JIPB Journal of Integrative Plant Biology

Journal of Integrative Plant Biology 2012, **54** (10): 773–789

Research Article

Pollen Morphology of the Platycodonoid Group (Campanulaceae s. str.) and Its Systematic Implications[F]

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Available online on 11 September 2012 at www.jipb.net and www.wileyonlinelibrary.com/journal/jipb

doi: 10.1111/j.1744-7909.2012.01164.x

Abstract

In this study, we examined the pollen morphology of the platycodonoid group in Campanulaceae s. str. using a scanning electronic microscope. We used pollen grains of 25 accessions representing 24 species of the *Codonopsis* complex (including *Campanumoea*, *Cyclocodon*, *Leptocodon*, and all three subgenera of *Codonopsis*), which is extremely controversial among authors for taxonomic treatment. Pollen morphology of all the other genera in the group observed by previous authors is taken into account in our discussion. A total of nine pollen types with two subtypes in the group were recognized and named for the first time. Molecular and morphological data imply that each pollen type corresponds to a natural group at generic level, and thus the mergence of *Leptocodon* with *Codonopsis* and the restoration of *Cyclocodon* as a separate genus are justifiable, and *Codonopsis* subg. *Pseudocodonopsis*, subg. *Obconicicapsula*, and two species of *Codonopsis* subg. *Codonopsis* (*C. purpurea* and *C. chimiliensis*) may be better classified as three independent genera separate from the core *Codonopsis*.

Keywords: Campanulaceae s. str.; platycodonoid group; *Codonopsis* complex; pollen morphology; pollen type; taxonomy; systematics.

Hong DY, Pan KY (2012) Pollen morphology of the platycodonoid group (campanulaceae s. str.) and its systematic implications. *J. Integr. Plant Biol.* **54**(10), 773–789.

Introduction

The Campanulaceae s. str. is a well-established family (Cosner et al. 1994; Gustafsson and Bremer 1995; Gustafsson et al. 1996; Lundberg and Bremer 2003). However, the subdivisions of the family and the relationships of genera were unclear before this century. Recent studies on molecular systematics of the Campanulaceae s. str. have resulted in more insight into infrafamilial phylogenetic relationships. Eddie et al. (2003) analyzed the phylogenetic relationships of the family using ITS sequences of nuclear ribosomal DNA. Borsch et al. (2009) conducted analyses of phylogenetic relationships using DNA sequences of the chloroplast gene *pet*D. In the same year, Haberle et al. (2009) used DNA sequences of three chloroplast genes, *atp*B, *mat*K, and *rbc*L, to analyze the phylogenetic relationships of the Campanulaceae s. str. These three studies tell us that the Campanulaceae s. str. consists of three major monophyletic groups: campanuloids, platycodonoids and wahlenbergioids.

On the other hand, the three studies mentioned above also indicate that the phylogenetic relationships within each of the three major groups are far from well established. They all clearly show that *Campanula* and *Wahlenbergia* each is a polyphyletic genus in the traditional sense. Eddie et al. (2003) and Haberle et al. (2009) have convinced us that the genus *Codonopsis* Wall. in the platycodonoid group is also polyphyletic.

Pollen morphology has been used as a very powerful tool to consider systematics of the Campanulaceae. Erdtman (1952) conducted a rather extensive survey on pollen of the Campanulaceae s. lat. and pointed out that Schönland's "subtribes of Campanulaceae – Campanuloideae do not seem to be homogeneous." For example, Schönland's Platycodinae is

a mixture of *Platycodon* with pollen having elongate apertures, and *Microcodon* and *Musschia* with porate pollen. Thulin (1975) further emphasized the significance of pollen morphology in taxonomy, stating that for the arrangement of genera in the Campanulaceae s. str., the first consideration should be pollen morphology. Morris and Lammers' (1997) work is the most extensive on the *Codonopsis* complex. They observed pollen morphology of 23 accessions representing 17 species, and took into account the results of previous investigators. As a result, they recognized four pollen types, and revealed their close relationships with taxonomy.

Molecular phylogenetic results clearly show that there is a major dichotomy between the colpate/colporate pollen alliance (platycodonoid taxa) and the porate pollen alliance (wahlenbergioid and campanuloid taxa). The wahlenbergioid and campanulioid groups both have porate pollen, but the former possesses capsules dehiscent by apical valves (loculicidal), while capsules of the latter are dehiscent by lateral pores (poricidal).

In the platycodonoid group, the circumscription of *Codonopsis* has been controversial among authors. Moeliono and Tuyn (1960) merged *Campanumoea* s. lat. (including the genus *Cyclocodon*) with *Codonopsis*. This circumscription of *Codonopsis* was accepted by Grey-Wilson (1990) and Lammers (1992). Grey-Wilson (1990) merged *Leptocodon* into *Codonopsis*, and this action was followed by Lammers (1999). Thus, Lammers adopted the broadest concept of *Codonopsis*, accommodating all four genera mentioned above. However, Hong (1983) recognized *Campanumoea* s. lat. (including sect. *Cyclocodon*) and *Leptocodon* each as an independent genus. Hong and Pan (1998) restored *Cyclocodon* at generic rank based on data from the pollen and seed-coat, which are distinct from those in *Codonopsis* and *Campanumoea* sect. *Campanumoea*. In "Flora of China" (Hong et al. 2011: p. 505), readers can find controversial views on how to treat the group under discussion. Hong recognized four genera, but the two co-authors, Lammers and Klein, stated their belief that *Campanumoea* and *Leptocodon* should be merged into *Codonopsis*. Another issue involved in the taxonomic treatment of the group under study is whether *Codonopsis* s. str. is monophyletic or polyphyletic. According to the current designation (Shen and Hong 1983; Hong in Hong et al. 2011), *Codonopsis* consists of three subgenera, subg. *Codonopsis*, subg. *Obconicicapsula* D. Y. Hong (1980) and subg. *Pseudocodonopsis* Kom. (1908). Two molecular trees, one based on the ITS sequence of nuclear ribosomal DNA (Eddie et al. 2003), and the other based on three cpDNA genes, *atp*B, *mat*K and *rbc*L (Haberle et al. 2009), indicate that the only species of subg. *Obconicicapsula*, *Codonopsis dicentrifolia* (C. B. Clarke) W. W. Smith, does not join the *Codonopsis* clade, but instead forms a clade with *Cyananthus lobatus*. Based on their observations, Morris and Lammers (1997) revealed that three species in subg. *Pseudocodonopsis* have pollen morphology distinctly different from that of most species of subg. *Codonopsis*. If the other species of subg. *Pseudocodonopsis* are the same as these three species in terms of pollen morphology, one could question the justification for keeping them in *Codonopsis*, considering the sharp differences in external morphology between two subgenera.

Consequently, we think that it is worthwhile to conduct more extensive observations on pollen morphology of the platycodonoid group, and to conduct a comprehensive analysis because of the diverse pollen morphology of the group and the controversial views on taxonomy among authors as mentioned above.

Since previous authors have already conducted extensive observations on the pollen morphology of *Canarina* (Erdtman 1952; Dunbar 1975a; Avetisjan 1986), *Cyananthus* (Erdtman 1952; Dunbar 1975a; Shrestha and Tarasevich 1992), *Ostrowskia* (Erdtman 1952; Dunbar 1975a; Avetisjan 1986), *Platycodon* (Dunbar 1975a; Avetisjan 1986;), and *Echinocodon* (Hong 1984), we intend to pay special attention to *Campanumoea*, *Codonopsis*, *Cyclocodon* and *Leptocodon*. Morris and Lammers' (1997) observation strongly implies that pollen in *Codonopsis* s. lat. (Shen and Hong 1983) is polytypical, and Eddie et al. (2003) and Haberle et al. (2009) use molecular data to show that the taxon is polyphyletic. Therefore, we greatly expanded sampling in *Codonopsis*, including the type species *C. viridis*.

Results and Discussion

The results of our observations are presented in **Figures 1–6** and **Table 1**, where previous observations are also provided to give adequate information for discussion purposes. From **Table 1**, the pollen of *Platycodon* is 5–6-colporate with medium-high sexine spinules (1.5 μm), and is thus a distinct type of pollen called the Platycodon Type. The pollen of genus *Ostrowskia* is very special, and is 6–7-colpate with large-verrucose sexine. It is deserving of being named an independent pollen type, the Ostrowskia Type. The genus *Echinocodon* D. Y. Hong possesses 4–5-colpate pollen with short colpi (colpus length/polar axis length ≅ 0.36) and basally divided sexine spinules. Such pollen cannot be assigned to any of the above-mentioned types, and thus is here designated as the Echinocodon Type. According to Dunbar (1975a) and Shrestha & Tarasevich (1992), the pollen of the genus *Cyananthus* is 6–9-colpate with long colpi, verrucose colpus membrane, and verrucose sexine (**Table 1**). It should also be designated an independent pollen type, the Cyananthus Type.

Based on previous observations (Erdtman 1952; Dunbar 1975a; Avetisjan 1967, 1986; Hong 1984; Morris and Lammers

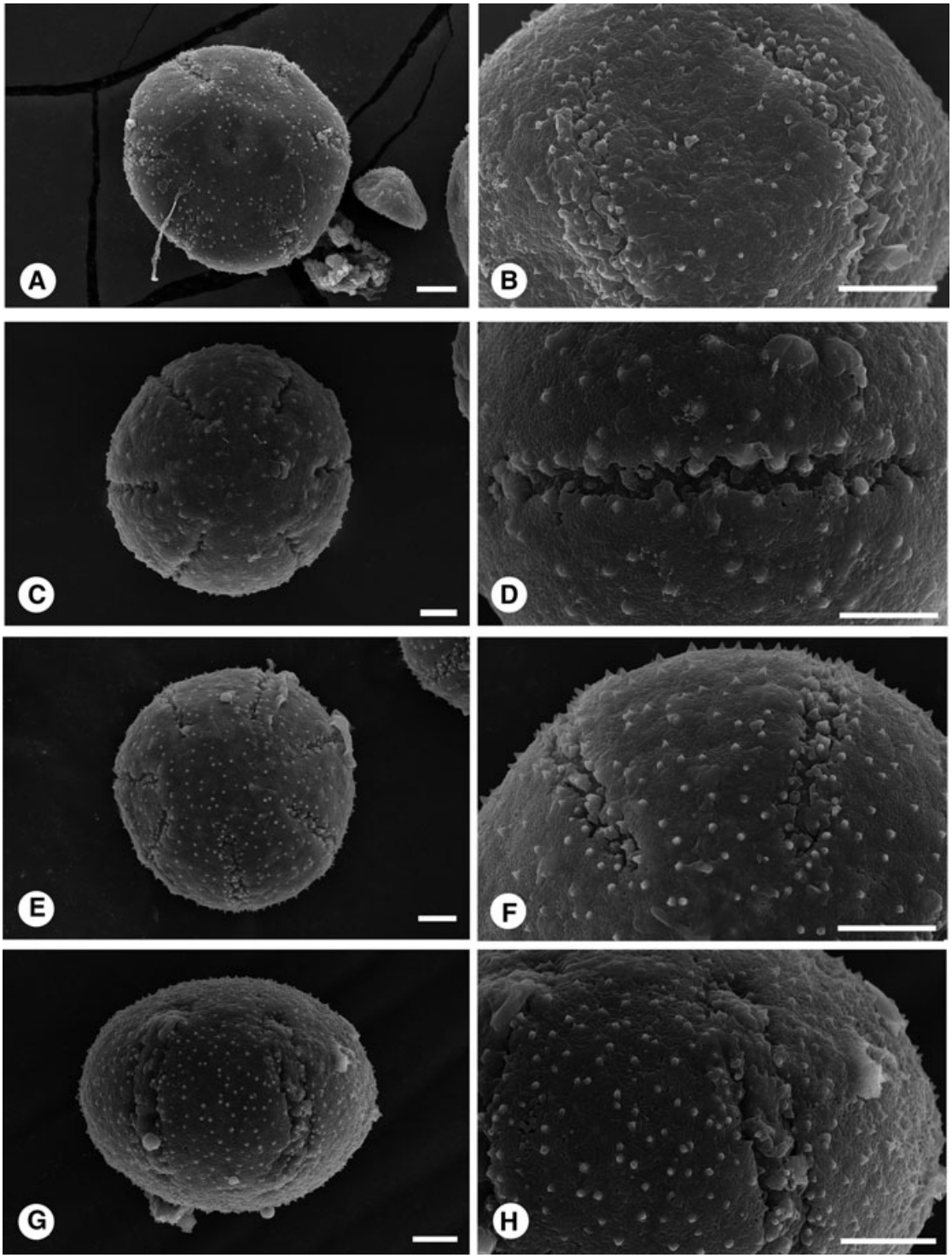

Figure 1. SEM photographs of pollen grains in *Codonopsis*

(A, B) *Codonopsis benthamii* Hook. f. & Thomson
(C, D) *Codonopsis farreri* J. Anthony
(E, F) *Codonopsis meleagris* Diels
(G, H) *Codonopsis subglobosa* W. W. Smith
Scale bars = 5 μm

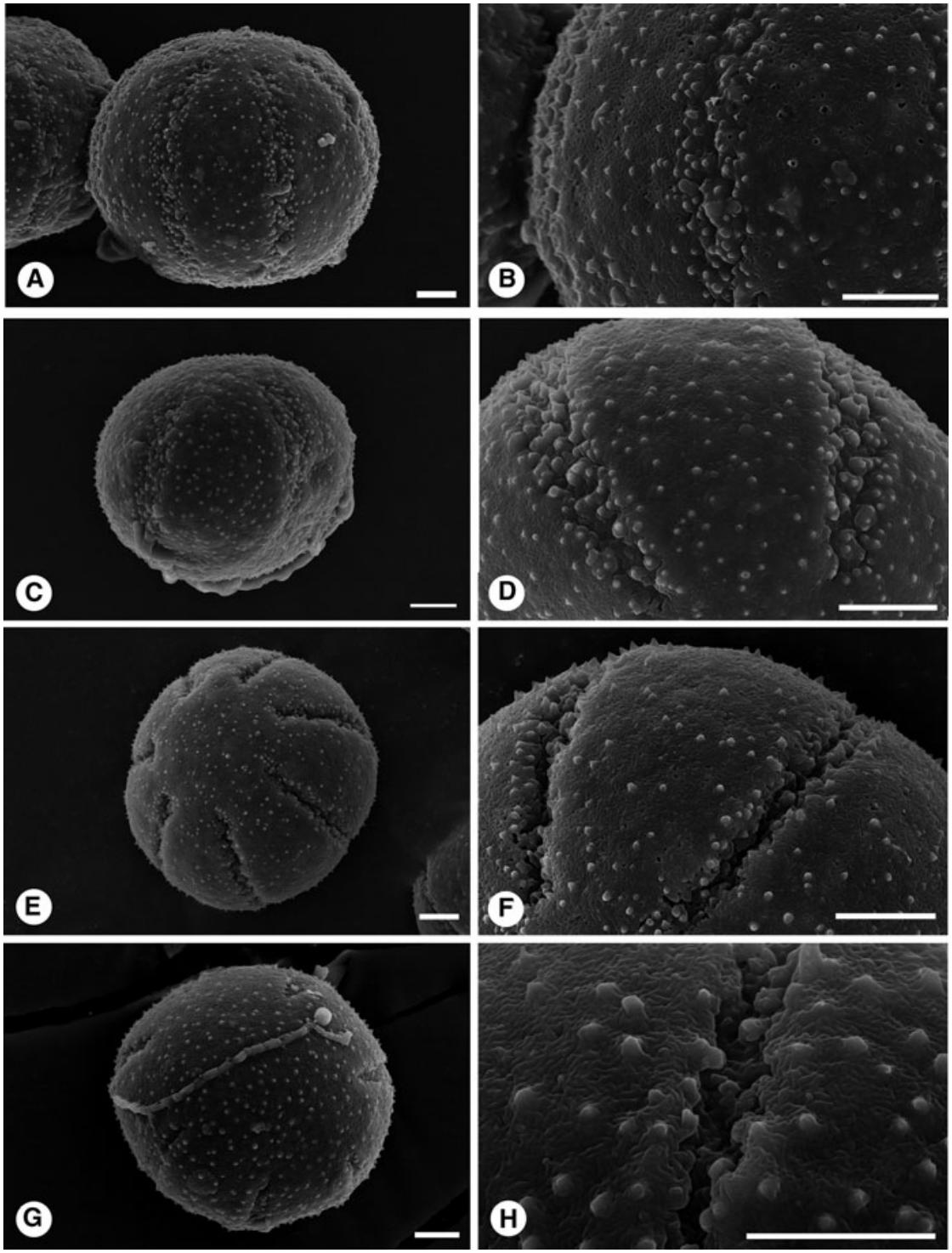

Figure 2. SEM photographs of pollen grains in *Codonopsis*

(A, B) *Codonopsis subscaposa* Kom.
(C, D) *Codonopsis subsimplex* Hook. f. & Thomson
(E, F) *Codonopsis thalictrifolia* Wall.
(G, H) *Codonopsis viridis* Wall.
Scale bars = 5 μm

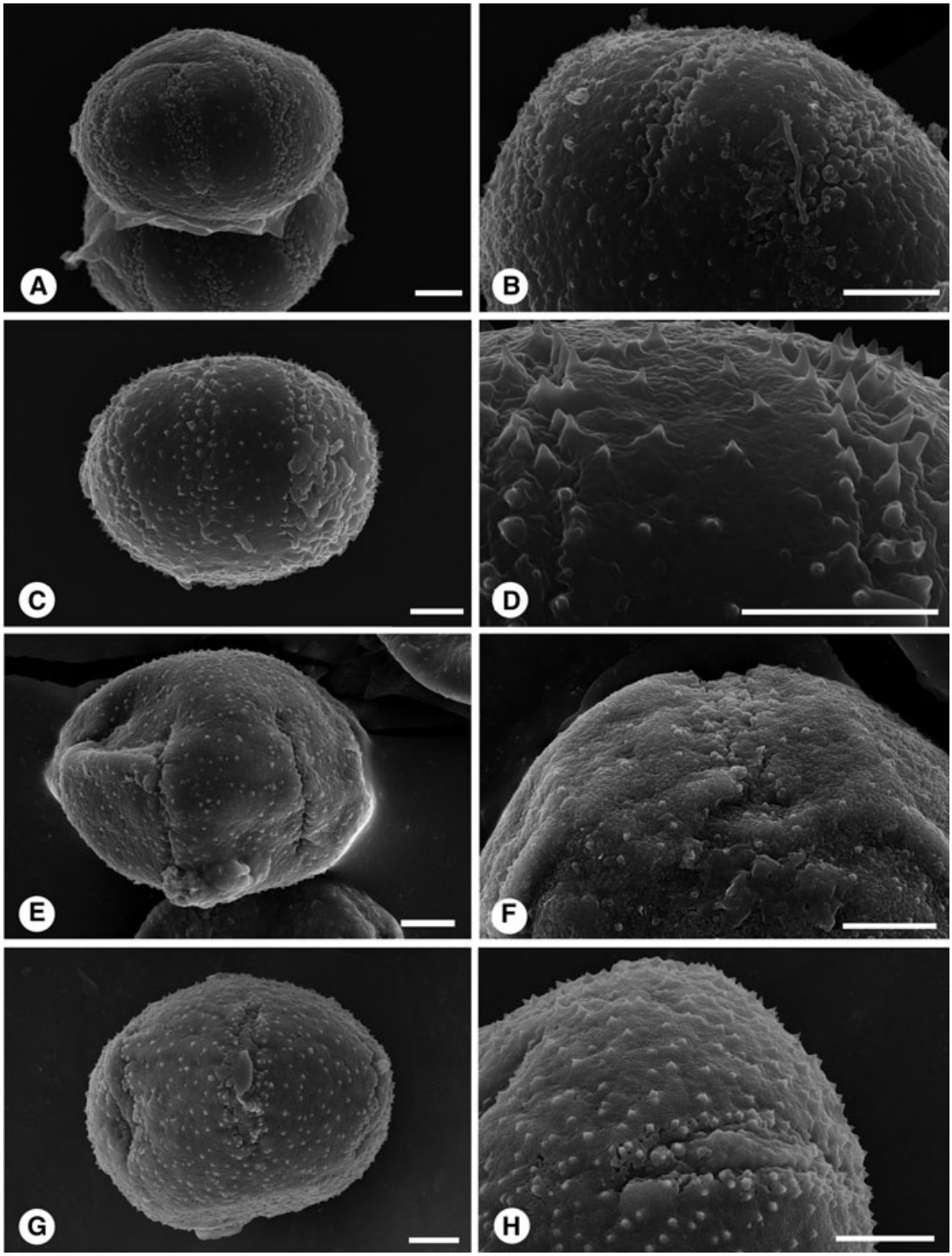

Figure 3. SEM photographs of pollen grains in *Leptocodon* and *Campanumoea*

(A, B) *Leptocodon gracilis* Hook. f. & Thomson
(C, D) *Leptocodon hirsutus* D. Y. Hong
(E, F) *Campanumoea inflata* Hook. f. & Thomson
(G, H) *Campanumoea javanica* Bl. subsp. *javanica*
Scale bars = 5 μm

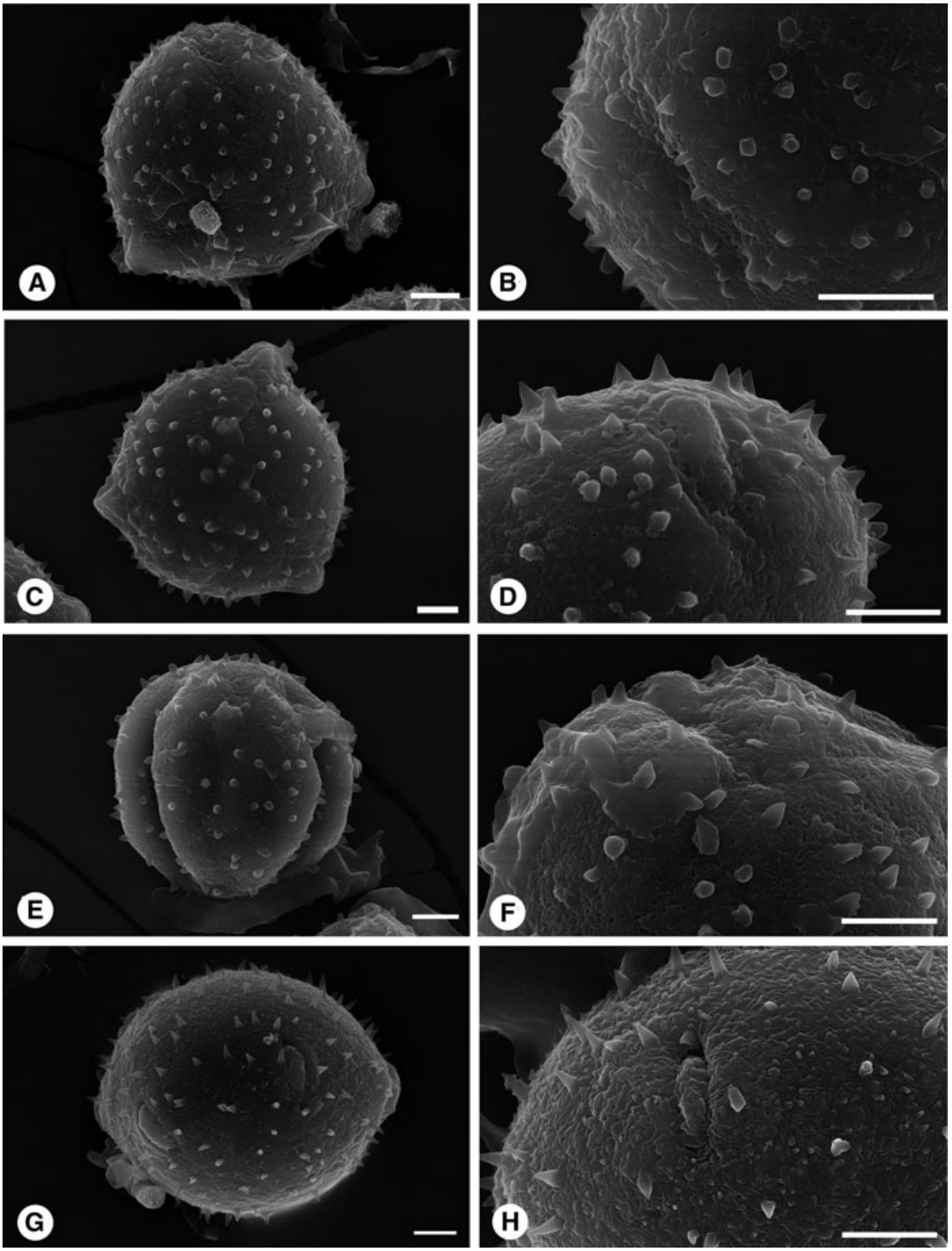

Figure 4. SEM photographs of pollen grains in *Cyclocodon* and *Codonopsis*

(A, B) *Cyclocodon celebicus* (Bl.) D. Y. Hong
(C, D) *Cyclocodon lancifolius* (Roxb.) Kurz
(E, F) *Cyclocodon parviflorus* (Wall. ex A. DC.) Hook. f. & Thomson
(G, H) *Codonopsis convolvulacea* Kurz subsp. *convolvulacea*
Scale bars = 5 μm

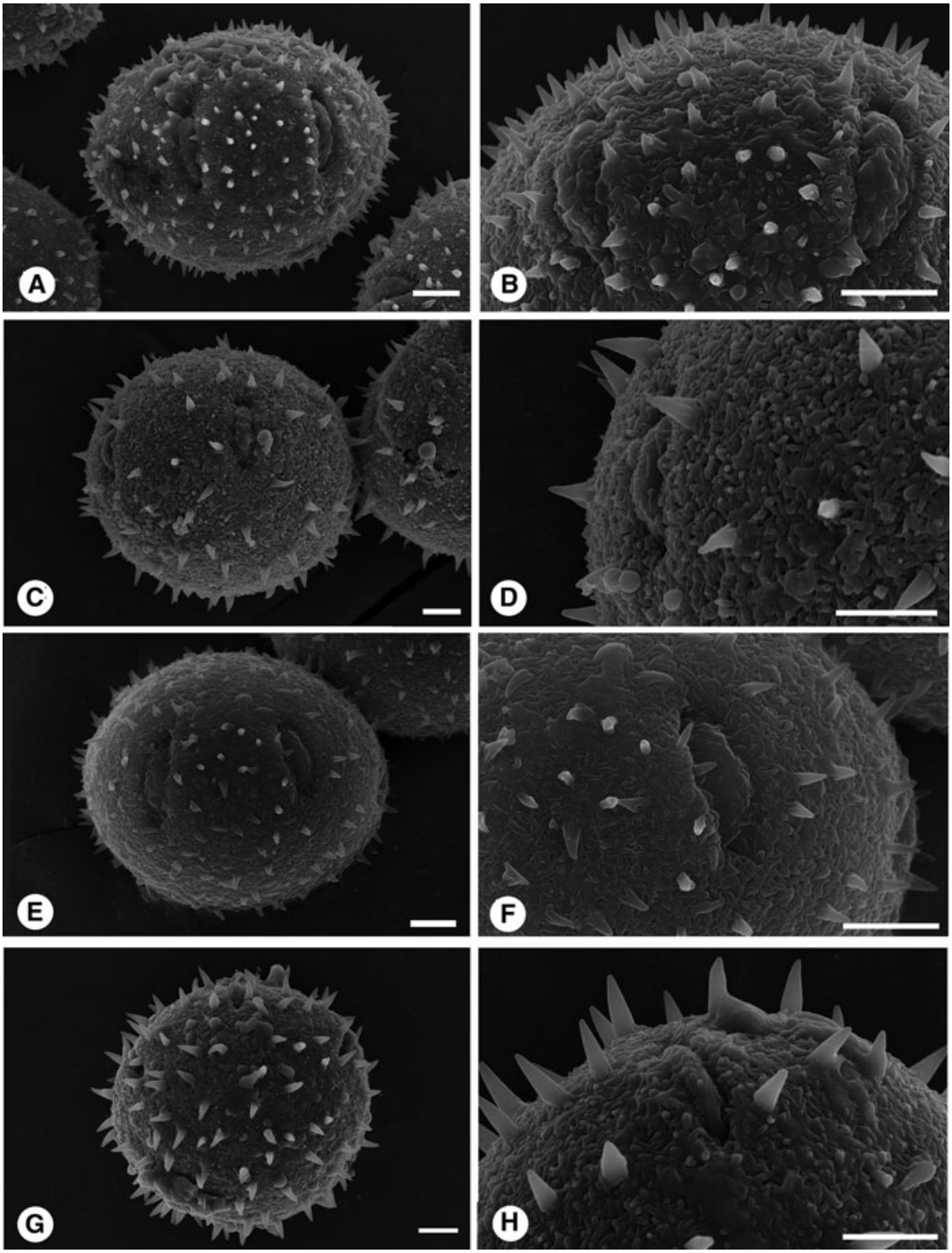

Figure 5. SEM photographs of pollen grains in *Codonopsis*

(A, B) *Codonopsis convolvulacea* subsp. *vinciflora* (Kom.) D. Y. Hong
(C, D) *Codonopsis convolvulacea* subsp. *grey-wilsonii* (J. M. H. Shaw) D. Y. Hong
(E, F) *Codonopsis graminifolia* H. Lév.
(G, H) *Codonopsis hirsuta* (Hand.-Mazz.) D. Y. Hong
Scale bars = 5 μm

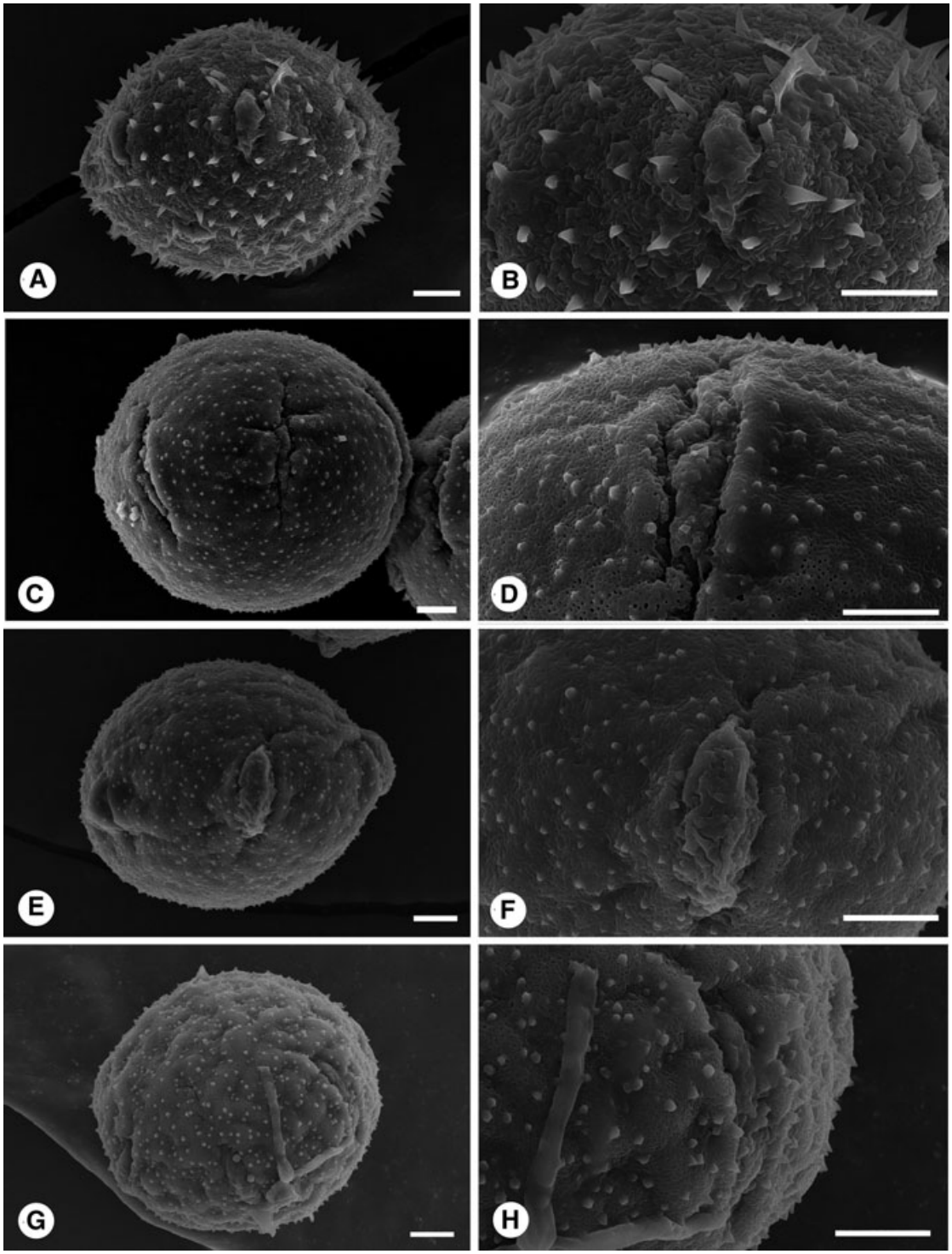

Figure 6. SEM photographs of pollen grains in *Codonopsis*

(A, B) *Codonopsis rosulata* W. W. Smith
(C, D) *Codonopsis dicentrifolia* (Wall.) W. W. Smith
(E, F) *Codonopsis purpurea* Wall.
(G, H) *Codonopsis chimiliensis* J. Anthony
Scale bars = 5 μm

Table 1. Pollen morphology of the platycodonoid group (Campanulaceae s. str.)(the classification of *Codonopsis* and its allies follows "Flora of China" Vol.19. 2011)

Taxon	Aperture	Colpus length[1)] long	Colpus length[1)] medium-long	Colpus length[1)] short	Ornamentation spinulose	Ornamentation verrucose	Colpus membrance	Spinule/verrucae height[2)] high	Spinule/verrucae height[2)] short	Spinule base divided	Spinule base entire	Reference/voucher
Campanumoea												
C. inflata	6(–8)-colpate		+		+		granulose		+		+	Murthy 1982; Morris & Lammers 1997; *Qinghai-Xizang Exped. 1588* (PE)
C. javanica												
ssp. *javanica*	5–6-colpate		+		+				+		+	Dunbar 1975a; Murthy 1982; Morris & Lammers 1997; *Z. Y. Cao 533* (PE)
ssp.*japonica*	4–6-colpate		+		+		granulose		+		+	Hong & Pan 1998
Canarina												
C. abyssinica	3-colporate	+				+		+			+	Dunbar 1975a
C. canariensis	3-colporate	+				+		+			+	Erdtman 1952; Avetisjan 1986
C. eminii	3(–4)-colporate			+		+		+			+	Erdtman 1952; Dunbar 1975a
Codonopsis subg. ***Codonopsis***												
C. affinis	5–6-colpate	+			+				+		+	Morris & Lammers 1997
C. benthamii	6-colpate	+			+		spinulose		+		+	*Gaoligong Shan Biod. Surv. 31195* (CAS)
C. bulleyana	6–8-colpate	+			+				+		+	Chapman 1967; Nowicke et al. 1992
C. cardiophylla	7–9-colpate	+			+				+		+	Erdtman 1952
C. chimiliensis	6-colpate			+	+		smooth		+		+	*G. Forrest 29890* (PE)
C. clematidea	6–8-colpate	+			+				+		+	Erdtman 1952; Dunbar 1975a & 1984; Morris & Lammers 1997
C. farreri	6-colpate	+			+		spinulose		+		+	*Gaoligong Shan Biod. Surv. 32059* (CAS)
C. kawakamii	6–7-colpate	+			+				+		+	Huang 1972
C. lanceolata	9–10-colpate	+			+				+		+	Chapman 1967; Lee et al. 1988; Yoo & Lee 1989; Morris & Lammers 1997
C. meleagris	7-colpate	+			+		spinulose		+		+	*R. C. Ching 30503* (PE)
C. micrantha	7 (9)-colpate	+			+				+		+	Morris & Lammer 1997; Wei 2001
C. ovata	7–8-colpate										+	Avetisjan 1986

Table 1. Continued

Taxon	Aperture	Colpus length[1)] long	Colpus length[1)] medium-long	Colpus length[1)] short	Ornamentation spinulose	Ornamentation verrucose	Colpus membrance	Spinule/verrucae height[2)] high	Spinule/verrucae height[2)] short	Spinule base divided	Spinule base entire	Reference/voucher
C. pilosula (*C. tangshen* & *C. volubilis*)	(5–)7(–9)-colpate	+			+				+		+	Erdtman 1952; Dunbar 1975a; Lee et al. 1988; Yoo & Lee 1989; Morris & Lammers 1997; Wei 2001
C. purpurea	6-colpate			+	+		smooth		+		+	*D. Y. Hong et al. H11003* (PE)
C. rotundifolia	7-colpate	+			+				+		+	Morris & Lammers 1997
C. subglobosa	6-colpate	+			+		spinulose		+		+	*D. Y. Hong et al. H11029* (PE)
C. subscaposa	8-colpate	+			+		spinulose		+		+	*Qinghai-Xizang Exped. 3981* (PE)
C. subsimplex: A	5–6-colpate	+			+				+		+	*G. H. Cave s. n,* (PE)
B	5–6-colpate	+			+		spinulose		+		+	*P. C. Tsoong 5931* (PE)
C. thalictrifolia	7-colpate	+			+		spinulose		+		+	Morris & Lammers 1997; *D. Y. Hong & Q. Wang XZ002* (PE)
C. tubulosa	6–7-colpate	+			+				+		+	Morris & Lammers 1997
C. ussuriensis (*C. minima*)	8–10-colpate	+			+				+		+	Lee et al. 1988; Yoo & Lee 1989; Morris & Lammers 1997
C. viridiflora	8-colpate	+			+				+		+	Dunbar 1975a
C. viridis	6-colpate	+			+		spinulose		+		+	*Strachey & WinterbottomNo3* (PE)
Codonopsis subg. ***Obconicicapsula***												
C. dicentrifolia	5–7-colpate			+	+				+		+	Morris & Lammers 1997; *D. Y. Hong et al. H11004* (PE)
Codonopsis subg. ***Pseudocodonopsis***												
C. convolvulacea												
ssp. *convolvulacea*	7(–9)-colpate			+	+		smooth	+			+	Nowicke et al. 1992; Wei 2001; *W. Q. Yin 1962* (PE)
ssp. *forrestii*	6-colpate			+	+		smooth	+			+	Morris & Lammers 1997
ssp. *grey-wilsonii*	5(6)-colpate			+	+		smooth	+			+	*D. Y. Hong et al. H11005* (PE)
ssp. *vinciflora*	(4)6-colpate			+	+		smooth	+			+	Erdtman 1952; *D. Y. Hong & Q. Wang H10003* (PE)
C. graminifolia	6-colpate			+	+		smooth	+			+	Morris & Lammers 1997; *D. Y. Hong et al. H11042* (PE)
C. hirsuta	6-colpate			+	+		smooth	+			+	*D. Y. Hong et al. H10029* (PE)
C. rosulata	6-colpate			+	+		smooth	+			+	*D. Y. Hong et al. H11039* (PE)

Table 1. Continued

Taxon	Aperture	Colpus length[1)]			Ornamentation		Colpus membrane	Spinule/verrucae height[2)]		Spinule base		Reference/voucher
		long	medium-long	short	spinulose	verrucose		high	short	divided	entire	
Cyananthus												
C. cordifolius	7-colpate										+	Shrestha & Tarasevich 1992
C. delavayi	7–8-colpate										+	Shrestha & Tarasevich 1992
C. fasciculatus	7–8-colpate	+									+	Shrestha & Tarasevich 1992
C. flavus	7–8-colpate	+									+	Shrestha & Tarasevich 1992
C. formosus	8-colpate										+	Avetisjan 1986; Shrestha & Tarasevich 1992
C. hookeri	8–12-colpate	+							+			Erdtman 1952; Shrestha & Tarasevich 1992
C. incanus (*C. dolichosceles*)	9-colpate	+				+	verrucose		+		+	Dunbar 1975a & 1984
C. inflatus	8–9-colpate	+				+	verrucose		+		+	Dunbar 1975a; Shrestha & Tarasevich 1992
C. integer	7–9-colpate	+							+			Shrestha & Tarasevich 1992
C. lickiangensis	7–8-colpate	+							+			Shrestha & Tarasevich 1992
C. lobatus (*C. hayanus*)	6–10-colpate	+				+	verrucose		+		+	Dunbar 1975a; Shrestha & Tarasevich 1992
C. longiflorus	9-colpate	+							+			Shrestha & Tarasevich 1992
C. macrocalyx (*C. leiocalyx; C. spathulifolius*)	8-colpate	+							+		+	Shrestha & Tarasevich 1992
C. microphyllus	7–8-colpate	+				+			+		+	Dunbar 1975a
C. pedunculatus (*C. himalaicus*)	7–8-colpate											Shrestha & Tarasevich 1992
C. sherriffii	8-colpate	+							+		+	Shrestha & Tarasevich 1992
C. wardii	8–9-colpate	+							+		+	Shrestha & Tarasevich 1992

Table 1. Continued

Taxon	Aperture	Colpus length[1)]			Ornamentation		Colpus membrance	Spinule/verrucae height[2)]		Spinule base		Reference/voucher
		long	medium-long	short	spinulose	verrucose		high	short	divided	entire	
Cyclocodon												
C. celebicus	3-colporate	+			+		granulose	+		+		Murthy 1982; Morris & Lammers 1997; Hong & Pan 1998; *Z. C. Ni et al. 0650* (PE)
C. lancifolius	3-colporate	+			+			+		+		Huang 1972; Dunbar 1975a; Hong & Pan 1998; *D. Y. Hong et al. H11021* (PE)
C. parviflorus	3-colporate	+			+			+		+		Erdtman 1952; Murthy 1982; Avetisjan 1986; Morris & Lammers 1997; *J. D. Hooker s. n.* (PE)
Echinocodon												
E. lobophyllus	4–5-colpate			+	+		smooth		+	+		Hong 1984
Leptocodon												
L. gracilis	8–9(–10)-colpate	+			+				+		+	Erdtman 1952; Avetisjan 1986; Shrestha & Tarasevich 1992; Morris & Lammers 1997; *G. Forrest 22158* (PE)
L. hirsutus	7-colpate	+			+				+		+	Morris & Lammers 1997; *D. Y. Hong et al. H11009* (PE)
Ostrowskia												
O. magnifica	6–7-colpate					+		+				Avetisjan 1986; Dunbar 1975a; Tarasevich & Shrestha 1992
Platycodon												
P. grandiflorus	5–6-colporate	+			+				±		+	Erdtman 1952; Dunbar 1975a; Avetisjan 1986; Lee et al. 1988; Shrestha & Tarasevich 1992; Wei 2001

1) colpus length/polar axis length: long (0.70–0.78); medium-long (0.54–0.57); short (0.2–0.4)

2) spinule/verrucae height: high ($\geq$ 2 μm); short ($\leq$ 1 μm).

1997; Hong and Pan 1998), **Figures 1–6**, and **Table 1**, we thus recognize and name nine pollen types and two subtypes in the platycodonoid group of the Campanulaceae s. str. for the first time. Of them, the Codonopsis Type, the Cyclocodon type, the Pseudocodonopsis Type and the Obconicicapsula Type correspond to Morris and Lammers' (1997) Types I, II, III and IV, respectively. Pollen types are highly correlated with previously reported external morphology and molecular data (Eddie et al. 2003; Borsch et al. 2009; Haberle et al. 2009), and generally with taxonomic designation in the group.

These nine pollen types can be keyed out without much difficulty.

Key to pollen types of the platycodonoid group (Campanulaceae s. str.) (The distribution of each type is indicated in parentheses):

1a. Pollen colporate.
  2a. Pollen 5–6- colporate . . . . . Platycodon Type (*Platycodon*)
  2b. Pollen 3- colporate.
    3a. Sexine verrucose . . . . . Canarina Type (*Canarina*)
    3b. Sexine spinulose . . . . . Cyclocodon Type (*Cyclocodon*)

1b. Pollen colpate.
  4a. Apertures elongate (colpus length/polar axis length $\geq$ 0.54).
    5a. Sexine verrucose, verrucae rounded or elongated, ca. 2 μm high . . . . . Ostrowskia Type (*Ostrowskia*)
    5b. Sexine spinulose or verrucose, spinules or verrucae not rounded, $\leq$ 1.5 μm high.
      6a. Sexine verrucose; colpus membrane verrucose . . . . . Cyananthus Type (*Cyananthus*)
      6b. Sexine spinulose; colpus membrane spinulose or granulose . . . . . Codonopsis Type
        7a. Apertures long (colpus length/polar axis length $\geq$0.7); colpus membrane spinulose . . . . . Codonopsis subtype (*Codonopsis* s. str. + *Leptocodon*)
        7b. Apertures medium-long (colpus length/polar axis length = 0.54–0.57); colpus membrane granulose . . . . . Campanumoea subtype (*Campanumoea*)
  4b. Apertures short (colpus length/polar axis length $\leq$0.4).
    8a. Spinules divided at base . . . . . Echinocodon Type (*Echinocodon*)
    8b. Spinules entire at base.
      9a. Sexine spinules $\geq$2 μm high . . . . . Pseudocodonopsis Type (*Codonopsis* subg. *Pseudocodonopsis*)
      9b. Sexine spinules $\leq$ 1 μm high . . . . . Obconicicapsula Type
        10a. Colpus membrane granulose . . . . . Obconicicapsula subtype (*Codonopsis* subg. *Obconicicapsula*)
        10b. Colpus membrane smooth . . . . . Purpurea subtype (*Codonopsis purpurea* & *C. chimiliensis*)

We examined pollen of the type species of *Codonopsis*, *C. viridis* (**Figure 2G, H**), which is 7-colpate with long colpi, spinulose colpus membrane, and short exine spinules. The pollen morphology of all the species of *Codonopsis* subg. *Codonopsis* listed in **Table 1**, except for *C. chimiliensis* and *C. purpurea*, and shown in **Figure 1** and **Figure 2A–F**, is similar to that of *C. viridis*, and distinctly different from all the others observed here. It is here designated as the Codonopsis Type, and corresponds to Morris and Lammers' (1997) Type I.

The genus *Canaria* is homogeneous in pollen and has a pollen type of its own, and its generic status has never been questioned. Pollen grains of 17 species of *Cyananthus* (nearly all) have been observed, illustrating that the genus is homogeneous in pollen morphology. The genera *Platycodon*, *Ostrowskia*, and *Echinocodon* each is morphologically distinct, and each has a separate pollen type of its own. Their generic positions have also not be brought into question.

All the three species of *Cyclocodon* are uniform in pollen morphology and share the same pollen type: the Cyclocodon Type. Moeliono and Tuyn (1960) merged *Campanumoea* s. lat. (including *Cyclocodon*) with *Codonopsis*, ignoring the sharp differences in pollen between *Campanumoea parviflora* (= *Cyclocodon parviflorus* (Wall. ex A. DC.) Hook. f. & Thomson) (3-colporate) and three species of *Codonopsis* (7–9-colpate) as already revealed by Erdtman (1952). Not only do molecular (Borsch et al. 2009; Wang et al. unpublished) and morphological data support the restoration of *Cyclocodon* to generic status based on pollen and seed-coat morphology as proposed by Hong and Pan (1998), but these data also indicate that the closest relative of *Cyclocodon* is the genus *Platycodon*, not *Codonopsis*. *Leptocodon* was merged with *Codonopsis* by Grey-Wilson (1990), which was followed by Lammers (1999, 2001). The present work shows that *Leptocodon* is of the same pollen type as that of the core *Codonopsis*. In our molecular tree constructed using sequences of four chloroplast DNA regions and the ITS sequences of nuclear ribosomal DNA (Wang et al. unpublished), *Leptocodon* is nested among the species of the core *Codonopsis*. Therefore, palynological and molecular data

Table 2. Pollen morphology and external morphological characters in the *Codonopsis* complex (including *Campanumoea*, *Cyclocodon* and *Leptocodon*) (the classification system follows "Flora of China", Vol.19. 2011)

Taxon	Pollen type		Pollen morphology	External morphological characters
	Present assignment	Morris and Lammers' (1997) assignment		
Codonopsis subg. ***Codonopsis*** p. p. maj. & ***Leptocodon***	Codonopsis Type A (Figures 1, 2, 3A–D)	Type I	5–10-colpate; colpi long; colpus membrane spinulose; exine spinules short (≤ 1 μm).	Roots carrot-shaped, rarely tuberous; disc/glands present; stigma 3-fid, with lobes ovate or oblong; fruits a capsule; corolla campanulate or tubular.
Campanumoea	Codonopsis Type B (Campanumoea subtype) (Figure 3E–H)	Type I	(4–)5–6(–8)-colpate; colpi medium-long; colpus membrane granulose; exine spinules short (≤ 1 μm).	Roots attenuate; disc/glands present; ovary 3–6-locular, stigma 3–6-fid, stigma lobes ovate; fruits a berry; seed-coat reticulate, muri with beadlike processes
Codonopsis subg. ***Pseudocodonopsis***	Pseudocodonopsis Type (Figures 4G, H, 5, 6A, B)	Type III	4–6-colpate; colpi short; colpus membrane smooth; exine spinules high (≥ 2 μm).	Roots globose; disc/glands absent; ovary 3-locular, stigma 3-fid, stigma lobes oblong; fruits a capsule; corolla rotate, 5-fid to near base.
Codonopsis subg ***Obconicicapsula***	Obconicicapsula Type A (Figure 6C,D)	Type IV	5–7- colpate; colpi short; colpus membrane granulose; exine spinules short (≤ 1 μm).	Roots attenuate, smooth; corolla campanulate; disc/glands absent; stigma 3-fid; stigma lobes elliptic; fruits a capsule; seeds compressed.
Codonopsis subg. ***Codonopsis*** p. p. (*C. purpurea* & *C. chimiliensis*)	Obconicicapsula Type B (Purpurea subtype) (Figure 6E–H)	—	6-colpate; colpi short; colpus membrane smooth; exine spinules short (≤ 1 μm).	Roots attenuate; corolla campanulate; disc/glands absent; stigma 3-fid with lobes elliptic; fruits a capsule; seeds lunate, strongly compressed, wide-winged.
Cyclocodon	Cyclocodon Type (Figure 4A–F)	Type II	3-colporate; sexine spinules high (≥ 2 μm).	Roots attenuate; disc/glands absent; ovary 5-locular, stigma (4–)5–6-fid, stigma lobes linear; fruits a berry; corolla rotate.

Table 3. Materials for observations on pollen morphology of the platycodonoid group (Campanulaceae s. str.) (the classification system follows "Flora of China", Vol.19. 2011)

Taxon	Origin	Voucher
Campanumoea		
C. inflata (Hook. f. & Thomson) C. B. Clarke	Mêdog, Tibet, China	*Qinghai-Xizang Exped. 1588* (PE)
C. javanica Bl. ssp. *javanica*	Ceheng, Quizhou, China	*Z. Y. Cao 533* (PE)
Codonopsis subg. ***Codonopsis***		
C. benthamii Hook. f. & Thomson	Gongshan, Yunnan, China	*Gaoligong Shan Biodiversity Survey 31195* (CAS)
C. chimiliensis J. Anthony	Gaoligong Shan	*G. Forrest 29890* (PE)
C. farreri J. Anthony	Fugong, Yunnan, China	*Gaoligong Shan Biodiversity Survey 32059* (CAS)
C. meleagris Diels	Lijiang, Yunnan, China	*R. C. Ching 30503* (PE)
C. purpurea Wall.	Nyalam, Tibet, China	*D. Y. Hong et al. H11003* (PE)
C. subglobosa W. W. Smith	Muli, Sichuan, China	*D. Y. Hong et al. H11029* (PE)
C. subscaposa Kom.	Xiangcheng, Sichuan, China	*Qinghai-Xizang Exped. 3981* (PE)
C. subsimplex Hook. f. & Thomson	Sikkim, India	*G. H. Cave s. n.* (PE)
ditto	Yadong, Tibet, China	*P. C. Tsoong 5931* (PE)
C. thalictrifolia Wall.	Nyalam, Tibet, China	*D. Y. Hong & Q. Wang XZ002* (PE)
C. viridis Wall.	Kumaon, India	*R. Strachey & J. E. Winterbottom No.3* (PE)
Codonopsis subg. ***Obconicicapsula***		
C. dicentrifolia (C.B. Clarke.) W. W. Smith	Nyalam, Tibet, China	*D. Y. Hong et al. H11004* (PE)
Codonopsis subg. ***Pseudocodonopsis***		
C. convolvulacea Kurz		
ssp. *convolvulacea*	Yuanjiang, Yunnan, China	*W. Q. Yin 1962* (PE)
ssp. *grey-wilsonii* (J. M. H. Shaw) D. Y. Hong	Nyalam, Tibet, China	*D. Y. Hong et al. H11005* (PE)
ssp. *vinciflora* (Kom.) D. Y. Hong	Gongbo'gyamda, Tibet, China	*D. Y. Hong & Q. Wang H10003* (PE)
C. graminifolia H. Lév.	Yanbian, Sichuan, China	*D. Y. Hong et al. H11042* (PE)
C. hirsuta (Hand. -Mazz.) D. Y. Hong	Lijiang, Yunnan, China	*D. Y. Hong et al. H10029* (PE)
C. rosulata W. W. Smith	Yanbian, Sichuan, China	*D. Y. Hong et al. H11039* (PE)
Cyclocodon		
C. celebicus (Bl.) D. Y. Hong	Mêdog, Tibet, China	*Z. C. Ni et al. 0650* (PE)
C. lancifolius (Roxb.) Kurz	Emei Shan, Sichuan, China	*D. Y. Hong et al. H11021* (PE)
C. parviflorus (Wall. ex A. DC.) Hook. f. & Thomson	Sikkim, India	*J. D. Hooker s.n.* (PE)
Leptocodon		
L. gracilis Hook. f. & Thomson	Muli, Sichuan, China	*G. Forrest 22158* (PE)
L. hirsutus D. Y. Hong	Cona, Tibet, China	*D. Y. Hong et al. H11009* (PE)

both support Grey-Wilson's (1990) mergence of *Leptocodon* into *Codonopsis*. It is rather hard to classify *Campanumoea* Bl. s. str. in taxonomy from a palynological view. The taxon was merged with *Codonopsis* by Moeliono and Tuyn (1960), and this action was followed by Grey-Wilson (1990) and Lammars (1992). However, Hong (1983b, 1995, in Hong et al. 2011) kept it separate from *Codonopsis*. The pollen of the taxon is recognized here as a subtype of the Codonopsis Type. Morphologically, it differs from *Codonopsis* in having baccate fruits. In our molecular tree mentioned above, *Campanumoea* forms a clade sister to the core *Codonopsis*. Therefore, whether to retain the taxon as an independent genus or to merge it with *Codonopsis* are two options that can both be considered, but we prefer for it to have generic status.

Although it seems that we have clarified the taxonomy of the platycodonoid group, we have still not reached that point. The pollen of *Codonopsis* s. str., as circumscripted by Hong (1983b, 1995, in Hong et al. 2011), is highly heterogeneous. *Codonopsis* subg. *Pseudocodonopsis* Kom. is a distinct pollen type of its own: the Pseudocodonopsis Type. *Codonopsis* subg. *Obconicicapsula* D. Y. Hong, with only one species, also has a distinct pollen type: the Obconicicapsula Type. Even *Codonopsis* subg. *Codonopsis* itself is heterogeneous in pollen morphology. Pollen grains of two species, *C. chimiliensis* and *C. purpurea*, are uniform, but differ distinctly from those of all the other species examined in the subgenus. It is named here as the Obconicicapsula Type Purpurea subtype. If, for the convenience of discussion, we use the *Codonopsis* complex

to cover all the taxa with controversial views regarding their taxonomic treatments, then the complex includes *Codonopsis* s. str., *Campanumoea*, *Cyclocodon*, *Leptocodon*. **Table 2** shows that the pollen in the complex is polytypical, containing four types with two subtypes. The pollen of *Campanumoea*, *Cyclocodon*, and *Leptocodon* and their taxonomical classifications have been discussed above. From **Table 2**, the genus *Codonopsis* s. str. (Shen and Hong 1983; Hong in Hong et al. 2011) possesses three pollen types, which have been recognized by Morris and Lammers (1997), and two subtypes. The above discussion implies that a particular pollen type generally corresponds to generic status. Therefore, *Codonopsis* subg. *Pseudocodonopsis* and subg. *Obconicicapsula*, each possessing their own pollen type, should be separated as an independent genus. The typical subgenus, subg. *Codonopsis*, is still heterogeneous in pollen morphology, containing two types, the Codonopsis Type and the Obconicicapsula Type Purpurea subtype. Taking morphological and molecular data (**Table 2**; Wang et al. unpublished) into consideration, the two species *Codonopsis purpurea* and *C. chimiliensis*, with the Obconicicapsula Type Purpurea subtype of pollen, should be separated from *Codonopsis* and given generic status.

Materials and Methods

We sampled 17 species of *Codonopsis* and all the species of *Campanumoea* (2), *Cyclocodon* (3), and *Leptocodon* (2). All the materials and vouchers are listed in **Table 3**. Pollen grains from herbarium specimens were treated according to Hong (1983a), being first soaked in warm water for 5 min, and then dehydrated in 100% alcohol for a few minutes. They were coated before being observed under a Scanning Electronic Microscope (Hitachi S-4800). Because lengths of apertures and spinules on the sexine surface are constant within species and even within pollen types (Dunbar 1975a; Morris and Lammers 1997), we used measurements from only one or two pollen grains. The terminology here used follows Dunbar (1975a).

Slightly different from previous observations, we paid special attention to type of aperture (colpate or colporate), colpus length, colpus membrane, type of sexine sculpture (spinulose or verrucose), spinule length, and morphology of the spinule base. We believe that these six characters are stable, and can thus reflect the differences and relationships between the taxa being studied.

Acknowledgements

We are grateful to Mr. MA Xin-Tang, Mr. YUE Ji-Pei, Mr. WANG Huan-Chong and Dr. WANG Qiang for contributing to our field work, and to Mr. XIAO Yin-Hou for the SEM operation and Mr. LI Min and Miss GONG Xiao-Lin for assistance in preparation of the manuscript. This work was supported by the National Natural Science Foundation of China (31170175).

Received 5 Jul. 2012 Accepted 27 Aug. 2012

References

Avetisjan EM (1967) Morfologiya Pyl'cy sem. Campanulaceae i blizkikh k nemu semeystv (Sphenocleaceae, Lobeliaceae, Cyphiaceae) v svyazi s voprosami ikh sistematiki i filogenii –Flora i rastitel "nost" armyanskoy SSR. *Trudy Bot. Instit.* 16. –Erevan.

Avetisjan EM (1986) Palynomorphology of the families Campanulaceae, Sphenocleaceae and Pentaphragmataceae. *Bot. Zhurn.* **71,** 1003–1010.

Borsch T, Korotkova N, Raus T, Lobin W, Löhne C (2009) The *petD* group II intron as a species level marker: Utility for tree inference and species identification in the diverse genus *Campanula* (Campanulaceae). *Willdenowia* **39,** 7–33.

Chapman JL (1967) Comparative palynology in Campanulaceae. *Trans. Kansas Acad. Sci.* **69,** 197–200.

Dunbar A (1975a) On pollen of Campanulaceae and related families with special reference to the surface ultrastructure I. Campanulaceae subfam. Campanuloideae. *Bot. Notiser* **128(1),** 71–101.

Dunbar A (1975b) On pollen of Campanulaceae and related families with special reference to the surface ultrastructure II. Campanulaceae subfam. Cyphioideae and subfam. Lobelioideae; Goodeniaceae; Sphenocleaceae. *Bot. Notiser* **128,** 102–118.

Dunbar A (1984) Pollen morphology in Campanulaceae IV. *Nord. J. Bot.* **4,** 1–19.

Eddie WMM, Shulkina T, Gaskin J, Haberle RG, Jansen RK (2003) Phylogeny of Campanulaceae s. str. inferred from ITS sequences of nuclear ribosomal DNA. *Ann. Missouri Bot. Gard.* **90,** 554–575.

Erdtman G (1952) Pollen morphology and plant taxonomy. *Geolog. Fören. Stoc. Förhan.* **74(4),** 526–527.

Grey-Wilson C (1990) A survey of *Codonopsis* in cultivation. *The Plantsman* **12(2),** 64–99.

Haberle RC, Dang A, Lee T, Peñaflor C, Cortes-Burns H, Oestreich A, Raubeson L, Cellinese N, Edwards EJ, Kim S, Eddie WMM, Jansen RK (2009) Taxonomic and biogeographic implications of a phylogenetic analysis of the Campanulaceae based on three chloroplast genes. *Taxon* **58(3),** 715–734.

Hong DY (1980) New taxa of Campanulaceae from Xizang. *Acta Phytotax. Sin.* **18(2),** 245–247.

Hong DY (1983a) On pollen shape in some groups of dicotyledons. *Grana* **22,** 73–78.

Hong DY (1983b) Campanulaceae. *Fl. Reipubl. Popularis Sin*. **73(2),** 1–177. Science Press, Beijing.

Hong DY (1984) *Echinocodon* Hong, a new genus of Campanulaceae

and its systematic position. *Acta Phytotax. Sin*. **22(3),** 181–184, pl. 1& 2.

Hong DY (1995) The geography of the Campanulaceae: On the distribution centres. *Acta Phytotax. Sin.* **33,** 521–536.

Hong DY, Ma LM (1991) Systematics of the genus *Cyananthus* Wall. ex Royle. *Acta Phytotax. Sin*. **29(1),** 25–51.

Hong D Y, Pan KY (1998) The restoration of the genus *Cyclocodon* (Campanulaceae) and its evidence from pollen and seed-coat. *Acta Phytotax. Sin*. **36(2),** 106–110, pl. I & II.

Hong DY, Lammers TG, Klein LL (2011) *Codonopsis*. In Wu ZY, Raven PH, Hong DY, eds. *Flora of China Volume 19*. Science Press and Missouri Bot. Gard, Beijing & St. Louis, pp. 513–526.

Huang TC (1972) *Pollen Flora of Taiwan*. National Taiwan University, Taipei.

Lammers TG (1992) Systematics and biogeography of the Campanulaceae of Taiwan. In: Peng CI ed. Phytogeography and Botanical Inventory of Taiwan. *Inst. Bot. Acad. Sin. Monogr. Ser*. No. 12, Taipei. pp. 43–61.

Lammers TG (1999) A new *Lobelia* from Mexico, with additional new combination in world Campanulaceae. *Novon* **9,** 381–389.

Lammers TG (2001) Further nomenclatural innovation in the world Campanulaceae. *Novon* **11,** 67–67.

Lee S, Chung YJ, Lee J (1988) A palynotaxonomic study of the Korean Campanulaceae. *Korean J. Pl. Taxon.* **18,** 115–131.

Moeliono B, Tuyn P (1960) Campanulaceae. In: van Steenis CGGJ ed. *Flora Malesiana*, ser. 1, vol. 6, part 1. Noordhoff-Kolff, Djakarta, pp. 107–141.

Morris KE, Lammers TG (1997) Circumscription of *Codonopsis* and the allied genera *Campanumoea* and *Leptocodon* (Campanulaceae: Campanuloideae) I. Palynological data. *Bot. Bull. Acad. Sin*. **38,** 277–284.

Murthy GVS (1982) Pollen morphology of Indian *Campanumoea* Bl. – a revision of the genus. *J. Palynol*. **18(1&2),** 55–59.

Nowicke JW, Shetler SG, Morin N (1992) Exine structure of pantoporate *Campanula* (Campanulaceae) species. *Ann. Missouri Bot. Gard.* **79,** 65–80. 45. Engelmann, Leipzig. pp. 4070

Schönland S (1889) Campanulaceae. In: Engler A, Prantl K eds. *Die Natrlichen Pflanzenfamilien* **4–5**. Engelmann, Leipzig. pp. 40–70.

Shrestha KK, Tarasevich VF (1992) Comparative pollen morphology of genus *Cyananthus* in relation to its systematics and its position within the family Campanulaceae. *Bot. Zhurn.* **77(10),** 1–13.

Tarasevich VF, Shrestha KK (1992) Palynological data on the position of the genus *Ostrowskia* within the Campanulaceae family. *Bot. Zhurn.* **77(9),** 27–30.

Thulin M (1975) The genus *Wahlenbergia* s. lat. (Campanulaceae) in tropical Africa and Madagascar. *Acta Univ. Uppsal. Symb. Bot. Uppsal*. **21(1),** 1–223.

Wei ZX (2001) Pollen morphology of some *Codonopsis* and related species of Campanulaceae. *Acta Bot. Yunnan*. **23(3),** 335–335.

Yoo KO, Lee WT (1989) A taxonomic study of the genus *Codonopsis* in Korea. *Korean. J. Pl. Taxon*. **19(2),** 81–102.

(Co-Editor: Chun-Ming Liu)