seeds of *C. arvensis*. Based on this observation, he speculated that the hilar fissure is the water gap in this species. In contrast, the present studies show that slits around the bulges are the initial site for water entry into seeds of *C. arvensis* and of those of most other species in the family that produce physically dormant seeds.

Only in *Cuscuta* does the water gap differ from that of seeds of the other physically dormant species in the family.



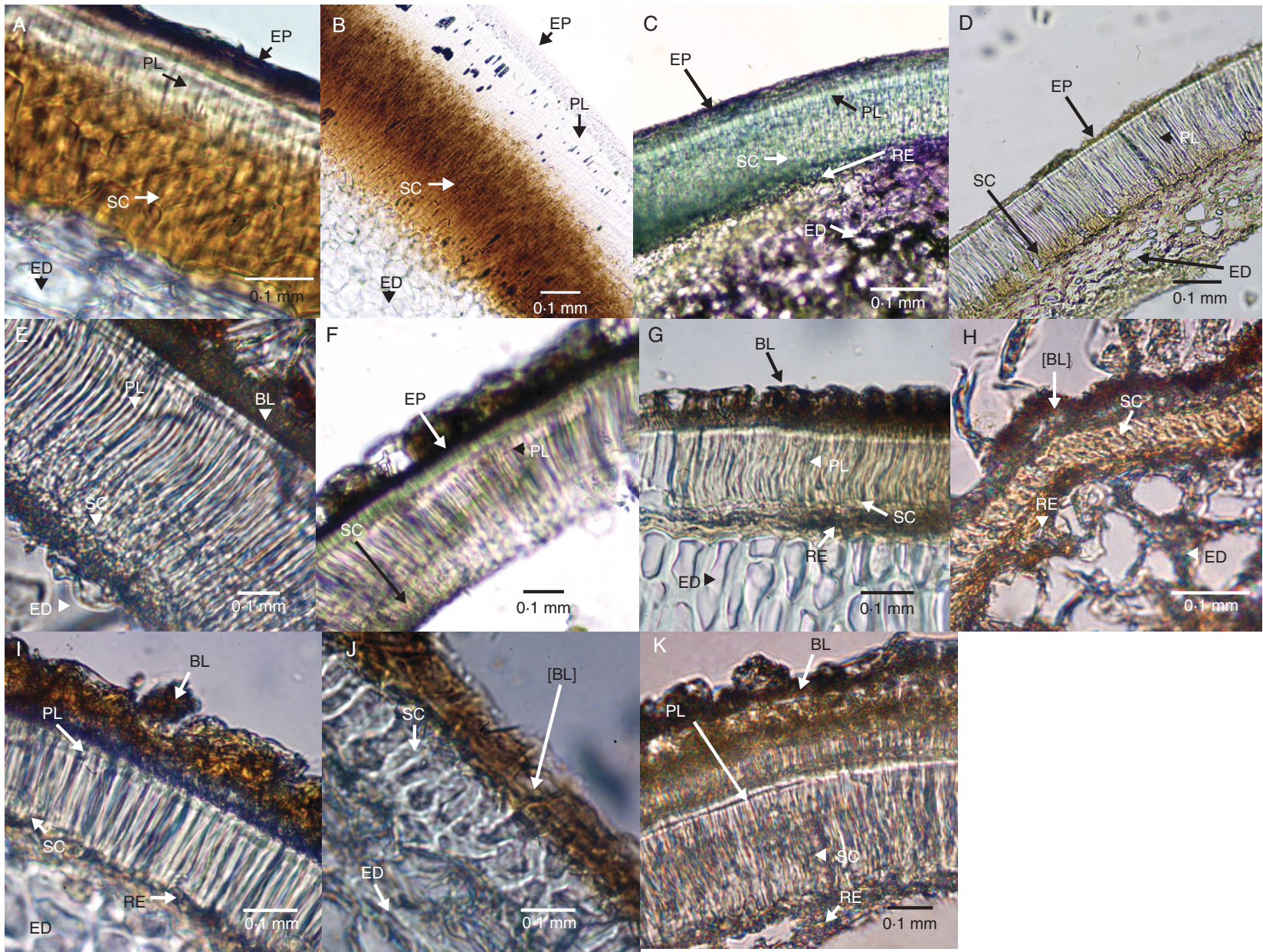


FIG. 5. Photomicrographs of seed coat away from bulge of seeds of *Ipomoea lacunosa* (A), *Merremia dissecta* (B), *Convolvulus arvensis* (C), *Aniseia martinicensis* (D), *Jaquemontia ovalifolia* (E), *Evolvulus nuttallianus* (F), *Dichondra micrantha* (G), *Maripa panamensis* (H), *Cuscuta australis* (I), *Erycibe henryi* (J) and *Cardiochlamys madagascariensis* (K). Abbreviations: BL, bulging cell layer; [BL], remnants of bulging cell layer; ED, endodermal layer; EP, epidermis; PL, palisade layer; RE, remnants of endodermal and nucellar cells; SC, sclereid layer.



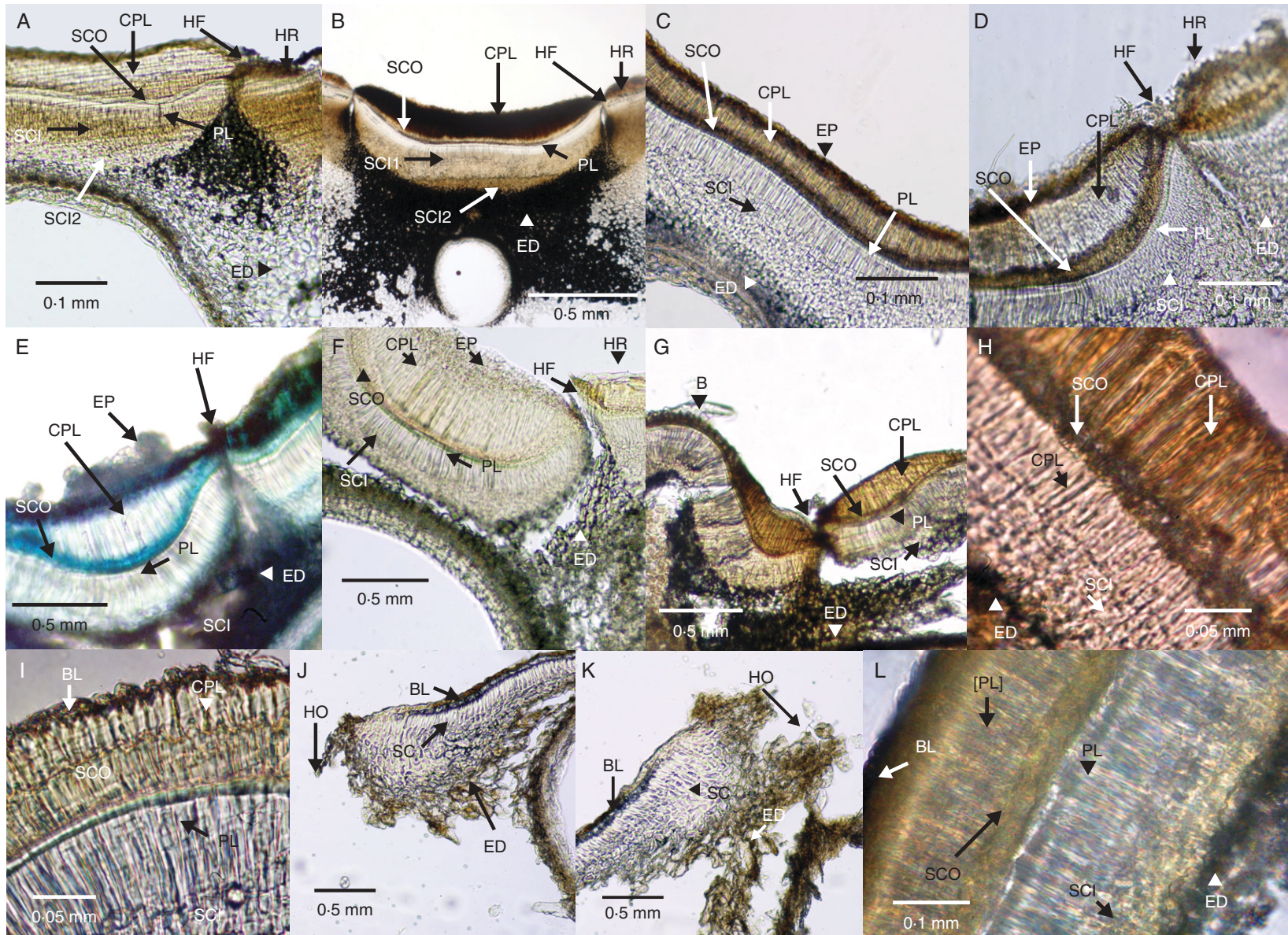


FIG. 6. Photomicrographs of hilar pad of seeds of *Ipomoea lacunosa* (A), *Merremia dissecta* (B), *Convolvulus arvensis* (C), *Aniseia martinicensis* (D), *Jaquemontia ovalifolia* (E), *Evolvulus nuttallianus* (F), *Dichondra micrantha* (G), *Maripha panamensis* (H), *Cuscuta australis* (I), *Erycibe henryi* (left side) (J), *Erycibe henryi* (right side) (K) and *Cardiochlamys madagascariensis* (L) seeds. Abbreviations: B, bulge; BL, bulging cell layer; CPL, counter palisade layer; ED, endodermal cells; EP, epidermis; HF, hilar fissure; HO, hilar opening; HR, hilar ring; PL, palisade layer; SCI, inner sclereid layer; SCI1, inner sclereid layer 1; SCI2, inner sclereid layer 2; SCO, outer sclereid cell layer.



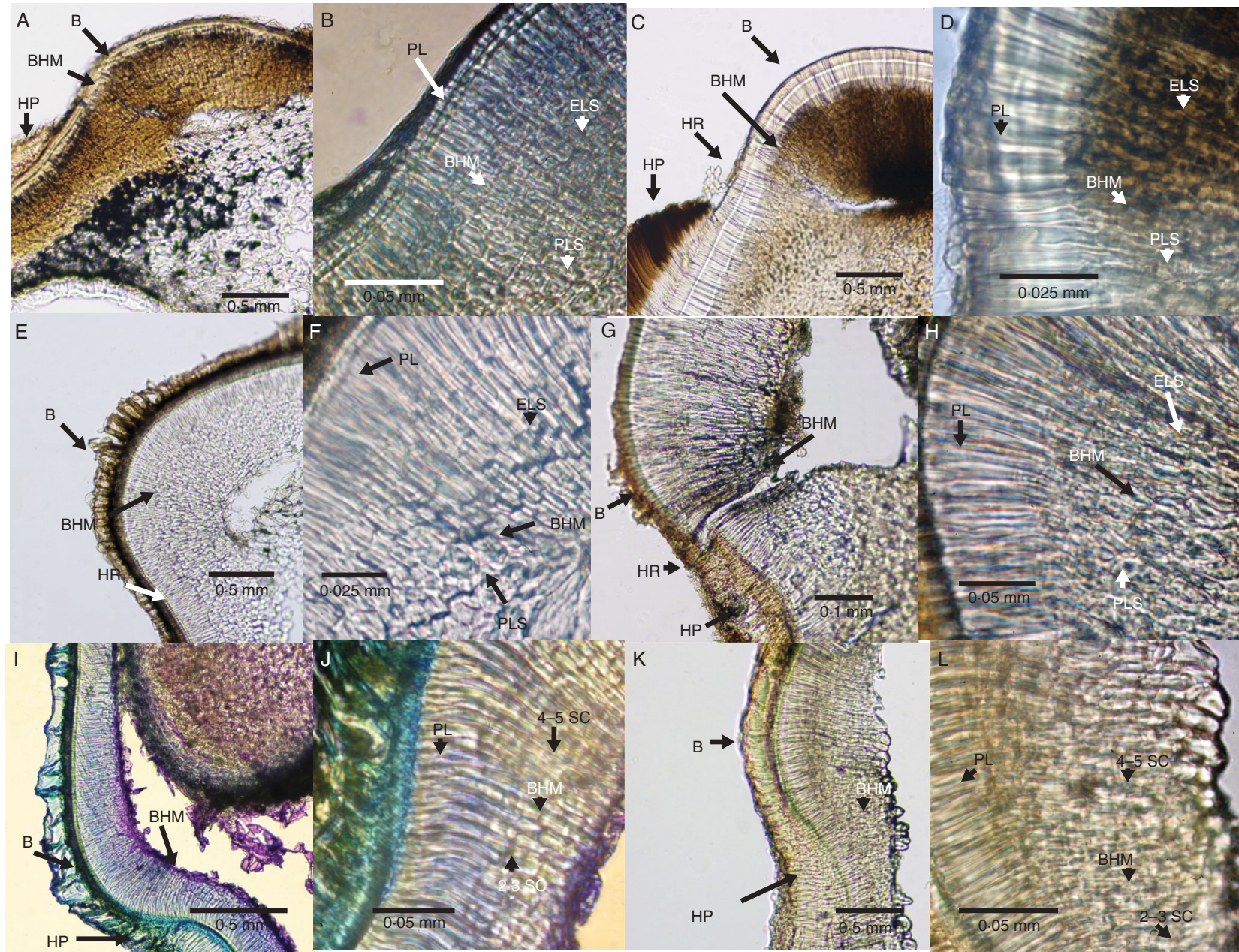


FIG. 7. Photomicrographs of 'water gap' in seeds of Convolvulaceae. Bulge of *Ipomoea lacunosa* (A, B), *Merremia dissecta* (C, D), *Convolvulus arvensis* (E, F), *Aniseia martinicensis* (G, H), *Jaquemontia ovalifolia* (I, J), *Evolvulus nuttallianus* (K, L), *Dichondra micrantha* (M, N), *Maripa panamensis* (area of seed corresponding to the water gap of other species) (O, P), hilum fissure of *Cuscuta australis* (Q) and bulge of *Cardiochlamys madagascariensis* (R, S). Abbreviations: B, bulge; BHM, bulge-hilum margin; BL, bulging cell layer; CPL, counter palisade layer; ED, endodermal cells; ELS, elongated sclereid cells; HF, hilar fissure; HP, hilar pad; HR, hilar ring; NOPL, no palisade layer; PL, palisade layer; PLS, polygonal shaped sclereid cells; 2-3 SC, 2-3 sub-layered sclereid layer; 3-4 SC, 3-4 sub-layered sclereid layer; 4-5 SC, 4-5 sub-layered sclereid layer; 6-8 SC, 6-8 sub-layered sclereid layer; 8-10 SC, 8-10 sub-layered sclereid layer.



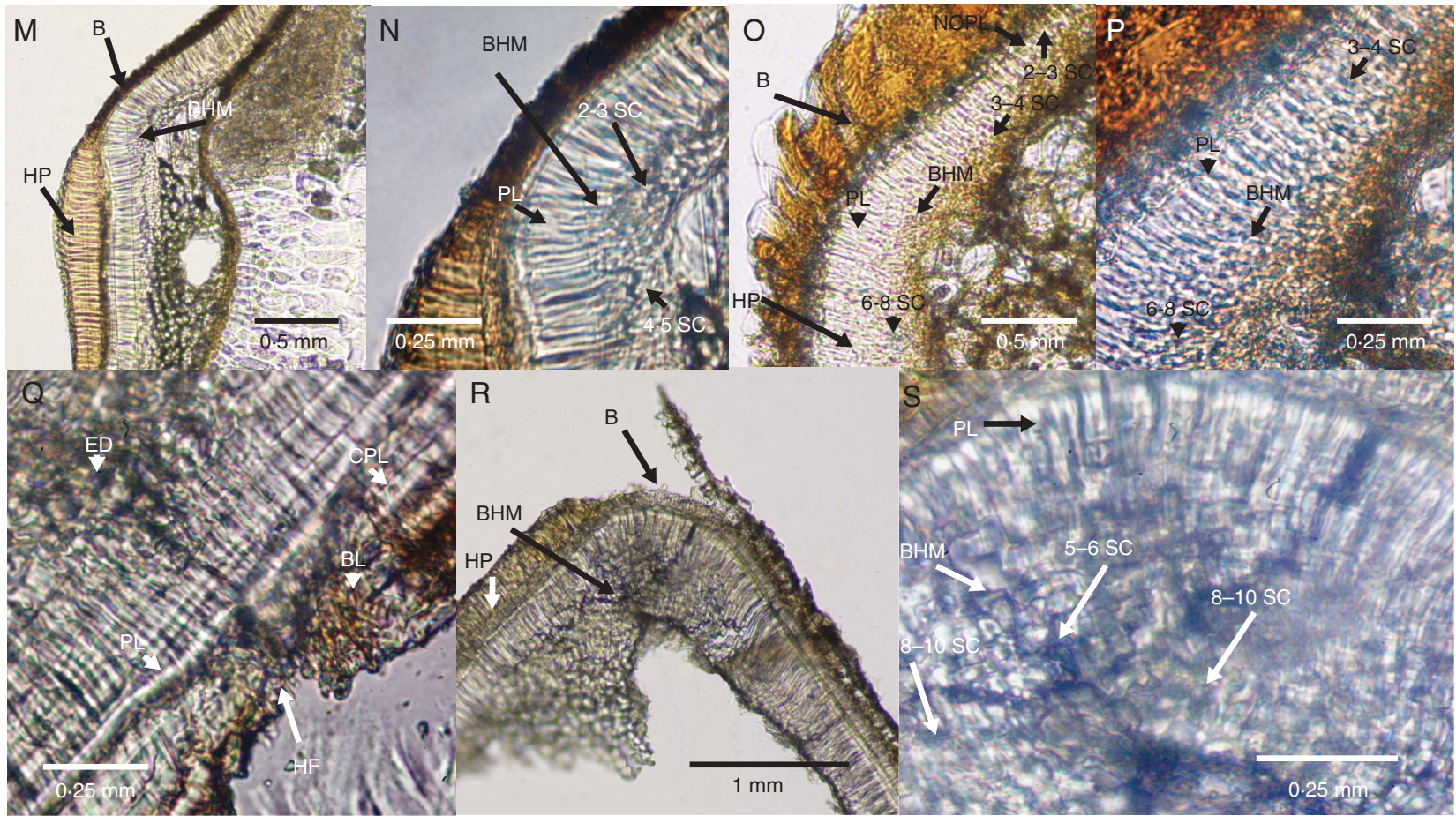


FIG. 7. Continued.



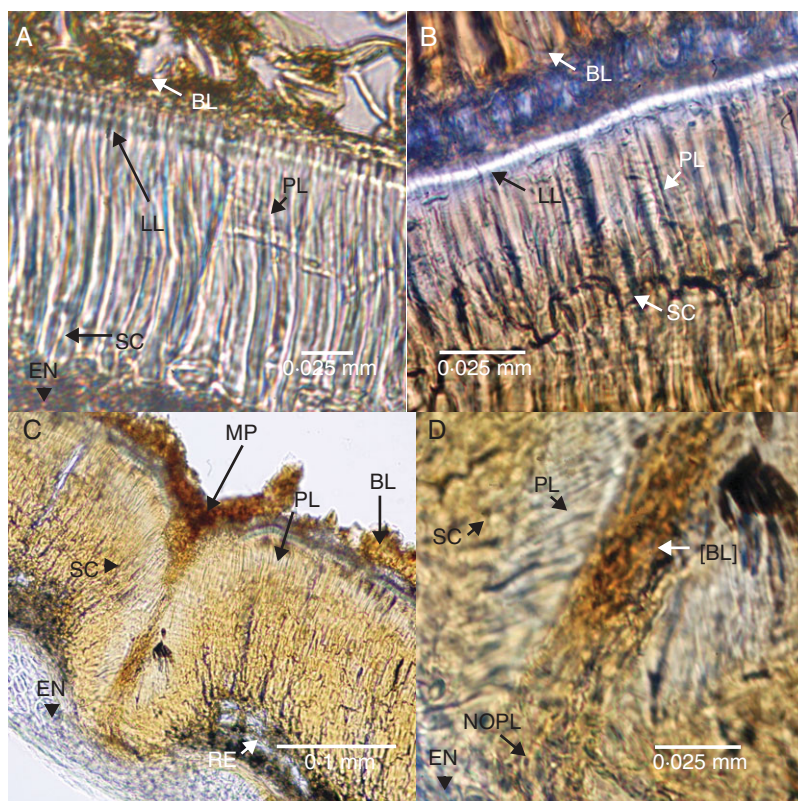


FIG. 8. Photomicrographs of seed coat (A), hilar pad (B), micropylar opening (C) and bottom part of the micropylar opening (D) of seeds of *Bonamia menziesii*. Abbreviations: BL, bulging cell layer; [BL], remnants of bulging cells; EN, endosperm; LL, light line; MP, micropyle; NOPL, no palisade; PL, palisade; RE, remnants of endodermis and nucellus cells; SC, sclereid layer.

Jayasuriya *et al.* (2008b) showed that the hilar fissure is the initial route of water entry into *C. australis* seeds, and studies on the water gap of *C. gronovii* seeds (Jayasuriya, 2008) support this finding. Hutchison and Ashton (1979) and Lyshede (1984) claim that water enters non-dormant seeds of *C. campestris* through the entire seed coat. Hutchison and Ashton (1979) and Lyshede (1984) used concentrated sulfuric acid and mechanical abrasion, respectively, to make seeds of this species non-dormant. These treatments are not similar to those that break dormancy in nature, and it seems possible that they might have damaged the seed coat throughout. However, in the present study dry storage at ambient laboratory temperature followed by 1 week storage at 100 % relative humidity at 35/20 °C was used to break dormancy in seeds of *C. australis*, and dry storage at 35/20 °C for 6 months to break dormancy in those of *C. gronovii*. These treatments closely simulate the conditions that these seeds are exposed to in their natural environment, and thus would be the natural dormancy-breaking treatment for this species.

Most species in the Convolvulaceae produce orthodox seeds with PY (Jayasuriya *et al.*, 2008c). However, non-treated seeds (all four collections) of *B. menziesii* were non-dormant and orthodox, those of *E. henryi* and *M. panamensis* non-dormant and recalcitrant (Jayasuriya *et al.*, 2008c), those of *Erycibe griffithii* non-dormant (and recalcitrant?) (Ng, 1991), and those of *C. europea* (Gaertner, 1956), *C. approximata* (Tingey and Allred, 1961) and *C. epithymum* (Meulebrouck *et al.*, 2008) combinationally dormant and orthodox. The

species that do not produce physically dormant orthodox seeds are located in a basal tribe of the family (Erycibeae), or in a tribe that is closely related to a basal tribe (Maripeae; *sensu* Stefanovic *et al.*, 2003) (Jayasuriya *et al.*, 2008c), except for *B. menziesii*. Anatomy and morphology of seed dormancy in Convolvulaceae follows this same phylogenetic pattern.

Using morphology and anatomy of the hilar pad and water gap, the species studied here can be divided into two major clusters and two individual tribes (Fig. 9). These two clusters closely resemble the Convolvuloideae and Dicanostyloideae clades identified by Stefanovic *et al.* (2003). Cluster one consists of tribes Ipomoeae, Merremieae, Convolvuleae and Aniseieae, which were included in the Convolvuloideae clade. This clade is characterized by a morphologically prominent bulge, change in cell shape in the transition at the bulge–hilar pad and bulge–seed coat away from hilum margin, and a horseshoe-shaped hilar fissure that encircles two-thirds of the hilar pad. Aniseieae seems to be the most basal tribe of this group (Stefanovic *et al.*, 2003). The representative species in this tribe can be separated from the species in the other tribes. During dormancy break, the bulge in Aniseieae is detached from the seed, forming a dentate margin that is not morphologically prominent like it is in seeds of other tribes in the clade. *Merremia* is suggested to be the representative genus for the type of morphoanatomy of dormancy in the clade Convolvuloideae. Anatomy of dormancy in seeds of *M. dissecta* is illustrated to represent the clade (Fig. 10).

Linkage method: NEAREST NEIGHBOR  
 Distance measure: Sorensen (Bray-Curtis)

Percent chaining - 53.85

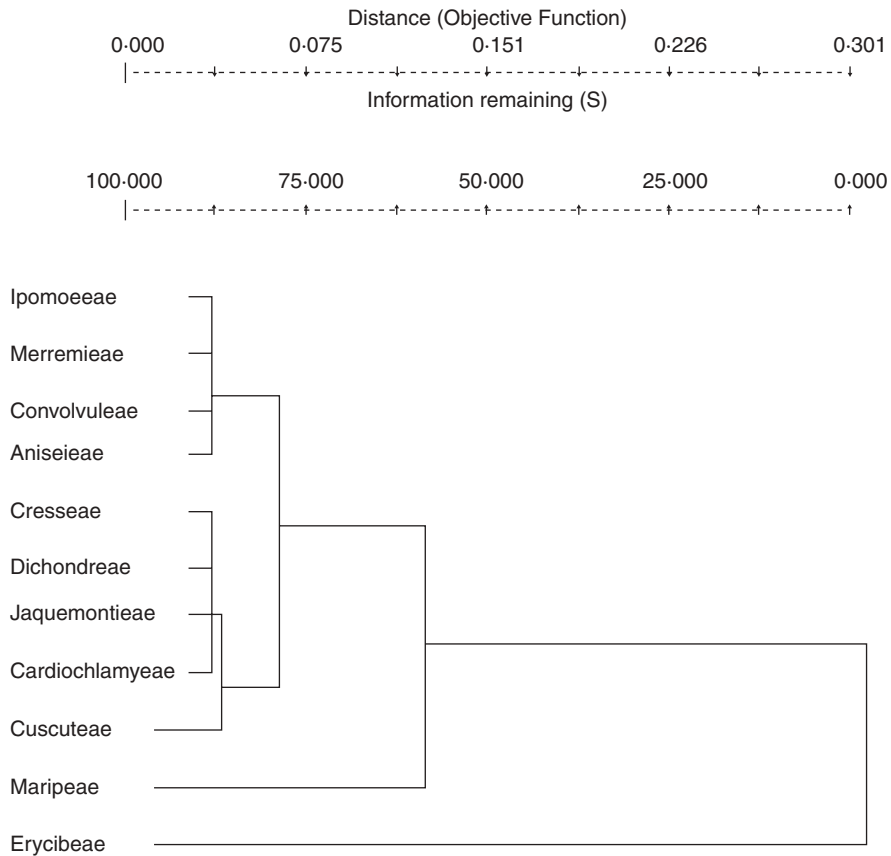


FIG. 9. Dendrogram of Convolvulaceae, subfamily Convolvuloideae seeds based on seed anatomical, morphological, dormancy and storage characters.

According to Stefanovic *et al.* (2003), the Dicranostyloideae clade consists of tribes Jacquemontieae, Maripeae, Cresseae and Dichondreae. However, the second cluster identified here also includes Cardiochlamyaeae, based on similarity in anatomy and morphology of seed dormancy of *C. madagascariensis* to that of the four tribes (Jacquemontieae, Maripeae, Cresseae and Dichondreae) placed in the clade by Stefanovic *et al.* (2003). The clusters identified here [Dicranostyloideae (of Stefanovic *et al.*, 2003) + Cardiochlamyaeae] are characterized by a linear-shaped hilar fissure located in the centre of the hilar pad, a morphologically non-prominent bulge and change in cell number in the transition at the bulge–hilar pad and bulge–seed coat away from hilum margins. The present cluster analysis separates Maripeae from this cluster, which is due to not recognizing recalcitrant behaviour of the tribe as a reversal by the similarity analysis.

Austin (1998) grouped all the genera that belong to the new Cardiochlamyaeae (Stefanovic *et al.*, 2003) and some of the genera that belong to the new Dichondreae (Stefanovic *et al.*, 2003) into tribe Poraneae. Although there are clear similarities in seed morphology of *C. madagascariensis* and *R. tiliifolia* that belong to Austin's (1998) Poraneae, Stefanovic *et al.*

(2003) included *R. tiliifolia* in Dichondreae. However, Cardiochlamyaeae can be separated from the rest of the tribes since the seeds do not have a hilar ring, and thus the bulge and hilar pad share a common margin. Cresseae and Dichondreae can be considered as basal tribes of Dicranostyloideae. The representative species of these tribes can be separated (Fig. 9) from other tribes since they have a third prominent protrusion between the two bulges. This protrusion is the enlarged radicular protrusion that encapsulates the radicle. However, this character can also be considered to be ancestral, since the most advanced clade, Convolvuloideae, does not have a third protrusion between the bulges. *Jacquemontia* is suggested to be the representative genus for this type of morphoanatomy of seed dormancy in clade Dicranostyloideae. The anatomy of seed dormancy in *J. ovalifolia* is illustrated to represent the Dicranostyloideae (Fig. 11).

*Bonamia menziesii* is an unusual member of tribe Cresseae in that it produces non-dormant seeds. However, *B. menziesii* seeds have the same anatomy as those of its physically dormant relatives. The entire seed coat is water impermeable, except the micropylar opening and the hilar fissure. These two openings are filled with crushed cell material, and they allow



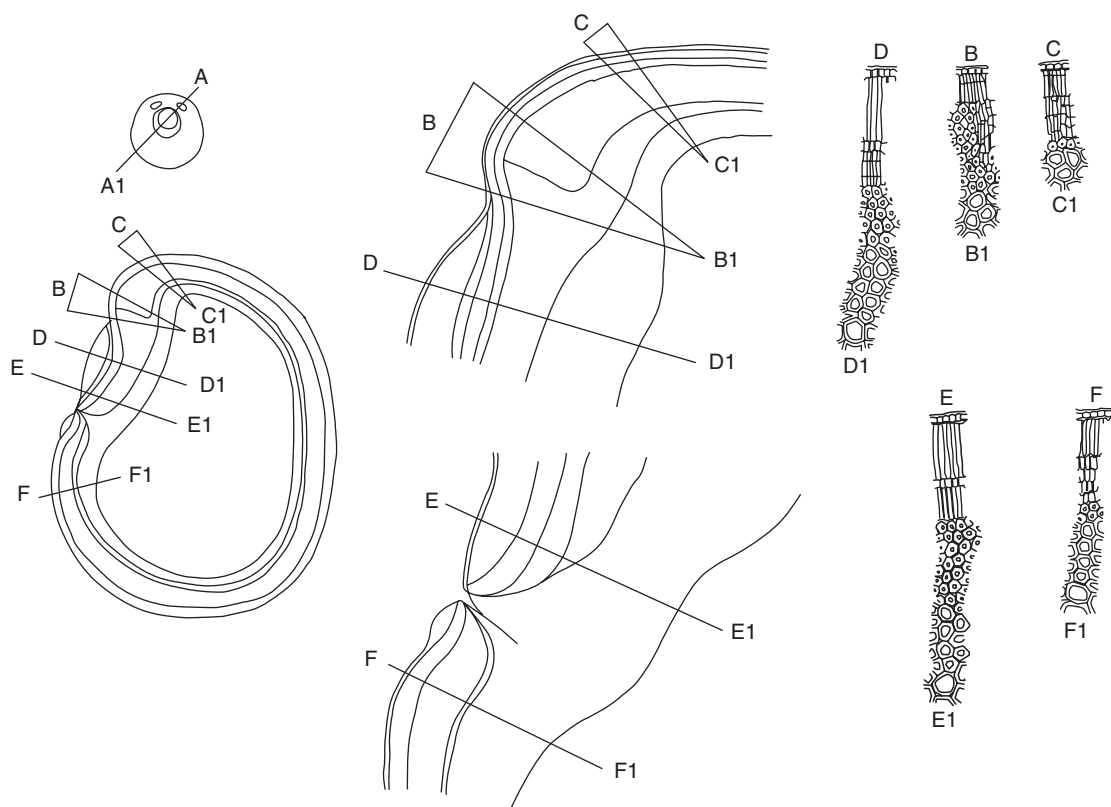


FIG. 10. Hand-drawings (not to scale) of the seed coat of *Merremia dissecta* to depict the Convolvuloid clade-type bulge anatomy. The seed coat : seed ratio is not proportionate. Seed coat layers are proportionate to each other.

the seed to imbibe water slowly. This change may have developed as an adaptation to its dry and mesic forest habitats in Hawaii (Wagner *et al.*, 1999). On the other hand, *B. menziesii* is atypical of the genus. The species has been isolated in Hawaii for so long that it has accumulated a number of traits not found in other species of the genus, and this is one reason Degener (1932) put it in his *Perispermum* Degener, when he described the species.

Maripeae, with recalcitrant seeds, appears to be a tribe that differs from other tribes in the cluster. However, there are close similarities between seeds of Maripeae and those of other tribes. In contrast to seeds of the other recalcitrant tribe, Erycibeae, those of Maripeae have some morphological and anatomical similarities to those of tribes in Dicranostyloideae, where Stefanovic *et al.* (2003) placed Maripeae. The linear-shaped hilar fissure at the centre of the hilar pad, the morphologically non-prominent but anatomically distinguishable bulge and the reduced number of sclereid cell layers at the hilum–bulge transition are similarities between seeds of Maripeae and those of other tribes in Dicranostyloideae. Further, *Maripa* has palisade cells only in the hilum area. Palisade cells disappear at the bulge–seed coat away from hilum transition. These similarities provide more evidence to support classifying Maripeae under Dicranostyloideae (Stefanovic *et al.*, 2003), rather than including *Maripa* together with *Erycibe* and *Humbertia* in tribe Erycibeae (Austin 1998). Therefore, as Jayasuriya *et al.* (2008c) suggested, the recalcitrant behaviour of *Maripa* is a reversal from the orthodox physically dormant behaviour of

the ancestor. Species of Maripeae are distributed in neotropical and neosub-tropical habitats (Austin, 1973). Therefore, this recalcitrant behaviour may be an adaptive advantage to species of this tribe for tropical and sub-tropical habitats. This agrees with the conclusion that orthodox behaviour has been lost several times during the evolution of angiosperms (Von Tiechman and Van Wyk, 1994; Pammenter and Berjak, 2000; Farnsworth, 2000; Dickie and Prichard, 2002).

*Cuscuta* is a noteworthy genus that has been placed in a separate monogeneric tribe (Austin, 1998; Stefanovic *et al.*, 2003). Both of these classifications agree with our identification of tribe Cuscutae as a separate group. Morphological and anatomical absence of bulges separates this tribe from all other physically dormant tribes of the Convolvulaceae. The hilar fissure functions as the water gap in seeds of Cuscutae [observed in both *C. australis* (Figs 1V and 4H) and *C. gronovii* (images not shown)]. According to Stefanovic *et al.* (2003), Cuscutae is derived from the common ancestor of Convolvuloideae and Dicranostyloideae. Austin (1998) suggested Cuscutae as the basal tribe of Convolvulaceae, and some plant taxonomists have excluded Cuscutae from Convolvulaceae and placed it in its own family Cuscutaceae (Takhtajan, 1980, 1997; Dahlgren, 1991). However, morphology and anatomy of seed dormancy support the opinion of Stefanovic *et al.* (2003) that Cuscutae should be included in Convolvulaceae and that it is derived from an ancestral tribe common to all physically dormant tribes and Maripeae.

Cardiochlamyaeae, Erycibeae and Humbertieae are the basal tribes of Convolvulaceae according to Stefanovic *et al.* (2003),

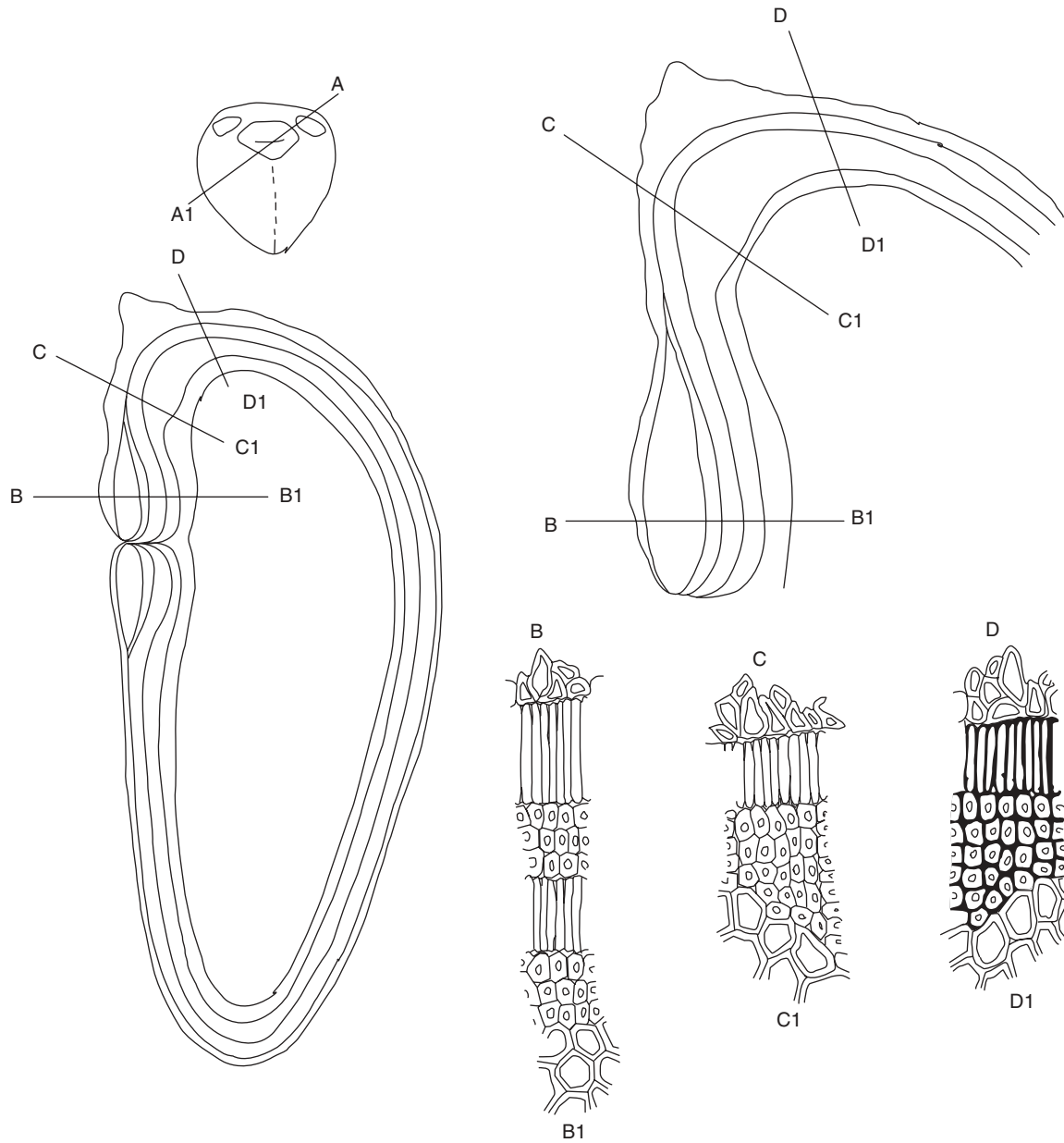


FIG. 11. Hand-drawings (not to scale) of the seed coat of *Jacquemontia ovalifolia* to depict the Dicranostyloid clade-type bulge anatomy. The seed coat:seed ratio is not proportionate. Seed coat layers are proportionate to each other.

Humberteae being the most basal tribe in the family according to these authors. It was not possible to obtain seeds of *H. madagascariensis* and thus seed dormancy in this rare species could not be studied. Stefanovic *et al.* (2003) placed Cardiochlamyaeae and Erycibeae as tribes derived from a common ancestor that was derived from an ancestor closely related to *Humbertia*. Austin (1998) also suggests that *Erycibe* is derived from an ancestor closely related to *Humbertia*.

If *Humbertia* is a recalcitrant (a state suggested by its relatively large seeds and thinly fleshy fruit) species, *Erycibe* seems to have conserved the recalcitrant trait, in which case Cardiochlamyaeae is the most basal tribe in Convolvulaceae

with PY. Stefanovic *et al.* (2002) divide Convolvulaceae into two subfamilies, Humbertioidae and Convolvuloideae. Humbertioidae is a monospecific subfamily with only *H. madagascariensis*. Erycibeae and Cardiochlamyaeae are the most basal tribes of subfamily Convolvuloideae. According to the morphoanatomy of seed dormancy, as previously discussed, Cardiochlamyaeae is more closely related to Dicranostyloideae than to Erycibeae. Thus, it is suggested that Cardiochlamyaeae is derived from an ancestral species with PY that was derived from a non-dormant species closely related to Erycibeae. Therefore, it can be suggested that PY in subfamily Convolvuloideae evolved from an ancestor with non-dormant recalcitrant seeds. Erycibeae seems to



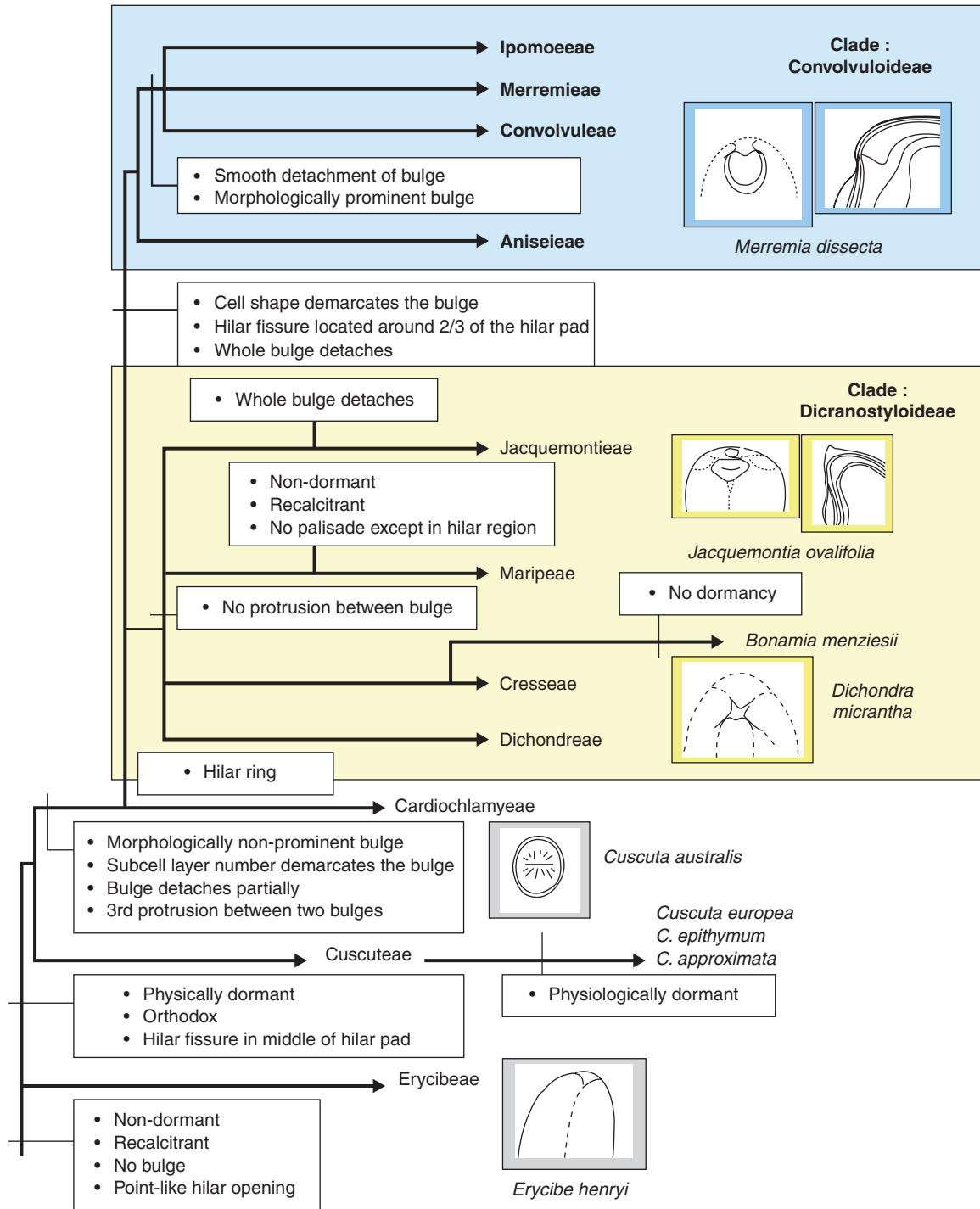


FIG. 12. Proposed phylogenetic tree of seed dormancy for Convolvulaceae, subfamily Convolvuloideae, with illustrations of the hilum area of seeds of some taxa. The tree was constructed by combining the morphoanatomy of seed dormancy data and seed storage characteristics with proposed phylogeny of Convolvulaceae by Stefanovic *et al.* (2003).

be the most closely related tribe to this ancestor. The proposed phylogeny of seed dormancy in subfamily Convolvuloideae is illustrated in Fig. 12.

Species of Erycibeae are distributed in Asian and Australian tropics, and Cardiochlamyaeae occurs in the dry parts of Asia. Therefore, it is hypothesized that PY evolved and thrived with

adaptive advantage in a seasonally dry/seasonally wet climate (Fig. 13). The bulge, which is the most important structure in controlling germination by acting as the environmental signal detector, has developed from a morphologically non-prominent structure demarcated anatomically by a change in the number of cell layers in the seed coat to a morphologically

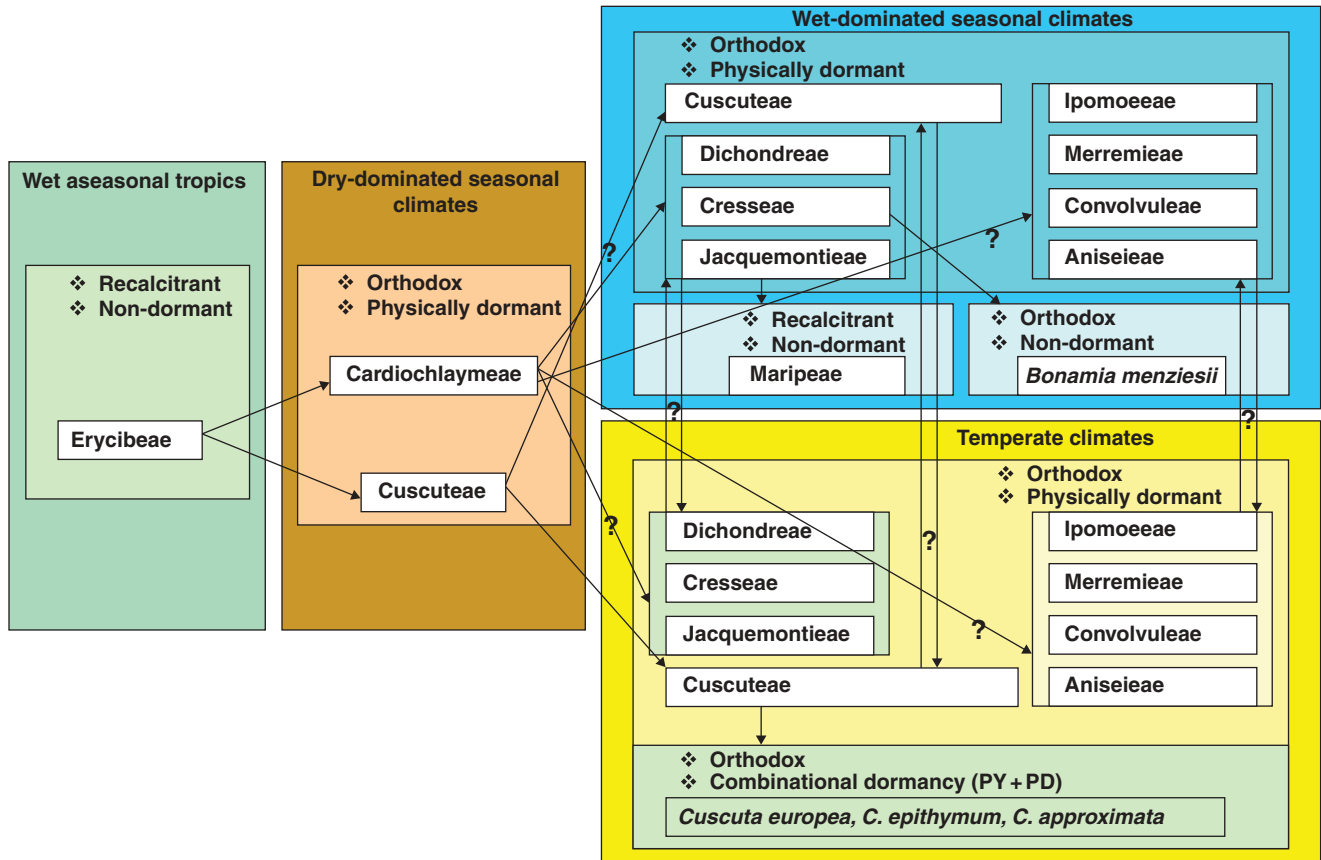


FIG. 13. Proposed evolutionary change of seed dormancy with change in climate and geographical distribution in Convolvulaceae. A question mark (?) indicates that there was no information to predict whether the species in wet-dominated climates evolved from species in dry-dominated seasonal climates or in temperate climates, or vice versa.

prominent protuberance anatomically demarcated by a change in cell shape of the sclereid layer. However, later, physically dormant Convolvulaceae species again may have become distributed throughout the tropics. Most of these species have retained the trait of PY. However, in some species, reversal in seed storage behaviour and dormancy from dormant orthodox to non-dormant recalcitrant, as in *M. panamensis*, or from dormant orthodox to non-dormant orthodox, as in *B. menziesii*, may have occurred as Convolvulaceae species became redistributed in the tropics.

Convolvulaceae is also distributed throughout temperate regions. Most Convolvulaceae species did not change their dormancy traits as they (probably) spread gradually (Fig. 13). However, *Cuscuta* is an exception. In seeds of *Cuscuta* species such as *C. europea*, *C. approximata* and *C. epithymum*, PD has been added to PY. This combinational dormancy may be an adaptive advantage in an aseasonal climate with temperature fluctuations. Baskin *et al.* (2000) hypothesized that combinational dormancy (PY + PD) in *Rhus* evolved from PY (i.e. PD was added to PY) during climate change from tropical dry to temperate, and fossil evidence seems to support this conclusion. However, according to Finch-Savage and Leubner-Metzger (2006), combinational dormancy evolved from PD. There are no taxa in Cuscutaeae or in sister tribes of Cuscutaeae known to produce seeds with

PD. Therefore, it does not seem likely that combinational dormancy evolved from PD in Convolvulaceae.

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