The stem anatomy of the *Clematis* species (Ranunculaceae) in Taiwan

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Abstract

Background

Studies on the stem anatomical characteristics of Taiwanese species from the *Clematis* genus (Ranunculaceae) are scarce. This study aimed to investigate and compare the patterns of secondary growth in stems of 22 *Clematis* species.

Results

The rhytidome is composed of periderm and non-conducting phloem and formed either cogwheel-like or continuous segment bark. Key features of the genus were stem with an irregular conformation, wedge-like phloem and rays, indentations in the axial parenchyma, ray dilatation, and narrow rays. Approximately eight *Clematis* species formed bark arc shape, which developed the cogwheel- like rhytidome. There were with approximately 27% of the *Clematis* species in Taiwan having 12 vascular bundles. The vessels dispersed throughout the stem were semi-ring-porous in most species but were ring-porous in others. No species had diffuse-porous vessels. The vessel restriction pattern was only found in the two shrubs, *C. psilandra* and *C. tsugetorum*. The primary xylem ring was located around the pith in *C. uncinata* var. *uncinata*, making its pith cavity hexagon in shape. Four species had the pith cavity feature. Narrow rays that occurred in the secondary xylem increased with increasing stem diameter.

Conclusions

The cambial variants described in this study provide a foundation for further morphological studies of the *Clematis* genus.

Background

The vascular cambium of climbing plants produces xylem and phloem under normal conditions, and both types of tissue have large amounts of parenchyma cells. These cells can be re-differentiated into meristematic cells, which may give rise to secondary vascular tissue, cork cambia, dilatation tissue, or adventitious buds (Mauseth 1988). Due to the uneven deposition of secondary xylem, stems are generally irregularly shaped after secondary growth (Carlquist 1991; Rajput et al. 2014) as diverse cambial variants, or secondary growth patterns are formed. The stems examined generally developed axial vascular elements in segments separating by the wider xylem and phloem rays, forming xylem in plate type. This type is derived from a single cambium, according to Angyalossy et al. (2012, 2015). Many families develop this variant, including Ranunculaceae (Carlquist 2001).

The Ranunculaceae family comprises approximately 60 genera and 2500 species, including approximately 300 *Clematis* species worldwide (Wang and Bartholomew 2007), of which 22 are found in Taiwan (Yang and Huang 1996). The life forms of *Clematis* species include shrubs, herbs, and perennial climbers (lianas). Within the Ranunculaceae, several cambial variants have been reported (Angyalossy et al. 2012, 2015).

Previous anatomical studies of Ranunculaceae have mainly concentrated on the genus *Clematis* (Smith 1928; Sieber and Kucera 1980; Gregory 1994; Carlquist 1995). Few secondary rays begin abruptly and occur in *C. cirrbosa*, *C. flammula*, *C. montevidensis*, *C. vitalba*, and *C. viticella* (Schweingruber et al. 2011) with increasing the stem diameter. Carlquist (1995) indicated that new rays are wide multiseriate from their origin, and are initiated abruptly rather than as narrow rays that gradually increase in width in *Clematis* genus. Beck (2011) described that closely spaced, narrow rays occurred in groups will simulate large rays which terms as aggregate rays. The secondary or new rays also occur in woody climbers in two families, Cucurbitaceae (Carlquist 1992) and Aristolochiaceae (Carlquist 1993). The cambium in the secondary vascular tissue forms increasing ray-like parenchyma in *Clematis flammula* var. *maritima* (Isnard et al. 2003a). Interfascicular cambia ray width increases with increasing stem diameter in *C. alpina* subsp. *alpina*, that the rays are wedge-like in shape (Schweingruber et al. 2011).

The phellogen of *C. alpina* and *C. vitalba* produces cork cells and forms the phellem (cork) layers is located outside the secondary phloem (Schweingruber et al. 2011). The complex tissue region of the periderm and enclosed non-conducting phloem is called the rhytidome (Beck, 2011), which is generally comprised of successive cork and non-conducting phloem. According to the arrangement and detachment degree of rhytidome, two types were formed: cogwheel-like rhytidome (ring bark) and continuous segment rhytidome (scale bark) (Esau 1958; Sieber and Kucera 1980; Evert 2006). *C. vitalba* is a vine with big and small vascular bundles (six each) in its primary state. It forms the pith cavity, its bark tissue develops an arc, and the stem appears in a cogwheel-like form due to the different activities in the fascicular and interfascicular regions (Sieber and Kucera 1980).

In *C. vitalba*, the cambium is dented towards the pith in the broad rays region (Sieber and Kucera 1980), and in *C. alpina* and *C. pickerringii*, the interfascicular areas always have indentations (Carlquist 1995). Owing to the presence of thin-walled axial parenchyma in latewood adjacent to the thin-walled ray cells, wedge-shaped indentations can be seen in the interfascicular region (Sieber and Kucera 1980; Carlquist 1995). However, the indentations are strands of thin-walled axial parenchyma near the broader rays. Isnard et al. (2003a, b) defined four developmental stages of *C. flammula* var. *maritima*, *C. recta*, and *C. vitalba* by the appearance of the periderms, phloem fibers, rhytidome, and wide rays. The axial parenchyma of *C. alpina*, *C. columbiana*, *C. hirsutissima*, and *C. recta* are paratracheal with semi-ring-porous wood and distinct annual rings, while *C. flammula*, *C. vitalba*, and *C. viticella* have ring-porous wood with annual rings (Schweingruber et al. 2011).

The xylem in plate variant was found in the genus *Clematis* (Carlquist 1995, Isnard et al. 2003b, Yang and Chen 2015), but the information about the patterns of secondary growth or cambial variants in Taiwan 22 *Clematis* species were scarce. As cambial variants constitute an extremely diverse morphology, the present study aimed to 1) provide detailed photographs of the features discussed and 2) provide a bracketed key based on the anatomical characteristics of the stems to facilitate the identification of irregular cambial activity in the *Clematis* genus in Taiwan.

Methods

Research Materials

Multiple samples of 22 *Clematis* species of the family Ranunculaceae were collected. The habits of these species included annual and perennial herbs, shrubs, and lianas growing in different forests. The dataset included species scientific name, collector, herbarium, and voucher number. The voucher species information of all *Clematis* species is presented in Table 1. Approximately 48% of the species were endemic to Taiwan. Among them, *Clematis psilandra* and *C. tsugetorum* are shrubs growing at high elevations of approximately 2,300 m and 3,200 m, respectively. The species *C. pseudootophora* is an herb rarely found in the field. The remaining 19 species are climbing vines in which *C. montana* grows at the highest elevation of approximately 3,200 m. This species consists of only a few populations located at an elevation of 1,500–1,900 m in eastern Taiwan.

Table 1
Voucher specimens of the 22 Taiwanese *Clematis* species investigated

Species	Collector	Herbarium and voucher number
Clematis akoensis Hayata	Po-Hao Chen	PPI75993
Clematis chinensis Osbeck var. *chinensis*	Po-Hao Chen	PPI73776
Clematis chinensis Osbeck var. *tatushanensis* T.Y.A. Yang	Po-Hao Chen	PPI76140
Clematis crassifolia Benth.	Chien-Fan Chen	TAIF449023
Clematis formosana Kuntze	Po-Hao Chen	PPI76748
Clematis gouriana Roxb. ex DC. subsp. *lishanensis* T.Y.A. Yang & T.C. Huang	Sheng-Zehn Yang	PPI57118
Clematis grata Wall.	Po-Hao Chen	PPI79191
Clematis henryi Oliv. var. *henryi*	Po-Hao Chen	PPI76097
Clematis henryi Oliv. var. *morii* (Hayata) T.Y.A. Yang & T.C. Huang	Her-Long Chiang	PPI65589
Clematis lasiandra Maxim.	Sheng-Zehn Yang	PPI57133
Clematis leschenaultiana DC.	Chien-Fan Chen	PPI63232
Clematis meyeniana Walp.	Chien-Fan Chen	PPI60238
Clematis montana Buch.-Ham. ex DC.	Chien-Fan Chen	PPI63771
Clematis parviloba Gard. ex Champ. subsp. *bartlettii* (Yamam.) T.Y.A. Yang & T.C. Huang	Guang-Pu Hsieh	PPI61169
Clematis pseudootophora M.Y. Fang	Chien-Fan Chen	TAIF455630
Clematis psilandra Kitag.	Guang-Pu Hsieh	PPI60043
Clematis tamurae T.Y.A. Yang & T.C. Huang	Po-Hao Chen	PPI79723
Clematis tashiroi Maxim.	Po-Hao Chen	PPI78815
Clematis terniflora DC. var. *garanbiensis* (Hayata) M.C. Chang	Po-Hao Chen	PPI78668
Clematis tsugetorum Ohwi	Guang-Pu Hsieh	PPI68569
Clematis uncinata Champ. ex Benth. var. *kinawensis* (Ohwi) Ohwi	Kun-Ping Lo	PPI69701
Clematis uncinata Champ. ex Benth. var. *uncinata*	Jyuen-Jyie Chen	PPI63904
*: Endemic species in Taiwan		

Research Methods

Multiple samples of each species were collected, stems with thick bark and secondary growth characteristics were selected in the field. To keep the materials fresh and retain humidity, the stems were stored in a collecting bag. Different diameters of each plant were collected to compare various developmental stages and accurately assess the position of various vascular bundle tissues. One or three samples with obvious and easy-to-observe cambial variants were selected per species for photographs and the scoring of morphological characteristics. The morphological features of stem cross-sections in the investigated species were used to construct a comparison table.

In the laboratory, the fresh materials were cut into pieces approximately 5 cm long, and a freehand cross-section of each stem was made with a razor blade. The stem cross-section was immediately photographed using a Nikon D7100 SLR digital camera with a 1:1 lens (Lens AF Micro Nikon 60 mm 1:2.8D; Nikon Corporation, Tokyo, Japan). Cambial characteristics were measured and described. Quantitative anatomical traits, such as stem diameter, bark thickness, mean xylem width, and mean primary ray width, were determined using Image-J software (Ferreira and Rasband 2011). All specimens were oven-dried at 60°C

for 4–5 days and then stored at -20°C for 3–4 days. They were then deposited in the Provincial Pingtung Institute (PPI) herbarium at the National Pingtung University of Science and Technology, Pingtung, Taiwan, for subsequent identification. The nomenclature follows the Flora of Taiwan volume II (Yang and Huang 1996). The materials of *C. pseudootophora* were permanently preserved in 75% aqueous alcohol because of its rarity.

The following stem features of each species were investigated (Table 2): locality; elevation (m); stem diameter (mm); phloem fibers (+/−); arc-like bark (+/−); cogwheel-like rhytidome (+/−); continuous segment rhytidome (+/−); rhytidome layer number; rhytidome thickness (mm); wedge-like rays (+/−); indentation of the axial parenchyma near the broader rays (+/−); restricted vessel pattern (+/−); ray dilatations (+/−); vascular bundle number; xylem width (mm) (mean ± SD, n = xylem number); ray width (μm) (mean ± SD, n = ray number); wide rays number; narrow rays number; ring-porous vessels (+/−); semi-ring-porous vessels (+/−); and developmental stage. The vessel restriction pattern, ray types, and axial parenchyma types were classified based on Carlquist (2001) and the IAWA Committee on Nomenclature (1964). Species were grouped based on the number of vascular bundles they contained (Smith 1928), with the 'central type' having 12 bundles, the 'many type' having > 12 bundles, and the 'few type' having < 12 bundles.

Table 2
Morphological characteristics determined from stem cross-sections of Taiwanese *Clematis* species and each line for each spec...

Characters / Scientific name	LO	EL (m)	SD (mm)	PF	AR	COG	SE	RHL (no.)	RHT (mm)	WER	ID	VR	RD	VB (no.)	XW (mm)	RW (μm)
*C. akoensis	Shouka, Pingtung County	350	3.8	+	−	−	−	−	−	−	−	−	−	14	0.31 ± 0.08	205 ± 95
*C. akoensis	..	..	6.0	−	−	−	−	−	−	+	+	−	+	12	0.41 ± 0.11	441 ± 32
C. chinensis var. chinensis	Lising, Nantou County	1800	4.3	+	−	−	−	−	−	−	−	−	−	20	0.41 ± 0.11	104 ± 36
C. chinensis var. chinensis	..	..	11.9	−	+	+	−	3–5	0.3–0.7	−	+	−	+	22	0.83 ± 0.19	232 ± 79
C. chinensis var. tatushanensis	Shalu, Taichung City	150	2.2	−	−	−	−	−	−	−	−	−	−	20	0.21 ± 0.05	25 ± 13
C. crassifolia	Henglingshan, Taichung City,	1700	4.1	+	−	−	−	−	−	−	−	−	+	20	0.29 ± 0.06	127 ± 34
C. crassifolia	Manabangshan, Miaoli County	1200	8.9	−	−	−	+	3–6	1.8–3.4	−	+	−	+	20	0.47 ± 0.11	186 ± 66
*C. formosana	Shoushan, Kaohsiung City	200	3.4	+	−	−	+	1	0.1–1.3	−	+	−	+	12	0.41 ± 0.12	90 ± 16
*C. formosana	..	..	11.3	−	−	−	+	2	0.3–1.8	−	+	−	+	12	1.48 ± 1.00	138 ± 46
*C. gouriana subsp. lishanensis	Hehuanshan, Nantou County	2600	6.2	−	−	−	+	1–2	0.2–0.7	−	+	−	+	12	0.81 ± 0.34	108 ± 51
C. grata	Dapu, Chiayi County	500	3.9	+	−	−	−	−	−	−	−	−	−	12	0.46 ± 0.14	89 ± 29
C. grata	..	..	3.5	−	−	+	−	1	0.1–0.3	+	−	−	−	12	0.49 ± 0.13	108 ± 22
C. grata	..	..	14.1	−	+	+	−	4	0.3–1.1	−	+	−	+	12	1.69 ± 0.69	642 ± 208
C. henryi var. henryi	Beidawushan, Pingtung County	1500	5.5	−	−	−	+	1–3	0.4–0.9	+	+	−	+	10	0.38 ± 0.07	449 ± 131
*C. henryi var. morii	Sinjhongheng, Chiayi County	2400	4.0	−	−	−	+	1	< 0.1	+	+	−	+	10	0.58 ± 0.15	283 ± 55
*C. henryi var. morii	..	..	7.3	−	−	−	+	1	0.1–0.2	+	+	−	+	11	0.90 ± 0.21	553 ± 116
C. lasiandra	Lingmingshan, Taichung City	3000	2.0	−	−	−	−	−	−	−	−	−	−	12	0.34 ± 0.03	96 ± 52
C. lasiandra	Hehuanshan, Nantou County	2800	19.7	−	+	+	−	2–5	0.5–2.7	−	+	−	−	14	2.76 ± 0.52	230 ± 178
C. leschenaultiana	Duona trail, Kaohsiung City	700	6.4	−	−	−	+	2	0.2–0.3	−	+	−	+	21	0.45 ± 0.14	175 ± 61

Noted: *: endemic species; LO: locality; EL: elevation; SD: stem diameter; PF: phloem fibers in dense bundles or strands; AR: arc-like bark; COG: cogwheel-like r... rhytidome; RHL: rhytidome layer number; RHT: rhytidome thickness; WER: wedge-like rays; ID: indentation of the axial parenchyma near the wider rays; VR: ve... dilatations; VB: number of vascular bundle; XW: xylem width (mean ± SD, n = xylem number); RW: ray width (mean ± SD, n = ray number); WR: wide rays initiat... initiated abruptly; RP: ring-porous vessel; SRP: semi-ring-porous vessel; DS: developmental stage (1: first stage, collenchyma or primary phloem fibres present... beneath the primary phloem, secondary phloem fibers present. 3: third stage, sequent periderm develop within the secondary phloem, wider rays and larger ve... sequent periderm developed in deeper layers in the secondary phloem, narrow rays present).

Characters / Scientific name	LO	EL (m)	SD (mm)	PF	AR	COG	SE	RHL (no.)	RHT (mm)	WER	ID	VR	RD	VB (no.)	XW (mm)	RW (µm)
C. leschenaultiana	..	..	7.8	−	−	−	+	2	0.2–0.5	−	+	−	+	16	0.69 ± 0.32	145 ± 52
C. leschenaultiana	..	..	15.6	−	−	−	+	4	0.2–1.6	−	+	−	+	19	1.34 ± 0.64	225 ± 46
C. meyeniana	Duona trail, Kaohsiung City	700	7.3	−	−	−	+	3	0.4–0.8	−	+	−	+	21	0.58 ± 0.12	117 ± 39
C. montana	Hehuanshan, Nantou County	3200	3.1	+	−	−	+	1	0.2–0.3	−	−	−	+	12	0.34 ± 0.06	202 ± 29
*C. parviloba subsp. bartlettii	Hehuan river, Nantou County	2600	2.4	−	−	−	+	1	0.1–0.2	−	−	−	+	12	0.32 ± 0.10	64 ± 25
*C. parviloba subsp. bartlettii	..	..	3.9	−	−	−	+	2	0.1–0.3	−	+	−	+	12	0.49 ± 0.13	147 ± 50
C. pseudootophora	Sihyuanyakou, Yilan County	1800	3.5	−	+	+	−	1	0.1–0.2	+	+	−	−	6	0.93 ± 0.10	393 ± 87
*C. psilandra	Alishan, Chiayi County	2300	4.3	−	−	−	−	−	−	−	−	+	−	20	0.33 ± 0.11	387 ± 99
*C. psilandra	..	..	4.9	−	−	−	−	−	−	−	−	+	−	23	0.20 ± 0.08	218 ± 79
*C. tamurae	Fonggangshan, Kaohsiung City	1600	2.1	+	−	−	−	−	−	−	−	−	−	12	0.22 ± 0.05	107 ± 23
*C. tamurae	..	..	4.5	−	+	+	−	1	0.1–0.3	−	+	−	+	12	0.52 ± 0.14	167 ± 49
*C. tamurae	..	..	8.3	−	+	+	−	3–4	0.1–1.2	−	+	−	+	12	0.91 ± 0.13	393 ± 61
*C. tashiroi	Rueisuei trail, Hualien County	2400	4.0	+	−	−	−	−	−	−	−	−	−	14	0.32 ± 0.07	289 ± 116
*C. tashiroi	Beizihtong forest trail, Chiayi county	1600	8.8	−	−	−	−	−	−	−	+	−	+	20	0.58 ± 0.10	343 ± 155
*C. tashiroi	..	..	17.8	−	−	−	−	−	−	−	+	−	+	20	1.44 ± 0.30	450 ± 187
*C. terniflora var. garanbiensis	Hengchun, Pingtung County	100	2.7	+	−	−	−	−	−	−	−	−	−	14	0.33 ± 0.09	63 ± 29

Noted: *****: endemic species; LO: locality; EL: elevation; SD: stem diameter; PF: phloem fibers in dense bundles or strands; AR: arc-like bark; COG: cogwheel-like r... rhytidome; RHL: rhytidome layer number; RHT: rhytidome thickness; WER: wedge-like rays; ID: indentation of the axial parenchyma near the wider rays; VR: ve... dilatations; VB: number of vascular bundle; XW: xylem width (mean ± SD, n = xylem number); RW: ray width (mean ± SD, n = ray number); WR: wide rays initiate... initiated abruptly; RP: ring-porous vessel; SRP: semi-ring-porous vessel; DS: developmental stage (1: first stage, collenchyma or primary phloem fibres present... beneath the primary phloem, secondary phloem fibers present. 3: third stage, sequent periderm develop within the secondary phloem, wider rays and larger ve... sequent periderm developed in deeper layers in the secondary phloem, narrow rays present).

Characters Scientific name	LO	EL (m)	SD (mm)	PF	AR	COG	SE	RHL (no.)	RHT (mm)	WER	ID	VR	RD	VB (no.)	XW (mm)	RW (µm)
*C. terniflora var. garanbiensis	..	..	2.8	+	+	+	−	1	0.1−0.2	−	+	−	+	12	0.34 ± 0.05	77 ± 31
*C. terniflora var. garanbiensis	..	..	4.3	−	+	+	−	1−2	0.2−0.5	−	+	−	+	19	0.36 ± 0.10	77 ± 34
*C. tsugetorum	Hehuanshan, Nantou County	3200	3.9	−	−	−	−	−	−	−	−	+	−	17	0.17 ± 0.08	374 ± 131
C. uncinata var. okinawensis	Dahanshan, Pingtung County	900	4.0	+	−	−	−	−	−	−	−	−	−	14	0.31 ± 0.08	195 ± 84
C. uncinata var. okinawensis	..	..	9.7	−	+	+	−	1−3	0.5−1.9	−	+	−	+	12	0.12 ± 0.20	332 ± 83
C. uncinata var. uncinata	Syuejian, Miaoli County	2000	5.7	+	−	−	−	−	−	−	−	−	+	12	0.73 ± 0.07	260 ± 61
C. uncinata var. uncinata	Shihkejianshan, Pingtung County	1300	12.4	−	+	+	−	3−5	0.5−1.5	−	+	−	+	14	1.38 ± 0.19	386 ± 62
Sum				12	8	8	9			5	18	2	15			

Noted: *: endemic species; LO: locality; EL: elevation; SD: stem diameter; PF: phloem fibers in dense bundles or strands; AR: arc-like bark; COG: cogwheel-like rhytidome; RHL: rhytidome layer number; RHT: rhytidome thickness; WER: wedge-like rays; ID: indentation of the axial parenchyma near the wider rays; VR: vascular dilatations; VB: number of vascular bundle; XW: xylem width (mean ± SD, n = xylem number); RW: ray width (mean ± SD, n = ray number); WR: wide rays initiated abruptly; RP: ring-porous vessel; SRP: semi-ring-porous vessel; DS: developmental stage (1: first stage, collenchyma or primary phloem fibres present beneath the primary phloem, secondary phloem fibers present. 3: third stage, sequent periderm develop within the secondary phloem, wider rays and larger vessels sequent periderm developed in deeper layers in the secondary phloem, narrow rays present).

The ontogenetic stage of all 22 *Clematis* species was determined based on two lianas and one herb reported in Isnard et al. (2003a, b). During the first ontogenetic stage, stems have a cortex and a vascular cylinder, collenchyma and primary sclerenchyma fibers (primary phloem fibers) as a continuous ring or dense bundles in liana, but as separate bundle sheaths in herb. The primary phloem fibers are located between the collenchyma and secondary phloem. During the second stage, the stem has secondary tissue and an initial periderm (Esau 1958) formed beneath the primary phloem in liana, but cortex suberisation and parenchyma lignified in herb. Secondary phloem fibers are produced along the secondary phloem. In the third stage, sequent periderms develop within the secondary phloem; the cortex and primary phloem are detached into a ringbark or a continuous segment (Esau 1958); possess a wide ray and forms many larger vessels; secondary phloem fibers increases. In the last stage, sequent periderm is produced in progressively deeper layers in the secondary phloem; periderms are detached after forming decorticated tissues.

Results

Bark Morphologies

The anatomical and morphological details of stems for the 22 species investigated in this study are listed in Tables 2 and presented in Figs. 1 to 7. Seventeen species had wrinkled rhytidome; among them, nine had continuous segment rhytidome, and eight had cogwheel-like rhytidome, which peeled and teared easily. The rhytidome of *C. crassifolia* (Fig. 2A) was a continuous segment and was the thickest (1.8−3.4 mm), that of *C. lasiandra* (Fig. 3F) was cogwheel-like with 0.5−2.7 mm thickness (Table 2). The remaining five species, *C. akoensis* (Fig. 1B), *C. chinensis* var. *tatushanensis* (Fig. 1E), *C. psilandra* (Fig. 5C-D), *C. tashiroi* (Fig. 6B-D), and *C. tsugetorum* (Fig. 7B), were without rhytidome.

Cambial Variant Types

The stems of the 22 species investigated were shallowly grooved or angulated, and the stems were round (Fig. 1A-B) or hexagon and deeply grooved in shape (Figs. 2E, 3E, 5B, 5E) in the younger stem. Only *C. henryi* var. *morii* (Fig. 3D) was a lobe with asymmetrical conformation. The stem of *C. formosana* (Fig. 2C) was eccentric to oval or elliptical. *C. gouriana* subsp. *lishanensis* (Figs. 2D) formed discontinuous wedge-like phloem. The interfascicular cambia made the rays wider and wedge-like (Schweingruber et al. 2011); this character was apparent in five of the *Clematis* species studied, such as *C. akoensis* (Fig. 1B), *C. grata* (Fig. 3A), *C. henryi* var. *henryi* (Fig. 3B), *C. henryi* var. *morii* (Fig. 3D), and *C. pseudootophora* (Fig. 5B) (Table 2, WER column). For example, *C. henryi* var. *henryi* and *C. grata* had the widest rays, with a maximum width of 668 µm and 642 µm, respectively (Table 2, RW column). The rays size of five *Clematis* species, *C. chinensis* var. *chinensis* (Fig. 1D), *C. crassifolia* (Fig. 2A), *C. grata*, *C. leschenaultiana* (Fig. 4C), and *C. tashiroi* (Fig. 6D), became wider which called as abrupt wide rays which defined in Carlquist (1995). The remaining 13 *Clematis* species examined generally developed axial vascular elements in segments, and separated by the wider xylem and phloem rays, forming xylem in plate variant.

Variation in Vascular Elements

There was an obvious indentation in the region of the wider rays, except in *C. chinensis* var. *tatushanensis* (Fig. 1E), *C. montana* (Fig. 4E), *C. psilandra* (Fig. 5D), and *C. tsugetorum* (Fig. 7B). Perhaps, this is because the stem diameters of *C. chinensis* var. *tatushanensis*, *C. montana* were too small to develop wider rays. The wedge-shaped indentations developed in the interfascicular region were only found in *C. henryi* var. *morii* due to its thin-walled axial parenchyma adjacent to the thin-walled ray cells (Sieber and Kucera 1980). The axial parenchyma of *C. psilandra* and *C. tsugetorum* were very scarce, and vessel distribution was limited to the central portions of the fascicular area with growth rings. Almost all *Clematis* species in this study had semi-ring-porous vessels, except for *C. crassifolia* (Fig. 2A) and *C. lasiandra* (Fig. 3F), which had ring-porous vessels with distinct annual rings. None of the species had diffuse-porous vessels, which is consistent with previous reports (Schweingruber et al. 2011).

An arc-like bark developed in eight species, *C. chinensis* var. *chinensis* (Fig. 1D), *C. grata* (Fig. 2F, 3A), *C. lasiandra* (Fig. 3F), *C. pseudootophora* (Fig. 5B), *C. tamurae* (Fig. 6A), *C. terniflora var. garanbiensis* (Figs. 6E-F, 7A), *C. uncinata* var. *okinawensis* (Fig. 7C-D), and *C. uncinata* var. *uncinata* (Fig. 7E-F) (Table 2, AR column). *C. uncinata* var. *uncinata* (Fig. 7E-F) had a primary xylem ring located around the pith. The pith cavity formed in *C. parviloba* subsp. *bartlettii* (Fig. 4F, 5A), *C. psilandra* (Fig. 5C), *C. uncinata* var. *okinawensis* (Fig. 7C), and *C. uncinata* var. *uncinata* (Fig. 7F), among them, *C. uncinata* var. *uncinata* formed a hexagon shape.

Stem cross-sections showed that nine *Clematis* species formed one to three narrow rays within some secondary xylem tissues [*C. chinensis* var. *chinensis* (Fig. 1D), *C. formosana* (Fig. 2B-C), *C. gouriana* subsp. *lishanensis* (Figs. 2D), *C. grata* (Fig. 3A), *C. lasiandra* (Fig. 3F), *C. leschenaultiana* (Fig. 4C), *C. tashiroi* (Fig. 6D), *C. uncinata* var. *okinawensis* (Fig. 7C-D), and *C. uncinata* var. *uncinata* (Fig. 7E-F)] (Table 2, NR column).

In this study, the number of vascular bundles observed in *Clematis* species ranged from 6–21. Of which, three were classified as few type (14%), 13 as many type (59%), and six as central type (27%) (Table 2, VB column). Only one species, *C. pseudootophora* (Fig. 5B), had six vascular bundles.

Developmental Stages

Based on the characteristics observed in stem cross-sections of 42 samples from the 22 species, stem developments were divided into four ontogenetic stages (Table 2, DS column). If multiple samples of the same species were collected (which was the case for 15 species), each sample was investigated separately. Therefore, these species, such as *C. grata* (Figs. 2E-F, 3A) and *C. leschenaultiana* (Figs. 4A-C), were assigned one to four developmental stages. The remaining seven species with only one sample were assigned with one developmental stage. No rhytidomes were found in six samples consisting of three species, *C. psilandra* (Figs. 5C-D), *C. tashiroi* (Figs. 6B-D), and C. *tsugetorum* (Fig. 7B).

Nine samples consisting of nine species had dense or strand bundles of primary phloem fibers within the cortex and were identified as being in the first developmental stage (Figs. 1A, 1C, 1E, 1F, 2E, 5E, 6E, 7C, 7E). In the second stage, seven samples consisting of seven species had the ring-like initial periderm and secondary phloem fibers (Figs. 1B, 2B, 2F, 3E, 4E-F, 6F). In the third stage, seven samples consisting of seven species had loss of primary fibers; sequent periderms were produced within the secondary phloem; cork and non-conducting phloem were detached into a rhytidome; wide rays, larger vessels (Figs. 3B-C, 4A, 4D, 5A, 5B, 5F). In the fourth stage, thirteen samples consisting of twelve species had sequent periderms produced continuously with progressively deeper layers, and narrow rays were formed (Figs. 1D, 2A, 2C-D, 3A, 3D, 3F, 4B-C, 6A, 7A, 7D, 7F).

Rhytidomes were not observed in three samples of *C. tashiroi*, and its epidermis was deep green in color and glabrous; the stem cross-section was hexagon in shape. However, according to the stem features, three developmental stages of *C. tashiroi* can be divided. In the first stage, the stem had primary phloem fibers as strands along the secondary phloem (Figs. 6B). In the second stage, secondary phloem became triangular with secondary phloem fibers, and a wider ray and many larger vessels were formed (Figs. 6C). In the third stage, rays became unequal in width, and narrow rays were formed (Figs. 6D).

Bracket key to 22 clematis species

A key to the 22 C*lematis* species in Taiwan, based on the characteristics of bark and vascular bundles, is provided below.

1. Vessels restricted to the central portions of the fascicular area…………………………2

1. Vessels unrestricted to the central portions of the fascicular area……………………3

2. Vascular bundles (20), pith cavity……………………………… *Clematis psilandra*

2. Vascular bundles (17), pith without cavity………………………*Clematis tsugetorum*

3. Wedge-like phloem …………………………*Clematis gouriana* subsp. *lishanensis*

3. Non-wedge-like phloem …………………………………………… …………..4

4. Wedge-like rays …. …………………………………………………………………5

4. Non-wedge-like rays………………………………. …………………………9

5. Vascular bundles (6)…………………. ………………*Clematis pseudootophora*

5. Vascular bundles (> 6)……………………………………………………………6

6. Arc-like bark …………………………………………. …………*Clematis grata*

6. Non-arc-like bark …. …………………………………………. …………………7

7. Stem polygonous lobe………………………………… *Clematis henryi* var. *morii*

7. Stem round……………………………………………………………8

8. Bark without rhytidome layers………………………………*Clematis akoensis*

8. Bark with one to three rhytidome layers…………*Clematis henryi* var. *henryi*

9. Bark without rhytidome layers……………………………………………10

9. Bark with one to five rhytidome layers……………………………………11

10. Vascular bundles (20)………………… *Clematis chinensis var. tatushanensis*

10. Vascular bundles (14–20)…………………………………*Clematis tashiroi*

11. Pith cavity…………………………………*Clematis parviloba* subsp. *bartlettii*

11. Pith without cavity……………………………………………………12

12. Non-arc-like bark…………………………………………………………13

12. Bark arc-like……………………………………………………17

13. Vascular bundles (12)………………………………… ………………………14

13. Vascular bundles (16–21)……………………………………… …………………15

14. Stem, eccentric to oval or elliptical……………………………*Clematis formosana*

14. Stem, round……………………………………………*Clematis montana*

15. Rhytidome thickness (0.4–0.8 mm)……………………*Clematis meyeniana*

15. Rhytidome thickness (> 0.9 mm)………………………………… ……………16

16. Rhytidome thickness (0.2–1.6 mm)…………………*Clematis leschenaultiana*

16. Rhytidome thickness (1.8–3.4 mm)………………………*Clematis crassifolia*

17. Vessels, ring-porous………………………………………… *Clematis lasiandra*

17. Vessels, semi-ring-porous…………………………………………………18

18. Pith cavity, hexagon in shape………………*Clematis uncinata* var. *uncinata*

18. Pith without cavity………………………………………………………19

19. Vascular bundles (20–22) …………………*Clematis chinensis* var. *chinensis*

19. Vascular bundles (12–19)……………………………………………20

20. Constant vascular bundles (12)………………………………*Clematis tamurae*

20. Varying vascular bundles………………………………… ………………21

21. Narrow rays not formed………………*Clematis terniflora* var. *garanbiensis*

21. Narrow rays approximately 13…………*Clematis uncinata* var. *okinawensis*

Discussion

The rhytidome is one of the diagnostic characteristics of the *Clematis* genus. The texture and detachment of the rhytidome result from an irregular appearance in the developmental stages. It is easier to correctly identify the structure of rhytidome from fresh materials than from dry materials. Among the 22 species, the rhytidome was not observed in *C. akoensis*, *C. chinensis* var. *tatushanensis*, *C. psilandra, C. tashiroi,* and *C. tsugetorum* (Table 2) due to their smaller stem diameters or earlier production of periderm establishing inner to the cortex. The stem diameters of some *Clematis* species were smaller than 8.8 mm and can form a rhytidome, but that of *C. tashiroi* was approximately 17.8 mm and did not appear to have a rhytidome. We simply suggested that rhytidome was not observed even in some species with smaller stems already having this feature. Perhaps there is an evolutionary mechanism (e.g., heterochrony) leading to the delayed formation of rhytidome in *C. tashiroi.* However, their bark may continue to develop into one of the rhytidome types; therefore, continued observations are necessary.

The stem cross-section of *C. henryi* var. *morii* and *C. formosana* were a lobe with asymmetrical conformation and eccentric to oval or elliptical, respectively. This generates as an irregular conformation, found in some Apocynaceae and Malpighiaceae (Angyalossy et al. 2015). *Clematis gouriana* subsp. *lishanensis* forms discontinuous wedge-like phloem, a common characteristic of the family Bignoniaceae (Pace et al. 2011). In summary, except for xylem in plate variant, two cambial variants are found in the Ranunculaceae family—stems with an irregular conformation, and phloem arcs/wedges.

Indentation toward the pith in the wider rays region is obvious in *Clematis* species (Sieber and Kucera 1980; Carlquist 1995). This feature was found in most *Clematis* species investigated in this study, except for those with a small stem diameter (Table 2, ID column). The bark texture formed an arc shape outside the fascicular region in *C. vitalba* (Sieber and Kucera 1980); this feature was also found in eight species apparently (Table 2, AR column). The arc feature comprised several layers of periderm combined with the non-conducting phloem (e.g., Fig. 1D. The characteristic of arc-like bark indeed is diagnostic evidence for *Clematis* species comparison.

The vessel restriction pattern was found in *Xanthorhiza apiifolia* of Ranunculaceae (Carlquist 1995), *Valeriana* of Valerianaceae (Carlquist 1983), *Launea* of Astraceae (Carlquist 1988), and *Dicentra* and *Hunnemannia* of Papaveraceae. This pattern appeared in *Clematis psilandra* (Fig. 5C-D) and *C. tsugetorum* (Fig. 7B) studied, but few vessels were in contact with the rays; this phenomenon also appeared in the *Clematis* genus (Carlquist and Zona 1988). Carlquist and Zona (1988) suggested that the species clearly showed vessel restriction patterns; stems had limited diameter and duration, and they were either woody herbs or short-lived shrubs. This unusual wood feature of *Clematis psilandra* and *C. tsugetorum* was useful as a potential indicator of relationship (Carlquist 1995).

Clematis species often have 12 vascular bundles, with Smith (1928) finding this feature in 67% of 138 species studied. This is generally considered the central type for this genus. In Taiwan, only 27% of the 22 *Clematis* species had 12 vascular bundles. Five of the species in our study had inconsistent numbers of vascular bundles compared with the findings of Smith (1928). In our study, *Clematis henryi* var. *henryi* (Fig. 3B) had 10 vascular bundles, but only six were recorded by Smith (1928). Moreover, *C. chinensis* var. *chinensis* (Fig. 1C-D) had 20 – 23 bundles, *C. lasiandra* (Fig. 3F) had 12 – 14, *C. meyeniana* (Fig. 4D) had 21, and both *C. uncinata* var. *okinawensis* (Fig. 7C, D) and *C. uncinata* var. *uncinata* (Fig. 7E, F) had 12–14, but Smith (1928) recorded only 12 vascular bundles in these species. The inconsistent results might be due to different sample sizes, or developmental stages; further investigation is required to elucidate this discrepancy.

Vascular bundle numbers are usually constant for a given species, but the numbers can vary in some species. We examined three samples of *C. leschenaultiana* (Fig. 4A-C) collected from the same branch, finding that the number of vascular bundles varied in different growth stages. The vascular bundle number of *C. leschenaultiana* is from 21 (Figs. 4A) to 16 (Figs. 4B) to 19 (Figs. 4C). We checked that within 21 vascular bundles in the second stage, 10 might aggregate closely into five groups, each group was composed of a big and a small vascular bundle, and the vascular bundle seems to be 16. In the fourth stage, some groups gradually separated, the number of vascular bundles seems to change to be 19. These processes could indicate that the bigger vascular bundle and fascicular zone were more active than the smaller vascular bundle and interfascicular zone. To confirm this, future work should focus on collecting and observing more specimens from different stem positions of the same species.

Clematis henryi is taxonomically treated as *C. henryi* var. *henryi* and *C. henryi* var. *morii* (Yang and Huang 1996), but *C. henryi* var. *morii* is sometimes treated as a species, *C. morii* (Wang and Bartholomew 2007). In this study, these two taxa had wedge-like rays and a continuous rhytidome segment, but the stem of *C. henryi* var. *henryi* was round while that of *C. henryi* var. *morii* was an irregular conformation variant. These stem shape characteristics provide a way to distinguish these two taxa.

The site of phellogen initiation is often in an outer layer of cortical parenchyma cells, one or two layers beneath the epidermis (Beck, 2011). However, the phellogen location was highly variable and formed deep within the cortex, in the outer secondary phloem, or even within the secondary phloem (Isnard et al. 2003a), such as the *Clematis* genus. Compared with the characteristics of the developmental stage described in Isnard et al. (2003a, b), some features were different or could not be found in the present study. In the first stage, the stem had the primary phloem fibers in dense bundles or strands, not in a continuous ring; and in the fourth stage, narrow rays were found.

The one to two samples of two shrub species, *C. psilandra*, and *C. tsugetorum*, were not enough to assess their developmental stages. Assessing their development stage by the features of lianas and herb (Isnard et al. 2003a, b), we suggested they should belong to the fourth stage due to develop the narrow rays. However, further study collecting specimens of these species and examining their characteristics of different developmental stages is needed, especially species without the rhytidome.

In the present study, different narrow rays were observed in nine *Clematis* species (Table 2, NR column). These rays had been recorded in the species *Aristolochia macrophylla* (Aristolochiaceae) (Carlquist 1993; Schweingruber et al. 2011), in the Cucurbitaceae family (Carlquist 1992), and the *Cyclea* genus (Menispermaceae) (Yang and Chen 2016). The characteristics of narrow rays and activity of the bigger vascular bundles and the fascicular areas of *C. grata* are similar to those of *C. vitalba*, showing that they could be related to stem bark thickness and the formation of arc-like or cogwheel-like bark. However, the narrow rays were not found in all of the species investigated in our study, and its presence might be related to stem diameter size, but this needs further investigation.

The pith cavity resulted from non-lignified walls in the inner-most pith parenchyma cells; this characteristic was found in four species, *Clematis parviloba* subsp. *bartlettii*, *C. psilandra*, *C. uncinata* var. *okinawensis*, and *C. uncinata* var. *uncinata.*

Moreover, the stem features of the *Clematis* genus, such as the arc-like bark, rhytidome, wedge-like phloem, ray, and indentations, restricted vessel pattern, secondary phloem fibers, and narrow rays, are very important diagnostic characteristics used to identify the species in the *Clematis* genus. The stem anatomy of the 22 *Clematis* species described here provided evidence for systematic problems within this genus.

Conclusions

The objectives of this study were to understand the stem anatomy of the 22 Taiwanese *Clematis* species in the Ranunculaceae family. Our results showed that due to successive layers of periderm combined with non-conducting phloem, the cogwheel-like and continuous segments rhytidome were formed in most *Clematis* species. Three cambial variants of the *Clematis* genus were found—stems with an irregular conformation, wedge-like phloem, and xylem in plate. Most *Clematis* species had the indentation of the axial parenchyma near the wider rays, ray dilatation, and varied in the numbers and sizes of vascular bundles. The vessel restriction pattern was found in two shrubs, *C. psilandra* and *C. tsugetorum*, which were different from the other vine *Clematis* species. The narrow rays occurred in some secondary xylem, and no diffuse-porous vessels dispersed throughout the stems. Further fresh materials collection and observations of different developmental stages are still needed. In conclusion, the data presented here provide important basic information on lianas addressing *Clematis* taxonomy to understand their morphology and ensure the conservation of their diversity.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests

Authors' contributions

SZY conceived of and designed the experiments and wrote the paper. PHC conducted the fieldwork and collected the plant specimens and performed the taxonomical study. CFC analyzed and interpreted the plants growth stage regarding the patterns of secondary growth of stem cross-section. Three authors read and approved the final manuscript

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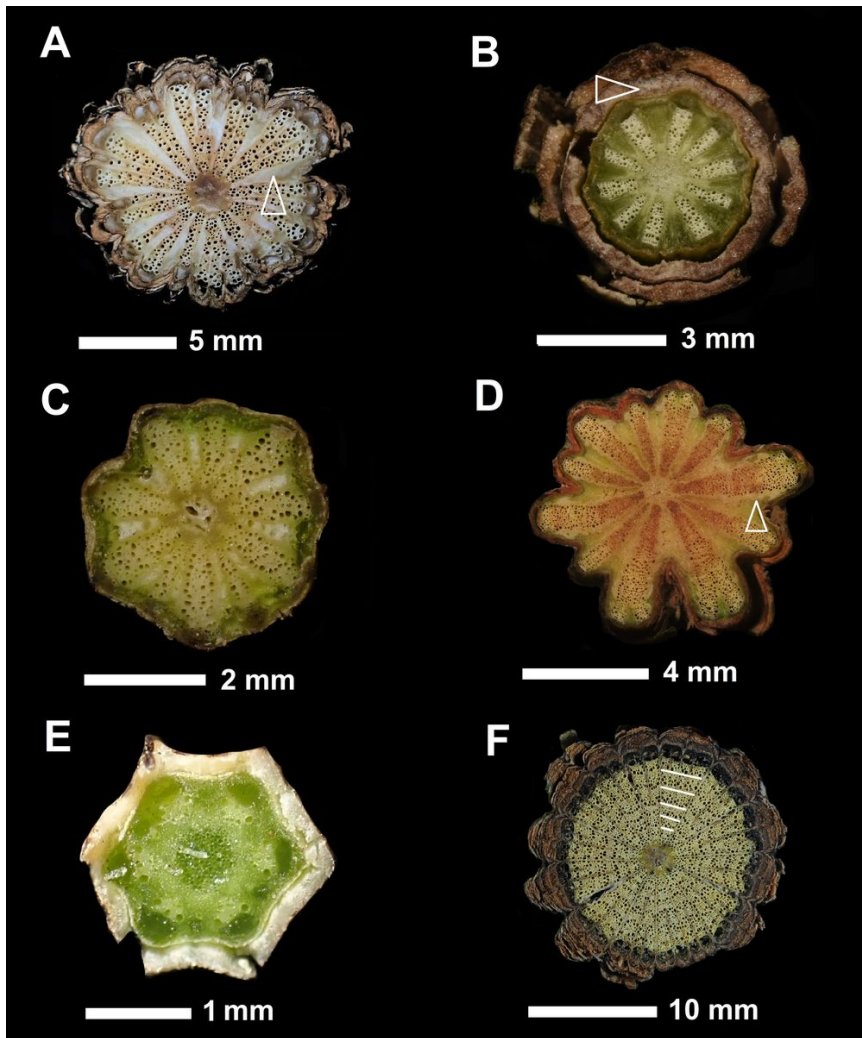
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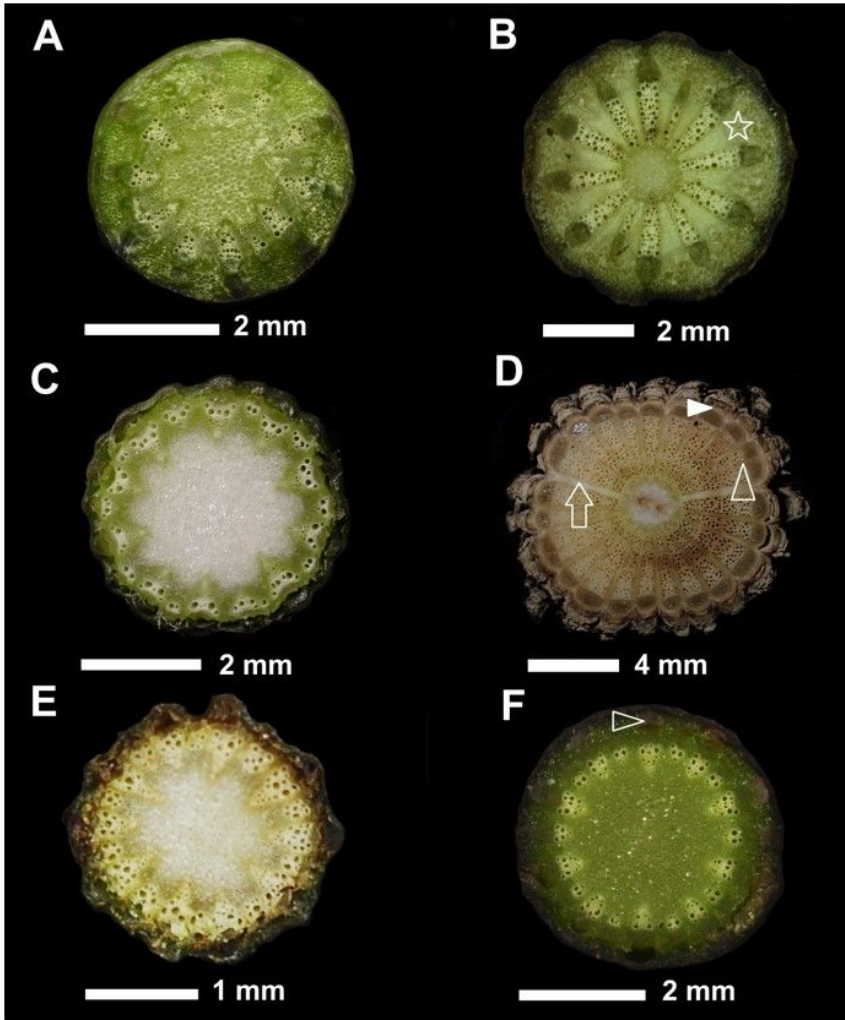
Figure 1

Stem transverse sections of Clematis species. A. C. akoensis; collenchyma strands and primary phloem fibers in dense bundles. B. C. akoensis; secondary phloem triangular in shape, wedge-like rays (open star). C. C. chinensis var. chinensis; phloem fiber strands, 10 big and 10 small vascular bundles. D. C. chinensis var. chinensis; cogwheel-like rhytidome with 3–4 layers, arc of bark (solid arrowhead), sequent periderm above semi-circular secondary phloem, 22 vascular bundles, 3 narrow rays (open arrowhead), two wide rays (open arrow). E. C. chinensis var. tatushanensis; stem with 10 shallow grooves, 10 big and 10 small vascular bundles. F. C. crassifolia; primary phloem fibers in dense bundles (open arrowhead), 10 big and 10 small vascular bundles.
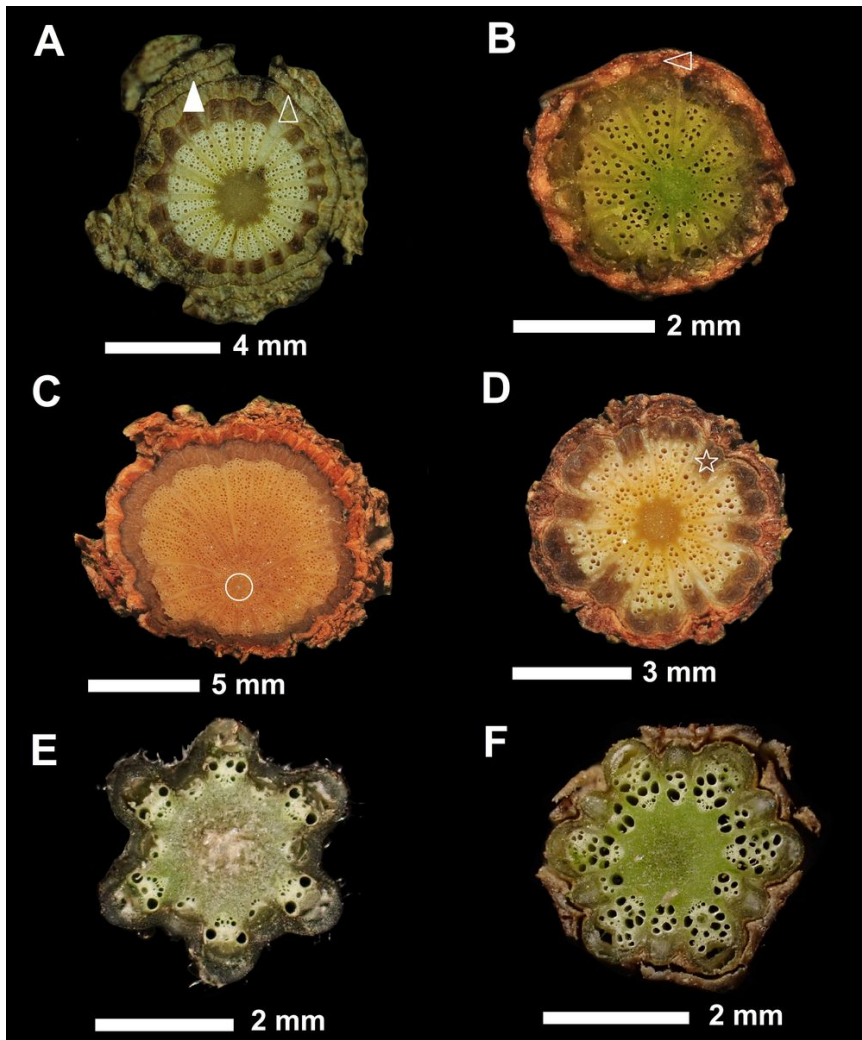
Figure 2

Stem transverse sections of Clematis species. A. C. crassifolia; sequent periderm produced in deeper layers within the secondary phloem, continuous segment rhytidome with 3–6 layers, each layer composed periderm (open arrowhead) and non-conducting phloem (solid arrowhead), ray dilatation, 6 wide rays. B. C. formosana; secondary phloem fiber strands (open arrowhead). C. C. formosana; secondary phloem fibers strands, black, continuous segment rhytidome with 2 layers, 32 narrow rays, eccentric pith (circle). D. C. gouriana subsp. lishanensis; ring-like sequent periderm, discontinuous wedge-like phloem (star), 12 wide rays, 14 narrow rays. E. C. grata; stem with 6 shallow grooves, phloem fiber in dense bundles beneath the angular extension of stem, 6 big and 6 small vascular bundles. F. C. grata; ring-like initial periderm, yellow, cogwheel-like rhytidome with 1 layer.

Figure 3

Stem transverse sections of Clematis species. A. C. grata; ray dilatation, wedge-like rays, 14 wide rays (open arrowhead), 11 narrow rays. B. C. henryi var. henryi; ring-like sequent periderm (open arrowhead), continuous segment rhytidome with 2 layers, wedge-like rays. C. C. henryi var. morii; stem shallowly lobed, wedge-like rays, ray dilatation, indentation near the wider rays, 10 vascular bundles. D. C. henryi var. morii; stem irregularly lobed, wedge-like rays, wedge-like indentation near the wider rays (open arrowhead), 11 vascular bundles. E. C. lasiandra; hexagon-shaped stem, 2 mm in diameter, 12 vascular bundles. F. C. lasiandra; arc-like bark, cogwheel-like rhytidome with 2–5 layers, 30 narrow rays, ring-porous vessels (lines).
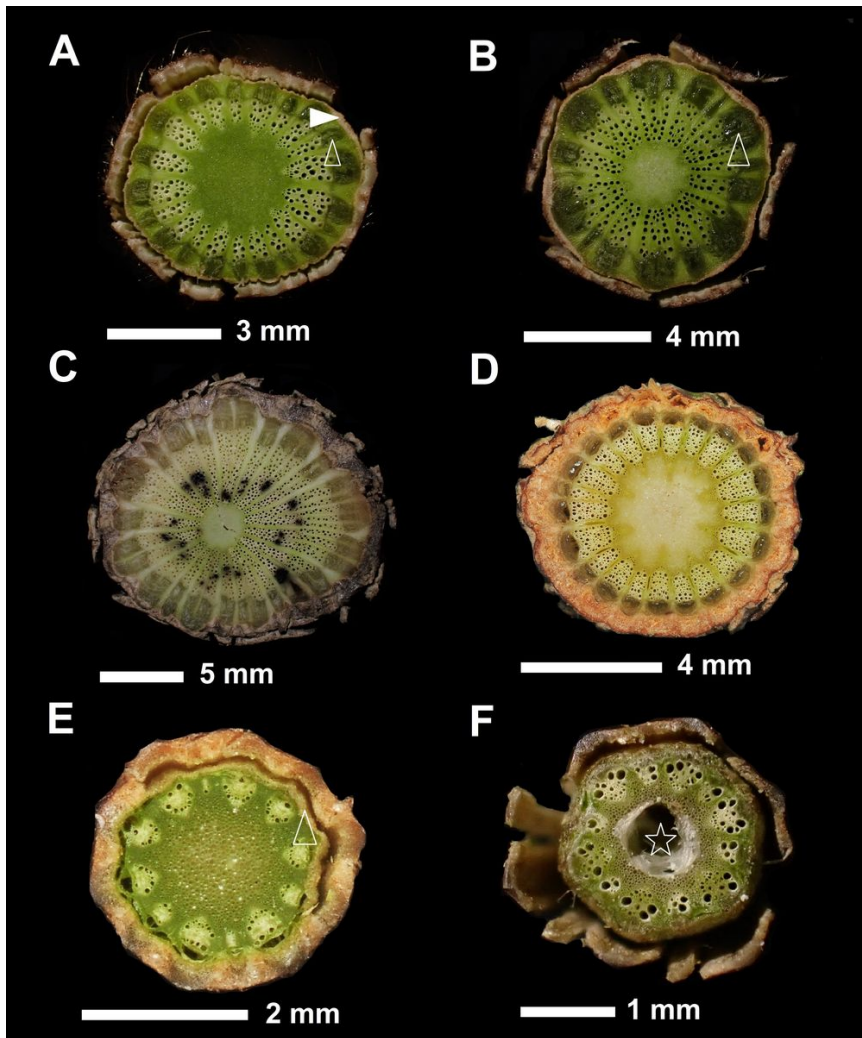
Figure 4

Stem transverse sections of Clematis species. A. C. leschenaultiana; semi-circular secondary phloem (open arrowhead), sequent periderm (solid arrowhead), continuous segment rhytidome with 2 layers, ray dilatation, 21 vascular bundles. B. C. leschenaultiana; aggregated secondary phloem (open arrowhead), 6 narrow rays, 16 vascular bundles. C. C. leschenaultiana; sequent periderms within the secondary phloem, continuous segment rhytidome with 4 layers, ray dilatation, 15 narrow rays, 19 vascular bundles. D. C. meyeniana; continuous segment rhytidome with 3 layer, sequent periderm within the secondary phloem, ray dilatation, indentation near the wider rays. E. C. montana; primary phloem fibers strands, ring-like initial periderm (open arrowhead), yellow. F. C. parviloba subsp. bartlettii; continuous segment rhytidome with 1 layer, 6 big and 6 small vascular bundles, pith cavity (open star).

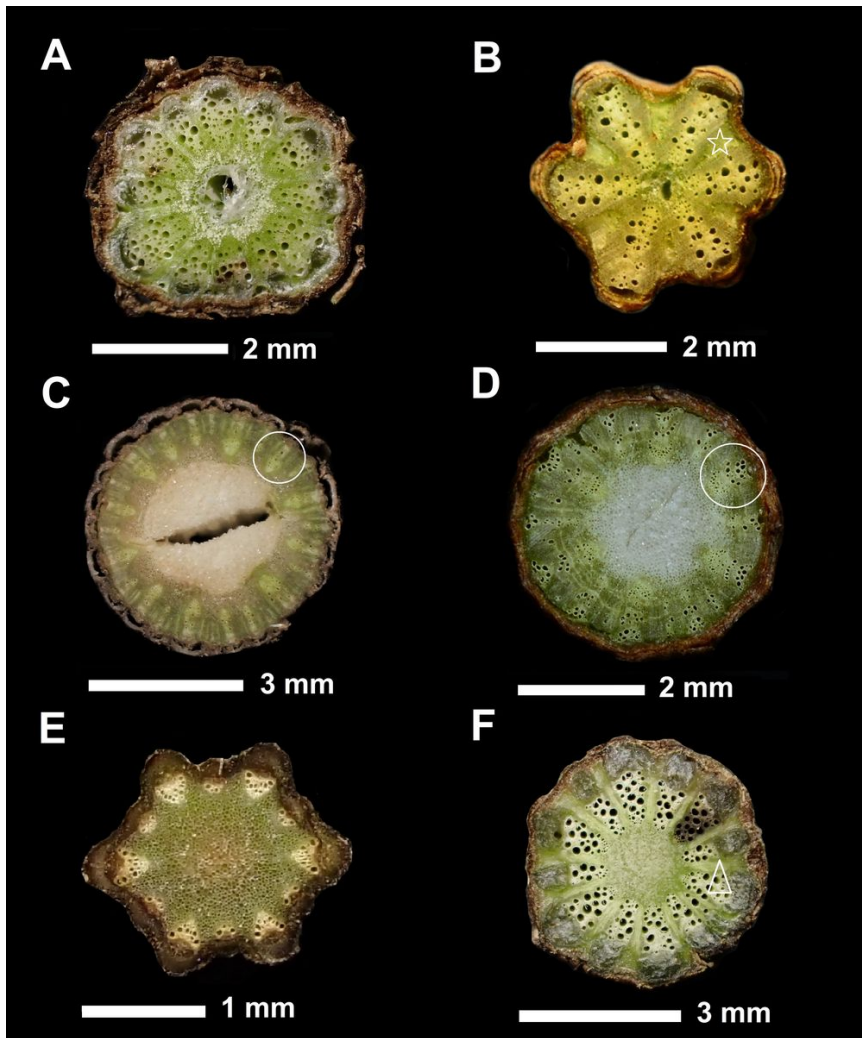**Figure 5**

Stem transverse sections of Clematis species. A. C. parviloba subsp. bartlettii: ring-like sequent periderm, continuous segment rhytidome with 2 layers. B. C. psendotophora; stem with 6 shallow grooves, cogwheel-like rhytidome with 2 layers, wedge-like rays (open star), 6 vascular bundles. C-D. C. psilandra; vessel limited to the central portions of fascicular area (circle), 20–23 vessel bundles, pith cavity. E. C. tamurae; stem with 6 shallow grooves, primary phloem fiber ring-like, 6 big and 6 small vascular bundles. F. C. tamurae; secondary phloem dark-green and rectangular, ring-like sequent periderm, cogwheel-like rhytidome with 1 layer, ray dilatation, indentation of the axial parenchyma near the wider rays (open arrowhead).
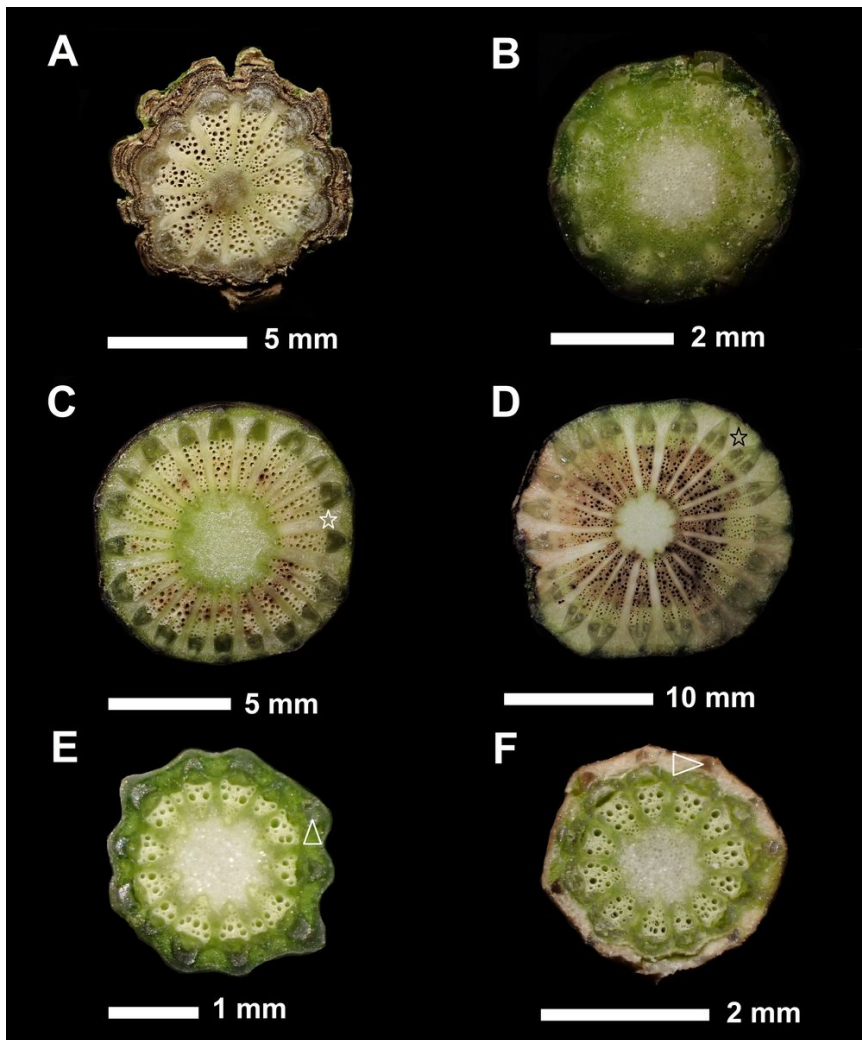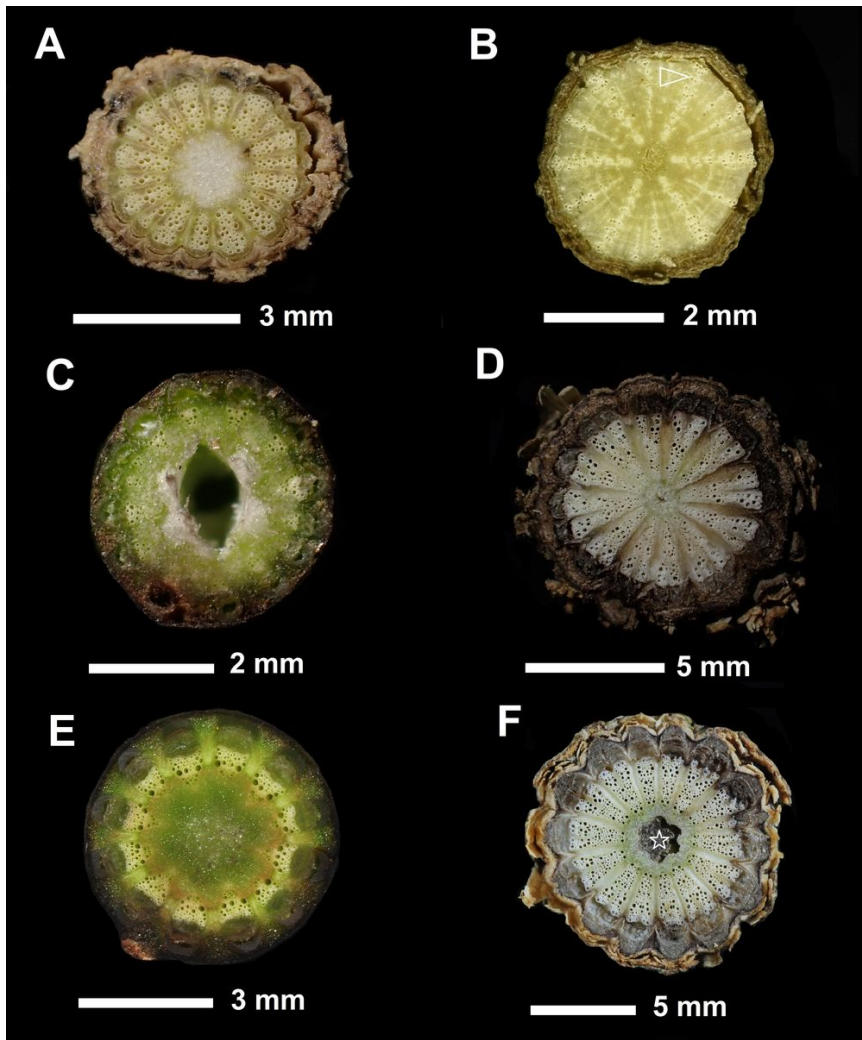
Figure 6

Stem transverse sections of Clematis species. A. C. tamurae; arc-like bark, sequent periderm within the secondary phloem, cogwheel-like rhytidome with 3–4 layers, ray dilatation, indentation near the wider rays. B. C. tashiroi; primary phloem fiber stands, 14 vascular bundles. C. C. tashiroi; secondary phloem triangular in shape and deep-green, ray dilatation, 4 wide rays (open star), 6 narrow rays. D. C. tashiroi; obvious ray dilatation (open star), 6 wide rays, 18 narrow rays. E. C. terniflora var. garanbiensis; collenchyma strands, primary phloem fibers bundles (open arrowhead), 14 vascular bundles. F. C. terniflora var. garanbiensis; primary phloem fibers triangular (open arrowhead), black, ring-like initial periderm.

Figure 7

Stem transverse sections of Clematis species. A. C. terniflora var. garanbiensis; sequent periderm in the secondary phloem, cogwheel-like rhytidome with 2 layers. B. C. tsugetorum; vessels restricted to the central portions of fascicular area, vessel as growth rings (open arrowhead). C. C. uncinata var. okinawensis; primary phloem fibers strands, pith cavity. D. C. uncinata var. okinawensis; arc-like bark, cogwheel-like rhytidome with 1-3 layers, ray dilatation, 13 narrow rays. E. C. uncinata var. uncinata; primary phloem fibers strands, ray dilatation. F. C. uncinata var. uncinata; arc-like bark, 11 narrow rays, pith cavity, hexagon in shape (open star), primary xylem ring located around the pith.