Bark and Cambial Variation in the Genus Clematis (Ranunculaceae) in Taiwan

Sheng-Zehn Yang (yangsz@mail.npust.edu.tw)
National Pingtung University of Science and Technology https://orcid.org/0000-0001-8648-7507
Po-Hao Chen
Graduate Institute of bioresources
Chien-Fan Chen
Taiwan Forestry Research Institute

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Abstract

Background

Studies on the anatomical characteristics of stems of Taiwanese species from the *Clematis* genus (Ranunculaceae) are scarce. The aim of this study was to investigate and compare cambial variation in stems of 22 *Clematis* species.

Results

The rhytidome (outer bark) was either cogwheel-like or continuous, except for in the species *Clematis tashiroi*. Key features of the genus were eccentric to elliptical or polygonous-lobed stems, wedge-like phloem, wedge-like rays, indentations in the axial parenchyma, and ray dilatation. The cortical sclerenchyma fibers were embedded in the phloem rays with approximately 23% of the *Clematis* species. Both *C. psilandra* and *C. tsugetorum* had restricted vessels. There were three vascular bundle patterns, 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. Only two species had a primary xylem ring located around the pith. Secondary xylem rays split the secondary xylem into parts, increasing stem diameter. The developmental stage of each sample was determined, with the initial ring-like periderm being produced in the primary phloem during the second stage.

Conclusions

The cambial variations 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. All parenchyma cells in climbing plants can be redifferentiated into meristematic cells, which may give rise to vascular bundles, 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 1991a; Rajput et al. 2014) as diverse cambial variants are formed. One cambial variant consists of xylem parts that are separated by wider rays (the 'xylem in plate' variant). 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), 22 of which 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; Rajput and Gondaliya 2017; Rajput et al. 2017).

Previous anatomical studies of Ranunculaceae have mainly concentrated on the genus *Clematis* (Gregory 1994; Smith 1928; Carlquist 1995; Sieber and Kucera 1980). 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). Secondary xylem rays are initiated in *C. flammula*, *C. hirsutissima*, and *C. viticella* (Isnard et al. 2003b; Schweingruber et al. 2011). Interfascicular cambia ray width increases with increasing stem diameter in *C. alpina* and *C. viticella* (Isnard et al. 2003b), and rays are wedge-like in shape (Schweingruber et al. 2011). In *C. alpina* and *C. vitalba*, the phellogen produces cork cells and the phellem (cork) layers outside the phloem form rhytidome (outer bark), which is composed of cork and dead phloem (Sieber and Kucera 1980). In *C. vitalba*, 12 vascular bundles are divided into two types in the primary state, the pith cavity forms, secondary phloem are composed of parenchyma cells, an arc of sclerenchyma phloem fibers develop, and rhytidome appears in a cogwheel-like form (Sieber and Kucera 1980).

In *C.vitalba*, the cambium is dented towards the pith in the region of the broad rays (Sieber and Kucera 1980), and in *C. alpina* and *C. pickerringii* the fascicular areas always have indentations (Carlquist 1995). The indentations are strands of thin-walled axial parenchyma near the broader rays. Isnard et al. (2003a, 2003b) defined four developmental stages of *C. flammula* var. *maritima*, *C. recta*, and *C. vitalba* by the appearance of cambial characteristics, such as the initial periderms and phloem fibers.

The xylem in plate variant is one of the cambial variants found in the Ranunculaceae (Yang and Chen 2015), but other stem characteristics of the *Clematis* genus in Taiwan have not been described. As cambial variations 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 Ranunculaceae family in Taiwan.

Methods

Research Materials

Multiple samples of 22 *Clematis* species of the family Ranunculaceae recorded in the Flora of Taiwan (Yang and Huang 1996) 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, and collection locality. The voucher species information of all *Clematis* species is presented in Table 1. Approximately 48% of the species were endemic to Taiwan. Among them, *C. psilandra* and *C. tsugetorum* are shrubs growing at high elevations of approximately 2,000 m and 3,500 m, respectively. The remaining 20 species are climbing vines in which the species *C. montana* grows at the highest elevation, approximately 3,600 m. *Clematis pseudootophora* is a herb and rarity in the field. This species consists of only a few populations located at an elevation of 1,500–1,900 m, in eastern Taiwan.

Research Methods

Multiple samples of each species were collected, and stems with thick bark and visible secondary growth characteristics were selected in the field. To keep the material 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 to accurately assess the position of various vascular bundle tissues. One or two samples with obvious and easy-to-observe cambial variations were selected per species for photographs and the scoring of morphological characteristics. Cambial variations 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 anomalous structures of each species were investigated: stem diameter (mm); cortex (+/−); sclerenchyma fibers, including primary phloem fibers or secondary phloem fibers (+/−); cogwheel-like rhytidome (+/−); continuous segment rhytidome (+/−); rhytidome layer number; rhytidome thickness (mm); wedge-like rays (+/−); wedge-like phloem (+/−); indentation of the axial parenchyma near the wider rays (+/−); cortical sclerenchyma connected with the plate of sclerenchyma fibers embedded in the phloem rays (+/−); restricted vessel pattern (+/−); ray dilatations (+/−); vascular bundle number; mean width of xylem (mm) (mean ± SD, n = xylem number); mean width of primary ray (μm) (mean ± SD, n = ray number); secondary xylem rays (+/−); ring-porous vessels (+/−); semi-ring-porous vessels (+/−); pith cavity (+/−); and developmental stage. The vessel restriction pattern, ray types, and axial parenchyma types were classified based on Carlquist (1988) and the IAWA Committee on Nomenclature (1964).

The ontogenetic stage of all 22 *Clematis* species was determined based on Isnard et al. (2003a, b). During the first ontogenetic stage, stems have an epidermis, a cortex, a vascular cylinder ring, and dense or strand bundles of primary phloem fibers. During the second stage, stems have an initial periderm that is linked with dead and collapsed cortical parenchyma, and secondary phloem fibers formed from the vascular cambium. In the third stage, sequent periderms develop, which initiate within the secondary phloem; the cortex and primary phloem are detached into a continuous bark segment or into cogwheel-like bark; and a wider ray and many larger vessels are formed. In the last stage, sequent periderm is produced in progressively deeper layers in the secondary phloem, periderms are detached after forming decorticated tissue (rhytidome), and secondary xylem rays are formed.

Results

Bark Morphologies

The anatomical and morphological details of stems for the 22 species investigated in this study are listed in Tables 2 and 3 and are presented in Figures 1 to 8. The stem bark contained inner bark and rhytidome (outer bark). The inner bark was located under the rhytidome and comprised the cortex and secondary phloem. Among the 22 species investigated, only *C. akoensis* (Fig. 1B), *C. chinensis* var. *tatushanensis* (Fig. 1E), and *C. tashiroi* (Fig. 7A, B) had inner bark due to their smaller stem diameters (3.8–6.0 mm) (Table 2). The bark of *C. tashiroi* was deep green in color and glabrous without any rhytidome in spite of large stem sizes (4.0–17.8 mm), and its stem cross-section was hexagonous. Rhytidome is comprised of successive cork and dead phloem to form dead outer bark. According to the arrangement and detachment degree of rhytidome, it can be divided into two forms: cogwheel-like rhytidome (ring bark) and continuous segment rhytidome (scale bark) (Esau 1958; Sieber and Kucera 1980; Evert 2006). The rhytidome did not last long on the bark in the 22 *Clematis* species. Nineteen species had wrinkled rhytidome; among them, 11 species had continuous segment rhytidome and eight species had cogwheel-like rhytidome, which peeled and teared easily. The rhytidome of *C. crassifolia* (Fig. 2B) was continuous segment and was the thickest (1.8–3.4 mm). That of *C. lasiandra* (Fig. 3E) was cogwheel-like and 0.5–2.7 mm in thickness. The remaining species were thinner than the above two species.

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. 2F, 6A, 6D). Only *C. henryi* var. *morii* (Fig. 3F) had an irregular conformation, forming a deeply polygonous lobe. The stem of *C. formosana* (Fig. 2D) was eccentric to oval or elliptical at the last stage. The *Clematis* stems examined generally developed axial vascular elements in segments, and the xylem were separated by wider rays, forming the xylem in plate type. This type is derived from a single cambium according to Angyalossy et al. (2012). Except for the xylem in plate type, *C. gouriana* subsp. *lishanensis* (Figs. 2D) formed discontinuous wedge-like phloem.

Variation in Vascular Elements

The secondary rays were always linear, but that of *C. akoensis* (Fig. 1B), *C. grata* (Fig. 3C), *C. henryi* var. *henryi* (Fig. 3D), *C. henryi* var. *morii*, *C. pseudootophora* (Fig. 6A), and *C. tsugetorum* (Fig. 8B) were wedge-like (Table 2, RWL column). The interfascicular cambia made the rays of *C. alpina* wider and wedge-like (Schweingruber et al. 2011); this character was apparent in six of the *Clematis* species studied. The wedge-like ray form could be referred from the average width of the primary rays. For example, *C. henryi* var. *henryi* and *C. grata* had the widest primary rays, with a maximum width of 668 μm and 642 μm, respectively.

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 (Carlquist 1995). The fascicular areas of stem cross-sections of *Clematis* species were investigated. There was an obvious indentation in the region of the wider rays (Fig. 5B), except in *C. akoensis* (Fig. 1B), *C. chinensis* var. *tatushanensis* (Fig. 1E), *C. montana* (Fig. 5C), *C. psilandra* (Fig. 6C), and *C. tsugetorum* (Fig. 8B). This is because the stem diameters of these species were too small to develop wider rays. The cortical sclerenchyma fibers of five species, *C. chinensis* var. *chinensis* (Fig. 1C, D), *C. grata* (Fig. 3C), *C. lasiandra* (Fig. 4B), *C. uncinata* var. *okinawensis* (Fig. 8C, D), and *C. uncinata* var. *uncinata* (Fig. 8E, F), were connected with the plate of sclerenchyma fibers that were embedded in the phloem rays and formed an arc outside the fascicular regions. The axial parenchyma of *C. psilandra* (Fig. 5E) and *C. tsugetorum* (Fig. 8B) were very scarce, and vessel distribution was limited to the central portions of the fascicular xylem area with growth rings. The vessels of the remaining 20 species were distributed along the edge of the fascicular areas.

Species were grouped based on the number of vascular bundles they contained, with the 'central type' having 12 bundles, the 'many type' having >12 bundles, and the 'few type' having <12 bundles (Smith 1928). In this study, the number of vascular bundles observed in *Clematis* species ranged from 6–21. Among them, three species were classified as few type (14%), 13 species were classified as many type (59%), six species were classified as central type (27%) (Table 2, VB column). Only one species, *C. pseudootophora* (Fig. 6A), had six vascular bundles (Table 2).

The *Clematis* species in this study almost all had semi-ring-porous vessels, except for *C. crassifolia* (Fig. 2B), *C. lasiandra* (Fig. 4B), *C. psilandra* (Fig. 6C), and *C. tsugetorum* (Fig. 8B), which had ring-porous vessels with distinct annual rings. None of the species had diffuse-porous vessels. *Clematis parviloba* subsp. *bartlettii* (Fig. 5E) and *C. uncinata* var. *uncinata* (Fig. 8E, F) had a primary xylem ring located around the pith.

The secondary xylem rays evolve near the periphery and split the secondary xylem by parenchyma proliferation, which can continuously increase stem diameters (Schweingruber et al. 2011). Stem cross-sections showed that eight *Clematis* species formed one to three secondary xylem rays within some vascular bundles [*C. chinensis* var. *chinensis* (Fig. 1D), *C. formosana* (Fig. 2C, D), *C. gouriana* subsp. *lishanensis* (Figs. 2E), *C. grata* (Fig. 3B, C), *C. lasiandra* (Fig. 4B), *C. leschenaultiana* (Fig. 5A), *C. tashiroi* (Fig. 7D), *C. uncinata* var. *okinawensis* (Fig. 8C, D), and *C. uncinata* var. *uncinata* (Fig. 8E, F)] (Table 2, SR column). The remaining 14 *Clematis* species had thick-walled cells with lignified walls.

Developmental Stages

Based on the characteristics observed in stem cross-sections of 50 samples from 22 species, samples were divided into four ontogenetic stages (Table 2). If multiple samples were collected of the same species (which was the case for 16 species), each sample was investigated separately. Therefore, these species were assigned 2–3 developmental stages.

Twelve 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, 1F, 2C, 2F, 4C, 6D, 7A, 7E, 8C, 8E),. In the second stage, the ring-like initial periderm and secondary phloem fibers were formed (Figs. 3A, 5C, 6D, 7F). In the third stage, the wider rays and many larger vessels formed centripetally; sequent periderms were produced within the secondary phloem; cork and dead phloem were detached into a continuous segment (Figs. 2B, 2D-F, 3F, 5A, 5B-C, 5D, 6C, 8B) or cogwheel-like form (Figs.1D, 3C, 4B, 6A, 6F, 8A); and few secondary xylem rays were formed. In the fourth stage, sequent periderms were produced continuously with progressively deeper layers forming rhytidomes successively, and many secondary xylem rays were formed (Figs. 1D, 2D-E, 3C, 4B, 5A, 8D, 8F).

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 xylem area……………..2

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

2. Vascular bundles 20, pith large, white……………………….. *Clematis psilandra*

2. Vascular bundles 17, pith small, grey………………...........*Clematis tsugetorum*

3. Phloems wedge-like ……………..............*Clematis gouriana*subsp. *lishanensis*

3. Phloems without wedge-like…………….......................................…. ……...4

4. Rays wedge-like…. …………........…………….........………….……..............5

4. Rays without wedge-like…. ………..............…………………. ………...............9

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

5. Vascular bundles > 6……………..........………………………. ……. …..................6

6. Cortical sclerenchyma embedded in the phloem rays…………….*Clematis grata*

6. Cortical sclerenchyma unembedded in the phloem rays…. ……….... ……. ……..7

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

7. Stem round…………………………... …..……..8

8. Bark with cortex… …………….. …………………....... ……….*Clematis akoensis*

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

9. Bark with cortex and vascular cylinder……………….....................……….. ……..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. Primary xylem ring-like………………*Clematis parviloba* subsp. *bartlettii*

11. Primary xylem not ring-like……………..….12

12. Cortical sclerenchyma embedded in the phloem rays…….............................13

12. Cortical sclerenchyma unembedded in the phloem rays……………..............17

13. Vascular bundles 20-22………………….......................... ……..….........14

13. Vascular bundles 12-14…………….......................……. …...........................15

14. Rhytidome cogwheel-like……………………*Clematis chinensis* var. *chinensis*

14. Rhytidome continuous segments. . ……………..……. …*Clematis crassifolia*

15. Vessels ring-porous............. . ………………….…....... *Clematis lasiandra*

15. Vessels semi-ring-porous………….................................. ……...…..16

16. Pith cavity hexagon in shape..... ... ……………*Clematis uncinata* var. *uncinata*

16. Pith without cavity . ...*Clematis uncinata* var. *okinawensis*

17. Vascular bundles 16-21……………….. ………….18

17. Vascular bundles 12-19…….. ……...................19

18. Rhytidome thickness 0.4-0.8 mm……………….*Clematis leschenaultiana*

18. Rhytidome thickness 0.2-0.4 mm……………..……….*Clematis meyeniana*

19. Secondary xylem rays numerous……………….............*Clematis formosana*

19. Secondary xylem rays few or none..20

20. Rhytidome continuous segments. . ……………..……….*Clematis montana*

20. Rhytidome cogwheel-like……………..…………21

21. Vascular bundles with definite number……………………..*Clematis tamurae*

21. Vascular bundles with indefinite number…*Clematis terniflora* var. *garanbiensis*

Discussion

Sequent periderms develop in deeper layers within the secondary phloem, and periderm layers gradually develop centrifugally and result in rhytidome successively (Carlquist 1995). The rhytidome is one of the diagnostic characteristics of the *Clematis* genus. The texture and detachment of rhytidome results 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. Due to smaller stem diameters, three species did not appear to have a rhytidome. However, their bark may continue to develop into either cogwheel-like or continuous segment; therefore, continued observations are necessary. The stem diameters of some *Clematis* species are 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 suggest that *C. tashiroi* is the only species that does not form a rhytidome among the 22 Taiwanese *Clematis* species.

Stem cross-sections of *C. henryi* var. *morii* showed that it forms a deeply polygonous lobe (Angyalossy et al. 2012). *Clematis gouriana* subsp. *lishanensis* forms a discontinuous wedge-like phloem, which is a common characteristic of the the family Bignoniaceae (Pace et al. 2011). The stem of *C. formosana* is eccentric to oval or elliptical in shape due to abnormal production in conformation (Angyalossy et al. 2015). In summary, three cambial variants are found in the Ranunculaceae family—eccentric stems, stems with a deeply polygonous lobe, and wedge-like phloem.

Indentation toward the pith in the region of the wider rays 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 cortical sclerenchyma fibers embedded in the phloem rays formed an arc, which is characteristic of Lardizabalaceae and Sabiaceae (Carlquist 1984, 1991b; Yang et al. 2019). This feature has not been previously reported in the Ranunculaceae family.

Clematis psilandra (Fig. 6C) and *C. tsugetorum* (Fig. 8B) had few axial parenchyma and had vessel restriction. They also had growth in a different location than the other *Clematis* species studied, but similar to that of *Xanthorhiza apiifolia* (Carlquist 1995), which grows in temperate regions. *Xanthorhiza* is a primitive genus according to its vessel restriction pattern (Carlquist 1995). This pattern was also found in *C. psilandra* and *C. tsugetorum,* suggesting that they might be more primitive than the other Taiwanese *Clematis* species.

Clematis species often have 12 vascular bundles, with Smith (1928) finding this feature in 67% of 138 species studied. Therefore, this is generally considered the central type for this genus. Conversely, 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). *Clematis henryi* var. *henryi* (Fig. 3D) had ten vascular bundles in our study, but only six were recorded by Smith (1928). Moreover, *C. chinensis* var. *chinensis* (Fig. 1C, D) had 20 bundles in our study, *C. lasiandra* (Fig. 4B) had 14, *C. meyeniana* (Fig. 5B) had 21, and both *C. uncinata* var. *okinawensis* (Fig. 8C, D) and *C. uncinata* var. *uncinata* (Fig. 8E, F) had 12–14. Conversely, Smith (1928) recorded only 12 vascular bundles in these species. The inconsistent results might be due to different sample sizes or environmental factors; further investigation is required.

Vascular bundle numbers are usually constant for a given species, but in some species the numbers can vary. We examined multiple samples from *C. leschenaultiana* (Fig. 4C, 4D-F, 5A), *C. terniflora* var. *garanbiensis* (Fig. 7E, F, 8A), *C. uncinata* var. *uncinata* (Fig. 8E, F), and *C. uncinata* var. *okinawensis* (Fig. 8C, D) and found that the number of vascular bundles varied between growth stages. This might be related to the differentiation of meristematic cells or the action of interfascicular cambium. 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* (Fig. 3D) and *C. henryi* var. *morii* (Fig. 3F) (Yang and Huang 1996), but *C. henryi* var. *morii* is sometime treated as a species, *C. morii* (Wang and Bartholomew 2007). In this study, these two taxa had wedge-like rays and continuous segment of rhytidome, but the stem of *C. henryi* var. *henryi* was round while that of *C. henryi* var. *morii* was a deeply polygonous lobe. These stem shape characteristics provide a way to distinguish these two taxa. The vessel arrangement of the *Clematis* species in this study was semi-ring-porous in almost all species, with no species having diffuse-porous vessels, which is consistent with previous reports (Schweingruber et al. 2011).

In the present study, one to three secondary xylem rays were observed in some *Clematis* species. This has been recorded in the species *Aristolochia macrophylla* (Aristolochiaceae) (Carlquist 1993; Schweingruber et al. 2011), in the Cucurbitaceae (Carlquist 1992), and in the genus *Cyclea* (Menispermaceae) (Yang and Chen 2016). According to Carlquist (1995), secondary xylem rays are new rays that originate abruptly and are relative to the vine habit. Secondary xylem rays split the secondary xylem into two or more parts and the amount of bark increases as some of the splits are near the secondary phloem. Secondary xylem rays increased stem diameter and significantly increased the amount of bark. However, secondary xylem rays were not found in all of the species investigated in our study. The presence of secondary xylem rays might be related to stem diameter size, but this needs further investigation.

The pith cavity in *C. vitalba* results from non-lignified walls in the inner-most pith parenchyma cells (Sieber and Kucera 1980). However, we only observed this characteristic in *C. uncinata* var. *uncinata* (Fig. 8E, F); the pith cavity formed a hexagon shape around the primary xylem ring. Isnard et al. (2003a, b) used stem size to define four ontogenetic stages of three *Clematis* species. However, in this study we failed to observe the different developmental stages of each species due to low abundance, limited localities, and small stem diameters in some species. Further work collecting specimens and examining the characteristics of different developmental stages of these species is needed, especially species that only contain inner bark. However, the diagnostic features, such as the rhytidome, wedge-like phloem, xylem in plate type, restricted vessel pattern, indentations, and secondary phloem fibers, could be used to identify the species in the *Clematis* genus. The characteristics of the 22 *Clematis* species described here and provide evidence for systematic problems within this genus.

Conclusions

The bark and cambial variations in the Ranunculaceae family are diverse. The habits of Taiwanese *Clematis* species include two shrubs and twenty vines, in which three species grow at high altitudes ranging from 2,000 m to 3,600 m. Our results showed that the rhytidome (outer bark) is a key diagnostic characteristic for *Clematis* species identification, and can either take the form of continuous segments bark or cogwheel-like bark. Among the 22 Taiwanese *Clematis* species, *C. tashiroi* was the only species that did not form rhytidome. Three cambial variants of the *Clematis* genus were found—stems with polygonous lobes, wedge-like phloem, and the xylem in plate type. Most *Clematis* species had ray dilatation and indentation of the axial parenchyma near the wider rays. The cortical sclerenchyma fibers embedded in the phloem rays and numbers and sizes of vascular bundles varied among the *Clematis* species. The vessels of *C. psilandra* and *C. tsugetorum* were restricted to the central portions of the fascicular xylem, which was different from the other *Clematis* species. The xylem vessels dispersed throughout the stem were mostly semi-ring-porous, but a few were ring-porous with annual rings. No diffuse-porous stems were observed. The secondary xylem rays split the vascular elements into different segments, increasing stem diameters. Further collection of fresh materials and observations of different developmental stages are still needed. Interestingly, we found unusual wood features, such as indentation near the wider rays, vessel restriction, and the presence of secondary xylem rays. In conclusion, bark and cambial variations could facilitate future studies addressing *Clematis* taxonomy.

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 cambial variation of stem cross-section. Three authors read and approved the final manuscript

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Tables

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

Species	Collector	Herbarium and voucher number	Collection localities
**Clematis akoensis* Hayata	Po-Hao Chen	PPI75993	Tajen, Taitung County
Clematis chinensis Osbeck var. *chinensis*	Po-Hao Chen	PPI73776	Shihkechienshan, Pingtung County
**Clematis chinensis* Osbeck var. *tatushanensis* T.Y.A. Yang	Po-Hao Chen	PPI76140	Shalu, Taichung City
Clematis crassifolia Benth.	Chien-Fan Chen	TAIF449023	Yuanshan Township, Ilan County
**Clematis formosana* Kuntze	Po-Hao Chen	PPI76748	Lilungshan, Pingtung County
**Clematis gouriana* Roxb. ex DC. subsp. *lishanensis* T.Y.A. Yang & T.C. Huang	Sheng-Zehn Yang	PPI57118	Chungchihkuan, Kaohsiung City
Clematis grata Wall.	Po-Hao Chen	PPI79191	Siangyang, Taitung County
Clematis henryi Oliv. var. *henryi*	Po-Hao Chen	PPI76097	Peitawushan, Pingtung County
**Clematis henryi* Oliv. var. *morii* (Hayata) T.Y.A. Yang & T.C. Huang	Her-Long Chiang	PPI65589	Ligavon Trail, Taitung County
Clematis lasiandra Maxim.	Sheng-Zehn Yang	PPI57133	Chungchihkuan, Kaohsiung City
Clematis leschenaultiana DC.	Chien-Fan Chen	PPI63232	Tahanshan, Pingtung County
Clematis meyeniana Walp.	Chien-Fan Chen	PPI60238	Rtangjhen, Pingtung County
Clematis montana Buch.-Ham. ex DC.	Chien-Fan Chen	PPI63771	Jenai, Hualien County
**Clematis parviloba* Gard. ex Champ. subsp. *bartlettii* (Yamam.) T.Y.A. Yang & T.C. Huang	Guang-Pu Hsieh	PPI61169	Mwilan Trail, Kaohsiung City
Clematis pseudootophora M.Y. Fang	Chien-Fan Chen	TAIF455630	Ssuchi Village, Ilan County
**Clematis psilandra* Kitag.	Guang-Pu Hsieh	PPI60043	Wutai, Pingtung County
**Clematis tamurae* T.Y.A. Yang & T.C. Huang	Po-Hao Chen	PPI79723	Shizaitoushan, New Taipei City
Clematis tashiroi Maxim.	Po-Hao Chen	PPI78815	Shouka, Pingtung County
**Clematis terniflora* DC. var. *garanbiensis* (Hayata) M.C. Chang	Po-Hao Chen	PPI78668	Hengchun, Pingtung County
**Clematis tsugetorum* Ohwi	Guang-Pu Hsieh	PPI68569	Nenggao cross-ridge, Nantou County,
Clematis uncinata Champ. ex Benth. var. *okinawensis* (Ohwi) Ohwi	Kun-Pin Lo	PPI69701	Tahanshan, Pingtung County
Clematis uncinata Champ. ex Benth . var. *uncinata*	Jyuen-Jyie Chen	PPI63904	Yitingshan, Pingtung County

*: Endemic species in Taiwan

Table 2. Morphological characteristics determined from stem cross-sections of 22 Taiwanese *Clematis* species

Characters / Scientific name	SD	CO	SC	COG	SE	RHL	RHT	RWL	PW	ID	CS	VR	RD	VB	XW	PRW	SR	RP
*C. akoensis	3.8	<0.1	+	–	–	–	–	–	–	–	–	–	–	14	0.31 ± 0.08	205 ± 95	–	–
*C. akoensis	6.0	0.15	–	–	–	–	–	+	–	–	–	–	+	12	0.41 ± 0.11	441 ± 32	–	–
C. chinensis var. chinensis	4.3	<0.1	+	–	–	–	–	–	–	–	–	–	–	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. crassifolia	4.1	<0.1	+	–	–	–	–	–	–	–	–	–	+	20	0.29 ± 0.06	127 ± 34	–	–
C. crassifolia	7.8	–	–	–	+	1–4	0.5–1.6	–	–	+	–	–	+	20	0.45 ± 0.09	160 ± 49	–	–
C. crassifolia	8.9	–	–	–	+	3–6	1.8–3.4	–	–	+	–	–	+	20	0.47 ± 0.11	186 ± 66	–	+
*C. formosana	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. grata	3.9	<0.1	+	–	–	–	–	–	–	–	–	–	–	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	11.5	–	–	+	–	1-4	0.4–1.3	–	–	+	+	–	+	12	1.42 ± 0.55	428 ± 191	+	–
C. grata	14.1	–	–	+	–	2	0.3–1.1	+	–	+	+	–	+	12	1.69 ± 0.69	642 ± 208	+	–
*C. henryi var. morii	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	2.0	<0.2	–	–	–	–	–	–	–	–	–	–	–	9	0.34 ± 0.03	96 ± 52	–	–
C. lasiandra	19.7	–	–	+	–	2–5	0.5–2.7	–	–	+	+	–	–	14	2.76 ± 0.52	230 ± 178	+	+
C. leschenaultiana	4.1	<0.1	+	–	–	–	–	–	–	–	–	–	–	19	0.26 ± 0.12	111 ± 37	–	–
C. leschenaultiana	5.6	–	–	–	+	1	0.2–0.4	–	–	+	–	–	+	19	0.37 ± 0.12	113 ± 43	–	–
C. leschenaultiana	6.4	–	–	–	+	1	0.2–0.3	–	–	+	–	–	+	21	0.45± 0.14	175 ± 61	–	–
C. leschenaultiana	7.8	–	–	–	+	2	0.2–0.5	–	–	+	–	–	+	16	0.69 ± 0.32	145 ± 52	+	–

Characters Scientific name	SD	CO	SC	COG	SE	RHL	RHT	RWL	PW	ID	CS	VR	RD	VB	XW	PRW	SR	RP
C. leschenaultiana	15.6	–	–	–	+	2–3	0.2–1.6	–	–	+	–	–	+	19	1.34 ± 0.64	225 ± 46	+	–
**C. parviloba* subsp. *bartlettii*	2.4	–	–	–	+	1	0.1–0.2	–	–	–	–	–	+	12	0.32 ± 0.10	64 ± 25	–	–
**C. parviloba* subsp. *bartlettii*	3.9	–	–	–	+	1	0.1–0.2	–	–	+	–	–	+	12	0.50 ± 0.09	164 ± 55	–	+
**C. parviloba* subsp. *bartlettii*	3.9	–	–	–	+	2	0.1–0.3	–	–	+	–	–	+	12	0.49 ± 0.13	147 ± 50	–	–
C. pseudootophora	1.7	<0.1	+	–	–	–	–	–	–	–	–	–	–	6	0.32 ± 0.01	54 ± 12	–	–
C. pseudootophora	3.5	–	–	+	–	1	0.1–0.2	+	–	+	–	–	–	6	0.93 ± 0.10	393 ± 87	–	–
**C. psilandra*	4.9	–	–	–	+	1	0.1–0.2	–	–	–	–	+	+	23	0.20 ± 0.08	218 ± 79	–	–
**C. psilandra*	4.3	–	–	–	+	1	0.1–0.2	–	–	–	–	+	+	20	0.33 ± 0.11	387 ± 99	–	+
**C. tamurae*	2.1	<0.1	+	–	–	–	–	–	–	–	–	–	–	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*	4.0	<0.1	+	–	–	–	–	–	–	–	–	–	–	14	0.32 ± 0.07	289 ± 116	–	–
**C. tashiroi*	6.8	<0.1	+	–	–	–	–	–	–	+	–	–	+	21	0.49 ± 0.12	207 ± 58	–	–
**C. tashiroi*	8.8	<0.1	+	–	–	–	–	–	–	+	–	–	+	20	0.58 ± 0.10	343 ± 155	–	–
**C. tashiroi*	11.1	<0.1	–	–	–	–	–	–	–	+	–	–	+	20	0.91 ± 0.20	373 ± 117	+	–
**C. tashiroi*	17.8	<0.1	–	–	–	–	–	–	–	+	–	–	+	20	1.44 ± 0.30	450 ± 187	+	–
**C. terniflora* var. *garanbiensis*	2.7	<0.1	+	–	–	–	–	–	–	–	–	–	–	14	0.33 ± 0.09	63 ± 29	–	–
**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. uncinata var. *okinawensis*	4.0	<0.1	+	–	–	–	–	–	–	–	–	–	–	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	+	–

Characters Scientific name	SD	CO	SC	COG	SE	RHL	RHT	RWL	PW	ID	CS	VR	RD	VB	XW	PRW	SR	RP
C. uncinata var. *uncinata*	5.7	<0.1	+	–	–	–	–	–	–	–	–	–	+	12	0.73 ± 0.07	260 ± 61	–	–
C. uncinata var. *uncinata*	12.4	–	+	+	–	3-5	0.5–1.5	–	–	+	+	–	+	14	1.38 ± 0.19	386 ± 62	+	–
Clematis chinensis var. *tatushanensis*	2.2	<0.1	–	–	–	–	–	–	–	–	–	–	–	20	0.21 ± 0.05	25 ± 13	–	–
******C. gouriana* subsp. *lishanensis*	6.2	–	–	–	+	1–2	0.2–0.7	–	+	+	–	–	+	12	0.81 ± 0.34	108 ± 51	+	–
C. henryi var. *henryi*	5.5	–	–	–	+	1–3	0.4–0.9	+	–	–	–	–	+	10	0.38 ± 0.07	449 ± 131	–	–
C. meyeniana	7.3	–	–	–	+	1	0.4–0.8	–	–	+	–	–	+	21	0.58± 0.12	117 ± 39	–	–
C. montana	3.1	–	+	–	+	1	0.2–0.3	–	–	–	–	–	+	12	0.34 ± 0.06	202 ± 29	–	–
******C. tsugetorum*	3.9	–	–	–	+	1	0.2–0.3	+	–	–	–	+	–	17	0.17 ± 0.08	374 ± 131	–	+
Sum		12		8	11			6	1	17	5	2	15				9	4

Noted: *****: endemic species; SD: stem diameter, mm; CO: cortex; SC: sclerenchyma fibers, including phloem fibers or secondary phloem; COG: cogwheel-like rhytidome; SE: continuous segment rhytidome; RHL: rhytidome layer number; RHT: rhytidome thickness, mm; RWL: wedge-like rays; PW: wedge/arc-like phloem; ID: indentation of the axial parenchyma near the wider rays; CS: cortical sclerenchyma connected with the plate of sclerenchyma that embeded in the phloem rays; VR: vessel restriction pattern; RD: ray dilatations; VB: number of vascular bundle; XW: mean xylem width (mean ± SD, n = xylem number), mm; PRW: mean primary ray width (mean ± SD, n = ray number), μm; SR: secondary xylem rays; RP: ring-porous vessel; SRP: semi-ring-porous vessel; PC: pith cavity; DS: developmental stage (1: first stage, 2: second stage, 3: third stage, 4: fourth stage); present/absent (+/–).

Table 3. Collection localities and altitude from stem samples of 22 Taiwanese *Clematis* species

Information Scientific name	Figure	Location	Altitude (m)
Clematis akoensis	1A, 1B	Shouka, Pingtung County	350
C. chinensis var. *chinensis*	1C, 1D	Lising, Nantou County	1800
Clematis chinensis var. *tatushanensis*	1E	Shalu, Taichung City	150
C. crassifolia	1F, 2A	Henglingshan, Taichung City	1700
C. crassifolia	2B	Manabangshan, Miaoli County	1200
C. formosana	2C, 2D	Shoushan, Kaohsiung City	200
C. gouriana subsp. *lishanensis*	2E	Hehuanshan, Nantou County	2600
C. grata	2F, 3A, 3B, 3C	Dapu, Chiayi County	500
C. henryi var. *henryi*	3D	Beidawushan, Pingtung County	1500
C. henryi var. *morii*	3E, 3F	Sinjhongheng, Chiayi County	2400
C. lasiandra	4A	Lingmingshan, Taichung City	3000
C. lasiandra	4B	Hehuanshan, Nantou County	2800
C. leschenaultiana	4C	Huaguoshan, Kaohsiung City	800
C. leschenaultiana	4D	Lijia forest trail, Taitung County	1000
C. leschenaultiana	4E, 4F, 5A	Duona forest trail, Kaohsiung City	700
C. meyeniana	5B	Duona forest trail, Kaohsiung City	700
C. montana	5C	Hehuanshan, Nantou County	3200
C. parviloba subsp. *bartlettii*	5D, 5F	Hehuan river, Nantou County	2600
C. parviloba subsp. *bartlettii*	5E	Sihyuanyakou, Yilan County	1800
C. pseudootophora	6A	Sihyuanyakou, Yilan county	1800
C. psilandra	6B, 6C	Alishan, Chiayi County	2300
C. tamurae	6D, 6E, 6F	Fonggangshan, Kaohsiung City	1600
C. tashiroi	7A	Rueisuei forest trail, Hualien County	2400
C. tashiroi	7B	Shouka, Pingtung County	300
C. tashiroi	7C, 7D	Beizihtong forest trail, Chiayi county	1600
C. terniflora var. *garanbiensis*	7E, 7F, 8A	Hengchun, Pingtung County	100
C. tsugetorum	8B	Hehuanshan, Nantou County	3200
C. uncinata var. *okinawensis*	8C, 8D	Dahanshan, Pingtung County	900
C. uncinata var. *uncinata*	8E	Syuejian, Miaoli county	2000
C. uncinata var. *uncinata*	8F	Shihkejianshan, Pingtung County	1300

Figures

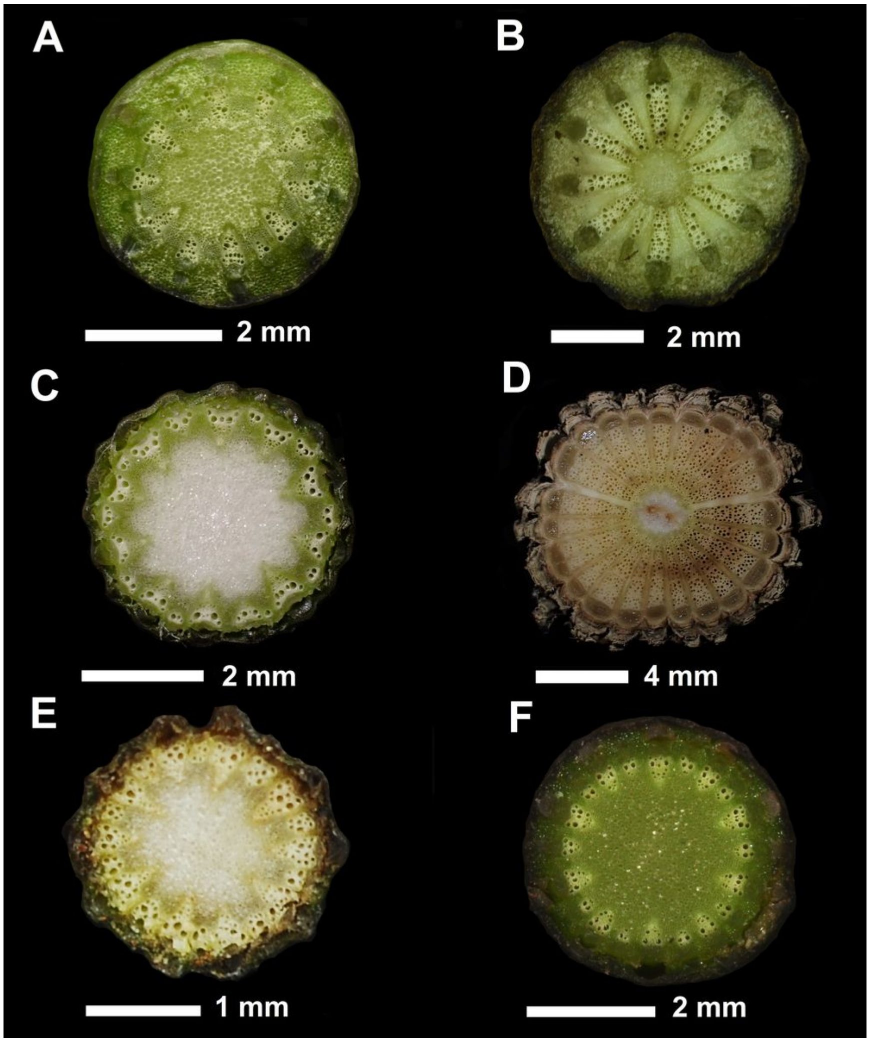

Figure 1

Stem transverse sections of Clematis species. A. C. akoensis; collenchyma and primary phloem fibers in dense bundles. B. C. akoensis; secondary phloem triangular in shape, wedge-like rays. 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, cortical sclerenchyma connected with a plate of sclerenchyma embedded in the phloem rays, 22 vascular bundles, few secondary xylem rays with growth rings. 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.

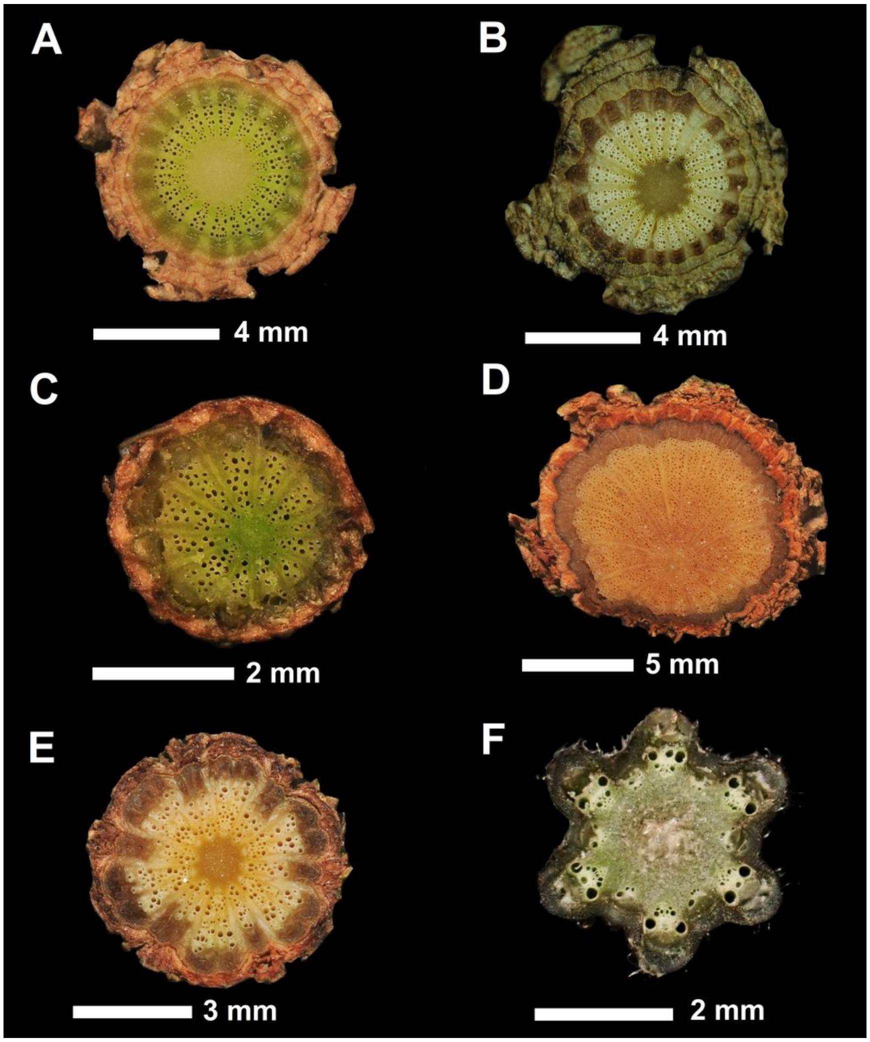

Figure 2

Stem transverse sections of Clematis species. A. C. crassifolia; continuous segment rhytidome with 1–4 layers, ray dilatation. B. C. crassifolia; sequent periderm produced in deeper layers within the secondary phloem, continuous segment rhytidome with 3–6 layers, ray dilatation, ring-porous. C. C. formosana; primary phloem fiber strands. D. C. formosana; secondary phloem fibers obvious, continuous segment rhytidome with 2 layers, 32 secondary xylem rays, eccentric pith. E. C. gouriana subsp. lishanensis; ring-like initial periderm, discontinuous wedge-like phloem, 14 secondary xylem rays. F. C. grata; stem with 6 shallow grooves, primary phloem fibers in dense bundles, 6 big and 6 small vascular bundles.

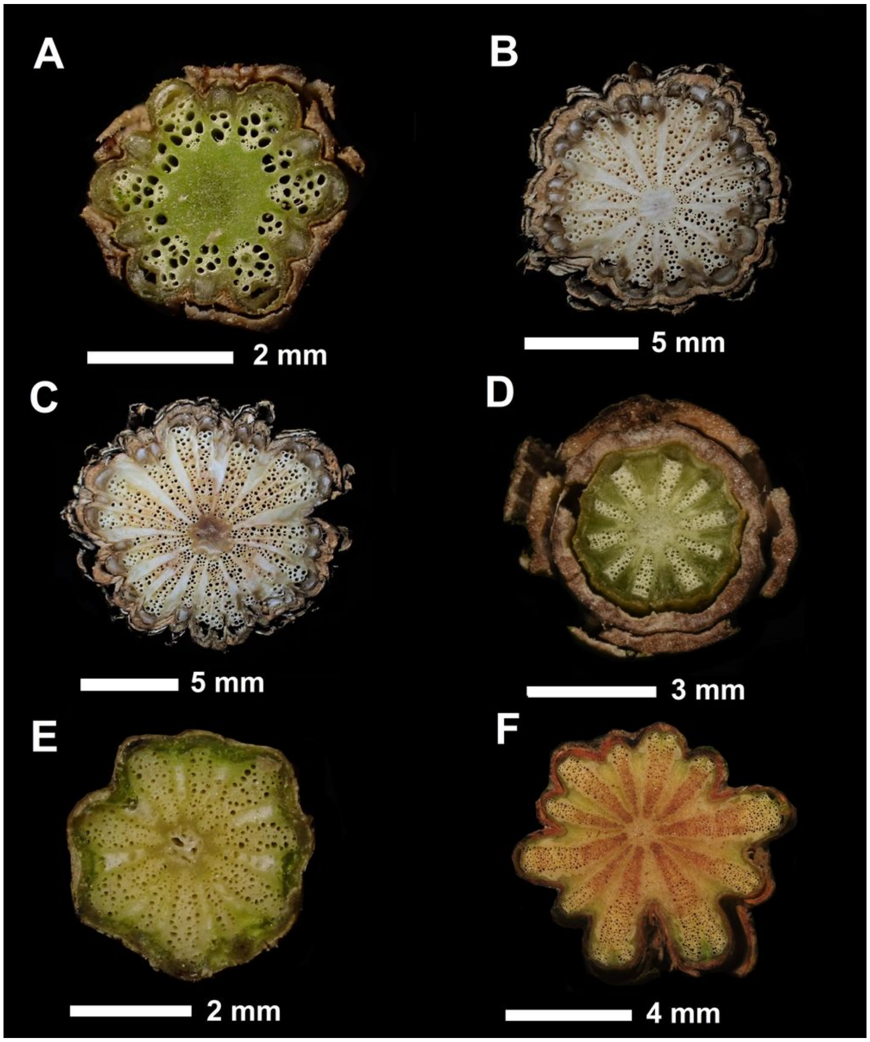

Figure 3

Stem transverse sections of Clematis species. A. C. grata; ring-like initial periderm, yellow, cogwheel-like rhytidome with 1 layer, 6 big and 6 small vascular bundles. B. C. grata; cogwheel-like rhytidome with 2 layers, cortical sclerenchyma connected with the plate of sclerenchyma embedded in the phloem rays, ray indentation, 9 secondary xylem rays. C. C. grata; ray dilatation, wedge-like rays, 11 secondary xylem rays. D. C. henryi var. henryi; ring-like initial periderm, continuous segment rhytidome with 2 layers, wedge-like rays. E. C. henryi var. morii; stem shallowly lobed, wedge-like rays, ray indentation and dilatation, 10 vascular bundles. F. C. henryi var. morii; stem irregularly lobed, wedge-like rays, ray indentation, 11 vascular bundles.

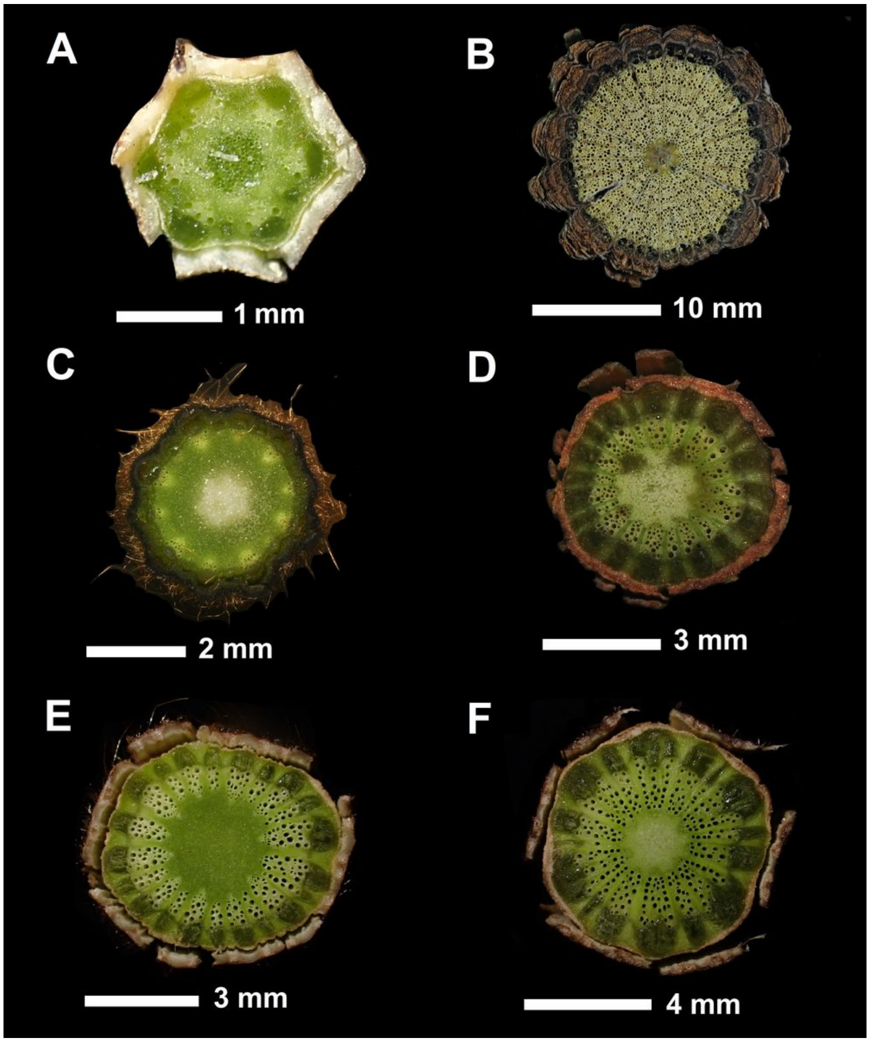

Figure 4

Stem transverse sections of Clematis species. A. C. lasiandra; hexagon-shaped stem (2 mm in diameter), 0.1–0.2 mm cortex, 6–9 vascular bundles. B. C. lasiandra; cogwheel-like rhytidome with 2–5 layers, 30 secondary xylem rays, ring-porous. C. C. leschenaultiana; ring-like primary phloem fibers, 19 vascular bundles. D. C. leschenaultiana; rectangular secondary phloem, black in color, continuous segment rhytidome with 1 layer, ray dilatation. E. C. leschenaultiana; ring-like initial periderm, irregular phloem fibers within the secondary phloem, continuous segment rhytidome with 1 layer, ray dilatation. F. C. leschenaultiana; continuous segment rhytidome with 2 layers, 6 secondary xylem rays.

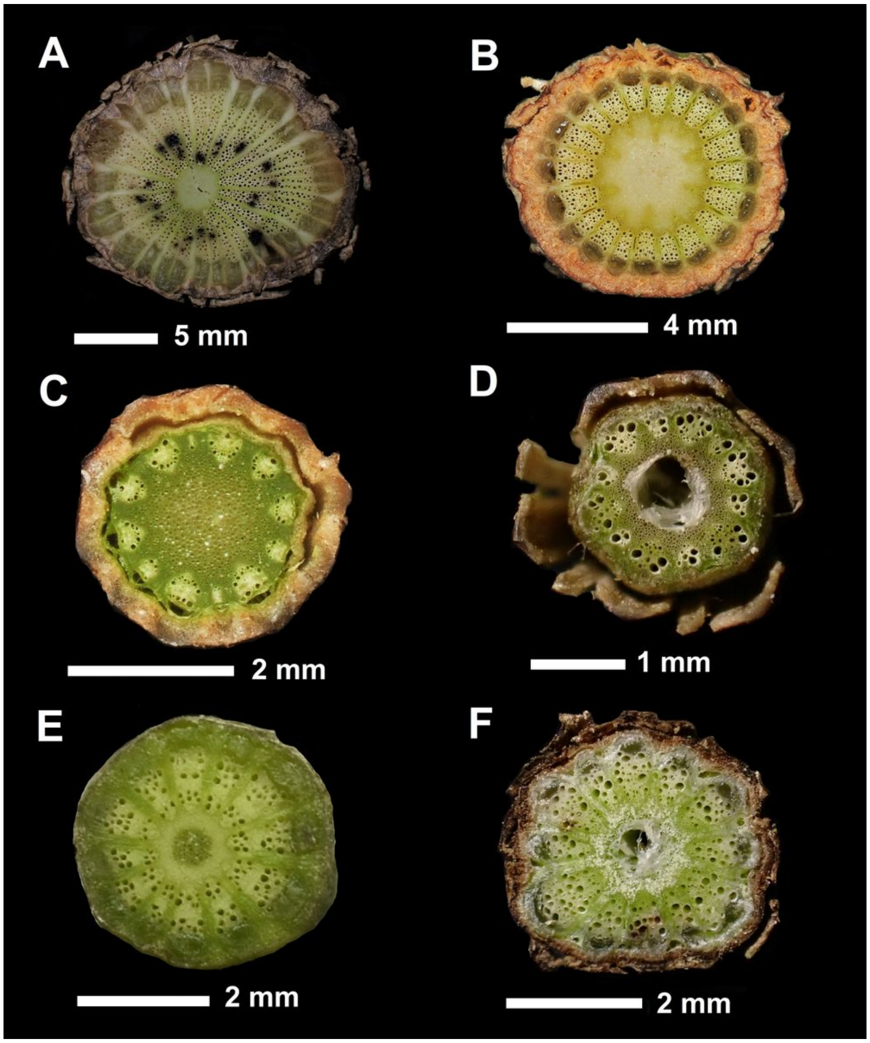

Figure 5

Stem transverse sections of Clematis species. A. C. leschenaultiana; linear phloem fibers within the secondary phloem, continuous segment rhytidome with 2 layers, ray dilatation, 15 secondary xylem rays. B. C. meyeniana; ring-like initial periderm, continuous segment rhytidome with 1 layer, ray dilatation, ray indentation. C. C. montana; primary phloem fibers in strands, ring-like initial periderm, yellow. D. C. parviloba subsp. bartlettii; continuous segment rhytidome with 1 layer, 6 big and 6 small vascular bundles. E. C. parviloba subsp. bartlettii; cortex lacerated and falling off, primary xylem ring located around the pith, growth ring present. F. C. parviloba subsp. bartlettii: ring-like initial periderm, continuous segment rhytidome with 2 layers.

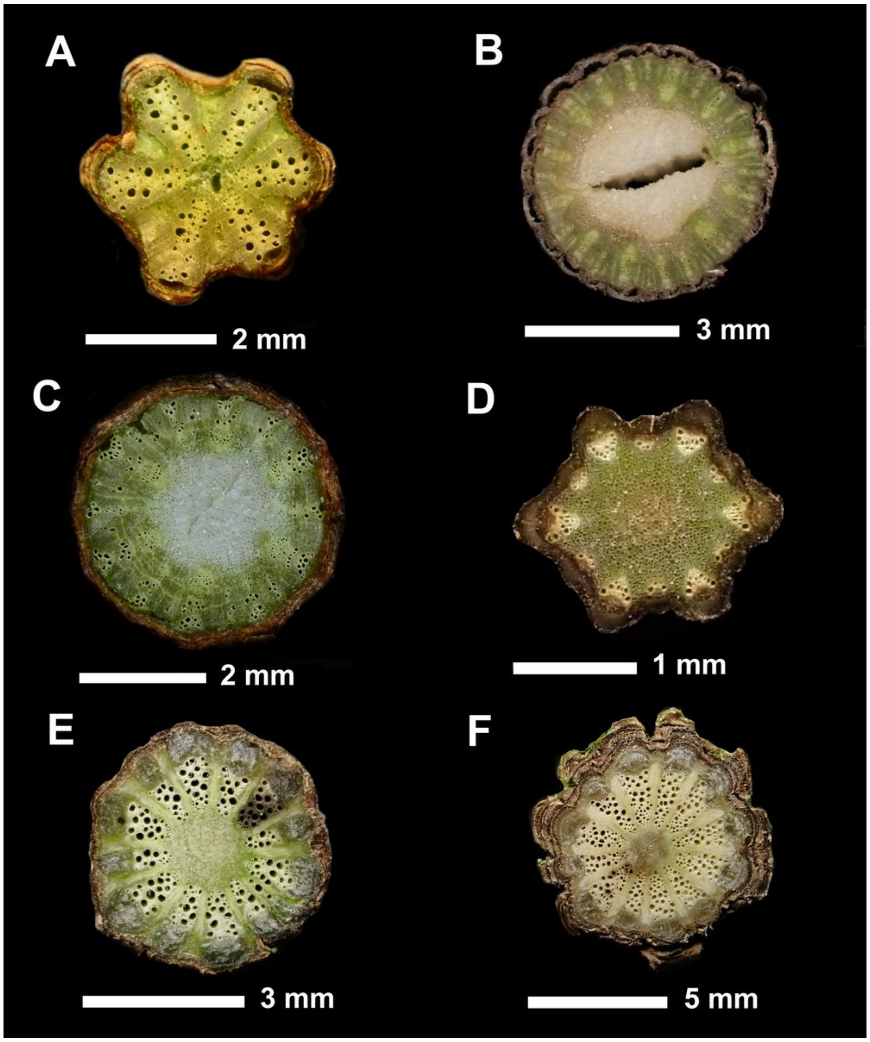

Figure 6

Stem transverse sections of Clematis species. A. C. psendotophora; stem with 6 shallow grooves, cogwheel-like rhytidome with 1 layer, wedge-like rays, 6 vascular bundles. B. C. psilandra; continuous segment rhytidome with 1 layer, vessel limited to the central portions of fascicular xylem, 23 vessel bundles. C. C. psilandra; continuous segment rhytidome with 1 layer, vessels limited to the central portions of fascicular xylem, vessel as growth rings. D. C. tamurae; stem with 6 shallow grooves, primary phloem fiber in strands, 6 big and 6 small vascular bundles. E. C. tamurae; secondary phloem dark-green and rectangular, ring-like sequent periderm, cogwheel-like rhytidome with 1 layer, ray dilatation. F. C. tamurae; linear phloem fibers within the secondary phloem, cogwheel-like rhytidome with 3–4 layers, ray dilatation.

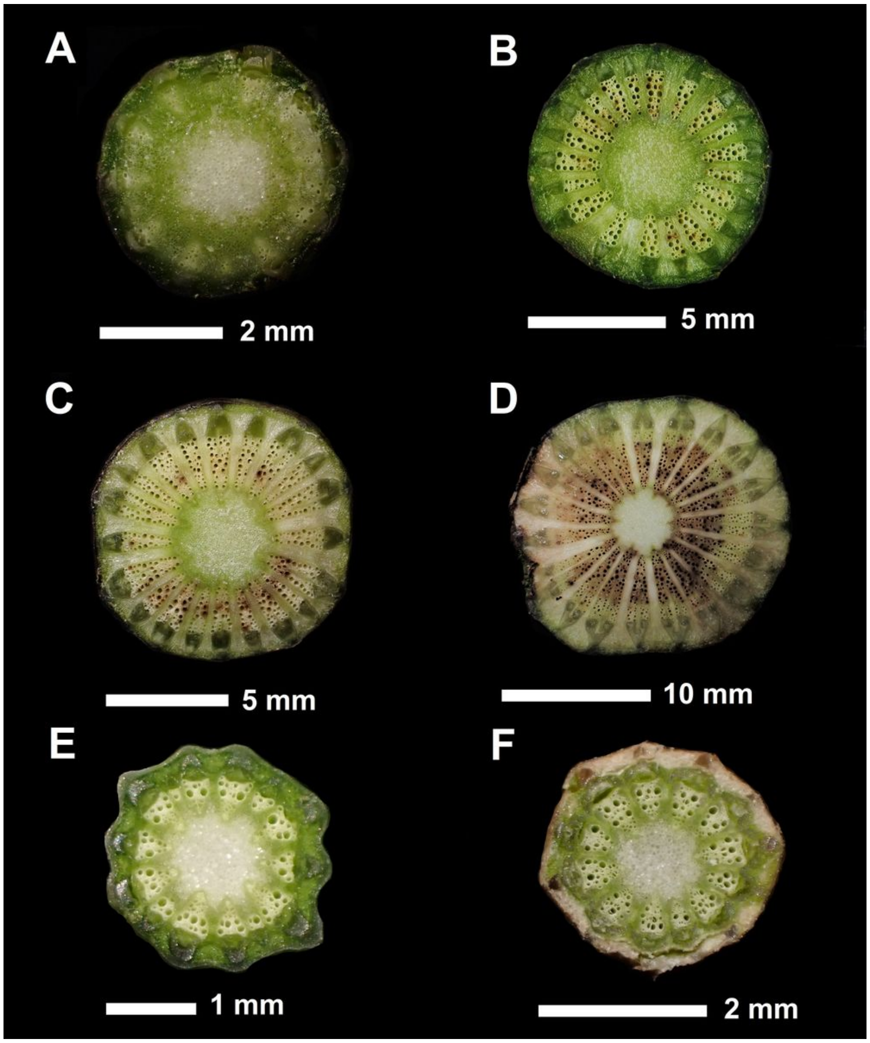

Figure 7

Stem transverse sections of Clematis species. A C. tashiroi; primary phloem fiber stands, 14 vascular bundles. B. C. tashiroi; primary phloem fiber strands, secondary phloem triangular in shape and deep-green, ray dilatation, rays unequal in width, secondary phloem fiber strands. C. C. tashiroi; secondary phloem triangular in shape and deep-green, ray dilatation, rays unequal in width. D. C. tashiroi; obvious ray dilatation, rays unequal in width, secondary xylem rays. E. C. terniflora var. garanbiensis; ring-like collenchyma, ring-like initial periderm, primary phloem fibers strands, 14 vascular bundles. F. C. terniflora var. garanbiensis; primary and secondary phloem fibers present, ring-like initial periderm.

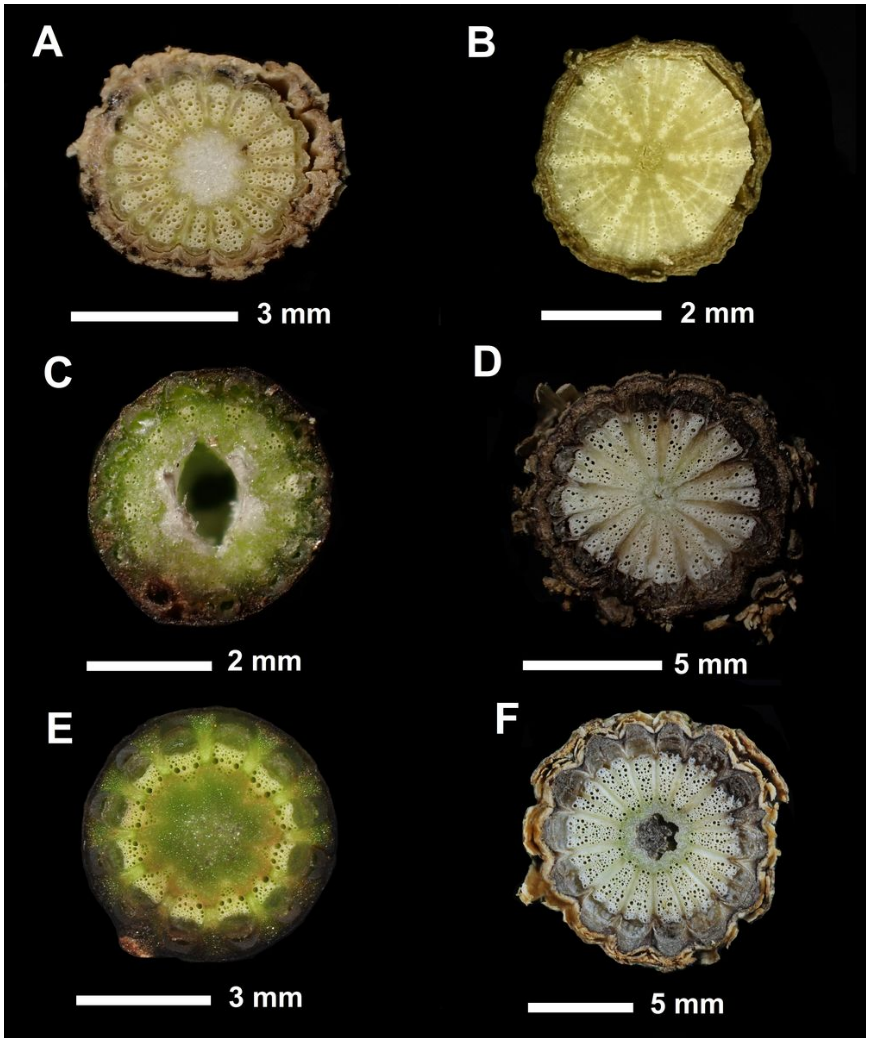

Figure 8

Stem transverse sections of Clematis species. A. C. terniflora var. garanbiensis; secondary phloem fibers in bands, ring-like initial periderm, cogwheel-like rhytidome with 2 layers. B. C. tsugetorum; wedge-like rays, vessel distribution restricted to the central portions of fascicular xylem, vessel as growth rings. C. C. uncinata var. okinawensis; primary phloem fibers in strands. D. C. uncinata var. okinawensis; primary phloem fibers in strands, cogwheel-like rhytidome, cortical sclerenchyma connected with plate of sclerenchyma embedded in the phloem rays, ray dilatation. E. C. uncinata var. uncinata; primary phloem fibers in strands, ray dilatation. F. C. uncinata var. uncinata; cortical sclerenchyma connected with plate of sclerenchyma embedded in the phloem rays, 11 secondary xylem rays, pith cavity, hexagon in shape, primary xylem ring clearly located around the pith.