



The taxonomic significance of leaf anatomical characters in *Cussonia* and related genera (Araliaceae)

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The anatomy of the petiole, petiolule and leaf lamina of 20 *Cussonia* spp. is described and taxonomically evaluated. Comparisons are made with related taxa of Araliaceae: two Afro-Malagasy *Schefflera* spp., two Asian *Schefflera* spp. and *Seemannaralia gerrardii*. Transverse sections and epidermal peels (lamina only) were studied. The continuous collenchyma layer in the midrib, the absence of a hypodermis and papillate epidermal cells are characters shared between Afro-Malagasy *Schefflera* and *Seemannaralia*, and *Cussonia* and *Seemannaralia* share adaxial stomata, abaxial papillae on the lamina and similarly sized secretory canals in the petiole. Infrageneric groupings suggested by three types of petiolar vascular bundles correspond to one of the two existing infrageneric classification systems. Anatomical characters also contribute to a better understanding of species delimitation. The study has revealed six new generic apomorphies for *Cussonia*, namely the discontinuous, subepidermal collenchyma layer and the associated stomata in the petiole and petiolule, the adaxially invaginated and flattened ring of separate or connected bundles forming a narrow arc in the petiolules and the occurrence of arm palisade in the lamina. Leaf anatomical characters have greatly increased our understanding of relationships and circumscriptions at generic, infrageneric and specific levels in the taxa studied. © 2010 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2010, **164**, 246–263.

ADDITIONAL KEYWORDS: *Cussonia* subgenus *Paniculata* – *Cussonia* subgenus *Protocussonia* – lamina – petiole – petiolule – *Schefflera* – *Seemannaralia gerrardii* – subepidermal collenchyma – trichomes – vascular bundles.

INTRODUCTION

Cussonia Thunb. (Araliaceae, Apiales *sensu* APG III, 2009) is a genus of 22 species (one currently undescribed) found in grasslands, woodlands and forests of sub-Saharan Africa, the Arabian Peninsula (Yemen) and the Comoro Islands (Frodin & Govaerts, 2003). They are generally trees or shrubs, or occasionally subshrubs. The stems and underground parts are succulent. The leaves have relatively long petioles and are spirally arranged on the end of the branches or stems. They may be either simple (entire or lobed), or compound, with the leaflets simple, lobed or vertebrate (if vertebrate, then uni-digitate, bi-digitate or multi-digitate). The inflorescences are spikes,

racemes or umbels, which are borne terminally on the branches. The leaves usually die off before or during the formation of the inflorescence. The calyx is almost completely lost and the petals are fleshy and senesce early. There is no definite flowering season; plants flower sporadically, apparently in response to environmental conditions (mainly rainfall and temperature). The flowers are sessile or shortly stalked and subtended by one to three bracts. The fruits are fleshy and almost round or subglobose (Cannon, 1970, 1978; Strey, 1973, 1981; Bamps, 1974a,b; Reyneke, 1981, 1982). Previous studies of *Cussonia* (Harms, 1914; Strey, 1973, 1981; Bamps, 1974a,b; Reyneke, 1981, 1982) have shown it to be taxonomically complex and morphological characters to be variable and often confusing when used to distinguish species.

Leaf anatomical characters of Araliaceae, such as petiolar anatomy, have been used by various authors

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for taxonomic purposes (review; Ostroumova & Oskolski, 2010). Grushvitzky & Skvortsova (1969) found the structure of the stomata and epidermis of the leaf to be taxonomically useful in their studies of the Asian members of the genus *Schefflera* J.R.Forst. & G.Forst. In another study, the distribution pattern of the palisade parenchyma and the shape of the vascular tissue in the midrib aided in differentiating between *Panax japonicus* C.A.Mey., *Panax major* (Burk.) Ting., *Panax pseudo-ginseng* Wall. and *Panax trifolius* L. (Grushvitzky *et al.*, 1975). The taxonomic rank of various species of *Aralia* L. was determined on the basis of the type of petiolar medullary vascular bundles (Viguier, 1906). Hà Thi Dung, Grushvitzky & Skvortsova (1975) found that the structure of the petiolar vascular bundles supported the assignment of *Pentapanax fragrans* (D.Don) T.D.Ha var. *longipedunculatus* (N.S.Bui) T.D.Ha to a variety of *P. fragrans*, rather than to *Panax verticillatus* Dunn [= *Aralia* L. (Wen, 1993)]. Apart from these examples, leaf anatomical characters have been largely ignored when investigating the phylogeny of the family (Ostroumova & Oskolski, 2010). Little information is available on the anatomy of African Araliaceae, not to mention comparative studies of the genera. Solereder (1908) and Metcalfe & Chalk (1950), in their monumental works, included only a single representative of *Cussonia* and none of the Afro-Malagasy species of *Schefflera*. Viguier (1906) investigated the anatomy of *Cussonia*, but almost all of the species studied are now considered to be part of *Schefflera*; for example, *S. racemosa* Harms and *S. vantsilana* (Baker) Bernardi. *Schefflera* was shown to be polyphyletic, with the Afro-Malagasy and Asian species forming two out of five main lineages (Plunkett *et al.*, 2005). In their revision of the genus *Seemannaralia*, Burt & Dickison (1975) included a detailed anatomical study of the juvenile root, stem, wood, node, leaf and flower (vasculature). Because of a lack of data on other taxa, they were only able to compare and taxonomically evaluate their findings in relation to those of Metcalfe & Chalk (1950) and Rodriguez (1957).

Various authors have speculated that the monotypic *Seemannaralia* and/or Afro-Malagasy *Schefflera* are close relatives of *Cussonia*, especially as *Seemannaralia gerrardii* (Seem.) R.Vig and *Schefflera umbellifera* (Sond.) Baill. were formerly included in *Cussonia* (Viguier, 1906; Harms, 1914; Strey, 1973, 1981; Burt & Dickison, 1975; Reyneke, 1981; Plunkett, Wen & Lowry, 2004). As these taxa display morphological similarities to *Cussonia*, such as leaves borne on the end of branches, a reduction in leaf complexity in the developing inflorescence and an inflorescence carried on terminal branches or stems, it seems clear that a leaf anatomical study should include members of

these two genera. To allow an even broader evaluation, we have also included two members of the Asian palmate clade of *Schefflera*, with the expectation that their leaf anatomy may prove to be distinct from that of the Afro-Malagasy clade.

Over the years, a few infrageneric divisions of *Cussonia* have been attempted. Harms (1898) recognized two sections, namely *Eucussonia* Harms [= *Cussonia*], which included all the racemose or spicate species, and *Neocussonia* Harms, comprising all the umbellate species. All the species currently accepted as belonging to *Cussonia* were included in *Eucussonia*, while *Cussonia gerrardii* Seem. (now *Seemannaralia gerrardii*) and *Cussonia umbellifera* Sond. (now *Schefflera umbellifera*) were included in *Neocussonia*. Strey (1973) divided the genus into three subgenera, namely *Cussonia*, *Paniculata* Strey and *Protocussonia* Strey, based on leaf and inflorescence morphology. Subgenus *Protocussonia* is circumscribed as having simple, palmately lobed leaves, a panicle of umbels, compressed unilocular fruits and an absence of bracts. Subgenus *Paniculata* is characterized by palmately compound leaves with coarsely dentate to pinnatifid or sometimes entire leaflets and fruits, which are small globose berries with a single bract. Palmately compound leaves with entire or pinnatifid to palmatifid leaflets and rounded fruits with a single bract are diagnostic for subgenus *Cussonia*. Section *Capitata* Strey (of subgenus *Cussonia*) has single umbels, racemes or spikes, whereas section *Cussonia* has once-compound umbels of spikes. Reyneke (1981, 1982) proposed some changes to Strey's (1973) system, including elevation of section *Capitata* Strey to a subgenus and the creation of two sections within subgenus *Capitata* (Strey) Reyneke, namely *Sessiliflora* Reyneke and *Pedicellata* Reyneke. Reyneke (1981, 1982) placed more emphasis on the inflorescence morphology. Subgenus *Capitata* was circumscribed as having simple umbels, section *Sessiliflora* by sessile flowers, and section *Pedicellata* has stalked flowers. Species with once-compound umbels, raceme-like spikes or double racemes fall within the circumscription of subgenus *Paniculata*. Subgenus *Cussonia* contains all the species having once-compound umbels terminating in spikes, short internodes and cuneate fruits. Simple uni-digitate leaves, once-compound umbels, well developed internodes, spherical fruits and short flowers characterise subgenus *Protocussonia*. This differs from Strey's (1973, 1981) diagnoses: subgenus *Protocussonia* with palmately compound leaves; subgenus *Paniculata* with paniculate spikes and palmate leaves; subgenus *Cussonia* with palmate to bi-digitate leaves. These two systems were unfortunately based only on the southern African members of the genus.

In this study, the taxonomic value of leaf anatomical characters is investigated. The anatomy of the

petiole, petiolules and lamina, including the midrib, is described and characters of taxonomic value are identified. Leaf structure is explored and interpreted in relation to existing hypotheses of generic and intergeneric relationships and to species circumscriptions in *Cussonia* and its putative relatives. The present study is the first comparative leaf anatomical study of *Cussonia* spp. and is based on samples from 20 of the 22 known species. The monotypic *Seemannaralia* and four species of *Schefflera* were included for comparative purposes.

MATERIAL AND METHODS

Material was obtained from herbarium specimens or fresh leaves preserved in ethanol : acetic acid : formaldehyde : water (FAA), 2:1:10:7 (Sass, 1958) and is listed in Table 1. The only two *Cussonia* spp. not studied were *C. jatrophoides* Hutch. & E.A. Bruce and *C. ostinii* Chiov. because of unavailability of material. The petiole and primary leaflet petiolules (if available) were studied by removing material halfway between the point of attachment and the start of the lamina or petiolules. Small portions were cut from the midrib area of the lamina, approximately one half of the distance between the junction with the petiolule and the leaf apex. Similarly, other sections of the lamina were cut at the same distance from the petiolules and approximately halfway between the midrib and the margin. At least two samples of each species were studied. For convenience, in the present anatomical investigation, the terms 'lamina' and 'leaf blade' are used to indicate the area excluding the midrib area, although we are aware that the midrib is part of the lamina. In addition, in this study the term stoma is used to include not only the opening in the epidermis bordered by two guard cells but also any associated epidermal cells that may be ontogenetically and/or physiologically related to the guard cells.

Dry material was first rehydrated before placing in FAA for at least 24 h. All material was then dehydrated, infiltrated and embedded in glycol methacrylate (GMA) according to a slightly modified method of Feder & O'Brien (1968). This modification involves infiltrating the material for a longer period (7 days) before placing in gelatin capsules. Transverse sections, 3–5 µm thick, were cut and stained according to the periodic acid-Schiff's/toluidine blue (PAS/TB) procedure (Feder & O'Brien, 1968). Using this staining method, the druse crystals in crystal-containing cells are usually dissolved by the periodic acid, leaving empty cells (here referred to as 'ghost' cells). Slides were therefore studied before and after staining. They were then mounted with entellan.

To study the epidermis, and especially the distribution, abundance and type of stomata, epidermal

and/or cuticular peels of the upper and lower leaf surfaces were prepared using Jeffrey's Fluid (Sass, 1958). These were rinsed, stained with toluidine blue and mounted in glycerol.

Digital photographs of all the slides were taken with a Leitz Wetzlar compound microscope and JVC KY-F1030 digital camera and prepared for printing with Adobe Photoshop CS version 8 (Adobe Systems Inc., San Jose, CA, USA).

RESULTS

Descriptions of all parts of the leaf (petiole, petiolule, lamina and midrib) are given below. These are based on transverse sections, except for the epidermal cells of the lamina and midrib, which were also studied in surface view. Authors of plant names below are given in Table 1.

THE PETIOLE (Figs 1, 2)

Outline round to oval, not ribbed or ribbed [*Cussonia* sp. nov., *C. arenicola*, *C. gamtoosensis*, *C. paniculata*, *C. spicata* (De Villiers *et al.* 65), *C. thyrsoflora*, *C. zuluensis*, Fig. 1A]. Cuticle thick (2–7 µm) or thin (*c.* 1 µm; *C. bancoensis*, *C. brieyi*, *C. sphaerocephala*, *C. thyrsoflora*). Trichomes absent. Epidermal cells square to rounded or oval (Fig. 1E), rarely papillate (*C. transvaalensis*); outer periclinal cell walls thickened (Fig. 1E) and with surface striae present [*C. gamtoosensis*, *C. sphaerocephala*, *C. spicata* (excluding De Villiers & De Villiers 56; De Villiers *et al.* 65, 70), *C. transvaalensis*] or absent; stomata only associated with subepidermal chlorenchyma (Fig. 1E), level with the surface or sunken (*C. gamtoosensis*, *C. transvaalensis*). Ground tissue of subepidermal chlorenchyma discontinuous, interrupted with collenchyma (Fig. 1A, E) or mostly continuous with patches of collenchyma (collenchyma only in ribs of *C. arenicola*); intercellular spaces large; collenchyma nearly always eight to 14 cell layers thick (six in *C. brieyi*), outer layers lamellar, inner layers angular; parenchyma cells without chloroplasts to interior of collenchyma/chlorenchyma. Vascular bundles arranged in one (*C. brieyi*, *C. holstii*, *C. thyrsoflora*), two or more concentric circles (Fig. 1A, B); peripheral bundles collateral (Figs 1A, 2A–C) or bicollateral; sclerenchyma cap, together with or without a complete or incomplete bundle sheath (Fig. 2A–C); phloem of each bundle as a flat arc or inverted 'U', connected or discrete (*C. brieyi*, *C. paniculata*, *C. transvaalensis*); xylem of each bundle as a flat arc or 'U'-shaped (Fig. 2C), connected (*C. angolensis*, *C. arborea*, *C. bancoensis*, *C. corbisieri*, *C. holstii*, *C. natalensis*, *C. zimmermannii*; Fig. 2A) or discrete (*C. arenicola*, *C. brieyi*, *C. gamtoosensis*,

Table 1. Taxa sampled for the leaf anatomical study of *Cussonia* and some related genera. The taxa names and authority abbreviations are according to Brummitt & Powell (1992) and the herbaria abbreviations follow Holmgren, Holmgren & Barnett (1990). Transverse sections were made of the petiole (P), petiolules (Pt) and lamina (L). Epidermal peels (Ep) were made of the lamina to study the surface view

Taxon	Voucher specimen(s)
<i>Cussonia angolensis</i> (Seem.) Hiern.	<i>Dechamps, Murta and Da Silva 1023</i> , BR (P, Pt, L); <i>Estes 54c</i> , PRE (P, Pt, L)
<i>C. arborea</i> Hochst. ex. A.Rich.	<i>Angus 1474</i> , PRE (L); <i>Coates Palgrave s.n.</i> , JRAU (P, Pt, L); <i>Dümmer 1375</i> , PRE (Ep, P, L); <i>Hornby 2435</i> , PRE (P, L); <i>Jansen 7952</i> , PRE (Ep, P, Pt, L); <i>Jansen 7980</i> , PRE (P, Pt); <i>Santos and Henriques 392</i> , PRE (L); <i>Torre and Correira 14947</i> , PRE (L); <i>Williams 120</i> , PRE (P, Pt, L)
<i>C. arenicola</i> Strey	<i>De Villiers and Oskolski 104</i> , JRAU (P, Pt, L); <i>De Villiers, Tilney and Oskolski 78</i> , JRAU (Ep, P, Pt, L); <i>79</i> , JRAU (P, Pt, L); <i>80</i> , JRAU (L); <i>103</i> , JRAU (L); <i>Brenan et al., 713</i> , PRE (P, Pt, L)
<i>C. bancoensis</i> Aubrév. & Pellegr.	<i>Enti R850</i> , PRE (Ep, P, Pt, L); <i>Hall 2777</i> , BR (P, Pt, L)
<i>C. brieiyi</i> De Wild.	<i>Bamps, Martins and Da Silva 4230</i> , BR (P, Pt, L); <i>Toussaint 433</i> , BR (P, Pt, L)
<i>C. corbisieri</i> De Wild.	<i>Bingham s.n.</i> , BR (P, Pt, L); <i>Brenan 8374</i> , PRE (Ep, P, Pt, L); <i>Symoens 14126</i> , BR (P, Pt, L)
<i>C. gamtoosensis</i> Strey	<i>De Villiers, De Villiers, Magee and Le Roux 20</i> , JRAU (P, Pt, L); <i>De Villiers 51</i> , JRAU (Ep, P, Pt, L); <i>117</i> , JRAU (P, Pt, L)
<i>C. holstii</i> Harms ex Engl.	<i>Greenway and Kanuri 12129</i> , PRE (Ep, P, Pt, L); <i>Greenway, Turner and Harvey 10213</i> , PRE (Ep, P, Pt, L); <i>Perdue et al. 9103</i> , PRE (L); <i>Polhill 140</i> , PRE (L)
<i>C. natalensis</i> Sond.	<i>De Villiers, Tilney and Oskolski 72</i> , JRAU (Ep, P, Pt, L); <i>Norrgram 434</i> , PRE (P, Pt, L); <i>Roberts 2627</i> , PRE (L)
<i>C. nicholsonii</i> Strey	<i>De Villiers, Tilney and Oskolski 77</i> , JRAU (Ep, P, Pt, L); <i>De Villiers, Oskolski and Van Wyk 98</i> , JRAU (P, Pt, L)
<i>C. paniculata</i> Eckl. & Zeyh. subsp. <i>paniculata</i>	<i>De Villiers and De Villiers 47</i> , JRAU (P, Pt, L); <i>De Villiers and Van Wyk 110</i> , JRAU (P, Pt, L)
<i>C. paniculata</i> subsp. <i>sinuata</i> (Reyneke & Kok) De Winter	<i>De Villiers 2</i> , JRAU (Ep, P, Pt, L); <i>De Villiers and De Villiers 63</i> , JRAU (P, Pt, L); <i>De Villiers, Tilney and Oskolski 82</i> , JRAU (Ep, P, Pt, L); <i>De Villiers and O'Brien 83</i> , JRAU (Ep, P, Pt, L); <i>De Villiers 85</i> , JRAU (P, Pt, L); <i>Codd s.n.</i> , PRE (L)
<i>Cussonia</i> sp. nov.	<i>Abbott 8159</i> , PRU (P, Pt, L); <i>8834</i> , PRU (P, Pt, L); <i>De Villiers, Van Wyk and Abbott 113</i> , JRAU (P, Pt, L); <i>114</i> , JRAU (P, Pt, L); <i>115</i> , JRAU (P, Pt, L)
<i>C. sessilis</i> Lebrun	<i>Liben 3905</i> , BR (P, Pt, L); <i>Thiebaud 271</i> , BR (P, Pt, L)
<i>C. sphaerocephala</i> Strey	<i>De Villiers, Tilney and Oskolski 76</i> , JRAU (P, Pt, L); <i>De Villiers and Oskolski 81</i> , JRAU (Ep, P, Pt, L); <i>87</i> , JRAU (P, Pt, L); <i>100</i> , JRAU (L); <i>101</i> , JRAU (L)
<i>C. spicata</i> Thunb.	<i>De Villiers 1</i> , JRAU (Ep, P, Pt, L); <i>De Villiers and De Villiers 37</i> , JRAU (P, Pt, L); <i>56</i> , JRAU (P, L); <i>De Villiers 60</i> , JRAU (L); <i>De Villiers, Tilney and Oskolski 65</i> , JRAU (P, Pt, L); <i>67</i> , JRAU (Ep, P, Pt, L); <i>70</i> , JRAU (P, Pt, L); <i>73</i> , JRAU (P, Pt, L); <i>Kerfoot 3270</i> , PRE (P, L)
<i>C. thyrsoiflora</i> Thunb.	<i>De Villiers and De Villiers 4</i> , JRAU (Ep, P, Pt, L); <i>57</i> , JRAU (Ep, P, Pt, L); <i>De Villiers, Oskolski and Van Wyk 64</i> , JRAU (P, Pt, L)
<i>C. transvaalensis</i> W.F. Reyneke	<i>De Villiers 34</i> , JRAU (Ep, P, Pt, L); <i>De Villiers, De Villiers and De Villiers 58</i> , JRAU (Ep, P, Pt, L)
<i>C. zimmermannii</i> Harms	<i>Drummond and Hemsley 1198</i> , PRE (P, Pt, L); <i>Schlieben 5557</i> , PRE (Ep, P, Pt, L); <i>Semsei 4000</i> , PRE (Ep, P, Pt, L)
<i>C. zuluensis</i> Strey	<i>De Villiers and De Villiers 43</i> , JRAU (Ep, P, Pt, L); <i>De Villiers and Oskolski 92</i> , JRAU (P, Pt, L); <i>99</i> , JRAU (P, Pt, L); <i>Borle 196</i> , PRE (P)
<i>Schefflera actinophylla</i> (Endl.) Harms	<i>De Villiers, Tilney and Oskolski 68</i> , JRAU (Ep, P, Pt, L)
<i>Schefflera arboricola</i> (Hayata) Merr.	<i>De Villiers and Van Wyk 108</i> , JRAU (Ep, P, Pt, L)

Table 1. Continued

Taxon	Voucher specimen(s)
<i>S. barteri</i> (Seem.) Harms	<i>Leeuwenberg 4449</i> , PRE (P, Pt, L); <i>Magbagboela et al. 43</i> , PRE (Ep)
<i>S. umbellifera</i> (Sond.) Baill.	<i>De Villiers, Tilney and Oskolski 75</i> , JRAU (Ep, P, Pt, L); <i>102</i> , JRAU (P, Pt, L); <i>De Villiers and Van Wyk 109</i> , JRAU (P, Pt, L); <i>De Villiers, Abbott, Le Roux and Van Wyk 116</i> , JRAU (L)
<i>Seemannaralia gerrardii</i> (Seem.) R.Vig.	<i>De Villiers and De Villiers 41</i> , JRAU (Ep, P, Pt, L); <i>De Villiers, Oskolski and Van Wyk 97</i> , JRAU (Ep, P, Pt, L); <i>De Villiers and Oskolski 107</i> , JRAU (P, Pt, L); <i>Hoffman 75</i> , PRE (P, Pt, L); <i>Story 4002</i> , PRE (Ep, P, Pt, L)

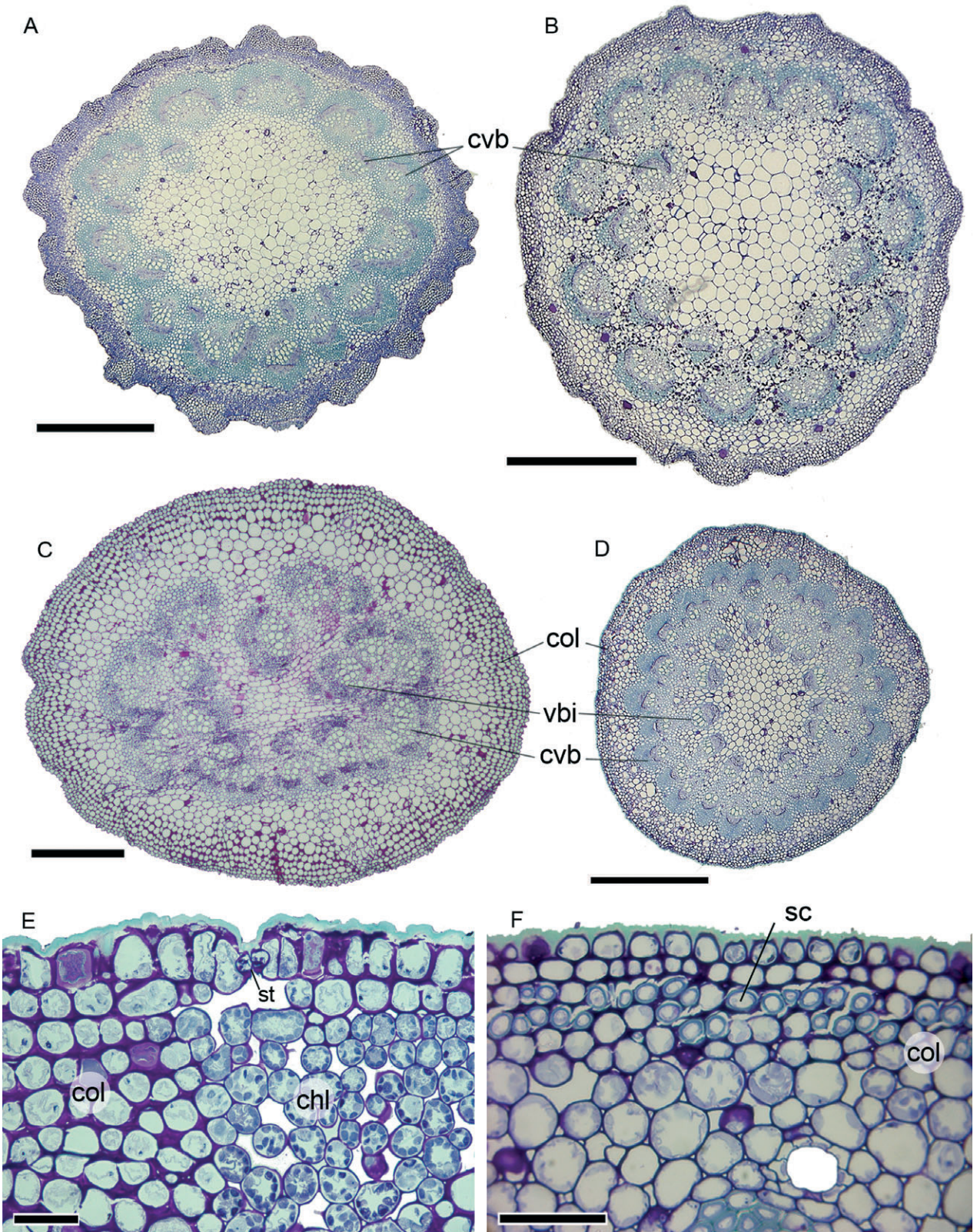
Figure 1. Petiole anatomy (in transverse section) of *Cussonia* and related genera. A, typical *Cussonia* petiole (*C. arenicola*; *De Villiers and Oskolski 104*) showing the oval or rounded shape, concentric rings of vascular bundles and collateral medullary vascular bundles (cvb). B, concentric rings of vascular bundles and collateral medullary vascular bundles in *Schefflera umbellifera* (*De Villiers et al. 75*). C, vascular tissue arranged as a dissected arc with invaginated ends, inverted medullary vascular bundles (vbi) and continuous collenchyma (col) in *Seemannaralia gerrardii* (*De Villiers et al. 97*). D, continuous collenchyma (col) and inverted medullary vascular bundles (vbi) in *Schefflera actinophylla* (*De Villiers et al. 68*). E, stoma (st) associated with the chlorenchyma (chl) and the interruption of the collenchyma (col) by chlorenchyma (chl) in *C. gamtoosensis* (*De Villiers et al. 20*). F, subepidermal collenchyma (col) continuous and a lack of stomata in *Schefflera actinophylla* (*De Villiers et al. 68*). Scale bars, 0.8 mm (A, C); 1 mm (B); 0.5 mm (C); 0.07 mm (E).

C. nicholsonii, *Cussonia* sp. nov., *C. sessilis*, *C. sphaerocephala*, *C. spicata*, *C. thyrsiflora*, *C. transvaalensis*, *C. zuluensis*; Fig. 2B); medullary bundles collateral (*C. arenicola*, *C. gamtoosensis*, *C. natalensis*; Fig. 1A), perpendicular to surface (*C. arenicola*, *C. sphaerocephala*, *C. transvaalensis*) or rarely inverse (*Cussonia* sp. nov., *C. gamtoosensis*, *C. sphaerocephala*, *C. transvaalensis*); phloem of each bundle as a flat arc or 'U'-shaped (*C. paniculata*); xylem of each bundle as a flat arc (*C. sphaerocephala*), semi-amphivasal (*Cussonia* sp. nov., *C. paniculata*; rarely *C. gamtoosensis*, *C. spicata*, *C. transvaalensis*, *C. zuluensis*), amphivasal (*C. paniculata*, rarely *Cussonia* sp. nov., *C. transvaalensis*, Fig. 2D) or rarely amphicribal (*C. paniculata*, rarely *Cussonia* sp. nov., *C. transvaalensis*; Fig. 2E). Resin ducts in ground tissue present, large (c. 90 µm in diameter) or absent (*C. briei*); in sclerenchyma or phloem (*C. angolensis*, *C. arborea*, *C. bancoensis*, *C. corbisieri*, *C. holstii*, *C. natalensis*, *C. zimmermannii*; Fig. 2A), small (c. 45 µm in diameter) or absent; with single layer of epithelial cells. Crystals druse, in parenchyma without chloroplasts.

THE PETIOLULE (Fig. 3)

Petiolule absent in *C. arborea* (*Angus 1474*; *Coates Palgrave s.n.*; *Jansen 7952*; *Jansen 7980*; *Santos & Henriques 392*; *Torre & Correira 14947*), *C. corbisieri*, *C. natalensis*. Outline round to oval (*C. arenicola*, *C. holstii*, *C. paniculata*) frequently adaxially flat-

tened (Fig. 3A); smooth, not ribbed. Epidermal cells round to oval, rarely papillate (Fig. 3C; entirely papillate in *C. holstii*), outer periclinal cell walls thickened (Fig. 3C–D) and with small (c. 2.5 µm) surface striae present (e.g. *C. sphaerocephala*; Fig. 3D) or absent (e.g. *C. arborea*); stomata only associated with subepidermal chlorenchyma, level with surface or sometimes sunken (*C. gamtoosensis*, *C. transvaalensis*; Fig. 7C). Ground tissue of subepidermal chlorenchyma discontinuous, interrupted with collenchyma (Fig. 3A); intercellular spaces large; collenchyma prominent in corners, nearly always 6–8 cell layers thick; outer layers lamellar, inner layers angular; parenchyma without chloroplasts to interior of collenchyma/chlorenchyma (Fig. 3A). Vascular bundles adaxially, flattened ring of more or less connected or dissected (*C. arenicola*, *C. briei*, *C. gamtoosensis*, *C. paniculata*, *C. thyrsiflora*, *C. transvaalensis*, *C. zuluensis*; Fig. 3A) bundles forming a narrow arc; peripheral bundles collateral, medullary bundles inverse, amphivasal (*C. paniculata*), semi-amphicribal (*C. nicholsonii*, *C. spicata*) or scattered (*C. nicholsonii*); sclerenchyma cap, with or without complete or incomplete sheath; phloem of each bundle connected (*C. holstii*, *Cussonia* sp. nov.) or dissected, xylem of each bundle connected. Resin ducts rarely present in collenchyma; present in chlorenchyma and parenchyma without chloroplasts, large (c. 80 µm in diameter), with single layer of epithelial cells. Crystals druse, in parenchyma without chloroplasts, in collenchyma.



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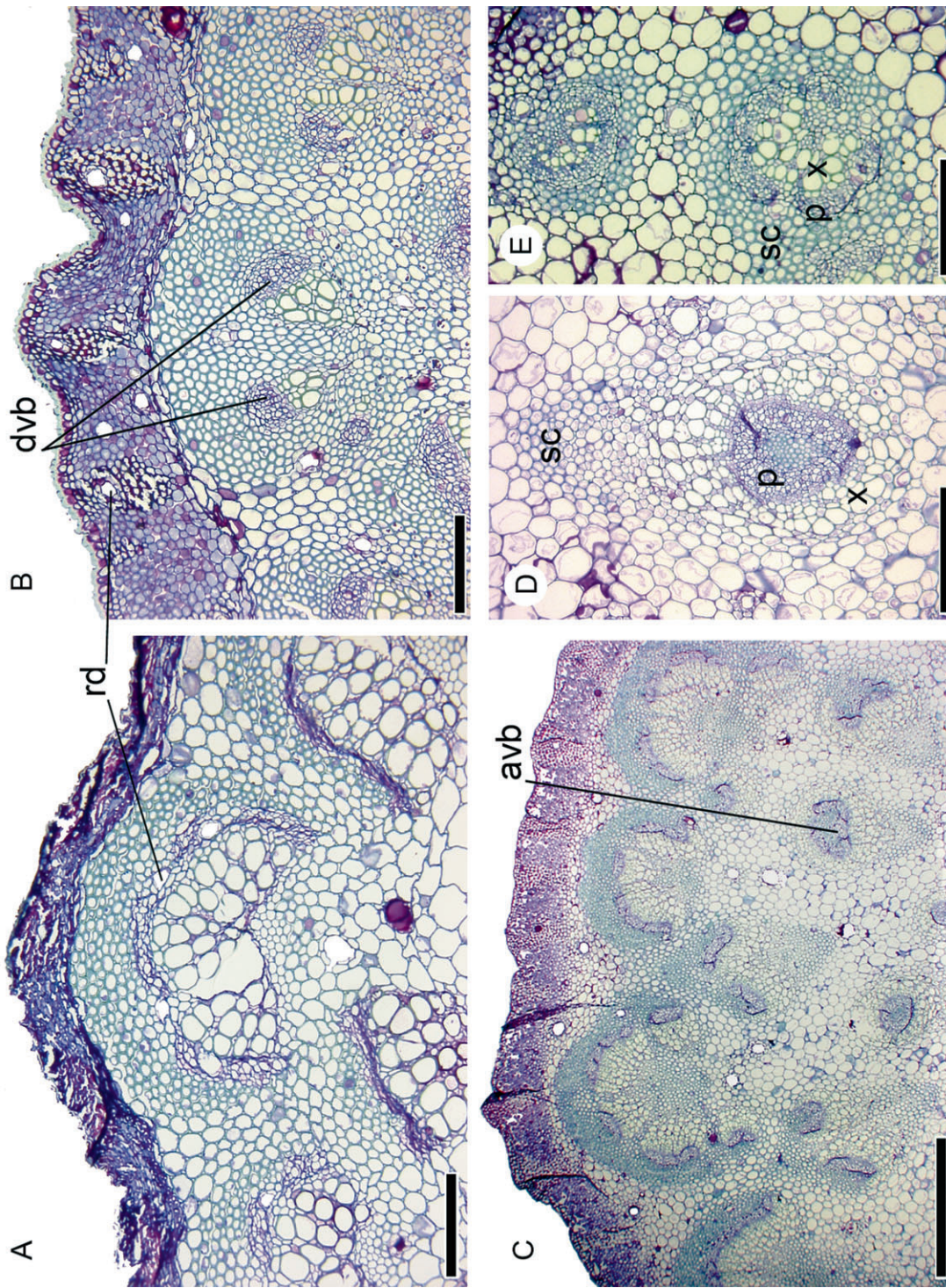


Figure 2. The different types of vascular bundles (in transverse section) in the petioles of *Cussonia* species (p, phloem; sc, sclerenchyma; x, xylem). A, *C. bancocensis* (Enri 1850) with collateral peripheral vascular bundles and the fused bundles connected by a sheath of sclerenchyma (rd) are present in the sclerenchyma or phloem. B, discrete peripheral vascular bundles (dvb) surrounded by a sheath of sclerenchyma in *C. gamtoosensis* (De Villiers 51). C, *C. paniculata* (De Villiers *et al.* 82) with the disconnected phloem parts of the peripheral vascular bundles not forming a continuous layer (as in A) and the abundance of semi-amphivasal and amphivasal (avb) vascular bundles. D, amphivasal vascular bundle in *C. paniculata* (De Villiers *et al.* 83). E, a rare amphicribal vascular bundle in *C. spicata* (De Villiers *et al.* 67). Scale bars, 0.2 mm (A, B, D, E); 0.8 mm (C).

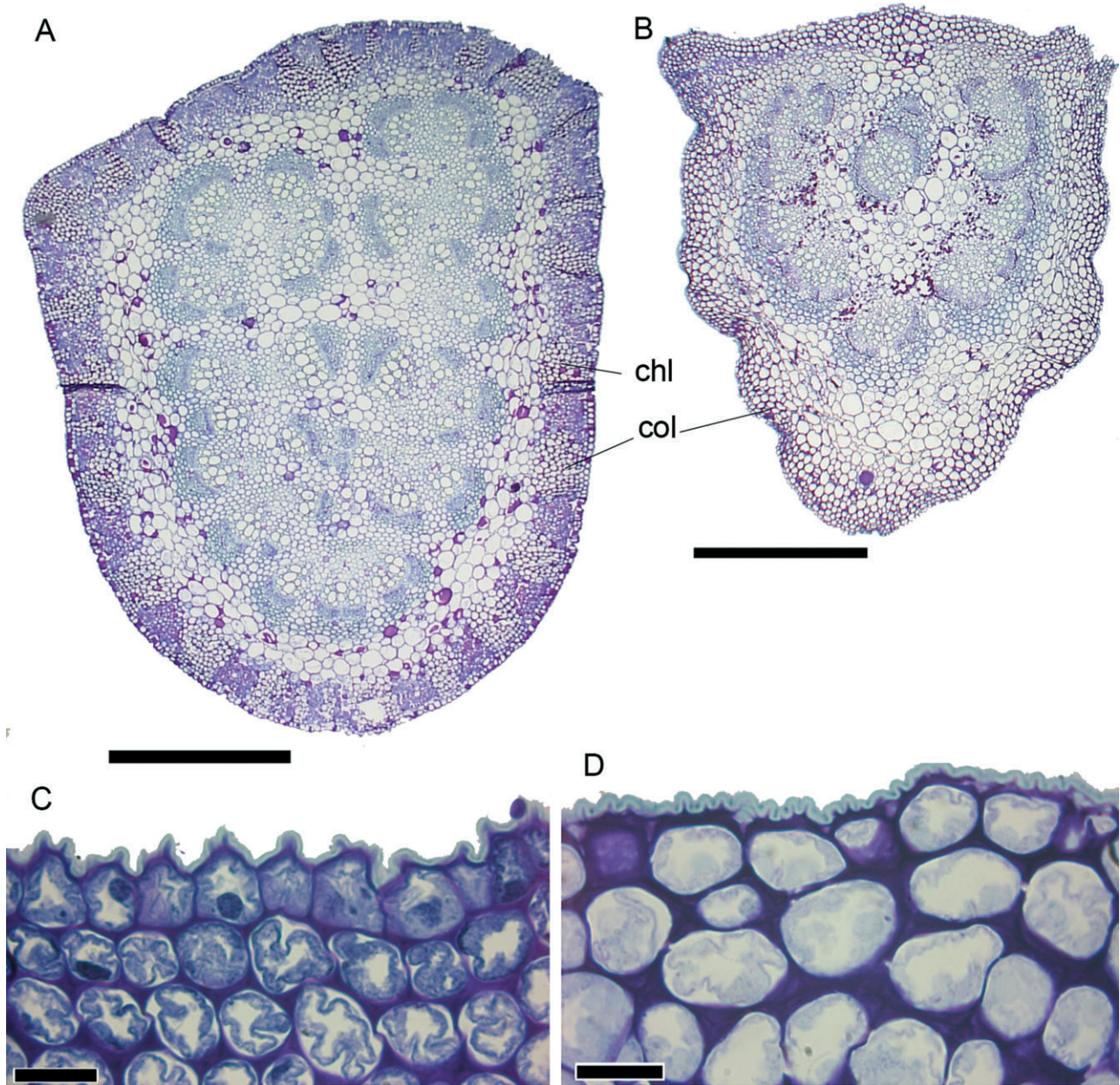


Figure 3. Petiolute anatomy (in transverse section) of *Cussonia* and related genera. A, discontinuous collenchyma (col) interrupted with chlorenchyma (chl), the vascular bundles are arranged in an invaginated ring of separate or connected bundles, forming a narrow arc in *C. transvaalensis* (De Villiers *et al.* 58). B, vascular bundles are in the form of a crescent with the concave side facing the upper surface of the petiolute, the tips are invaginated towards the centre and additional bundles are located at the tips of the strands in *Schefflera umbellifera* (De Villiers *et al.* 75). C, papillate epidermal cells in *C. spicata* (De Villiers 1), with surface striae (visible as small superficial projections on the outer cell wall). D, numerous and small surface striae on the epidermal cells in *C. sphaerocephala* (De Villiers & Oskolski 100). Scale bars, 1 mm (A); 0.8 mm (B); 0.02 mm (C, D).

THE LAMINA (Figs 4–6)

Bifacial, 360–420 μm thick. Cuticle smooth, abaxially thin (c. 1.8 μm), adaxially thicker (c. 3.7 μm). Trichomes absent (Fig. 5B–C) or, when present

(*C. arborea*, *C. corbisieri*; Fig. 4A–D), multicellular, unbranched, sparser adaxially, more common on veins (Figs 4A, 8C), but also rarely variably branched abaxially (*C. corbisieri*; Fig. 4D). Epidermal cells abaxially mostly square to oval, sometimes papillate

Figure 4. Trichomes of *Cussonia* and *Seemannaralia* on leaf surfaces (abaxial unless otherwise stated). A, *C. arborea* (Van Wyk BSA1039) showing the trichomes on the major and minor veins. B, unbranched multicellular trichomes of *C. arborea* (Van Wyk BSA1039). C, *C. corbisieri* (Bingham 6847) with an abundance of trichomes on the veins and lamina. D, unbranched and branched (arrow) multicellular trichomes of *C. corbisieri* (Bingham 6847). E, branched, multicellular trichomes of *S. gerrardii* (De Villiers & Oskolski 107). F, unbranched multicellular trichomes of *S. gerrardii* (De Villiers & Oskolski 107) on the adaxial surface. Scale bars, 500 µm (A, B); 1 mm (D, E, F).

[*C. arenicola*, *C. natalensis*, *C. spicata* (De Villiers et al. 60), *C. transvaalensis*; (Fig. 7A)] in transverse section, c. 15 µm long, square to polygonal in surface view (Fig. 5A–C); adaxially mostly rectangular to oval (Figs 6B, 7A), sometimes papillate (*C. corbisieri*, *C. paniculata* ssp. *sinuata*, *C. spicata*, *C. transvaalensis*; Fig. 7A) in transverse section, with sinuous anticlinal walls in surface view, c. 25 µm long; outer periclinal and anticlinal cell walls thickened (Fig. 7A–C); upper epidermal cells conspicuously larger than lower ones (Fig. 7A); hypostomatic (Fig. 5B–C) or amphistomatic [*C. arborea* (Hornby 2435), *C. corbisieri*, *C. gamtoosensis*, *C. transvaalensis*; Fig. 7A]; anomocytic (60–80%) or anisocytic and paracytic; level with surface (Fig. 7B) or sometimes sunken (*C. gamtoosensis*, *C. transvaalensis*; Fig. 7C). Hypodermal cells present adaxially (Figs 5D, 6B) or absent (*C. angolensis*, *C. transvaalensis*), sinuous in surface view (Fig. 5D); normally 1 (–3) cell layers thick. Mesophyll of arm palisade parenchyma (Fig. 6C), in 3–5 (–7) (*C. gamtoosensis*, *C. transvaalensis*) cell layers; spongy parenchyma approximately two-thirds of leaf thickness, intercellular spaces large, sclerenchyma forming incomplete sheaths around main bundles; bundle sheaths collenchymatous, mostly associated with larger veins, mainly adaxial (Fig. 7A), occasionally abaxial and adaxial. Vascular bundles collateral (Fig. 7A). Resin ducts common in mesophyll (in both palisade and spongy) and collenchyma of smaller veins. Crystals druse, in mesophyll, rarely in hypodermis.

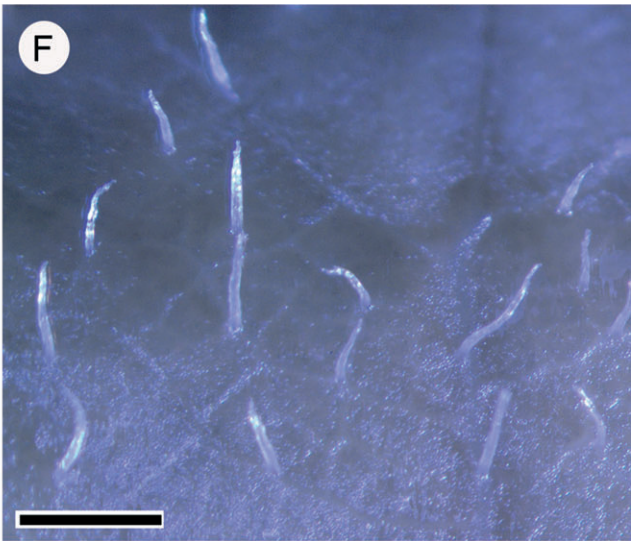
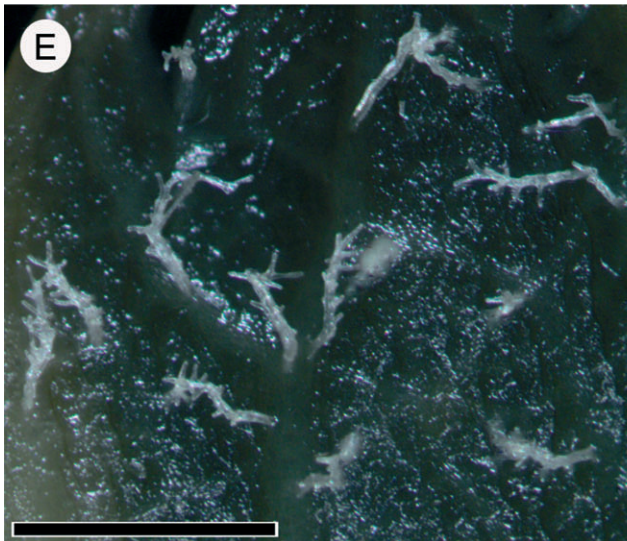
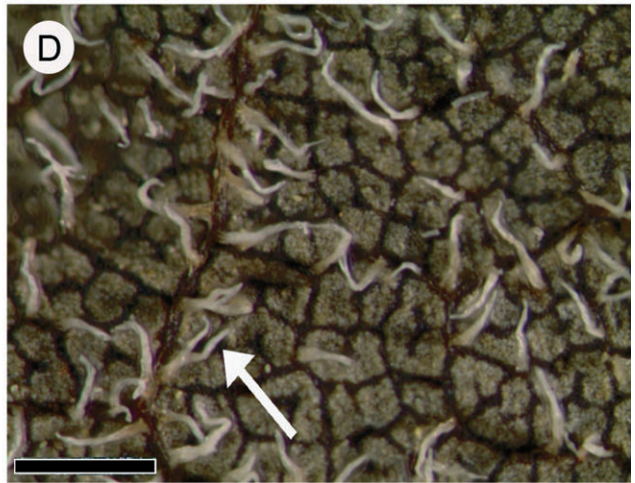
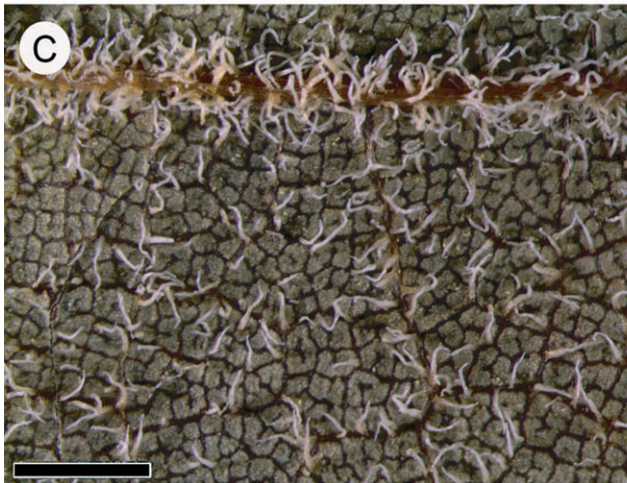
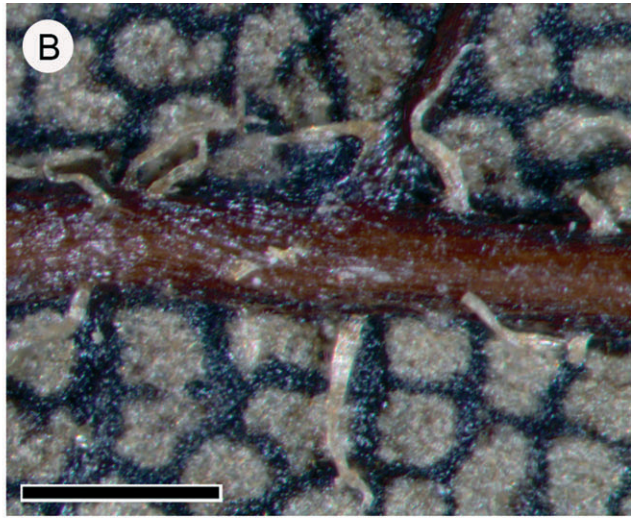
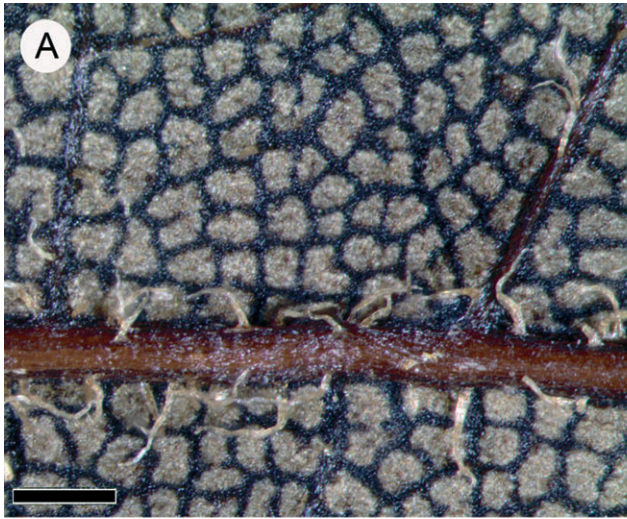
THE MIDRIB (Figs 5 and 8)

Conspicuously raised below and less conspicuously raised above, varying from more or less flat (*C. gamtoosensis*) to fairly raised, oval (*C. arborea*, *C. bancoensis*, *C. gamtoosensis*, *C. holstii*, *C. natalensis*, *C. paniculata*, *C. spicata*, *C. transvaalensis*, *C. zuluensis*; Fig. 8D, F) or ovate with an acuminate narrow end (*C. angolensis*, *C. arborea*, *C. arenicola*, *C. corbisieri*, *C. nicholsonii*, *C. sessilis*, *C. sphaerocephala*, *C. spicata*, *C. thyrsoflora*, *C. zimmermannii*; Fig. 8A–B). Cuticle abaxially thinner (c. 7.6 µm), adaxially thicker (c. 8.2 µm). Trichomes absent (Fig. 5C) or, when present (*C. arborea*, *C. corbisieri*; Fig. 4A–D), multicellular, unbranched, sparser adaxially, more common on veins, but also rarely variably branched

abaxially (*C. corbisieri*; Fig. 4D). Epidermal cells abaxially mostly round to oval or papillate [*C. arenicola*, *C. natalensis*, *C. spicata* (De Villiers, Tilney & Oskolski 60), *C. transvaalensis*]; adaxially mostly square to oval or papillate (*C. arenicola*, *C. zimmermannii*) in transverse section; elongated in surface view; abaxially conspicuously smaller (c. 13 µm in length) than adaxially; outer periclinal and anticlinal cell walls thickened; hypostomatic or amphistomatic (*C. angolensis*, *C. arborea*, *C. corbisieri*, *C. gamtoosensis*, *C. natalensis*, *C. nicholsonii*, *C. paniculata*, *C. spicata*, *C. transvaalensis*, *C. zuluensis*; Fig. 5E–F), mostly anomocytic (c. 90%), anisocytic or paracytic; level with surface or sunken (*C. gamtoosensis*, *C. transvaalensis*). Hypodermal cells rectangular to square, larger than epidermal cells, c. 70 µm long; 1–2 (–3) cell layers thick; extend into midrib, not continuous (Fig. 8A–F). Ground tissue of subepidermal collenchyma, collenchyma lamellar and/or angular; 6–10 cell layers thick, thicker adaxially (Fig. 8F); mesophyll of arm palisade parenchyma, not extending into midrib from leaf blade (*C. corbisieri*, *C. gamtoosensis*, *C. sessilis*, *C. zimmermannii*; Fig. 8C), present in the midrib only as a medial patch in adaxial collenchyma (*C. holstii*, *C. natalensis*, *C. nicholsonii*; Fig. 8D), extending a short distance into the midrib (*C. angolensis*, *C. arborea*, *C. arenicola*, *C. bancoensis*, *C. spicata*, *C. transvaalensis*; Fig. 8E), extending into the midrib and with two patches sub-medially in adaxial collenchyma (*C. paniculata*, *C. spicata*, *C. zuluensis*; Fig. 8F), or forming a continuous layer through the midrib, except for a medial patch of adaxial collenchyma (*C. briei*, *C. sphaerocephala*, *C. thyrsoflora*; Fig. 8A). Vascular bundles forming an invaginated flattened arc of separate or connected bundles; additional bundle(s) may occur outside the arc (Fig. 8A, C, F), collateral. Resin ducts present, 30–70 µm, single layer of epithelial cells, in collenchyma and parenchyma without chloroplasts. Crystals druse, in chlorenchyma, collenchyma and parenchyma without chloroplasts.

DIAGNOSTIC VALUE OF LEAF CHARACTERS

A large number of taxonomically valuable characters were identified during the study of the leaf anatomy.



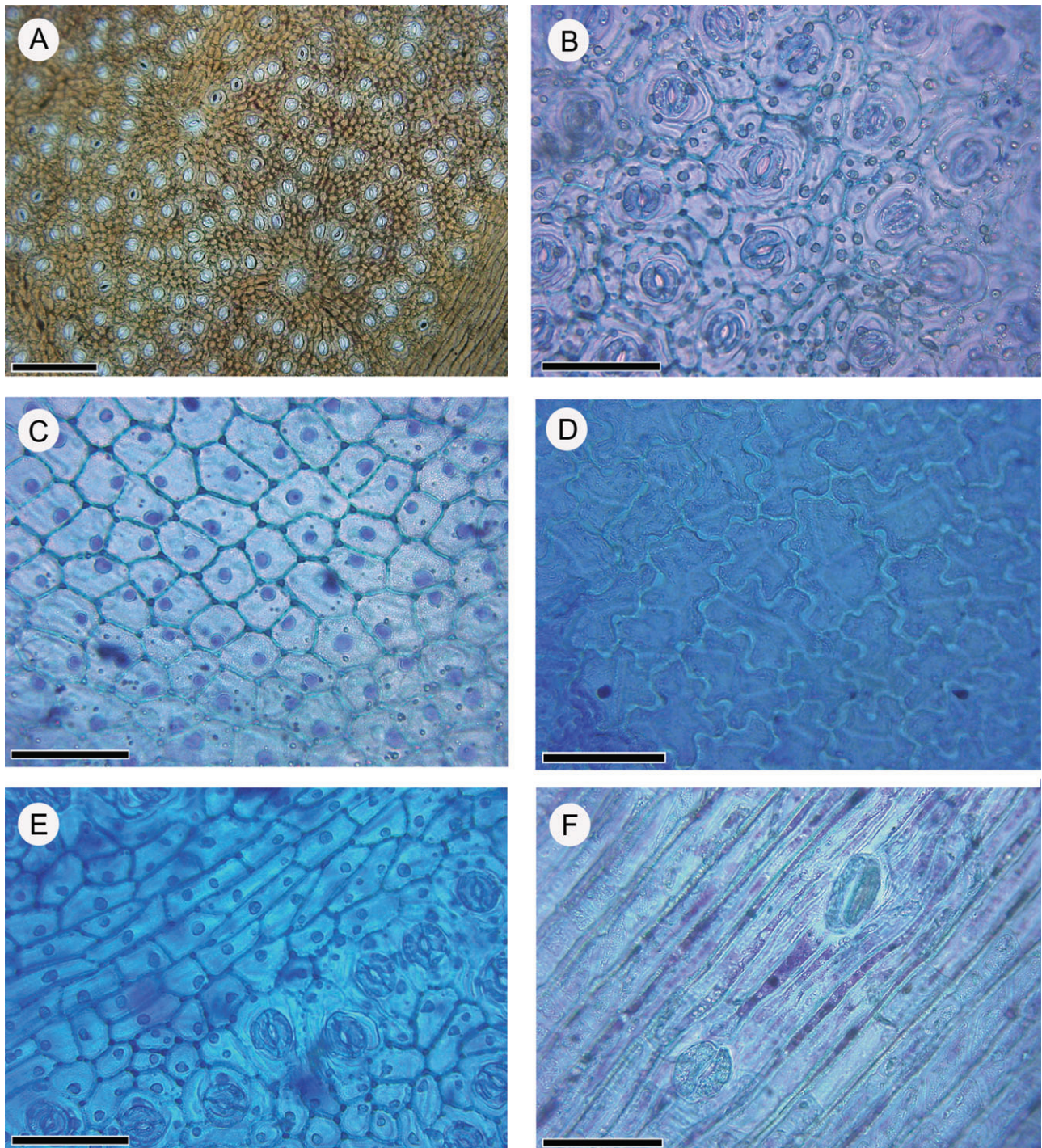


Figure 5. Lamina and/or midrib surfaces of various species of *Cussonia*. A, numerous anomocytic stomata on the abaxial lamina surface of *C. thyrsoiflora* (De Villiers & De Villiers 57). B, anomocytic stomata on the abaxial lamina surface of *C. paniculata* (De Villiers 2). C, square to rectangular epidermal cells on the adaxial leaf surface of *C. paniculata* (De Villiers 2). D, highly sinuous adaxial hypodermal cells of *C. zimmermannii* (Schlieben 5557). E, stomata on the adaxial surface of the lamina around the midrib of *C. paniculata* (De Villiers 2). F, stomata on the adaxial surface of the midrib of *C. natalensis* (De Villiers *et al.* 72). Scale bars, 1 mm (A); 0.2 mm (B, C); 0.07 mm (D–H).

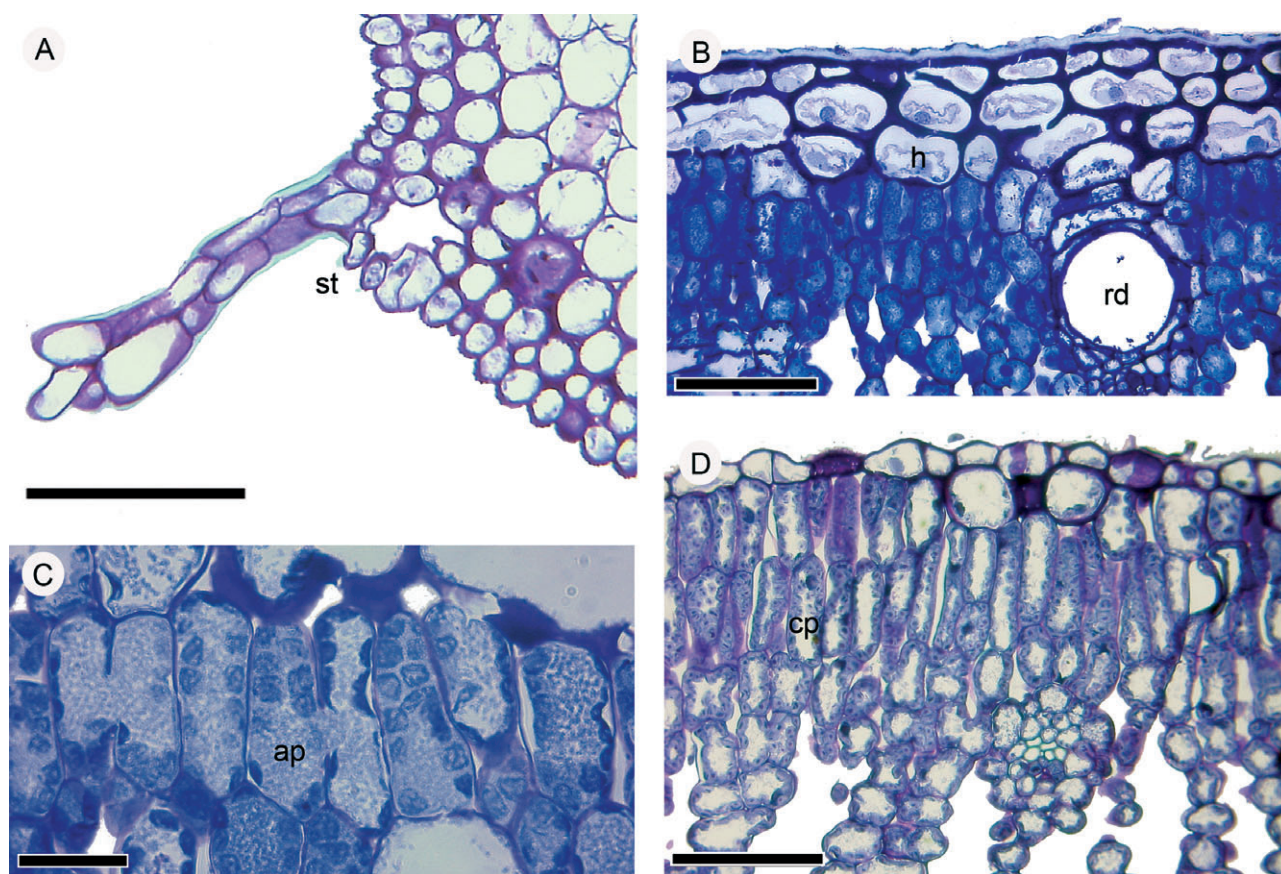


Figure 6. Leaf blade anatomy (in transverse section) of *Cussonia* and *Seemannaralia*. A, branched, multicellular trichome and stoma (st) on the lower surface of *S. gerrardii* (De Villiers *et al.* 97). B, hypodermis (h) and resin duct (rd) present in *C. natalensis* (De Villiers *et al.*, 72). C, arm palisade (ap) parenchyma in *C. spicata* (De Villiers 60). D, columnar palisade (cp) parenchyma in *S. gerrardii* (De Villiers & Oskolski 107) and the lack of a hypodermis can also be seen. Scale bars, 0.1 mm (A); 0.07 mm (B, D); 0.02 mm (C).

These characters can be used to distinguish genera, groups within *Cussonia* and species. The findings are discussed below, in the order in which they are described, and compared with those of previous researchers.

CUTICLE

Viguier (1906) studied the cuticle thickness of *Cussonia vantsilana* Baker (now *Schefflera vantsilana* (Baker) Bernardi) and found it to be approximately 20 μm thick. Burt & Dickison (1975) noted that *Seemannaralia* has a thin cuticle but did not report the actual thickness. In the present study, it was found that the cuticle of the lamina in *Seemannaralia* is thinner (20–28 μm) than that in *Cussonia* and *Schefflera* (c. 45–65 μm). When compared with previous authors' findings, the *Cussonia* and *Schefflera* species studied here have a much thicker cuticle. The

difference in thickness clearly distinguishes *Seemannaralia* from the other two genera.

TRICHOMES

Viguier (1906) stated that the distribution and structure of trichomes are extremely useful in the classification of the family. Burt & Dickison (1975) reported the occurrence of simple, unbranched multiseriate trichomes in *Seemannaralia*. However, in all the *Seemannaralia* specimens examined in the present study, unbranched trichomes (Figs 4F, 6A) were only observed on the adaxial surface, whereas on the abaxial surface the trichomes were branched (Fig. 4E). The occurrence of trichomes proved to be of importance for species identification. Most *Cussonia* spp. are glabrous, but *C. arborea* and *C. corbisieri* are glabrescent to hairy, especially in the juvenile phase. The occurrence of variably branched trichomes on the abaxial

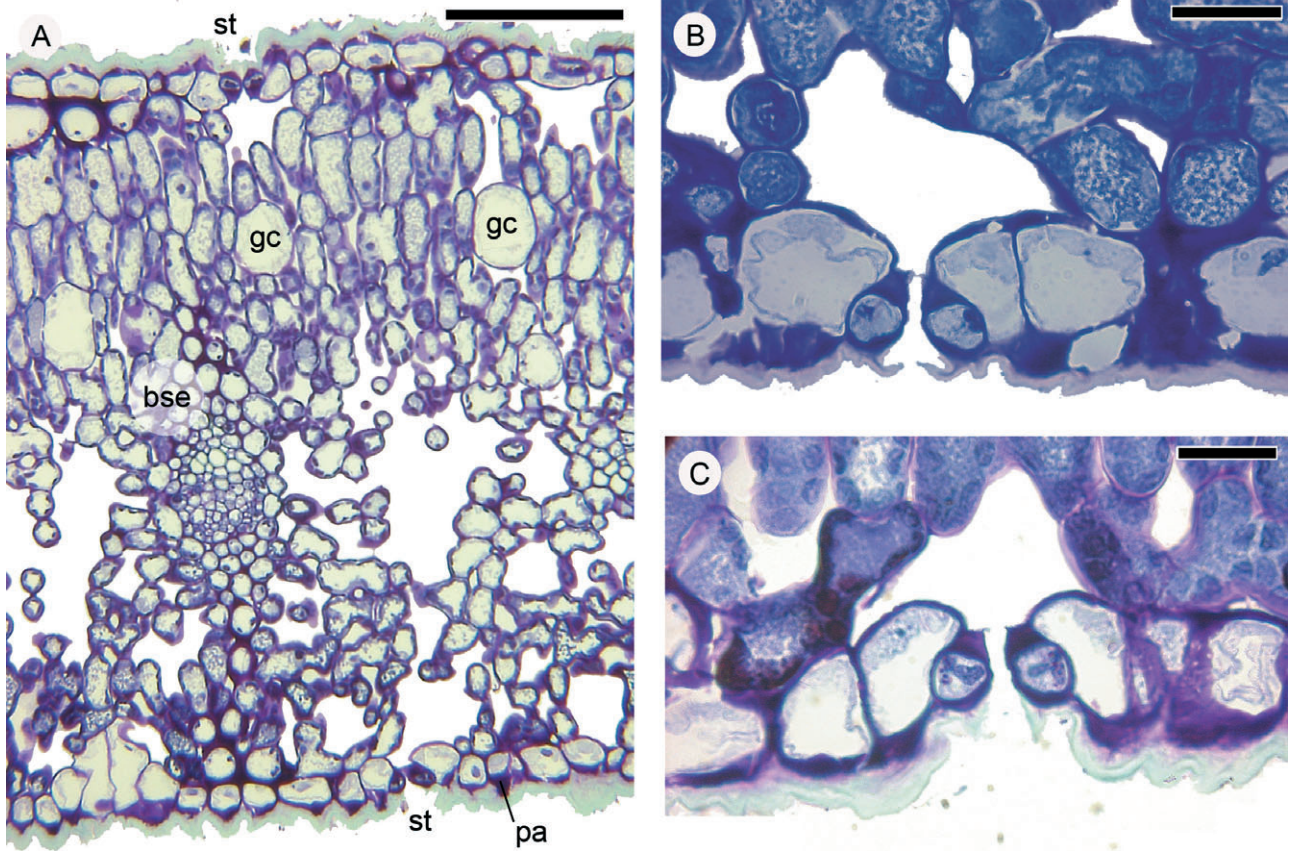


Figure 7. Stomatal distribution and structure (in transverse section) in *Cussonia*. A, abaxial and adaxial stomata (st), abaxial papillate epidermal cells (pa), collenchymatous bundle sheath extension (bse) and 'ghost' cells (gc) in which the druse crystals were present in *C. transvaalensis* (De Villiers *et al.* 58). B, abaxial stoma with the guard cells level with the subsidiary cells in *C. spicata* (De Villiers 60). C, abaxial stoma with the guard cells sunken relative to the subsidiary cells in *C. transvaalensis* (De Villiers *et al.* 58). Scale bars, 0.1 mm (A); 0.02 mm (B, C).

surface of the leaf blade and midrib of *C. corbisieri* anatomically distinguishes it from *C. arborea*, which only has simple, unbranched trichomes. Metcalfe & Chalk (1950) made no mention of the existence of forked trichomes in Araliaceae. No trichomes were observed in any of the *Schefflera* species studied.

EPIDERMAL CELLS

Solereder (1908) reported that papillose differentiation of the lower leaf epidermal cells is common in Araliaceae and Burt & Dickison (1975) also mentioned that these cells are associated with the major veins in *Seemannaralia*. The dull surface of the petiole, petiolules and lamina is generally caused by these cells (Solereder, 1908). Metcalfe & Chalk (1950) reported that papillate cells affect the sculpturing of the cuticular ridges and can have taxonomic value. Reyneke (1981, 1982) investigated these ridges but reported that they lacked taxonomic value at the

infrageneric level in *Cussonia*. In some *Cussonia* species and *Seemannaralia* the uniseriate epidermal cells are moderately papillate on the petiole but were absent in the *Schefflera* species studied (Fig. 1B, D, F). This species difference has not been reported before. *Cussonia sphaerocephala* and *C. arenicola*, which have papillate cells on the adaxial surface of the midrib, can be distinguished from *C. spicata* (most specimens) and *C. nicholsonii*, which lack them.

STOMATA

Viguié (1906) did not find the distribution and type of stomata taxonomically useful. He and Metcalfe & Chalk (1950) stated that stomata are confined to the abaxial surface in *Cussonia*, *Schefflera* and *Seemannaralia*. Burt & Dickison (1975) also found that adaxial stomata were absent in *Seemannaralia*. Adaxial stomata (here reported for the first time) occur in *C. arborea* (rarely), *C. corbisieri*, *C. gam-*

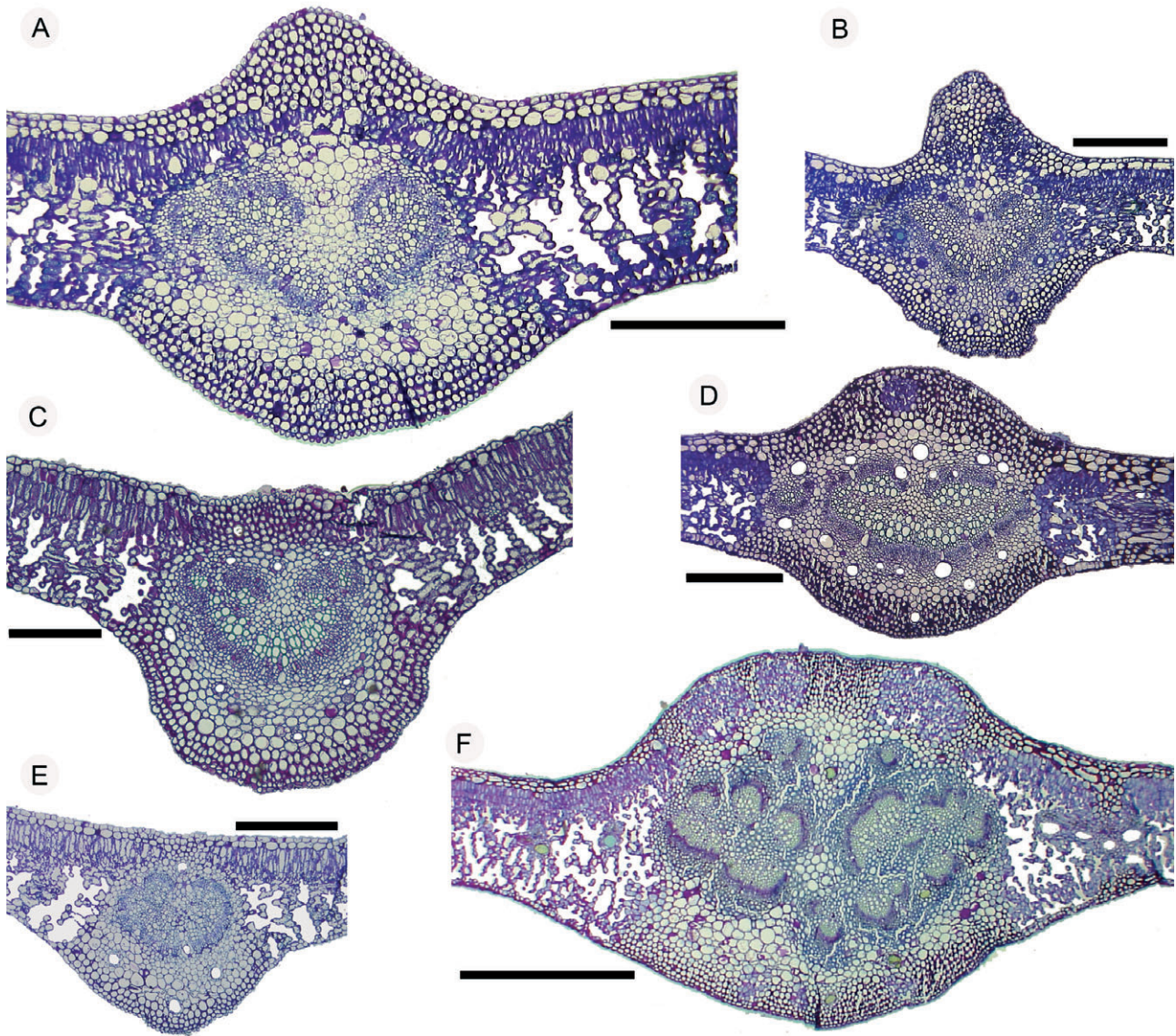


Figure 8. Midrib anatomy (in transverse section) of *Cussonia* and related genera. A, the palisade parenchyma is a continuous layer through the midrib in *C. sphaerocephala* and the midrib is conspicuously raised above the lamina (De Villiers & Oskolski 100). B, *C. arenicola* (De Villiers et al. 103) showing the ovate midrib (with an acute narrow end), with the hypodermis and palisade parenchyma extending into the midrib. C, *Seemannaralia gerrardii* (De Villiers et al. 97) illustrating the dissected arc with invaginated ends; palisade parenchyma absent from midrib. The midrib is not conspicuously raised on the adaxial surface. D, *C. natalensis* (De Villiers et al. 72) showing the palisade parenchyma absent from the midrib, except for a patch medially in the collenchyma. E, *Schefflera umbellifera* (De Villiers et al. 116) having a midrib which is not conspicuously raised above, a hypodermis and palisade parenchyma extending into the midrib. F, in *C. paniculata* (De Villiers 2) the palisade parenchyma extends into the midrib with patches in the adaxial collenchyma. Scale bars, 0.8 mm (A, F); 0.4 mm (B–E).

toosensis and *C. transvaalensis*. Various differences were found in the distribution of stomata on the lamina and the midrib. These were confirmed by making epidermal peels. Species with adaxial stomata also have abaxial stomata on the surface of both the lamina and midrib. Adaxial stomata can be

used to distinguish *C. transvaalensis* from the morphologically similar *C. spicata*. Adaxial stomata were observed on the midrib only of a few species. The most noteworthy of these are *C. spicata* and *C. nicholsonii* (Fig. 5E), as this distinguishes them from *C. sphaerocephala* and *Cussonia* sp. nov., which have no adaxial

stomata. This difference aids in species identification. In the present study, petiolar stomata were found to be associated with the subepidermal collenchyma (see below) in *Cussonia*. Stomata were absent in both the petiole and petiolules of *Schefflera* (Fig. 1B, D) and *Seemannaralia* (Fig. 1C). This is an apomorphy for the genus and has not been reported before.

SUBEPIDERMAL COLLENCHYMA

Viguié (1906) found the collenchyma to be continuous in the petiole of three *Cussonia* spp., but these are now known to be African *Schefflera* spp. Metcalfe & Chalk (1950) made no mention of any interruption in the distribution of the collenchyma, but Burt & Dickison (1975) reported an uninterrupted, subepidermal collenchyma layer in *Seemannaralia*. In the present study, the subepidermal collenchyma in both the petiole and petiolule was found to show clear and constant differences between the species. In *Cussonia* it is interrupted with chlorenchyma (Fig. 1E), whereas in all the *Schefflera* spp. (Fig. 1B, D, F) studied and *Seemannaralia* (Fig. 1C) it is continuous. The interrupted subepidermal collenchyma is an apomorphy for *Cussonia* when compared with the other genera.

SUBCOLLENCHYMAL SCLERENCHYMA

Viguié (1906) studied 12 species of *Schefflera* (mostly from Africa) and did not find subcollenchymal sclerenchyma. The petioles of the two Asian *Schefflera* spp. studied (not examined by Viguié) have a layer of subcollenchymal sclerenchyma (Fig. 1F).

HYPODERMIS

The presence of a hypodermis in the leaf blade of *Cussonia*, Afro-Malagasy *Schefflera* and *Seemannaralia* spp. was identified by Viguié (1906), Metcalfe & Chalk (1950) and Burt & Dickison (1975). Most of the species examined exhibit a hypodermis of varying degrees of differentiation. A hypodermis is absent in *C. angolensis*, *C. transvaalensis* and *C. zimmermannii*. This character can then be used to distinguish anatomically between *C. spicata* and *C. transvaalensis*, and *C. angolensis* and *C. brieyi*. In *Seemannaralia*, a small portion of hypodermis may be present (up to 7 cells wide in transverse section), which is usually associated with veins (Fig. 6D). Approximately 90% of the lamina lacks a hypodermis. This does not correspond with the report of Burt & Dickison (1975). In our study, the Asian *Schefflera* spp. studied were found to have a hypodermis 3–4 layers thick, whereas

that of the Afro-Malagasy *Schefflera* spp. studied was 2 or 3 layers thick. This warrants further study.

MESOPHYLL

Solereder (1908) and Metcalfe & Chalk (1950) reported the occurrence of arm palisade parenchyma in the *Cussonia* species they investigated. Our study confirmed its presence in all the species (Fig. 6C). The other genera studied have columnar palisade parenchyma. This is the first time that arm palisade parenchyma has been identified as a generic apomorphy.

The number of palisade layers can be used to distinguish between *C. transvaalensis* and *C. spicata*. *Cussonia transvaalensis* usually has a relatively large number of layers, ranging from 5 to 7, while *C. spicata* has a consistently lower number of 3 or 4.

The distribution of palisade parenchyma in the midrib can also aid in species identification. *Cussonia arenicola* has palisade parenchyma that extends from the lamina into the midrib (Fig. 8B), whereas in *C. nicholsonii* it does not extend into the midrib, except for two patches. In the case of *C. zuluensis*, a close relative of the aforementioned (Reyneke, 1981, 1982), the palisade parenchyma extends approximately a third of the width into the midrib and also occurs as two patches in the midrib. *Cussonia sphaerocephala* has a continuous palisade parenchyma layer through the midrib (Fig. 8A), whereas *C. spicata* (mostly) has the same pattern as *C. nicholsonii*. Thus, the arrangement of the palisade parenchyma can be used to distinguish between these morphologically similar species. Most of the palisade parenchyma distribution types has also been found in *Panax* L. (Grushvitzky *et al.*, 1975). The palisade parenchyma extends into the midrib in *P. ginseng* L., and *P. trifolius* L., whereas it forms a continuous layer through the midrib in *P. japonicus* C.A.Mey. and *P. pseudo-ginseng* Wall.

VASCULAR BUNDLES

Both Viguié (1906) and Metcalfe & Chalk (1950) suggested that the number and arrangement of the vascular bundles could be of taxonomic value. Burt & Dickison (1975) recorded the vascular bundle arrangement of the midrib as a dissected arc with invaginated ends in *Seemannaralia*. Viguié (1906) reported a normal orientation of the bundles (collateral) in some of the Afro-Malagasy *Schefflera* such as *S. boivini* and *S. vantsilana*. Metcalfe & Chalk (1950) and Tomlinson *et al.* (2005) observed an irregular distribution with inversely orientated bundles in the petiole of species belonging to the Asian clade of *Schefflera* (Plunkett *et al.*, 2005). Inversely orientated petiolar medullary bundles have been reported

in *Aralia* (Viguier, 1906) and in *Delarbrea* Vieill. (Ostroumova & Oskolski, 2010).

Many differences in the arrangement of vascular bundles and the position of the xylem and phloem within the bundles were found in the genera studied. In all of the taxa studied, the peripheral vascular bundles of the petiole are collateral. The petiolar medullary bundles are inversely orientated in the Asian *Schefflera* (Fig. 1D) and *Seemannaralia* (Fig. 1C) spp. studied, whereas the Afro-Malagasy *Schefflera* (Fig. 1B) and *Cussonia* spp. have normally orientated medullary bundles. *Schefflera* have been shown to be paraphyletic (Plunkett *et al.*, 2005) and our limited investigation indicates that the orientation of the petiolar medullary bundles (of *Schefflera*) agrees, with the Asian and Afro-Malagasy species being two distinct groups (but further study is needed). The vascular bundles in *Schefflera* and *Seemannaralia* spp. are different from those in *Cussonia*, in that they are smaller and more numerous.

Amphivasal or ampicribal bundles have been found in genera including *Mackinlaya* F.Muell., *Meryta* J.R.Forst. & G.Forst. and *Teighemopanax* Baill. [nom. inval., incert. sed.; = *Polyscias* J.R.Forst & G.Forst (Frodin & Govaerts, 2003)] (Viguier, 1906) and *Pentapanax verticillatus* Dunn (Hà Thi Dung *et al.*, 1975). Petiolar medullary bundles that are orientated perpendicular to the surface were also found in *Pentapanax* Seem. [= *Aralia* L. (Wen, 1993)] by Hà Thi Dung *et al.* (1975).

Three different types of medullary bundles were observed in the petioles of *Cussonia*: Type 1 – collateral bundles with phloem orientated towards the adaxial surface, with the xylem of the larger peripheral bundles forming a continuous patch and numerous resin ducts in the phloem; Type 2 – peripheral bundles divided into 2 (sometimes up to 4) sub-bundles, either because of the division of the phloem or xylem and few resin ducts associated with the phloem; Type 3 – xylem forming an incomplete sheath around the phloem (semi-amphivasal), or completely surrounding the phloem (amphivasal). These three types are of infrageneric importance. Type 1 is found in most of the East and West African members of the genus, namely *C. angolensis*, *C. arborea*, *C. bancoensis*, *C. corbisieri*, *C. holstii* and *C. natalensis*. Most of the southern African and two central African species have the Type 2 arrangement, namely *C. arenicola*, *C. brieyi*, *C. gamtoosensis*, *C. nicholsonii*, *Cussonia* sp. nov., *C. sessilis*, *C. sphaerocephala*, *C. spicata*, *C. thyrsoflora*, *C. transvaalensis* and *C. zuluensis*. Even although Type 3 is mostly found in *C. paniculata*, in rare cases one or two of the medullary bundles of the other species show one of the variations of Type 3. *Cussonia gamtoosensis*, *C. spicata*, *C. transvaalensis* and *C. zuluensis* can all have a few

bundles where the xylem forms an incomplete sheath around the phloem. In the case of a complete sheath, only *Cussonia* sp. nov. and *C. transvaalensis* show this type of arrangement. However, only in *C. paniculata* do the medullary bundles have a majority of Type 3 vascular bundles. All the species examined showed some of the medullary bundles in a perpendicular position to the surface. In rare cases in *C. sphaerocephala* and *C. transvaalensis*, the vascular bundles were turned to an inverse position. These different medullary vascular bundle types have not been reported before for *Cussonia* and are of infrageneric significance.

Three types of vascular bundles were observed in the petiole: (1) an adaxially flattened and invaginated ring of separate or connected bundles, forming a narrow arc as in *Cussonia* spp. (Fig. 3A), (2) connected or discrete vascular bundles forming a crescent with the concave side facing the adaxial side of the petiole, the tips invaginated towards the centre and additional bundles located at the tips of the strands as in Afro-Malagasy *Schefflera* spp. (Fig. 3B); and (3) an adaxially invaginated and flattened ring of separate or connected bundles, forming a flat arc, with one or two strands of medullary bundles, normally (towards the centre) orientated bundles in the concave side of the arc as in Asian *Schefflera* spp. The arrangement of the vascular bundles is an apomorphy for *Cussonia* and has not been reported previously for the genus. The vascular bundles can either be connected or in separate/discrete bundles. Variation in this character can be used for species identification. *Cussonia arenicola* and *C. zuluensis* both have discrete bundles in a ring, whereas *C. nicholsonii* and *Cussonia* sp. nov. have connected bundles. The connected bundles of *C. sphaerocephala* and *C. spicata* enable these species to be distinguished to be distinguished from *C. transvaalensis*, and *C. holstii* from *C. zimmermannii*.

RESIN DUCTS

Viguier (1906) placed great importance on the distribution of the resin ducts in Araliaceae; for example, in his placement of the genus *Arthropphyllum* Blume in the family. He stated that the ducts are numerous and in some cases very large in the *Cussonia* spp. studied (which are now *Schefflera* spp.). All of the taxa have resin ducts in various parts of the leaf. In the petiole they are associated with the phloem in some species. This character groups certain species and can be used to support infrageneric relationships (see Vascular bundles). The smaller ducts are usually associated with the vascular tissue and the larger ducts with the parenchymatous areas of the petiole, petiolules, lamina and midrib.

TAXONOMIC AND PHYLOGENETIC CONSIDERATIONS

This study shows that anatomical characters can greatly contribute to a clearer understanding of relationships between closely related genera. *Cussonia* and *Seemannaralia* share the occurrence of adaxial stomata and abaxial papillae on the lamina and secretory canals of a similar size in the petiole. Anatomically, the genus *Seemannaralia* has more in common with Afro-Malagasy *Schefflera* than with *Cussonia*. The continuous collenchyma layer in the petiole, the absence of a hypodermis in the lamina and the papillate epidermal cells on the main veins of the abaxial leaf surface, are shared characters. The particular vascular tissue arrangement of the petiole and petiolules, and the thin cuticle, are characters associated with *Seemannaralia* only. This genus also differs from *Cussonia* in that the petal aestivation is imbricate and the fruit is dry, bicarpellate and strongly flattened.

The investigation of leaf anatomy has resulted in the identification of apomorphies for the genus *Cussonia*. The discontinuous, subepidermal collenchyma layer and the associated stomata in the petiole and petiolule are previously unreported characters. Similarly, the adaxially invaginated and flattened ring of separate or connected bundles forming a narrow arc in the petiolules is another new character. Our study has revealed that all the *Cussonia* spp. investigated have arm palisade parenchyma.

The three types of vascular bundle arrangements correspond to the groupings or subgenera as suggested by Strey (1973, 1981). Type 1 vascular bundles are found in *Protocussonia*, Type 2 in subgenus *Cussonia* and Type 3 in subgenus *Paniculata*. If the East and West African *Cussonia* species, which were not considered by Strey (1973, 1981), are included, then the leaf anatomical results correspond to the infrageneric divisions. Thus, the leaf anatomical characters are congruent with the infrageneric relationships as proposed by Strey (1973, 1981), but not Reyneke (1981, 1982).

Several leaf anatomical characters support the species rank proposed by Strey (1973) for *C. arenicola*, *C. nicholsonii* and *C. zuluensis*. In his thesis, Reyneke (1981) proposed that the *C. arenicola* should be regarded as varieties of *C. zuluensis*. The connected vascular bundles in the petiolules separate *C. nicholsonii* from *C. arenicola* and *C. zuluensis*. The absence of adaxial stomata on the midrib distinguishes *C. arenicola* from the other two species. The distribution pattern of the palisade parenchyma in the midrib is another difference. These three species can clearly be identified by a unique combination of morphological (e.g. the sessile or subsessile fruits of *C. nicholsonii*) and anatomical character states.

Reyneke (1981) also proposed that *C. sphaerocephala* should merely be considered a variety of *C. spicata*. However, *C. sphaerocephala* differs from *C. spicata* in the shape of the epidermal cells of the petiolules and midrib, the lack of adaxial stomata on the midrib and the different type of palisade parenchyma distribution pattern in the midrib. These characters support the species rank as proposed by Strey (1973). However, the connected vascular bundles (as opposed to dissected) of the petiolules of *C. sphaerocephala* and *C. spicata* support a close relationship.

CONCLUSIONS

The leaf anatomy of *Cussonia* and its close relatives provides a wealth of taxonomically useful characters that reveal new insights into circumscriptions and relationships at the specific, infrageneric and, especially, the generic level. The data presented here will allow for rigorous comparisons at the generic level and may prove to be valuable in future studies to gain a better understanding of infrafamilial and infrageneric relationships in Araliaceae.

Anatomical characters contribute to a better understanding of species delimitations in *Cussonia* and are also valuable for species identification, especially with superficially similar species that were variously interpreted by previous authors. The three subgenera proposed by Strey (1973, 1981) on the basis of leaf and inflorescence morphology (and a limited number of species) are strongly supported by the difference in the vascular bundles of the petiole.

At the generic level, the results are particularly informative. The vascular tissue forming a dissected arc with invaginated ends in the petiole and the thinner cuticle of the lamina support the distinctiveness of the genus *Seemannaralia*, when compared with *Cussonia* and *Schefflera*. Four convincing generic apomorphies for *Cussonia* have been identified, namely the discontinuous, subepidermal collenchyma layer in the petiole and petiolule, the presence of stomata on the petiole and petiolule, the adaxially invaginated and flattened ring of dissected or connected bundles forming a narrow arc in the petiolules, and the presence of arm palisade parenchyma in all the species.

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