Taxonomic relevance of anatomical and morphological characteristics of petioles of *Clematis* L. (Ranunculaceae) taxa from South Korea (#56114)

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Taxonomic relevance of anatomical and morphological characteristics of petioles of *Clematis* L. (Ranunculaceae) taxa from South Korea

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We assessed the morphological and anatomical structure of the 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 results of this study showed that the presence/absence and abundance of trichomes, petiole cross-section outlines, upper surface wings and grooves, and the number of vascular bundles were proved to be useful for species discrimination in *Clematis*. Among the studied taxa, *C. hexapetala* was the only species with a glabrous petiole surface. Two types of trichomes were observed in the other 18 taxa: long, non-glandular and flagelliform trichomes and short, glandular capitate trichomes. We found four to six major vascular bundles and a maximum of eight interfascicular vascular bundles (*C. heracleifolia* and *C. urticifolia*) in the 19 taxa. A cluster analysis based on UPGMA identified 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, exhibited 93.36% similarity in petiole features whereas *C. hexapetala* and *C. serratifolia* share only 52.7% similar petiole features with the rest of the species. Although the numbers of taxa investigated was limited, taxa from the sections *Tubulosae*, *Viorna*, and *Astragene* clustered with each other in the UPGMA phenogram due to the overall similarity of petiole features. As a single source of information, this study may not be useful for resolving infrageneric relationships; however, the data obtained could be used as descriptive and/or diagnostic features for particular taxa in the genus.

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ABSTRACT

We assessed the morphological and anatomical structure of the 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 results of this study showed that the presence/absence and abundance of trichomes, petiole cross-section outlines, upper surface wings and grooves, and the number of vascular bundles were proved to be useful for species discrimination in *Clematis*. Among the studied taxa, *C. hexapetala* was the only species with a glabrous petiole surface. Two types of trichomes were observed in the other 18 taxa: long, non-glandular and flagelliform trichomes and short, glandular capitate trichomes. We found four to six major vascular bundles and a maximum of eight interfascicular vascular bundles (*C. heracleifolia* and *C. urticifolia*) in the 19 taxa. A cluster analysis based on UPGMA identified 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, exhibited 93.36% similarity in petiole features whereas *C. hexapetala* and *C. serratifolia* share only 52.7% similar petiole features with the rest of the species. Although the numbers of taxa investigated was limited, taxa from the sections *Tubulosae*, *Viorna*, and *Astragene* clustered with each other in the UPGMA phenogram due to the overall similarity of petiole features. As a single source of information, this study may not be useful for resolving infrageneric relationships; however, the data obtained could be used as descriptive and/or diagnostic features for particular taxa in the genus.

Keywords: Petiole morphology, *Clematis*, Trichomes, Vascular bundle, Infrageneric relationship, Taxonomy

INTRODUCTION

Ranunculaceae is one of the larger families of eudicots comprising nearly 2500 species within 50–60 genera (*Tamura, 1993*; *Hoot et al., 2012*; *Wang et al., 2013*). The family has been classified under the early branching order of eudicot Ranunculales (*APG IV, 2016*). Ranunculaceae are distributed across the world and exhibit their greatest diversity in the temperate and cold regions of the Northern and Southern Hemispheres (*Tamura, 1993*). A number of classification models that consider morphological characters, molecular sequencing, and a combination of both morphological and molecular data have been proposed for this family (*Hutchinson, 1923*; *Janchen, 1949*; *Johansson & Jansen, 1993*; *Tamura, 1995*; *Hoot, 1995*; *Jensen et al., 1995*; *Ro et al., 1997*; *Wang et al., 2009, 2013*; *Emadzade et al., 2010*; *Hoot et al., 2012*; *Wang et al., 2014*; *Zhai et al., 2019*). In addition to the various morphological criteria, the basic chromosome numbers and type of chromosome have became ~~the most~~ reliable reference that is compatible with the molecular phylogeny of Ranunculaceae (*Gregory, 1941*; *Tamura, 1987*; *Ro et al., 1997*; *Wang et al., 2009*).

61   Within Ranunculaceae, *Clematis* L. is classified under the tribe Anemoneae DC. in the

62   subfamily Ranunculoideae Hutch. (*Tamura, 1995*). *Clematis* is one of the largest genera in the family,

63   which comprises approximately 280–350 cosmopolitan species (*Tamura, 1987, 1995*; *Wang & Li, 2005*).

64   In Korea, *Nakai (1952)* reported 21 species and 14 varieties of *Clematis* in a synoptical sketch of Korean

65   flora, but *Lee (1967)* later identified 16 species, 11 varieties, and five forma in the genus. In the book

66   New Flora of Korea, *Lee (2007)* described 18 taxa including *C. taeguensis* Y. Lee, which was first

67   described by *Lee (1982)*. The *Korea National Arboretum (2017)* recently listed 17 species and five

68   varieties of *Clematis* in the Checklist of Vascular Plants in Korea, whereas *Kim (2017)* described 13

69   species and seven varieties within the genus in The Flora of Korea. After a careful review of *Lee (2007)*,

70   *Chang et al. (2011)*, the *Korea National Arboretum (2017),* and *Kim (2017)* we included 16 species and

71   three varieties in this study.

72   Due to the vast morphological disparity among its species*, Clematis* has been a subject of

73   investigation since the early 19th century and has been subjected to several infrageneric revisions. Several

74   ==systematic studies on the anatomy== of different parts, palynology, and cytology of *Clematis* have been

75   carried out (*Tobe, 1974, 1980a, 1980b, 1980c, 1980d*; *Essig, 1991*; *Zhang, 1991*; *Yano, 1993*; *Yang &*

76   *Moor, 1999*; *Shi & Li, 2003*; *Xie & Li, 2012*; *Ghimire et al., 2020*). The morphological characters that

77   have been extensively studied and considered in the infrageneric classifications of *Clematis* include habit,

78   seed germination, seedling phyllotaxy, leaf structure, ==inflorescence,== ~~the sexuality of flowers,~~ floral

79   morphology, and pollen and achene morphology (see *Wang & Li, 2005*).

80   The importance of nodal and petiolar anatomy in intergeneric and familial taxonomy has been

81   extensively studied (*Howard, 1962, 1979*; *Schofield, 1968*; *Dickison, 1969, 1980*; *Datta & Dasgupta,*

82   *1979*). The middle portion of the petiole is considered to be the most stable zone, from which even a

83   single section can be taken for comparative purposes (*Metcalfe & Chalk, 1979*). In addition, the complex

84   vascular systems of the petiole provide a range of diagnostic structures that can be useful for taxonomic

85   treatment at any rank (*Solereder, 1908*; *Metcalfe & Chalk, 1979*; *Ashton, 1982*; *Dehgan, 1982*; *Rojo,*

86   *1987*; *Pardi et al., 1991*; *Kamel & Loutfy, 2001*; *Kocsis & Borhidi, 2003*; *Noraini et al., 2016,, 2017*).

87   Within Ranunculaceae, few taxonomic-related ==investigations of petiole and nodal anatomy that include==

88   ==stems== have been carried out (*Worsdell, 1908*; *Tamura, 1962*; *Oh, 1971*; *Kavathekar & Pilli, 1976*; *Tobe,*

89   *1979, 1980*; *Kökdil et al., 2006*; *Gostin, 2011*; *Novikoff & Mitka, 2015*). Unfortunately, studies pertaining

90   to the petiole morphology and anatomy of *Clematis*, one of the most morphologically diverse and

91   taxonomically complicated genera within Ranunculaceae, are very rare in the literature. In an anatomical

92   study of *Clematis* in Korea, only *Oh (1971)* made some remarks on the petiole anatomy of nine Korean

93   species.

94   In this study, we provide a comprehensive investigation of the petiole morphology and anatomy

95   of 19 *Clematis* taxa distributed in Korea. The primary objective of this study was to investigate the

96   morphological structure of the petiole in detail as well as the anatomy of the included taxa and to evaluate

97   the implications of petiolar characters for species delimitation. We attempted to compare our results with

98   those for previously studied species and summarized them in order to make taxonomic conclusions.

99

100   **MATERIALS AND METHODS**

101   **Plant materials**

102   The names of the investigated species and their voucher numbers are provided in Table 1. The voucher

103   specimens were deposited in the herbarium of Korea National Arboretum (KH). Data were collected as

104   previously described in *Ghimire et al. (2020).*

105   **Stereo and scanning electron microscopy**

106   Petiole morphology including indumentum, trichome type and abundance, and upper surface groove was

107   observed under a stereomicroscope and a scanning electron microscope (SEM). A Leica MZ16 FA

108   microscope (Leica Microsystems GmbH, Wetzlar, Germany) was used for the observations and digital

109   images of the best-represented part of the petiole were taken with a Leica DFC420 C multifocal camera

110   attached to the microscope. Before SEM imaging, petiole pieces were immersed in 100% ethanol and

111   sputter-coated with gold in a KIC-IA COXEM Ion-Coater (COXEM. Co., Ltd., Daejeon, Korea). SEM

112   imaging was carried out with a COXEM EM-30 PLUS+ table scanning electron microscope (COXEM) at

113   20 kV, at the seed testing laboratory of the Korea National Arboretum.

114   **Microtome and light microscopy**

115   At least three petioles of each taxon were subjected to microtome sectioning according to the following

116   procedure as used in *Ghimire et al., 2020.* Freshly collected leaf petioles were fixed in formalin, acetic

117   acid, and 50% ethyl alcohol (FAA) at a ratio of 90:5:5 for a week and preserved in 50% ethyl alcohol.

118   During the experiment, the preserved petioles were cut into small pieces (approximately 2 mm) and

119   dehydrated with an ethanol series (50, 70, 80, 90, 95 and 100%). After complete dehydration, the petiole

120   pieces were infiltrated with ethanol/Technovit mixtures (3:1, 1:1, 1:3, and 100% Technovit) and then

121   embedded in Technovit 7100 resin. The embedded materials were cut into serial sections of 4–6 μm

122   thickness using a Leica RM2255 rotary microtome (Leica Microsystems GmbH, Wetzlar, Germany) with

123   disposable blades and attached to a glass slide. The slides were dried using an electric slide warmer for 12

124   h. The dried slides were stained with 0.1% toluidine blue 'O' for 60–90 s, rinsed with water and dried

125   again with the slide warmer for at least 6 h to remove any remaining water (*Johansen, 1940*). The stained

126   slides were then mounted with Entellan (Merck Co., Darmstadt, Germany) and later examined under a

127   Leica DM3000 LED (Leica Microsystem, Wetzlar, Germany). Photomicrographs were taken with a

128   scientific CMOS camera. Multiple image alignment was performed using Photoshop CS for Windows

129   2010.

Morphometry and data analysis

131   Thirteen quantitative characters were categorized and coded with binary and/or multistate coding.

132   Principal component analysis (PCA) and cluster analysis using the unweighted pair group (UPGMA)

133   clustering method using the Gower general similarity coefficient were carried out with MultiVariate

134   Statistical Package 3.1 software (MVSP Version 3.1) (Kovach, 1999). The character states and their codes

135   are provided in Supplementary File S1.

RESULTS

137   The morphological and anatomical characters of *Clematis* petioles observed in this study include the

138   petiole indumentum, trichome type and abundance, petiole outline in cross-section (CS), upper surface

139   wings and groove, sclerenchyma region, and vascular bundles. All the characters are summarized in Table

140   2. Selected images of the petioles are provided in Figures 1-6. The morphological and anatomical features

141   of the petiole are comprehensively described below.

Petiole surface and trichomes

143   The petiole surface of the studied species is pubescent except in *Clematis hexapetala*, which has an

144   almost glabrous surface (a few trichomes occur in the region from which leaflets arise) (Table 2, Fig. 1A-

145   S). *Clematis taeguensis* and *C. serratifolia* have subglabrous petiole surfaces, and only a few trichomes

146   occur on the petioles of these species. Two types of trichomes are observed on the petiole; long, non-

147   glandular, flagelliform trichomes and short, glandular, capitate trichomes (Figs. 1A-S, 2A-J). Glandular

148   trichomes are usually distributed in the upper surface groove. In some species such as *C. terniflora*, *C.*

149   *terniflora* var. *mandshurica*, *C. brachyura*, *C. fusca* var. *fusca*, and *C. fusca* var. *violacea*, non-glandular

150   trichomes are concentrated only on the upper surface groove. The pubescent species can be categorized as

151   'villous', covered with long, soft, and dense hairs (i.e., *C. apiifolia*, *C. brevicaudata*, *C. heracleifolia*, *C.*

152   *urticifolia*, and *C. takedana*), or 'pilose', covered with soft, weak, thin, and separated hairs, as found in

153   rest of the species (i.e., *C. trichotoma*, *C. terniflora*, *C. terniflora* var. *mandshurica*, *C. patens*, *C.*

154   *brachyuran*, *C. fusca* var. *fusca*, *C. fusca* var. *flabellata*, *C. fusca* var. *violacea*, *C. koreana*, and *C.*

155   *ochotensis*). The non-glandular trichomes are either unicate (*C. apiifolia*, *C. brevicaudata*, *C.*

156   *heracleifolia*, *C. urticifolia*, and *C. takedana*) or flabelliform (all other species except *C. hexapetala*).

157   Based on the trichome density per unit area, the trichome abundance is categorized as high, medium, or

158   low.

Petiole outline and upper surface groove

160   The studied species show considerable variation in their CS petiole outline (Table 2). The species can be

161   divided into three categories based on the shape of their prtiole in CS: pentagonal petioles (seven species),

162   U-shaped petioles (nine species), and U-shaped or semi-circular petiole (only *C. terniflora*) (shown in

163   Figures 3, 4, 5, and 6). *Clematis hexapetala* and *C. serratifolia* exhibit both pentagonal and semi-circular

164   petioles in CS. Out of the 19 taxa, 13 taxa exhibit two noticeable upper or dorsal surface wings, while six

165   species do not have noticeable wings. Based on the shape of the upper surface groove, the petioles can be

166   categorized as flattened (three species), sub-flattened (11 species), U-shaped (three species), or V-shaped

167   (three species). The formation of the upper surface groove is due primarily to the dorsal surface wings,

168   although some species with inconspicuous wings have a slight groove in the petiole (*C. brevicaudata, C.*

169   *trichotoma*, and *C. heracleifolia*).

Petiole epidermis and cortex

171   The epidermis is single-layered and cutinized in all species (Figures 3, 4, 5, and 6). The cells are small,

172   rounded, cuboidal, narrow, or slightly elongated. In some places, the continuation of the epidermis is

173   interrupted by the presence of stomata. The epidermis is underlain by the cortex, which is 3-5 cells thick.

174   The cortical cells are loosely arranged and parenchymatous with abundant air spaces and are rounded,

175   ovoid, elongated, or irregular in shape. The cortex is collenchymatous above the phloem fibre, where the

176   cells are thick-walled and closely packed.

Vascular bundles

178   The vascular bundles are of the open, conjoint, collateral type. There is remarkable variation in the

179   number of vascular bundles (ranging from 5 to 14) among *Clematis* species. The 19 taxa have four to six

180   major vascular bundles and a maximum of eight interfascicular vascular bundles (*C. heracleifolia* and *C.*

181   *urticifolia*). Twelve species have five major vascular bundles, six species have six and only *C. patens* has

182   four (Table 2). The major vascular bundles are ovoid with the phloem oriented towards the cortex and the

183   xylem oriented towards the pith. The xylem and phloem are separated by 2-4 layers of the cambial cells.

184   Each major vascular bundle is overlain with a cluster of thick-walled fibrous cells, i.e., the phloem fibre

185   cap. The quantity of phloem fibres within the studied species is variable. Based on its height in cell layers

186   the phloem cap is categorized as: large (more than ten cells high), medium (five to ten cells high), or

187   small (less than five cells high). Three species *C. terniflora, C. fusca* var. *fusca*, and *C. koreana*, have

188   small fiber caps, while seven and nine species have medium and large fibre caps, respectively. There is a

189   permanent sclerenchymatous strand between the adjacent vascular bundles in all species.

190   The petiole of each species has a large region of the ground tissue, i.e., the pith. The cells in the

191   pith are thin-walled, rounded, ovoid, or angular and parenchymatous and are comparatively larger than

192   those in the cortex (Figures 3, 4, 5, and 6).

193   **Statistical analysis**

194   The similarities among the species based on the 13 petiole features were revealed using PCA and cluster

195   analysis. The first three components of the PCA explained 74.01% of the total variation in the analysed

196   data. The first axis of the first complete set explained 42.66% of the total variation and showed strong

197   positive loadings for trichome abundance and the number of vascular bundles (TA, IV, VB, and VG) (Fig.

198   7). The second axis explained 16.86% of the total variation and showed strong positive loadings for

199   trichome type and upper surface groove (TT and UG) and strong negative loadings for phloem fibre cap

200   height and interfascicular sclerenchyma (PF and SC). The cluster analysis based on UPGMA using the

201   Gower similarity coefficient identified six clusters with 18 nodes (Fig. 8). *Clematis fusca* var. *fusca* and

202   *C. fusca* var. *violacea*, representing the first node in the sixth cluster of the phenogram showed 93.6%

203   similarity in petiole features, whereas *C. hexapetala* and *C. serratifolia*, representing the 18th node in the

204   first cluster in the phenogram shared only 52.7% similarity in petiole features with the rest of the species.

205   **DISCUSSION**

206   The genus *Clematis* is morphologically diverse in terms of leaf phyllotaxy, types of compound leaves,

207   and leaflet number. The anatomy of the petiole, therefore, is expected to be equally diverse. Previously,

208   *Oh (1971)* found variation in the number of vascular bundles in the petiole of some *Clematis* species.

209   Thus, the reasonable diversity of petiole anatomical features, including variation in the number of

210   vascular bundles, is certainly useful for the taxonomic treatment of the genus. The overall anatomical

211   organization of the petioles in the investigated species was comparable to that described in *Oh (1971)*. In

212   addition, this study provides a comprehensive anatomical description of the petiole of all Korean *Clematis*

213   species and a discussion of their taxonomic relevance within the genus, which have not been included in

214   previous studies.

215   The results of this study showed that *Clematis* species can be differentiated based on the petiole

216   indumentum. The presence/absence and/or type of trichomes in the petiole have also been used for

217   species differentiation in other taxa (*Solereder, 1908*; *Metclafe & Chalk, 1979*; *Noraini et al., 2017*).

218   Among the 19 species investigated, *Clematis hexapetala* is the only species with glabrous petioles

219   (although sparsely distributed trichomes can be observed at the base of the leaflets), whereas *C.*

220   *taeguensis* and *C. serratifolia* have subglabrous or sparsely pubescent indumentum. The remaining

221   species have sparsely or thickly pubescent petioles. In sparsely pubescent taxa such as *C. terniflora*, *C.*

222   *terniflora* var. *mandshurica*, *C. fusca* var. *fusca*, and *C. fusca* var. *violacea*, the trichomes are restricted to

223   mainly the upper groove region. Additionally, the stem of these taxa is either subglabrous or is

224   puberulous only at nodes (*Wang & Bartholomew, 2001*; *Kim, 2017*). On the other hand, species such as

225   *C. apiifolia*, *C. heracleifolia*, *C. urticifolia*, and *C. takedana*, which have thickly pubescent petioles, also

226   have heavily pubescent stems and branches (*Wang & Bartholomew, 2001*; *Kadota, 2006*; *Moon et al.,*

227   *2013*; *Kim, 2017*). This indicates that the trichomes in the petiole are generally continuous with those on

228   the stem in the *Clematis* species.

229   Most of the species have both types of trichomes although glandular trichomes are very scarce

230   and restricted to the upper surface groove of the petiole. We observed both glandular capitate and simple

231   non-glandular trichomes on the leaf surface of most of the investigated *Clematis* species (*Ghimire et al.*

232   unpublished report). Glandular pubescence on the stem, petiole and leaf of *C. gattingeri* Small has been

233   reported in a few older studies (Svenson, 1941; Dennis, 1978). Petiole features that are considered to be

234   useful for taxonomic discrimination in various taxa (*Solereder, 1908*; *Metcalfe & Chalk, 1979*; *Dehgan,*

235   *1982*; *Rojo, 1987*; *Pardi et al., 1991*; *Kamel & Loutfy, 2001*; *Kocsis & Borhidi, 2003*; *Noraini et al.,*

236   *2016,, 2017*) are generally neglected in morphological studies of *Clematis* because no recent reports have

237   considered the systematic utility of such pubescence for the infra-generic classifications of this genus

238   (*Tamura, 1995*; *Wang & Li, 2005*; *Lehtonen et al., 2016*; *Wang & Bartholomew, 2001*; *Kadota, 2006*;

239   *Kim, 2017*). In a taxonomic study of *C. gattingeri*, Dennis (1978) confirmed the presence of glandular

240   pubescence on the stem, petiole, and leaf which had never been observed in other species of subsection

241   *Viornae*. The recognition of *C. gattingeri* as a species has been based primarily on the glandular

242   pubescence and small flower size of Gattinger's specimens. The results from this study also revealed that

243   the petiole indumentum and types of trichomes appear to have taxonomic value for species delimitation in

244   the *Clematis*.

245   In addition to the surface indumentum, some other petiole features that can contribute to the

246   identification of a particular species in *Clematis* include the petiole outline in CS, the upper surface

247   groove and wings, and the phloem fibre cap. Of these features, the petiole outline and upper surface

248   groove and wings have already been proven to be useful in the taxonomic discrimination of species in

249   some eudicot genera (*Kocsis & Borhidi, 2003*; *Noraini et al., 2017*; *Abeysinghe & Scharaschkin, 2019*).

250   *Oh (1971)* reported pentagonal and/or horseshoe or rounded horseshoe-shaped petiole in nine *Clematis*

251   species and the results of this study corroborated those reports. We observed that the petiole of *Clematis*

252   in CS is dorsiventral, pentagonal with five visibly and/or weakly represented ridges or semicircular and

253   ridgeless. *Clematis hexapetala* and *C. serratifolia* appear to have both pentagonal and U-shaped petioles,

254   whereas some petioles of *C. terniflora* are semi-circular. Species with pentagonal petioles have

255   conspicuous upper surface wings that form an upper surface groove, while some of the species with U-

256   shaped petioles have inconspicuous upper surface wings. Species with inconspicuous wings such as *C.*

257   *brevicaudata*, *C. trichotoma*, *C. heracleifolia*, and *C. terniflora* still form a slight upper surface groove.

258   Of the three taxa, *C. fusca* var. *fusca*, *C. fusca* var. *flabellata*, and *C. fusca* var. *violacea*, the first has

259   pentagonal petiole, while the latter two have U-shaped petioles with noticeable upper wings. Interestingly,

260   these three taxa showed dissimilar upper surface groove characteristics: *C. fusca* var. *violacea* has a sub-

261   flattened groove, whereas *C. fusca* var. *fusca* and *C. fusca* var. *flabellata*, have a U-shaped groove.

262   Although reports on such upper wing extensions and adaxial grooves on the petiole are lacking in the

263   literature, our study suggests the possibility of using these features for species identification in *Clematis*.

264   In petiole anatomy, the vascular system of the petiole has received the most attention (*Kocsis &*

265   *Borhidi, 2003*; *Noraini et al., 2016*; *2017*; *Long & Oskolski, 2018*; *Abeysinghe & Scharaschkin, 2019*).

266   According to *Hare (1942)*, various arrangements of vascular bundles in the petiole can be used as

267   diagnostic characteristics in some taxonomic groups. *Howard (1962, 1974)* suggested that the vascular

268   structure of the petiole is most useful at the generic level and sometimes at the family level, although the

269   intensity of taxonomic value may vary from one taxonomic group to another. The *Clematis* petiole

270   showed remarkable consistency in the arrangement of the vascular system, although the studied species

271   differ from each other by the number of major and interfascicular vascular bundles. There are tipically

272   five major vascular bundles, which possibly correspond to the five ridges of the petiole; however, *C.*

273   *patens*, with exclusively U-shaped petioles have no ridges, wings, or upper surface grooves, has only four

274   major vascular bundles. In some taxa such as *C. apiifolia*, *C. taeguensis*, *C. hexapetala*, *C. terniflora* var.

275   *mandshurica*, *C. heracleifolia*, and *C. takedana*, the vascular bundle in the upper groove regions develops

276   in the same way as the vascular bundles in the edges, resulting in a total of six major vascular bundles in

277   these taxa.

278   Regarding the number of vascular bundles, the results of this study are almost congruent with

279   those of *Oh (1971)* for *C. apiifolia*, *C. trichotoma*, and *C. koreana* but are slightly different for *C.*

280   *brachyura* and *C. patens*, in which he described six major and four interfascicular vascular bundles and

281   six major vascular bundles, respectively. Instead, we observed five major and four interfascicular vascular

282   bundles in *C. brachyura* and four major and one or two interfascicular bundles in *C. patens*. We observed

283   variation in the number and position of interfascicular bundles even in the different samples of the same

284   species including in *C. patens*: thus this feature may have only a slight taxonomic value for discriminating

285   among *Clematis* species. On the other hand, the number and position of the major vascular bundles,

286   which showed remarkable consistency among the investigated samples of all species, may have

287   significant taxonomic value for species discrimination within the genus. At this point, our results suggest

288   that the pre-existing data presented by *Oh (1971)* on the number of vascular bundles, specifically the

289   number of major bundles, in the petioles of *Clematis* species should be corrected.

290   The cluster analysis based on 13 petiole features generated at least six clusters. Of these,

291   *Clematis serratifolia* and *C. hexapetala* formed the first cluster, separate from the rest of the species (Fig.

292   8). The sixth ==cluster was represented the largest cluster== and comprised seven taxa; *C. calcicola, C.*

293   *ochotensis, C. fusca* var. *fusca, C. fusca* var. *violacea, C. fusca* var. *flabellata, C. koreana,* and *C.*

294   *brachyura.* According to ~~the infrageneric classification of~~ *Johnson (2001)* and *Wang & Li (2005)* these

295   seven taxa belong to the sections *Viorna* (*C. fusca* var. *fusca, C. fusca* var. *violacea, C. fusca* var.

296   *flabellata*), section *Atragene* (*C. ochotensis, C. calcicola,* and *C. koreana*), and *Pterocarpa* (*C.*

297   *brachyura*). In a recent phylogenetic classification of the genus, *C. fusca* var. *fusca, C. fusca* var.

298   *violacea,* and *C. fusca* var. *flabellata* were classified under clade L; *C. ochotensis, C. calcicola,* and *C.*

299   *koreana* were classified under clade H, and *C. brachyura* was classified under clade K (*Lehtonen et al.,*

300   *2016*). Additionally, *C. urticifolia, C. takedana,* and *C. heracleifolia,* which made up the third cluster in

301   the UPGMA phenogram in this study, belong to section *Tubulosae* in the *Johnson (2001)* and *Wang & Li*

302   *(2005)* classification and to clade C in *Lehtonen et al. (2016).* According to infrageneric classifications by

303   *Tamura (1995), Wang & Li (2005)* and *Xie et al. (2011), C. brachyura* is considered to be closer to

304   section *Flammula* (*C. taeguensis, C. hexapetala, C. terniflora* var. *mandshurica,* and *C. terniflora*).

305   However, in this study, it remained connected in the *Viorna* and *Atragene* sections. In fact, this

306   uncertainty is a common and typical interpretation for this large genus, as previous morphological and

307   molecular studies also found a similar tendency (*Grey-Wilson, 2000; Wang & Li, 2005; Miikeda et al.,*

308   *2006; Xie et al., 2011; Xie & Li, 2012; Lehtonen et al., 2016; Ghimire et al., 2020*).

309   Previous studies have suggested that neither molecular analyses nor morphological data strongly

310   support the infrageneric classification of *Clematis.* We agree with most authors that performing

311   infrageneric classification in *Clematis* based on morphological characters is extremely difficult. In our

312   analysis, out of three taxa from section *Viorna, C. fusca* var. *flabellata* was grouped with *C. koreana*

313   (section *Atragene*) and *C. brachurya* (section *Pterocarpa*), whereas *C. fusca* var. *fusca* and *C. fusca* var.

314   *violacea* remains together in a separate sub-cluster. The petioles of *C. fusca* var. *flabellata* differ from

315   those of *C. fusca* var. *fusca* and *C. fusca* var. *violacea* in terms of trichome abundance, phloem fibre cap

316   height, and number of vascular bundles. In terms of morphology, *C. fusca* var. *flabellata* is an erect herb

317   with ternate leaves, whereas *C. fusca* and *C. fusca* var. *violacea* are woody vines with pinnately foliate

318   leaves. In addition out of the three species in section *Clematis, C. trichotoma* and *C. brevicaudata* form a

319   sub-cluster with *C. patens* (section *Viticella*) while *C. apiifolia* allied with ==*C. terniflora*== (section

320   *Flammula*) are in another sub-cluster. Morphologically, the petiole of *C. apifolia* differs from those of the

321   other two species in its trichome abundance, outline in CS, phloem fibre cap, intrafascicular

322 sclerenchyma, and number of vascular bundles. Notably, petiole features alone can be more useful for

323 species delimitation than for distinguishing infrageneric relationships and thus are not indicative of

324 infrageneric classifications. Similar explanations were provided by *Ghimire et al. (2020)* and *Xie & Li*

325 *(2012)* based on the achene morphology and pollen morphology of *Clematis*, respectively.

326   In conclusion, the presence/absence and abundance of trichomes, petiole outline in CS, upper

327 surface wings and groove, and number of vascular bundles were determined to be useful for species

328 discrimination in Korean *Clematis*. The results of this study also indicated that taxa from the sections

329 *Tubulosae*, *Viorna*, and *Atragene* could be grouped based on the overall similarity of their petiole

330 features. Moreover, we found that many of the petiole characters that were neglected in taxonomic and

331 systematic considerations within and among *Clematis* taxa were actually useful for species delimitation.

332 We do not consider this petiole morphological data to be strong enough to provide useful information on

333 infrageneric relationships with the *Clematis* genus; however, our results provide new and interesting

334 insight into these data, which can be used as a source of descriptive and/or diagnostic features for

335 particular taxa in the genus.

336

337 **ADDITIONAL INFORMATION AND DECLARATION**

338 **Author Contributions**

339 • BKP conceived the study, performed the experiment, analysed the data, and wrote and reviewed
340   drafts of the manuscript.

341 • BG and DCS conceived the study and designed the experiment, analysed the data, and wrote and
342   reviewed the 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.
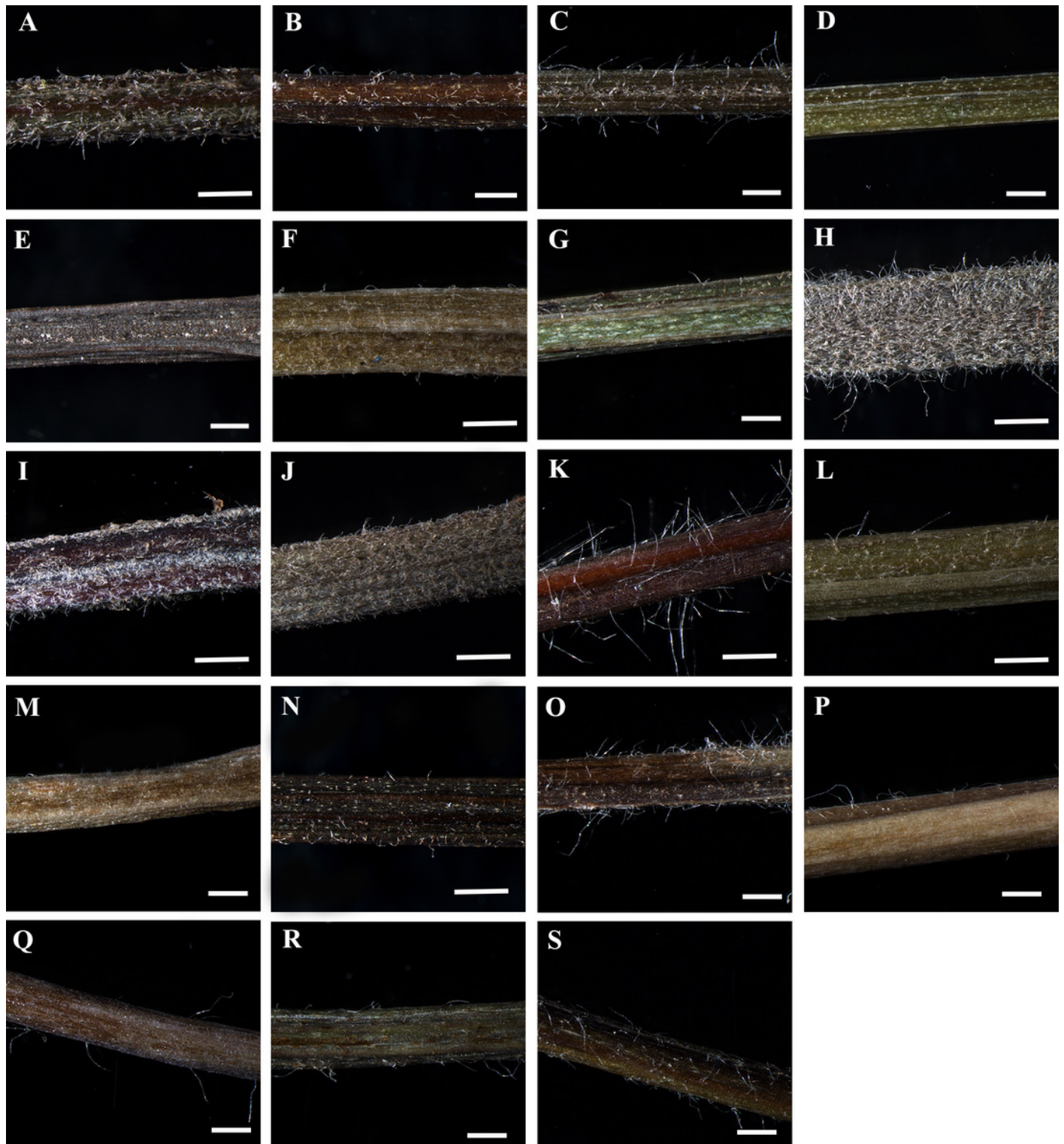
Figure 2

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).
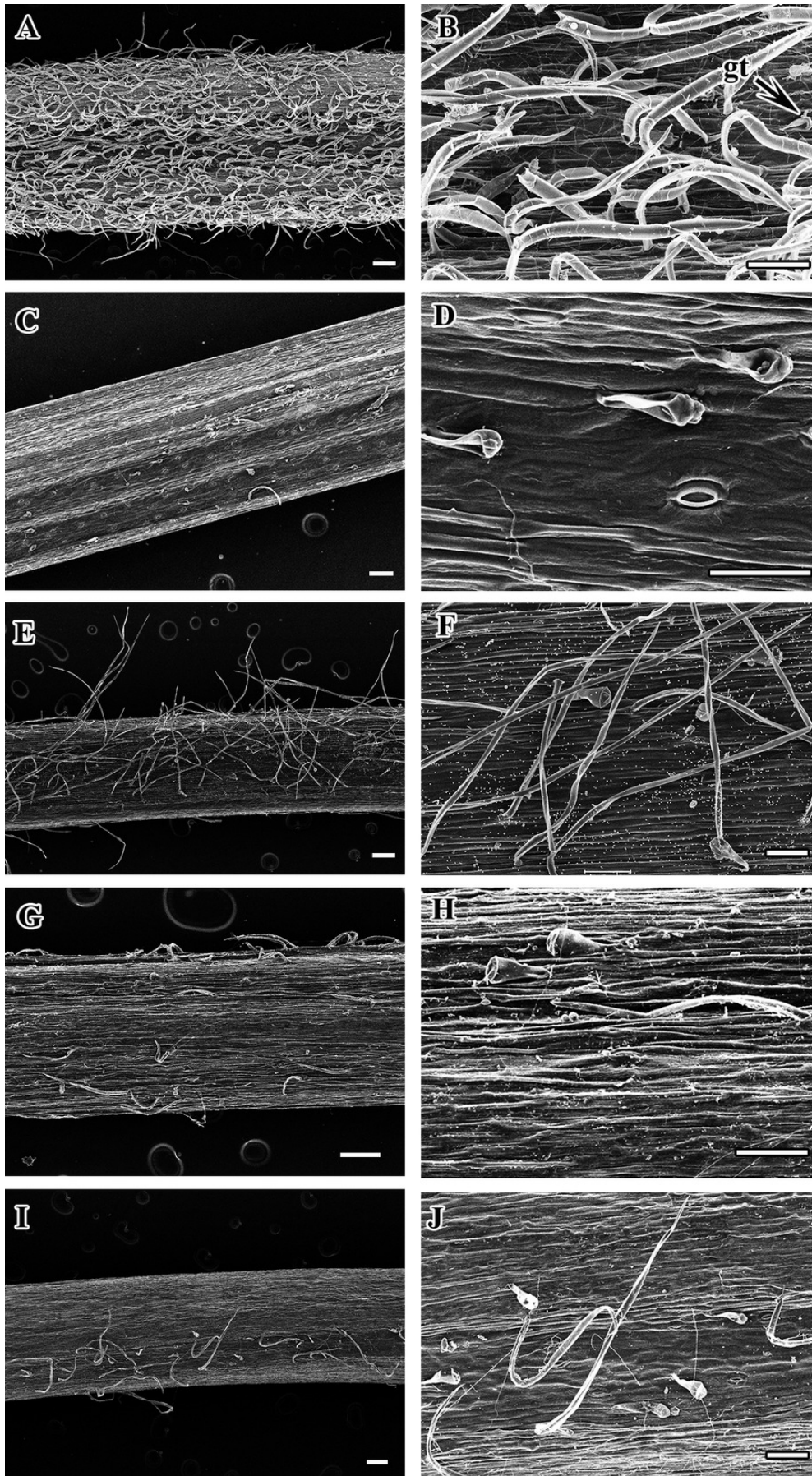
Figure 3

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).
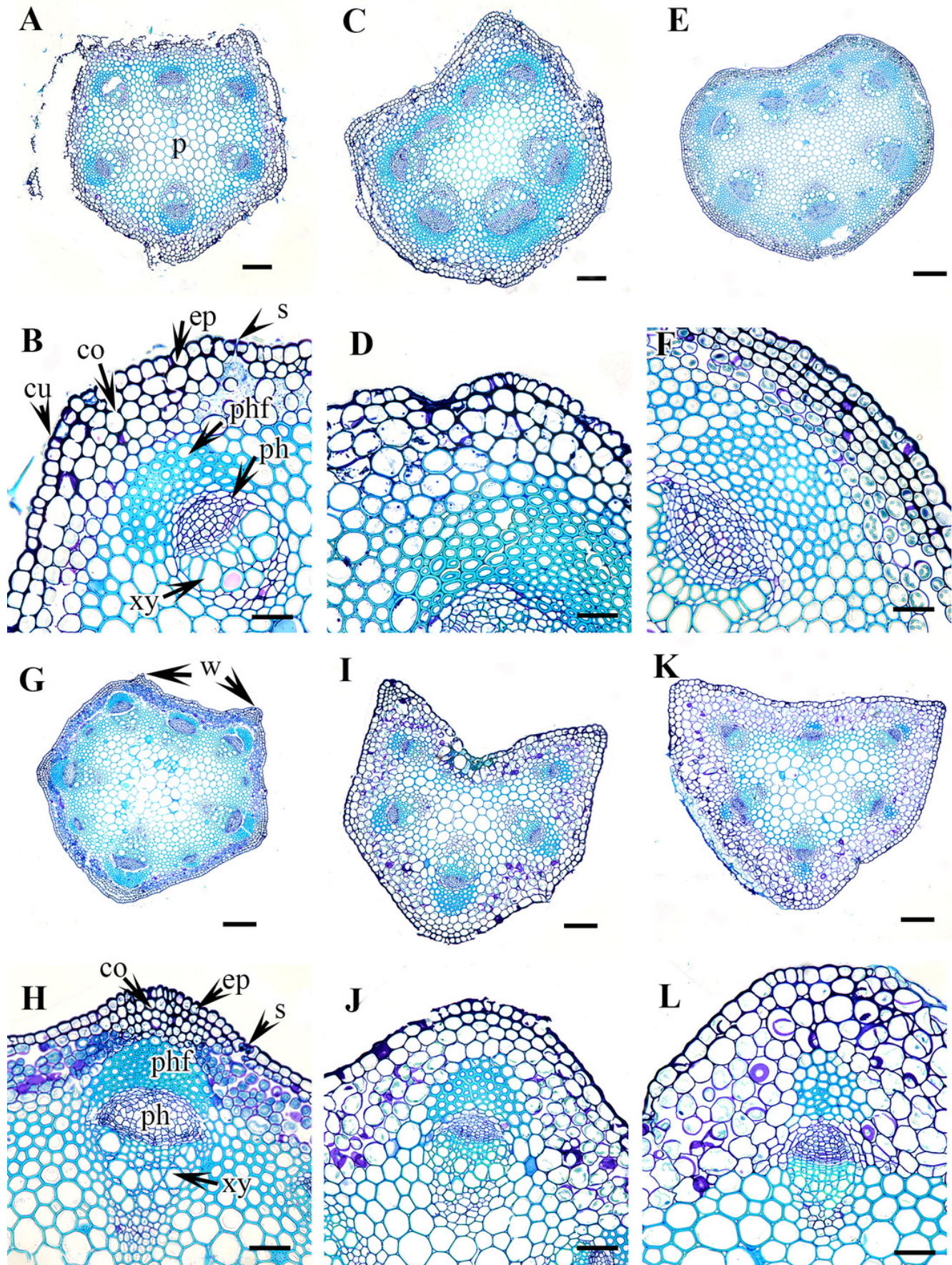
Figure 4

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).
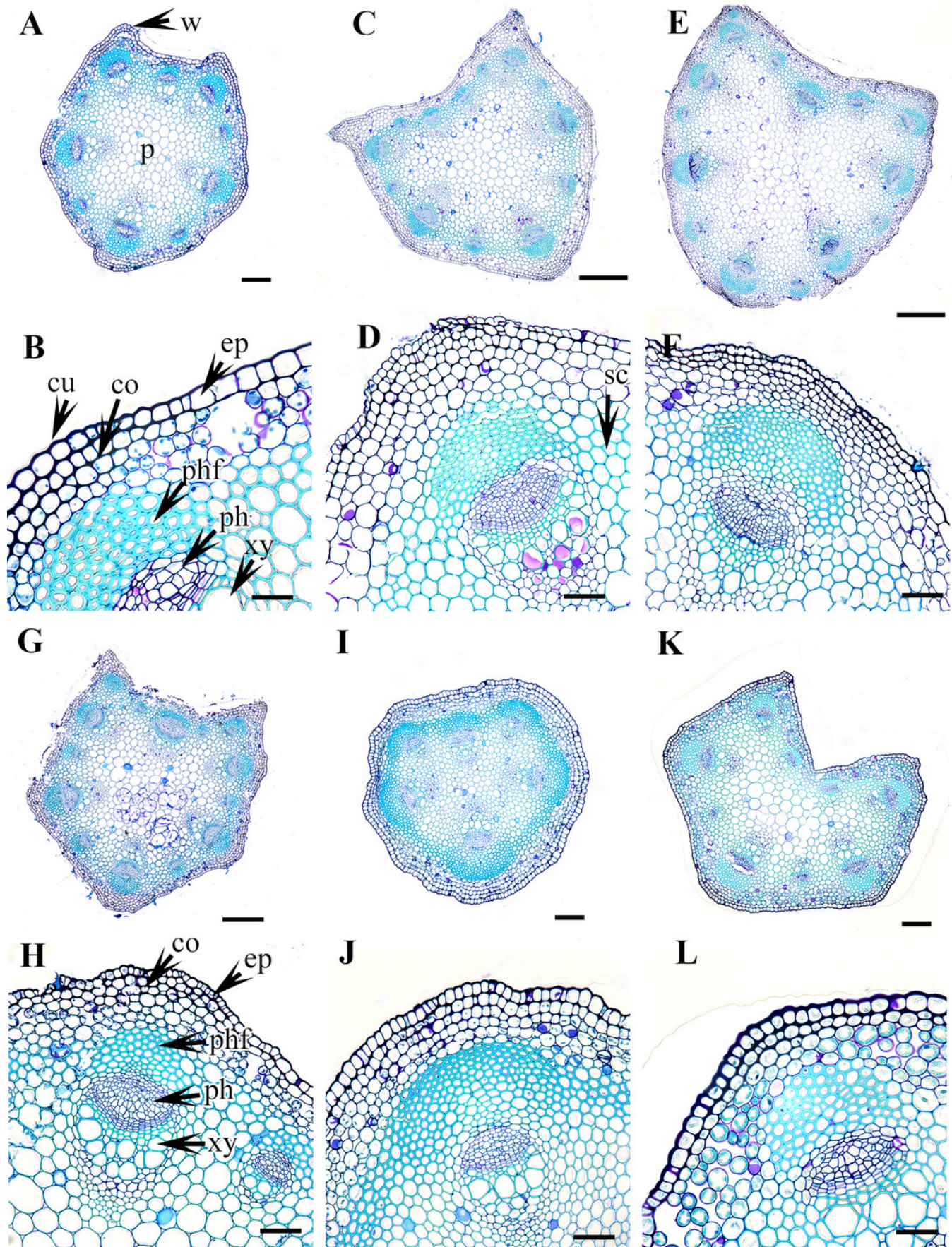
Figure 5

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).
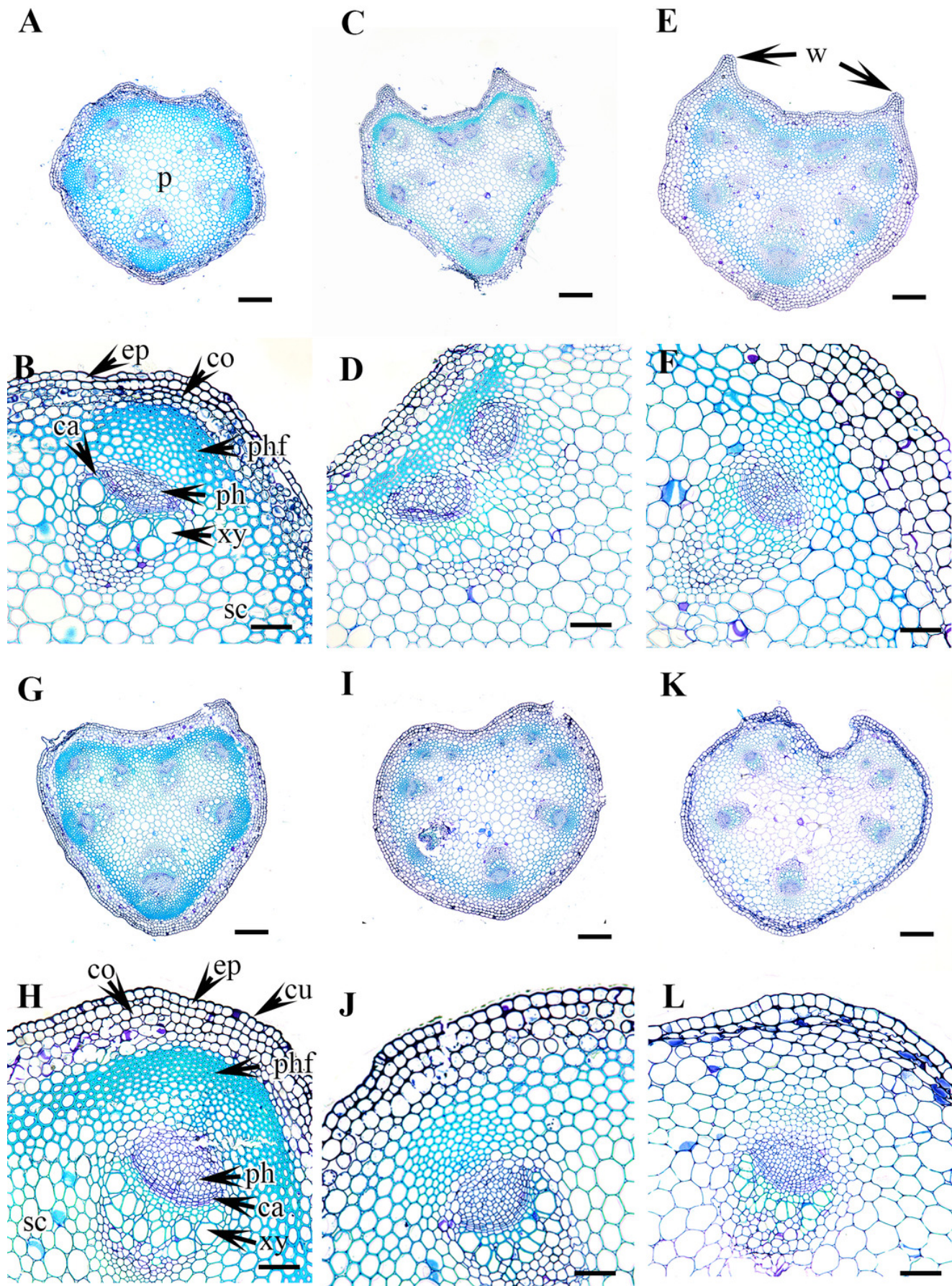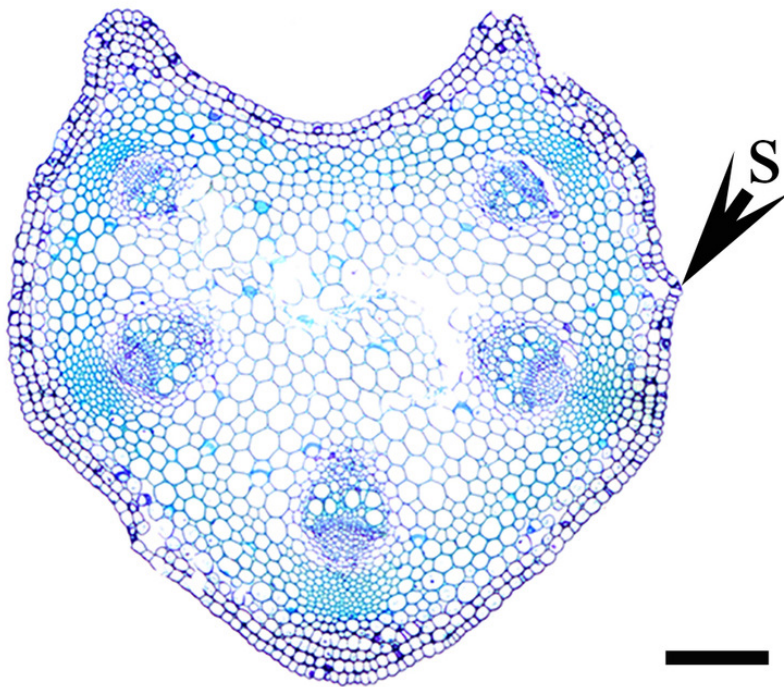
Figure 6

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).

A

S
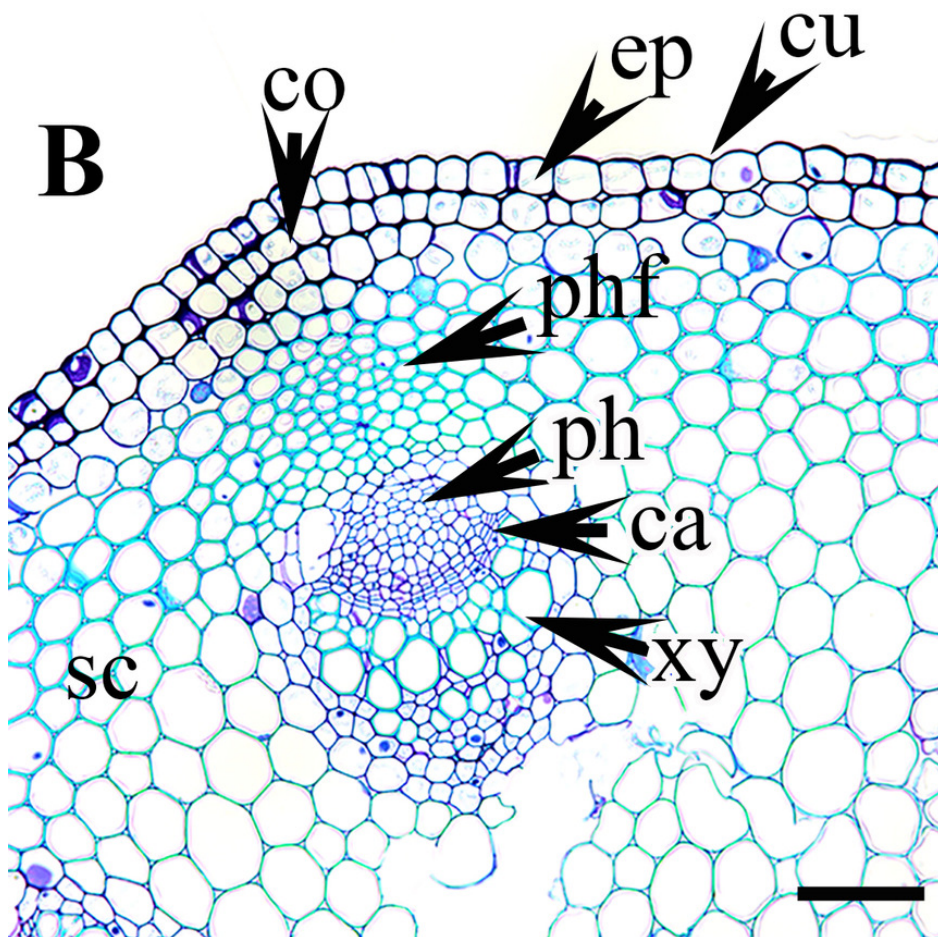
B

co ep cu

phf

ph

ca

sc xy

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.
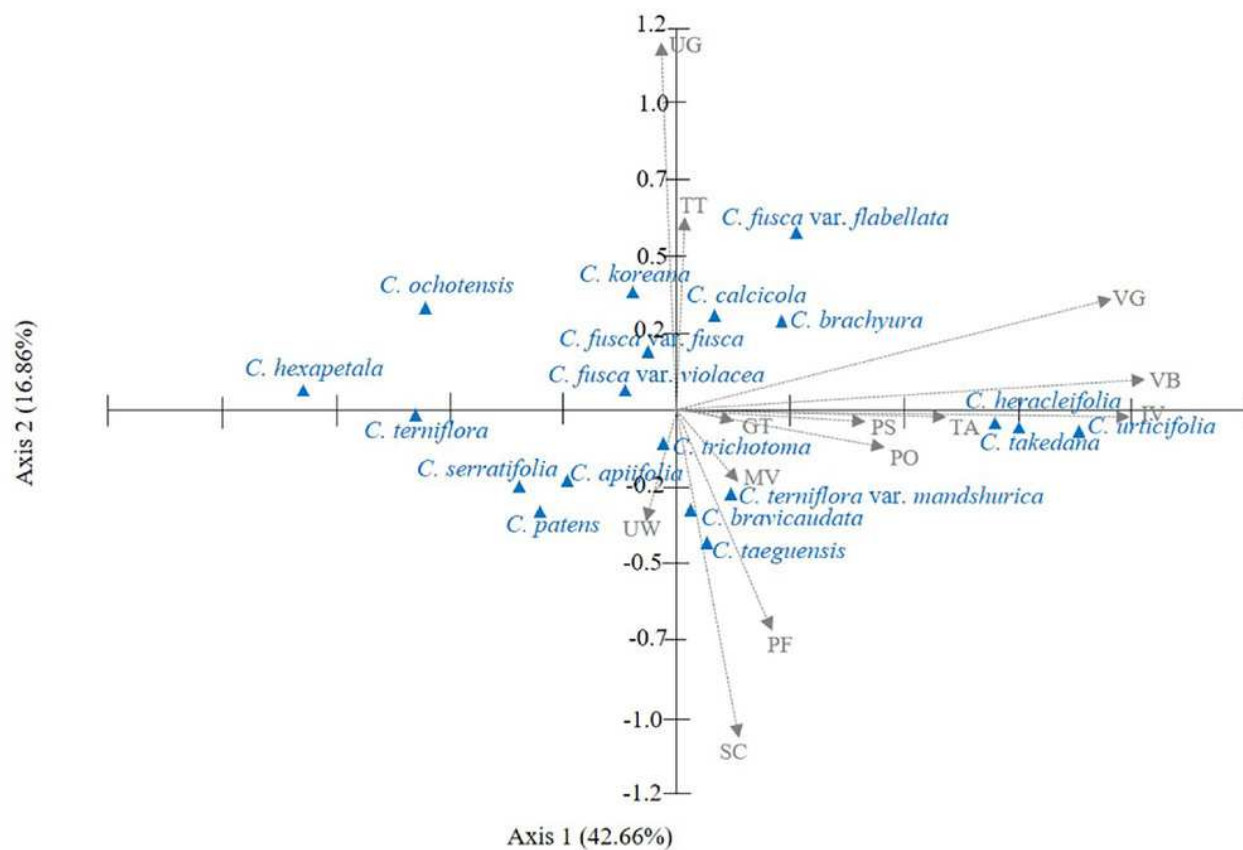
Figure 8

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

C. hexapetala
C. serratifolia
C. heracleifolia
C. takedana
C. urticifolia
C. terniflora var. mandshurica
C. taeguensis
C. calcicola
C. ochotensis
C. fusca var. violacea
C. fusca var. fusca
C. koreana
C. fusca var. flabellata
C. brachyura
C. patens
C. trichotoma
C. bravicaudata
C. terniflora
C. apiifolia

0.4   0.5   0.6   0.7   0.8   0.9   1
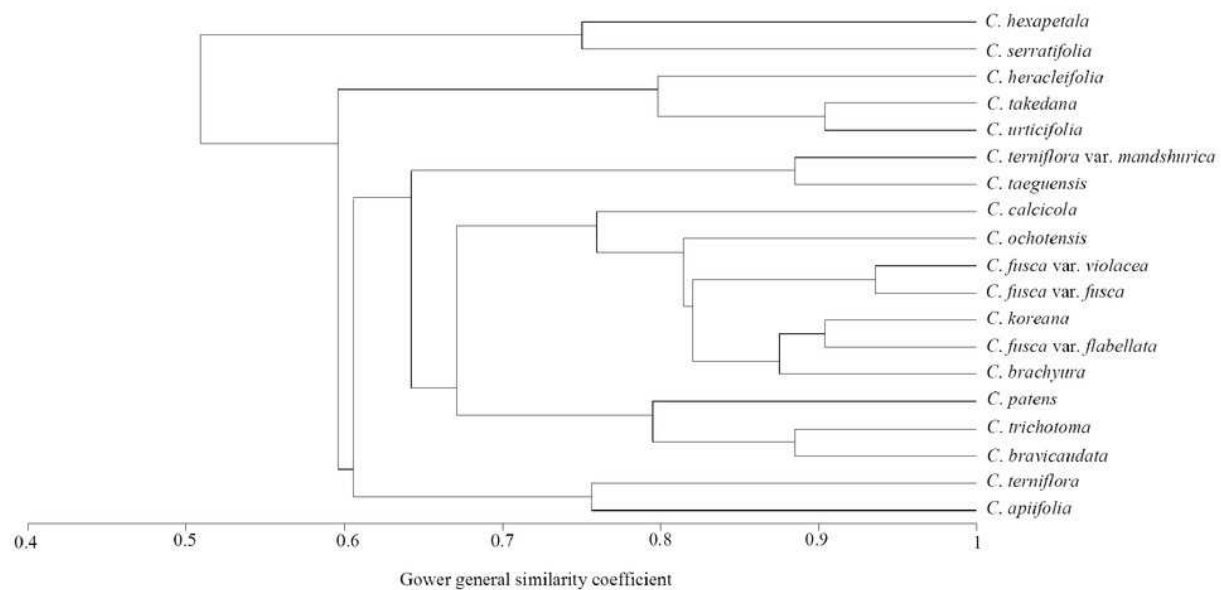
Gower general similarity coefficient

Table 1(on next page)

Name of taxa with voucher number and collection information.

1 **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

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Table 2(on next page)

Table 2

1 **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	U-shaped	Inconspicuous
C. trichotoma	Pilose	Flagelliform	Present	Medium	U-shaped	Inconspicuous
C. taeguensis	Subglabrous/pilose	Flagelliform	Present	Low	Pentagonal	Conspicuous
C. hexapetala	Glabrous	Absent	Absent	None	U-shaped /pentagonal	Conspicuous
C. terniflora	Pilose	Flagelliform	Present	Low	Semi-circular/ U-shaped	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	U-shaped	Inconspicuous
C. takedana	Villous	Unicate	Present	High	Pentagonal	Conspicuous
C. patens	Pilose	Flagelliform	Present	Medium	U-shaped	Inconspicuous
C. brachyura	Pilose	Flagelliform	Present	Medium (in upper surface groove)	Pentagonal	Conspicuous
C. serratifolia	Subglabrous/pilose	Flagelliform	Absent	Low	U-shaped /pentagonal	Conspicuous
C. fusca var. *fusca*	Pilose	Flagelliform	Present	Low	Pentagonal	Conspicuous
C. fusca var. *flabellata*	Pilose	Flagelliform	Present	Medium	U-shaped	Conspicuous
C. fusca var. *violacea*	Pilose	Flagelliform (in groove)	Present	Low	U-shaped	Conspicuous
C. calcicola	Subglabrous/pilose	Flagelliform	Absent	Low	U-shaped	Inconspicuous/conspicuous
C. koreana	Pilose	Flagelliform	Present	Medium	U-shaped	Conspicuous
C. ochotensis	Pilose	Flagelliform	Present	Medium	U-shaped	Conspicuous

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6 **Table 2. Cont.**

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

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

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