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Bark and wood anatomy of *Leucosidea* and *Cliffortia* (Sanguisorbeae, Rosaceae)

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ABSTRACT

The wood and bark structure of *Leucosidea sericea* and two species of *Cliffortia*, the South African members of the tribe Sanguisorbeae (Rosaceae) are described. These two genera share few anatomical traits (the presence of schizo-rhexigenous intercellular spaces in the cortex, almost exclusively simple perforation plates, small alternate intervessel pits, etc.) with other Rosaceae. However, *Leucosidea* shows a distinct storied structure of the secondary phloem and wood as well as stratification of the secondary phloem, with conductive elements and non-sclerified crystalliferous axial parenchyma arranged into alternating bands. These conditions are recorded for the first time for the family Rosaceae. In contrast to *Leucosidea*, two species of *Cliffortia* show neither storied structure of secondary phloem and xylem, nor stratification of secondary phloem.

Keywords: Storied structure, stratification, pit membranes, pseudotori, reticulate wall thickenings.

INTRODUCTION

The Rosaceae are represented in southern Africa by a single species of *Prunus* L. (the well-known *P. africana* (Hook. f.) Kalkman which has a wide distribution in tropical Africa) and two endemic genera of the tribe Sanguisorbeae: the monotypic *Leucosidea* Eckl. & Zeyh. (subtribe Agrimoniinae) and *Cliffortia* L. (subtribe Sanguisorbinae) (Jordaan 2000). The Sanguisorbeae consist of two subtribes (Agrimoniinae and Sanguisorbinae) and about 12 genera (Potter *et al.* 2007). Species of this tribe are distributed in cool temperate or alpine regions on all continents. Their habit varies from herbs and shrubs (*Leucosidea*, *Margyricarpus* Ruiz & Pav., some *Cliffortia*, *Polylepis* Ruiz & Pav. and *Poterium* L.) to trees (*Hagenia* J.F. Gmel., *Leucosidea*, *Polylepis*). Members of the Sanguisorbeae are distinguished from the rest of subfamily Rosoideae by their cup-shaped hypanthium that entirely encloses the carpel(s), resulting in a perigynous position of the flower. They are also characterized by a reduction in the number of stamens and carpels (in many cases to a single carpel). The leaves of Sanguisorbeae are stipulate and mostly pinnate, although exceptions are found within the genus *Cliffortia* (Kerr 2004).

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Cliffortia comprises about 132 shrubby species, most of which are endemic to the Cape Floristic Region (Whitehouse 2002, 2004; Whitehouse & Fellingman 2007). *Leucosidea* is a monotypic genus with a single species, *L. sericea* Eckl. & Zeyh. This is a shrub or small tree that is distributed in the Eastern Cape, Free State and KwaZulu-Natal provinces of South Africa (Van Wyk *et al.* 2008) as well as in Zimbabwe (Kalkman 2004).

Information on the wood and bark anatomy of these two genera is scanty. To date, published anatomical results are available only for the bark of *Cliffortia falcata* Spreng. *ex* Eckl. & Zeyh., *C. grandifolia* Eckl. & Zeyh. and *C. ilicifolia* L. (Lotova & Timonin 2005).

With the exception of a comment by Solereder (1908), noting the presence of spiral thickening of the pitted vessels in *Cliffortia*, the structure of the wood in *Cliffortia* and of the stem tissues in *Leucosidea* remains unknown. The aim of this study is to describe the bark and wood anatomy of *Leucosidea sericea*, as well as *C. ruscifolia* L. and *C. strobilifera* L.

MATERIALS AND METHODS

Samples of bark and wood of *Cliffortia* were collected during field trips in the Western Cape in 2014. *Leucosidea* material was collected on the campus of the University of Johannesburg.

Species sampled and voucher specimens (all in JRAU) are as follows (abbreviations: BEVW = B.-E. van Wyk; KK = E. L. Kotina): *C. ruscifolia* (KK&BEVW95-14), *C. strobilifera* (KK&BEVW96-14); *L. sericea* (KK85-14 A, B, C).

We collected bark and wood from young stem tips, and also from the very base of the stems in *Cliffortia,* and from the thickest available stem in *Leucosidea* (see the sample diameters in Tables 1 and 2). Material was fixed in FAA (Johansen 1940) and sectioned with a freezing microtome (Ernst Leitz GmbH, Wetzlar, Germany). Transverse, radial and tangential longitudinal sections from fixed material were stained with a 1 : 1 alcian blue/safranin mixture (Jansen *et al.* 2004) and mounted in Euparal. Jeffrey's solution (Johansen 1940) was used to macerate secondary phloem and wood, soaked for 24 hours and then mounted in glycerol. Sections were studied using light microscopy; digital images were taken with an Olympus ColorView Soft Imaging System, and measurements made with the Olympus Analysis Imaging Solutions (OASIS) programme.

The wood structure and crystals in bark were investigated by scanning electron microscopy (SEM, TESCAN, soft – VegaTS): FAA-fixed samples were dehydrated through an alcohol series of increasing concentration, followed by amyl acetate and critical point drying. Samples were mounted on aluminum stubs with double-sided carbon tape and coated with gold. Energy-dispersive X-ray spectroscopy (EDS) was performed using an Oxford Instruments apparatus with AZtec software. Terminology follows Trockenbrodt (1990), Junikka (1994), the IAWA Committee (1989) and Jansen *et al.* (2007).

RESULTS

Leucosidea sericea

Bark structure — The surface of young stems is covered with a dense, prominent indumentum. The epidermis on young parts of stems is composed of a single layer of isodiametric thin-walled cells covered by a thin cuticle (<1 μm in thickness). Trichomes are unicellular and of different sizes (short <1 mm and long >1 mm) (Fig. 1A–C). The long trichomes are located on emergences (Fig. 1A). Glandular trichomes composed of unicellular heads on multicellular (2–4 cells) stalks occur on the epidermal surface (Fig. 1B).

The cortex is composed of collenchyma and parenchyma. Cortical collenchyma is angular to annular and occurs in one ring of three to five cell layers. These cells are 13–35 μm in tangential diameter and 500–750 μm in length; they contain chloroplasts but lack crystals. Cortical parenchyma is composed of six to twelve layers of isodiametric, thin-walled cells of 35–85 μm in tangential diameter and 150–250 μm in length. They sometimes contain druses, and chloroplasts occur in the cells of the outermost regions. Dilatation of the cortical tissue is effected by tangential stretching of cells with their separation along middle lamella and rupturing of cell walls that result in formation of schizogenous and rhexigenous intercellular spaces (Fig. 1C). Perivascular fibers are in groups of 2–40.

The mature bark is brownish-grey to reddish, with scaling in the form of stringy flakes (on the oldest stems also by shaggy flakes). The initiation of first-formed periderm occurs in the deeper layers of the cortex (Fig. 1C). The phellem is composed of four or five layers of radially-flattened cells with thin to thick (sometimes sclerified) cell walls.

Table 1. Bark anatomical characters of southern African Rosaceae.

	Leucosidea sericea	*Cliffortia ruscifolia*	*Cliffortia strobilifera*
Diameter sample (mm)	170	50	18
Length of sieve tubes (mean/min–max, μm)	281 ± 5.3 205–331	351 ± 18.0 212–570	390 ±1 2.2 244–500
Diameter of sieve tubes (μm)	13–26	12–21	12–20
Length fiber-like sclereids and fibers	651 ± 56.8 130–1045	512 ± 36.3 217–1228	460 ± 21.9 247–745
Width of multiseriate rays, number of cells	2–5	2–4	2–5
Width of multiseriate rays (min–max, μm)	40–105	31–73	30–115
Height of multiseriate rays (min–max, μm)	129–320	113–500	284–1562
Number of multiseriate rays per mm (mean)	5.9	4.2	3.4
Number of uniseriate rays per mm (mean)	1.9	1.8	3.2

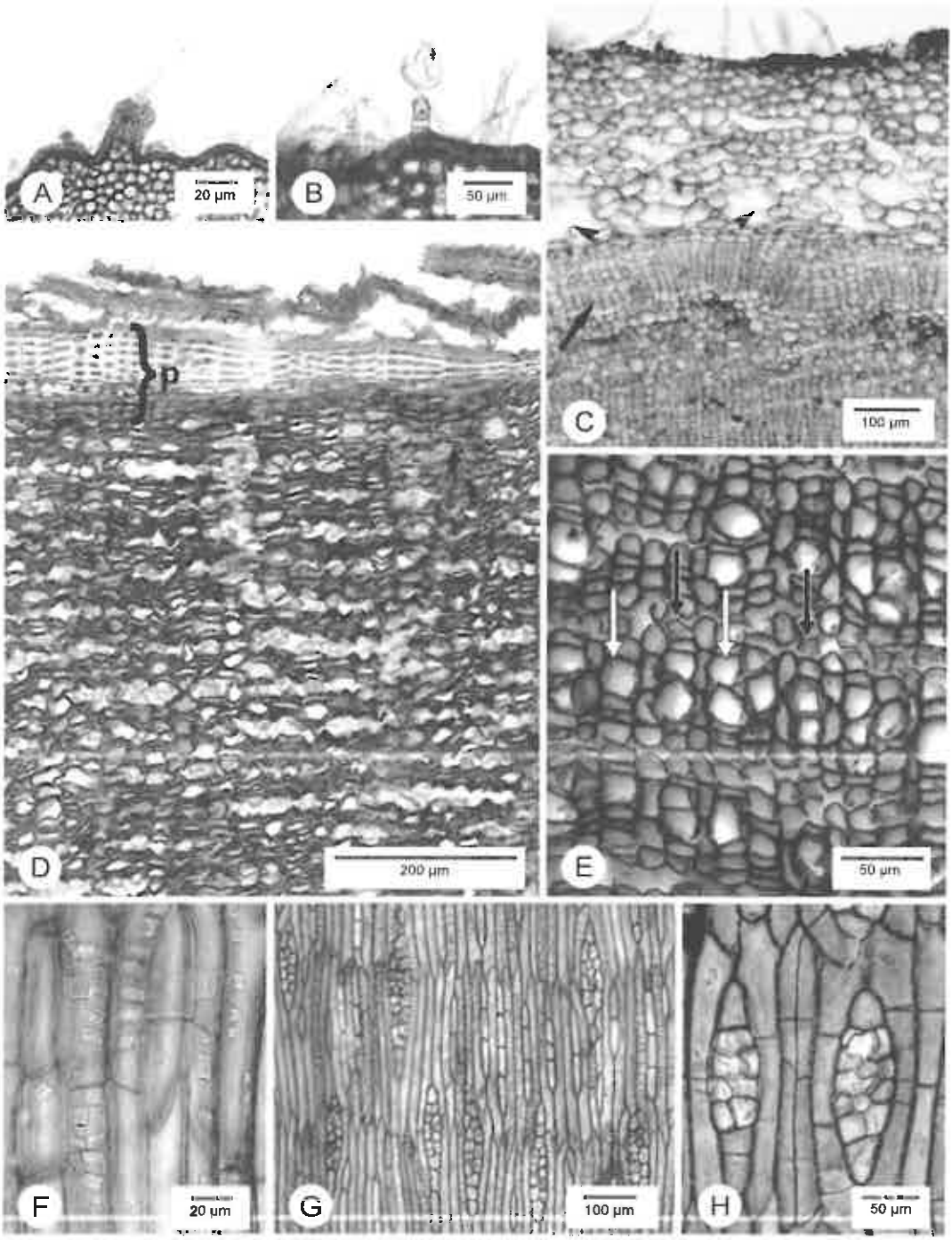

Figure 1. Bark structure of *Leucosidea sericea*. – A–C: TS. Young stem. – A: Long trichome on emergence. – B: Glandular trichome and short trichomes. – C: Cortex with schizo-rhexigenous intercellular spaces, first-formed periderm (arrow), ruptured cell walls (arrowheads). – D & E: TS. Mature bark. – D: Subsequent periderm (p) and stratified non-conducting secondary phloem showing bands of crystalliferous axial parenchyma. – E: Conducting secondary phloem; sieve tubes (white arrows) and axial parenchyma with relatively thick walls alternate with bands of crystalliferous axial parenchyma (black arrows). – F–H: TLS. – F: Prismatic crystals in axial parenchyma cells. – G: Storied arrangement of rays and axial parenchyma in secondary phloem. – H: Anticlinal division of axial parenchyma cells.

The phelloderm comprises three to five layers of isodiametric to radially-flattened, thin-walled cells. No crystalliferous cells or sclereids occur. Subsequent periderm consist of one to three layers of phelloderm and three to eight layers of phellem cells with sclerified walls. The secondary phloem is stratified in transverse section, *i.e.* it shows an alternation of 3- or 4-seriate tangential bands of conductive elements accompanied by relatively thick-walled (occasionally also sclerified) non-crystalliferous axial parenchyma with uni- to biseriate lines of thin-walled crystalliferous axial parenchyma (Fig. 1D, E). Sieve tube members are 13–26 μm wide; their length varies from 205 to 331 μm (Table 1). Sieve plates are mostly compound with two to eight sieve areas, or rarely simple, located on slightly oblique cross walls. Sometimes sieve areas are present on the lateral walls. Non-crystalliferous axial parenchyma consists of relatively thick-walled (cell walls 1–3 μm thick) fusiform cells and strands of 2–6 cells; chambered cells also occur. Crystalliferous axial parenchyma is composed of very thin-walled (cell walls less than 1 μm thick), mostly chambered, fusiform cells and strands of 2–4 cells containing prismatic crystals (Fig. 1F) and rarely also crystal sand. Crystalliferous cells usually bear a single crystal per chamber, but sometimes two or three crystals co-occur. Examination of these crystals with X-ray microanalyses (EDS) showed large calcium, carbon, and oxygen peaks, suggesting these to be calcium oxalate crystals.

The transition from conducting to non-conducting secondary phloem is gradual. Non-conducting secondary phloem is also stratified in transverse section (Fig. 1D), *i.e.* it differs from conducting secondary phloem by the obliteration of conductive elements, the occurrence of more numerous crystalliferous cells and by sclerification of some parenchyma cells with the formation of fiber-like sclereids or fibers with tapered tips.

Axial parenchyma and rays in the secondary phloem show a storied arrangement (Fig. 1G). Secondary phloem rays are uniseriate and multiseriate (up to 5-seriate). Uniseriate rays are composed mostly of square and upright cells while multiseriate rays have procumbent cells or also square and upright cells forming one or two marginal rows. Prismatic crystals occur in ray cells. No sclerified ray cells were observed.

Dilatation of secondary phloem is effected mostly by expansion and anticlinal divisions of axial parenchyma cells (Fig. 1H), to a lesser degree also by tangential expansion of ray cells. Rays in dilated secondary phloem remain straight or become slightly wavy.

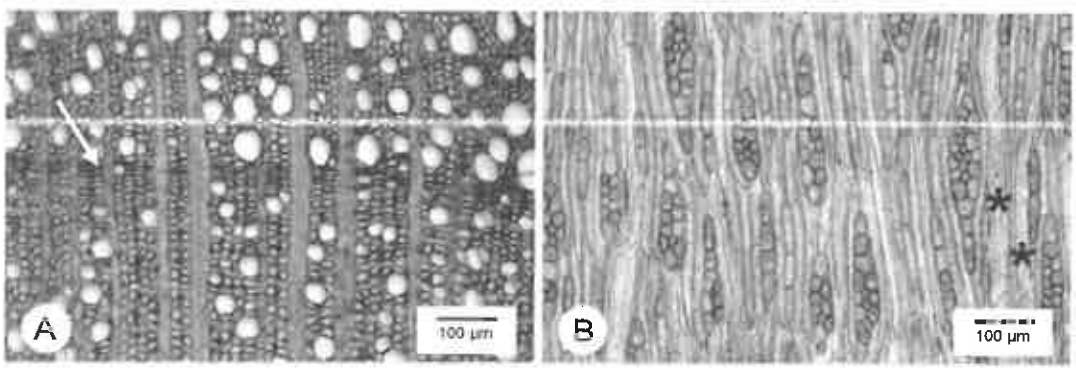

Figure 2. Wood structure of *Leucosidea sericea*. – A: TS. Growth ring boundary (arrow). – B: TLS. Partially storied arrangement of axial parenchyma and vessel elements (asterisks).

Table 2. Wood anatomical characters of southern African Rosaceae.

	Leucosidea sericea	*Cliffortia ruscifolia*	*Cliffortia strobilifera*
Diameter sample (mm)	16; 27; 48	50	18
Length of vessel elements (mean/min–max, μm)	306 ± 5.2 220–400	462 ± 12.3 335–590	282 ± 9.0 110–390
Tangential diameter of vessels (mean/min–max, μm)	32 ± 1.3 20–50	44 ± 3.2 15–79	33 ± 1.8 17–50
Vertical size of intervessel pits (mean/min–max, μm)	3.7 ± 0.2 2.3–6.5	3.6 ± 0.08 2.8–4.0	3.9 ± 0.2 2.7–6.0
Vessel frequency (per mm^2)	150–210	44–102	64–144
Length of libriform fibers (mean/min–max, μm)	593 ± 14.0 337–911	1094 ± 31.2 760–1495	665 ± 20.9 335–935
Width of multiseriate rays, number of cells	2–4 (5)	2–4	2–4 (6)
Width of multiseriate rays (mean/min–max, μm)	26 ± 2.6 7–58	34 ± 1.6 17–52	36 ± 1.9 15–70
Height of multiseriate rays (min–max, μm)	154–442	73–418	73–1445
Number of multiseriate rays per mm (mean)	3.2	2.2	3.2
Number of uniseriate rays per mm (mean)	5.0	1.6	2.7

Wood structure — Growth rings are distinct, marked by differences in radial diameter of the fibers (Fig. 2A). Vessels are mostly rounded (sometimes angular) in outline, narrow (up to 50 μm in tangential diameter) and numerous (vessel frequency in the range 150–210 per mm^2), solitary and in groups of two to four (Table 2). Vessel walls are 0.5–2.5 μm thick. The length of vessel elements ranges from 220–400 μm.

Perforation plates are mostly simple (Fig. 3A), rarely scalariform or reticulate (Fig. 3B) with 1–4 bars. Intervessel pitting is alternate to opposite; pits are rounded to oval with slit-like apertures and are minute to small (vertical size 2.3–6.5 μm). Pit membranes are solid (Fig. 3C), sometimes bearing pseudotori (Fig. 3D). Coarse reticulate (rarely also helical) thickenings that are commonly associated with pit apertures are present on the vessel walls (Fig. 3E). Vessel-ray and vessel-axial parenchyma pits are distinctly to indistinctly bordered and are similar to intervessel pits in size and shape. It is noteworthy that vascular tracheids with helical thickenings are also present.

Fibers with very thin walls (1.3–3.6 μm thick), and minutely to distinctly bordered pits of 2.5–5.1 μm in diameter are common in both radial and tangential walls. Septate fibers are not found. Coarse reticulate thickenings occur on the walls of some fibers (Fig. 3E).

Axial parenchyma is diffuse-in-aggregates to diffuse; it consists of fusiform cells and strands of 2–3(–4) cells.

Rays 3–8 per mm, uni- and multiseriate, 2–4 (up to 5) cells in width (Fig. 2B). Uniseriate rays are composed mostly of square and upright cells. Multiseriate rays are

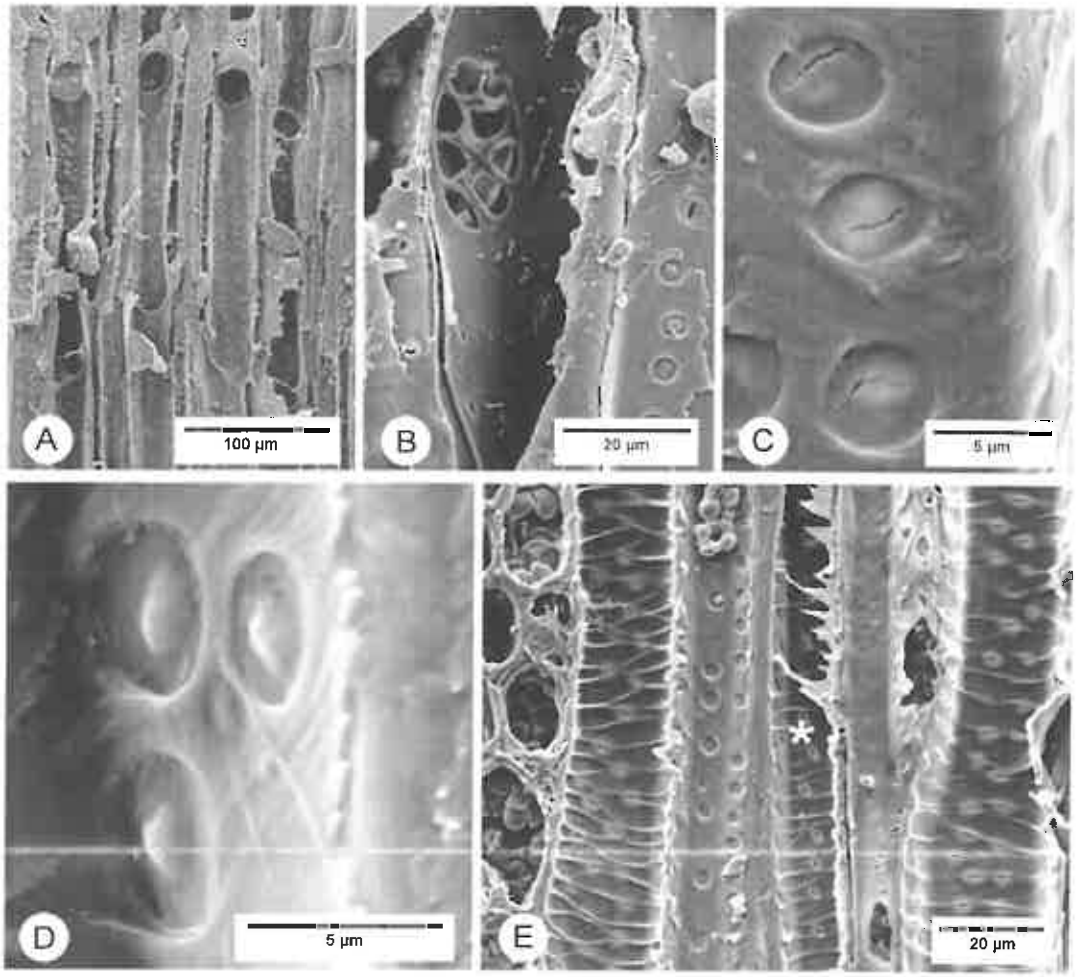

Figure 3. Wood structure of *Leucosidea sericea*. SEM. – A: Simple perforation plates. – B: Reticulate perforation plate. – C: Solid membranes of intervessel pits. – D: Pseudotori on intervessel pit membranes. – E: Vessel elements and fiber (asterisk) with reticulate to helical thickenings on their walls.

composed of procumbent cells, with 1–3 marginal rows of square and upright cells. Crystals are not found in the ray cells.

A storied arrangement of axial parenchyma is distinctive in the wood and is sometimes also visible in the rays and vessel elements (Fig. 2B). The storied structure is visible at a distance of more than 8 mm from the pith [as was shown by the examination of three samples having radii of 8 mm (KK85-14 A), 14 mm (KK85-14 B) and 24 mm (KK85-14 C) respectively], but not at distances closer than 7 mm from the pith.

Cliffortia

Bark structure — The surface of young stems is smooth in *C. strobilifera*, and covered by a dense indumentum in *C. ruscifolia*. The epidermis on young stems is composed of a single layer of isodiametric thin-walled cells covered by a rather thick cuticle (3–10 µm). Uni- or bicellular trichomes occur on the epidermal surface of *C. ruscifolia*; some of them are located on emergences. Glandular trichomes with unicellular heads on multicellular (4–8 cells) stalks are very rarely present.

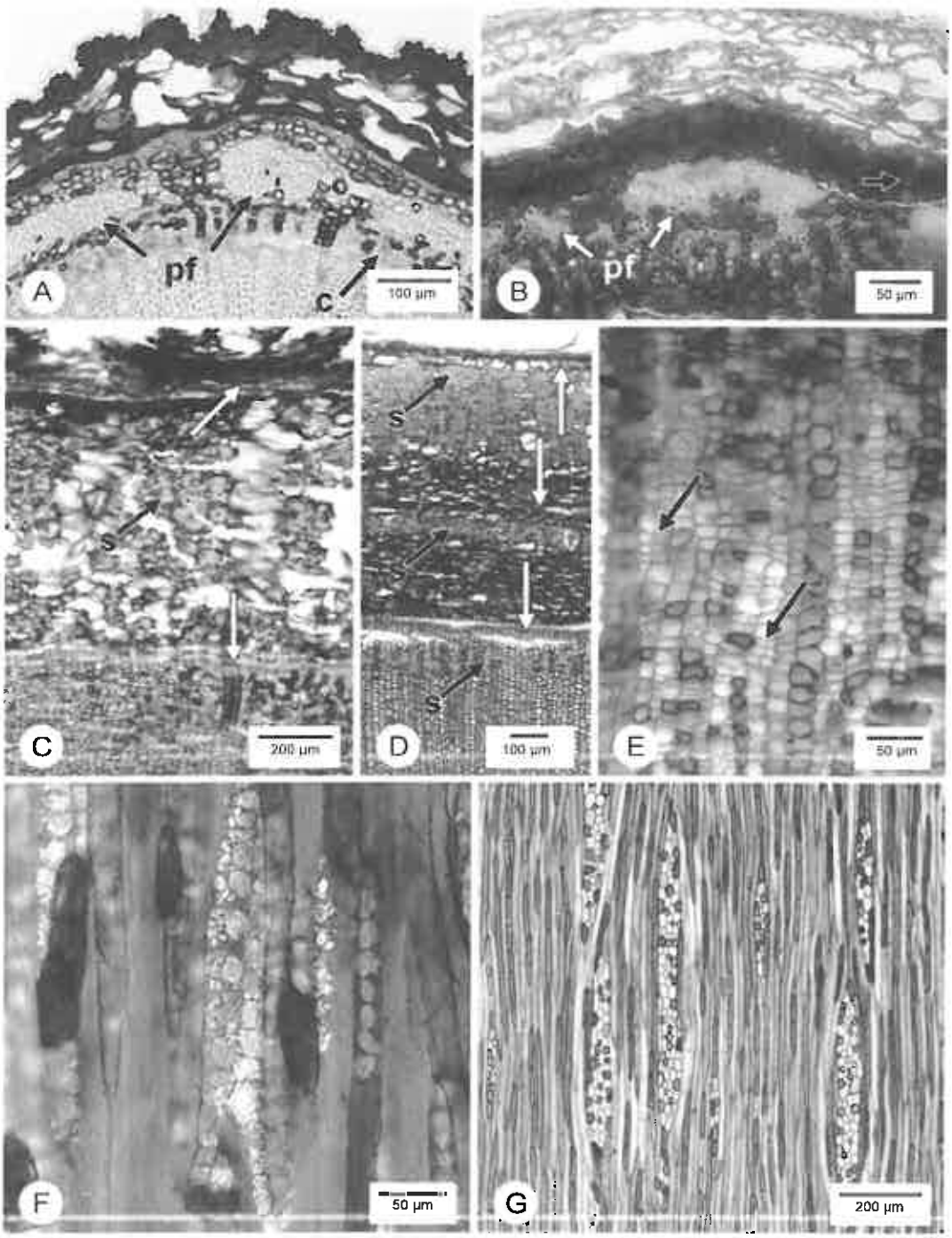

Figure 4. Bark structure of *Cliffortia*. – A &B: TS. Young stem showing the cortex with schizorhexigenous intercellular spaces and perivascular fibers (pf) in groups. – A: *C. ruscifolia*. Cambium (c). – B: *C. strobilifera*. First-formed periderm (arrow) in the deeper layers of cortex. – C & D: TS. Mature bark: non-conducting secondary phloem and part of secondary phloem between subsequent periderms (white arrows). – C: *C. ruscifolia*. Sclereids (s) in groups. – D: *C. strobilifera*. Sclereids (s) in tangential bands. – E: TS. Secondary phloem of *C. ruscifolia*, anticlinal divisions of axial parenchyma cells (arrows). – F & G: TLS. – F: *C. ruscifolia*. Axial parenchyma with colored content and crystals. – G: *C. strobilifera*. Rays and axial parenchyma with colored content.

The cortex consists of six to 10 layers of axially-elongated thin-walled parenchyma cells (Fig. 4A, B). Two types of cells can be distinguished, *viz.* smaller cells of 6–22 μm (up to 58 μm in *C. ruscifolia*) in tangential diameter containing orange-reddish (as seen in unstained sections) to raspberry-red (in sections stained with a mixture of safranin and alcian blue) content, and larger cells of 8–59 μm (up to 88 μm in *C. ruscifolia*) in tangential diameter. Cells of the former type are scattered throughout the cortex in *C. ruscifolia* (Fig. 4A), or aggregated in the innermost region of the cortex in *C. strobilifera*. Prismatic crystals were observed only in a few parenchyma cells in *C. ruscifolia*. Dilatation of the cortical tissue is effected by tangential stretching of cells with their separation along middle lamella and rupturing of cell walls that result in formation of schizogenous and rhexigenous intercellular spaces (Fig. 4A, B). Perivascular fibers are in large tangentially-elongated clusters of 10–70 (up to 100 in *C. ruscifolia*).

The mature bark is brownish-grey, with stringy scaling. The initiation of first-formed periderm occurs in the deeper layers of the cortex in very young stems. Both phellem and phelloderm are composed of one or two layers of radially-flattened, thin-walled cells. Subsequent periderms are initiated as concentric rings. Phellem consists of two layers of radially-flattened, thin-walled cells. Phelloderm consists of two to four layers of radially-flattened, thin-walled cells (Fig. 4C, D). Crystals and sclereids were not observed.

The secondary phloem shows no distinctive patterns in the arrangement of its conductive elements and/or axial parenchyma (Fig. 4E). Sieve-tube members are 8–30 μm wide; their length varies from 210 to 570 μm (Table 1). Sieve plates are compound with 4 to 16 (up to 21 in *C. ruscifolia)* sieve areas, located on slightly oblique cross walls. Sometimes sieve areas are present on the lateral walls. Axial parenchyma consists of fusiform cells and strands of 2–8 cells scattered between the conductive elements. Orange-reddish (as seen in unstained sections) or raspberry-red (when stained with a mixture of safranin and alcian blue) content or prismatic crystals (in *C. ruscifolia* also crystal sand) occur in the axial parenchyma cells (Fig. 4F). Crystaliferous parenchyma cells are mostly chambered. Examination of these crystals with X-ray microanalyses (EDS) showed large calcium, carbon and oxygen peaks, suggesting these to be calcium oxalate crystals.

The transition from conducting to non-conducting secondary phloem is gradual. Non-conducting secondary phloem differs from conducting secondary phloem by the occurrence of more numerous crystalliferous cells and the presence of fiber-like sclereids and fibers with tapered tips. The sclereids are mostly in groups of two to ten cells (*C. ruscifolia*) (Fig. 4C), or are aggregated into tangential bands in the outermost region of the secondary phloem (*C. strobilifera*) (Fig. 4D).

Secondary phloem rays are uniseriate and multiseriate (up to 5-seriate). Uniseriate rays are composed mostly of square and upright cells while multiseriate rays have procumbent cells with or without upright and square cells forming up to three marginal rows. The height of the multiseriate rays is 110–500 μm in *C. ruscifolia* and 280–1560 μm in *C. strobilifera* (Fig. 4G) (Table 1). Prismatic crystals occur in the ray cells. A few sclerified ray cells are found in the outermost region of the non-conducting secondary phloem in *C. strobilifera*.

Dilatation of secondary phloem is effected mostly by expansion and anticlinal divisions of axial parenchyma cells (Fig. 4C–E) and, to a lesser degree, also by tangential expansion (and very rare anticlinal divisions) of ray cells. Rays in the dilated secondary phloem remain straight.

A storied arrangement is not found for any elements of the secondary phloem.

Wood structure — Growth rings are absent (Fig. 5A, B). Vessels are mostly rounded (sometimes angular) in outline, narrow (up to 80 μm in tangential diameter) and rather numerous (44–102 per mm^2 in *C. ruscifolia* and 64–144 per mm^2 in *C. strobilifera*), solitary and in radial multiples of two to four. Vessel walls are 1.5–4.5 μm thick. The length of the vessel elements ranges between 110–390 μm in *C. strobilifera* and 335–590 μm in *C. ruscifolia* (Table 2).

Perforation plates are simple (Fig. 6A, B); a few reticulate perforation plates with 3–5 bars are found in *C. ruscifolia*. Intervessel pitting is alternate or transitional between opposite and scalariform, pits are rounded with slit-like apertures, and are minute to small (vertical size 2.8–4.0 μm in *C. ruscifolia* and 2.7–6.0 μm in *C. strobilifera*). Intervessel pit apertures in *C. ruscifolia* are located in shallow grooves (Fig. 6C) which are sometimes interconnecting (Fig. 6D). Pit membranes are mostly solid (in

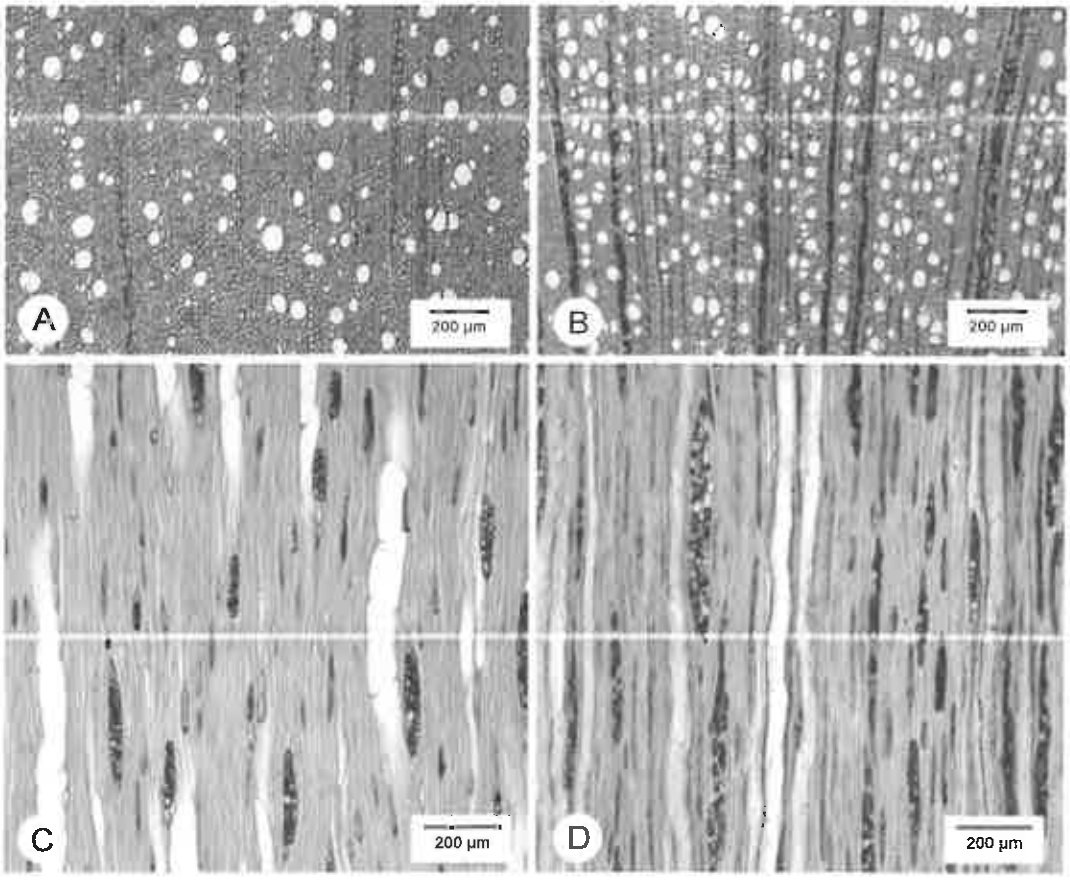

Figure 5. Wood structure of *Cliffortia*. – A & B: TS. – A: *C. ruscifolia*. – B: *C. strobilifera*. – C & D: TLS. – C: *C. ruscifolia*. – D: *C. strobilifera*.

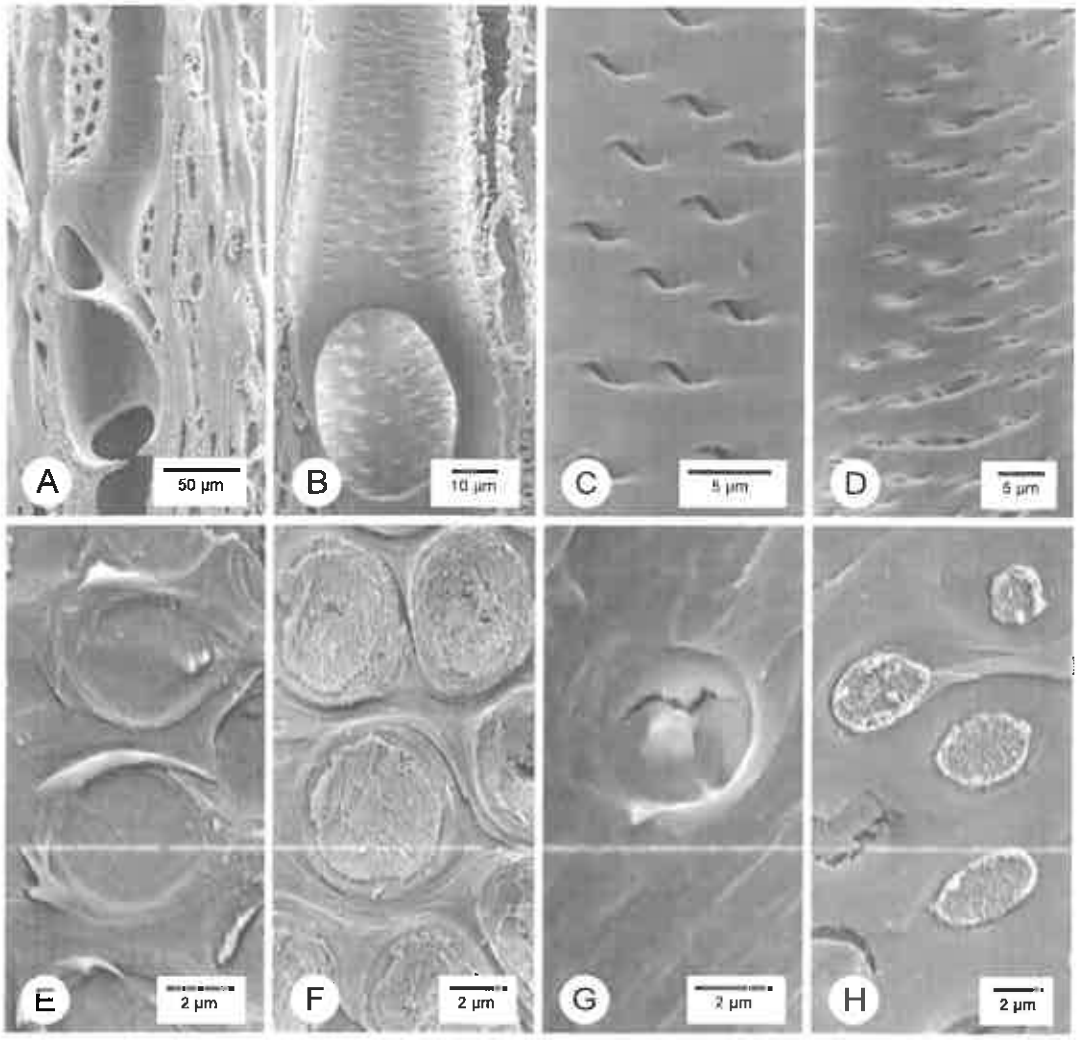

Figure 6. Wood structure of *Cliffortia*. SEM. – A–E: *C. ruscifolia*. – A: Vessel elements with simple perforation plates. – B: Simple perforation plate and alternate intervessel pitting. – C: Intervessel pit apertures in interconnecting grooves. – D: Intervessel pit apertures in shallow grooves. – E: Solid membranes of intervessel pits. – F–H: *C. strobilifera*. – F: Fibrillar membranes of intervessel pits. – G: Pseudotorus on membrane of intervessel pit. – H: Incrustations in chambers of intervessel pits.

C. strobilifera also fibrillar) (Fig. 6E, F) to perforated, sometimes bearing pseudotori (Fig. 6G). Pit membranes in the chambers of some intervessel pits in *C. strobilifera* are heavily incrusted (Fig. 6H). Vessel-ray and vessel-axial parenchyma pits are distinctly to indistinctly bordered and are similar to the intervessel pits in size and shape. Helical or reticulate thickenings were not found on the vessel walls. No vascular tracheids were observed.

Fibers are thin- to thick-walled (3–6 µm thick, up to 9 µm in *C. ruscifolia*), with minutely to distinctly bordered pits of 2.0–4.7 µm in diameter that are common in both radial and tangential walls. Septate fibers were not found.

Axial parenchyma is diffuse to diffuse-in-aggregates and scanty paratracheal, and occurs as solitary cells and strands near the vessels; it consists of fusiform cells and

strands of 2 or 3 (or 4) cells. Raspberry-red content (as seen in stained sections) commonly occurs in the axial parenchyma cells.

Rays 2–6 per mm (up to 9 in *C. strobilifera*), uni- and multiseriate of 2–4 (up to 6 in *C. strobilifera*) cells in width (Fig. 5C, D). Some rays in *C. strobilifera* exceed 1 mm (up to 1.4 mm) in height (Table 2). Uniseriate rays are composed mostly of square and upright cells. In *C. ruscifolia*, multiseriate rays are composed of procumbent cells, with 1–4 marginal rows of square and upright cells. In *C. strobilifera* multiseriate rays are composed of procumbent, square and upright cells mixed throughout the ray. Crystals are not found in the ray cells.

A storied arrangement is not found in any of the wood elements.

DISCUSSION

Species of *Cliffortia* and *Leucosidea* share only a few traits with other Rosaceae, such as the presence of schizo-rhexigenous intercellular spaces in the cortex, almost exclusively simple perforation plates, alternate intervessel pits, and vessel-ray and vessel-axial parenchyma pits similar to intervessel pits in size and shape (Zhang 1992a, b; Zhang & Baas 1992; Lotova & Timonin 2005). These two genera are rather distinctive in their wood structure and differ from each other as well as from other members of the tribe Sanguisorbeae (Kerr 2004; Potter *et al.* 2007). *Hagenia*, *Polylepis* and *Sarcopoterium* Spach (*Poterium* according Potter *et al.* 2007) have previously been examined by wood anatomists (Zhang 1992b; InsideWood 2004-onwards). Apart from the above mentioned traits of most Rosaceae, the five genera of the tribe share only the presence of diffuse and diffuse-in-aggregates axial parenchyma in 2–4-celled strands (Table 3). Each genus, however, shows clear-cut differences from other members of Sanguisorbeae in its unique combination of wood traits, such as vessel element length, fiber length, F/V ratio, intervessel pit diameter and shape, occurrence of helical thickenings, presence or absence of septate fibers, type and width of rays, number of rays per mm and occurrence of crystals in ray cells (Table 3). We could find no congruence between phylogenetic relationships based on molecular data (Kerr 2004) and the similarities and dissimilarities in wood anatomy summarized in Table 3.

Cliffortia and *Leucosidea* are also rather distinctive from each other in their bark and wood structure and similar only in the initiation of first-formed phellogen in deeper parts of the cortex, the occurrence of prismatic crystals in the axial and radial parenchyma of non-conducting secondary phloem and in the stringy rhytidome formed by thin (5–8-seriate) subsequent periderms. No data on bark anatomy of other members of the tribe Sanguisorbeae have been reported to date.

The storied wood and secondary phloem found in *Leucosidea* is noteworthy, as it has never been reported in Rosaceae. This feature is characteristic of some angiosperm families (Den Outer 1986; Carlquist 2001), but the origin of this trait in a single monotypic genus within a relatively large plant family provides an interesting case of the evolutionary shift from a non-storied to a storied pattern. It is notable that the genera *Hagenia, Polylepis* and *Sarcopoterium*, which are the closest woody relatives of *Leucosidea* (Potter *et al.* 2007), show no signs of a storied arrangement in their wood

Table 3. Comparison of the wood structure in the tribe Sanguisorbeae (Rosaceae).

	Hagenia	*Polylepis*	*Sarcopoterium*	*Leucosidea sericea*	*Cliffortia ruscifolia*	*Cliffortia strobilifera*
Length of vessel elements (mean/min–max, μm)	650 (375–1500)	350 (250–550)	250 (140–360)	306 (220–400)	462 (335–590)	282 (110–390)
Length of fibers (mean/min–max, μm)	1640 (625–1825)	730 (550–880)	340 (240–440)	593 (337–911)	1094 (760–1495)	665 (335–935)
F/V ratio	2.5	2.1	1.4	1.9	2.4	2.4
Diameter of intervessel pits (min–max, μm)	6.0–11.0	4.0–8.0	4.0–6.0	2.3–6.5	2.8–4.0	2.7–6.0
Intervessel pits shape	Rounded	Rounded to oval	Rounded to polygonal	Rounded to oval	Rounded	Rounded
Septate fibers	–	–	+	–	–	–
Axial parenchyma: abundance and type	Common. Diffuse and diffuse-in-aggregates	Abundant. Diffuse and diffuse-in-aggregates, in 2–4-celled strands	Very scanty Apotracheally diffuse and diffuse-in-aggregates	Scanty. Diffuce, diffuse-in-aggregates and scanty paratracheal	Scanty. Diffuse and scanty paratracheal	Scanty Diffuse and scanty paratracheal
Number of cells in axial parenchyma strands	2–4	2–4	2–3	2–4	2–4	2–4
Maximum width of rays, number of cells	9	4	12	5	4	6
Number of rays per mm	2–6	7–13	5–14	3–8	2–6	2–9
Rays composition	Procumbent cells only, or with 1 marginal row of square cells	Procumbent cells only, or with 1 row of square marginal cells	Mostly square (rarely to weakly procumbent) and upright cells	Procumbent cells with 1–3 marginal rows of square and upright cells	Procumbent cells with 1–4 marginal rows of square and upright cells	Procumbent, square and upright cells mixed throughout the ray
Helical thickening	–	+	–	+	–	–
Prismatic crystals in ray cells	–	–	+	–	–	–
Source	Zhang (1992b)	Zhang (1992b)	Zhang (1992b)	present study	present study	present study

structure (Zhang 1992a,b; InsideWood 2004-onwards). As both axial elements and rays in the secondary phloem and secondary xylem of *L. sericea* are arranged in apparent storeys, this species has double-storied cambium. For the strands and fusiform cells of the axial parenchyma, as well as for the vessel elements, this pattern results from the active anticlinal divisions of fusiform cambial initials combined with the absence of intrusive elongation of daughter cells (Bailey 1923; Iqbal & Ghouse 1990). As for the regular tiers of rays, they can be achieved by different mechanisms, such as the initiation of rays within the storeys of fusiform initials, the controlled vertical migration of rays and/or the vertical splitting of high rays between the storeys (Myśkow & Zagórska-Marek 2004, 2008, 2013). The impact of each of these processes in the formation of the double-storied pattern in *L. sericea* needs special investigations. It should be noted that this pattern is absent in the earliest stages of the cambial ontogeny of *L. sericea*: it appears at a distance of c. 7 mm from the pith. The late transition from non-storied to double-storied cambium has been reported also for *Hippophae rhamnoides* L. (Eleagnaceae) and *Aesculus turbinata* Blume (Sapindaceae), but the mechanisms that trigger this shift remain unknown (Myśkow & Zagórska-Marek 2004, 2008).

Leucosidea sericea also differs from the two *Cliffortia* species in its stratified secondary phloem, *i.e.* the alternation of tangential lines of conductive elements with bands of axial parenchyma with relatively thick cell walls, and the uni- to biseriate lines of thin-walled axial parenchyma containing prismatic crystals. Among Rosaceae, the alternation of tangential multiseriate bands of sclereids with bands of conductive elements and axial parenchyma has been reported in the secondary phloem of *Crataegus* L., *Cydonia* Tourn. *ex* Mill., *Pyrus* L., *Sorbus* L. and *Malus* Mill., the members of subtribe Pyrineae Dumont (tribe Pyreae of the subfamily Spiraeoideae) that correspond to the long-recognized subfamily Maloideae (Liu & Gao 1993; Schweingruber *et al.* 2011); it occurs also in *Exochorda* Lindl. (tribe Osmaronieae, subfamily Spiraeoideae) and *Purshia* DC. (subfam. Dryadoideae) (Lotova & Timonin 2005). Within the Rosoideae, this trait has been reported only in *Rosa* L. (Schweingruber *et al.* 2011). In contrast to these genera, *L. sericea* shows more regular alternation of conductive elements and crystalliferous axial parenchyma arranged into lines. This pattern of stratification of the secondary phloem has not yet been reported for the Rosaceae.

On the vessel walls of *L. sericea* there are mostly reticulate (rather than helical) coarse thickenings that are commonly associated with intervessel pits. These thickenings are usually absent in Rosoideae, but irregular ones occur in *Rosa* (Zhang 1992a,b). Within the closest relatives of *L. sericea* (*i.e.* woody members of tribe Sanguisorbeae), only *Polylepis* species show fine helical thickenings in some vessel elements (Zhang 1992a,b).

Pseudotori on pit membranes were observed in some intervessel pits of *C. strobilifera* and *L. sericea,* but we did not find these in *C. ruscifolia.* This feature occurs in many Rosaceae genera, including *Prunus* L. (Jansen *et al.* 2007), but it has never been reported in *Cliffortia* or *Leucosidea*. Pseudotori are thickenings on pit membranes that can be distinguished from genuine tori by their irregular shape and localization. Unlike tori, that are typical for bordered pits of conifers and also found in some members of a few angiosperm families [including the genus *Cercocarpus* Kunth in Rosaceae (Jansen

et al. 2007)], pseudotori are associated with plasmodesmata during their formation. Mature pseudotori are covered by secondary cap-like thickenings whereas the material of the primary cell wall layers undergoes autolysis (Rabaey *et al.* 2008). The function of pseudotori remains unknown.

The incrustations found in chambers of some intervessel pits in *Cliffortia strobilifera* resemble the coating within or around the pit membranes that has been reported in many tree species (*e.g.* Wheeler 1981; Sano & Fukuzava 1994; Sano 2005; Schmitz *et al.* 2012). The deposition of these materials is considered as a way to reduce the water permeability of the heartwood (Sano & Fukuzava 1994), or to prevent water loss during stressful periods (Wheeler 1981; Schmitz *et al.* 2012). As *C. strobilifera* shows no traits of heartwood formation, these incrustations in its intervessel pit membranes are more likely formed temporarily in response to seasonal droughts.

The unique structure of the wood and secondary phloem of *Leucosidea* provokes questions about the phylogenetic origin of this monotypic genus. The detailed anatomical descriptions presented here can be useful for future studies of phylogenetic and evolutionary relationships in the Sanguisorbeae, as well as for the identification of bark and wood material.

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