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Taxonomic relevance of petiole morphology in *Clematis* L. (Ranunculaceae)

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We assessed the morphological and anatomical structure of petioles of 19 *Clematis* taxa from South Korea with the help of stereomicroscopy and scanning electron microscopy for surface features and microtomy and light microscopy for anatomical features. The result of this study showed that presence/absence and abundance of trichomes, petiole outline in cross-section, upper surface wings and groove, and the number of vascular bundles proved to be useful for species discrimination in the *Clematis*. Among the studied taxa, *C. hexapetala* was the only species with a glabrous petiole surface. Two types of trichomes are observed, long non-glandular and flagelliform and short glandular capitate in the other 18 taxa. We found four to six major vascular bundles and a maximum of eight interfascicular vascular bundles (*C. heracleifolia* and *C. urticifolia*) in 19 species. The cluster analysis based on the UPGMA determined six clusters with 18 nodes. *Clematis fusca* var. *fusca* and *C. fusca* var. *violacea* representing the first node in the sixth cluster of the phenogram show 93.36% similarity in petiole features whereas *C. hexapetala* and *C. serratifolia* representing the 18th node and first cluster in the phenogram share only 52.7% similar petiole features with rest of the species. Although the numbers of investigated taxa are limited, the overall similarity of petiole features indicated that taxa from the sections *Tubulosae*, *Viorna*, and *Astragene* grouped in the UPGMA phenogram. As a single source of information, the result of this may not be useful for resolving the infrageneric relationship, however, the obtained data could be used as a descriptive and/or diagnostic feature of the particular taxa in the genus.

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ABSTRACT

We assessed the morphological and anatomical structure of petioles of 19 *Clematis* taxa from South Korea with the help of stereomicroscopy and scanning electron microscopy for surface features and microtomy and light microscopy for anatomical features. The result of this study showed that presence/absence and abundance of trichomes, petiole outline in cross-section, upper surface wings and groove, and the number of vascular bundles proved to be useful for species discrimination in the *Clematis*. Among the studied taxa, *C. hexapetala* was the only species with a glabrous petiole surface. Two types of trichomes are observed, long non-glandular and flagelliform and short glandular capitate in the other 18 taxa. We found four to six major vascular bundles and a maximum of eight interfascicular vascular bundles (*C. heracleifolia* and *C. urticifolia*) in 19 species. The cluster analysis based on the UPGMA determined six clusters with 18 nodes. *Clematis fusca* var. *fusca* and *C. fusca* var. *violacea* representing the first node in the sixth cluster of the phenogram show 93.36% similarity in petiole features whereas *C. hexapetala* and *C. serratifolia* representing the 18th node and first cluster in the phenogram share only 52.7% similar petiole features with rest of the species. Although the numbers of investigated taxa are limited, the overall similarity of petiole features indicated that taxa from the sections *Tubulosae*, *Viorna*, and *Astragene* grouped in the UPGMA phenogram. As a single source of information, the result of this may not be useful for resolving the infrageneric relationship, however, the obtained data could be used as a descriptive and/or diagnostic feature of the particular taxa in the genus.

Keywords: Petiole morphology, *Clematis*, trichomes, vascular bundle, infrageneric relationship, taxonomy

INTRODUCTION

The Ranunculaceae is one of the larger families of eudicots comprising nearly 2500 species within 50–60 genera (*Tamura, 1993*; *The Plant List, 2013*). The family has been classified under the early branching order of eudicot Ranunculales where it remains sister to the Berberidaceae (*APG IV, 2016*). Ranunculaceae are distributed across the world with the greatest diversity in the temperate and cold region of the northern and southern hemisphere (*Tamura, 1993*). A number of classification models by considering ~~several~~ morphological characters, molecular sequencing, and combination of both morphological and molecular dataset have been proposed for the family (*Hutchinson, 1923*; *Janchen, 1949*; *Johansson & Jansen, 1993*; *Tamura, 1995*; *Hoot,1995*; *Jensen et al., 1995*; *Ro et al., 1997*; *Wang et al., 2009*). Among the various morphological criteria, basic chromosome numbers and the type of chromosome became the most reliable reference compatible with the molecular phylogeny of the family (*Gregory, 1941*; *Tamura, 1987*; *Ro et al., 1997*; *Wang et al., 2009*).

Within Ranunculaceae, *Clematis* L. is classified under the subtribe Clematidinae Lotsy of tribe

Anemoneae DC. in the subfamily Ranunculoideae Hutch. (*Tamura, 1995*). It is one of the largest genera in the family which comprises about 280–350 cosmopolitan species (*Tamura, 1987, 1995*; *Wang & Li, 2005*). Due to the vast morphological disparity among the species the genus has become the subject of investigation form the early 19th century and been subjected to several infrageneric ~~classifications~~ (*De Candolle, 1818*; *Spach, 1839*; *Kuntz, 1885*; *Prantl, 1888*; *Tamura, 1987*; *Johnson, 1997, 2001*; *Grey-Wilson, 2000*; *Wang & Li, 2005*). ~~As morphological and anatomical characters are considered to be a useful source for taxonomic interpretations, s~~several systematic studies on the anatomy of different parts, palynology, and cytology of the *Clematis* have been carried out (*Tobe, 1974, 1980a, 1980b, 1980c, 1980d*; *Essig, 1991*; *Zhang, 1991*; *Yano, 1993*; *Yang & Moor, 1999*; *Shi & Li, 2003*; *Xie & Li, 2012*; *Ghimire et al., 2020*). Morphological characters which are extensively studied and considered for the infrageneric classifications in the *Clematis* include~~d~~ habit, seed germination, seedling phyllotaxy, ~~types of compound leaves,~~ inflorescence, the sexuality of flowers, ~~aestivation and spreading direction of sepals, presence or absence of petals, indumentum in the filaments,~~ and pollen and achene morphology (see *Wang & Li, 2005*).

~~The petiole is part of a leaf that links the lamina with the stem.~~ The importance of nodal and petiolar anatomy in the taxonomic treatment at intergeneric-familial levels has been extensively studied (*Howard, 1962, 1979*; *Schofield, 1968*; *Dickison, 1969, 1980*; *Datta & Dasgupta, 1979*). The middle portion of the petiole is considered to be the most stable zone from which even a single section can be taken for the comparative purpose (*Metcalfe & Chalk, 1979* ~~and reference therein~~). Besides, the complex vascular systems of the petiole provide a range of diagnostic structure which can be useful in the taxonomic treatment at any rank (*Solereder, 1908*; *Metcalfe & Chalk, 1979*; *Ashton, 1982*; *Dehgan, 1982*; *Rojo, 1987*; *Pardi et al., 1991*; *Kamel & Loutfy, 2001*; *Kocsis & Borhidi, 2003*; *Norani et al., 2016*; *Talip et al., 2017*). Within Ranunculaceae, few petioles and nodal anatomical investigations including stem in relation to taxonomy have been carried out (*Worsdell, 1908*; *Tamura, 1962*; *Oh, 1971*; *Kavathekar & Pilli, 1976*; *Tobe, 1979, 1980*; *Kökdil et al., 2006*; *Gostin, 2011*; *Novikoff & Mitka, 2015*). Unfortunately, studies pertaining to the petiole morphology and anatomy of *Clematis*, one of the morphologically diverse and taxonomically complicated genera within the family, are very rare in the literature. In an anatomical study of *Clematis* in Korea, *Oh (1971)* made some remarks on the petiole anatomy of nine Korean species.

In this study, we provide a comprehensive investigation of petiole morphology and anatomy of 19 *Clematis* taxa distributed in Korea. The primary objective of this study was to investigate the detailed structure of petiole morphology and the anatomy of the included taxa and evaluate the implication of petiolar characters for species delimitations. We ~~will~~ attempt to compare our results with previously studied species and summarize them to make ~~some~~ taxonomic conclusions.

MATERIALS AND METHODS

Plant materials

~~Petioles of 19 taxa of *Clematis* from Korea were investigated.~~ The names of the investigated species with their voucher numbers are listed in Table 1. The voucher specimens are deposited in the herbarium of Korea National Arboretum (KH).

Stereo microscopy and scanning electron microscopy

Petiole morphology including indumentum, trichome type and abundance, and upper surface groove were observed under stereo microscope and scanning electron microscope. A Leica MZ16 FA microscope (Leica Microsystems GmbH, Wetzlar, Germany) was used for the observation and digital images of best represented part of the petiole were taken with Leica DFC420 C multifocal camera attached to the microscope. Before SEM imaging, petiole pieces were immersed in 100% ethanol and were sputter coated with gold in a KIC-IA COXEM Ion-Coater (COXEM. Co., Ltd., Daejeon, Korea). SEM imaging was carried out with a COXEM EM-30 PLUS+ table scanning electron microscope (COXEM) at 20 kv, at the seed testing laboratory of the Korea National Arboretum. ~~The scale bars in the images were added manually.~~

Light microscopy

At least three petioles of each taxon were used for microtome sectioning according to the following procedure. Freshly collected leaf petioles were fixed in FAA (formalin, acetic acid, and 50% ethyl alcohol with the ratio of 90:5:5 ~~per 100 ml~~) for a week and preserved in 50 % ethyl alcohol. During experiment, the preserved petioles were cut into small pieces (about 2 mm) and dehydrated through an ethanol series (50, 70, 80, 90, 95 and 100%). After complete dehydration, the petiole pieces were infiltered with ethanol/Technovit combinations (3:1, 1:1, 1:3, and 100% Technovit) and then embedded in Technovit 7100 resin. The embedded materials were cut into serial sections of 4–6 µm thickness using a Leica RM2255 rotary microtome (Leica Microsystems GmbH, Wetzlar, Germany) with disposable blades, stuck onto a slide glass, and dried using an electric slide warmer for 12 h. The dried slides were stained with 0.1% Toluidine Blue 'O' for 60–90 s, rinsed with water and again dried with a slide warmer for at least 6 h to remove water. The stained slides were then mounted with Entellan (Merck Co., Darmstadt, Germany) and ~~permanent slides were prepared which were~~ examined under a Leica DM3000 LED (Leica Microsystem, Wetzlar, Germany). Photomicrographs were taken with a scientific CMOS camera. Multiple image alignment was performed using Photoshop CS for Windows 2010.

Morphometry and data analysis

Thirteen quantitative characters were categorized and coded with binary and/or multistate coding. Principal component analysis (PCA) and cluster analysis using the un-weighted pair group (UPGMA) clustering method was carried out using the computer program MultiVariate Statistical Package 3.1 (MVSP Version 3.1). The character states and their coding are provided in Supplementary File S1.

RESULTS

Petiole morphology and anatomical characteristics of *Clematis* observed in this study include petiole indumentum, trichomes type and abundance, petiole outline in cross-section, upper surface wings and groove, sclerenchyma region, and vascular bundles. All the characters are summarized in Table 2. ~~A~~ selected ~~stereomicroscopic and light microscopic~~ image of the petiole are shown in Figures 1-6. The morphological and anatomical features of the petiole are comprehensively described below.

Petiole surface and trichomes

The petioles surface of the studied species is pubescent except *Clematis hexapetala* which has an almost glabrous surface (few trichomes are observed in the region from where leaflets arise) (Table 2, Fig. 1A-S). *Clematis taeguensis* and *C. serratifolia* have subglabrous petiole surface, only a few trichomes are observed in the petiole of these species. Two types of trichomes are observed, long non-glandular and flagelliform and short glandular capitate (Figs. 1A-S, 2A-J). Glandular trichomes are usually distributed in the upper surface groove. In some species like *C. terniflora*, *C. terniflora* var. *mandshurica*, *C. brachyura*, *C. fusca* var. *fusca*, and *C. fusca* var. *violacea* non-glandular trichomes are only concentrated on the upper surface groove. Pubescent species are categorized into 'villous' covered with long, soft, and dens hairs~~, as found in~~ *C. apiifolia*, *C. brevicaudata*, *C. heracleifolia*, *C. urticifolia*, and *C. takedana* and 'pilose' covered with soft, weak, thin, and separated hairs, as found in rest of the species. The non-glandular trichomes are either unicate (*C. apiifolia*, *C. brevicaudata*, *C. heracleifolia*, *C. urticifolia*, and *C. takedana*) or flabelliform (rest of the species except *C. hexapetala*). Based on the density per unit area trichome abundance is categorized into high, medium, and low.

Petiole outline and upper surface groove

Studied species showed considerable variation in petiole outline in cross-section (CS) (Table 2). Based on the shape of petiole in CS the species are divided into three categories: pentagonal petioles in CS represented by seven species, semicircular petioles represented by nine species, and half- to semicircular petiole represented by sole *C. terniflora* (Figs. 3A, C, E, G, I, K, 4A, C, E, G, I, K, 5A, C, E, G, I, K, 6A). *Clematis hexapetala* and *C. serratifolia* have both pentagonal and semicircular petioles in the cross-section. Out of 19, 13 species exhibit two noticeable upper or dorsal surface wings while six species are without noticeable wings. Based on the upper surface groove, the petioles are categorized into flattened (three species), sub flattened (11 species), U-shaped (three species), and V-shaped (three species). The dorsal surface wings are particularly responsible for the formation of upper surface groove although some species with inconspicuous wings had a slight groove in the petiole (*C. brevicaudata*, *C. trichotoma*, and *C. heracleifolia*).

Petiole epidermis and cortex

The epidermis is single-layered and cutinized in all species (Figs. 3B, D, F, H, J, L, 4B, D, F, H, J, L 5B, D, F, H, J, L, 6B). The cells are small rounded, cuboidal, narrow, or slightly elongated. In some places, the continuation of the epidermis is interrupted by the presence of stomata. The epidermis is followed by the cortex which is 3-5 cells layered thick. The cortical cells are loosely arranged parenchymatous with abundant air spaces and rounded, oval, elongated, or irregular in shape. The cortex is collenchymatous above the phloem fiber where the cells are thick-walled and closely packed.

Vascular bundles

The vascular bundles are conjoint, collateral~~, and open type~~. There is a remarkable variation in the number of vascular bundles (ranging from 5 to 14) in *Clematis* species. Four to six major vascular bundles and a maximum of eight interfascicular vascular bundles (*C. heracleifolia* and *C. urticifolia*) are observed in 19 species. Twelve species have five, six species have six and only *C. patens* has four major vascular bundles (Table 2). The major vascular bundles are oval-shaped with phloem facing towards the cortex and xylem towards the pith. The xylem and phloem are separated by 2-4 layers of the cambial cell. Each major vascular bundle is overlaid with a bunch of thick-walled fibrous cells, the phloem fiber cap. The quantity of phloem fibers is variable within the studied species. Based on the number of cell layers in vertical height, the phloem cap is categorized into: very large with more than ten cells high, large with five to ten cells high, and small with less than five cells high. Three species *C. terniflora*, *C. fusca* var. *fusca*, and *C. koreana* have small fiber cap while seven and nine species have large and very large fiber cap, respectively. There is a permanent sclerenchymatous strand between the adjacent vascular bundles in all species.

The petiole of each species has a large ground region, the pith. The cells in the pith are thin-walled, rounded, oval, or angular parenchymatous and comparatively larger than the cortex (Figs. 3A, C, E, G, I, K, 4A, C, E, G, I, K, 5A, C, E, G, I, K, 6A).

Statistical analysis

The similarities among the species based on the 13 petiole features were revealed using PCA and cluster analysis. The first three components of the PCA explains 74.01% of the total variation in the analyzed data. The first axis of the first complete set explains 42.66% of the total variation and shows strong positive loading for trichome abundance and number of vascular bundles (TA, IV, VB, and VG) (Fig. 7). The second axis explains 16.86% of the total variation and shows strong positive loading for trichome type and upper surface groove (TT and UG) and strong negative loading for phloem fiber cap and interfascicular sclerenchyma (PF and SC). The cluster analysis based on the UPGMA using the Gower similarity coefficient determined six clusters with 18 nodes (Fig. 8). *Clematis fusca* var. *fusca* and *C. fusca* var. *violacea* representing the first node in the sixth cluster of the phenogram show 93.6% similarity in petiole features whereas *C. hexapetala* and *C. serratifolia* representing the 18th node and first cluster in the phenogram share only 52.7% similar petiole features with rest of the species.

DISCUSSION

The genus *Clematis* is morphologically diverse in terms of leaf phyllotaxy, types of compound leaves, and a number of ~~leaf lamina~~. Anatomy of the petiole, therefore, is expected to be equally diverse. Previously, *Oh (1971)* found variation in the number of vascular bundles in the petiole of some *Clematis* species. Thus, reasonable diversity in petiole anatomical features including the numerical variation in the vascular bundles is certainly useful for the taxonomic treatment of the genus. The over-all anatomical organization of the petiole of investigated species was comparable to that of *Oh (1971)*. Apart from that, we provided a comprehensive anatomical description of the petiole of all the Korean *Clematis* species and also prepared and analyzed the morphological data set and discussed its taxonomic relevance in the genus which is missed in the previous report.

The result of this study showed that *Clematis* species can be differentiated based on the petiole indumentum. The presence/absence and/or type of trichomes in the petiole has been used for the species differentiation in other taxa as well (*Solereder, 1908*; *Metcalf & Chalk, 1979*; *Talip et al., 2017*). Among the 19 species investigated, *Clematis hexapetala* is the only species with glabrous petiole ~~indumentum~~ (although sparsely distributed trichomes can be observed ~~in the region from where~~ leaflets ~~originated~~) whereas *C. taeguensis* and *C. serratifolia* have subglabrous or sparsely pubescent indumentum. The remaining species have sparsely or thickly pubescent petiole ~~indumentum~~. In sparsely pubescent species like *C. terniflora*, *C. terniflora* var. *mandshurica*, *C. fusca* var. *fusca*, and *C. fusca* var. *violacea* the trichomes are mainly restricted in the upper groove region. Also, the stem of these ~~species~~ is either subglabrous or only nodes are puberulous (*Wang & Bartholomew, 2001*; *Kim, 2017*). On the other hand, the species like *C. apiifolia*, *C. heracleifolia*, *C. utriticifolia*, and *C. takedana* which have thickly pubescent petioles also ~~are~~ heavily pubescent stems and branches (*Wang & Bartholomew, 2001*; *Kadota,* *2006*; *Moon et al., 2013*; *Kim, 2017*). This indicated that the trichomes in the petiole are generally continued from the stem in the *Clematis* species.

Most of the species have both types of trichomes although glandular trichomes are very scarce and restricted on the upper surface groove of the petiole. We observed both glandular capitate and simple non-glandular trichomes in the leaf surface of most of the *Clematis* species (unpublished report by same authors), however, none of the previous reports revealed the occurrence of glandular capitate trichomes in the petiole and/or leaf of any *Clematis* species (*Tamura, 1995*; *Wang & Li, 2005*; *Lehtonen et al., 2016*; *Wang & Bartholomew, 2001*; *Kadota, 2006*; *Kim, 2017*). Petiole features which are considered to be useful for the taxonomic discrimination in various taxa (*Solereder, 1908*; *Metcalfe & Chalk, 1979*; *Dehgan, 1982*; *Rojo, 1987*; *Pardi et al., 1991*; *Kamel & Loutfy, 2001*; *Kocsis & Borhidi, 2003*; *Norani et al., 2016*; *Talip et al., 2017*) are generally neglected in previous morphological studies of the *Clematis*. The results from this study showed that petiole indumentum and types of trichomes appear to have taxonomic value for species delimitation in the genus.

Along with the surface indumentum, some other petiole features which can contribute to the identification of a particular species in *Clematis* include petiole outline in cross-section, upper surface groove and wings, and phloem fiber cap. Among these petiole outline and upper surface groove and wings have already been proved to be useful in taxonomic discrimination of the species in some eudicot genera (*Kocsis & Borhidi, 2003*; *Talip et al., 2017*; *Abeysinghe & Scharaschkin, 2019*). *Oh (1971)* reported pentagonal and/or horseshoe or rounded horseshoe-shaped petiole in nine *Clematis* species and he result of this study is not different either. We observed that the petiole of *Clematis* in the cross-section is dorsiventral, pentagonal with five visibly and/or weakly represented ridges or semicircular without ridges. *Clematis hexapetala* and *C. serratifolia* appear to have both pentagonal and semicircular petiole whereas some petioles of *C. terniflora* have half-circular in outline. Species with pentagonal petioles have conspicuous upper surface wings forming an upper surface groove while some of the semicircular members have inconspicuous upper surface wings. Species with inconspicuous wings like *C. bravicaudata*, *C. trichotoma*, *C. heracleifolia*, and *C. terniflora* still form a slight upper surface groove. Among the three taxa, *C. fusca* var. *fusca*, *C. fusca* var. *flabellata*, and *C. fusca* var. *violacea*, theformer one has pentagonal while later two have semicircular petiole shape with noticeable upper wings. Interestingly, these three taxa showed dissimilar character on upper surface groove as *C. fusca* var. *violacea* has sub flattened groove whereas *C. fusca* var. *fusca* and *C. fusca* var. *flabellata*, have U-shaped groove. Although reports on such upper wing extensions and adaxial groove on the petiole are lacking in the literature, our study suggested the possibility of using these features for species identification in *Clematis*.

In petiole anatomy, the vascular system has received the most attention (*Kocsis & Borhidi, 2003*;

Noraini et al., 2016; *Talip et al. 2017*; *Long & Oskolski, 2018*; *Abeysinghe & Scharaschkin, 2019*). According to *Hare (1942)*, various arrangements of vascular bundle in the petiole can be used as a diagnostic character in some taxonomic groups. *Howard (1962, 1974)* suggested that the vascular structure of the petiole can be most useful at the generic level and sometimes at the family level, although, the intensity of taxonomic value may vary from one taxonomic group to another. *Clematis* petiole showed remarkable consistency in the arrangement of the vascular system although studied species differ each other by the number of major and interfascicular vascular bundles. There are mostly five major vascular bundles which are possibly corresponding to five ridges of the petiole, however, *C. patens* which are exclusively semicircular without ridges, wings, and upper surface groove has only four major vascular bundles. In some species such as *C. apiifolia*, *C. taeguensis*, *C. hexapetala*, *C. terniflora* var. *mandshurica*, *C. heracleifolia*, and *C. takedana*, the vascular bundle in the upper groove regions also develop equally like vascular bundles in the edges and thus have six major vascular bundles.

Regarding the number of vascular bundles, the result of this study almost congruent with that of *Oh (1971)* for *C. apiifolia*, *C. trichotoma*, and *C. koreana* but slightly differs for *C. brachyura* and *C. patens* in which he described six major and four interfascicular and six major vascular bundles, respectively. Instead, we observed five major and four interfascicular vascular bundles in *C. brachyura* and four major and one or two interfascicular bundles in *C. patens.* We found variation in number and position of interfascicular bundles even in the different samples of the same species and *C. patens* is one of such species, thus this feature may have only a little taxonomic value in *Clematis* species. On the other hand, the number and position of the major vascular bundles which showed remarkable consistency among the investigated samples of all species may have a significant taxonomic value for species discrimination within the genus. At this point, our results suggested the correction of preexisting data on the number of vascular bundles, specifically the major bundles, in the petiole of *Clematis* species presented by *Oh (1971)*.

Cluster analysis based on 13 petiole features has generated at least six clusters among which *Clematis serratifolia* and *C. hexapetala* formed the first cluster separated from the rest of the species (Fig. 8). The sixth cluster represented the largest one comprising seven taxa, *C. calcicola*, *C. ochotensis*, *C. fusca* var. *fusca*, *C. fusca* var. *violacea*, *C. fusca* var. *flabellata*, *C. koreana*, and *C. brachyura*. In an infrageneric classification of *Johnson (2001)* and *Wang & Li (2005)* these seven species belong to section *Viorna* (*C. fusca* var. *fusca*, *C. fusca* var. *violacea*, *C. fusca* var. *flabellata*), section *Astragene* (*C. ochotensis*, *C. calcicola*, and *C. koreana*), and section *Pterocarp* (*C. brachyura*). However, in recent phylogenetic classification of the genus *C. fusca* var. *fusca*, *C. fusca* var. *violacea*, and *C. fusca* var. *flabellata* have categorized under clade L, *C. ochotensis*, *C. calcicola*, and *C. koreana* under clade H, and *C. brachyura* under clade K (*Lehtonen et al., 2016*). Also, *C. urticifolia*, *C. takedana*, and *C. heracleifolia* representing the third cluster in the UPGMA phenogram in this study belong to section *Tubulosae* in *Johnson (2001)* and *Wang & Li (2005)* classification and clade C in *Lehtonen et al. (2016)*. *Clematis brachyura* which is considered to be closer with section *Flammula* (*C. taguensis*, *C. hexapetala*, *C. terniflora* var. *mandshurica*, and *C. terniflora*) in Tamura (1995), *Wang & Li (2005)* and *Xie et al. (2011)* reports remains connected with *Viorna* and *Astragene* in this study. However, this is a common and usual interpretation for this largest genus as previous morphological and molecular studies also found a similar tendency (*Grey-Wilson, 2000*; *Wang & Li, 2005*; *Miikeda et al., 2006*; *Xie et al., 2011*; *Xie & Li, 2012*; *Lehtonen et al., 2016*; *Ghimire et al., 2020*).

In conclusion, the presence/absence and abundance of trichomes, petiole outline in cross-section, upper surface wings and groove, and a number of vascular bundles proved to be useful for species discrimination in the Korean *Clematis*. The results of this study also indicated that taxa from the sections *Tubulosae*, *Viorna*, and *Astragene* grouped based on overall similarity of petiole features; however, the number of investigated taxa remain very low, and thus any interpretation made on this basis could be arbitrary. ~~We recommend more studies considering as many taxa as possible from the same section which will certainly provide a handful of data for truthful interpretation. Our understanding from this study is that p~~etiole morphology alone ~~as a single source of character~~ can not be ~~expected as~~ useful information for an infrageneric relationship, however~~, could~~ be used as a descriptive and/or diagnostic feature of the particular taxa in the genus.

ADDITIONAL INFORMATION AND DECLARATION

Author Contributions

- BKP conceived the study, performed the experiment, analyzed the data wrote ~~or~~ reviewed drafts of the manuscript.
- BG and DCS conceived the study and designed the experiment, analyzed the data, wrote ~~or~~ reviewed the draft ~~and approved the final draft~~.

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Figure 1

Petiole of *Clematis* under stereomicroscope.

A. *C. apiifolia*. B. *C. brevicaudata*. C. *C. trichotoma*. D. *C. taeguensis.* E. *C. hexapetala*. F. *C. terniflora*. G. *C. terniflora* var. *mandshurica*. H. *C. heraclefolia*. I. *C. urticifolia*. J. *C. takedana*. K. *C. patens*. L. *C. brachyura*. M. *C. serratifolia*. N. *C. fusca* var. *fusca*. O. *C. fusca* var. *flabellata*. P. *C. fusca* var. *violacea*. Q. *C. calcicola*. R. *C. koreana*. S. *C. ochotensis*. Scale bars: 1mm.

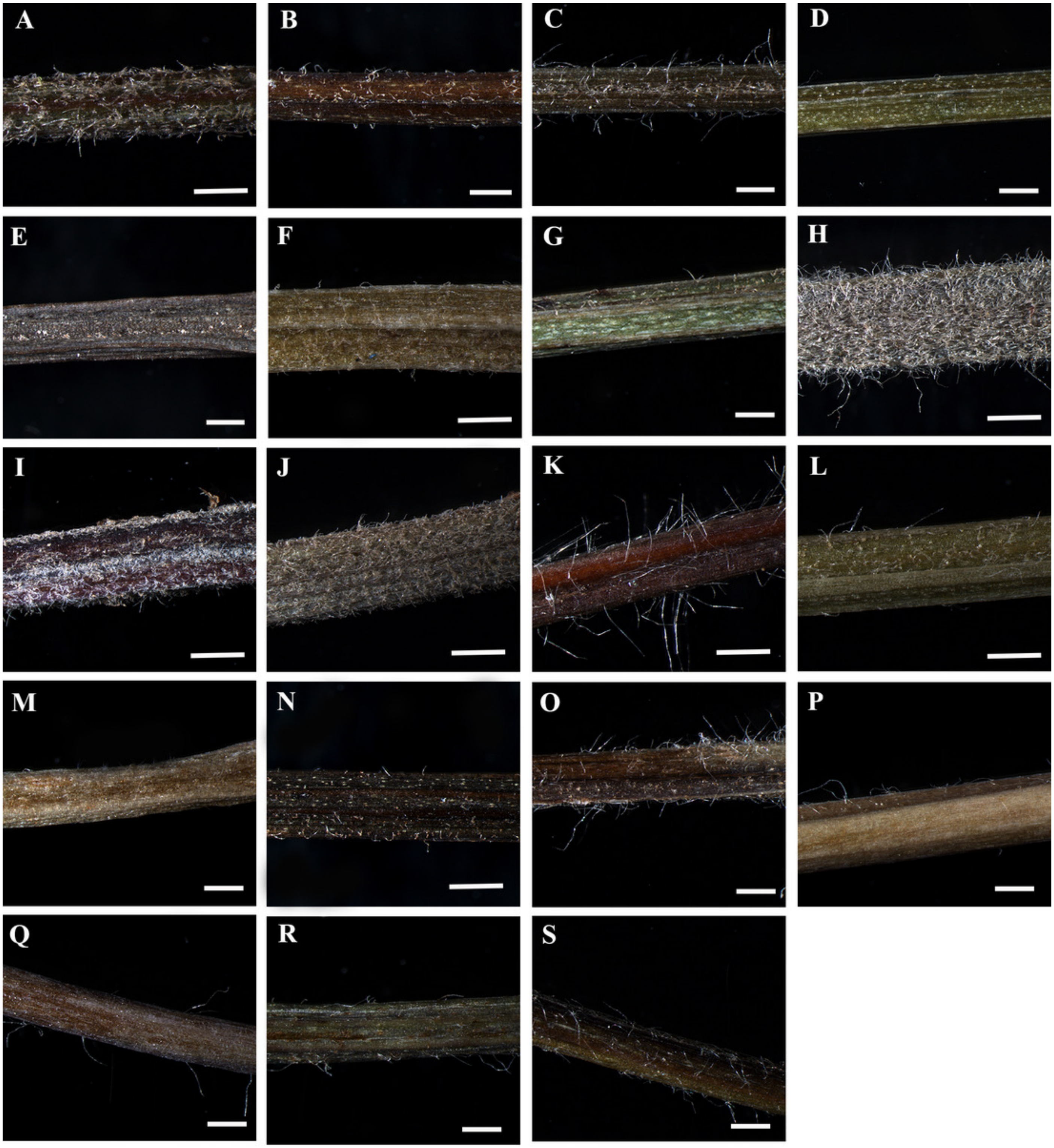
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Figure 2

Scanning electron micrograph of petiole of *Clematis*.

A-B. *C. heraclefolia*. C-D. *C. taeguensis.* E-F. *C. patens*. G-H. *C. brevicaudata*. I-J. *C. fusca* var. *violacea*. Abbreviations: gt, glandular trichome. Scale bar: 200 µm (A, C, E, G, I), 100 µm (B, D, F, H, J).

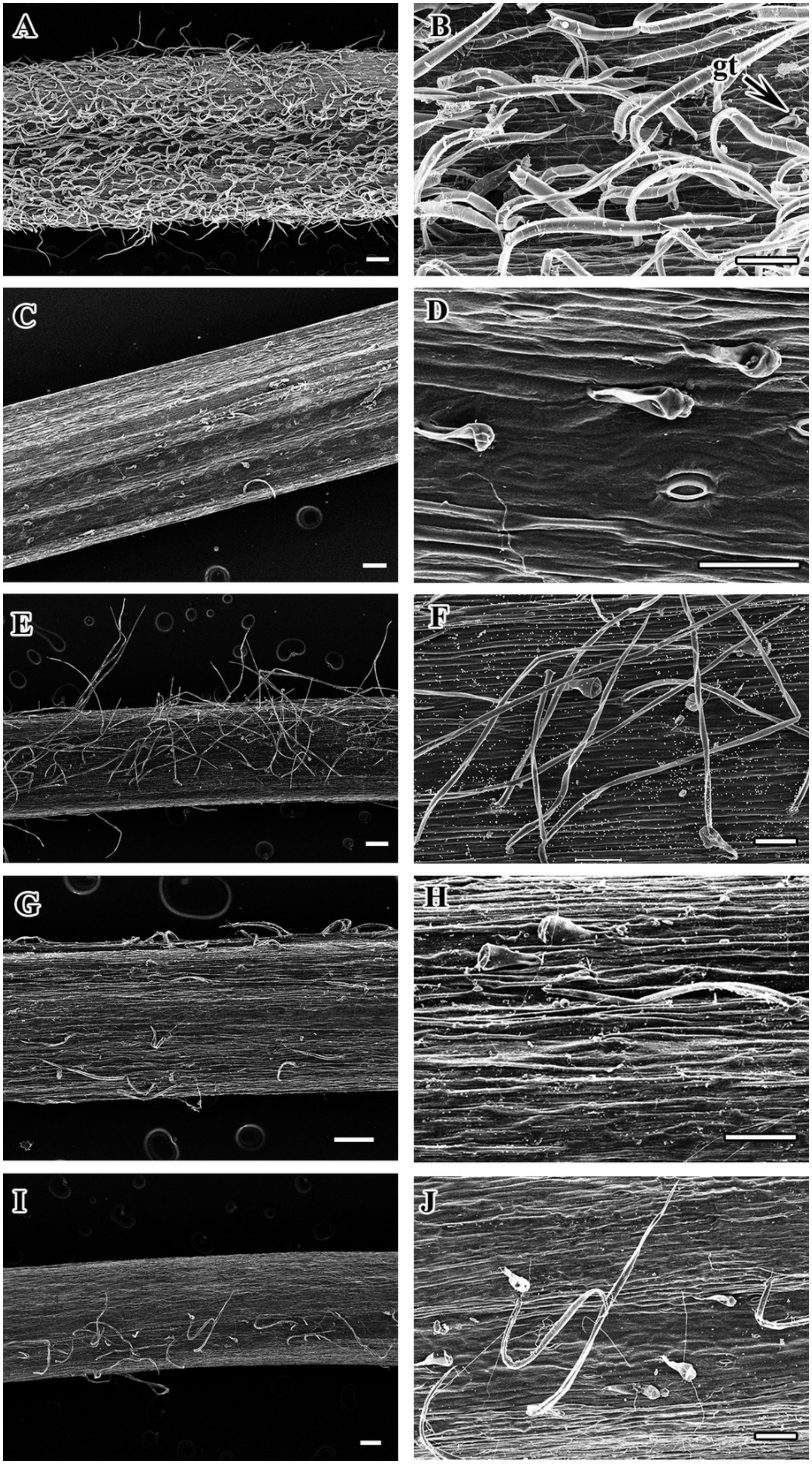
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Figure 3

Cross section of petiole of *Clematis*.

A-B. *C. apiifolia*. C-D. *C. brevicaudata*. E-F. *C. trichotoma*. G-H. *C. taeguensis* I-J. *C. hexapetala*. K-L. *C. terniflora*. Abbreviations: co, collenchyma; cu, cuticle; ep, epidermis; ph, phloem; phf, phloem fiber; s, stomata; xy, xylem. Scale bars: 50 µm (B, D, J), 75 µm (F, H, L), 100 µm (A, C, I), 200 µm (E, G, K).

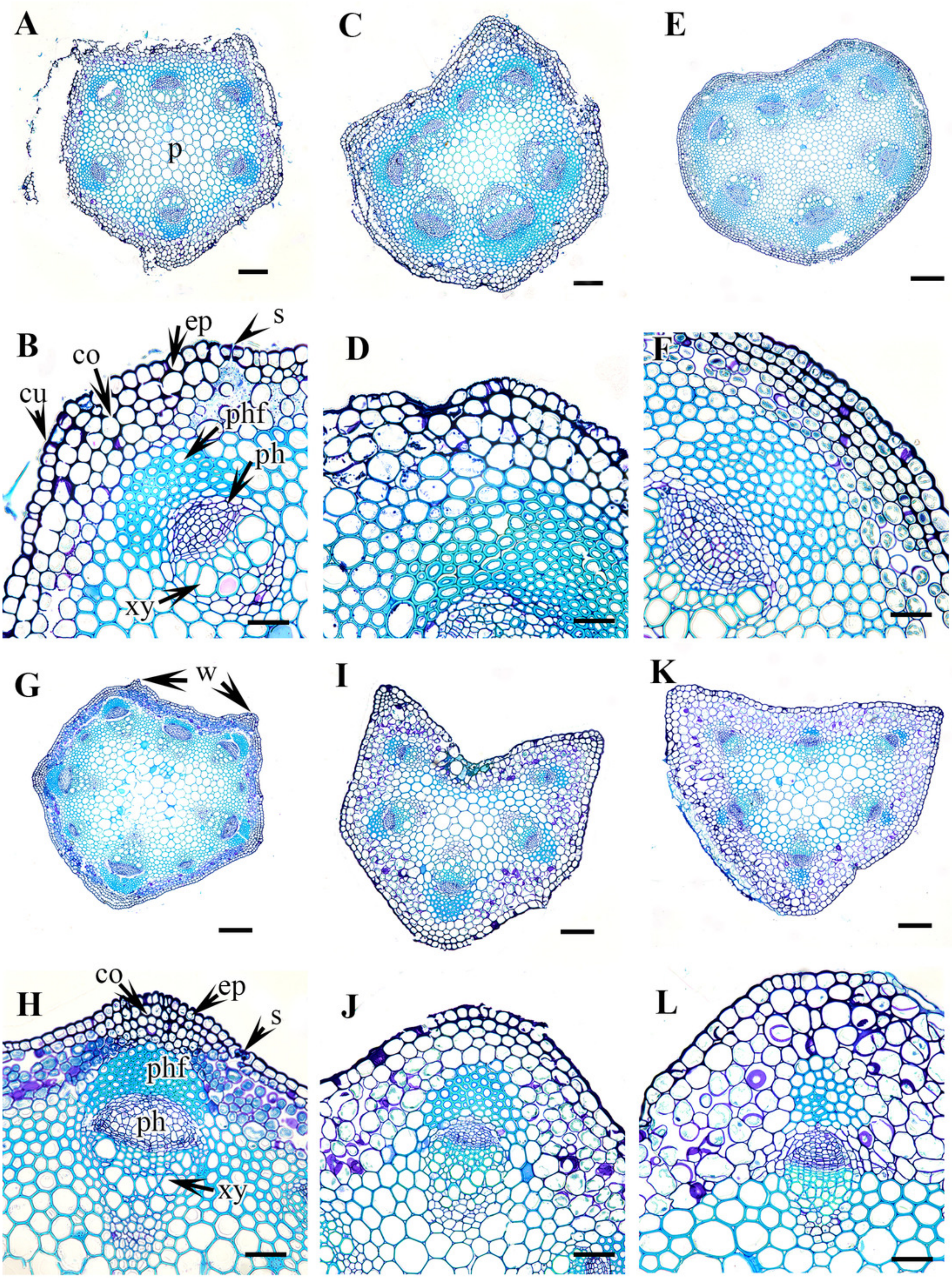
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Figure 4

Cross section of petiole of *Clematis*.

A-B. *C. terniflora* var. *mandshurica*. C-D. *C. urticifolia*. E-F. *C. heraclefolia*. G-H. *C. takedana*. I-J. *C. patens*. K-L. *C. brachyura*. Abbreviations: co, collenchyma; cu, cuticle; ep, epidermis; ph, phloem; phf, phloem fiber; s, stomata; sc, sclerenchyma; xy, xylem. Scale bars: Scale bars: 50 µm (B, J, L), 100 µm (A, I, K, F, D, H), 500 µm (C, E, G).

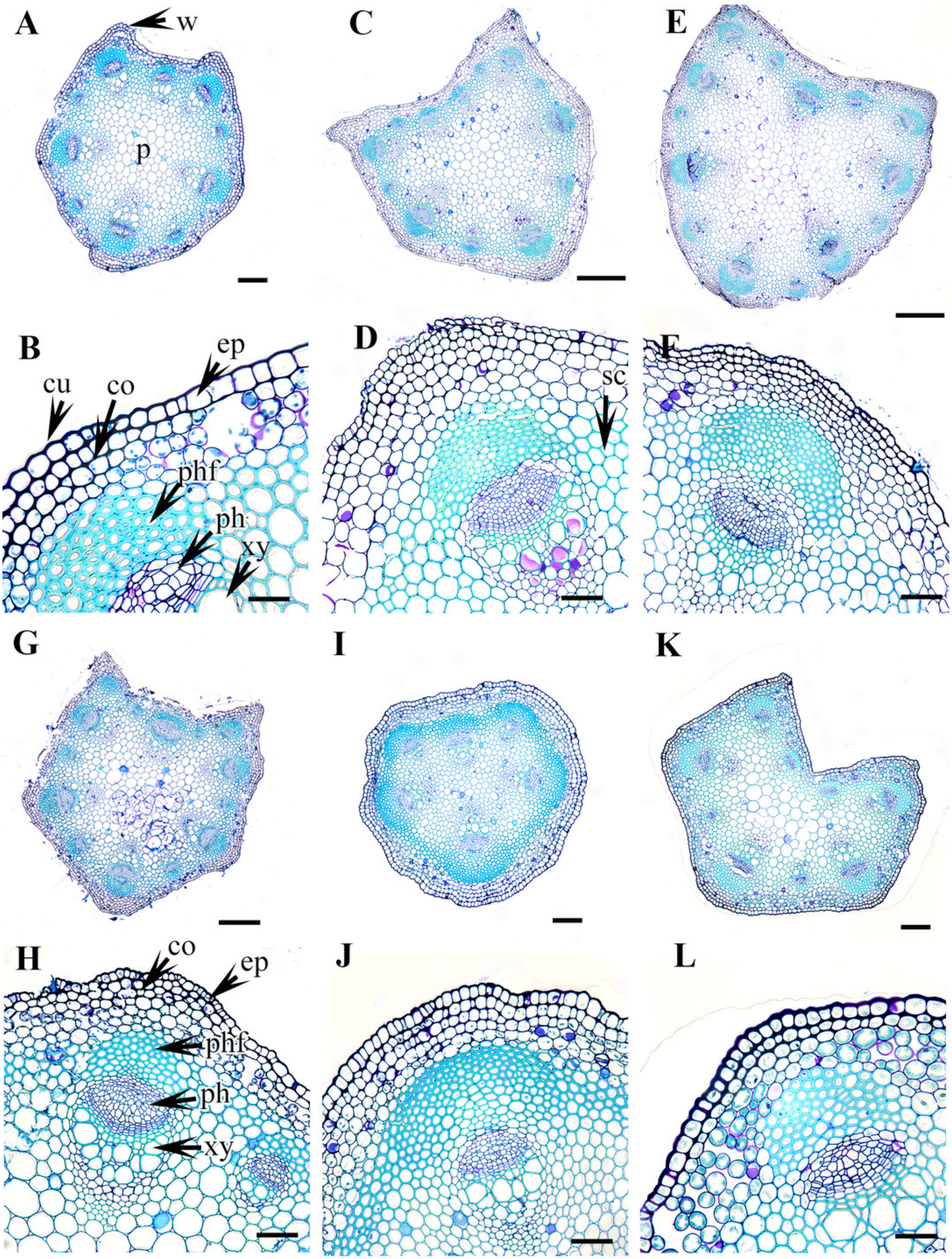
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Figure 5

Cross section of petiole of *Clematis*.

A-B. *C. serratifolia*. C-D. *C. fusca* var. *fusca*. E-F. *C. fusca* var. *flabellata*. G-H. *C. fusca* var. *violacea*. I-J. *C. calcicola*. K-L *C. koreana*. Abbreviations: co, collenchyma; cu, cuticle; ep, epidermis; ph, phloem; phf, phloem fiber; s, stomata; xy, xylem. Scale bars: 75 µm (B, D, F, H, J, L), 200 µm (A, C, E, G, I, K).

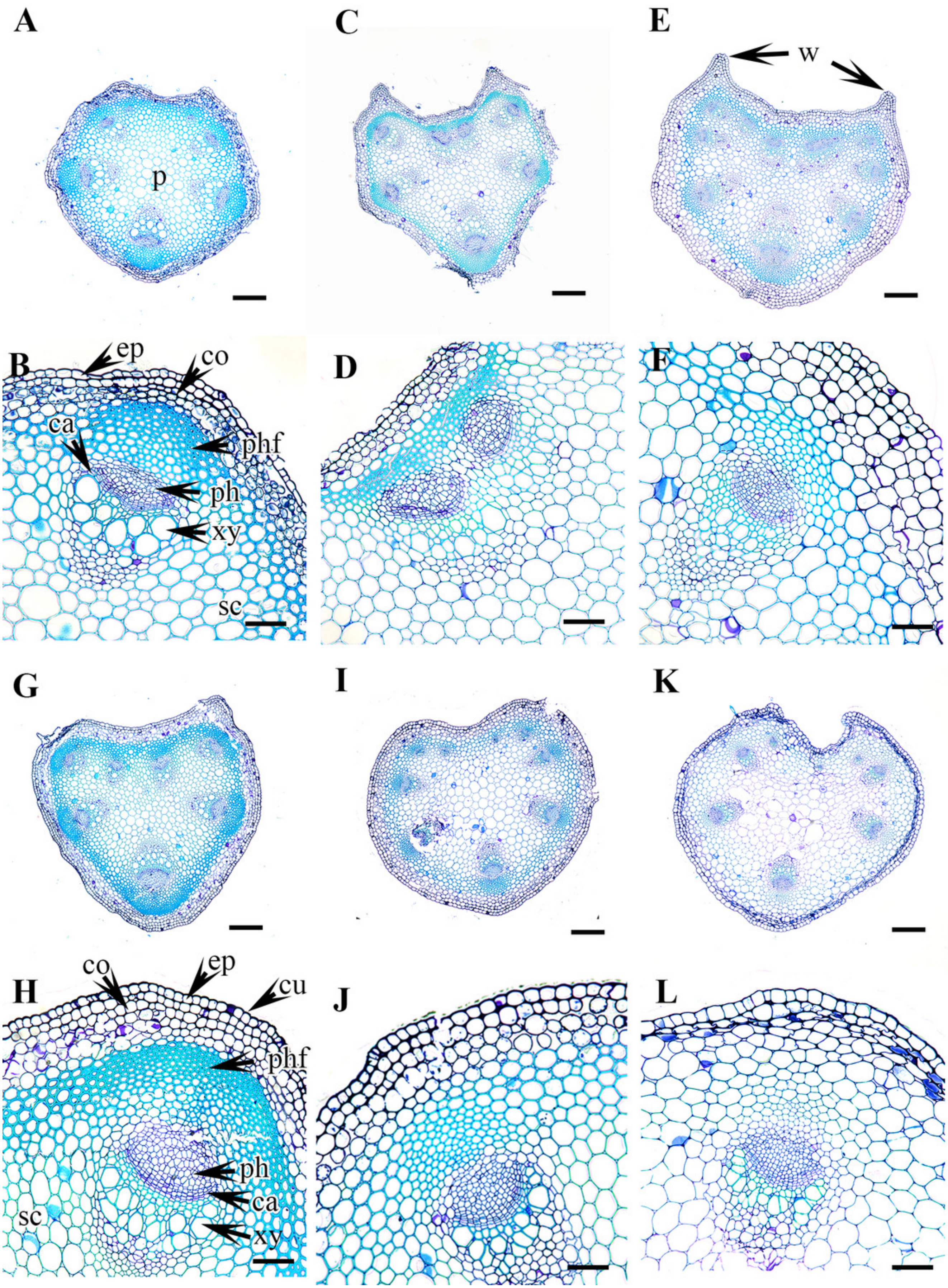
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Figure 6

Cross section of petiole of *Clematis*.

A-B. *C. ochotensis*. Abbreviations: co, collenchyma; cu, cuticle; ep, epidermis; ph, phloem; phf, phloem fiber; s, stomata; xy, xylem. Scale bars: 75 μm (B), 200 μm (A).

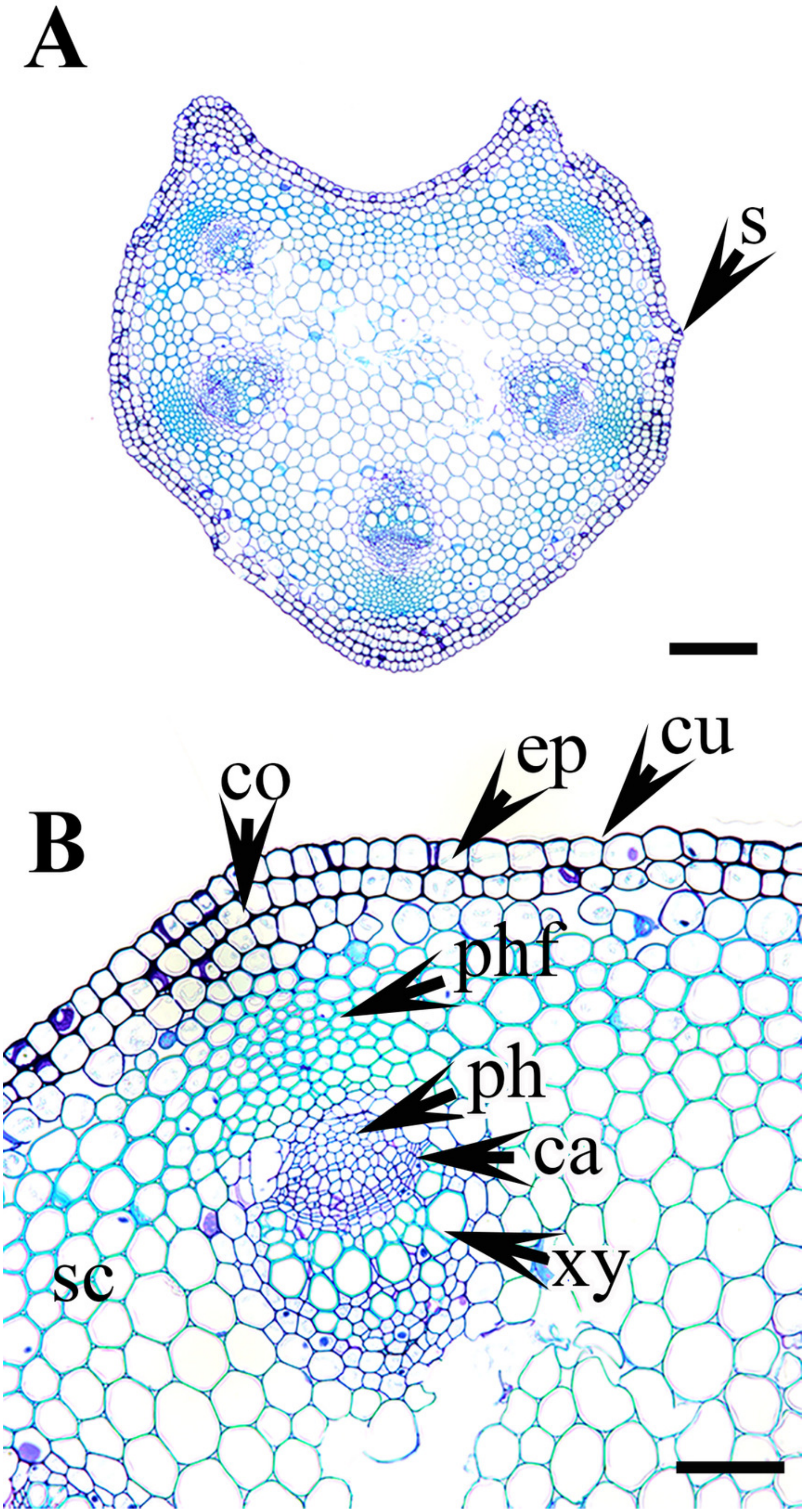
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Figure 7

Principal component analysis (PCA) of 13 petiole characters of *Clematis* taxa.

PS, petiole surface; TT, trichome type; GT, glandular trichome; TA, trichome abundance; PO, petiole outline in cross section; UW, upper surface wings; UG, upper surface groove; PF, phloem fiber cap; SC, interfascicular sclerenchyma; MV, major vascular bundles; IV, interfascicular vascular bundle; VB, total vascular bundle; VG, vascular bundles in groove.

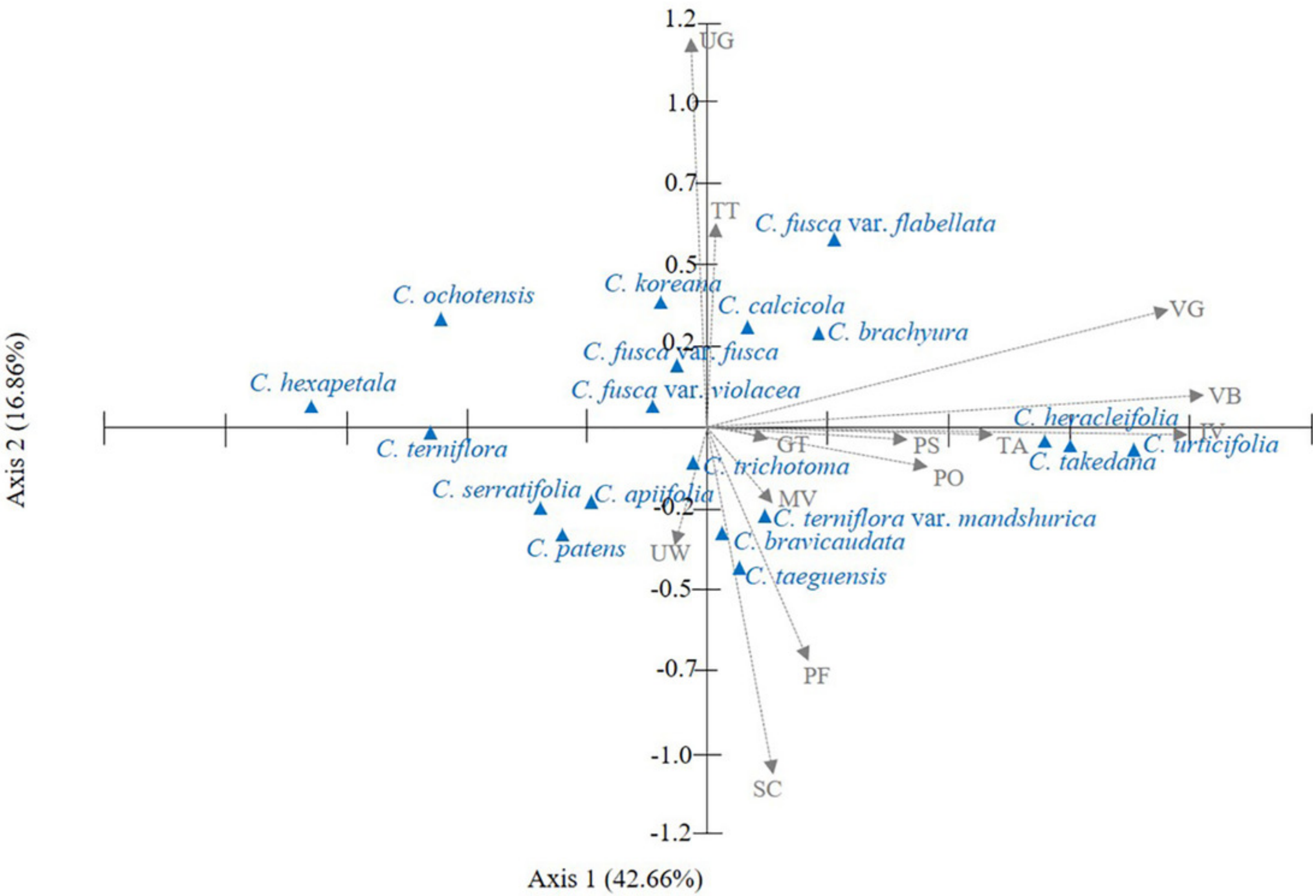

Figure 8

UPGMA cluster analysis based on petiole characters of *Clematis* taxa.

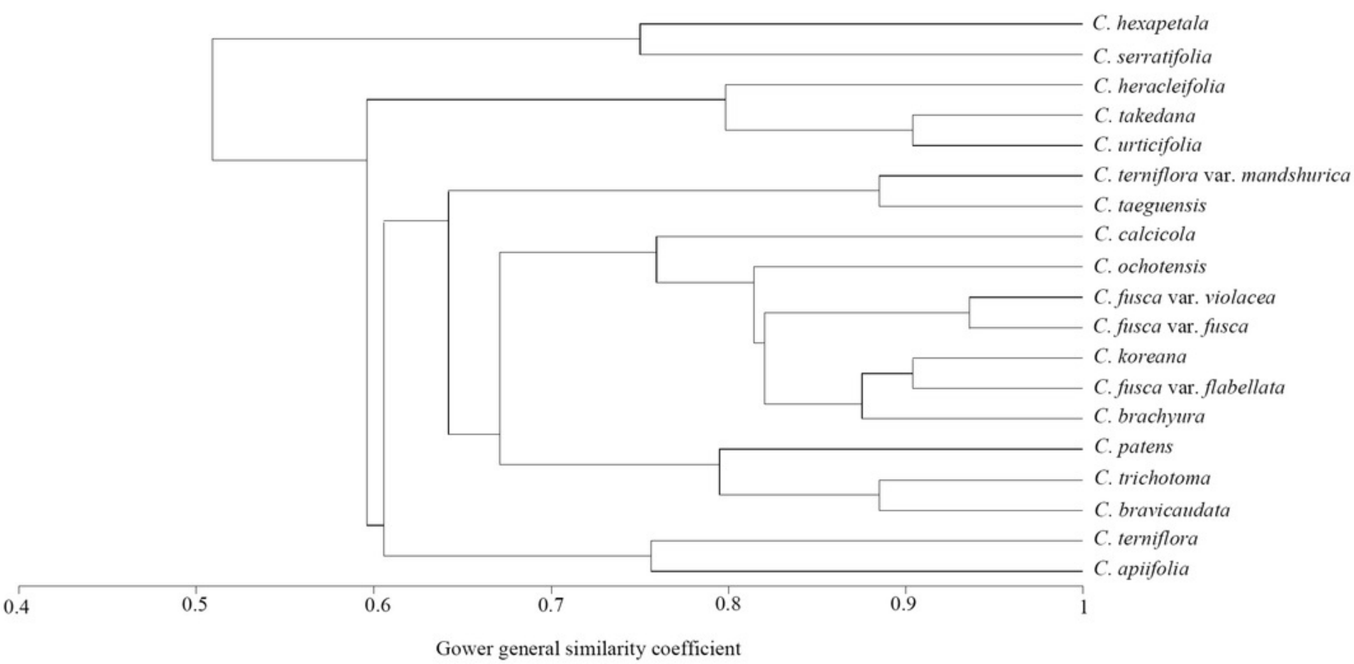

Table 1(on next page)

Name of taxa with voucher number and collection information.

Table 1. Name of taxa with voucher number and collection information.

Taxon	Collection sites	Voucher No.
Clematis apiifolia DC.	Mt. Sinbul, Icheon-ri, Sangbuk-myeon, Ulju-gun, Ulsan, Korea	Sinbulsan-190911-001
C. brevicaudata DC.	Unchi-ri, Sindong-eup, Jeongseon-gun, Gangwon-do, Korea	Unchiri-191007-001
C. trichotoma Nakai	Mt. Sinbul, Icheon-ri, Sangbuk-myeon, Ulju-gun, Ulsan, Korea	Sinbulsan-190911-001
C. taeguensis Y. Lee	Gyuram-ri, Jeongseon-eup, Jeongseon-gun, Gangwon-do, Korea	Gyuramri-190818-001
C. hexapetala Pall.	Ho-ri, Palbong-myeon, Seosan-si, Chungcheongnam-do, Korea	Hori-190809-001
C. terniflora DC.	Jukpo-ri, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea	Dolsando-191004-002
C. terniflora var. *mandshurica* (Rupr.) Ohwi	Namhansanseong Fortress, Sanseong-ri, Namhansanseong-myeon, Gwangju-si, Gyeonggi-do, Korea	Namhansanseong-190809-001
C. heracleifolia DC.	Sihwa Lake, Munho-ri, Namyang-eup, Hwaseong-si, Gyeonggi-do, Korea	Sihwaho-190921-016
C. urticifolia Nakai ex Kitag.	Mt. Gariwang, Sugam-ri, Bukpyeong-myeon, Jeongseon-gun, Gangwon-do, Korea	Gariwangsan-191007-001
C. takedana Makino	Sihwa Lake, Munho-ri, Namyang-eup, Hwaseong-si, Gyeonggi-do, Korea	Sihwaho-190921-001
C. patens C.Morren & Dence.	Mt. Johang, Samsong-ri, Cheongcheon-myeon, Goesan-gun, Chungcheongbuk-do, Korea	Johangsan-170831-049
C. brachyura Maxim.	Seondol, Bangjeol-ri, Yeongwol-eup, Yeongwol-gun, Gangwon-do, Korea	Seondol-190719-001
C. serratifolia Rehder	Gasong-ri, Dosan-myeon, Andong-si, Gyeongsangbuk-do, Korea	Gasongri-191007-001
C. fusca Turcz.	Mt. Cheongtae, Sapgyo-ri, Dunnae-myeon, Hoengseong-gun, Gangwon-do, Korea	Cheongtaesan-190819-001
C. fusca var. *flabellata* (Nakai) J. S. Kim	Eundae-bong, Gohan-ri, Gohan-eup, Jeongseon-gun, Gangwon-do, Korea	Eundaebong-190818-001
C. fusca var. *violacea* Maxim.	Mt. Baekhwa, Mawon-ri, Mungyeong-eup, Mungyeong-si, Gyeongsangbuk-do, Korea	Mawonri, Baekhwasan-150707-007
C. calcicola J. S. Kim	Mt. Deokhang, Daei-ri, Singi-myeon, Samcheok-si, Gangwon-do, Korea	Deokhangsan-190818-001
C. koreana Kom.	Mt. Hambaek, Gohan-ri, Gohan-eup, Jeongseon-gun, Gangwon-do, Korea	Hambaeksan-190818-001
C. ochotensis (Pall.) Poiret	Mt. Gariwang, Sugam-ri, Bukpyeong-myeon, Jeongseon-gun, Gangwon-do, Korea	Gariwangsan-190819-007

Table 2. Morphological and anatomical features of petiole of *Clematis* species.

Taxon	Petiole surface	Non-glandular trichomes	Glandular trichomes	Trichome abundance	Petiole outline in cross section	Upper surface wings
C. apiifolia	Villous	Unicate	Present	High	Pentagonal	Inconspicuous/conspicuous
C. brevicaudata	Villous	Unicate	Present	Medium	Semicircular	Inconspicuous
C. trichotoma	Pilose	Flagelliform	present	Medium	Semicircular	Inconspicuous
C. taeguensis	Subglabrous/pilose	Flagelliform	Present	Low	Pentagonal	Conspicuous
C. hexapetala	Glabrous	Absent	Absent	None	Semicircle/pentagonal	Conspicuous
C. terniflora	Pilose	Flagelliform	Present	Low	Half circular/semicircular	Inconspicuous/conspicuous
C. terniflora var. *mandshurica*	Pilose	Flagelliform	Present	Low	Pentagonal	Conspicuous
C. urticifolia	Villous	Unicate	Present	High	Pentagonal	Conspicuous
C. heracleifolia	Villous	Unicate	Present	High	Semicircular	Inconspicuous
C. takedana	Villous	Unicate	Present	High	Pentagonal	Conspicuous
C. patens	Pilose	Flagelliform	Present	Medium	Semicircular	Inconspicuous
C. brachyura	Pilose	Flagelliform	Present	Medium (in upper surface groove)	Pentagonal	Conspicuous
C. serratifolia	Subglabrous/pilose	Flagelliform	Absent	Low	Semicircle/pentagonal	Conspicuous
C. fusca var. *fusca*	Pilose	Flagelliform	Present	Low	Pentagonal	Conspicuous
C. fusca var. *flabellata*	Pilose	Flagelliform	Present	Medium	Semicircular	Conspicuous
C. fusca var. *violacea*	Pilose	Flagelliform (in groove)	Present	Low	Semicircular	Conspicuous
C. calcicola	Subglabrous/pilose	Flagelliform	Absent	Low	Semicircular	Inconspicuous/conspicuous
C. koreana	Pilose	Flagelliform	Present	Medium	Semicircular	Conspicuous
C. ochotensis	Pilose	Flagelliform	Present	Medium	Semicircular	Conspicuous

Table 2. Cont.

Taxon	Upper surface groove	Phloem fiber cap	Interfascicular sclerenchyma	MVB	IVB	TVB	BVG
C. apiifolia	Flattened/Sub-flattened	Large, 5-10 layers	<5 layers	6	0	6	1
C. brevicaudata	Sub flattened	Very large, >10 layers	>10 layers	5	2 to 3	7 to 8	2
C. trichotoma	Sub flattened	Very large, >10 layers	5-10 layers	5	2	7	2
C. taeguensis	Sub flattened	Very large, >10 layers	>10 layers	6	4	10	1
C. hexapetala	V-shaped	Large, 5-10 layers	<5 layers	5	0	5	0
C. terniflora	Flattened/Sub flattened	Small, <5 layers	<5 layers	6	0	6	1
C. terniflora var. *mandshurica*	Sub flattened	Very large, >10 layers	>10 layers	6	4	10	1
C. urticifolia	Sub flattened	Very large, >10 layers	5-10 layers	5	8	13	4
C. heracleifolia	Sub flattened	Very large, >10 layers	<5 layers	6	8	14	3
C. takedana	Sub flattened	Large, 5-10 layers	5-10 layers	6	7	13	3
C. patens	Flattened	Large, 5-10 layers	>10 layers	4	2	6	1
C. brachyura	V-shaped	Large, 5-10 layers	<5 layers	5	4	9	2
C. serratifolia	Sub flattened/U-shaped	Very large, >10 layers	5-10 layers	5	3	8	1
C. fusca var. *fusca*	U-shaped	Very large, >10 layers	<5 layers	5	2	7	2
C. fusca var. *flabellata*	U-shaped	Small, <5 layers	<5 layers	5	5	10	3
C. fusca var. *violacea*	Sub flattened	Very large, >10 layers	<5 layers	5	2	7	2
C. calcicola	Sub flattened/U-shaped	Large, 5-10 layers	<5 layers	5	4	9	2
C. koreana	V-shaped	Small, <5 layers	<5 layers	5	3	8	2
C. ochotensis	U-shaped	Large, 5-10 layers	<5 layers	5	0	5	0

Abbreviations: MVB, major vascular bundles; IVB, interfascicular vascular bundle; TVB, total vascular bundle; VBG, vascular bundles in groove.