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ORIGINAL ARTICLE

Variation of pollen morphology, and its implications in the phylogeny of *Clematis* (Ranunculaceae)

Lei Xie · Liang-Qian Li

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Abstract *Clematis* s.l. (including *Archiclematis* and *Naravelia*) is a genus of approximately 300 species with cosmopolitan distribution. The diversity of its pollen was surveyed in 162 taxa belonging to all infrageneric groups of *Clematis* s.l. Pollen morphology was investigated by use of scanning electron microscopy to identify useful characters, test taxonomic and systematic hypotheses, and elucidate pollen character evolution on the basis of the molecular phylogeny. *Clematis* pollen is small to medium (14.8–32.1 μm × 14.2–28.7 μm), oblate to prolate ($P/E = 0.9$–1.4) in shape. The apertures may be tricolpate and pantoporate sometimes with 4-zonocolpate and pantocolpate pollen grains as transitional forms. The tricolpate pollen grains are predominant and occur in all the sections of the genus, whereas pantoporate pollen grains can be found in sect. *Tubulosae*, sect. *Viorna*, sect. *Viticella*, and *Naravelia* only. Phylogenetic mapping of aperture types reveals that the pantoporate pollen type may be the apomorphy in the genus and evolved several times. The surface ornamentation in all taxa studied is similar and characterized by microechinae evenly distributed on the microperforate tectum. The size and density of spinules on the tectum vary greatly but successive in the whole genus. According to the character syndromes of the ornamentation, separating sect. *Brachiata* from sect. *Meclatis* is supported. Though pollen morphology may contribute to investigation of problematic taxa, the taxonomic value of pollen morphology is limited at the species level.

Keywords *Archiclematis* · *Clematis* · *Naravelia* · Phylogeny · Pollen morphology · Ranunculaceae · Scanning electron microscopy

Introduction

The genus *Clematis* L., with approximately 280–350 species, is one of the largest genera in Ranunculaceae. This cosmopolitan genus comprises climbing lianas, small shrubs, and erect sub-shrubs distributed predominantly in the temperate zone of both hemispheres but with some species distributed in tropical areas (Tamura 1995; Johnson 1997; Grey-Wilson 2000; Wang and Li 2005). The genus is extremely diverse in temperate and subtropical regions of the Northern Hemisphere, especially in eastern Asia, with 147 species reported in China, 93 of which are endemic to the country (Wang and Bartholomew 2001). Many *Clematis* species are of horticultural interest (e.g. *C. montana*, *C. patens*, and *C. viorna*), and some others are regarded as pharmaceutically important (e.g. *C. chinensis*, *C. henryi*, and *C. armandii*).

According to Tamura (1991), *Clematis* belongs to subtribe Clematidinae Lotsy (including *Clematis*, *Naravelia*, and *Archiclematis*) in the tribe Anemoneae DC. Molecular phylogenetic studies support the monophyly of both the tribe Anemoneae and the subtribe Clematidinae (Johansson and Jansen 1993; Johansson 1995; Hoot 1995; Ro et al. 1997; Wang et al. 2009). However, the genus *Clematis* had been defined differently by different authors. For example, the genus *Archiclematis* was recognized by Tamura (1987,

L. Xie
College of Biological Sciences and Biotechnology,
Beijing Forestry University, Beijing 100083, China
e-mail: xielei_si@126.com

L.-Q. Li (✉)
State Key Laboratory of Systematic and Evolutionary Botany,
Institute of Botany, The Chinese Academy of Sciences,
Beijing 100093, China
e-mail: lqli@ibcas.ac.cn

1991, 1995), whereas Johnson (1997), Grey-Wilson (2000), and Wang and Li (2005) reduced it into *Clematis*. Another example is *Naravelia*, which was often accepted as a separate genus (Tamura 1995; Wang and Li 2005), but Johnson (1997) merged it into *Clematis* as sect. *Naravelia*. Recent molecular phylogenetic studies (Miikeda et al. 2006; Xie et al. 2011) support the widest circumscription (*Clematis* s.l.) of the genus defined by Johnson (1997), which including *Archiclematis* and *Naravelia* in *Clematis*.

The taxonomy of *Clematis* is regarded as difficult because *Clematis* species are morphologically highly variable (Tamura 1995; Johnson 1997; Brandenburg 2000; Grey-Wilson 2000; Wang and Li 2005). Classifications of the genus differ from each other, mainly because of different morphological characters emphasized in each system. In addition to recent taxonomic revisions (Tamura 1995; Johnson 1997; Brandenburg 2000; Grey-Wilson 2000; Wang and Li 2005), systematic studies based on anatomy, seedling morphology, palynology, cytology, and molecular phylogeny have also been conducted (Tobe 1974, 1980a, b; Essig 1991; Zhang 1991; Yano 1993; Miikeda et al. 1999, 2006; Yang and Moore 1999; Shi and Li 2003; Xie et al. 2011). Among these, pollen morphology had potential systematic value within the genus, because of wide variation in aperture size, configuration, and number (Tobe 1974; Nowicke and Skvarla 1995).

Pollen morphology of a small number of *Clematis* species has been reported in some studies of the palynologic characters of Ranunculaceae (Wodehouse 1935, 1936; Kumazawa 1936; Erdtman 1952; Petrov and Ivanova 1975; Nowicke and Skvarla 1995; Santisuk 1979; Al-Eisawi 1986; Clarke et al. 1991; Blackmore et al. 1995). Several palynologic studies specifically on *Clematis* have also been conducted, mainly using light microscopy (LM) (Ikuse 1956; Vishnu-Mittre and Sharma 1963; Nair 1965; Tobe 1974; Petrov and Ivanova 1975; Kapoor et al. 1989; Yang and Huang 1992; Yano 1993; Yang and Moore 1999). Kumazawa (1936) reported pollen morphology of 28 taxa of *Clematis* by use of LM. Three pollen types were recognized, summarized, and illustrated in the study. Kapoor et al. (1989) observed the pollen grains of 32 taxa of *Clematis* and one species of *Naravelia* representing the South Asian species of the genus by use of LM. Three-zono-colpate pollen grains were observed in most species and only *C. cadmia* was reported to have panporate pollen grains. Nowicke and Skvarla (1995) sampled 13 *Clematis* species and examined pollen morphology by use of scanning electronic microscopy (SEM). They observed three aperture types within *Clematis*, tricolpate, pantoporate, and pantocolpate. However, until now, there has been no comprehensive, systematic survey of *Clematis* pollen, and there is still a lack of knowledge about pollen morphology of most species of *Clematis*, especially using SEM.

Recent molecular phylogenetic studies (Miikeda et al. 2006; Xie et al. 2011) showed that the previously recognized genera *Archiclematis* and *Naravelia* are nested within *Clematis* and most subgenera and sections defined by morphological characters (Tamura 1995; Wang and Li 2005) are not supported. The newly established phylogenetic framework enables the examination of pollen characters from an evolutionary perspective. The purpose of this study was to:

1. survey the diversity of pollen morphology across the genus by use of SEM;
2. place palynologic variation into an evolutionary context; and
3. assess the usefulness of pollen morphology for systematic study of *Clematis*.

For *Clematis* s.str., the classification by Tamura (1995) was followed in this study.

Materials and methods

In total, 162 taxa, including 145 species and 13 varieties from *Clematis*, one species from *Archiclematis*, and three from *Naravelia*, were examined (Appendix). Dried herbarium material was used for all the sampled species. The sampling covered all the currently recognized subgenera and sections (sensu Tamura 1995) throughout all the distribution areas of *Clematis*. Efforts were made to sample multiple accessions, particularly for those taxa spanning large biogeographical ranges or having a diverse morphology, to observe potential infraspecific variation.

Pollen samples of some *Clematis* species, e.g. *C. akebioides*, *C. pinnata*, and *C. henryi*, are fragile and the acetolysis method for pollen preparation (Erdtman 1960) is too drastic and damages the pollen grains for SEM observation (Hesse et al. 2009). Thus, in this study mature anthers were fragmented and mounted directly on the stubs with double-sided adhesive tape without acetolysis to preserve the exine and intine. This treatment for SEM observation was applied by Liu et al. (2010, 2011), Polevova et al. (2010), Welsh et al. (2010), among others. The samples were gold-coated and examined by use of an Hitachi S-800 SEM.

Morphological features of pollen grains were observed for each species and were analyzed to describe and categorize pollen types. Measurements were based on 20 pollen grains, the values of *P* (polar axis length), *E* (equatorial diameter), spinule height, and density were measured and the *P*/*E* ratio was calculated. Statistics of the palynologic characters were calculated by use of PAST 2.14 (Hammer et al. 2001). Descriptive terminology follows Clarke et al. (1991), Hesse et al. (2009), and Punt et al. (2007).

To evaluate character evolution, aperture types were mapped on to a simplified strict consensus tree of *Clematis* built in Mesquite 2.7 (Maddison and Maddison 2011), resulting from the phylogenetic analyses based on three plastid (*atp*B-*rbc*L spacer, *psb*A-*trn*H-*trn*Q spacer, and *rpo*B-*trn*C spacer) and nuclear ITS sequences (Xie et al. 2011). The strict consensus tree from Xie et al. (2011) was simplified by removing the duplicate samples from the same species. Clades with significant statistical support values (Bayesian posterior probability $\geq$0.95) were considered and are indicated in the tree. Aperture types were coded as tricolpate pollen (or, rarely, with pantocolpate pollen) = 0, and pantoporate pollen = 1. Other characters, for example polar axis length, equatorial diameter, and spinule feature, are quantitative characters and only analyzed statistically.

Results

Descriptions of *Clematis* s.l. pollen

Pollen grains of *Clematis* s.l. are monad, radially symmetrical, isopolar, spheroidal, oblate or suboblate to oblate spheroidal (*P*/*E* range: 0.9–1.0) with broad poles, to prolate (or subprolate) (*P*/*E* range: 1.1–1.4) with broad or relatively narrow poles. Pollen grains are small to medium (Hesse et al. 2009) in size, with $P \times E$ (polar length $\times$ equatorial diameter) of 14.8–32.1 μm × 14.2–28.7 μm (min–max) for tricolpate pollen, diameter from 14.8 to 36.6 μm for pantoporate pollen. Equatorial outline elliptical, circular (Table 1).

Aperture is tricolpate, occasionally 4-zonocolpate and pantocolpate, and pantoporate (Fig. 1a–d). Colpi sunken or open, wider in the middle, gradually acute at the ends. Colpus membrane is often covered with granular or microechinate elements usually the same size as or larger than those on the tectum. For pantoporate grains, pores are irregular in shape and distribution, and in pantocolpate pollen, the colpi are also arranged sporadically.

Aperture in *Clematis* s.l. can be divided into two major types: tricolpate and pantoporate. One-hundred and forty-five sampled taxa have the former pollen type, whereas all sampled species of *Naravelia* and three sections of *Clematis* s.str., sect. *Tubulosae*, sect. *Viorna*, and sect. *Viticella*, have the pantoporate pollen grains (Table 1). Pantocolpate pollen is rare and always accompanied by tricolpate or pantoporate grains.

In most cases, pollen surface is perforate, with microechinate ornamentation evenly distributed. Spinules vary in size (ranging from 0.08 to 0.40 μm) and density (ranging from 5 to 37 per 9 μm^2) in the genus (Figs. 2, 3, 4).

Evolution of aperture types

Although a molecular phylogenetic study by Xie et al. (2011) did not generate a robust phylogenetic framework for *Clematis*, especially in deep branches, analysis of ancestral pollen character reconstruction still provided some systematic information. Phylogenetic mapping of aperture types (Fig. 5) demonstrates that the tricolpate pollen type may be the ancestral type and the pantoporate pollen may be the apomorphy in the genus and evolved several times.

Discussion

Aperture types

Pollen of *Clematis* s.l. is heteromorphic (Fig. 1). This has also been reported after previous studies, for example those of Kumazawa (1936), Nowicke and Skvarla (1995), and Kapoor et al. (1989). The prevalent aperture type in *Clematis* s.l. is tricolpate; 89.5 % of the taxa examined can be characterized as this type. It can be found in all the sections of *Clematis* s.str. and *Archiclematis*. However, this apertural type may be accompanied in the same anther by a small proportion of 4-zonocolpate or pantocolpate grains (Fig. 1b, c). Pantoporate pollen is also occasionally accompanied by pantocolpate grains and are confined to sect. *Tubulosae*, sect. *Viorna*, sect. *Viticella*, and *Naravelia*. All the sampled *Naravelia* species have pantoporate pollen, but this pollen type is not section-specific in *Clematis* s.str. In sect. *Tubulosae*, two sampled Chinese species, *C. heracleifolia* and *C. pinnata* (Wang and Xie 2007), have tricolpate pollen, whereas the Japanese *C. stans* has pantoporate pollen. Another interesting distribution pattern was found in sect. *Viorna*. The American species of the section has pantoporate pollen whereas the Eurasian species (*C. integrifolia* and *C. fusca*) have tricolpate pollen. Both aperture types were also found in sect. *Viticella* but no distribution patterns were recognized.

Apertural heteromorphism is not rare in Ranunculaceae (Kumazawa 1936; Xi 1985; Nowicke and Skvarla 1995). An extreme example is *Anemone*, which has the most diverse aperture types including tricolpate, 5–8-zonocolpate, spiral, pantocolpate, and pantoporate grains (Huynh 1970; Nowicke and Skvarla 1995; Ehrendorfer et al. 2009). *Ranunculus* and *Pulsatilla* were also reported to have at least three aperture types (Xi 1985; Nowicke and Skvarla 1995). In eudicots, tricolpate pollen has been regarded as Plesiomorphic, whereas pantocolpate and pantoporate grains are regarded as progressively derived from tricolpate pollen (Wodehouse 1936; Xi 1985; Tellería and Daners

Table 1 Pollen characters in *Archiclematis*, *Clematis*, and *Naravelia*

	Taxon	Shape	Length (µm)	Width (µm)	*P*/*E*	Type of aperture	Spinule height	No. of spinules per 3 × 3 µm^2
Archiclematis (1/1) Sect. *Campanella* Tamura (24/40)	*Archiclematis alternata*	SP	23.3 (22.2–25.4)	21.9 (20.2–23.0)	1.06	Tricolpate	0.16 (0.13–0.18)	15–16
	Clematis urophylla	OS	22.5 (20.9–23.7)	23.0 (20.8–24.9)	0.98	Tricolpate	0.12 (0.08–0.16)	15–16
	C. qingchengshanica	OS	22.9 (20.7–24.1)	23.9 (21.0–24.8)	0.96	Tricolpate	0.11 (0.08–0.14)	13–14
	C. otophora	SP	24.3 (22.2–25.1)	21.1 (19.7–22.4)	1.15	Tricolpate	0.10 (0.08–0.12)	11–13
	C. kockiana	SP	26.6 (23.7–27.9)	25.6 (23.6–26.8)	1.04	Tricolpate	0.14 (0.12–0.17)	7–8
	C. pseudootophora	OS	22.1 (19.6–23.1)	23.0 (21.2–23.8)	0.96	Tricolpate	0.12 (0.10–0.15)	13–14
	C. pogonandra	SP	24.8 (22.7–25.6)	20.6 (18.9–22.1)	1.20	Tricolpate	0.09 (0.07–0.11)	6–7
	C. leschenaultiana	SP	23.8 (22.1–24.2)	22.2 (20.4–23.1)	1.08	Tricolpate	0.09 (0.08–0.11)	9–10
	C. leschenaultiana var. *rubifolia*	S	24.5 (22.6–26.0)	24.5 (22.7–25.9)	1.00	Tricolpate	0.17 (0.10–0.26)	18–20
	C. lasiandra	SP	24.8 (22.1–26.0)	21.8 (19.8–23.7)	1.14	Tricolpate	0.16 (0.13–0.18)	17–20
	C. dasyandra	SP	24.1 (22.2–26.0)	22.5 (21.1–23.8)	1.07	Tricolpate	0.17 (0.14–0.19)	28–30
	C. aethusifolia	S	24.5 (22.8–25.4)	24.5 (22.1–25.6)	1.00	Tricolpate	0.16 (0.13–0.19)	18–19
	C. ranunculoides	SP	26.3 (23.3–27.1)	21.3 (19.9–22.2)	1.23	Tricolpate	0.14 (0.12–0.17)	15–17
	C. yuanjiangensis	SP	24.0 (22.2–26.1)	22.5 (20.1–23.6)	1.07	Tricolpate	0.10 (0.08–0.12)	18–19
	C. rehderiana	SP	23.8 (21.5–24.7)	22.5 (20.4–24.1)	1.06	Tricolpate	0.16 (0.14–0.18)	13–15
	C. grewiiflora	S	24.7 (21.2–26.0)	24.4 (20.9–26.0)	1.01	Tricolpate	0.18 (0.12–0.28)	13–14
	C. siamensis	OS	23.3 (20.9–25.0)	23.7 (22.1–24.3)	0.98	Tricolpate	0.11 (0.08–0.14)	15–17
	C. yunnanensis	SP	24.7 (22.0–25.8)	20.7 (18.4–22.2)	1.19	Tricolpate	0.08 (0.06–0.10)	17–18
	C. henryi	S	23.6 (21.5–25.3)	23.1 (21.1–25.2)	1.02	Tricolpate	0.08 (0.07–0.10)	16–17
	C. repens	S	24.4 (22.7–25.8)	24.0 (22.9–26.0)	1.02	Tricolpate	0.09 (0.08–0.11)	23–25
	C. acuminata var. *longicaudata*	OS	23.0 (21.2–25.5)	24.0 (22.3–26.7)	0.96	Tricolpate	0.08 (0.06–0.10)	14–15
	C. jingdungensis	OS	24.0 (22.0–26.1)	24.5 (21.7–26.2)	0.98	Tricolpate	0.11 (0.08–0.14)	19–22
	C. kweichowensis	SP	24.8 (22.2–25.9)	22.0 (20.4–24.0)	1.13	Tricolpate	0.09 (0.08–0.11)	13–15
	C. buchananiana	S	24.3 (21.7–26.7)	24.3 (22.2–26.5)	1.00	Tricolpate	0.23 (0.20–0.25)	7–9
	C. connata	SP	23.3 (21.0–25.5)	22.2 (20.1–24.4)	1.05	Tricolpate	0.25 (0.20–0.31)	10–14
	C. pinchuanensis	SP	23.8 (21.1–25.9)	22.1 (20.1–24.2)	1.07	Tricolpate	0.14 (0.12–0.17)	15–17
Sect. *Tubulosae* Decne. (3/8)	*C. heracleifolia*	P	23.6 (20.1–24.5)	17.3 (15.4–19.1)	1.37	Tricolpate	0.21 (0.16–0.26)	6–7
	C. pinnata	SP	22.9 (21.3–24.8)	21.6 (20.2–22.8)	1.09	Tricolpate	0.26 (0.20–0.31)	5–6
	C. stans	S	21.1 (20.5–21.8)	21.1 (20.5–21.8)	1.00	Pantoporate*	0.27 (0.20–0.34)	8–10
Sect. *Bebaeanthera* Edgew. (3/4)	*C. japonica*	SP	23.4 (21.1–24.6)	21.1 (19.9–22.3)	1.11	Tricolpate	0.14 (0.12–0.17)	21–23
	C. tosaensis	SP	23.0 (20.8–24.1)	21.5 (20.3–22.4)	1.07	Tricolpate	0.15 (0.13–0.18)	18–20
	C. pseudopogonandra	OS	22.5 (20.8–23.6)	23.0 (21.1–24.8)	0.98	Tricolpate	0.16 (0.13–0.18)	16–19
Sect. *Atragene* (L.) DC. (2/9)	*C. macropetala*	S	24.6 (23.1–25.4)	24.6 (22.9–25.7)	1.00	Tricolpate	0.15 (0.12–0.18)	16–19
	C. sibirica	S	23.8 (22.1–24.6)	23.6 (21.9–24.7)	1.01	Tricolpate	0.16 (0.12–0.19)	17–19

Table 1 continued

	Taxon	Shape	Length (μm)	Width (μm)	*P*/*E*	Type of aperture	Spinule height	No. of spinules per 3 × 3 μm^2
Sect. *Meclatis* (Spach) Tamura (10/20)	*C. wightiana*	S	23.3 (22.1–24.7)	23.3 (22.2–24.9)	1.00	Tricolpate	0.30 (0.28–0.33)	8–9
	C. simensis	SP	27.2 (25.4–29.1)	23.1 (20.9–25.6)	1.18	Tricolpate	0.35 (0.29–0.42)	6–7
	C. hirsuta	SP	28.8 (26.6–30.0)	26.7 (24.3–28.7)	1.08	Tricolpate	0.26 (0.22–0.30)	9–11
	C. orientalis	SP	26.1 (24.6–27.1)	23.6 (22.1–24.1)	1.10	Tricolpate	0.14 (0.10–0.16)	21–24
	C. tangutica	SP	24.8 (22.1–26.2)	22.9 (20.3–24.2)	1.08	Tricolpate	0.13 (0.12–0.15)	19–20
	C. tangutica var. *obtusiuscula*	SP	24.3 (22.2–25.1)	23.3 (22.1–24.8)	1.04	Tricolpate	0.18 (0.09–0.25)	16–19
	C. akebioides	SP	23.1 (22.2–24.5)	22.4 (20.8–23.6)	1.03	Tricolpate	0.13 (0.10–0.15)	22–24
	C. serratifolia	OS	24.1 (22.8–25.3)	24.6 (22.6–25.4)	0.98	Tricolpate	0.14 (0.12–0.16)	20–21
	C. graveolens	SP	25.5 (23.7–26.4)	22.4 (20.8–24.1)	1.14	Tricolpate	0.16 (0.13–0.18)	20–21
	C. intricata	S	23.0 (21.8–24.3)	22.8 (21.2–24.1)	1.01	Tricolpate	0.17 (0.14–0.19)	17–19
	C. viridis	S	23.5 (22.2–24.6)	23.5 (22.5–24.5)	1.00	Tricolpate	0.17 (0.15–0.19)	28–29
Sect. *Pseudanemone* Prant (6/25)	*C. caleoides*	S	24.6 (22.8–25.7)	24.6 (22.7–25.6)	1.00	Tricolpate	0.30 (0.25–0.36)	14–16
	C. corniculata	S	25.0 (24.2–25.9)	25.0 (24.2–26.0)	1.00	Tricolpate	0.26 (0.20–0.31)	11–14
	C. trifida	S	24.8 (23.9–26.4)	24.8 (23.9–26.4)	1.00	Tricolpate or pantocolpate	0.32 (0.29–0.36)	5–6
	C. villosa	S	25.0 (24.4–25.8)	25.0 (24.6–25.7)	1.00	Tricolpate	0.33 (0.24–0.41)	8–10
	C. stanleyi	SP	30.5 (26.4–32.1)	23.3 (20.8–26.5)	1.31	Tricolpate	0.26 (0.20–0.32)	7–9
	C. chrysocarpa	S	25.5 (24.2–26.7)	25.5 (24.1–26.5)	1.00	Tricolpate	0.25 (0.20–0.31)	7–9
Sect. *Viorna* (Reichb.) Prantl (9/21)	*C. albicoma*	S	32.2 (31.1–33.4)	32.2 (31.1–33.4)	1.00	Pantoporate	0.16 (0.13–0.18)	17–18
	C. bigelovii		32.3 (30.0–34.5)	32.3 (30.0–34.5)	1.00	Pantoporate	0.18 (0.11–0.34)	13–14
	C. reticulata	S	31.0 (28.9–34.6)	31.0 (28.9–34.6)	1.00	Pantoporate	0.22 (0.11–0.39)	12–14
	C. pitcheri	S	34.2 (30.2–36.5)	34.2 (30.2–36.5)	1.00	Pantoporate	0.24 (0.10–0.38)	14–15
	C. glaucophylla	S	35.2 (34.5–36.5)	35.2 (34.5–36.5)	1.00	Pantoporate	0.23 (0.14–0.31)	14–16
	C. versicolor	S	31.6 (29.8–33.2)	31.6 (29.8–33.2)	1.00	Pantoporate	0.24 (0.12–0.36)	15–17
	C. crispa	S	31.7 (30.2–32.5)	31.7 (30.2–32.5)	1.00	Pantoporate	0.28 (0.14–0.42)	9–11
	C. fusca	S	24.5 (22.7–25.8)	24.5 (23.0–25.7)	1.00	Tricolpate	0.16 (0.10–0.28)	18–20
	C. integrifolia	SP	25.3 (23.7–26.9)	23.5 (22.1–24.7)	1.08	Tricolpate	0.15 (0.13–0.17)	20–22

Table 1 continued

	Taxon	Shape	Length (μm)	Width (μm)	*P*/*E*	Type of aperture	Spinule height	No. of spinules per 3 × 3 μm^2
Sect. *Clematis* Eichler (12/25)	*C. apiifolia*	S	20.1 (19.1–21.8)	18.5 (17.2–20.0)	1.00	Tricolpate	0.25 (0.20–0.31)	9–12
	C. grandidentata	SP	20.5 (18.9–21.8)	19.8 (17.7–21.4)	1.04	Tricolpate	0.28 (0.24–0.32)	7–10
	C. peterae	SP	21.5 (19.7–22.8)	17.7 (15.9–19.2)	1.21	Tricolpate	0.23 (0.16–0.30)	8–9
	C. peterae var. *trichocarpa*	SP	21.6 (19.5–22.9)	17.4 (16.0–19.4)	1.24	Tricolpate	0.24 (0.12–0.32)	8–9
	C. subumbellata	SP	19.1 (17.8–21.1)	16.2 (15.2–17.4)	1.18	Tricolpate	0.24 (0.22–0.28)	8–9
	C. ganpiniana	SP	19.6 (17.7–20.9)	18.6 (16.8–20.1)	1.05	Tricolpate	0.30 (0.20–0.46)	8–9
	C. ganpiniana var. *tenuisepala*	SP	19.5 (17.6–20.7)	18.7 (17.0–20.3)	1.04	Tricolpate	0.28 (0.16–0.45)	8–9
	C. gouriana	SP	17.6 (15.8–19.5)	15.3 (14.2–17.7)	1.15	Tricolpate	0.24 (0.20–0.32)	8–9
	C. parviloba	SP	21.6 (18.9–23.0)	20.0 (18.9–21.7)	1.08	Tricolpate	0.25 (0.22–0.31)	8–9
	C. parviloba var. *longianthera*	SP	21.5 (18.7–22.8)	19.2 (18.4–21.3)	1.12	Tricolpate	0.25 (0.21–0.32)	7–8
	C. mashanensis	SP	21.5 (18.9–23.0)	18.2 (17.1–19.7)	1.18	Tricolpate	0.28 (0.25–0.32)	8–9
	C. tsaii	S	18.7 (17.8–19.6)	18.7 (17.8–19.6)	1.00	Tricolpate	0.33 (0.29–0.46)	11–12
	C. puberula	OS	18.7 (16.8–19.9)	19.1 (17.8–21.0)	0.97	Tricolpate	0.30 (0.26–0.45)	8–9
	C. chingii	SP	19.6 (17.8–20.9)	19.1 (17.4–20.6)	1.03	Tricolpate	0.21 (0.16–0.32)	14–16
	C. tenuipes	S	20.1 (18.4–21.7)	20.1 (19.2–21.3)	1.00	Tricolpate	0.16 (0.14–0.18)	8–9
Sect. *Cheiropsis* DC. (13/13)	*C. laxistrigosa*	S	25.0 (23.6–26.5)	25.0 (23.5–26.5)	1.00	Tricolpate	0.16 (0.11–0.22)	12–14
	C. wenshanensis	OS	24.8 (23.6–25.7)	24.8 (23.5–26.0)	0.97	Tricolpate	0.24 (0.12–0.32)	10–13
	C. venusta	S	18.2 (16.8–19.7)	18.2 (16.5–19.9)	1.00	Tricolpate	0.25 (0.21–0.32)	15–17
	C. chrysocoma	S	22.4 (20.5–24.1)	22.1 (20.8–23.9)	1.01	Tricolpate	0.17 (0.13–0.22)	16–19
	C. gracilifolia	SP	22.5 (20.7–24.3)	19.4 (17.9–21.6)	1.16	Tricolpate	0.20 (0.16–0.32)	17–19
	C. gracilifolia var. *dissectifolia*	SP	22.7 (20.5–23.9)	19.6 (17.7–21.4)	1.16	Tricolpate	0.21 (0.14–0.30)	16–18
	C. montana	SP	21.6 (20.1–23.0)	19.4 (17.7–21.2)	1.11	Tricolpate	0.25 (0.17–0.32)	17–18
	C. acerifolia	SP	23.8 (21.4–25.1)	18.6 (16.6–20.0)	1.28	Tricolpate	0.14 (0.10–0.17)	15–17
	C. glabrifolia	S	24.1 (22.8–26.1)	24.0 (22.5–25.8)	1.00	Tricolpate	0.25 (0.19–0.30)	19–21
	C. fasciculiflora	SP	26.4 (24.1–27.9)	22.7 (20.7–24.1)	1.16	Tricolpate	0.16 (0.13–0.20)	11–12
	C. napaulensis	OS	27.6 (25.3–29.0)	28.2 (26.6–29.5)	0.98	Tricolpate	0.17 (0.13–0.22)	9–10
	C. cirrhosa	S	25.1 (24.3–26.2)	25.1 (24.2–26.2)	1.00	Tricolpate	0.25 (0.17–0.32)	18–20
	C. potaninii	SP	25.8 (23.9–27.1)	19.7 (18.2–21.8)	1.31	Tricolpate	0.28 (0.25–0.33)	12–13
	C. williamsii	SP	25.7 (23.8–27.2)	24.1 (22.5–26.2)	1.07	Tricolpate	0.14 (0.10–0.17)	17–18

Table 1 continued

	Taxon	Shape	Length (μm)	Width (μm)	*P*/*E*	Type of aperture	Spinule height	No. of spinules per 3 × 3 μm^2
Sect. *Naraveliopsis* Hand–Mazz. (7/13)	*C. metouensis*	S	16.7 (15.2–18.1)	16.7 (15.2–18.1)	1.00	Tricolpate or pantocolpate	0.32 (0.19–0.47)	12–13
	C. menglaensis	S	16.6 (14.8–17.2)	16.6 (14.8–17.2)	1.00	Tricolpate or pantocolpate	0.30 (0.25–0.36)	10–12
	C. smilacifolia	S	16.5 (14.9–17.6)	16.5 (14.9–17.6)	1.00	Tricolpate	0.32 (0.20–0.45)	12–14
	C. tashiroi	S	16.6 (15.2–17.1)	16.6 (15.3–17.3)	1.00	Tricolpate	0.25 (0.17–0.32)	19–22
	C. loureiriana	S	16.8 (15.4–17.5)	16.6 (15.4–17.2)	1.01	Tricolpate	0.16 (0.13–0.20)	16–17
	C. pianmaensis	S	16.0 (14.8–17.2)	16.0 (14.8–17.2)	1.00	Tricolpate or pantocolpate	0.30 (0.27–0.33)	15–18
	C. crassipes	S	17.3 (15.9–18.7)	17.3 (15.9–18.7)	1.00	Tricolpate or pantocolpate	0.16 (0.11–0.24)	12–14
Sect. *Aspidanthera* Spach (24/72)	*C. aristata*	SP	21.0 (18.9–22.6)	18.7 (16.7–20.0)	1.12	Tricolpate	0.32 (0.22–0.46)	12–14
	C. ibarensis	SP	28.0 (26.7–29.2)	21.3 (20.1–23.0)	1.31	Tricolpate	0.32 (0.24–0.39)	8–9
	C. virginiana	SP	23.3 (21.7–25.2)	21.1 (20.0–23.2)	1.11	Tricolpate	0.40 (0.32–0.48)	6–8
	C. ligusticifolia	OS	23.6 (21.8–25.0)	24.0 (22.3–26.4)	0.98	Tricolpate	0.30 (0.26–0.36)	7–8
	C. drummondii	SP	24.0 (22.2–26.1)	23.0 (21.3–24.5)	1.04	Tricolpate	0.25 (0.21–0.30)	6–7
	C. dioica	SP	24.7 (22.3–26.2)	24.0 (22.2–27.1)	1.03	Tricolpate	0.32 (0.16–0.48)	6–7
	C. acapulcensis	SP	24.8 (22.1–26.2)	19.6 (17.4–21.3)	1.27	Tricolpate	0.35 (0.26–0.45)	5–6
	C. bonariensis	OS	23.1 (21.5–24.9)	23.5 (21.4–25.2)	0.98	Tricolpate	0.30 (0.27–0.32)	6–7
	C. campestris	SP	28.2 (26.4–30.3)	22.1 (20.5–24.2)	1.28	Tricolpate	0.25 (0.17–0.32)	7–8
	C. alborosea	SP	28.5 (26.4–30.2)	22.8 (20.2–24.9)	1.25	Tricolpate	0.29 (0.17–0.39)	9–11
	C. populifolia	S	26.5 (24.7–28.1)	26.5 (25.3–28.0)	1.00	Tricolpate	0.28 (0.26–0.32)	9–11
	C. grossa	SP	26.2 (22.3–28.1)	21.2 (19.8–23.4)	1.24	Tricolpate	0.32 (0.30–0.36)	8–9
	C. grahami	SP	27.2 (25.5–28.8)	24.6 (22.7–26.3)	1.11	Tricolpate	0.33 (0.30–0.35)	7–8
	C. affinis	OS	25.4 (23.3–27.1)	25.8 (22.9–27.6)	0.98	Tricolpate	0.26 (0.19–0.31)	5–6
	C. peruviana	S	26.4 (24.2–28.5)	26.4 (24.5–28.7)	1.00	Tricolpate	0.27 (0.24–0.31)	8–9
	C. seemanni	S	24.5 (22.8–26.2)	24.5 (22.8–26.2)	1.00	Tricolpate	0.29 (0.25–0.34)	6–7
	C. glycinoides	SP	27.7 (24.6–29.1)	21.6 (19.8–23.4)	1.28	Tricolpate	0.36 (0.30–0.46)	9–10
	C. haenkeana	S	21.3 (19.6–23.4)	21.3 (19.7–23.5)	1.00	Tricolpate	0.32 (0.16–0.45)	9–10
	C. millefoliata	SP	31.1 (28.9–33.0)	26.6 (24.7–27.5)	1.17	Tricolpate	0.28 (0.25–0.30)	9–10
	C. viridiflora	SP	25.3 (23.1–27.6)	19.6 (17.6–22.1)	1.29	Tricolpate	0.21 (0.12–0.39)	9–11
	C. dissecta	S	25.1 (22.3–27.4)	25.1 (23.1–27.2)	1.00	Tricolpate	0.31 (0.27–0.33)	14–15
	C. foetida	SP	22.6 (19.8–24.3)	18.3 (16.8–21.1)	1.23	Tricolpate	0.25 (0.19–0.29)	8–9
	C. microphylla	S	25.0 (24.1–26.3)	25.0 (24.0– 26.1)	1.00	Tricolpate	0.28 (0.20–0.40)	9–11
	C. paniculata	S	26.3 (23.2–28.1)	26.3 (23.2–28.1)	1.00	Tricolpate or pantocolpate	0.35 (0.29–0.49)	5–6

Table 1 continued

	Taxon	Shape	Length (μm)	Width (μm)	*P*/*E*	Type of aperture	Spinule height	No. of spinules per 3 × 3 μm^2
Sect. *Lasiantha* Tamura (2/2)	*C. lasiantha*	SP	25.2 (23.1–27.6)	22.5 (20.7–24.8)	1.12	Tricolpate	0.30 (0.24–0.36)	8–10
	C. pauciflora	S	26.0 (24.8–28.5)	26.1 (24.9–28.3)	1.00	Tricolpate or pantocolpate	0.31 (0.27–0.33)	6–7
Sect. *Flammula* DC. (17/25)	*C. flammula*	SP	23.2 (20.9–25.2)	20.6 (18.7–22.1)	1.13	Tricolpate	0.09 (0.08–0.10)	13–16
	C. dilatata	SP	22.5 (20.1–24.3)	19.1 (17.3–21.4)	1.18	Tricolpate	0.22 (0.18–0.28)	12–15
	C. recta	SP	24.4 (22.2–26.1)	21.7 (19.6–23.4)	1.12	Tricolpate	0.21 (0.14–0.29)	15–18
	C. chinensis	SP	23.1 (21.1–25.3)	20.6 (18.4–22.5)	1.12	Tricolpate	0.18 (0.14–0.21)	13–15
	C. obscura	S	22.7 (20.2–24.3)	22.5 (20.4–24.1)	1.01	Tricolpate	0.16 (0.13–0.19)	23–24
	C. meyeniana	SP	23.1 (21.2–25.4)	22.2 (20.8–24.5)	1.04	Tricolpate	0.17 (0.11–0.26)	19–20
	C. terniflora	S	25.0 (23.8–26.4)	25.0 (24.0–26.1)	1.00	Tricolpate	0.16 (0.13–0.19)	20–21
	C. finetiana	S	24.6 (22.1–25.7)	24.6 (22.0–26.0)	1.00	Tricolpate	0.17 (0.14–0.20)	17–19
	C. mandshurica	S	25.9 (23.6–27.1)	25.9 (23.7–27.3)	1.00	Tricolpate	0.17 (0.13–0.22)	22–24
	C. jialasaensis	S	25.1 (23.3–27.4)	25.1 (23.3–27.2)	1.00	Tricolpate	0.21 (0.14–0.29)	10–12
	C. uncinata	P	27.3 (25.5–28.6)	19.7 (17.4–21.3)	1.39	Tricolpate	0.29 (0.25–0.34)	9–10
	C. akoensis	SP	23.0 (21.8–25.2)	22.6 (20.7–24.4)	1.02	Tricolpate	0.24 (0.19–0.28)	9–11
	C. armandii	SP	22.6 (20.2–24.5)	21.7 (19.8–23.0)	1.04	Tricolpate	0.21 (0.14–0.29)	9–11
	C. armandii var. *hefengensis*	SP	22.2 (20.1–24.2)	21.9 (19.9–23.5)	1.01	Tricolpate	0.21 (0.15–0.28)	9–12
	C. quinquefoliolata	S	22.7 (20.2–24.9)	22.5 (20.4–24.8)	1.01	Tricolpate	0.29 (0.25–0.34)	14–15
	C. shensiensis	SP	22.9 (20.8–25.2)	22.3 (20.6–25.1)	1.03	Tricolpate	0.24 (0.19–0.28)	8–11
	C. kirilowii	SP	24.5 (22.1–26.8)	18.3 (16.4–20.5)	1.34	Tricolpate	0.22 (0.16–0.29)	15–16
	C. crassifolia	SP	18.8 (16.4–20.2)	18.4 (16.1–20.0)	1.02	Tricolpate	0.12 (0.08–0.14)	13–15
Sect. *Angustifolia* (Tamura) Serov (1/1)	*C. hexapetala*	SP	22.9 (20.2–24.4)	21.8 (19.1–23.3)	1.05	Tricolpate	0.16 (0.10–0.23)	18–19
	C. hexapetala var. *tchefouensis*	SP	21.5 (20.2–23.4)	20.5 (19.2–22.1)	1.20	Tricolpate	0.19 (0.12–0.28)	20–21
Sect. *Fruticella* Tamura (6/7)	*C. songarica*	S	18.7 (16.9–20.0)	18.7 (17.0–20.0)	1.00	Tricolpate	0.16 (0.13–0.18)	19–20
	C. songarica var. *aspleniifolia*	SP	22.9 (20.1–24.4)	20.6 (18.2–22.1)	1.11	Tricolpate	0.09 (0.08–0.10)	17–18
	C. lancifolia	SP	23.8 (21.1–25.4)	18.2 (16.6–19.7)	1.31	Tricolpate	0.19 (0.14–0.25)	10–12
	C. lancifolia var. *ternata*	SP	26.6 (24.4–28.6)	19.7 (17.7–21.6)	1.32	Tricolpate	0.18 (0.13–0.23)	10–11
	C. delavayi	SP	25.5 (22.1–28.2)	23.2 (21.2–25.3)	1.10	Tricolpate	0.16 (0.08–0.23)	12–13
	C. delavayi var. *calvescens*	SP	22.9 (20.2–25.0)	19.7 (17.1–22.2)	1.16	Tricolpate	0.17 (0.11–0.22)	11–12
	C. nanophylla	SP	23.0 (20.9–25.5)	20.6 (17.8–22.9)	1.12	Tricolpate	0.13 (0.09–0.17)	35–37
	C. fruticosa	OS	23.1 (21.7–25.3)	23.6 (20.8–25.9)	0.98	Tricolpate	0.20 (0.14–0.25)	14–15
	C. tomentella	OS	20.0 (18.8–22.3)	21.0 (18.9–22.8)	0.95	Tricolpate	0.17 (0.13–0.22)	17–19

Table 1 continued

	Taxon	Shape	Length (μm)	Width (μm)	*P*/*E*	Type of aperture	Spinule height	No. of spinules per 3 × 3 μm^2
Sect. *Viticella* (Moench) DC. (7/10)	*C. cadmia*	S	26.5 (23.4–28.7)	26.5 (23.4–28.7)	1.00	Pantoporate	0.24 (0.19–0.28)	19–20
	C. lanuginosa	S	26.0 (24.2–28.4)	26.0 (24.2–28.4)	1.00	Pantoporate	0.38 (0.16–0.48)	10–12
	C. hancockiana	S	27.3 (25.1–29.0)	27.3 (25.1–29.0)	1.00	Pantoporate	0.17 (0.13–0.20)	17–18
	C. patens var. *tientaiensis*	S	25.5 (24.1–27.2)	25.5 (24.1–27.2)	1.00	Pantoporate	0.28 (0.20–0.34)	11–12
	C. courtoisii	S	26.2 (24.8–28.1)	26.2 (24.8–28.1)	1.00	Pantoporate	0.25 (0.19–0.29)	18–20
	C. huchouensis	SP	26.6 (24.5–28.6)	22.7 (20.2–25.1)	1.17	Tricolpate	0.16 (0.14–0.18)	8–10
	C. viticella	SP	25.5 (23.1–28.0)	21.4 (19.8–23.3)	1.19	Tricolpate	0.16 (0.12–0.19)	9–12
Sect. *Pterocarpa* Tamura (1/1)	*C. brachyura*	SP	18.2 (16.7–20.2)	17.5 (15.4–19.7)	1.04	Tricolpate	0.19 (0.11–0.27)	15–16
Naravelia DC. (3/7)	*N. zylanica*	S	16.6 (15.2–18.0)	16.6 (15.2–18.0)	1.00	Pantoporate	0.19 (0.12–0.26)	18–20
	N. pilurifera	S	17.7 (16.4–18.8)	17.7 (16.4–18.8)	1.00	Pantoporate	0.21 (0.12–0.30)	22–23
	N. laurifolia	S	16.2 (14.9–18.7)	16.2 (14.9–18.7)	1.00	Pantoporate	0.20 (0.11–0.30)	23–25

S spheroidal, *SP* subprolate, *OS* oblate spheroidal, *P* prolate

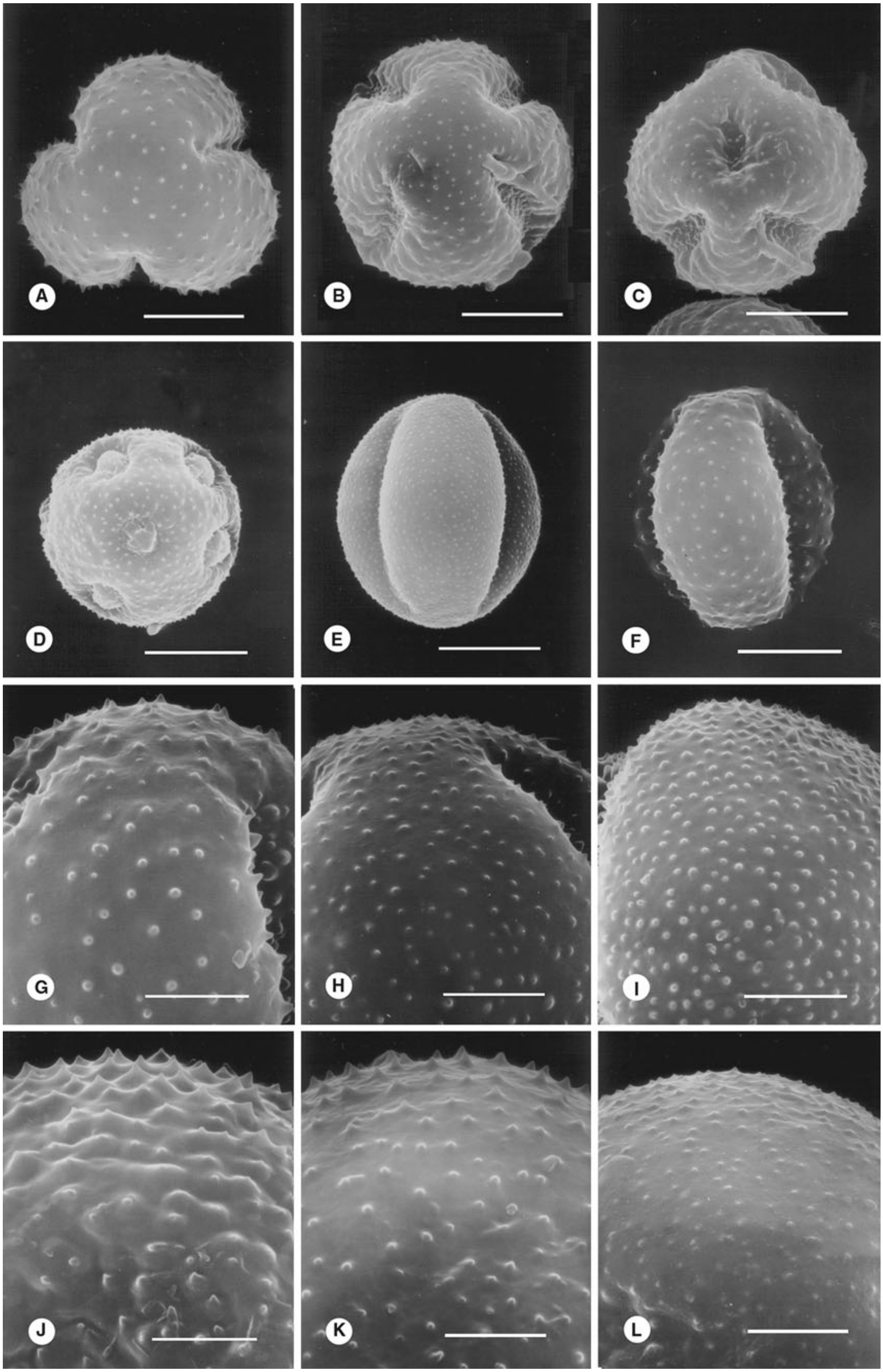
2003). If this evolutionary trend is applied to *Clematis* s.l., Japanese species in sect. *Tubulosae*, North American species of sect. *Viorna*, most species of sect. *Viticella*, and *Naravelia* can be inferred as derived groups, although the most primitive groups of the genus cannot be inferred from aperture types.

Tectal variation

Like most genera of Ranunculaceae, for example *Caltha*, *Cimicifuga*, *Thalictrum*, *Anemone*, *Pulsatilla*, and *Ranunculus*, pollen grains of *Clematis* s.l. have spinulose tecta that are usually punctate or microperforate (Kumazawa 1936; Xi 1985; Nowicke and Skvarla 1995). Although it has uniformity in tectal ornamentation in *Clematis*, the size and density of sculpture elements vary substantially (Fig. 1e–l). The spinules are very small and indistinct (<20 μm) in some sections, for example sect. *Campanella* and sect. *Atragene*, whereas they are larger (up to 40 μm) in sect. *Clematis*, sect. *Tubulosae*, and sect. *Aspidanthera* (Fig. 2a).

The density of the spinules also varied substantially (Figs. 1g–i, 2b). It is noteworthy that smaller spinules are often more densely distributed than larger ones. Although exceptional pollen grains may be observed, pollen of sect. *Aspidanthera*, sect. *Tubulosae*, sect. *Lasiantha*, sect. *Clematis*, and sect. *Pseudanemone* tends to have larger sculpture elements that are more sparsely distributed than in sect. *Campanella*, sect. *Bebaeanthera*, sect. *Atragene*, sect. *Fruticella*, and *Archiclematis* (Figs. 2, 3).

Combining these two characters, pollen grains of sect. *Campanella*, sect. *Bebaeanthera*, sect. *Atragene*, and sect. *Fruticella* can be distinguished from those of sect. *Aspidanthera*, sect. *Lasiantha*, sect. *Tubulosae*, and sect. *Clematis* (Fig. 1f, i). Thus, the combined characters provide some systematic information. For example, two groups of pollen grains in sect. *Meclatis* (sensu Tamura 1995) can be recognized (Fig. 3, green cross). *Clematis wightiana*, *C. hirsuta*, and *C. simensis* have pollen with large and sparse spinules which is very similar to the pollen in sect. *Clematis* and sect. *Aspidanthera*, whereas other species in sect. *Meclatis* (*C. tangutica*, *C. akebioides*, etc.) have pollen grains similar to those of sect. *Campanella*. This result supports Snoeijer's (1992) treatment separating the former species as a section *Brachiata*. These species have often been placed in sect. *Meclatis* (e.g. Tamura 1991, 1995) mainly because they all have hairy filaments. They differ from those of sect. *Meclatis* s.str. (sensu Snoeijer 1992) in their spreading, white sepals and narrowly linear filaments. In species of sect. *Meclatis* s.str., the sepals are usually ascending and yellow in color, and the filaments are widened below. Wang (2004) hypothesized that sect. *Brachiata* may be closely related to sect. *Clematis* on the


A
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◀ **Fig. 1** Variation of aperture types (**a**–**d**). **a** *Clematis grahami*, *scale bar* 6 µm, **b**, **c** *C. trifida*, *scale bars* 10 µm, **d** *C. patens*, *scale bar* 10 µm. Tectum variation (**e**–**f**). **e** *C. nanophylla scale bar* 8.6 µm, **f** *C. gouriana scale bar* 6 µm. Variation of spinule density (**g**–**i**). **g** *C. gouriana*, **h** *C. finetiana*, **i** *C. dasyandra*. Variation of spinule height (**j**–**l**). **j** *C. stans*, **k** *C. villosa*, **l** *C. japonica*. *Scale bars* (**g**–**l**) 3 µm

basis of gross resemblance. Our palynologic data support this point. Both sections have pollen grains with sparse and large spinules. However, in the whole genus these two quantitative characters of ornamentation are continuous and cannot be used to delimitate sectional groups.

Pollen size

Pollen of *Clematis* is generally small to medium, on the basis of the standard of Hesse et al. (2009). Our measurements largely agree with the size range described by Kapoor et al. (1989) but are slightly smaller in most of the species sampled. Kumazawa (1936) considered that different wet and dry conditions or different preparations for pollen samples may substantially affect pollen size. We agree with Kumazawa (1936) and attribute the difference between our study and that of Kapoor et al. (1989) to the

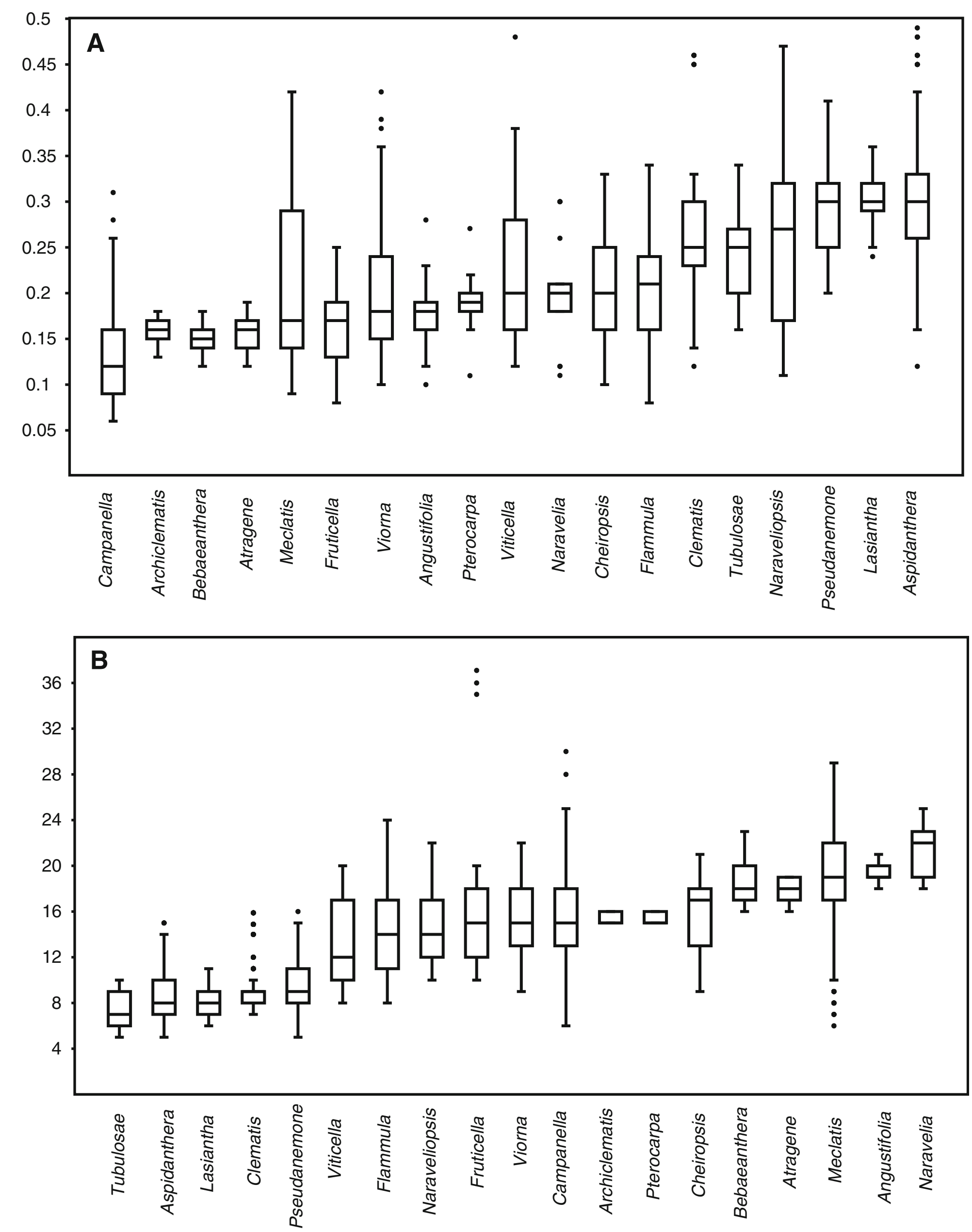

Fig. 2 Box plot showing variation of spinule height (**a**) and spinule density (**b**) of *Clematis* s.l. *Rectangles* define 25–75 %; *horizontal lines* show median; *whiskers* show 5–95 %; *circles* indicate extreme values

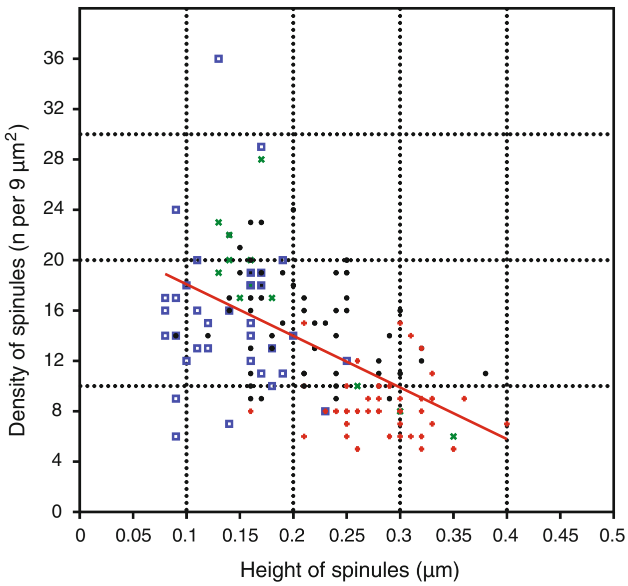

Fig. 3 Scatter diagram illustrating the relationship between the height and density of the sculpture element. *Squares*: sect. *Campanella*, sect. *Bebaeanthera*, sect. *Atragene*, sect. *Fruticella*, and *Archiclematis*; *red crosses*: sect. *Tubulosae*, sect *Aspidanthera*, sect. *Lasiantha*, sect. *Clematis*, and sect. *Pseudanemone*; *points*: samples from sect. *Viticella*, sect. *Flammula*, sect. *Naraveliopsis*, sect. *Viorna*, sect. *Pterocarpa*, sect. *Cheiropsis*, sect. *Angustifolia*, and *Naravelia*; *green crosses*: sect. *Meclatis*

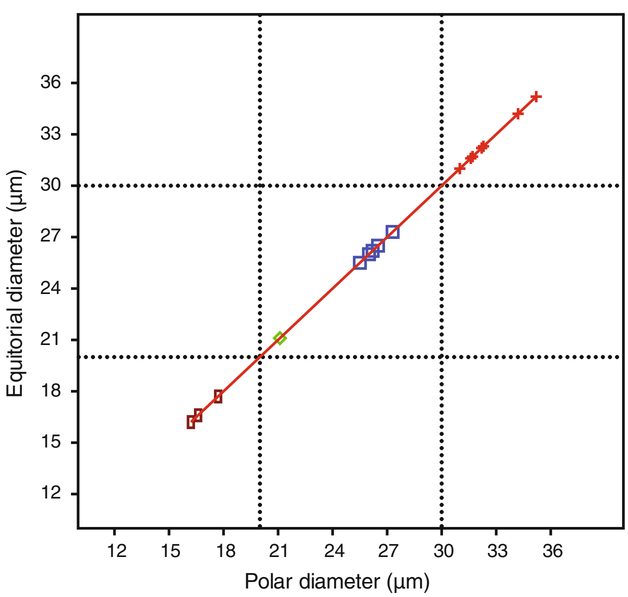

Fig. 4 Scatter diagram illustrating the size of pantoporate pollen. *Crosses*: sect. *Viorna*; *squares*: sect. *Viticella*; *diamond*: sect. *Tubulosae*; *rectangles*: *Naravelia*

preparation and observation methods. Kapoor et al. (1989) acetolyzed the pollen and observed them under LM, whereas this study mounted grains without treatment and observed them under SEM.

In this study, there was little regular variation of the size of pollen grains, especially the tricolpate type, in *Clematis* s.l. However, the size of pantoporate pollen provides some information. Pantoporate pollen grains of *Naravelia*, sect. *Tubulosae*, sect. *Viticella*, and sect. *Viorna* can be clearly separated by their size (Fig. 4). Sect. *Viorna* has the largest grains. Pollen of sect. *Viticella* is smaller than that of Sect. *Viorna* and larger than that of sect. *Tubulosae*. *Naravelia* has the smallest pantoporate pollen. This indicates that although only a small proportion is of the pantoporate type, it may have evolved several times independently.

Pollen and phylogeny of *Clematis*

Clematis is one of the most difficult groups taxonomically. There are major conflicts among currently published classifications (Tamura 1995; Johnson 1997; Grey-Wilson 2000; Wang and Li 2005; reviewed by Xie et al. 2011). Furthermore, recent molecular phylogenetic studies (Miikeda et al. 1999, 2006; Xie et al. 2011) are largely inconsistent with the above mentioned classifications. These studies did not generate a robust phylogenetic framework for *Clematis*, especially in deep branches, which indicated that the crown group of *Clematis* diverged recently, and a recent species radiation may have happened in the genus.

Similar to other characters, for example seedling morphology (Essig 1991) and leaf epidermis (Shi and Li 2003), pollen morphology alone is insufficient to reconstruct phylogenetic relationships within the genus. In *Clematis* s.l., palynologic characters are rather conservative, and only two major aperture types are recognized in the genus. We mapped this character on the simplified phylogeny (Fig. 5) resulting from Xie et al. (2011) and confirmed that the pantoporate type is derived and has evolved several times in the genus. At least three significantly supported clades have derived this character.

Naravelia has been recognized as a distinctive genus by most taxonomists (Eichler 1963; Tamura 1995; Grey-Wilson 2000; Wang and Li 2005). This genus was found nested within *Clematis* and closely related with sect. *Naraveliopsis* by recent molecular phylogenetic analyses (Miikeda et al. 2006; Xie et al. 2011). The aperture type of sect. *Naraveliopsis* (*C. loureiriana* in Fig. 5) is tricolpate, whereas *Naravelia* has pantoporate pollen. The result indicates that *Naravelia* may be derived from sect. *Naraveliopsis* and extends its distribution to tropical areas in South and South East Asia.

Pantoporate pollen also occurs in sect. *Tubulosae*. The molecular phylogenetic study by Xie et al. (2011) revealed that *C. pinnata* from northern China is more closely related to sect. *Clematis* than to sect. *Tubulosae*, and *C. heracleifolia* from China and *C. stans* from Japan are sibling species (Fig. 5). The Chinese species was found to have tricolpate pollen and its Japanese ally has pantoporate

Fig. 5 Aperture types optimized on to a tree resulting from molecular phylogenetic study of *Clematis* based on the combined data of nrITS, *atp*B-*rbc*L, *psb*A-*trn*H-*trn*Q, and *rpo*B-*trn*C sequences (Xie et al. 2011). *Vertical bars* indicate the clades supported by ≥0.95 Bayesian posterior probability values

pollen. The dispersal route of sect. *Tubulosae* from China to Japan may be inferred from our palynologic observation.

In previous classifications, sect. *Viorna* and sect. *Viticella* are thought to be distantly related groups in *Clematis*, because of their different floral characters (Tamura 1995; Johnson 1997; Grey-Wilson 2000; Wang and Li 2005). However, these two sections were clustered together and their relationships were not clearly supported in the molecular phylogenetic study by Xie et al. (2011). It is interesting that both sections have the two aperture types. Sect. *Viorna* is disjunctly distributed in North America and North Eurasia. All the North American species have pantoporate pollen and Eurasian species have tricolpate species. Sect. *Viticella* is distributed in Eurasia and most of the sampled species from East Asia have pantoporate pollen except European *C. viticella* and East Asian *C. huchouensis*. Because the relationships of these two sections are not resolved, the evolution of the pantoporate pollen of this clade cannot be inferred. The size of the pantoporate pollen in sect. *Viorna* is larger than that in sect. *Viticella* (Fig. 4) indicating that this aperture type may have evolved more than once in this clade.

Previous molecular phylogenetic studies (Miikeda et al. 2006; Xie et al. 2011) suggested recent species radiation in the genus. Molecular dating analysis indicated that the current species of *Clematis* may have diverged in the late Miocene (Xie et al. 2011). Approximately 300 species with highly diverse morphological characters evolved within a relatively short period of time. However, it is noteworthy that there is no evidence for the adaptive evolution of pollen morphology in *Clematis*, because the aperture types in the genus are rather simple. Tricolpate pollen grains are predominant in the genus and accompany all kinds of diverse morphological characters, for example floral structure (Jiang et al. 2010), inflorescence type, seedling phyllotaxy, sexuality of flowers, etc. (Xie et al. 2011).

Conclusions

Different morphotypes of pollen maximize the chances of successful fertilization under different conditions (Welsh et al. 2010). Tricolpate, 4-6-zonocolpate, pantocolpate, and pantoporate grains are regarded as progressively derived in

Angiosperms (Wodehouse 1936; Muller 1970; Tellería and Daners 2003; Welsh et al. 2010), and this "successiformy" (Van Campo 1976) also can be observed in Ranunculaceae (Nowicke and Skvarla 1995). Pollen of *Clematis* is heteromorphic. Tricolpate pollen is predominant and may be the primitive type and pantoporate pollen may be derived but is not synapomorphic among the sections which have this type of pollen. Size and ornamentation characters are variable and can provide some systematic information about the genus; however, the variation of these characters is quantitative, continuous within and among sections. Although evolutionary trends can be inferred from palynologic characters in some groups of *Clematis* s.l., the taxonomic value of pollen morphology is limited in *Clematis* at species level.

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Appendix

Vouchers used for scanning electron microscopy. Species (arranged in alphabetical order), country, collector, collector number, herbarium acronym from index Herbariorum.

Clematis acapulcensis Hook. et Arn.: Costa Rica, *A. Smith A656* (GH); ***C. acerifolia*** Maxim.: China, Beijing, Shangfangshan, *K.J. Guan 75003* (PE); ***C. acuminata*** var. ***longicaudata*** W. T. Wang: China, Yunnan, Binchuan Xian, *T. N. Liou 01776* (PE); ***C. aethusifolia*** Turcz.: China, Hebei, Zhuolu Xian, *Z. G. Yang 1471* (PE); ***C. affinis*** A. St.-Hil.: Brazil, Parana, *Koczzcki 53* (P); ***C. akebioides*** (Maxim.) Hort. ex Veitch.: China, Gansu, Lanzhou, *Y. Q. He 5759* (PE); ***C. akoensis*** Hayata: China, Taiwan, Pingbian, *T. Y. A. Yang 0271* (PE); ***C. albicoma*** Wherry: US, Virginia, *Heller 842* (MO); ***C. alborosea*** Ulbr.: Peru, *C. Vargas 5563* (GH); ***C. alternata*** Kitam. et Tamura: China, Tibet, Jilong, *Tibet Exped. 6889* (PE); ***C. apiifolia*** DC.: China, Zhejiang, Putuoshan, *Anonym 1187* (PE); ***C. aristata*** R. Br.: Australia, New S. Wales, *W. V. Dorrigo s.n.* (US); ***C. armandii*** Franch.: China, Guizhou, *M. Z. Zhang 350* (PE); ***C. armandii*** var. ***hefengensis*** (G. F. Tao) W. T. Wang: China, Hubei, Hefeng Xian, *J.X. Hong 1227* (PE); ***C. bigelovii*** Torr.: US, New Mexico, Sandia Mountains, *C. C. Ellis 18* (MO); ***C. bonariensis*** Juss. ex DC.: Argentina, *T. M. Pederson 3658* (US); ***C. brachyura*** Maxim.: Korea, *Faurie 14* (P); ***C. buchananiana*** DC.: China, Yunnan, *K. M. Feng 12912* (PE); ***C. cadmia*** Buch.-Ham. ex Wall.: China, Jiangxi, Nanchang, *Anonym 1688* (PE); ***C. caleoides*** Standl. et Steyerm.: Guatemala, *A.F. Skutch 105* (US); ***C. campestris*** Hil.: Argentina, Tucumán, *S. Venturi 686* (US); ***C. chinensis*** Osbeck: China, Guangxi, *N. K. Liang 2108* (PE); ***C. chingii*** W. T. Wang: China, Taiwan, Taizhou, *T. Y. A. Yang 02232* (PE); ***C. chrysocarpa*** Welw. ex Oliv.: Tanzania, Iringa, *Brigood* et al. *1306* (UPS); ***C. chrysocoma*** Franch.: China, Yunnan, Luquan Xian, *Kunming working station 1361* (PE); ***C. cirrhosa*** L.: Europe: *Anonym s. n.* (PE); ***C. connata*** DC.: China, Sichuan, Baoxing Xian, *G. L. Qu 3835* (PE); ***C. corniculata*** W. T. Wang: China, Xinjiang, Yecheng, *D. Zheng k102* (PE); ***C. courtoisii*** Hand.-Mazz.: China, Henan, Shangcheng Xian, *Anonym 276* (PE); ***C. crassifolia*** Benth.: China, Guangdong, *Anonym 16498* (PE); ***C. crassipes*** Chun et How: China, Hainan, Ledong, *Z. L. Chen 30349* (PE); ***C. crispa*** L.: US, Arkansas, Mississippi valley, *Loudewyks, 249* (MO); ***C. dasyandra*** Maxim.: China, Hubei, Badong Xian, *G. X. Fu and Z. S. Zhang 1066* (PE); ***C. delavayi*** Franch.: China, Sichuan, Daocheng Xian, *Sichuan Exped. 2095* (PE); ***C. delavayi*** var. ***calvescens*** Schneid.: China, Sichuan, Derong Xian, *Sichuan Exped. 4117* (PE); ***C. dilatata*** Péi: China, Zhejiang, *M. C. Chang 4933* (PE); ***C. dioica*** L.: US, *E. L. Ekman H13943* (US); ***C. dissecta*** Baker: Madagascar, Fianarantsoa, *Malcomber* et al. *1390* (UPS); ***C. drummondii*** Torr.: US, New Mexico, *E. O. Woaton 150* (MO); ***C. fasciculiflora*** Franch.: China, Yunnan, Dali Xian, *T. N. Liou 017915* (PE); ***C. finetiana*** Lévl. et Vant.: China, Guangdong, Pingyuan Xian, *L. Deng 4237* (PE); ***C. flammula*** L.: France, Corse, *P. Aellen 2462* (US); ***C. foetida*** Raoul.: New Zealand, *H. H. Allai s. n.* (IBSC); ***C. fruticosa*** Turcz.: China, Hebei, Zhuolu Xian, *Z. G. Yang et Z. Q. Zhang 441*(PE); ***C. fusca*** Turcz.: China, Heilongjiang, *X. N. Guan 6* (PE); ***C. ganpiniana*** (Lévl. et Vant.) Tamura: China, Guangxi, Hexian, *Z. T. Li 604134* (PE); ***C. ganpiniana*** var. ***tenuisepala*** (Maxim.) C. T. Ting: China, Shanxi, Ruicheng Xian, *S. Y. Bao 1536* (PE); ***C. glabrifolia*** K. Sun et M. S. Yan: China, Gansu, Wen Xian, *K. Sun* et al. *0251* (PE); ***C. glaucophylla*** Small: US, Florida, *Godfrey 84152* (GH); ***C. glycinoides*** DC.: Australia, New S. Wales, *M. Evans 2659* (GH); ***C. gouriana*** Roxb. ex DC.: China, Guizhou, *Sichuan and Guizhou Exped. 1602* (PE); ***C. gracilifolia*** Rehd. et Wils.: China, Sichuan, Barkam, *X. Li 70904* (PE); ***C. gracilifolia*** var. ***dissectifolia*** W. T. Wang: China, Sichuan, Kangding Xian, *Q. Y. Wang 4427* (PE); ***C. grahami*** Benth.: US, *E. Lyonnet 1315* (US);

C. grandidentata (Rehd. et Wils.) W. T. Wang: China, Zhejiang, Changhua, *Anonym 28596* (PE); ***C. graveolens*** Lindl.: India, *B. Khan s. n.* (PE); ***C. grewiiflora*** DC.: China, Tibet, Jilong Xian, *S. Jiang et K. F. Zhao 393* (PE); ***C. grossa*** Benth.: Guatemala, *A. F. Skutch 2029* (GH); ***C. haenkeana*** C. Presl.: Peru, *R. Ferreyra 2016* (P); ***C. hancockiana*** Maxim.: China, Henan, Xinyang Xian, *K. J. Guan 119* (PE); ***C. henryi*** Oliv.: China, Chongqing, Nanchuan Xian, *P. Tan 2677* (PE); ***C. heracleifolia*** DC.: China, Tianjin, Jixian *T. N. Liou 4521* (PE); ***C. hexapetala*** Pall.: China, Inner Mongolia, *Inner Mongolia et Ningxia Exped. 841* (PE); ***C. hexapetala*** var. ***tchefouensis*** (Debeaux) S. Y. Hu: China, Shandong, Qingdao Laoshan, *T. Y. Zhou 1239* (PE); ***C. hirsuta*** Guill. et Perr.: Uganda, Albert Nyanza, *E. A. Mearns 2550* (US); ***C. huchouensis*** Tamura: China, Zhejiang, Haining Xian, *L. Yuan 12* (PE); ***C. ibarensis*** Baker: Madagascar, Tananarive, *Anonym 2520* (P); ***C. integrifolia*** L.: China, Xinjiang, Burjin Xian, *Anonym 00074* (PE); ***C. intricata*** Bunge: China, Hebei, Fuping Xian, *K. M. Liu 3279* (PE); ***C. japonica*** Thunb.: Japan, *K. Hisauti 2729* (PE); ***C. jialasaensis*** W. T. Wang: China, Tibet, Nyingchi Xian, *W. L. Zheng 1589* (PE); ***C. jingdungensis*** W. T. Wang: China, Yunnan, Shunning Xian, *C. W. Wang 72020* (PE); ***C. kirilowii*** Maxim.: China, Beijing, *Zhongde Exped. 583* (PE); ***C. kockiana*** Schneider: China, Sichuan, Mabian Xian, *D. Y. Hong* et al. *s. n.* (PE); ***C. kweichowensis*** Péi: China, Guizhou, Panxian, *Anshun Exped. 1245* (PE); ***C. lancifolia*** Bur. et Franch.: China, Sichuan, *T. T. Yu 1163* (PE); ***C. lancifolia*** var. ***ternata*** W. T. Wang: China, Sichuan, Miyi Xian, *Anonym s. n.* (PE); ***C. lanuginosa*** Lindl.: China, Zhejiang, *Anonym 0737* (PE); ***C. lasiandra*** Maxim.: China, Sichuan, Pingwu Xian, *L. Xie 2004175* (PE); ***C. lasiantha*** Fisch.: Costa Rica, *C. F. Baker 499* (GH); ***C. laxistrigosa*** (W. T. Wang et M. C. Chang) W. T. Wang: China, Sichuan, *T. P. Wang 9614* (PE); ***C. leschenaultiana*** DC.: China, Guangxi, Fengshan Xian, *L. W. Xu 1411* (PE); ***C. leschenaultiana*** var. ***rubifolia*** Wight.: China, Guizhou, *J. Cavalerie s. n.* (PE); ***C. ligusticifolia*** Nutt.: Canada, *B. Boivin 9436* (GH); ***C. loureiriana*** DC.: China, Fujian, *G. S. He 9546* (PE); ***C. macropetala*** Ledeb.: China, Shannxi, Taibaiding, *J. X. Xiang 1041* (PE); ***C. mandshurica*** Rupr.: China, Heilongjiang, Dailing, *T. N. Liou 7171* (PE); ***C. mashanensis*** W. T. Wang: China, Guangxi, Mashan Xian, *X. Z. Zheng 112* (PE); ***C. menglaensis*** M. C. Chang: China, Yunnan, Mengla Xian, *C. W. Wang 80841* (PE); ***C. metouensis*** M. Y. Fang: China, Tibet, Mêdog Xian, *W. L. Chen 10876* (PE); ***C. meyeniana*** Walp.: China, Guangxi, Longsheng Xian, *Guangfu Exped. 0900a* (PE); ***C. microphylla*** DC.: Austrilia, *E. N. S. Jackson 4310* (IBSC); ***C. millefoliata*** Eichl.: Peru, *F. W. Pennell 13263* (US); ***C. montana*** Buch.-Ham. ex DC.: China, Sichuan, Kangding Xian, *Anonym s. n.* (PE); ***C. nanophylla*** Maxim.: China, Gansu, Lanzhou, *Y. K. Lian 96912* (PE); ***C. napaulensis*** DC.: China, Yunnan, *M. G. Li 2897* (PE); ***C. obscura*** Maxim.: China, Shanxi, Huanqu Xian, *S. Y. Bao 92* (PE); ***C. orientalis*** L.: China, Xinjiang, Turpan, *Z. M. Zhang 294* (PE); ***C. otophora*** Franch. ex Finet. et Gagnep.: China, Hubei, Shennongjia, *Shennongjia Exped. 11549* (PE); ***C. paniculata*** Gmelin: New Zealand, *L. Tibell n220* (IBSC); ***C. parviloba*** Gard. et Champ.: China, Jiangxi, Longnan Xian, *263 Mission group 1353* (PE); ***C. parviloba*** var. ***longianthera*** W. T. Wang: China, Sichuan, Emeishan, *T. T. Yu 547* (PE); ***C. patens*** var. ***tientaiensis*** M. Y. Fang: China, Zhejiang, Tiantaishan, *X. Y. He 1* (PE); ***C. pauciflora*** Nutt. ex Torr. et A. Gray: US, San Diego, *W. Deane s. n.* (GH); ***C. peruviana*** DC.: Peru, *R. Ferreyra 7591* (US); ***C. peterae*** Hand-Mazz.: China, Shanxi, Puxian, *X. Y. Liu 21057* (PE); ***C. peterae*** var. ***trichocarpa*** W. T. Wang: China, Gansu, Wenxian, *Z. Y. Zhang 14413* (PE); ***C. pianmaensis*** W. T. Wang: China, Guizhou, *Libo Exped. 81-6-0012* (PE); ***C. pinchuanensis*** W. T. Wang et M. Y. Fang: China, Yunnan, Binchuan Xian, *T. N. Liou 017699* (PE); ***C. pinnata*** Maxim.: China, Beijing, Baihuashan, *S. Y. Ho 15037* (PE); ***C. pitcheri*** Torr. et A. Gray: US, Kansas, *Norton 1* (GH); ***C. pogonandra*** Maxim.: China, Gansu, Wenxian, *X. L. Chen and Y. F. Wang 911693* (PE); ***C. populifolia*** Turcz.: South America, *F. J. Breteler 4666* (US); ***C. potaninii*** Maxim.: China, Sichuan, Zhegushan, *X. Li 74387* (PE); ***C. pseudootophora*** Finet et Gagnep.: China, Hunan, Shimen Xian, *Hupingshan Exped. A111* (PE); ***C. pseudopogonandra*** Finet et Gagnep.: China, Sichuan, Heishui Xian, *X. Li 73058* (PE); ***C. puberula*** Hook. et Thoms.: China, Yunnan, Gongshan Xian, *Anonym 7067* (PE); ***C. qingchengshanica*** W. T. Wang: China, Sichuan, Qingchengshan, *T. P. Wang 10009* (PE); ***C. quinquefoliolata*** Hutch.: China, Guizhou, Jiangko Xian, *Z. S. Zhang* et al. *401911* (PE); ***C. ranunculoides*** Franch.: China, Yunnan, Lijiang Xian, *Anonym 22656* (PE); ***C. recta*** L.: Austria, *D. Y. Hong s. n.* (PE); ***C. rehderiana*** Craib.: China, Sichuan, Dajin Xian, *X. Li 75591* (PE); ***C. repens*** Finet et Gagnep.: China, Sichuan, Honghua Xian, *Anonym 1476* (PE); ***C. reticulata*** Walter: US, Alabama, *Palmer 27234* (GH); ***C. seemanni*** Kuntze: US, *J. Soukup 3578* (US); ***C. serratifolia*** Rehd.: China, Jilin, Antu Xian, *T. N. Liou 3785* (PE); ***C. shensiensis*** W. T. Wang: China, Shanxi, Xiexian, *Yellow River Exped. 344* (PE); ***C. siamensis*** Drummond et Craib.: China, Yunnan, *China-Russia Exped. s. n.* (PE); ***C. sibirica*** (L.) Mill.: China, Xinjiang, *Xinjiang Exped. 10544* (PE); ***C. simensis*** Fresen: Ethiopia, Addis Ababa, *F. G. Meyer 7533* (US); ***C. smilacifolia*** Wall.: China, Yunnan, Puer Xian, *Y. Jiang 12935* (PE); ***C. songarica*** Bunge: China, Xinjiang, Wenquan, *Xinjiang and Tibet Exped. 3553* (PE); ***C. songarica*** var. ***aspleniifolia*** (Schrenk) Trautv.: China, Xinjiang, *L. Yang 88-085* (PE); ***C. stanleyi*** Hook.: Africa, *Bayliss 2002* (GH); ***C. stans*** Sieb. et Zucc.: Japan, Shizuoka, *F. Chikata*

15778 (PE); ***C. subumbellata*** Kurz.: China, Yunnan, Fengqing Xian, *T. T. Yu 18254* (PE); ***C. tangutica*** (Maxim.) Korsh.: China, Tibet, Gêrzê Xian, *F. Z. Li 23* (PE); ***C. tangutica*** var. ***obtusiuscula*** Rehd.et Wils.: China, Qinghai, *Tibet Exped. 177* (PE); ***C. tashiroi*** Maxim.: China, Taiwan, Taibei, *W. F. He, 648* (PE); ***C. tenuipes*** W. T. Wang: China, Yunnan, *C. W. Wang* et al. *87086* (PE); ***C. terniflora*** DC.: China, Anhui, Xiaoxian, *M. Chen and Zhang 19* (PE); ***C. tomentella*** (Maxim.) W. T. Wang: China, Shanxi, Yulin Xian, *P. Licent 7078* (PE); ***C. tosaensis*** Makino: Japan, *Miyoshi Furuse 27217* (PE); ***C. trifida*** Hook.: Madagascar, *Fortyth-Major 714* (US); ***C. tsaii*** W. T. Wang: China, Yunnan, *B. Y. Qiu 52651* (PE); ***C. uncinata*** Champ.: China, Chongqing, Nanchuan Xian, *Z. Y. Liu 4519* (PE); ***C. urophylla*** Franch.: China, Hubei, Hefeng Xian, *H. J. Li 8437* (PE); ***C. venusta*** M. C. Chang: China, Yunnan, Yulongshan, *SW Exped. 164* (PE); ***C. versicolor*** Small: US, Texas, *Correll 37401* (GH); ***C. villosa*** DC.: Tanzania, Hemit, *P. J. Greenway 7741* (US); ***C. virginiana*** L.: US, Texas, San Augustine, *E. J. Palmer 10622* (MO); ***C. viridiflora*** Bert.: Mozambique, Delagoa Bay, *H. Junod 370* (US); ***C. viridis*** (W. T. Wang et M. C. Chang) W. T. Wang: China, Sichuan, Sêrtar Xian, *Lu 06565* (PE); ***C. viticella*** L.: Slovenija, *Tauno Ulvinen s. n.* (UPS); ***C. wenshanensis*** W. T. Wang: China, Yunnan, Wenshan Xian, *Y. M. Shui 002714* (PE); ***C. wightiana*** Wall.: India, Kodaikanal Region, *Anglade s. n.* (GH); ***C. williamsii*** A. Gray ex Perry: Japan, Kyshu Fukuoka, *Sadao Tsutsui 29574* (IBSC); ***C. yuanjiangensis*** W. T. Wang: China, Yunnan, Yan-shan Lung-tang-tzai-shan, *C. W. Wang 83560* (PE); ***C. yunnanensis*** Franch.: China, Yunnan, Binchuan Xian, *T. N. Liou 017847* (PE); ***Naravelia laurifolia*** Wall.: Thailand, Kow Kiee Se Buhn Beung District, *J. F. Maxwell 76-691* (PE); ***N. pilurifera*** Hance: China, Yunnan, Mengkuan, *C. W. Wang 79972* (PE); ***N. zylanica*** (L.) DC. China, *Anonym s. n.* (PE).

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