



The systematic significance of bark structure in southern African genera of tribe Heteromorpheae (Apiaceae)

EKATERINA L. KOTINA^{1,2}, BEN-ERIK VAN WYK^{1*}, PATRICIA M. TILNEY¹ and ALEXEI A. OSKOLSKI²

¹Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, Johannesburg, South Africa

²Botanical Museum, V.L. Komarov Botanical Institute of the Russian Academy of Science, Prof. Popov Str. 2, 197376 St Petersburg, Russia

Received 18 May 2011; revised 9 October 2011; accepted for publication 8 December 2011

The bark anatomy of five southern African genera of tribe Heteromorpheae (Apiaceae) has been investigated: *Anginon* (eight species), *Glia* (one species), *Heteromorpha* (two species), *Polemannia* (two species) and *Dracosciadium* (one species). They are all similar to other members of the order Apiales and share with them the presence of secretory canals in both the cortex and secondary phloem, the presence of two types of axial parenchyma and the absence of fibres in the secondary phloem. The bark of Heteromorpheae typically has a narrow cortex with secretory canals arranged in one ring and radial dilatations of the secondary phloem, but these character states also occur in other genera of the family. The most remarkable differences between the genera are the appearance of the bark surface, the periderm structure, the presence of primary phloem fibres, the length of sieve tubes and sclerification of the axial parenchyma in collapsed secondary phloem. The trunks of *Heteromorpha* and *Polemannia* have translucent phellem and chloroplasts in the phelloderm cells, allowing the stems to photosynthesize. The shape of the epidermal cells, the presence of trichomes and the occurrence of secretory canals in the secondary phloem rays can be used as diagnostic characters for species identification. © 2012 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2012, 169, 677–691.

ADDITIONAL KEYWORDS: anatomy – Apiales – cortex – chloroplasts – epidermis – periderm – secondary phloem – secretory canals – stem.

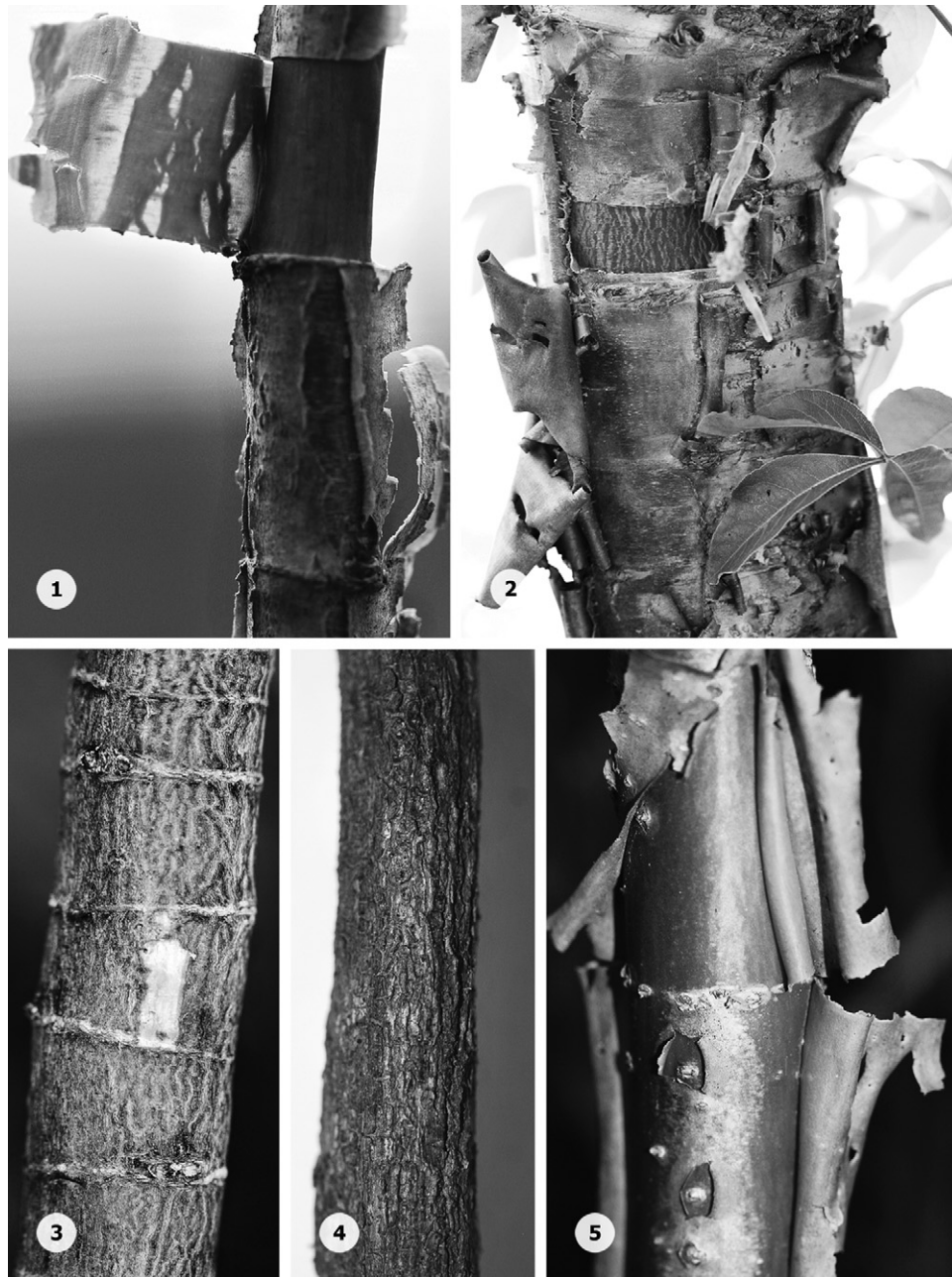
INTRODUCTION

Bark surface characters have often been used for the identification of trees and for taxonomic and phylogenetic studies (Whitmore, 1962; Roth, 1981; Yunus, Yunus & Iqbal, 1990). The external appearance of stems bearing periderm or rhytidome is highly variable (Evert, 2006) and therefore of diagnostic value. In the same way, anatomical characters of the bark have taxonomic value and can be used for clarification of the systematic position of anomalous or problematic taxa (Metcalf & Chalk, 1950; Chattaway, 1953; Junikka & Koek-Noorman, 2007; Kotina & Oskolski, 2010). However, few studies are available in which

both external and internal features of the bark have been included; notable exceptions are the studies of *Eucalyptus* L'Hérit. by Chattaway (1953) and Annonaceae by Junikka & Koek-Noorman (2007).

Heteromorpha arborescens (Spreng.) Cham. & Schlttdl. (Apiaceae, tribe Heteromorpheae) is a woody tree ≤ 15 m tall with a thick trunk (≤ 25 cm) and a distinctive smooth, peeling bark surface. When this parchment-like layer is removed, a bright green layer is revealed, even on the trunk of large trees (Figs 1, 2). Species of the related genus *Polemannia* Eckl. & Zeyh. have a similar bark surface (Fig. 5), but other genera such as *Anginon* Raf. and *Glia* Sond. have a fissured and brittle bark surface (Figs 3, 4). These observations prompted us to study the relation between surface morphology and anatomical structure

*Corresponding author. E-mail: bevanwyk@uj.ac.za



Figures 1–5. Appearance of bark surface. Fig. 1. Translucent phellem and green phelloderm in *Heteromorpha arbore-scens* var. *arbore-scens*. Fig. 2. Peeling, flexible bark surface and green phelloderm in *H. arbore-scens* var. *abyssinica* [KK30-10]. Fig. 3. Non-peeling, brittle, mature bark with shallow-fissured surface in *Anginon difforme* [KK23-10]. Fig. 4. Non-peeling, brittle, mature bark with shallow-fissured surface in *Glia prolifera*. Fig. 5. Smooth, peeling bark with a waxy parchment-like surface in *Polemanna simplicior* [BEVW4230].

of the bark of *Heteromorpha* Cham. & Schtdl. and related southern African genera. These plants have been the subject of several recent taxonomic studies by Allison & Van Wyk (1997) and Van Wyk, Allison & Tilney (1997) on *Anginon*, Van Wyk, Tilney & Magee (2010) on *Glia* and Winter & Van Wyk (1995) on

Heteromorpha, but the systematic value of bark characters has not yet been evaluated.

Apiaceae (Umbelliferae) are mostly herbs; trees and shrubs are unusual and most occur in Africa. One predominantly woody group is tribe Heteromorpheae, described recently (Downie *et al.*, 2001), originally to

accommodate five African genera that formed a distinct, early-branching clade in several molecular systematic studies of Apiaceae subfamily Apioideae (Plunkett, Soltis & Soltis, 1996a, b; Downie & Katz-Downie, 1999; Downie *et al.*, 2001). These genera are *Anginon*, *Dracosciadium* Hilliard & B.L.Burt, *Glia*, *Heteromorpha* and *Polemanna*.

Anginon comprises 13 species of shrubs or small trees endemic to southern Africa (from the Eastern, Western and Northern Cape Provinces of South Africa northwards to southern Namibia). *Dracosciadium* is a genus of two species of perennial herbs with woody rhizomes endemic to the eastern region of South Africa (KwaZulu-Natal and the Mpumalanga Province). Until recently *Glia* was considered to be monotypic [*G. prolifera* (Burm.f.) B.L.Burt only], but two new species were recently described (Van Wyk *et al.*, 2010). The plants are suffruticose (the lower parts of the stems are persistent and woody) with tuberous roots. *Heteromorpha* is a genus of seven species of shrubs and trees (≤ 15 m in height) with a wide distribution in Africa, from South Africa northwards to West and East Africa and extending into the Arabian Peninsula (Yemen only). *Polemanna* is a genus of three closely related shrubs or small trees from the eastern parts of South Africa and Lesotho. Based on molecular evidence, the tribe has since been expanded to include *Aframmi* C.Norman, *Andriana* B.-E.van Wyk, *Anisopoda* J.G.Baker, *Cannaboides* B.-E.van Wyk, '*Oreofraga*', *Pseudocannaboides* B.-E.van Wyk, *Pseudocarum* C.Norman and *Tana* B.-E.van Wyk (Calviño *et al.*, 2006).

The wood anatomy of the tribe was studied in detail by Oskolski & Van Wyk (2008), but information about bark anatomy is scant. Metcalfe & Chalk (1950) noted in *Heteromorpha arborescens* the presence of secretory canals in the cortex and secondary phloem and subepidermal initiation of the periderm. The revision of *Heteromorpha* by Winter & Van Wyk (1995) did not include descriptions of the anatomy (Winter's unpublished thesis of 1994 includes a description of the anatomy of young stems, but no information about secondary tissue).

The aim of this paper is therefore to describe bark structural characters of the woody members of Heteromorpheae from southern Africa and to assess their usefulness in systematics at all taxonomic levels. This work is part of a general study of the diversity and evolution of bark structure in Apiales (Kotina & Oskolski, 2007, 2010; Nilova & Oskolski, 2010; Oskolski & Van Wyk, 2010; Oskolski, Rossouw & Van Wyk, 2010).

MATERIAL AND METHODS

The origin of the material used is listed in Table 1. Three pieces of stem (20–100 mm long) were cut: (1)

from branch tips without a visible periderm layer; (2) from lower parts of the stem where periderm was starting to form; and (3) from thicker stems with mature bark that had more or less thick periderm. These were fixed in formalin–acetic acid–alcohol (FAA) (Johansen, 1940). Sections of *Heteromorpha arborescens* var. *abyssinica* (A.Rich.) H.Wolff and *P. montana* Schltr. & H.Wolff were prepared from fresh material (temporarily stored in water, with no fixing fluid). Fresh samples of *Anginon difforme* (L.) B.L.Burt [KK37-11] and *A. rugosum* (Thunb.) Raf. were kept for a few days in 10% alcohol before sectioning.

Transverse, radial and tangential sections (15–30 μ m thick) were cut with a freezing microtome (Ernst Leitz GMBH, Wetzlar, Germany). The sections were then stained with a 1:1 alcian blue/safranin mixture. Stained and unstained sections of *H. arborescens* var. *abyssinica*, *P. montana*, *A. difforme* [KK37-11] and *A. rugosum* were examined and mounted in Euparal or glycerol. Maceration of secondary phloem was carried out in Jeffrey's solution for 24 h. Lengths of sieve-tube members were determined from the macerated material mounted in glycerol.

Sections were photographed with an Olympus Color View Soft Imaging System and measurements were taken with the Olympus Analysis Imaging Solutions (OASIS) programme.

The descriptive terminology for bark structure follows Trockenbrodt (1990) and Junikka (1994). Parenchyma associated with the axial secretory canals is termed 'sheath parenchyma' after Roth (1981), whereas the term 'axial phloem parenchyma' is used for parenchyma associated with the conductive elements (sieve tubes and companion cells).

Evolutionary pathways for bark features were reconstructed by mapping of their character states onto a portion of the strict consensus tree derived from maximum parsimony (MP) analyses of nuclear rDNA internal transcribed spacer (ITS) (Calviño *et al.*, 2006). Evolutionary traces of the character states along the tree branches were visualized by the parsimony reconstruction method using the Character History Tracing option in the Mesquite 2.74 package (Maddison & Maddison, 2010).

RESULTS

Detailed descriptions of the bark of all genera of southern African Heteromorpheae are provided below.

The surface of young stems is smooth, green (sometimes reddish) and covered by a waxy layer in *Anginon paniculatum* (Thunb.) B.L.Burt, *A. rugosum*, *A. wellendamense* (Eckl. & Zeyh.) B.L.Burt, or by prominent [*A. verticillatum* (Sond.) B.L.Burt and *Polemanna montana*] or scant (*Heteromorpha* spp.) indumentum.

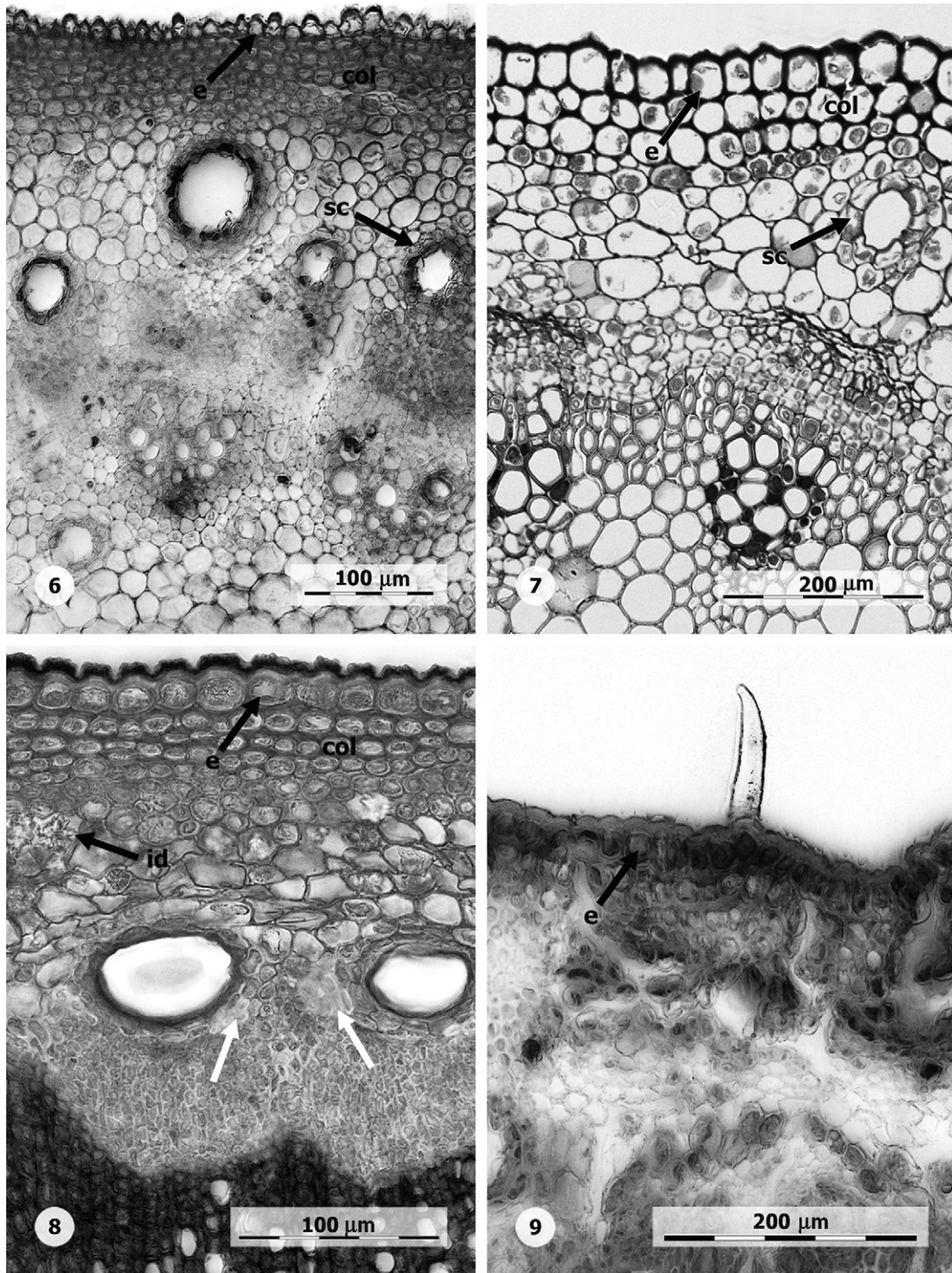
Table 1. Bark samples of members of tribe Heteromorphae. Provinces in South Africa are abbreviated (EC, Eastern Cape; FS, Free State; G, Gauteng; KZN, KwaZulu-Natal; LP, Limpopo; MP, Mpumalanga; NC, Northern Cape; WC, Western Cape)

Taxon	Collector's number and herbarium (all in JRAU unless stated otherwise)	Locality
<i>Anginon difforme</i> (L.) B.L.Burttt	I. Allison 186 [IA186]	NC, top of Nababiepsberg
<i>Anginon difforme</i>	A. Oskolski 14-09 [AO14-09] (LE)	WC, Kirstenbosch Botanical Garden
<i>Anginon difforme</i>	K. Kotina 23-10 [KK23-10]	NC, Namaqualand, Nourivier
<i>Anginon difforme</i>	K. Kotina 37-11 [KK37-11]	EC, near Alexandria
<i>Anginon fruticosum</i> I.Allison & B.-E.van Wyk	I. Allison 86 [IA86]	WC, top of Hex River Pass
<i>Anginon jaarsveldii</i> B.L.Burttt	I. Allison 162 [IA162]	NC, Pella Mountain
<i>Anginon paniculatum</i> (Thunb.) B.L.Burttt	B.-E. van Wyk 3337 [BEVW3337]	WC, Matsikamma Mountain
<i>Anginon paniculatum</i>	K. Kotina 28-10 [KK28-10]	WC, Clanwilliam Dam
<i>Anginon pumilum</i> I.Allison & B.-E.van Wyk	I. Allison 155 [IA155]	WC, De Hoop road, 2 km from turn-off to Ouplaas
<i>Anginon pumilum</i>	I. Allison 157 [IA157]	WC, De Hoop road, 3 km from main road
<i>Anginon rugosum</i> (Thunb.) Raf.	K. Kotina 47-11 [KK47-11]	EC, Paterson turn-off on N2 road
<i>Anginon swellendamense</i> (Eckl. & Zeyh.) B.L.Burttt	A. Oskolski 32-06 [AO32-06] (LE)	WC, Karoo Desert National Botanical Garden, Worcester
<i>Anginon verticillatum</i> (Sond.) B.L.Burttt	I. Allison 174 [IA174]	NC, top of Numeesberg
<i>Anginon verticillatum</i>	K. Kotina 24-10 [KK24-10]	NC, Namaqualand, Nourivier
<i>Glia prolifera</i> (Burm.f.) B.L.Burttt	A. Oskolski 38-06 [AO38-06] (LE)	WC, Citrusdal, Middelberg
<i>Heteromorpha arborescens</i> (Spreng) Cham. & Schlttdl. var. <i>abyssinica</i> (A.Rich.) H.Wolff	K. Kotina 30-10 [KK30-10]	GP, Johannesburg, University of Johannesburg Campus
<i>Heteromorpha arborescens</i> var. <i>abyssinica</i>	K. Kotina 31-10 [KK31-10]	GP, Pretoria, Pretoria National Botanical Garden
<i>Heteromorpha arborescens</i> var. <i>abyssinica</i>	P.J.D. Winter 53 [PW53]	LP, 49 km from Pietersburg to Tzaneen
<i>Heteromorpha arborescens</i> var. <i>abyssinica</i>	P.J.D. Winter 71 a [PW71a]	GP, Western Melville Ridge
<i>Heteromorpha arborescens</i> var. <i>arborescens</i>	B.-E. van Wyk 3313 [BEVW3313]	WC, Oudtshoorn, Cango Caves
<i>Heteromorpha arborescens</i> var. <i>frutescens</i> P.J.D.Winter	P.J.D. Winter 57 b [PW57b]	MP, Pilgrim's Rest
<i>Heteromorpha arborescens</i> var. <i>montana</i> P.J.D.Winter	A. de Castro 149 [AC149]	Zimbabwe, Chimanimani National Park
<i>Heteromorpha involucrata</i> Conr.	P.J.D. Winter 67 [PW67]	MP, Weltevrede
<i>Polemanna montana</i> Schltr. & H.Wolff	K. Kotina 34-11 [KK34-11]	FS, Golden Gate National Park
<i>Polemanna simplicior</i> Hilliard & B.L.Burttt	B.-E. van Wyk 4230 [BEVW4230]	FS, Golden Gate National Park
<i>Dracosciadium saniculifolium</i> Hilliard & B.L.Burttt	B.-E. van Wyk 4236 [BEVW4236]	KZN, Witsieshoek, road to Sentinel

The epidermis on young parts of stems is composed of a single layer of dome-like (Fig. 6) (in *A. difforme*, *A. fruticosum* I.Allison & B.E.van Wyk, *A. jaarsveldii* B.L.Burttt, *H. arborescens* var. *arborescens*) or rectangular (Fig. 7) (in *A. paniculatum*, *A. swellendamense*, *H. arborescens* var. *abyssinica*, *H. involucrata* Conr., *Dracosciadium saniculifolium* Hilliard & B.L.Burttt) to isodiametric (Fig. 8) (other species) thin-walled cells. Uni- or bicellular trichomes (Fig. 9) are present on the epidermal surface of *A. verticillatum*, *H. arborescens* var. *arborescens*, *H. arborescens* var. *abyssinica* [PW71a, KK30-10], *H. involucrata* and *P. montana*.

The cortex is composed of collenchyma and parenchyma. Cortical collenchyma is lamellar or angular-lamellar and occurs in one ring of one to six (up to eight in *A. paniculatum*, *A. swellendamense*, *A. jaarsveldii* and *Polemanna*) cell layers (Figs 6–8) or in an interrupted ring (*A. rugosum* and *D. saniculifolium*).

Collenchyma cells are 8–25 µm (≤ 35 µm in *A. paniculatum*, *A. pumilum* I.Allison & B.E.van Wyk and *A. verticillatum*) in tangential diameter and they contain chloroplasts. Druses are sometimes present in cells of the cortical collenchyma of *H. arborescens* var. *abyssinica* [PW 71a], *H. arborescens* var. *frutescens* and *H. involucrata*. Cortical parenchyma is formed from six to 20 layers of isodiametric to somewhat radially flattened, thin-walled cells (Figs 6–8). Within the cortical parenchyma, two types of cells can be distinguished, namely small cells of 7–30 µm in tangential diameter (≤ 35 µm in *Heteromorpha* and 40 µm in *G. prolifera*) containing chloroplasts, and larger cells (idioblasts) of 15–45 µm in tangential diameter (≤ 55 µm in *Polemanna* and 60 µm in *G. prolifera*). Many idioblasts contain druses (Fig. 8). Starch grains were observed in most of the cortical parenchyma cells. A single ring of schizogeneous axial



Figures 6–9. Transverse sections of portions of juvenile stems showing the epidermal cells (e), cortical collenchyma (col), secretory canals (sc) and idioblasts (id). Fig. 6. *Anginon difforme* [KK23-10] with dome-shaped epidermal cells, lamellar collenchyma in four layers, cortical parenchyma with axial secretory canals and primary vascular bundles. Fig. 7. *Heteromorpha arborescens* var. *abyssinica* [PW53] showing rectangular epidermal cells, angular-lamellar collenchyma in two layers, cortical parenchyma with an axial secretory canal and primary vascular bundles. Fig. 8. *Polemannia simplicior* [BEVW4230] with isodiametric to somewhat radially flattened epidermal cells, angular-lamellar collenchyma in three layers, cortical parenchyma with axial secretory canals and idioblasts with druses and primary phloem fibres (white arrows). Fig. 9. *H. arborescens* var. *abyssinica* [KK30-10] showing isodiametric epidermal cells with a bicellular trichome.

secretory canals is present in the cortical parenchyma (Fig. 6). These canals are lined with a single layer of six to 18 (up to 25 in *Polemannia*) epithelial cells and are surrounded by axial parenchyma sheaths of one or two cell layers. The lumina of the canals are commonly 15–110 μm ($\leq 55 \mu\text{m}$ in *G. prolifera*) in tangential diameter.

Dilatation of the cortical tissue is effected mostly by tangential stretching of cells and by anticlinal division of the cortical collenchyma and parenchyma cells, thus forming strands of two to four (up to eight in *Heteromorpha*) cells (Fig. 8). Fresh material of *H. arborescens* var. *abyssinica* showed that chloroplasts may occur in a few cortical cells even after dilatation. The cortical cells are not sclerified. The number of cells containing druses increases with age of the stem.

Primary phloem fibres have sclerified walls and occur as solitary cells or in groups of two to 15 (up to 30 in *A. fruticosum*, *A. paniculatum* and more than 100 in *A. difforme*). They are present in all genera except *Heteromorpha*. Chloroplasts were observed in cells of the axial parenchyma of primary xylem and in pith cells of *H. arborescens* var. *abyssinica* [KK30-10].

The mature bark of *Heteromorpha* and *Polemannia* is peeling and flexible, with a smooth, waxy parchment-like surface (Figs 1, 2, 5). In contrast, *Anginon*, *Glia* and *Dracosciadium* (Figs 3, 4) share non-peeling, brittle mature bark with shallow fissured surfaces.

The initiation of first-formed periderm is in the deeper layers of the cortex in most *Anginon* spp. [in the second or third cell layers beneath the epidermis in *A. jaarsveldii*, *A. pumilum* and *A. verticillatum*, or in deeper cell layers in *A. paniculatum* (Fig. 11)] or sub-epidermal in other studied species (Fig. 10). The phellem is composed of five to 30 (up to 40) layers of radially flattened cells with cell walls that are thin (0.3–0.9 μm) in *Anginon*, *G. prolifera* and *D. saniculifolium* (Figs 14, 16) or moderately thick (1.2–2.0 μm) in *Heteromorpha* and *Polemannia* (Figs 15, 17). The phelloderm comprises two to ten layers of radially flattened, thin-walled cells. Druses occur in the phelloderm cells of *Heteromorpha* and *Polemannia*.

Axial secretory canals are present in the phello-derm of *A. jaarsveldii*, *A. pumilum* and *A. verticillatum* (Figs 12, 13). They are lined by a single layer of four to six epithelial cells and have a tangential lumen diameter of 13–47 μm .

The first-formed periderm persists for several years in all studied species. Subsequent periderms are initiated in the outer region of the cortical parenchyma as scales in *A. difforme* [KK23-10], [KK37-11], *A. swellendamense* and *A. verticillatum* (Fig. 20) or as concentric rings in *H. arborescens* var. *abyssinica* and *P. montana*; no subsequent periderms were observed in other species examined. In *H. ar-*

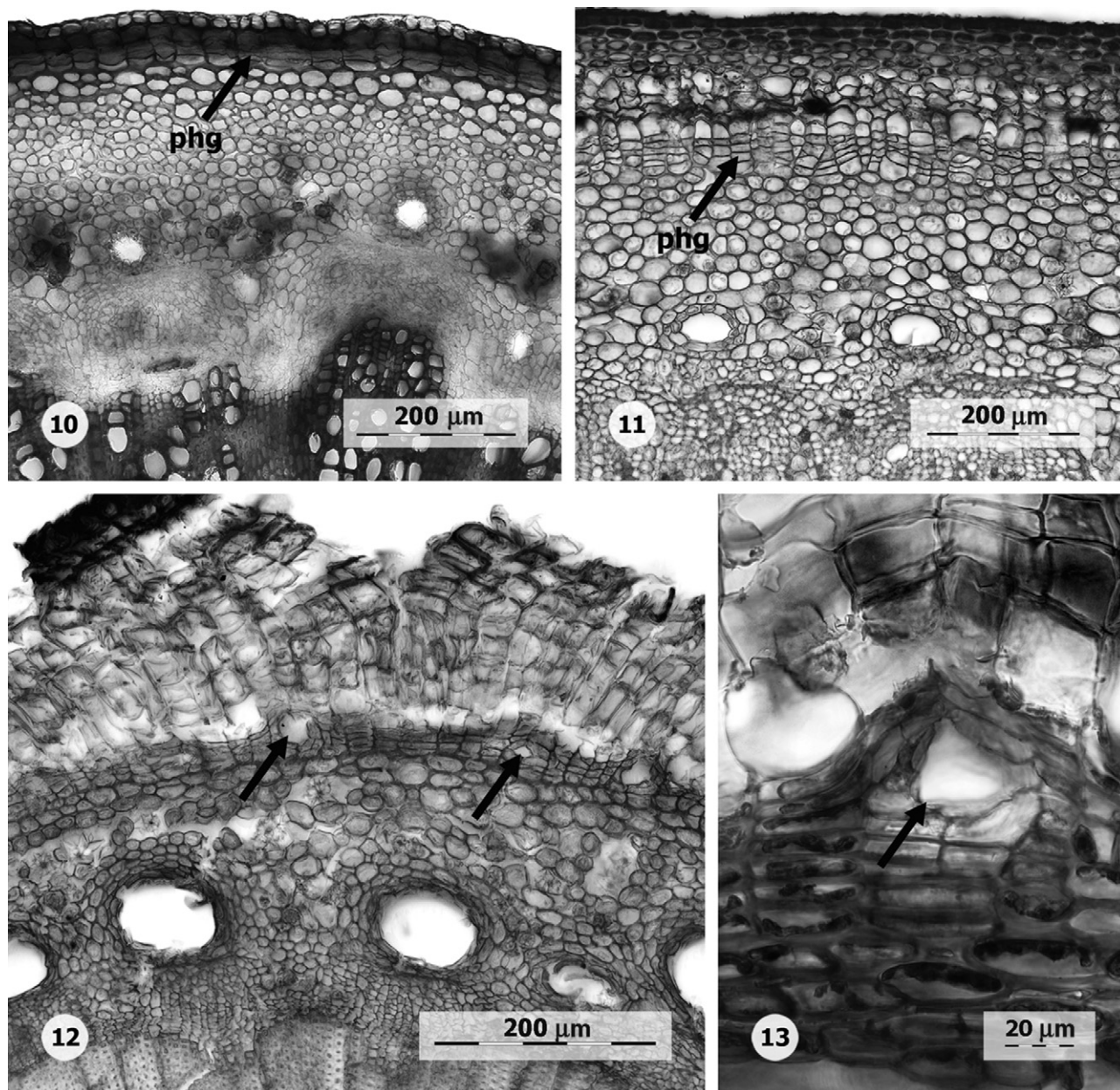
borescens var. *abyssinica* and *P. montana*, the phellogen persists for a long time, forming up to 100 layers of flattened phellem cells with moderately thickened walls, and 20 (up to 40 in *H. arborescens* var. *abyssinica*) layers of chloroplast-containing phelloderm cells that occasionally also contain druses (Fig. 21). In fresh material of *H. arborescens* var. *abyssinica*, *P. montana*, *A. difforme* [KK37-11] and *A. rugosum*, chloroplasts are clearly visible in the phelloderm cells. Numerous chloroplasts were observed in phelloderm cells and, to a lesser amount, in axial parenchyma cells of the outermost part of dilated secondary phloem in *H. arborescens* var. *abyssinica*, *P. montana*, *A. difforme* [KK37-11] and *A. rugosum*.

The pattern of secondary phloem is related to the arrangement of axial secretory canals surrounded by axial sheath parenchyma alternating with zones of sieve tubes, companion cells and axial phloem parenchyma. This pattern of alternating zones is obscured in the outermost region where phloem parenchyma is heavily dilated. Dilatation of secondary phloem is mostly radial (Figs 20, 21). Secretory canals are solitary or in tangential rows of two to eight (Figs 18, 21).

Sieve tube members are 6–30 μm wide; their mean length varies from 100–615 μm (Table 2). Sieve plates are compound with three to 15 (up to 23 in *Heteromorpha*) sieve areas, located on vertical or slightly oblique cross walls. Axial parenchyma cells associated with conducting elements occur as single fusiform cells and in strands of mostly two to nine cells (up to ten in *A. difforme*, *A. fruticosum* and *Heteromorpha*).

Axial secretory canals are present throughout the secondary phloem (Figs 18, 19, 21). They are lined by a single layer of four to six (rarely up to eight) epithelial cells and surrounded by axial parenchyma sheaths comprising one or two (sometimes three) cell layers. These sheaths consist of strands of two to seven (up to nine in *A. fruticosum* and 11 in *H. involucrata*) thin-walled cells. The lumina of the axial secretory canals vary from 10–55 μm ($\leq 60 \mu\text{m}$ *P. montana*) in diameter.

The transition from non-collapsed to collapsed secondary phloem is gradual (Fig. 18) (collapsed secondary phloem was not observed in the thin short-lived stem of *D. saniculifolium*). In the collapsed secondary phloem, some cells of the axial parenchyma associated with conducting elements are transformed into chambered crystalliferous cells containing druses and (in all studied species of *Anginon* except *A. rugosum*) also into sclereids with moderately thick walls (Fig. 19). In contrast, the axial parenchyma sheathing the secretory canals shows no evidence of obliteration, sclerification or formation of crystals in its cells.



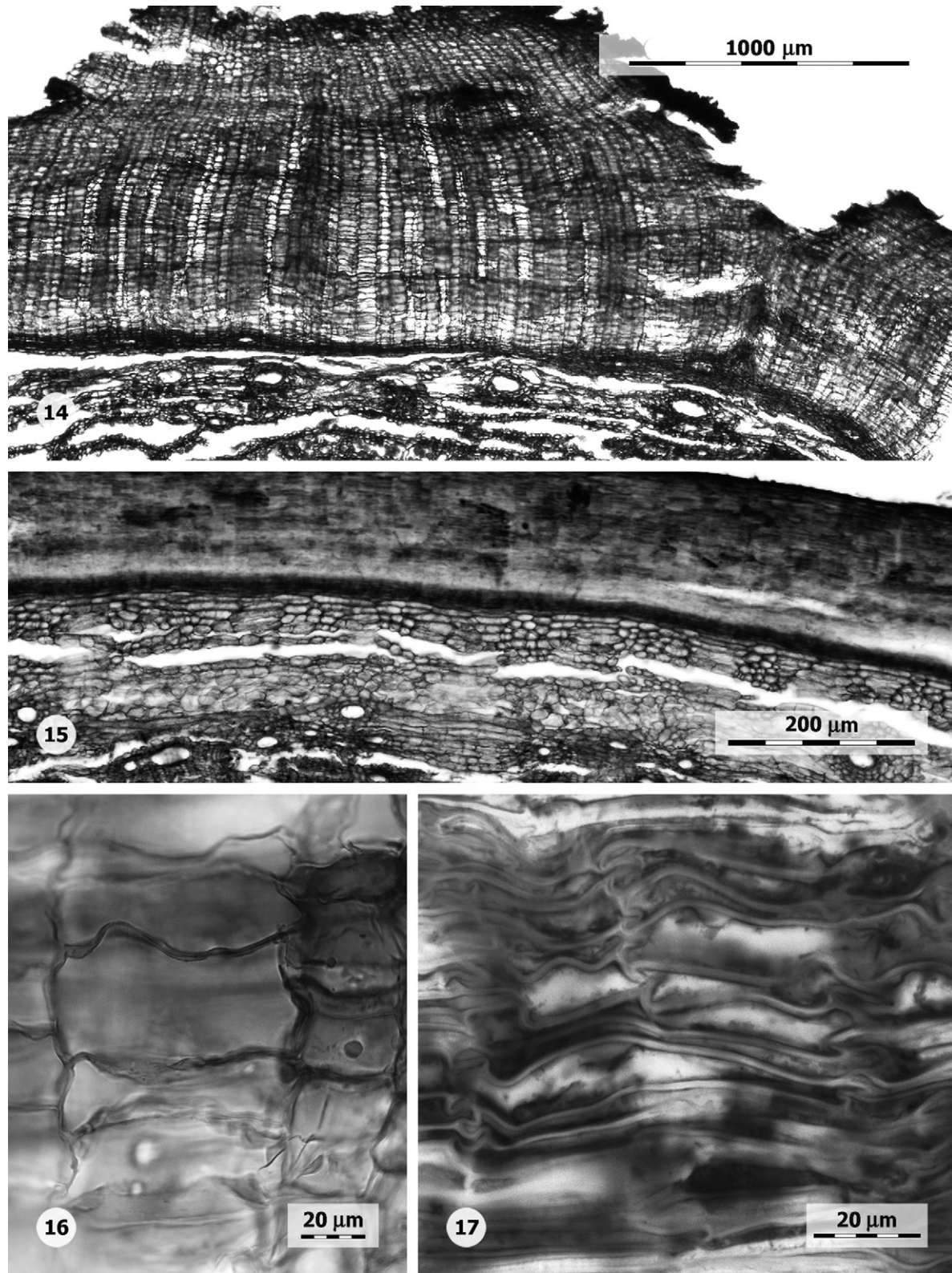
Figures 10–13. Figures 10–11. Transverse sections of portions of juvenile stems showing the initiation of the first-formed periderm (phg, phellogen). Fig. 10. *Heteromorpha arborescens* var. *abyssinica* [KK30-10], subepidermal. Fig. 11. *Anginon paniculatum* [KK28-10], beneath cortical collenchyma. Figures 12–13. Transverse sections showing young periderm with small secretory canals (black arrows) in the phellogen of *Anginon verticillatum* [KK24-10].

Secondary phloem rays are uniseriate and multiseriate (up to six-seriate in *Glia prolifera* and *H. arborescens* var. *abyssinica*). Uniseriate rays are composed mostly of square and upright cells, whereas multiseriate rays have procumbent cells or also upright and square cells forming one, two or more numerous (rarely up to six in *H. arborescens* var. *abyssinica*) marginal rows.

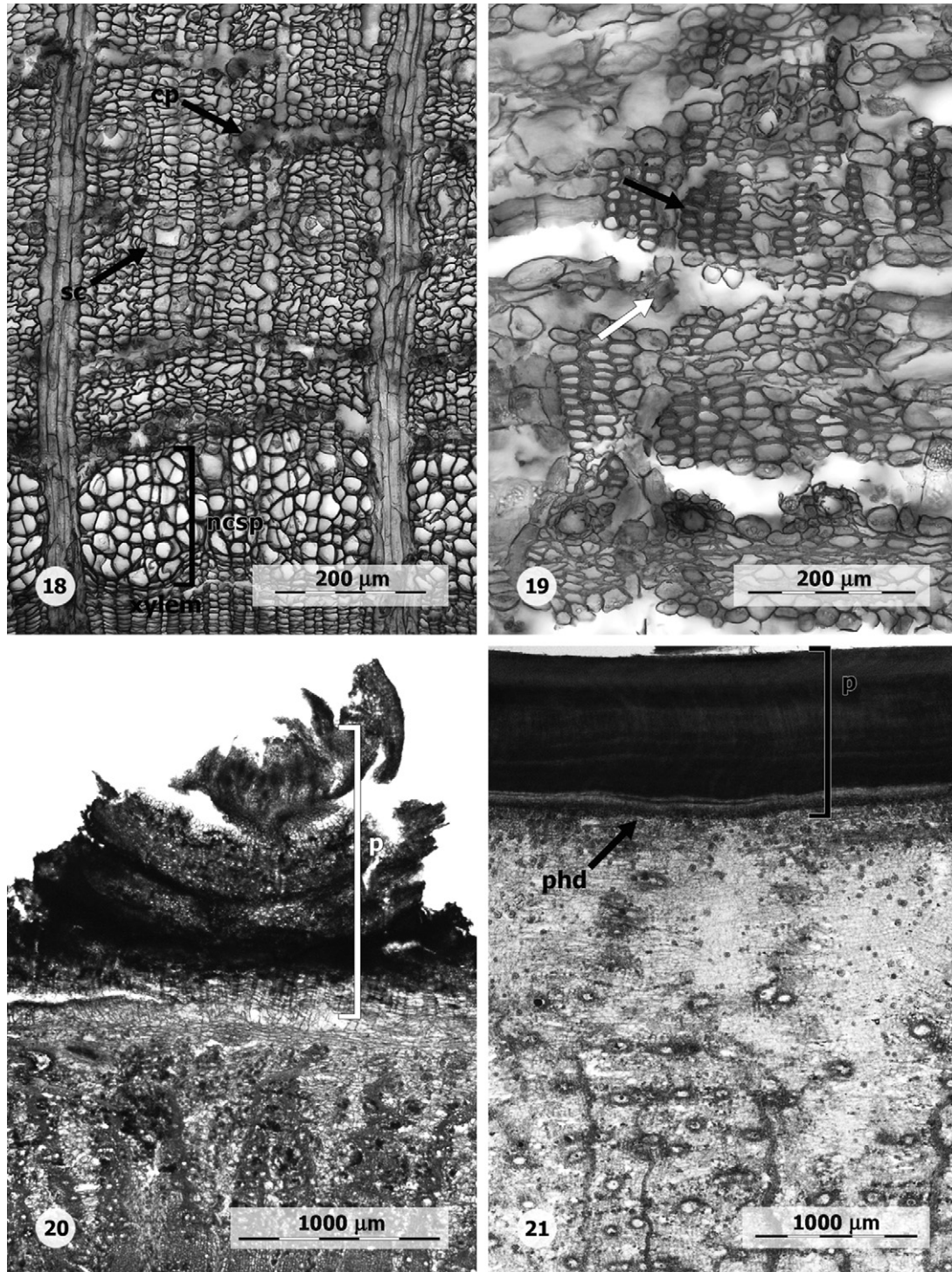
Dilated rays are extensively enlarged, mostly by tangential expansion and also by anticlinal divisions

of ray cells, resulting in rays up to ten cells wide (up to 14-seriate in *H. arborescens* var. *abyssinica*). Many of the parenchyma cells in the rays contain starch grains. Scattered druses are invariably present.

Radial secretory canals are found in *A. difforme*, *H. arborescens* var. *abyssinica*, *H. arborescens* var. *frutescens* and *D. saniculifolium*, but not in any of the other taxa studied. The lumen diameters of the radial secretory canals are 18–65 μm.



Figures 14–17. Transverse sections of portions of mature bark showing the first-formed periderm. Fig. 14. *Anginon verticillatum* [KK24-10], periderm with fissure. Fig. 15. *Heteromorpha arborescens* var. *abyssinica* [PW71a], smooth periderm with radially flattened phellem cells. Fig. 16. *Anginon verticillatum* [KK24-10], thin-walled phellem cells. Fig. 17. *Heteromorpha involucrata* [PW67], thick-walled phellem cells tangentially stretched.



Figures 18–21. Figures 18–19. Transverse sections of portions of mature bark to show the secondary phloem. Fig. 18. *H. arborescens* var. *abyssinica* [KK31-10], non-collapsed secondary phloem (ncsp) with distinct sieve tubes and companion cells; collapsed secondary phloem; axial secretory canal (sc) with sheath and crystalliferous axial parenchyma (cp). Fig. 19 *Anginon paniculatum* [KK28-10], collapsed secondary phloem with axial secretory canals and axial parenchyma cells: sclerified (black arrow) and crystalliferous (white arrow). Figures 20–21. Transverse sections of portions of mature bark showing subsequent periderms (p) and collapsed secondary phloem. Fig. 20. *Anginon difforme* [KK37-11], subsequent scale-like periderms and dilated secondary phloem. Fig. 21. *H. arborescens* var. *abyssinica* [KK31-10], subsequent periderms in concentric rings, phellem, green phelloderm (phd) and dilated secondary phloem.

Table 2. Bark anatomical characters of southern African members of tribe Heteromorphae

	Diameter sample (mm)	Length of sieve tubes (mean/min-max, μm)	Diameter of sieve tubes (μm)	Width of multiseriate rays, cells	Wide of multiseriate rays (min-max, μm)	Height of multiseriate rays (min-max, μm)	Number of multiseriate rays per mm (mean)	Number of uniseriate rays per mm (mean)
<i>Anginon difforme</i> [KK23-10]	20	324 \pm 7.5 253–406	6.8–16.2	1–4	24–131	100–896	3.9	3.8
<i>Anginon fruticosum</i> [IA86]	6	200 \pm 12 147–246	7.8–12.0	1–4	16–58	171–1091	4.4	2.5
<i>Anginon jaarsveldii</i> [IA162]	8	309.5 \pm 6.2 251–375	9.8–16.1	1–4	24–61	200–726	3.6	2
<i>Anginon paniculatum</i> [KK28-10]	20	295 \pm 12 184–390	9.7–16.6	1–4	26–83	111–1721	4.3	3.2
<i>Anginon pumilum</i> [IA155]	15	255.8 \pm 6.4 204.7–328	11.0–22.2	1–4	27–89	169–1325	3.9	1.8
<i>Anginon rugosum</i> [KK47-11]	12	261 \pm 11 198–427	9.5–17.5	1–5	24–57	103–1386	4.3	0.2
<i>Anginon swelldamense</i> [AO32-06]	15	266 \pm 8.9 180–378	9.4–18.3	1–4	33–92	165–1127	3.6	1.6
<i>Anginon verticillatum</i> [KK24-10]	12	262 \pm 6.3 194–400	8.6–15.7	1–4	35–105	246–1027	4.2	1.2
<i>Glia prolifera</i> [AO38-06]	15	172 \pm 6.3 120–270	7.5–18.0	1–6	35–135	227–1300	7.7	0.6
<i>Heteromorpha arborescens</i> var. <i>abyssinica</i> [KK30-10]	>50	388 \pm 13.7 204–527	10.1–20.0	1–6	22–78	110–704	3.5	0.5
<i>Heteromorpha arborescens</i> var. <i>abyssinica</i> [KK31-10]	>50	297–527	15.6–27.2	1–6	27–129 151–959	4.2	0.8	
<i>Heteromorpha arborescens</i> var. <i>abyssinica</i> [PW53]	20	427 \pm 13 305–614	11.6–30.0	1–4	14.7–50 111–649	3.9	0.8	
<i>Heteromorpha arborescens</i> var. <i>abyssinica</i> [PW71a]	16	389 \pm 12 268–527	10.2–22.4	1–3	12–47 157–882	3.2	0.7	
<i>Heteromorpha arborescens</i> var. <i>arborescens</i> [BEVW3313]	10	369 \pm 10 277–499	7.9–18.3	1–3	16.6–49 117–431	3.0	1.3	
<i>Heteromorpha arborescens</i> var. <i>frutescens</i> [PW57b]	17	340 \pm 7 248–403	8.0–17.2	1–3	20–49 105–498	3.2	1.9	
<i>Heteromorpha arborescens</i> var. <i>montana</i> [AC149]	8	367 \pm 11 203–468	8.7–14.2	1–3	17–43 186–711	6.2	1.8	
<i>Heteromorpha involucrata</i> [PW67]	8	328 \pm 9 245–437	11.0–24.9	1–2	10–30 102–734	2.4	2.8	
<i>Polemanita montana</i> [KK34-11]	15	190 \pm 9 167–209	8.7–19.5	1–5	16–44 95–536	4.2	0.8	
<i>Polemanita simplicior</i> [BEVW4230]	4	243 \pm 13 150–420	6.4–13.1	1–3	–	–	–	–
<i>Dracosciadium saniculifolium</i> [BEVW4236]	5–7	164 \pm 7 107–248	11.5–19.1	1–5	–	–	–	–

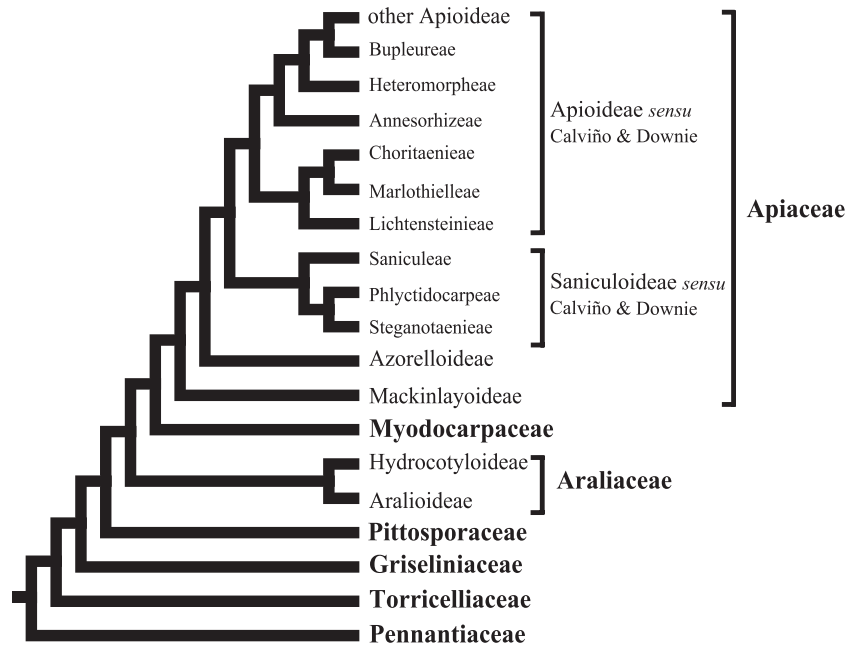


Figure 22. Simplified phylogenetic tree for the order Apiales (after Plunkett *et al.*, 2004; Calviño *et al.*, 2006; Nicolas & Plunkett, 2009; Stevens, 2001 onwards). The tribes of the early diverging Apioideae and their relationships follow Magee *et al.* (2010).

DISCUSSION

Anginon, *Dracosciadium*, *Glia*, *Heteromorpha* and *Polemanna* show the presence of secretory canals in the cortex (Figs 6–8) and secondary phloem (Figs 18, 19, 21), the presence of two types of axial parenchyma (sheath parenchyma surrounding secretory canals and phloem parenchyma accompanying sieve tubes) and the absence of fibres in the secondary phloem. These bark characters are typical of the large majority of woody Apiaceae and all Araliaceae, Myodocarpaceae and Pittosporaceae (Van Tieghem, 1884; Metcalfe & Chalk, 1950; Kotina & Oskolski, 2010; Nilova & Oskolski, 2010; Oskolski *et al.*, 2010) examined to date (Fig. 22), with the exception of *Centella* L. (Mackinlayoideae, Apiaceae), which has no secretory canals in the secondary phloem. Nevertheless, these bark features may be considered as synapomorphies supporting monophyly of suborder Apiineae Plunkett & Lowry (Plunkett *et al.*, 2004); then *Centella* shows a case of partial loss of the canals (which remain in the cortex, however).

All five genera under study share the radial type of dilatation of the secondary phloem (Fig. 20). This condition also occurs in all members of subfamilies Saniculoideae (*Eryngium* L.) and Mackinlayoideae (*Centella*, *Apiopetalum* Baill. and *Mackinlaya* F.Muell.) examined to date, whereas *Steganotaenia* Hochst. and *Polemanniopsis* B.L.Burt belonging to tribe Steganotaeniaceae (subfamily Apioideae) are dis-

tinctive in having a diffuse type of secondary phloem dilatation (Kotina & Oskolski, 2007; Nilova & Oskolski, 2010; Oskolski & Van Wyk, 2010; Oskolski *et al.*, 2010). As for other families, the radial type of dilatation occurs throughout Pittosporaceae (Nilova & Oskolski, 2010), in most Myodocarpaceae and in early branching lineages of Araliaceae and in the *Polyscias* J.R.Forst. & G.Forst. *s.l.* clade (Kotina & Oskolski, 2010). This distribution of secondary phloem dilatation types suggests that the radial type may be considered as an ancestral condition for Apiineae. Certainly, this bark character is of considerable taxonomic value that should be taken into consideration in further systematic studies of Apiaceae and other members of Apiales.

Anginon, *Dracosciadium*, *Glia*, *Heteromorpha* and *Polemanna* also share a narrow cortex (up to 25 cells in width), axial secretory canals arranged in a single ring in the innermost part of the cortical parenchyma (Figs 10–12), and radial dilatation of secondary phloem. Although these conditions occur separately in other groups within Apiineae, in combination they are otherwise only known in *Centella* and *Polemanniopsis* (Apiaceae) and in *Bursaria* Cav., *Hymenosporum* R.Br. ex F.Muell. and *Pittosporum* Banks ex Gaerth. (Pittosporaceae), which are different from the genera under study in other bark features, including the lack of druses in the phelloderm (Nilova & Oskolski, 2010; Oskolski & Van Wyk, 2010; Kotina & Oskolski, 2010; Oskolski *et al.*, 2010). Therefore, the combination of a narrow cortex with a single ring of secretory canals,

Table 3. Diagnostic and phylogenetically important bark characters of southern African members of tribe Heteromorpheae

	<i>Anginon</i>	<i>Glia</i>	<i>Heteromorpha</i>	<i>Polemanna</i>	<i>Dracosciadium</i>
Bark surface peeling	–	–	+	+	–
Bark texture	Brittle	Brittle	Flexible	Flexible	Brittle
Phellem cells with thick walls	–	–	+	+	–
Phellem cells becoming tangentially stretched	–	–	+	+	–
Primary phloem fibres in cortex	+	+	–	+	+
Types of successive periderm	Scale	?	Ring	Ring	?
Druses in phelloderm	–	–	+	+	–
Sclerification of axial parenchyma	+	–	–	–	–
Length of sieve tubes (μm)	140–410	120–270	200–615	150–420	100–250

secretory canals in the secondary phloem and radial dilatation of the secondary phloem is diagnostic for tribe Heteromorpheae.

Despite these similarities, many bark characters vary considerably within Heteromorpheae (Table 3). The most remarkable differences between the genera under study concern the appearance of the bark surface and the periderm structure. *Anginon*, *Glia* and *Dracosciadium* share non-peeling brittle bark with a shallow-fissured surface (Figs 3, 4) and thin-walled phellem cells (Fig. 16) and an absence of crystals in the phelloderm cells. In contrast, *Heteromorpha* and *Polemanna* have peeling flexible bark with a smooth parchment-like surface (Figs 1, 2, 5); their periderms form as concentric rings by long-persisting phellogens; the moderately thick-walled phellem cells are tangentially stretched during dilatation (Fig. 17) and druse crystals occur in the phelloderm cells. The long-persisting activity of phellogens and the capacity of phellem cells for marked stretching are probably responsible for the formation of the translucent phellem (Fig. 1) that transmits light into the phelloderm cells and outer secondary phloem. It is noteworthy that green phelloderm with numerous chloroplasts was observed not only in small stems of some *Anginon* spp. but also in large stems of *Heteromorpha* and *Polemanna* covered by thick (up to 100 cell layers) phellem (Fig. 21).

The presence of chloroplasts in the stem tissues beneath the phellem has been reported for a number of deciduous trees (e.g. Pearson & Lawrence, 1958; Foote & Schaedle, 1976; Kauppi, 1991). Experiments with *Populus tremuloides* Michx. showed that photosynthesis in the stem does not usually reach the level of net photosynthesis, but it is nevertheless regarded as significant for the energy balance of the stem itself, as a means of reducing losses attributable to respiration (Foote & Schaedle, 1976). The phellem is evidently of considerable importance as far as the penetration of light into the bark and the synthesis of chlorophyll in the cells of the phelloderm and secondary phloem are

concerned, as shown by the decreases in both light transmission and the chlorophyll content of the stem as the phellem thickened with age (Kauppi, 1991). The photosynthetic capacity of the stem may be considered to be an adaptation to survival in a climate with a short growing season, whether in seasonally cold regions (Pearson & Lawrence, 1958) or in regions with a Mediterranean climate (as it occurs in South Africa). Therefore, the formation of translucent phellem because of the persistence of circular phellogens and the conspicuous tangential stretching of phellem cells are suggested as preconditions for stem photosynthesis and related adaptations in South African Heteromorpheae.

The absence of primary phloem fibres in *Heteromorpha* is a distinctive character (Fig. 23). This condition rarely occurs in Apiaceae: it has been reported only in *Neopanax* Allan, *Oplopanax* (Torr. & A.Gray) Miq., a single species of *Polyscias*, *Bursaria* and *Pittosporum* (Kotina & Oskolski, 2010; Nilova & Oskolski, 2010) and in *Centella* (Oskolski & Van Wyk, 2010). This uncommon character supports the generic concept of *Heteromorpha* and indicates that *Polemanna* may be less closely related than the similarity in appearance of the bark would suggest.

Within the genera studied, the sclerification of the axial parenchyma in collapsed secondary phloem was found only in *Anginon* (Fig. 19). In all other genera studied, sclerification is absent. The lack of sclereids in the secondary phloem is characteristic of the majority of woody Apiaceae studied (*Centella*, *Polemanniopsis* and *Steganotaenia*) (Oskolski & Van Wyk, 2010; Oskolski *et al.*, 2010) and some Araliaceae and Pittosporaceae (Kotina & Oskolski, 2010; Nilova & Oskolski, 2010). It will be necessary to sample older stems of *Glia* to confirm this apparent unique generic character for *Anginon*.

The sieve tube members are distinctly longer in *Heteromorpha* (Tables 2 and 3) than in the other taxa studied. The vessel elements in the wood of this genus are also the longest in Heteromorpheae (Oskolski &

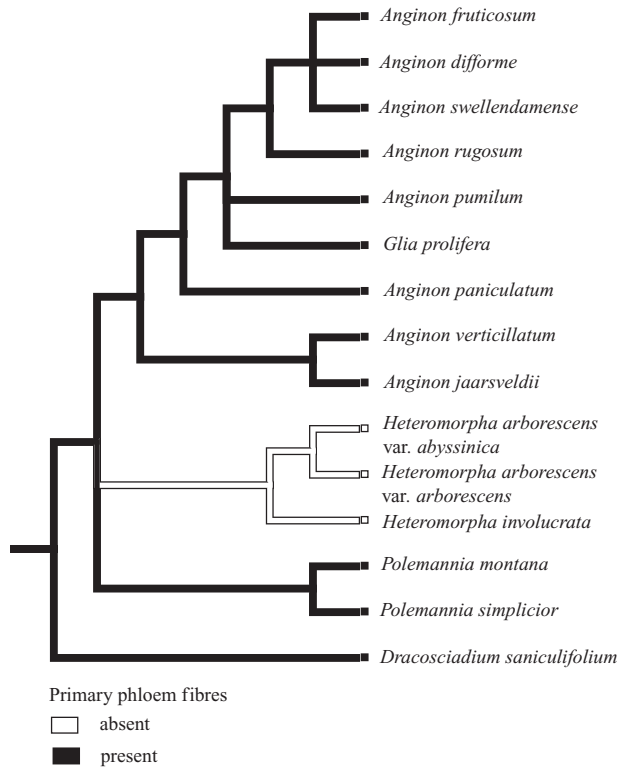


Figure 23. Mapping of the occurrence of primary phloem fibres on a portion of the strict consensus tree of southern African Apiaceae derived from maximum parsimony analyses of nuclear rDNA internal transcribed spacer (Calviño *et al.*, 2006).

Van Wyk, 2008). Our data are in agreement with a common correlation between plant size and vessel element length: these cells are longer in trees than in shrubs, perennial herbs and dwarf plants (Baas *et al.*, 1984; Carlquist, 2001). As the vessel elements are similar in length to the fusiform cambial initials (Bailey & Tupper, 1918), the sieve tube members as their derivatives should also show similar trends in size. In Araliaceae, however, no correlation between the average lengths of these two types of conducting elements taken from the same stem samples was revealed. This lack of correlation points to unequal changes in size for these cells during their differentiation from fusiform cambial initials (Kotina & Oskolski, 2010). However, the species of Heteromorphae show no marked differences in size between sieve tube members and vessel elements as found in Araliaceae. This is why the trends relating to the habit of the plants are manifested more clearly.

The shape of the epidermal cells, the presence of trichomes and the occurrence of secretory canals in the secondary phloem rays can be used as diagnostic characters for species identification. For example, *Anginon difforme*, *A. fruticosum*, *A. jaarsveldii* and

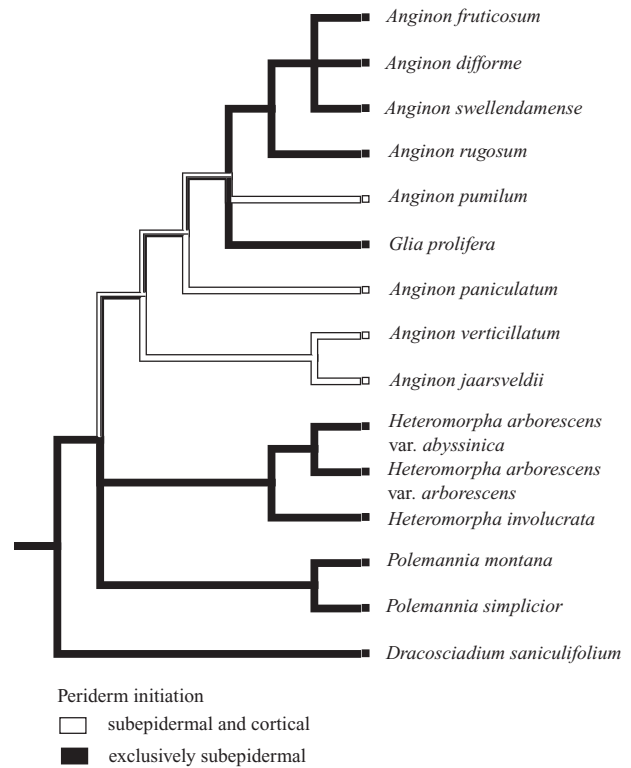


Figure 24. Mapping of the types of periderm initiation on a portion of the strict consensus tree of southern African Apiaceae derived from maximum parsimony analyses of nuclear rDNA internal transcribed spacer (Calviño *et al.*, 2006).

Heteromorpha arborescens var. *arborescens* share dome-shaped epidermal cells (Fig. 6), whereas most other studied species have isodiametric or square epidermal cells (or transitional forms between the two) (Figs 7, 8). *Anginon verticillatum*, *Polemannia montana* and both *Heteromorpha* spp. are distinctive in the presence of uni- to bicellular trichomes (Fig. 9). Secretory canals in the rays of the secondary phloem were found only in *Anginon difforme*, some varieties of *Heteromorpha arborescens* and in *Dracosciadium saniculifolium*. This character also has taxonomic value in tribe Steganotaeniae (Oskolski *et al.*, 2010), in *Centella* (Oskolski & Van Wyk 2010) and in some Araliaceae (Kotina & Oskolski, 2010).

In *Anginon jaarsveldii*, *A. pumilum*, *A. verticillatum* and *A. paniculatum* the periderm is initiated deep in the cortex (Fig. 24). The same type of initiation is also noted for *Steganotaenia araliacea* Hochst., *Polemanniopsis marlothii* (H. Wolff) B.L. Burtt and some other Apiaceae (Metcalf & Chalk, 1950; Oskolski *et al.*, 2010). Secretory canals were found in the phelloderm (Figs 12, 13) of all but one of the above-mentioned *Anginon* spp. (*A. paniculatum* is the exception). This character was found in *S. araliacea* (Oskolski *et al.*,

2010), but appears to be rare in angiosperms, as we could find only one previous report [*Lannea coromandelica* (Houtt.) Merrill of Anacardiaceae (Venkaiah & Shah, 1984)]. Therefore, both the cortical periderm initiation and the occurrence of secretory canals in the phelloderm are of considerable systematic value.

CONCLUSIONS

Bark anatomical characters are of systematic and phylogenetic value at all taxonomic levels. At the ordinal level, the following features are of diagnostic value: secretory canals in the cortex and secondary phloem, the presence of two types of axial parenchyma (sheath parenchyma surrounding secretory canals and phloem parenchyma accompanying sieve tubes) and the absence of fibres in the secondary phloem. At family level, the radial type of dilatation of the secondary phloem is typical. Members of Heteromorphaeae can be recognized by a narrow cortex and axial secretory canals arranged in a single ring in the innermost part of the cortical parenchyma. Character states of value at the generic level include the bark surface, periderm structure, the presence of primary phloem fibres, different types of successive periderm, sclerification of axial parenchyma and the length of sieve tubes. The formation of translucent phellem and the presence of chloroplasts in phelloderm cells are interesting adaptations allowing for photosynthetic activity under extreme conditions (e.g. after fire or during droughts when the plants are leafless). At species level, the shape of the epidermal cells, the presence of trichomes and the occurrence of secretory canals in the secondary phloem rays are all of taxonomic value. Our study suggests that bark anatomy can be useful for improving our understanding of taxonomic relationships in Apiaceae.

ACKNOWLEDGEMENTS

The University of Johannesburg, the National Research Foundation, the Russian Foundation of Basic Research (RFFI, grant 09-04-00618) and the Ministry of Education and Science of the Russian Federation (contract no. 16.518.11.7071 for A.A.O.) are gratefully acknowledged for financial support. We thank Khangela Baloyi for his help with the collection of samples in the Pretoria National Botanical Garden and Marina Toktarova for her help with the compilation of the plates.

REFERENCES

Allison I, Van Wyk B-E. 1997. A revision of the genus *Anginon* (Apiaceae). *Nordic Journal of Botany* **17**: 561–577.

- Baas P, Lee C, Zhang X, Cui K, Deng Y. 1984. Some effects of dwarf growth on wood structure. *IAWA Bulletin* **5**: 45–63.
- Bailey IW, Tupper WW. 1918. Size variation in tracheary cells. I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms and angiosperms. *Proceedings of the American Academy of Arts and Sciences* **54**: 149–204.
- Calviño IC, Tilney PM, Van Wyk B-E, Downie SR. 2006. A molecular phylogenetic study of southern African Apiaceae. *American Journal of Botany* **93**: 1828–1847.
- Carlquist S. 2001. *Comparative wood anatomy. Systematic, ecological, and evolutionary aspects of dicotyledon wood*. Berlin, Heidelberg: Springer-Verlag.
- Chattaway MM. 1953. The anatomy of bark. I. The genus *Eucalyptus*. *Australian Journal of Botany* **1**: 402–433.
- Downie SR, Katz-Downie DS. 1999. Phylogenetic analysis of chloroplast *rps16* intron sequences reveals relationships within the woody southern African Apiaceae subfamily Apioidae. *Canadian Journal of Botany* **77**: 1120–1135.
- Downie SR, Plunkett GM, Watson MF, Spalik K, Katz-Downie DS, Valiejo-Roman CM, Terentjeva EI, Troitsky AV, Lee BY, Lahham J, El-Oqlah A. 2001. Tribes and clades within Apiaceae subfamily Apioidae: the contribution of molecular data. *Edinburgh Journal of Botany* **58**: 301–330.
- Evert RF. 2006. *Esau's plant anatomy: meristems, cells and tissue of the plant body: their structure, function and development*. Hoboken, NJ: John Wiley & Sons.
- Footo KC, Schaedle M. 1976. Physiological characteristics of photosynthesis and respiration in stem of *Populus tremuloide* Michx. *Plant Physiology* **58**: 91–94.
- Johansen DA. 1940. *Plant microtechnique*. New York: McGraw-Hill.
- Junikka L. 1994. Survey of English macroscopic bark terminology. *IAWA Journal* **15**: 3–45.
- Junikka L, Koek-Noorman J. 2007. Anatomical structure of barks in neotropical genera of Annonaceae. *Annales Botanici Fennici* **44**: 79–132.
- Kauppi A. 1991. Seasonal fluctuations in chlorophyll content in birch stem with special reference to bark thickness and light transmission, a comparison between sprouts and seedlings. *Flora* **185**: 107–125.
- Kotina EL, Oskolski AA. 2007. Bark anatomy of *Apiopetalum* and *Mackinlaya* (Apiales). *Botanicheskii Zhurnal (St Petersburg)* **92**: 1490–1499.
- Kotina EL, Oskolski AA. 2010. Survey of the bark anatomy of Araliaceae and related taxa. *Plant Diversity and Evolution* **128**: 455–489.
- Maddison WP, Maddison DR. 2010. *Mesquite. A modular system for evolutionary analysis. Version: 2.74*. <http://mesquiteproject.org/mesquite/mesquite.html>
- Magee AR, Calviño CI, Liu M, Downie SR, Tilney PM, Van Wyk B-E. 2010. New tribal delimitations for the early diverging lineages of Apiaceae subfamily Apioidae. *Taxon* **59**: 567–580.
- Metcalf CR, Chalk L. 1950. *Anatomy of the dicotyledons*. Vol. II. Oxford: Clarendon Press.
- Nicolas AN, Plunkett GM. 2009. The demise of subfamily

- Hydrocotyloideae (Apiaceae) and the re-alignment of its genera across the entire order Apiales. *Molecular Phylogenetics and Evolution* **53**: 134–151.
- Nilova M, Oskolski AA. 2010.** Comparative bark anatomy of *Bursaria*, *Hymenoporum* and *Pittosporum* (Pittosporaceae). *Plant Diversity* **128**: 1–10.
- Oskolski AA, Rossouw AS, Van Wyk B-E. 2010.** Wood and bark anatomy of *Steganotaenia* and *Polemanniopsis* (tribe Steganotaenieae, Apiaceae) with notes on phylogenetic implications. *Botanical Journal of the Linnean Society* **163**: 55–69.
- Oskolski AA, Van Wyk B-E. 2008.** Systematic and phylogenetic value of wood anatomy in Heteromorpheae (Apiaceae, Apiales). *Botanical Journal of the Linnean Society* **158**: 569–583.
- Oskolski AA, Van Wyk B-E. 2010.** Wood and bark anatomy of *Centella*: scalariform perforation plates support an affinity with the subfamily Mackinlayoideae (Apiaceae). *Plant Systematics and Evolution* **289**: 127–135.
- Pearson LC, Lawrence DB. 1958.** Photosynthesis in aspen bark. *American Journal of Botany* **45**: 383–387.
- Plunkett GM, Chandler GT, Lowry II PP, Pinney SM, Sprenkle TS. 2004.** Recent advances in understanding Apiales and a revised classification. *South African Journal of Botany* **70**: 371–381.
- Plunkett GM, Soltis DE, Soltis PS. 1996a.** Higher level relationships of Apiales (Apiaceae and Araliaceae) based on phylogenetic analysis of *rbcL* sequences. *American Journal of Botany* **83**: 499–515.
- Plunkett GM, Soltis DE, Soltis PS. 1996b.** Evolutionary patterns in Apiaceae: inferences based on *matK* sequence data. *Systematic Botany* **21**: 477–495.
- Roth I. 1981.** *Structural patterns of tropical barks*. Berlin: Borntraeger Gebrüder.
- Stevens PF. 2001** (onwards). *Angiosperm Phylogeny Website*. Version 9, June 2008 [and more or less continuously updated since]. <http://www.mobot.org/MOBOT/research/APweb/>
- Trockenbrodt M. 1990.** Survey and discussion in the terminology used in bark anatomy. *IAWA Bulletin* **11**: 141–166.
- Van Tieghem MP. 1884.** Sur la structure et les affinités des *Pittisporées*. *Bulletin de la Société Botanique* **31**: 383–385.
- Van Wyk B-E, Allison I, Tilney PM. 1997.** Morphological variation and phylogenetic relationships in the genus *Anginon* (Apiaceae). *Nordic Journal of Botany* **17**: 511–526.
- Van Wyk B-E, Tilney PM, Magee AR. 2010.** A revision of the genus *Glia* (Apiaceae, tribe Heteromorpheae). *South African Journal of Botany* **76**: 259–271.
- Venkaiah K, Shah JJ. 1984.** Distribution, development and structure of gum ducts in *Lannea coromandelica* (Houtt.) Merrill. *Annals of Botany* **54**: 175–186.
- Whitmore TC. 1962.** Studies in systematic bark morphology. I. Bark morphology in Dipterocarpaceae. *New Phytologist* **61**: 208–220.
- Winter PJD, Van Wyk B-E. 1995.** A revision of the genus *Heteromorpha* (Apiaceae). *Kew Bulletin* **51**: 225–265.
- Yunus M, Yunus D, Iqbal M. 1990.** Systematic bark morphology of some tropical trees. *Botanical Journal of the Linnean Society* **103**: 367–377.