# Stem cambial variants of the *Clematis* species (Ranunculaceae) in Taiwan

**Sheng-Zehn YANG[1,*], Po-Hao CHEN[2], Chien-Fan CHEN[3]**

*1. Department of Forestry, National Pingtung University of Science and Technology, No. 1, Shuefu Rd., Neipu, Pingtung 91201, Taiwan.*
*2. Graduate Institute of Bioresources, National Pingtung University of Science and Technology, No. 1, Shuefu Rd., Neipu, Pingtung 91201, Taiwan. l26571583@yahoo.com.tw*
*3. Botanical Garden Division, Taiwan Forestry Research Institute, No. 53, Nanhai Rd., Taipei, 100051. Taiwan. chenc@tfri.gov.tw*
*\*Corresponding author's email: yangsz@mail.npust.edu.tw*

ABSTRACT: Studies on cambial variants of Taiwanese species from the genus *Clematis* (Ranunculaceae) are scarce. This study aimed to investigate and compare the patterns of secondary growth in stems of 22 *Clematis* taxa, including 19 species and three varieties. The experimental results showed that approximately 27% of the *Clematis* species in Taiwan had 12 vascular bundles in the stem. In three cambial variants of the genus, the stem was characterized by an irregular conformation, axial vascular elements in segments, and a wedge-like phloem. The rhytidome is composed of periderm and non-conducting phloem and forms either cogwheel-like or continuous segment bark. Eight *Clematis* species formed arc-like bark, which developed the cogwheel-like rhytidome. Most *Clematis* species had an indentation in the region of the wider rays, and the wedge-like indentations developed in the interfascicular region of *C. henryi* var. *morii* and *C. pseudootophora*. Narrow rays that occurred in the secondary xylem increased with increasing stem diameter. The vessels dispersed throughout the stem were semi-ring-porous in most species but ring-porous in others. No species had diffuse porous vessels. The vessel restriction pattern was only found in the two species, *C. psilandra* and *C. tsugetorum*. The primary xylem ring was located around the pith of *C. uncinata* var. *uncinata,* making its pith cavity hexagonal in shape. Five species had the pith cavity feature. This study provides important basic information about *Clematis* lianas and addresses their taxonomy, which will help to understand their morphology and to ensure the conservation of their diversity.

KEY WORDS: Ranunculaceae, ray indentation, ring bark, scale bark, vessel restriction, wedge phloem.

## INTRODUCTION

The vascular cambium of climbing plants produces xylem and phloem under normal conditions, and both types of tissue contain 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 (Putz and Mooney, 1991) as diverse cambial variants, or secondary growth patterns are formed. Stems generally develop axial vascular elements in segments separated by the wider xylem and phloem rays, forming xylem in plate type. This type is derived from a single cambium, as defined by Angyalossy *et al.* (2012, 2015). Several families have developed 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 taxa are found in Taiwan (Yang and Huang, 1996). The life forms of *Clematis* species include shrubs, herbs, and perennial climbers (lianas). Within the family Ranunculaceae, two cambial variants have been reported, namely axial vascular elements in segments (also known as xylem in plates), and stems with an irregular conformation (Angyalossy *et al.*, 2012; 2015).

Previous anatomical studies of the family Ranunculaceae have mainly focused on the genus *Clematis*, and concentrated on the secondary irregular growth of vascular elements (Smith, 1928; Sieber and Kucera, 1980; Gregory, 1994; Carlquist, 1995). For example, the ray developments varied in plant stem anatomy. In some species (such as *C. cirrbosa*, *C. flammula*, *C. montevidensis*, *C. vitalba*, and *C. viticella)* a few secondary rays begin abruptly and occur with increasing stem diameter in (Schweingruber *et al.,* 2011). Carlquist (1995) observed that new rays in the genus *Clematis* are wide and multiseriate from their origin and are initiated abruptly rather than as narrow rays that gradually increase in width. Beck (2011) described closely spaced, narrow rays that occur in groups, and are termed aggregate rays, and noted that these can simulate large rays. The cambium in the secondary vascular tissue generates broad ray-like parenchyma in *Clematis flammula* var. *maritima* (Isnard *et al.*, 2003a). The width of interfascicular cambial rays increases with increasing stem diameter in *C. alpina* subsp. *alpina*, with the result that the rays are wedge-like in shape (Schweingruber *et al.,* 2011).

The previous reports had described the features of rhytidome, numbers and sizes of vascular bundles, and the pith cavity formation. The phellogen of *C. alpina* and *C. vitalba* produces cork cells and forms 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 layers of cork and non-

conducting phloem. Two types of rhytidome have been recognized, based on arrangement and degree of detachment: cogwheel-like rhytidome (ring bark) and continuous segment rhytidome (scale bark) (Esau, 1958; Evert, 2006). In the genus *Clematis,* vascular bundles are characterized by size, as big or small (Smith, 1928). The big vascular bundles are major bundles developed from fascicular cambium, whereas the small bundles develop from interfascicular cambium, and occur in the stem with variable numbers (Beck, 2011). *Clematis vitalba* is a vine with big and small vascular bundles (six each) when young. With age, the pith cavity is formed, the 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 ray region (Sieber and Kucera, 1980), and in *C. alpina* and *C. pickerringii* the interfascicular areas always have indentations (Carlquist, 1995). Wedge-shaped indentations can be seen in the interfascicular region due to the presence of thin-walled axial parenchyma in latewood adjacent to the thin-walled ray cells. (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, whereas *C. flammula*, *C. vitalba*, and *C. viticella* have ring-porous wood with annual rings (Schweingruber *et al*., 2011).

Stems with an irregular conformation and variants in axial vascular elements in radial segments have been found in the genus *Clematis* (Carlquist, 1995; Isnard *et al*., 2003b; Yang and Chen, 2015; Angyalossy *et al.,* 2012; 2015); however, information about the patterns of secondary growth or cambial variants in Taiwan *Clematis* genus is scarce. As cambial variants constitute an extremely diverse morphology, the present study aimed to 1) investigate and compare the patterns of secondary growth and 2) provide a bracketed key based on the anatomical characteristics of the stems to facilitate the identification of irregular cambial activity in the genus *Clematis* in Taiwan.

## MATERIALS AND METHODS

Multiple samples of 22 *Clematis* taxa from the family Ranunculaceae were collected and each species had been examined at least five times. The habits of these taxa included annual and perennial herbs, shrubs, and lianas growing in forests. The voucher information for all *Clematis* species is presented in Table 1. Approximately 48% of these species are endemic to Taiwan. Among them, *C. psilandra* and *C. tsugetorum* are shrubs growing at high elevations of approximately 2,300 m and 3,200 m, respectively. *Clematis pseudootophora* is an herb that is rarely found in the field. The remaining 19 species are climbing vines, of which one, *C. montana*, grows up to

3,200 m. This species consists of only a few populations located at elevations of 1,500–1,900 m in eastern Taiwan.

Stems with thick bark, and secondary growth characteristics were selected in the field. The stems were stored to keep the materials fresh and retain humidity. Stems of different diameters were collected from each plant to enable accurate assessments of the positions of various vascular bundle tissues. One, two or three samples with obvious and easy-to-observe cambial variants were selected per taxa for photographs and scoring of morphological characteristics. Morphological features of the stem cross-sections were used to construct a comparison table of the investigated taxa.

The fresh materials were cut and a freehand cross-section of each stem was made with a razor blade. The stem cross-sections were immediately photographed using a Nikon D7100 SLR digital camera with a 1:1 lens (Lens AF Micro Nikkor 60 mm 1:2.8D; Nikon Corporation, Tokyo, Japan). Cambial characteristics were measured and described. The stem diameter, rhytidome 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 (Yang and Huang, 1996). Because of the rarity of *C. pseudootophora*, its materials were permanently preserved in 75% aqueous alcohol.

The investigated stem features are listed in the Table 2. The vessel restriction pattern, ray types, and axial parenchyma types were classified according to Carlquist (2001) and the IAWA Committee on Nomenclature (1964). Taxa were grouped according to 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.

## RESULTS

### Classification of vascular bundles

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

### Characteristics of the stem bark and phloem

The stems of the 22 taxa investigated were round or shallowly grooved to form hexagonal cross-sections in the younger stems. The stems of *C. grata* (Fig. 2E), *C. lasiandra* (Fig. 3E), *C. pseudootophora* (Fig. 5B), and *C. tamurae* (Fig. 5E) were hexagonal. Cork layers developed in the young stems of *C. formosana* (Fig. 2B), *C. lasiandra*

527

**Table 1**. Voucher specimens of the 22 Taiwanese *Clematis* taxa investigated. (*: Endemic species in Taiwan)

| Species | Collector | Herbarium digital number | Collection information |
|---|---|---|---|
| *Clematis akoensis* Hayata | Po-Hao Chen | PPI75993 | Shouka, Pingtung County (SP) |
| *Clematis chinensis* Osbeck var. *chinensis* | Po-Hao Chen | PPI73776 | Lising, Nantou County (LN) |
| *Clematis chinensis* Osbeck var. *tatushanensis* T.Y.A. Yang | Po-Hao Chen | PPI76140 | Shalu, Taichung City (ST) |
| *Clematis crassifolia* Benth. | Chien-Fan Chen | TAIF449023 | Henglingshan, Taichung City (HT); Manabangshan, Miaoli County (MM) |
| *Clematis formosana* Kuntze | Po-Hao Chen | PPI76748 | Shoushan, Kaohsiung City (SK) |
| *Clematis gouriana* Roxb. ex DC. subsp. *lishanensis* T.Y.A. Yang & T.C. Huang | Sheng-Zehn Yang | PPI57118 | Hehuanshan, Nantou County (HN) |
| *Clematis grata* Wall. | Po-Hao Chen | PPI79191 | Dapu, Chiayi County (DC) |
| *Clematis henryi* Oliv. var. *henryi* | Po-Hao Chen | PPI76097 | Beidawushan, Pingtung County (BP) |
| *Clematis henryi* Oliv. var. *morii* (Hayata) T.Y.A. Yang & T.C. Huang | Her-Long Chiang | PPI65589 | Sinjhongheng, Chiayi County (SC) |
| *Clematis lasiandra* Maxim. | Sheng-Zehn Yang | PPI57133 | Lingmingshan, Taichung City (LT); Hehuanshan, Nantou County (HN) |
| *Clematis leschenaultiana* DC. | Chien-Fan Chen | PPI63232 | Duona trail, Kaohsiung City (DK) |
| *Clematis meyeniana* Walp. | Chien-Fan Chen | PPI60238 | Duona trail, Kaohsiung City (DK) |
| *Clematis montana* Buch.-Ham. ex DC. | Chien-Fan Chen | PPI63771 | Hehuanshan, Nantou County (HN) |
| *Clematis parviloba* Gard. ex Champ. subsp. *bartlettii* (Yamam.) T.Y.A. Yang & T.C. Huang | Guang-Pu Hsieh | PPI61169 | Hehuan river, Nantou County (HRN) |
| *Clematis pseudootophora* M.Y. Fang | Chien-Fan Chen | TAIF455630 | Sihyuanyakou, Yilan County (SY) |
| *Clematis psilandra* Kitag. | Guang-Pu Hsieh | PPI60043 | Alishan, Chiayi County (AC) |
| *Clematis tamurae* T.Y.A. Yang & T.C. Huang | Po-Hao Chen | PPI79723 | Fonggangshan, Kaohsiung City (FK) |
| *Clematis tashiroi* Maxim. | Po-Hao Chen | PPI78815 | Rueisuei trail, Hualien County (RH); Beizihtong forest trail, Chiayi county (BC) |
| *Clematis terniflora* DC. var. *garanbiensis* (Hayata) M.C. Chang | Po-Hao Chen | PPI78668 | Hengchun, Pingtung County (HP) |
| *Clematis tsugetorum* Ohwi | Guang-Pu Hsieh | PPI68569 | Hehuanshan, Nantou County (HN) |
| *Clematis uncinata* Champ. ex Benth. var. *okinawensis* (Ohwi) Ohwi | Kun-Ping Lo | PPI69701 | Dahanshan, Pingtung County (DP) |
| *Clematis uncinata* Champ. ex Benth. var. *uncinata* | Jyuen-Jyie Chen | PPI63904 | Syuejian, Miaoli County (SM); Shihkejianshan, Pingtung County (SHP) |

(Fig. 3E), *C. leschenaultiana* (Fig. 4A), *C. montana* (Fig. 4E), *C. parviloba* subsp. *bartlettii* (Fig. 4F), and *C. terniflora* var. *garanbiensis* (Fig. 6F). However, *C. lasiandra* had both hexagonal stem, and cork layer. The stems of two species had an irregular conformation: the stem of *C. henryi* var. *morii* (Fig. 3D) was a lobe with an asymmetrical conformation, and that of *C. formosana* (Fig. 2C) was eccentric to oval or elliptical. The stem of *C. gouriana* subsp. *lishanensis* (Fig. 2D) displayed a discontinuous wedge-like phloem, as defined by Angyalossy *et al*. (2012; 2015).

Thirteen samples from 11 species (*C. akoensis* (Fig. 1A), *C. chinensis* var. *chinensis* (Fig. 1C), *C. crassifolia* (Fig. 1F), *C. formosana* (Figs. 2B–C), *C. grata* (Fig. 2E), *C. montana* (Fig. 4E), *C. tamurae* (Fig. 5A), *C. tashiroi* (Fig. 6B), *C. terniflora* var. *garanbiensis* (Figs. 6E–F), *C. uncinata* var. *okinawensis* (Fig. 7C), and *C. uncinata* var. *uncinata* (Fig. 7E)) had primary phloem fibers (referred to as primary sclerenchyma fibers) and were identified as being in an early stage of development (Isnard *et al.* 2003a, b).

Seventeen species had wrinkled rhytidome; among them, nine had continuous segment rhytidome and eight (for example, *C. parviloba* subsp. *bartlettii* (Fig. 4F)) had cogwheel-like rhytidome, which peeled and teared easily. An arc-like bark developed and gradually formed cogwheel-like bark in eight species: *C. chinensis* var. *chinensis* (Fig. 1D), *C. grata* (Figs. 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* (Figs. 7C–D), and *C. uncinata* var. *uncinata* (Figs. 7E–F; Table 2, AB column). The rhytidome of *C. lasiandra* (Fig. 3F) was cogwheel-like with 0.5–2.7 mm thickness and that of *C. crassifolia* (Fig. 2A) was a continuous segment and was the thickest of all (1.8–3.4 mm; Table 2). The remaining five species, *C. akoensis* (Figs. 1A–B), *C. chinensis* var. *tatushanensis* (Fig. 1E), *C. psilandra* (Figs. 5C–D)*, C. tashiroi* (Figs. 6B–D)*,* and *C. tsugetorum* (Fig. 7B), were without rhytidome.

In this study, different stem sizes, without rhytidomes, were observed in *C. tashiroi*. In the younger stems (4.4 mm in diameter; Fig. 6B), the cross-sections had primary phloem fibers as strands along the secondary phloem; in

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

| Scientific name | Lo | EL (m) | SD (mm) | VB (no.) | CO | PF | AB | CLR | CSR | RHL (no.) | RHT (mm) | WP | RD | WLR | WR (no.) | IN | WLI | NR (no.) | XW (mm) | RW (µm) | RP | SRP | VR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. akoensis* | SP | 350 | 3.8 | 14 | – | + | – | – | – | – | – | – | – | – | – | – | – | – | 0.31 | 205 | – | + | – |
| *C. akoensis* | .. | .. | 6.0 | 12 | – | – | – | – | – | – | – | – | + | + | – | + | – | – | 0.41 | 441 | – | + | – |
| C. chinensis var. chinensis | LN | 1800 | 4.3 | 20 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.41 | 104 | – | + | – |
| C. chinensis var. chinensis | .. | .. | 11.9 | 22 | – | + | + | + | – | 3–5 | 0.3–0.7 | – | – | – | 2 | + | – | 3 | 0.83 | 232 | – | + | – |
| C. chinensis var. tatushanensis | ST | 150 | 2.2 | 20 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.21 | 25 | – | + | – |
| C. crassifolia | HT | 1700 | 4.1 | 20 | – | + | – | – | – | – | – | – | + | – | – | – | – | – | 0.29 | 127 | – | + | – |
| *C. crassifolia* | MM | 1200 | 8.9 | 20 | + | – | – | – | + | 3–6 | 1.8–3.4 | – | + | – | 6 | + | – | – | 0.47 | 186 | + | + | – |
| *C. formosana* | SK | 200 | 3.4 | 12 | + | + | – | – | + | 1 | 0.1–1.3 | – | + | – | – | + | – | – | 0.41 | 90 | – | + | – |
| *C. formosana* | .. | .. | 11.3 | 12 | – | + | – | – | + | 2 | 0.3–1.8 | – | – | – | – | + | – | 32 | 1.48 | 138 | – | + | – |
| *C. gouriana subsp. lishanensis* | HN | 2600 | 6.2 | 12 | – | – | – | – | + | 1–2 | 0.2–0.7 | + | – | – | 12 | + | – | 14 | 0.81 | 108 | – | + | – |
| C. grata | DC | 500 | 3.5 | 12 | + | + | – | – | – | 1 | 0.1–0.3 | – | – | – | – | – | – | – | 0.46 | 89 | – | + | – |
| C. grata | .. | .. | 3.9 | 12 | – | – | + | + | + | 4 | 0.3–1.1 | – | + | + | 14 | + | – | 11 | 0.49 | 108 | – | + | – |
| C. grata | .. | .. | 14.1 | 12 | – | – | – | – | – | 1–3 | 0.4–0.9 | – | – | – | – | + | – | – | 1.69 | 642 | – | + | – |
| C. henryi var. henryi | BP | 1500 | 5.5 | 10 | – | – | – | – | + | 1 | <0.1 | – | – | + | – | + | – | – | 0.38 | 449 | – | + | – |
| *C. henryi var. morii* | SC | 2400 | 4.0 | 10 | – | – | – | – | + | 1 | <0.1 | – | – | – | – | – | – | – | 0.58 | 283 | – | + | – |
| *C. henryi var. morii* | .. | .. | 7.3 | 11 | – | – | – | – | + | 1 | 0.1–0.2 | + | + | + | – | + | + | – | 0.90 | 553 | – | + | – |
| C. lasiandra | LT | 3000 | 2.0 | 12 | + | + | – | – | – | – | – | – | – | – | – | – | – | – | 0.34 | 96 | – | + | – |
| C. lasiandra | HN | 2800 | 19.7 | 14 | – | – | + | + | + | 2–5 | 0.5–2.7 | – | + | + | – | – | – | 30 | 2.76 | 230 | + | + | – |
| C. leschenaultiana | DK | 700 | 6.4 | 21 | + | – | – | – | + | 1 | 0.2–0.3 | – | + | – | – | + | – | – | 0.45 | 175 | – | + | – |
| C. leschenaultiana | .. | .. | 7.8 | 16 | – | – | – | – | + | 2 | 0.2–0.5 | – | + | – | – | + | – | 6 | 0.69 | 145 | – | + | – |
| C. leschenaultiana | .. | .. | 15.6 | 19 | – | – | – | – | + | 3 | 0.2–1.6 | – | + | – | – | + | – | 15 | 1.34 | 225 | – | + | – |
| C. meyeniana | DK | 700 | 7.3 | 20 | – | – | – | – | + | 2 | 0.4–0.8 | – | + | – | – | + | – | – | 0.58 | 117 | – | + | – |
| C. montana | HN | 3200 | 3.1 | 12 | + | + | – | – | + | 1 | 0.2–0.3 | – | + | – | – | + | – | – | 0.34 | 202 | – | + | – |
| *C. parviloba subsp. bartlettii* | HRN | 2600 | 2.4 | 12 | + | – | – | – | + | 1 | 0.1–0.2 | – | + | – | – | + | – | – | 0.32 | 64 | – | + | – |
| *C. parviloba subsp. bartlettii* | .. | .. | 3.9 | 12 | + | – | – | – | + | 2 | 0.1–0.3 | – | + | – | – | + | – | – | 0.49 | 147 | – | + | – |
| C. pseudootophora | SY | 1800 | 3.5 | 6 | – | – | – | + | + | 1 | 0.1–0.2 | – | – | + | – | + | + | – | 0.93 | 393 | – | + | + |
| *C. psilandra* | AC | 2300 | 4.3 | 20 | – | – | – | – | – | 2 | 0.2–0.3 | – | – | – | – | – | – | – | 0.33 | 387 | + | + | + |
| *C. psilandra* | .. | .. | 4.9 | 23 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.20 | 218 | – | + | – |
| C. tamurae | FK | 1600 | 2.1 | 12 | + | + | – | – | – | – | – | – | – | – | – | – | – | – | 0.22 | 107 | – | + | – |
| C. tamurae | .. | .. | 4.5 | 12 | + | + | + | + | – | 1 | 0.1–0.3 | – | + | – | – | + | – | – | 0.52 | 167 | – | + | – |
| C. tamurae | .. | .. | 8.3 | 12 | + | + | + | + | – | 3–4 | 0.1–1.2 | – | + | – | – | + | – | – | 0.91 | 393 | – | + | – |
| C. tashiroi | RH | 2400 | 4.0 | 20 | – | + | – | – | – | – | – | – | – | – | – | – | – | – | 0.32 | 289 | + | + | – |
| C. tashiroi | BC | 1600 | 8.8 | 20 | + | – | – | – | – | – | – | – | + | – | 4 | + | – | 6 | 0.58 | 343 | – | + | – |
| *C. tashiroi* | .. | .. | 17.8 | 20 | + | – | – | – | – | – | – | – | + | – | 6 | + | – | 18 | 1.44 | 450 | – | – | – |
| *C. terniflora var. garanbiensis* | HP | 100 | 2.7 | 14 | + | + | – | – | – | – | – | – | – | – | – | – | – | – | 0.33 | 63 | – | + | – |
| *C. terniflora var. garanbiensis* | .. | .. | 2.8 | 12 | + | + | + | + | – | 1 | 0.1–0.2 | – | + | – | – | + | – | – | 0.34 | 77 | – | + | – |
| *C. terniflora var. garanbiensis* | .. | .. | 4.3 | 19 | + | + | + | + | – | 1–2 | 0.2–0.5 | – | + | – | – | + | – | – | 0.36 | 77 | – | + | – |
| *C. tsugetorum* | HN | 3200 | 3.9 | 17 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.17 | 374 | + | + | + |
| C. uncinata var. okinawensis | DC | 900 | 4.0 | 14 | – | – | – | – | – | – | – | – | + | – | – | – | – | – | 0.31 | 195 | – | – | – |
| C. uncinata var. okinawensis | .. | .. | 9.7 | 12 | – | + | – | – | – | 1–3 | 0.5–1.9 | – | + | – | – | + | – | 13 | 0.12 | 332 | – | + | – |
| C. uncinata var. uncinata | SM | 2000 | 5.7 | 12 | + | + | – | – | – | – | – | – | + | – | – | – | – | – | 0.73 | 260 | – | + | – |
| C. uncinata var. uncinata | SHP | 1300 | 12.4 | 14 | – | – | + | + | – | 2–3 | 0.5–1.5 | – | + | – | – | + | – | 11 | 1.38 | 386 | – | + | – |
| Sum (sp no.) | | | | | 7 | 11 | 8 | 8 | 9 | | | 1 | 11 | 5 | 5 | 17 | 2 | 9 | | | 4 | 18 | 2 |

Noted: *: endemic species; LO: locality (For detailed information see Table 1); EL: elevation; SD: stem diameter; VB: number of vascular bundle; CO: cork; PF: primary phloem fibers; AB: arc-like bark; CLR: cogwheel-like rhytidome; CSR: continuous segment rhytidome; RHL: rhytidome layer number; RHT: rhytidome thickness; WP: wedge-like phloem; RD: ray dilatations; WLR: wedge-like rays; WR: number of wide rays; IN: indentation of the axial parenchyma near the wider rays; WLI: wedge-like indentation; NR: narrow rays number; XW: xylem width (mean, n = xylem number); RW: ray width (mean, n = ray number); RP: ring-porous vessel; SRP: semi-ring-porous vessel; VR: vessel restriction pattern.
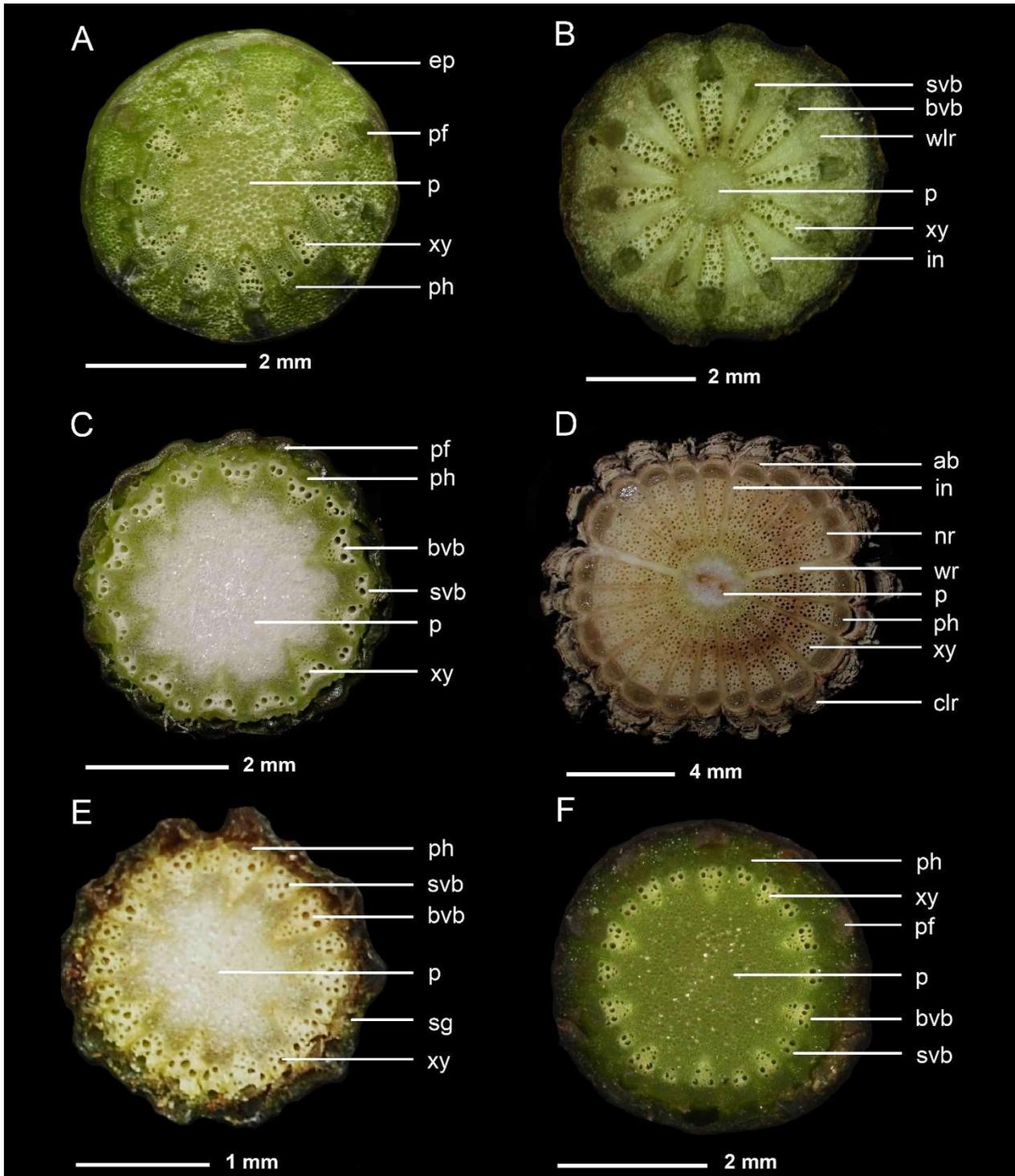
**Fig. 1.** Stem transverse sections of *Clematis* species. **A**. *C. akoensis*: stem diameter approximately 3.8 mm with thin epidermis (ep), primary phloem fibers (pf) in dense bundles, 14 vascular bundles with phloem (ph) and xylem (xy). **B**. *C. akoensis*: stem diameter approximately 6.0 mm, secondary phloem (ph) triangular in shape, wedge-like rays (wlr) obvious, 10 big vascular bundles (bvb), 2 small vascular bundles (svb), ray dilatation (rd), pith (p) round in shape, indentations (in) near the broader rays. **C**. *C. chinensis* var. *chinensis*: 10 big vascular bundles (bvb) and 10 small vascular bundles (svb), pith (p) white. **D**. *C. chinensis* var. *chinensis*: primary phloem fibers (pf) strands, cogwheel-like rhytidome (clr) with 3–4 layers formed by arc of bark (ab), sequent periderm above semi-circular secondary phloem, indentations (in) near the broader rays, 22 vascular bundles, 3 narrow rays (nr), 2 wide rays (wr). **E**. *C. chinensis* var. *tatushanensis*: stem with 10 shallow grooves (sg), 10 big vascular bundles (bvb) and 10 small vascular bundles (svb), pith (p) white. **F**. *C. crassifolia*: primary phloem fibers (pf) in dense bundles, 10 big vascular bundles (bvb) and 10 small vascular bundles (svb), pith (p) green.
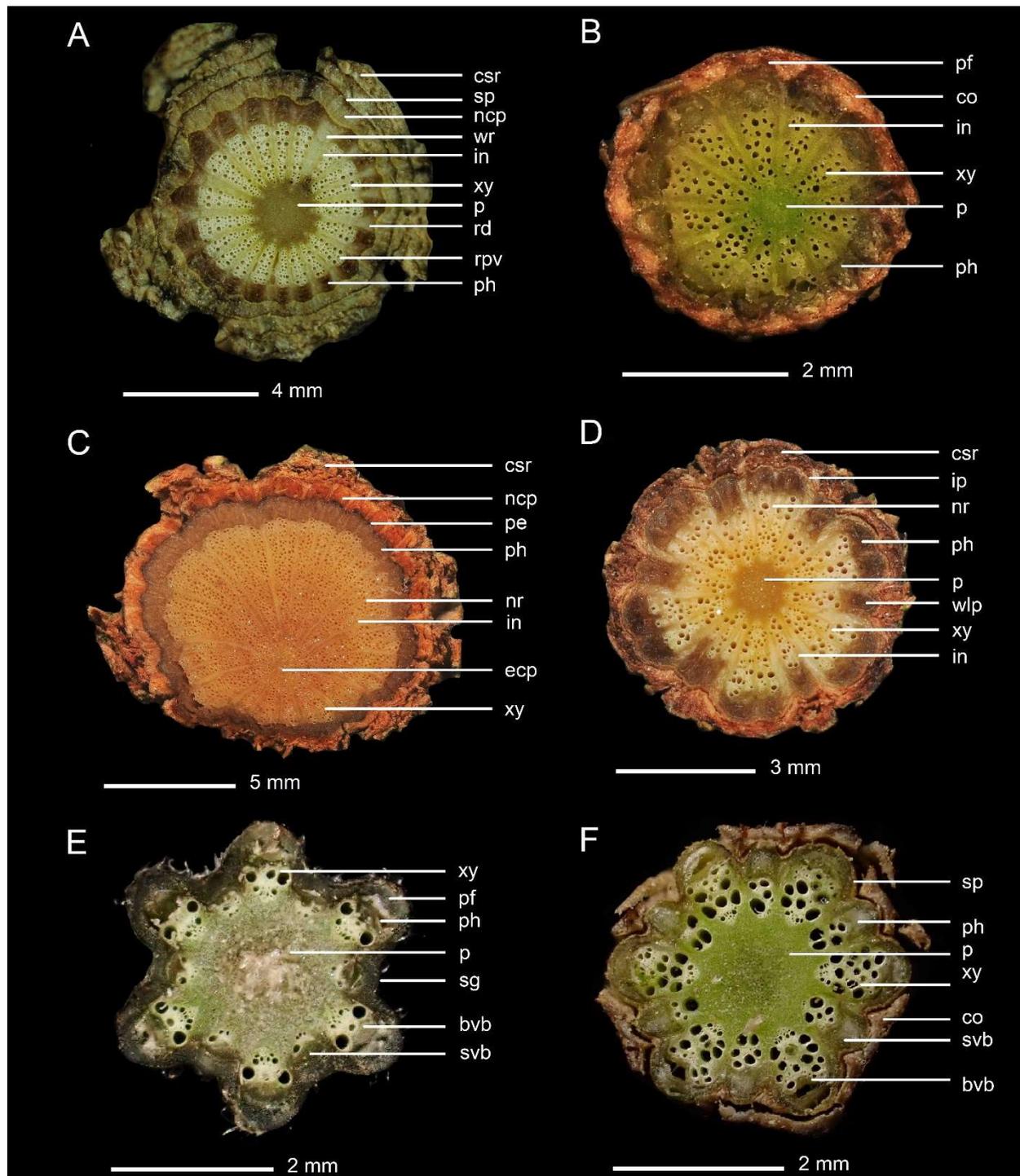
**Fig. 2.** Stem transverse sections of *Clematis* species. **A**. *C. crassifolia*: sequent periderm (sp) produced in deeper layers within the secondary phloem, continuous segment rhytidome (csr) with 3–6 layers, each layer composed periderm (pe) and non-conducting phloem (ncp), indentations (in) near the broader rays, ray dilatation (rd), 6 wide rays (wr) initial abruptly, ring-porous vessels (rpv). **B**. *C. formosana*: a pair of phloem fibers (pf) strands within the cork (co), indentations (in) near the broader rays, pith (p) green. **C**. *C. formosana*: stem eccentric to oval or elliptical, secondary phloem fibers strands, black, ring-like phloem (ph), continuous segment rhytidome (csr) with 2 layers, each layer composed periderm (pe) and non-conducting phloem (ncp), 32 narrow rays (nr), indentations (in) near the broader rays, eccentric pith (ecp). **D**. *C. gouriana* subsp. *lishanensis*: ring-like sequent periderm (sp), continuous segment rhytidome (csr), indentations (in) near the broader rays, discontinuous wedge-like phloem (wlp), 12 abrupt wide rays (wr), 14 narrow rays (nr). **E**. *C. grata*: stem diameter approximately 3.5 mm with 6 shallow grooves (sg), phloem fiber (pf) in dense bundles beneath the angular extension of stem, 6 big vascular bundles (bvb) and 6 small vascular bundles (svb). **F**. *C. grata*: stem diameter approximately 3.9 mm, ring-like initial periderm (ip), yellow, 6 big vascular bundles (bvb) and 6 small vascular bundles (svb), pith (p) green.
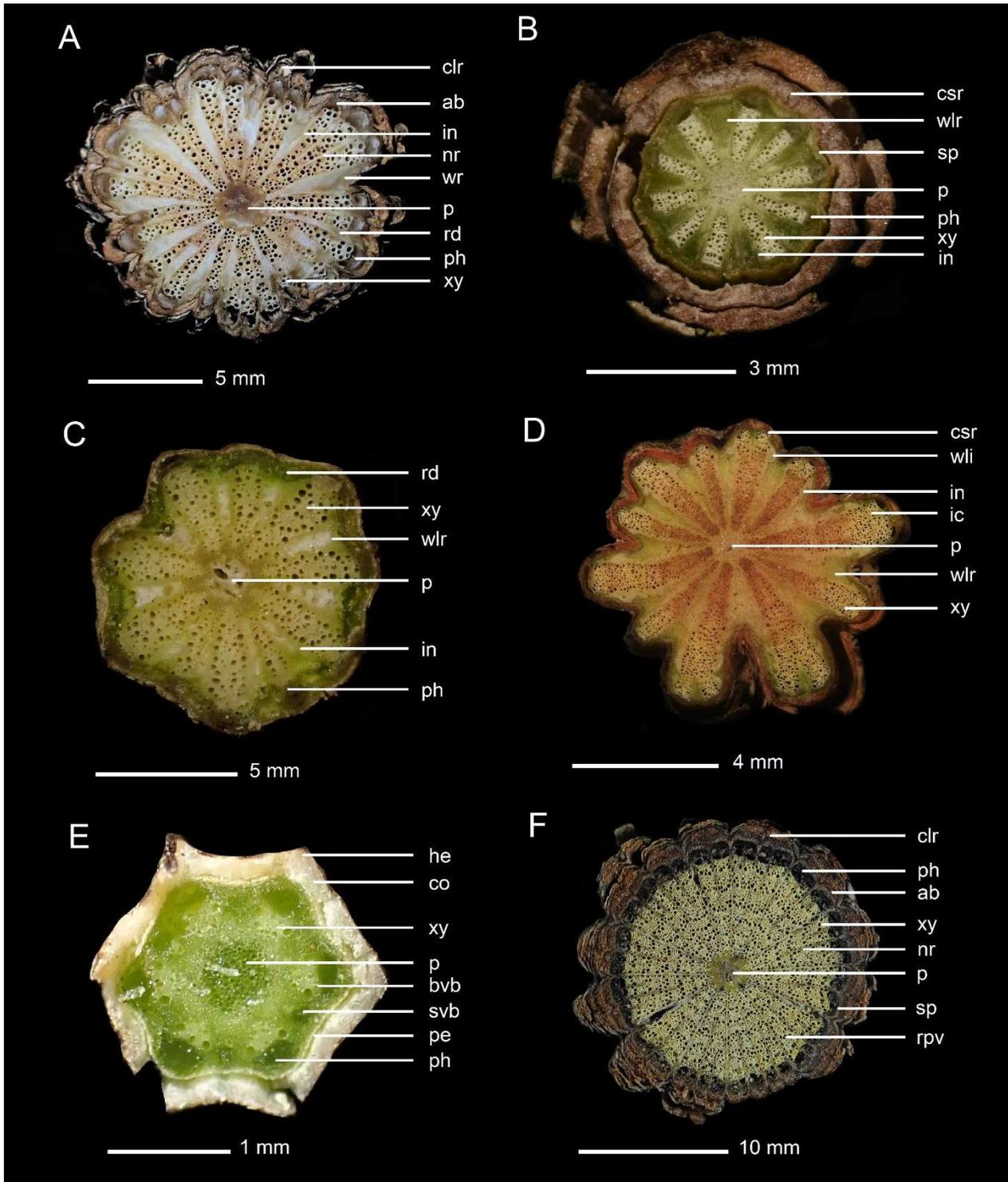
**Fig. 3.** Stem transverse sections of *Clematis* species. **A**. *C. grata*: arc of bark (ab) to form cogwheel-like rhytidome (clr), ray dilatation (rd), 14 wide rays (wr), indentations (in) near the broader rays, 11 narrow rays (nr). **B**. *C. henryi* var. *henryi*: stem round in shape, ring-like sequent periderm (sp), yellow in color, continuous segment rhytidome (csr) with 2 layers, indentations (in) near the broader rays, wedge-like rays (wlr). **C**. *C. henryi* var. *morii*: stem diameter approximately 4.0 mm with shallowly lobed, wedge-like rays (wlr), indentations (in) near the broader rays, ray dilatation (rd), 10 vascular bundles. **D**. *C. henryi* var. *morii*: stem diameter approximately 7.3 mm with irregularly conformation (ic), continuous segment rhytidome (csr) thin, wedge-like rays (wlr), wedge-like indentation (wli) near the wider rays, indentations (in) near the broader rays, 11 vascular bundles. **E**. *C. lasiandra*: hexagon-shaped stem (he) with cork (co) layer, 2 mm in diameter, 6 big vascular bundles (bvb) and 6 small vascular bundles (svb). **F**. *C. lasiandra*: arc of bark (ab) to form cogwheel-like rhytidome (clr) with 2–5 layers, ring-like sequent periderm (sp), 30 narrow rays (nr), ring-porous vessels (rpv).
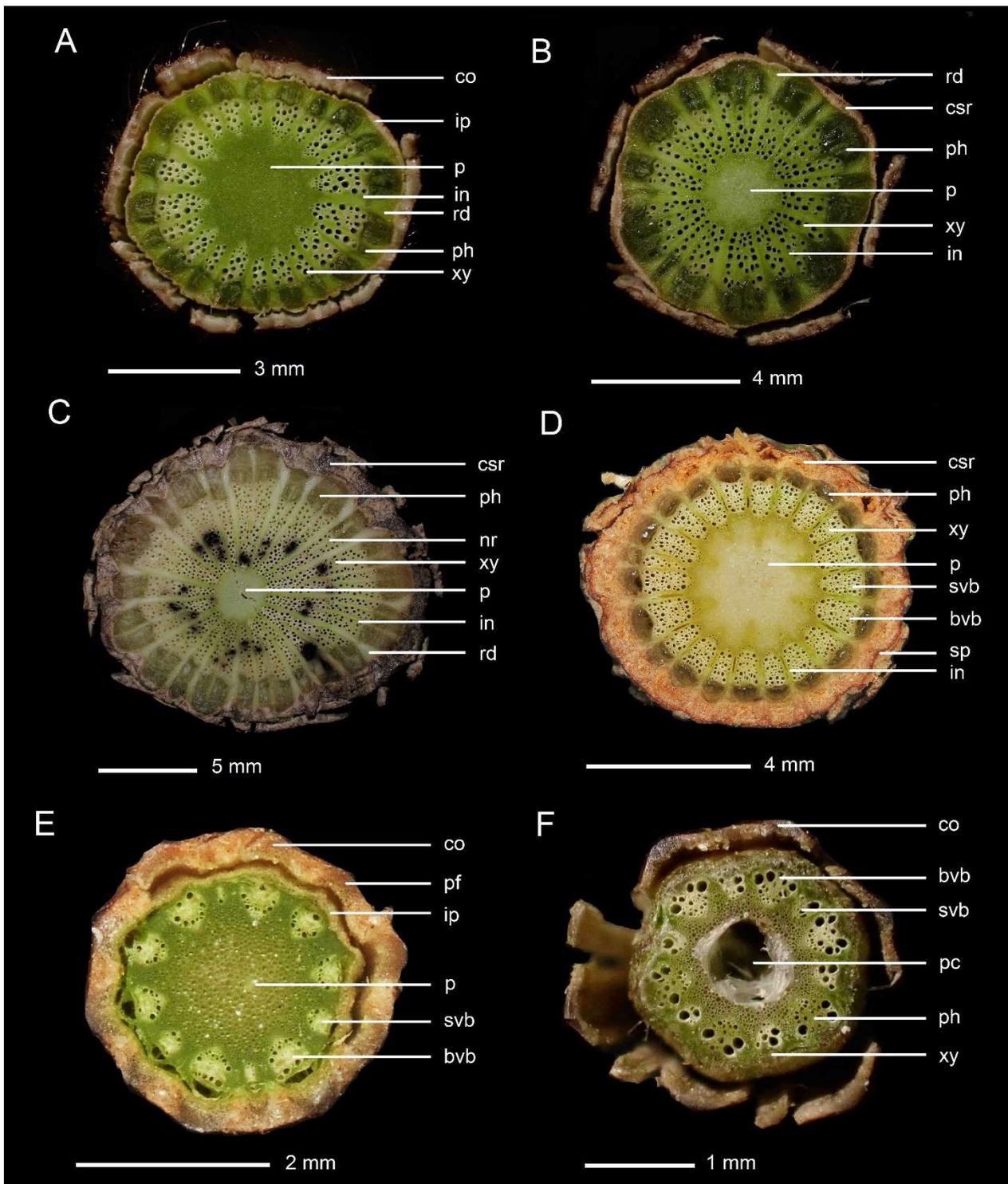
**Fig. 4.** Stem transverse sections of *Clematis* species. **A**. *C. leschenaultiana*: cork layer to form rhytidome, semi-circular secondary phloem (ph), initial periderm (ip), ray dilatation (rd), indentations (in) near the broader rays, 21 vascular bundles. **B**. *C. leschenaultiana*: aggregated secondary phloem (ph), continuous segment rhytidome (csr) with two-layer, ray dilatation (rd), indentations (in) near the broader rays, 16 vascular bundles. **C**. *C. leschenaultiana*: sequent periderms within the secondary phloem, continuous segment rhytidome (csr) with 3 layers, ray dilatation (rd), indentations (in) near the broader rays, 15 narrow rays (nr), 19 vascular bundles. **D**. *C. meyeniana*: ring-like sequent periderm (sp), continuous segment rhytidome (csr) with 2 layers, 10 big vascular bundles (bvb) and 10 small vascular bundles (svb), indentations (in) near the broader rays, pith (p) pale white. **E**. *C. montana*: cork with primary phloem fibers (pf) strands, ring-like initial periderm (ip), yellow, 10 big vascular bundles (bvb) and 2 small vascular bundles (svb). **F**. *C. parviloba* subsp. **bartlettii**: 1 layer cork, 6 big vascular bundles (bvb) and 6 small vascular bundles (svb), pith cavity (pc).
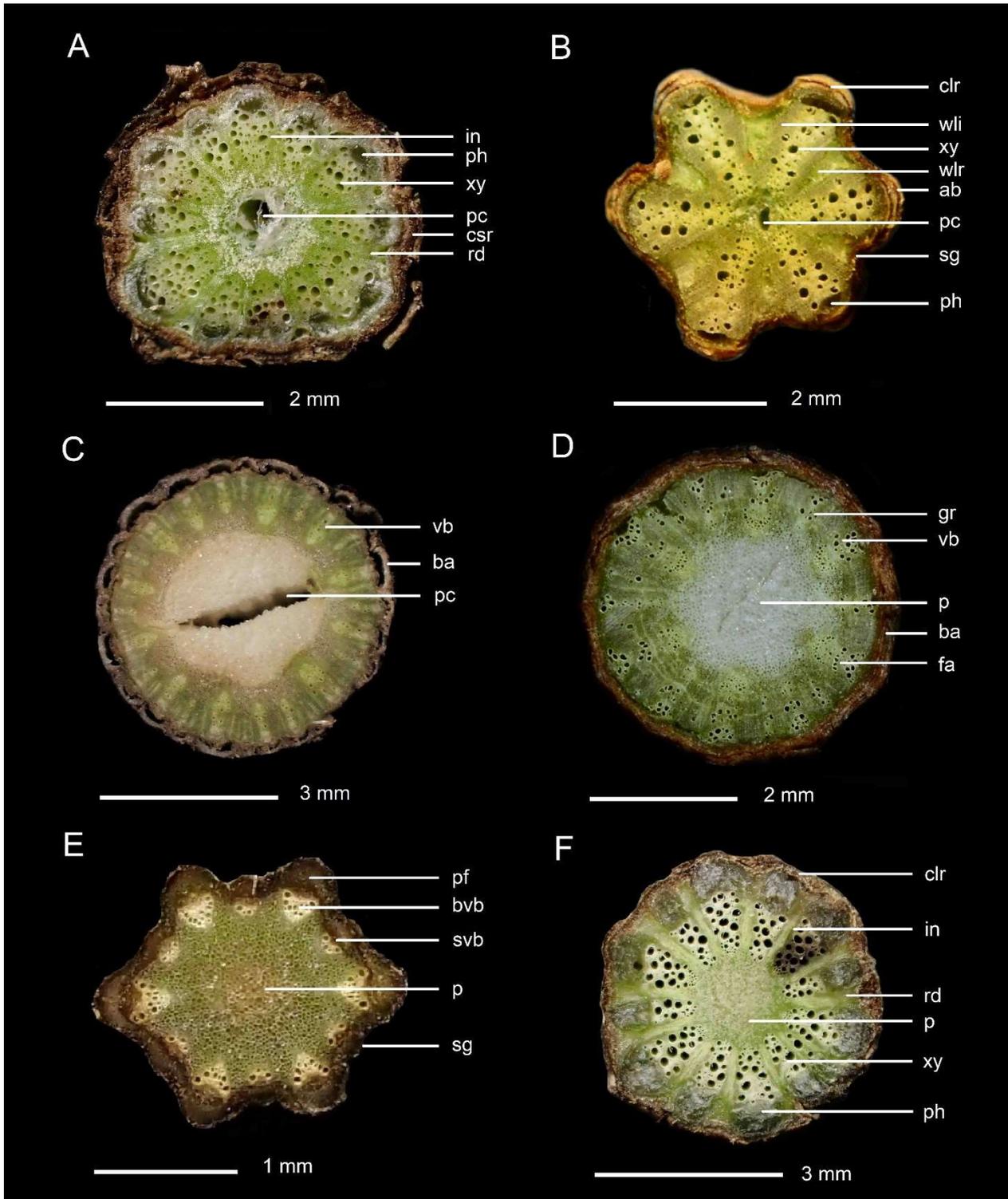
**Fig. 5.** Stem transverse sections of *Clematis* species. **A**. *C. parviloba* subsp. *bartlettii*: continuous segment rhytidome (csr) with 2 layers, ray dilatation (rd). **B**. *C. pseudootophora*: stem with 6 shallow grooves (sg), arc of bark (ab) to form cogwheel-like rhytidome (clr) with 2 layers, wedge-like rays (wlr) and wedge-like indentation (wli), only 6 vascular bundles, pith cavity (pc). **C**. *C. psilandra*: bark (ba) thin without rhytidome, 20–23 vessel bundles (vb), pith cavity (pc). **D**. *C. psilandra*: bark (ba) thick, vessel limited to the central portions of fascicular area (fa), vessel like ring-porous (rpv) to form growth ring (gr), 20–23 vessel bundles (vb), pith (p) white. **E**. *C. tamurae*: stem diameter approximately 2.1 mm with 6 shallow grooves (sg), primary phloem fiber (pf) ring-like, 6 big vascular bundles (bvb) and 6 small vascular bundles (svb). **F**. *C. tamurae*: stem diameter approximately 4.5 mm, secondary phloem (ph) dark-green and rectangular, cogwheel-like rhytidome (clr) with 1 layer, ray dilatation (rd), indentation (in) of the axial parenchyma near the wider rays.
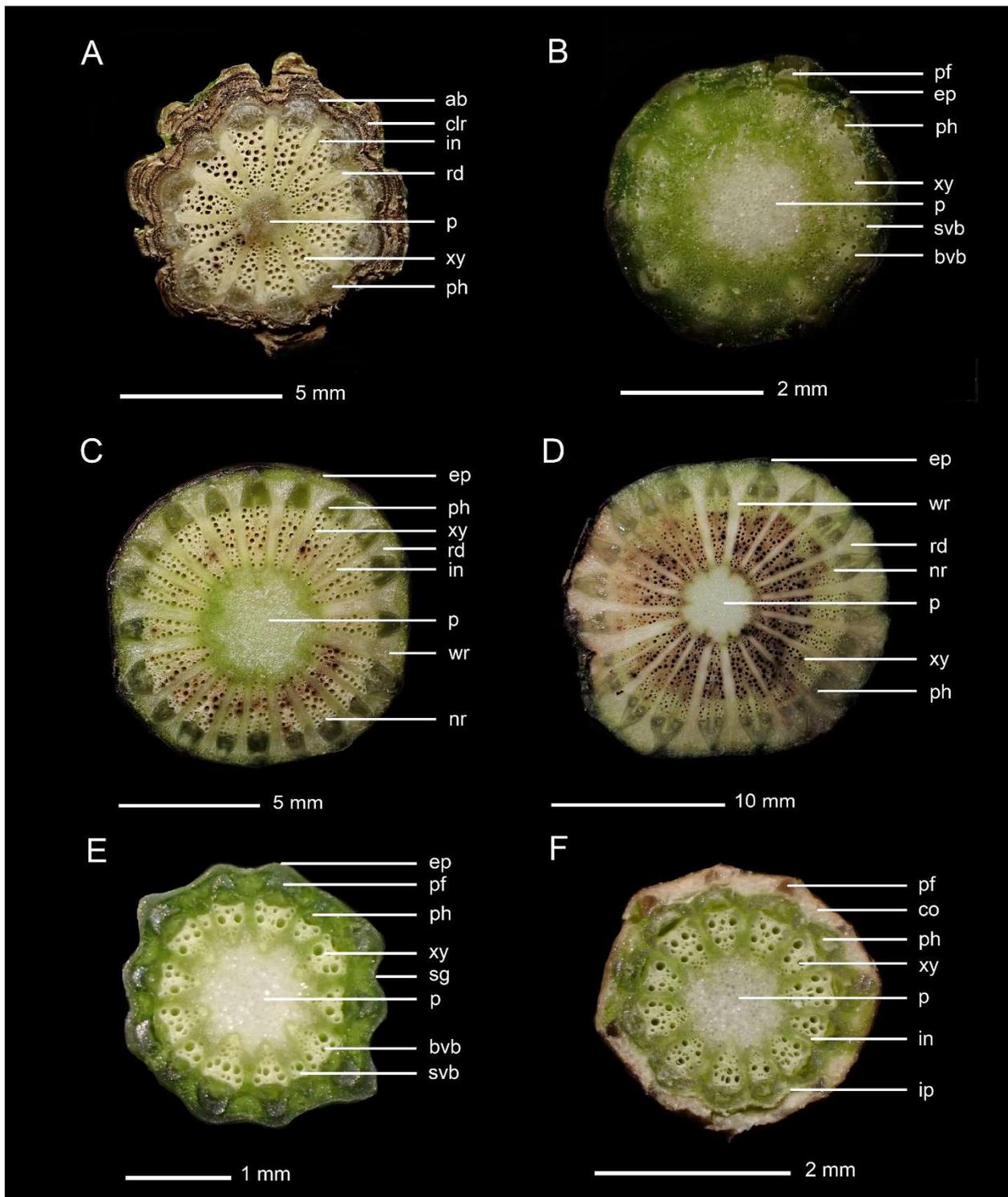
**Fig. 6.** Stem transverse sections of *Clematis* species. **A**. *C. tamurae*: arc-like bark (ab) to form cogwheel-like rhytidome (clr) with 3–4 layers, ray dilatation (rd), indentation (in) near the wider rays. **B**. *C. tashiroi*: stem diameter approximately 4.0 mm with thin epidermis (ep), primary phloem fiber (pf) stands, 10 big vascular bundles (bvb) and 10 small vascular bundles (svb). **C**. *C. tashiroi*: stem diameter approximately 8.8 mm, secondary phloem (ph) triangular in shape and deep-green, ray dilatation (rd), 4 wide rays (wr), 6 narrow rays (nr). **D**. *C. tashiroi*: stem diameter approximately 17.8 mm without rhytidome, obvious ray dilatation (rd), 6 wide rays (wr), 18 narrow rays (nr), pith (p) white. **E**. *C. terniflora* var. ***garanbiensis***: stem diameter approximately 2.7 mm with grooves (sg), epidermis (ep) green, primary phloem fibers (pf) in bundles, 9 big vascular bundles (bvb) and 5 small vascular bundles (svb). **F**. *C. terniflora* var. ***garanbiensis***: stem diameter approximately 2.8 mm; cork layer to form rhytidome, ring-like initial periderm (ip), primary phloem fibers (pf) triangular, black, 12 vascular bundles, pith (p) white.
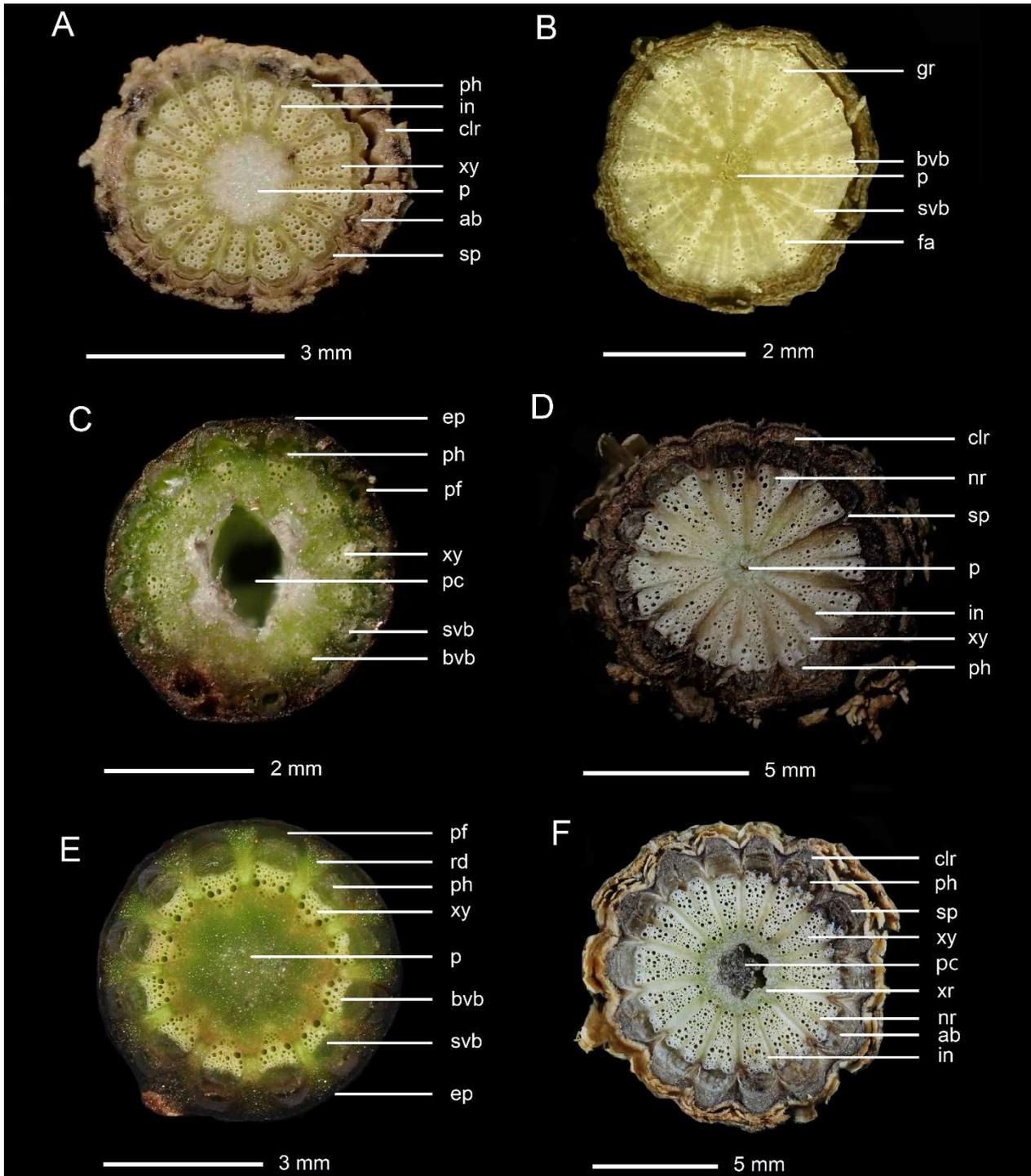
**Fig. 7.** Stem transverse sections of *Clematis* species. **A**. *C. terniflora* var. *garanbiensis*: sequent periderm (sp) above the secondary phloem (ph), arc-like bark (ab) to form cogwheel-like rhytidome (clr) with 2 layers, indentation (in) obvious, pith (p) white. **B**. *C. tsugetorum*: vessels limited to the central portions of fascicular area (fa), vessel like ring-porous (rpv) to form growth ring (gr), 6 big vascular bundles and 11 small vascular bundles. **C**. *C. uncinata* var. *okinawensis*: stem diameter approximately 4.0 mm, epidermis (ep) thin, primary phloem fibers (pf) strands, pith cavity (pc), 8 big vascular bundles and 4 small vascular bundles. **D**. *C. uncinata* var. *okinawensis*: stem diameter approximately 9.7 mm, arc-like bark (ab) to form cogwheel-like rhytidome (clr) with 1–3 layers, ray dilatation (rd), 13 narrow rays (nr), secondary phloem (ph) black. **E**. *C. uncinata* var. *uncinata*: stem diameter approximately 5.7 mm with thin epidermis (ep), primary phloem fibers (pf) strands, ray dilatation (rd), 6 big vascular bundles and 6 small vascular bundles. **F**. *C. uncinata* var. *uncinata*: stem diameter approximately 12.4 mm, arc-like bark (ab) to form cogwheel-like rhytidome (clr) with 2–3 layers, sequent periderm (sp) above the secondary phloem (ph), 15 vascular bundles, 11 narrow rays (nr), pith cavity (pc), hexagonal in shape, primary xylem ring (xr) located around the pith.

the medium sized stems (8.8 mm in diameter; Fig. 6C), secondary phloem became triangular with secondary phloem fibers and an abrupt wide ray and many larger vessels were observed; in the larger stems (17.8 mm in diameter; Fig. 6D), rays were unequal in width and many narrow rays were observed.

Phloem rays sometimes change their size due to longitudinal cell expansion; this feature is referred to as ray dilatation, or expansion tissue. Approximately 50% of the *Climatis* species in this study were observed to have this characteristic. Sometimes phloem rays located in the cortex might increase ray width and stem diameter. For example, three samples of *C. leschenaultiana* always possessed such a significant ray dilatation (Figs. 4A–C).

### Characteristics of the xylem

In species 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, WLR column) interfascicular cambia made the rays wider and wedge-like (Schweingruber, *et al.* 2011). For example, two species (*C. grata* and *C. henryi* var. *morii*) had the widest rays, with maximum widths of 642 µm and 553 µm, respectively (Table 2, RW column). The wider rays of five *Clematis* species (*C. chinensis* var. *chinensis* (Fig. 1D), *C. crassifolia* (Fig. 2A), *C. gouriana* subsp. *lishanensis* (Figs. 2D), *C. grata* (Fig. 3A), *C. leschenaultiana* (Fig. 4C), and *C. tashiroi* (Fig. 6D); Table 2, WR column), were recognized as abrupt wide rays, as defined by Carlquist (1995).

There was an obvious indentation in the region of the wider rays, except in *C. chinensis* var. *tatushanensis* (Fig. 1E), *C. lasiandra* (Fig. 3F), *C. montana* (Fig. 4E), *C. psilandra* (Figs. 5C–D), and *C. tsugetorum* (Fig. 7B). The stem diameters of *C. chinensis* var. *tatushanensis* and *C. montana* might have been too small to have wider rays. Wedge-like indentations developed in the interfascicular region were found in *C. henryi* var. *morii* (Fig. 3D) and *C. pseudootophora* (Fig. 5B; Table 2, WLI column) due to thin-walled axial parenchyma adjacent to the thin-walled ray cells (Sieber and Kucera, 1980).

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* (Figs. 2B–C), *C. gouriana* subsp. *lishanensis* (Fig. 2D), *C. grata* (Fig. 3A), *C. lasiandra* (Fig. 3F), *C. leschenaultiana* (Fig. 4C), *C. tashiroi* (Fig. 6D), *C. uncinata* var. *okinawensis* (Figs. 7C–D), and *C. uncinata* var. *uncinata* (Figs. 7E–F); Table 2, NR column).

Almost all *Clematis* in this study had semi-ring-porous vessels. The exceptions were *C. crassifolia* (Fig. 2A), *C. lasiandra* (Fig. 3F), *C. psilandra* (Figs. 5C–D), and *C. tsugetorum* (Fig. 7B), which had ring-porous vessels with distinct annual rings or growth rings (Table 2, RP column). None of the species had diffuse-porous vessels, which is consistent with previous reports

(Schweingruber *et al.*, 2011).

The axial parenchyma of *C. psilandra* (Figs. 5C–D) and *C. tsugetorum* (Fig. 7B) (Table 2, VR column) was very scarce, and vessel distribution was limited to the central portions of the fascicular area with growth rings, thus corresponding to the vessel restriction pattern described by Carlquist (1995).

### Characteristics of the pith

Pith cavities were formed in *C. parviloba* subsp. *bartlettii* (Figs. 4F; 5A), *C. pseudootophora* (Fig. 5B), *C. psilandra* (Fig. 5C), *C. uncinata* var. *okinawensis* (Fig. 7C), and *C. uncinata* var. *uncinata* (Fig. 7F). Among them, *C. uncinata* var. *uncinata* had a primary xylem ring located around the pith cavity which was hexagonal, and that of the other four species were irregular linear or triangular.

### Bracket key to 22 *Clematis* species

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 with cavity ....................…..... *C. psilandra*
2. Vascular bundles 17, pith without cavity …................…. *C. tsugetorum*
3. With wedge-like phloem …............. *C. gouriana* subsp. *lishanensis*
3. Without wedge-like phloem ………………………......…...…... 4
4. With wedge-like rays …......……………………....................... 5
4. Without wedge-like rays ……………………….................……...... 9
5. Vascular bundles 6 ……………………………... *C. pseudootophora*
5. Vascular bundles >6 …………………………………....................... 6
6. Stem polygonal lobed ……................……………... *C. henryi* var. *morii*
6. Stem rounded ………………………………………...…................…7
7. Bark without rhytidome layers ....….…….…................... *C. akoensis*
7. Bark with rhytidome layers …….............…………….….............. 8
8. Rhytidome cogwheel liked ……………....……………….. *C. grata*
8. Rhytidome continuous segmented ………........... *C. henryi* var. *henryi*
9. Bark without rhytidome ……………..…………………………… 10
9. Bark with one to five rhytidome layers ….……………….............. 11
10. Bark without primary fibers ……...…. *C. chinensis* var. *tatushanensis*
10. Bark with primary fibers …………………...…….……....... *C. tashiroi*
11. Pith with cavity ……………………..... *C. parviloba* subsp. *bartlettii*
11. Pith without cavity ……………….…………………………..…. 12
12. Rhytidome continuous segmented ………................................…… 13
12. Rhytidome cogwheel liked ……………........................................ 17
13. Vascular bundles 12, with cork ….........………………………..... 14
13. Vascular bundles 16–21 …………............ ………………......…… 15
14. Stem eccentric to oval or elliptical liked ...................... *C. formosana*
14. Stem rounded ......…….................…………………….... *C. montana*
15. Vessels, ring-porous, rhytidome thickness 1.8–3.4 mm . *C. crassifolia*
15. Vessels, semi-ring porous …………………………………......….16
16. Rhytidome thickness 0.2–0.3 mm ..…………...…. *C. leschenaultiana*
16. Rhytidome thickness 0.4–0.8 mm …………….……..... *C. meyeniana*
17. Vessels, ring-porous ......................……………………... *C. lasiandra*
17. Vessels, semi-ring-porous ……………………………….…............ 18
18. Pith cavity. …....….…………………………………..……............ 19
18. Pith without cavity …………………………………….…....……... 20
19. Pith with primary xylem ring around ….... *C. uncinata* var. *uncinata*
19. Pith without primary xylem ring around ……………….………….. …............……………………………. *C. uncinata* var. *okinawensis*
20. Vascular bundles 20–22 ………..……... *C. chinensis* var. *chinensis*
20. Vascular bundles 12–19 ……................................……………….. 21
21. Constant vascular bundles 12 ……...……….…...…... *C. tamurae*
21. Varying vascular bundles 12, 14, or 19 .... *C. terniflora* var. *garanbiensis*

## DISCUSSION

*Clematis* species often have 12 vascular bundles in the stem, which similar as Smith (1928) findings in 67% of the 138 species studied. This is generally considered the central type of the genus. In Taiwan, only 27% of the 22 *Clematis* species have 12 vascular bundles. Five of the species in our study had numbers of vascular bundles that were inconsistent with the findings of Smith (1928). In the present study, *C. henryi* var. *henryi* (Fig. 3B) had ten vascular bundles, but only six were recorded by Smith (1928). Moreover, *C. chinensis* var. *chinensis* (Figs. 1C−D) had 20−22 bundles, *C. lasiandra* (Fig. 3F) had 12−14, *C. meyeniana* (Fig. 4D) had 20, and both *C. uncinata* var. *okinawensis* (Figs. 7C−D), and *C. uncinata* var. *uncinata* (Figs. 7E–F) had 12–14, but Smith (1928) recorded only 12 vascular bundles in these taxa. The inconsistent results might be due to different sample sizes, developmental stages, or distances from the base to the upper portion of the stem; further investigation is required to elucidate this discrepancy.

Vascular bundle numbers are usually constant in all species. We examined three samples of *C. leschenaultiana* (Figs. 4A−C) collected from the same branch and found that the number of vascular bundles varied in different stem portions. The numbers of vascular bundles that were observed in *C. leschenaultiana* varied from 21 (Fig. 4A) to 16 (Fig. 4B) to 19 (Fig. 4C). To confirm this observation, future work should focus on collecting and observing more specimens from different stem positions of the same species.

The examined *Clematis* species generally developed axial vascular elements in segment types, separated by wider xylem and phloem rays. The stems of two species had an irregular conformation, such as a lobe with an asymmetrical conformation, and eccentric to oval or elliptical. Only one species formed a discontinuous wedge-like phloem. This can generate an irregular conformation, as found in some Apocynaceae and Malpighiaceae species (Angyalossy *et al*., 2015) or a discontinuous wedge-like phloem, which is a common characteristic of the family Bignoniaceae (Pace *et al*., 2011). In summary, apart from axial vascular elements in segments, two cambial variants were found in the Ranunculaceae family-stems with an irregular conformation, and phloem arcs/wedges.

Cork layers were related to rhytidome formation in stems of the genus *Clematis* (Table 2). Five of the studied taxa had no rhytidome, whereas the remaining 17 species developed cogwheel-like or continuous segment rhytidome, in which cork and non-conducting phloem were combined. Inspection of the samples of those taxa revealed that in seven species, this conspicuous cork layer was always observed in smaller stems; however, this cork feature needs to be investigated in different stem sizes in the future.

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 in the current study (Table 2, AB column). The arc feature comprised several layers of periderm combined with a non-conducting phloem (e.g., Fig. 1D). The characteristic of arc-like bark provides useful diagnostic evidence for distinguishing among *Clematis* species. 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 could be highly variable, as in the genus *Clematis*; phellogen could form deep within the cortex, in the outer secondary phloem, or even within the secondary phloem (Isnard *et al*., 2003a).

The rhytidome provides one of the diagnostic characteristics of the genus *Clematis*. The texture and detachment of the rhytidome result from an irregular appearance during the developmental stages. It is easier to correctly identify the structure of the rhytidome from fresh materials than from dry materials. Among the 22 taxa, the rhytidome was not observed in five species (Table 2) because of their smaller stem diameters or earlier production of periderm, which establishes the inner cortex. The epidermis of *C. tashiroi* was deep green in color and glabrous, which differed from the other *Clematis* species. The stem diameters of some other *Clematis* species studied were smaller than 8.8 mm and yet they could form a rhytidome, but stems of *C. tashiroi* were 8.8 mm (Fig. 6C) and 17.8 mm (Fig. 6D) in diameter and yet they did not appear to have a rhytidome. In this study, stem diameters of some plants were less than 10 mm already had rhytidome (see Table 2), but the bark of *C. tashiroi* had no rhytidome even the stem diameter was about 17.8 mm. Perhaps its bark might continue to develop into one of the rhytidome types or no rhytidome structure was its habit indeed, therefor, continued observations are necessary.

*Clematis henryi* is subdivided on *C. henryi* var. *henryi,* and *C. henryi* var. *morii* (Yang and Huang, 1996), but *C. henryi* var. *morii* is sometimes treated as an independent species (Wang and Bartholomew, 2007). In the present study, these two species 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* had an irregular conformation. Thus, investigating the stem shapes from different populations and different age stages were needed to provide evidences for distinguishing these two species.

Indentation toward the pith in the region of wider rays is obvious in *Clematis* species. This feature was found in most investigated taxa in this study, except for those with a small stem diameter (Table 2, IN column). In the present study, narrow rays were observed in nine *Clematis* species. Narrow rays have been recorded in the species *Aristolochia macrophylla* (Aristolochiaceae) by Carlquist

(1993) and Schweingruber *et al.* (2011); in the Cucurbitaceae family by Carlquist (1992), and in the *Cyclea* genus (Menispermaceae) by Yang and Chen (2016). The characteristics of narrow rays, activity of the bigger vascular bundles, and the fascicular areas of *C. grata* are similar to those of *C. vitalba*, suggesting that they could be related to stem bark thickness and the formation of cogwheel-like bark. However, narrow rays were not found in all of the taxa investigated in our study, and their presence might be related to stem diameter size, but this needs further investigation.

The vessel restriction pattern was described in another Ranunculaceae *Xanthorhiza apiifolia* by Carlquist (1995). This pattern was observed in *C. psilandra* (Figs. 5C–D) and *C. tsugetorum* (Fig. 7B), but few vessels were in contact with the rays; this phenomenon has previously been described in the genus *Clematis* by Carlquist and Zona (1988), who suggested that the *Clematis* species clearly showed vessel restriction patterns; stems had limited diameter and length, and they were either woody herbs or short-lived shrubs. This unusual woody feature of *C. psilandra* and *C. tsugetorum* was useful as a potential indicator of relationship with other *Clematis* species (Carlquist, 1995).

Pith cavities result from non-lignified walls in the inner-most pith parenchyma cells; this characteristic was found in five species studied. Among them, only *C. uncinata* var. *uncinata* had a primary xylem ring around the pith cavity and a hexagonal shape. Stem features of the genus *Clematis*, such as the arc-like bark, rhytidome, wedge-like phloem, rays and indentations, restricted vessel pattern, secondary phloem fibers, and narrow rays, are key diagnostic characteristics used to distinguish species in the genus *Clematis*. The cambial variants of the 22 *Clematis* species described here provide morphological characteristics of irregular secondary growth within this genus.

## CONCLUSIONS

In this study, we aimed to investigate the cambial variants of the 22 Taiwanese *Clematis* species for understanding the anomalous structure of stem. Our results showed that due to successive layers of periderm combined with non-conducting phloem, both cogwheel-like and continuous segments rhytidome were formed in most of the 22 *Clematis* species. Most of the *Clematis* species had indentation of the axial parenchyma near the wider rays, ray dilatation, and the species varied in the numbers and sizes of vascular bundles. Narrow rays occurred in some secondary xylem, and no diffuse-porous vessels were dispersed throughout the stems. The vessel restriction pattern was found in two shrubs, *C. psilandra* and *C. tsugetorum,* which were different from the other vine *Clematis* species. Three cambial variants of the genus *Clematis* were found: stems with an irregular

conformation, wedge-like phloem, and axial vascular elements in segments. Collection of further fresh materials, and more observations of different developmental conditions are needed. In conclusion, the data presented here provide important basic information about *Clematis* lianas, which will help to understand their morphology and to ensure the conservation of their diversity.

## ACKNOWLEDGMENTS

## LITERATURE CITED

**Angyalossy, V., G. Angeles, M.R. Pace, A.C. Lima, C.L. Dias-Leme, L.G. Lohmann and C. Madero-Vega.** 2012. An overview of the anatomy, development and evolution of the vascular system of lianas. Plant Ecol Divers **5(2)**: 167–182.

**Angyalossy, V., M.R. Pace and A.C. Lima.** 2015. Liana Anatomy: A broad perspective on structural evolution of the vascular system. In: Schnitzer, S.A., F. Bongers, R.J. Burnham and F.E. Putz (eds.) *Ecology of Lianas.* pp. 253–287. Wiley Blackwell, West Sussex, U.K.

**Beck, C.B.** 2011. An Introduction to Plant Structure and Development: Plant Anatomy for the Twenty-First Century. 2nd edn. Cambridge University Press, Cambridge.

**Carlquist, S.** 1992. Woody anatomy of selected Cucurbitaceae and its relationship to habit and systematics. Nordic J Bot **12(3)**: 347–355.

**Carlquist, S.** 1993. Wood and bark anatomy of Aristolochiaceae: systematic and habitual correlations. IAWA J **14(4)**: 341–357.

**Carlquist, S.** 1995. Wood and bark anatomy of Ranunculaceae (including *Hydrastis*) and Glaucidiaceae. Aliso **14(2)**: 65–84.

**Carlquist, S.** 2001. Cambial Variants (Anomalous Secondary Growth). In: Carlquist, S. (ed.) Comparative Wood Anatomy, 2nd edn. pp 271–295. Systematic, Ecological and Evolutionary Aspects of Dicotyledon Wood. Spinger-Verlag Press, Berlin, Germany.

**Esau, K.** 1958. Plants Anatomy. John Wiley & Sons, Inc. New York, USA.

**Evert, R.F.** 2006. Esau's Plant Anatomy. Meristems, Cells and Tissues of the Plant Body. Their Structure, Function and Development. Wiley and Sons. Hoboken, NJ, USA.

**Ferreira, T. and W. Rasband.** 2011. The ImageJ User Guide Version 1.44. http://rsbweb.nih.gov/ij/docs/user-guide.pdf

**Gregory, M.** 1994. Bibliography of Systematic Wood Anatomy of Dicotyledons. IAWA J Suppl **1**.

**IAWA Committee on Nomenclature.** 1964. Multilingual Glossary of Terms Used in Wood Anatomy. Verlagsbuchanstalt Konkordia, Winterthur, Switzerland. p. 186.

**Isnard, S., N. Rowe and T. Speck.** 2003a. Growth habit and mechanical architecture of the sand dune-adapted climber *Clematis flammula* L. var. *maritima* L. Ann. Bot. **91(4)**: 407–417.

**Isnard, S., T. Speck and N.P. Rowe.** 2003b. Mechanical architecture and development in *Clematis*: implications for canalised evolution of growth forms. New Phytol **158(3)**: 543–559.

**Mauseth, J.D.** 1988. Plant Anatomy. The Benjamin/Cummings Publishing Company, Menlo Park, CA.

**Pace, M.R., L.G. Lohmann and V. Angyalossy.** 2011. Evolution of disparity between the regular and variant phloem in Bignonieae (Bignoniaceae). Am. J. Bot. **98(4)**: 602–618

**Putz, F.E. and H.A. Mooney.** 1991. The Biology of Vines. pp 53–71. Cambridge University Press. UK.

**Schweingruber, F.H., A. Börner and E.D. Schulze.** 2011. Ranunculaceae. In: Schweingruber, F.H. *et al.* (eds.) Atlas of Stem Anatomy in Herbs, Shrubs and Trees. *1*: 352–371. Springer-Verlag, Berlin Heidelberg.

**Sieber, M. and L.J. Kucera.** 1980. On the stem anatomy of *Clematis vitalba* L. IAWA Bulletin **1(1-2)**: 49–54.

**Smith, P.** 1928. A comparative study of the stem structure of the genus *Clematis* with special reference to anatomical changes induced by vegetative propagation. Earth Environ. Sci. Trans. R. Soc. Edinb. **55(3)**: 643–664.

**Yang, S.Z. and P.H. Chen.** 2015. Classifying Taiwan lianas with radiating plates of xylem. Taiwania **60(4)**: 151–159

**Yang, S.Z. and P.H. Chen.** 2016. Cambial variants in the Family Menispermaceae in Taiwan. Am. J. Plant Sci. **7(6)**: 841–854

**Yang, T.Y.A. and T.C. Huang.** 1996. Ranunculaceae. In: Huang, T.-C. *et al.* (eds.), Flora of Taiwan, 2nd ed. 2: 694–714. Editorial Committee, Dept. Bot., NTU, Taipei, Taiwan.

**Wang, W. and B. Bartholomew.** 2007. *Clematis*. In: Wu, Z.Y., P.H. Raven and D.Y. Hong (eds.) Flora of China **6**: 333–360. Science Press & Missouri Botanical Garden.