Plant Syst Evol (2010) 289:127–135
DOI 10.1007/s00606-010-0338-6

ORIGINAL ARTICLE

Wood and bark anatomy of *Centella*: scalariform perforation plates support an affinity with the subfamily Mackinlayoideae (Apiaceae)

A. A. Oskolski · B.-E. Van Wyk

Received: 23 September 2009 / Accepted: 1 July 2010 / Published online: 1 August 2010

Abstract Detailed descriptions of the wood and bark anatomy of five representative *Centella* species are presented. The wood structure is reported for the first time for this genus. Scalariform perforation plates occur frequently in the secondary xylem of two *Centella* species (*Centella differmis* and *C. rupestris*) indicating a phylogenetic relationship with *Apiopetalum* and *Mackinlaya*, the only other genera of Apiaceae where this feature occurs regularly and not merely as an aberration. This character commonly occurs in two other families of Apiales, Araliaceae and Myodocarpaceae, supporting the presumed basal position of subfamily Mackinlayoidae within Apiaceae. Surprisingly, no axial secretory canals occur in the secondary phloem of *Centella* species, apparently a unique feature within the suborder Apiineae, which comprises Araliaceae, Myodocarpaceae, Pittosporaceae, and Apiaceae. Other noteworthy characters of *Centella* include subepidermal origin of the periderm, tangential expansion of rays in dilated secondary phloem, poorly sclerified or non-sclerified collapsed secondary phloem, small intervessel pits with groove-like sculptures near their apertures, diffuse and marginal axial parenchyma, and a predominance of upright and square cells in the ray composition, all of which may be of value in exploring infrageneric relationships.

A. A. Oskolski (✉)
Botanical Museum, Komarov Botanical Institute,
Prof. Popov Street 2, St Petersburg 197376, Russia
e-mail: aoskolski@gmail.com

B.-E. Van Wyk
Department of Botany and Plant Biotechnology,
University of Johannesburg, P.O. Box 524,
Auckland Park 2006, Johannesburg, South Africa

Keywords Apiaceae · *Apiopetalum* · Araliaceae · Bark anatomy · *Centella* · *Mackinlaya* · Phylogeny · Wood anatomy

Introduction

The genus *Centella* L. comprises some 45 species of perennial or, rarely, annual herbs, which are mostly (with the exception of four species) endemic to the fynbos region of South Africa (Schubert and Van Wyk 1997; Schubert 2000). In most classification schemes proposed for Apiaceae (Drude 1898; Pimenov and Leonov 1993) this genus has been placed in the subfamily Hydrocotyloideae, distinguished by Drude (1898) on the basis of a woody fruit endocarp and laterally compressed schizocarp. However, studies over the last decade using molecular data have provided new insight into the phylogenetics of *Centella* that is important for clarification of the evolutionary relationships between the families Araliaceae, Apiaceae, and other members of the order Apiales.

The first molecular phylogenetic analyses of relationships within Apiales (Plunkett et al. 1996a, b, 1997) revealed that Hydrocotyloideae are polyphyletic, with the type genus *Hydrocotyle* L. referred to Araliaceae, whereas other genera remained in Apiaceae. These studies have shown a close relationship of *Centella* to *Apiopetalum* Baill. and *Mackinlaya* F. Muell., two genera traditionally recognized as a separate group of Araliaceae, and to the hydrocotyloid *Micropleura* Lag. Results of subsequent studies (Plunkett and Lowry 2001; Chandler and Plunkett 2004; Andersson et al. 2006) confirmed the close relationships among these four genera and suggested that *Actinotus* Lab., *Xanthosia* Rudge and *Platysace* Bunge may be additional members of this assemblage. All these

genera belong to a clade that is sister group to the large clade that includes all other Apiaceae. In a recent classification of Apiales proposed by Plunkett et al. (2004), *Centella*, *Mackinlaya*, *Apiopetalum*, *Micropleura*, *Actinotus*, *Xanthosia*, and *Platysace* were recognized as a new subfamily Mackinlayoideae Plunkett & Lowry within Apiaceae. Based on plastid sequence data from the *rpl16* intron and the *trnD–trnT* regions, further clarification of the phylogenetic positions of practically all the genera that formerly belonged to Hydrocotyloideae was provided by Nicolas and Plunkett (2009). Mackinlayoideae consistently formed a well-supported clade in all the analyses and included the genera *Centella*, *Micropleura*, *Schoenolaena* Bunge, *Pentapeltis* Bunge, *Xanthosia*, *Chlaenosciadium* C. Norman, *Mackinlaya*, *Apiopetalum*, and *Actinotus*. The relationships of *Platysace* remained unclear (Andersson et al. 2006) but this study showed for the first time that *Platysace* and *Homalosciadium* form a separate lineage sister to the other Apioideae (excluding Mackinlayoideae).

The placement of *Centella* within Mackinlayoideae is well-supported by the molecular phylogenetic analyses of Plunkett et al. (1996a, b, 1997, 2004) and Nicolas and Plunkett (2009). In contrast, morphological evidence for a relationship of this genus to *Mackinlaya*, *Apiopetalum*, and other Mackinlayoideae is very poor. In that context, wood and bark anatomical characters may provide complementary data for assessing relationships among the genera of Araliaceae, Myodocarpaceae, and Apiaceae as shown by several previous studies (Oskolski 1996, 2001; Oskolski et al. 1997, 2007; Oskolski and Lowry 2000; Oskolski and Van Wyk 2008; Kolalite et al. 2003; Kotina and Oskolski 2007). Until now, no information on the wood anatomy of *Centella* has been available. Brief descriptions of bark structure of 17 species of *Centella* were provided by Lemesle (1926). As for other members of Mackinlayoideae, the wood structure of *Apiopetalum* and *Mackinlaya* has been studied by Oskolski and Lowry (2000) and the bark anatomy of these two genera by Kotina and Oskolski (2007). This study surveys the wood and bark anatomy of five South African species of *Centella*. The results are examined with regard to the suggested placement of this genus within Mackinlayoideae.

Materials and methods

All except one of the stem samples examined were collected by the first author in 2006 during a field trip in the Cape region, South Africa. The thickest stem portion taken from the basal part of above-ground shoots was fixed in formalin–acetic acid–alcohol (FAA) (Johansen 1940). A stem sample of *Centella rupestris* was collected by Mahalia Schubert and the second author at Hermanus in the South Western Cape Province of South Africa (Table 1). These five species of *Centella* were selected to represent the major groups within the genus (Schubert 2000). Authorities for names are given in Table 1. Voucher specimens are deposited in JRAU and LE.

Transverse, radial and tangential sections, 15–30 μm thick, were prepared by use of a freezing microtome and stained with a 1% aqueous solution of safranin and light blue (Barykina et al. 2004). The FAA-fixed samples were also treated according to a modification of the method of Feder and O'Brien (1968) for embedding in glycol methacrylate (GMA). This modification involves final infiltration in GMA for 5 days. Transverse, radial, and tangential sections, approximately 3 μm thick, were cut using a Porter–Blüm ultramicrotome. The sections were stained with toluidine blue.

Maceration of secondary phloem was carried out for 24 h in a mixture of equal volumes of acetic acid and hydrogen peroxide at 60°C, whereas the secondary xylem was macerated in a nitric acid–chromium dioxide mixture (Johansen 1940). Lengths of vessel elements and sieve tube members were determined from the macerated material mounted in glycerin. The bark of the dried stem sample of *C. rupestris* was damaged and could not be studied.

Specimens for scanning electron microscopy were prepared according to the methods of Exley et al. (1977). The standardized descriptive terminology for wood structure proposed by Carlquist (2001) and the IAWA list of microscopic features for hardwood identification (IAWA Committee 1989) were followed throughout, except that the vertical dimension of the diameter of intervessel pits was also recorded. The terminology used to describe bark structure follows Trockenbrodt (1990).

Results

Wood structure

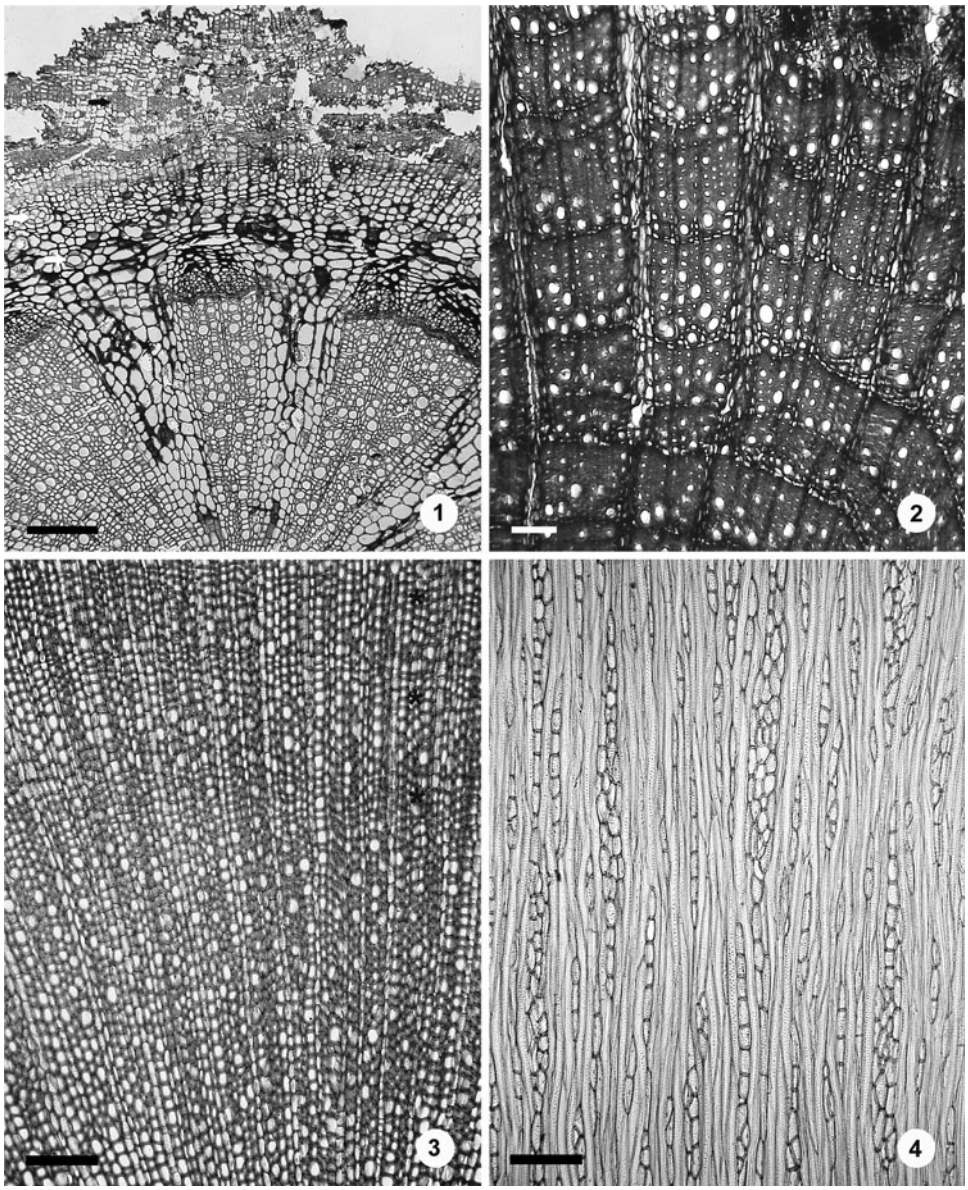
The stems of all five *Centella* species studied have very narrow rings of secondary phloem and xylem. These rings are interrupted by persistent medullary rays, which are especially wide in *C. difformis* (Fig. 1). The structure of the medullary rays is not described here.

Growth-ring boundaries are absent (*C. rupestris*, *C. triloba*, and *C. virgata*) to indistinct (Figs. 2, 3), marked by zones or rings of more numerous vessels (*C. difformis*, *C. rupestris*, and *C. villosa*) or/and by weak differences in vessel diameter between latewood and earlywood, and also by continuous lines of marginal parenchyma (*C. difformis* (Fig. 9) and *C. villosa*).

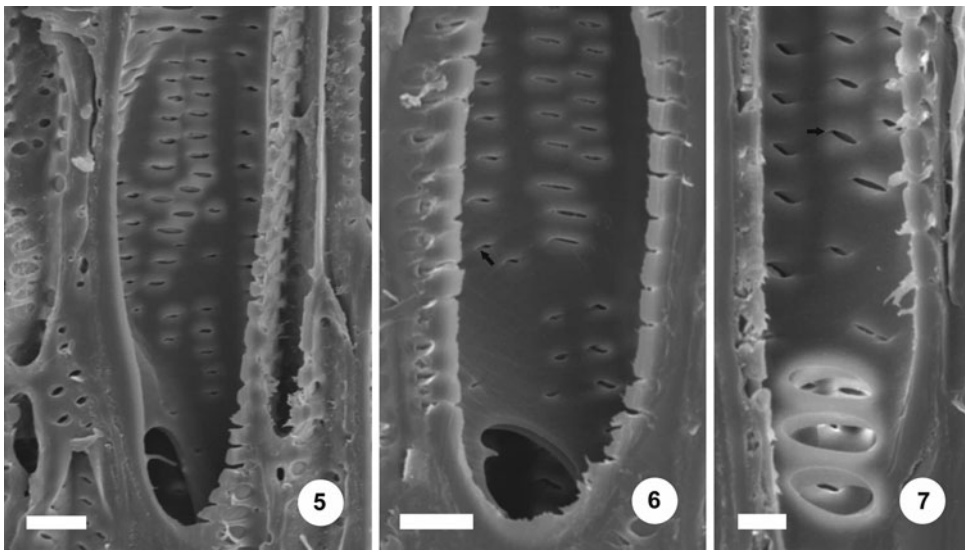
Vessels are rounded, rarely angular in outline, very narrow (tangential diameter up to 46 μm in *C. virgata*), and

Table 1 Voucher specimens and locality details of the wood and bark samples of *Centella* species examined

Species	Voucher specimen (Herbarium)	Locality	Habitat
Centella differmis (Eckl. & Zeyh.) Adamson	*Oskolski 11-06* (LE)	South Africa, Western Cape Province, Hermanus, Fernkloof Nature Reserve	Fynbos
C. rupestris (Eckl. & Zeyh.) Adamson	*Schubert & Van Wyk 55* (JRAU)	South Africa, Western Cape Province, Hermanus, Fernkloof Nature Reserve	Fynbos
C. triloba (Thunb.) Drude	*Oskolski 12-06* (LE)	South Africa, Western Cape Province, Hermanus, Fernkloof Nature Reserve	Fynbos
C. villosa L.	*Oskolski 37-06* (LE)	South Africa, Western Cape Province, Piekenierskloof Pass	Dry Fynbos
C. villosa L.	*Oskolski 41-06* (LE)	South Africa, Northern Cape Province, Cedarberg	Dry Fynbos
C. villosa L.	*Oskolski 42-06* (LE)	South Africa, Northern Cape Province, Cedarberg	Dry Fynbos
C. virgata (L.f.) Drude	*Oskolski s.n.* (LE)	South Africa, Western Cape Province, near Knysna	Afromontane Forest margin



Figs. 1–4 Wood and bark structure in some species of *Centella*. **1** Transverse section of stem of *C. differmis* showing wide medullary rays in wood, phellem with thick-walled sclerified cells (*black arrow*), axial secretory canals in cortex (*white arrows*), secondary phloem without axial secretory canals, **2** Transverse section of wood of *C. villosa* [AO42-06] showing indistinct growth rings marked by zones of wider and more numerous vessels and by lines of marginal axial parenchyma, **3**, **4** Wood structure of *C. rupestris*, **3** Transverse section showing very indistinct growth rings marked by zones of less numerous vessels (*asterisks*), **4** Tangential section showing rays composed mostly of square and upright cells with a few procumbent cells. *Scale bars* in 1, 3, 4 = 200 μm, in 2 = 100 μm



Figs. 5–7 Scanning electron micrographs of vessel elements in *Centella difformis*. **5** Simple perforation plate with vestigial bar, vessel-axial parenchyma pits, **6–7** Alternate intervessel pitting, groove-like wall sculptures near pit apertures (*arrows*), **6** Simple perforation plate, **7** Scalariform perforation plate with two bars. *Scale bars* in 5, 6 = 10 μm, in 7 = 5 μm

extremely numerous (vessel frequency varies in *C. villosa* from 277 per mm^2 [AO42-06] to 685 per mm^2 [AO37-06]), mostly solitary (Fig. 3), sometimes in small clusters and radial multiples of up to thirteen in *C. rupestris,* and up to six in other samples. Tyloses were not found. Vessel elements are (100–) 204–360 (–505) μm long.

Perforation plates are mostly simple (Figs. 5, 6); scalariform perforation plates with few (up to three) bars are quite common (ca. 20% of all perforation plates) in *C. difformis* (Fig. 7) and rarely also in *C. rupestris*. Intervessel pits alternate (rarely opposite and transitional from scalariform to alternate), 1.5–6.3 μm in vertical size, mostly with rounded margins and slit-like or lens-like apertures surrounded by shallow, groove-like wall sculpturing (Figs. 6, 7). Vessel-ray and vessel-axial parenchyma pits are simple or with indistinct borders, mostly similar to intervessel pits in size and shape, sometimes oval and scalariform (not found in *C. villosa*, and quite common in *C. difformis* and *C. rupestris*). Unilaterally compound pits (i.e. horizontally elongated pits) are present (along with diagonally to vertically elongated pits in *C. rupestris* and *C. trilobata*) on the ray cell walls. Up to three pits occur on the vessel walls (up to five in *C. rupestris*) in all the species studied except *C. villosa*. No helical thickenings were found on the vessel element walls. Vascular tracheids were not found.

Fibres are libriform, moderately thick-walled to thick-walled. The fibre walls are 1.4–4.5 μm thick, with few simple to minutely bordered pits, with slit-like apertures in the radial walls. Septate fibres were not found.

Axial parenchyma is diffuse to scanty vasicentric (Fig. 8) in solitary strands (in *C. villosa* [AO41-06, AO42-06] but rarely also aggregated into short lines), and banded (sometimes marginal) in continuous 1–2-seriate lines (in *C. difformis* (Fig. 9) and *C. villosa*) consisting of strands of 2–4 cells.

Rays (Fig. 4) are numerous (12–18 per mm), uni and multiseriate (mostly 1 to 3-seriate, up to 6-seriate); ray cells are small (up to 28 μm in tangential diameter). Ray height is commonly <1 mm, but a few rays >1 mm occur. Multiseriate rays are composed of upright, square cells and a few procumbent cells mixed throughout the ray, with long uniseriate portions (up to 18 marginal rows). Uniseriate rays are composed almost exclusively of square and upright cells. Radial canals are absent. Crystals were not found.

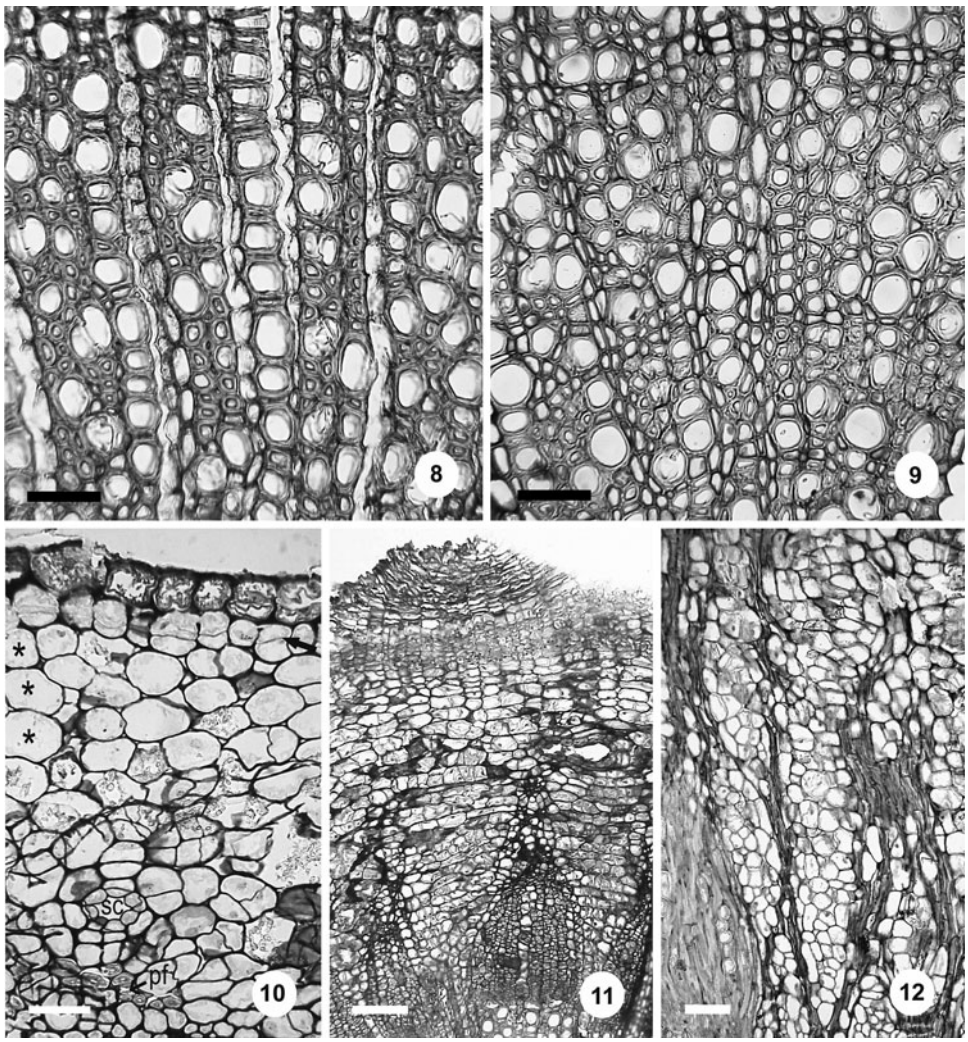
Bark structure

The epidermis is formed by a single layer of isodiametric (rarely somewhat radially flattened), thin-walled cells (with thicker outer walls in *C. triloba* and *C. villosa* [AO41-06]), rounded or rectangular in outline.

Periderm is of subepidermal origin (Fig. 10). The phellem is composed of 5–25 layers of thin-walled (or thin to thick-walled sclerified in *C. difformis* (Fig. 1) and *C. villosa* [AO41-06]), isodiametric to radially flattened cells (only radially flattened cells in *C. virgata* (Fig. 11); radially elongated cells occur in *C. villosa* [AO41-06, AO42-06]).

Phelloderm is composed of 5–15 layers of thin-walled, radially flattened cells. Druses of calcium oxalate occur in phelloderm cells in *C. difformis*, *C. villosa* [AO41-06], and *C. virgata*. Some crystalliferous phelloderm cells of *C. virgata* are 2–3-chambered, subdivided by radial walls.

The cortical collenchyma is lamellar and comprises one or two cell layers. Tangential diameter of collenchyma cells is 15–50 μm. Cortical parenchyma is formed by 12–20 layers of isodiametric or somewhat axially elongated, thin-walled parenchyma cells of 20–50 (up to 70) μm in tangential diameter. The outer 3–6 layers of



Figs. 8–12 Transverse sections of wood of *Centella* species showing different types of axial parenchyma. **8** *C. virgata*, scanty vasicentric and diffuse axial parenchyma, **9** *C. difformis*, axial parenchyma in 1–2-seriate lines and diffuse. Figs. **10–12** Bark structure in some species of *Centella*, **10** Transverse section of epidermis and cortex of *C. villosa* [AO37-06] showing subepidermal initiation of phellogen (*black arrow*), chlorenchyma cells in cortical parenchyma (*asterisks*), axial secretory canal (*sc*), primary phloem fibres (*pf*), **11** Transverse section of periderm, dilated cortex and secondary phloem in *C. virgata* showing the phellem composed of radially flattened thin-walled cells, secondary phloem without axial secretory canals, secretory phloem rays dilated by tangential expansion, **12** Tangential section of secondary phloem in *C. virgata* showing uniseriate and multiseriate secondary phloem rays. *Scale bars* in 8, 9, 12 = 100 μm, in 10 = 50 μm, in 11 = 200 μm

cortical parenchyma in *C. villosa* comprise chlorenchyma cells with large intercellular spaces (Fig. 10).

Axial secretory canals are present in the cortex of all the species examined (Fig. 10). Axial canals are lined by a single or double (most common in *C. triloba*) layer of 4–6 cells in *C. difformis* (Fig. 1) and 5–9 epithelial cells in other species. The lumina of the axial secretory canals in non-dilated cortex are 15–30 μm in diameter. Primary bark fibres are absent from *C. virgata* but in other species are thick-walled and aggregated into small groups or clusters, each 2–6 tangential layers deep (Fig. 10).

Dilatation of the cortical tissue is effected mostly by tangential stretching of cells (in *C. virgata* also by anticlinal division of cortical collenchyma and parenchyma cells). Axial secretory canals in dilated cortex are 20–70 μm in diameter (up to 130 μm in *C. virgata*). The number of epithelial cells in the canals increases to 15 in *C. virgata*. Druses are common in the cells of dilated cortex.

The secondary phloem is composed of tangential zones of 15–25 cell layers, comprising sieve elements, companion cells, and axial phloem parenchyma cells. The transition from non-collapsed to collapsed secondary phloem is gradual. Sieve tube members are 10–20 μm wide and 120–320 μm long (Table 2). Sieve plates are compound with 2–8 sieve areas located on oblique cross walls. Axial parenchyma cells are mostly fusiform or occur in strands of two or three. Druses and prismatic crystals are present in axial parenchyma cells of collapsed phloem. No axial secretory canals were found in the secondary phloem (Figs. 1, 11).

Secondary phloem rays (Fig. 12) are uniseriate and 2–8-seriate (up to 12-seriate in *C. villosa* [AO42-06] and 15-seriate in *C. virgata*). Multiseriate rays are composed of upright, square cells and a few procumbent cells mixed throughout the ray. Uniseriate rays are composed mostly of upright, square cells. Cells of dilated rays are extensively enlarged, mostly by tangential expansion and also by

Table 2 Wood and bark anatomical characters of *Centella* species

Species and voucher specimens	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Centella differmis [AO11-06]	3	273 ± 9.9 136–398	4.9/6.5	440	21 ± 0.7 12–30	1.6/5	44.3	417 ± 12.9 264–530	2.9/6	0.55/1.1	9.2	6.8	16.0	14.9/20	222 ± 11.8 155–320
C. rupestris [Schubert & Van Wyk 55]	6	363 ± 9.7 276–456	3.7/4.6	540	13 ± 0.5 6–19	2.7/13	12.7	456 ± 9.6 312–588	3.1/6	0.75/2.3	6.3	9.3	15.6	17.0/28	–
C. triloba [AO12-06]	3	263 ± 18.7 163–415	4.6/6.3	425	17 ± 0.8 10–25	2.0/5	21.2	365 ± 11.2 224–498	2.9/5	0.70/1.2	9.1	9.3	18.4	16.3/22	207 ± 6.2 170–245
C. villosa [AO37-06]	4	227 ± 11.6 98–315	4.7/5.8	685	14 ± 0.8 7–25	1.7/6	33.6	350 ± 11.0 259–433	3.0/6	0.41/1.1	7.4	5.2	12.6	11.0/17	203 ± 10.2 120–262
C. villosa [AO41-06]	3	204 ± 8.8 127–272	3.3/4.3	325	24 ± 1.1 14–39	1.7/5	33.8	400 ± 12.8 238–662	3.2/6	0.62/1.9	14.6	3.3	17.8	9.8/19	224 ± 8.4 145–295
C. villosa [AO42-06]	5	259 ± 15.0 117–505	5.1/7.0	277	16 ± 0.9 9–22	1.2/3	63.1	351 ± 10.5 249–537	3.3/6	0.47/2.5	8.9	9.3	18.2	13.9/20	244 ± 12.0 157–307
C. virgata [AO s.n.]	4	247 ± 11.6 148–344	3.6/4.7	357	29 ± 1.6 12–46	1.4/5	48.2	357 ± 10.7 227–482	2.8/6	0.68/1.6	8.7	8.3	17.0	12.2/19	210 ± 11.1 155–290

1, Radius of wood sample (mm); 2, length of vessel elements (mean/min–max, μm); 3, vertical size of intervessel pits (mean/maximum); 4, vessel frequency (per mm^2); 5, tangential diameter of vessels (mean/min–max, μm); 6, mean/greatest number of vessels in a vessel group; 7, percentage of solitary vessels; 8, mean length of libriform fibres (mean/min–max, μm); 9, width of multiseriate rays (mean/max, cells); 10, height of multiseriate rays (mean/max, mm); 11, number of multiseriate rays per mm; 12, number of uniseriate rays per mm; 13, total number of rays per mm; 14, tangential size of ray cells (mean/max, μm); 15, length of sieve tube members (mean/min–max, μm)

anticlinal divisions (ray width in dilated rays up to 16-seriate).

Radial secretory canals are found in the rays of *C. villosa* [AO41-06] and *C. virgata*. The lumen diameter of the radial secretory canals is mostly 30–70 μm (up to 130 μm in *C. virgata*). Druses, crystal sand, and sometimes prismatic crystals occur in the ray cells.

Discussion

The results of our anatomical study of the stem structure in selected species of *Centella* support the placement of this genus within the subfamily Mackinlayoideae and confirm its relationship to *Apiopetalum* and, especially, to *Mackinlaya*, as suggested by a recent classification system for the order Apiales based primarily on molecular phylogenetic analyses (Plunkett et al. 1996a, 2004; Andersson et al. 2006). *Centella* and *Mackinlaya* (Oskolski and Lowry 2000; Kotina and Oskolski 2007) share several wood and bark features, including subepidermal origin of the periderm, tangential expansion of rays in dilated secondary phloem, poorly sclerified or non-sclerified collapsed secondary phloem, the occurrence of scalariform perforation plates with a few bars, small intervessel pits (up to 6 μm in vertical diameter) with groove-like sculptures near their apertures, diffuse and marginal axial parenchyma (the latter occurring only in C. *differmis* and *C. villosa,* and in *Mackinlaya*), and a predominance of upright and square cells in the ray composition (heterogeneous rays of the IIA type, according to the classification of Kribs (1935)). *Apiopetalum* is more distinct from these two genera in its wood and bark structure but it also has most of these features. In contrast with *Centella* and *Mackinlaya*, however, *Apiopetalum* is characterized by sclerified collapsed secondary phloem, the occurrence of scalariform perforation plates with numerous bars (along with the simple ones) and by the presence of diffuse-in-aggregates axial parenchyma. Although each of these characters has also been observed in other genera of Apiaceae, Araliaceae, and/or Myodocarpaceae, their combined occurrence in *Centella*, *Mackinlaya*, and *Apiopetalum* is notable and supports the suggestion that these three genera are closely related.

The occurrence of scalariform perforation plates with a few bars in some *Centella* species is particularly noteworthy. Although this feature has been found in some genera of Apiaceae, for example *Aethusa* L., *Bupleurum* L., *Carum* L., *Oenanthe* L., *Peucedanum* L., and *Xanthosia* (Solereder (1908) as cited in Metcalfe and Chalk (1950)), it is important to note that all these reports referred to occasional aberrations and not to the perforation plates present regularly in the species studied. Such aberrant occurrences were also reported for other taxa with predominantly simple perforation plates (e.g. in many genera of Rosaceae (Zhang 1992)) but this feature is not commonly regarded as important for their phylogenetics or systematics. In contrast, ca. 20% of all perforation plates in *C. differmis* have one, two, or three bars, and this condition, therefore, cannot be regarded as aberrant. Among Apiaceae (sensu Plunkett et al. 2004), the frequent occurrence of scalariform perforation plates (with the simple plates) is also characteristic for *Mackinlaya* and *Apiopetalum* (Oskolski and Lowry 2000), but this feature has not been found in other woody members of the family (Rodriguez 1957; Oskolski 2001). Our finding provides the first morphological evidence of a relationship between *Centella*, *Mackinlaya*, and *Apiopetalum* to support the finding from molecular analyses. Scalariform perforation plates are also present in Araliaceae, Myodocarpaceae, and other families of the order Apiales (with the exception of Pittosporaceae) but not in any other genera of Apiaceae. Their occurrence in three genera of Mackinlayoideae is therefore congruent with the idea of a basal position for this lineage in relation to other clades within Apiaceae, suggesting that the loss of scalariform perforation plates may be a synapomorphy for all other Apiaceae.

These similarities notwithstanding, *Centella* is sharply distinct from *Mackinlaya* and, especially, from *Apiopetalum* by several features, including shorter vessel elements (their mean length is 200–360 μm in *Centella* vs. 520–770 μm in *Mackinlaya*) and fibres, much higher vessel frequency, absence of paratracheal axial parenchyma, and of axial secretory canals in secondary phloem. Most of this variation may be caused by ecological factors rather than reflecting phylogenetic relationships. The vessel characteristics of *Centella* are typical for its habit (small shrubs and perennial herbs) and for plants from dry environments (Baas and Schweingruber 1987; Wheeler and Baas 1993; Carlquist 2001). The lack of paratracheal axial parenchyma may be the result of the very high vessel frequency. It is difficult to distinguish diffuse axial parenchyma from the scanty paratracheal parenchyma in a wood with very numerous small vessels which have a diameter similar to that of the axial parenchyma cells.

The absence of axial secretory canals in the secondary phloem of *Centella* is very surprising because its presence has been reported in all the species of the suborder Apiineae (Plunkett et al. 2004), i.e. for all members of Araliaceae, Myodocarpaceae, Pittosporaceae, and Apiaceae examined to date (Viguier 1906; Metcalfe and Chalk 1950; Holdheide 1951; Rodriguez 1957; Zahur 1959; Roth 1981; Kolalite et al. 2003; Oskolski et al. 2007; Nilova and Oskolski 2010). Although Metcalfe and Chalk (1950) noted that "secretory canals present in the inner part of the primary cortex, as well as in pericycle and sometimes in secondary phloem of most if not of all members of the

family [Apiaceae]", these authors did not specify in which taxa axial secretory canals are absent from the secondary phloem. The lack of these secretory structures in *Centella* is probably caused by the reduced amount of secondary phloem in the stems.

Although the species of *Centella* studied are rather uniform in stem structure, there are also some conspicuous differences in their wood and bark. *Centella differmis* and *C. villosa* share the presence of sclerification of periderm cells and of marginal axial parenchyma—two features which are absent in other species. According to the infrageneric classification system proposed by Schubert (2000), these two species are closely related and belong to the type section of the genus. The absence of primary phloem fibers in *C. virgata* may be characteristic for section *Virgatae*. Our limited sampling does not enable any additional conclusions about relationships within the genus. A wider survey combined with an expanded molecular phylogenetic study is likely to reveal taxonomically useful anatomical characters for the genus *Centella*.

Acknowledgments The authors acknowledge financial support from the University of Johannesburg, the National Research Foundation of South Africa, and the Russian Foundation of Basic Research (grant 09-04-00618). The authors are also grateful to E. S. Chavchavadze for valuable consultations. Institutional support was provided to the first and second authors by the Botanical Museum, V. L. Komarov Botanical Institute, St Petersburg, Russia and the Department of Botany and Plant Biotechnology, University of Johannesburg, South Africa.

References

Andersson L, Kocsis M, Eriksson R (2006) Relationships of the genus *Azorella* (Apiaceae) and other hydrocotyloids inferred from sequence variation in three plastid markers. Taxon 55:270–280

Baas P, Schweingruber FH (1987) Ecological trends in the wood anatomy of trees, shrubs and climbers from Europe. IAWA Bull ns 8:245–274

Barykina RP, Veselova TD, Deviatov AG, Djalilova HH, Iljina GM, Chubatova NV (2004) Handbook of the botanical microtechniques (in Russ). Moscow University Press, Moscow

Carlquist S (2001) Comparative wood anatomy. Berlin, Springer

Chandler GT, Plunkett GM (2004) Evolution in Apiales: nuclear and chloroplast markers together in (almost) perfect harmony. Bot J Linn Soc 144:123–147

IAWA Committee (1989) IAWA list of microscopic features for hardwood identification. IAWA J 10:219–322

Drude O (1898) Umbelliferae. In: Engler A, Prantl K (eds) Die Natürlichen Pflanzenfamilien, vol 3(8). Wilhelm Engelmann, Leipzig, pp 63–250

Exley RR, Meylan BA, Butterfield BG (1977) A technique for obtaining clear cut surfaces on wood samples prepared for the scanning electron microscope. J Microsc 110:75–78

Feder N, O' Brien TP (1968) Plant microtechnique: some principles and new methods. Am J Bot 55:123–142

Holdheide W (1951) Anatomie mitteleuropäischer Gehölzrinden. Handbuch der Mikroskopie in der Technik 5(1):195–367

Johansen DA (1940) Plant microtechnique. McGraw–Hill Book Company, NY

Kolalite MR, Oskolski AA, Richter HG, Schmitt U (2003) Bark anatomy and intercellular canals in the stem of *Delarbrea paradoxa* (Araliaceae). IAWA J 24:139–154

Kotina EL, Oskolski AA (2007) Bark anatomy of *Apiopetalum* and *Mackinlaya* (Apiales) (in Russ). Bot Zhurn (St Petersburg) 92(10):1490–1499

Kribs DA (1935) Salient lines of structural specialization in the wood rays of dicotyledons. Bot Gaz 96:547–557

Lemesle R (1926) Contribution à l'étude structurale des ombellifères xérophiles. Ann Sci Nat Bot 10:1–138

Metcalfe CR, Chalk L (1950) Anatomy of the dicotyledons, vol 1. Clarendon Press, Oxford

Nicolas AN, Plunkett GM (2009) The demise of subfamily Hydrocotyloideae (Apiaceae) and the re-alignment of its genera across the entire order Apiales. Mol Phylogenet Evol 53:134–151

Nilova MV, Oskolski AA (2010) Comparative bark anatomy of *Bursaria*, *Hymenosporum* and *Pittosporum* (Pittosporaceae). Plant Div Evol 128/1 (in press)

Oskolski AA (1996) A survey of the wood anatomy of the Araliaceae. In: Donaldson LA, Singh AP, Butterfield BG, Whitehouse LJ (eds) Recent advances in wood anatomy. New Zealand Forest Research Institute, Rotorua, pp 99–119

Oskolski AA (2001) Systematic and phylogenetic wood anatomy of Apiales. Edinb J Bot 58:201–206

Oskolski AA, Lowry PP II (2000) Wood anatomy of *Mackinlaya* and *Apiopetalum* (Araliaceae) and its systematic implications. Ann Missouri Bot Gard 87:171–182

Oskolski AA, Van Wyk B-E (2008) Systematic and phylogenetic value of wood anatomy in Heteromorpheae (Apiaceae, Apioideae). Bot J Linn Soc 158:569–583

Oskolski AA, Lowry PP II, Richter H-G (1997) Systematic wood anatomy of *Myodocarpus*, *Delarbrea*, and *Pseudosciadium* (Araliaceae). Adansonia 3(19):61–75

Oskolski AA, Kotina EL, Fomichev IV, Tronchet F, Lowry PP II (2007) Systematic implications of wood and bark anatomy in the Pacific Island genus *Meryta* (Araliaceae). Bot J Linn Soc 153:363–379

Pimenov MG, Leonov MV (1993) The genera of the Umbelliferae: a nomenclator. Royal Botanic Gardens, Kew

Plunkett GM, Lowry PP II (2001) Relationships among "ancient araliads" and their significance for the systematics of Apiales. Mol Phylogenet Evol 19:259–276

Plunkett GM, Soltis DE, Soltis PS (1996a) Higher level relationships of Apiales (Apiaceae and Araliaceae) based on phylogenetic analysis of *rbcL* sequences. Am J Bot 83:499–515

Plunkett GM, Soltis DE, Soltis PS (1996b) Evolutionary patterns in Apiaceae: inferences based on *matK* sequence data. Syst Bot 21:477–495

Plunkett GM, Soltis DE, Soltis PS (1997) Clarification of the relationships between Apiaceae and Araliaceae based on *matK* and *rbcL* sequence data. Am J Bot 84:565–580

Plunkett GM, Chandler GT, Lowry PP II, Pinney SM, Sprenkle TS (2004) Recent advances in understanding Apiales and a revised classification. S Afr J Bot 70:371–381

Rodriguez RL (1957) Systematic anatomical studies on *Myrrhidendron* and other woody Umbellales. Univ Calif Publ Bot 29:145–318

Roth I (1981) Structural patterns of tropical barks. Gebrüder Borntraeger, Berlin

Schubert MTR (2000) A taxonomic study of the genus *Centella* (Apiaceae) Ph.D. Thesis. University of Johannesburg

Schubert MTR, Van Wyk B-E (1997) A revision of *Centella* series *Capenses* (Apiaceae). Nord J Bot 17:301–314

Solereder H (1908) Systematic anatomy of the dicotyledons. Clarendon Press, Oxford

Trockenbrodt M (1990) Survey and discussion in the terminology used in bark anatomy. IAWA Bull (New Series) 11(2):141–166

Viguier R (1906) Recherches botaniques sur la classification des Araliacées. Ann Sci Nat Bot 9(4):1–210
Wheeler EA, Baas P (1993) The potentials and limitations of dicotyledonous wood anatomy for climatic reconstructions. Paleobiology 19:487–498
Zahur MS (1959) Comparative study of secondary phloem of 423 species of woody dicotyledons belonging to 85 families. Mem Cornell Univ Agr Exp Sta 58:1–160
Zhang SY (1992) Wood anatomy of the Rosaceae. Rijksherbarium/Hortus Botanicus, Leiden