

WOOD ANATOMY OF RAUVOLFOIDEAE (APOCYNACEAE): A SEARCH FOR MEANINGFUL NON-DNA CHARACTERS AT THE TRIBAL LEVEL¹

FREDERIC LENS,² MARY E. ENDRESS,³ PIETER BAAS,⁴ STEVEN JANSEN,^{5,6} AND ERIK SMETS^{2,4}

²Laboratory of Plant Systematics, Institute of Botany and Microbiology, Kasteelpark Arenberg 31 Box 2437, K.U.Leuven, BE-3001 Leuven, Belgium; ³Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland; ⁴Nationaal Herbarium Nederland—Leiden University Branch, P.O. Box 9514, NL-2300 RA Leiden, The Netherlands; ⁵Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK

Wood anatomical studies in the economically important Apocynaceae or dogbane family are fragmentary. This study represents a first attempt to unravel the phylogenetic significance and major evolutionary trends in the wood of the family, using existing and new microscopic wood observations within the large subfamily Rauvolfioideae. On the basis of LM and SEM observations of 91 species representing all 10 currently recognized tribes, we found that most of the tribes are characterized by a unique combination of wood characters, such as vessel grouping, vessel element length, fiber type, frequency of uniseriate rays, and fused multiseriate rays. Climbing rauvolfioid taxa can generally be distinguished from erect species by their wider vessels, tendency to form paratracheal axial parenchyma, presence of tracheids, and occurrence of laticifers in rays. With respect to the entire family, there is a general phylogenetic trend toward shorter vessel elements, a higher proportion of vessels in multiples and more vessels per multiple, higher tracheid abundance, more paratracheal parenchyma, and fewer cells per axial parenchyma strand in the more derived Apocynaceae. Most of these evolutionary trends are likely to be triggered by drier environmental conditions and/or shifts from an erect to a climbing habit.

Key words: Apocynaceae; APSA clade; climbing vs. nonclimbing habit; Rauvolfioideae; systematic wood anatomy; tribal classification.

The Rauvolfioideae sensu Simões et al. (2007) are circumscribed as a paraphyletic subfamily within Apocynaceae s.l. and comprise about 980 species distributed among 84 genera and 10 tribes, representing about one-fourth to one-fifth of the species diversity within the family (Stevens, 2001 onward). Most species of the subfamily are small to medium-sized understory trees and shrubs growing in low altitude tropical forests. Exceptions to this general picture are found in some *Alstonia* and *Dyera* species that reach into the canopy or are even canopy emergents (up to 80 m), while a number of species in the genera *Alyxia*, *Carissa*, and *Vallesia* typically grow in drier scrub forests (Endress and Bruyns, 2000; Middleton, 2007). Lianescent genera are scattered throughout five tribes, with a main focus of diversity in Willughbeieae, in which 14 of 24 genera represent climbers. The economic use of the family as a whole is undoubtedly important. Especially in the field of medicine, extracts of apocynaceous plants have long been widely used to treat malaria, diarrhea, diabetes, skin diseases, and in cancer chemotherapies (Middleton, 2007). In addition, species of *Alstonia*, *Aspidosperma*, *Cerbera*, *Dyera*, and *Gonioma* are valuable timbers (Chalk et al., 1935; Record and Hess,

1943; Ingle and Dadswell, 1953; Soerianegara and Lemmens, 1993; Sosef et al., 1998).

Apocynaceae s.l. have always been placed within the order Gentianales and can be easily distinguished from other Gentianales families by the presence of latex (Middleton, 2007). Nonetheless, the exact taxonomic position of Apocynaceae within the order remains in dispute (Struwe et al., 1994; Endress et al., 1996; Backlund et al., 2000; Potgieter and Albert, 2001; Bremer et al., 2002). Also the higher level intrafamily relationships have been the subject of conflicting ideas. In the past, the subfamily Rauvolfioideae was placed with Apocynoideae in a narrowly defined Apocynaceae s.s., which was considered to be closely related to the former Asclepiadaceae (including the current subfamilies Asclepiadoideae, Periplocoideae, and Secamonoideae). Within Apocynaceae s.s., Rauvolfioideae was believed to be the “primitive” group and could be identified based on the sinistrorsely contorted corolla lobes in bud, unspecialized anthers that are free from the style head, and a broad array of fruit and seed types (Endress and Bruyns, 2000). Molecular phylogenetic analyses provided new insights into the higher level relationships of the study group: the traditional Rauvolfioideae as well as Apocynoideae (Apocynaceae s.s.) are now proven to be paraphyletic, while the former Asclepiadaceae are considered to be polyphyletic with its separate components all nested in Apocynoideae (Livshultz et al., 2007; Simões et al., 2007). Consequently, contemporary systematists favor the recognition of one broadly defined Apocynaceae s.l. family (Fig. 1; Sennblad and Bremer, 1996, 2002; Endress and Bruyns, 2000; Potgieter and Albert, 2001; Livshultz et al., 2007; Simões et al., 2007).

With the exception of the rauvolfioid tribes Tabernaemontaneae and Alyxieae, the former of which is characterized by lignified guide rails on the anthers (Endress and Bruyns, 2000) and the latter by 2–3-porate pollen grains with irregular shapes

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⁶ Author for correspondence (e-mail: frederic.lens@bio.kuleuven.be)

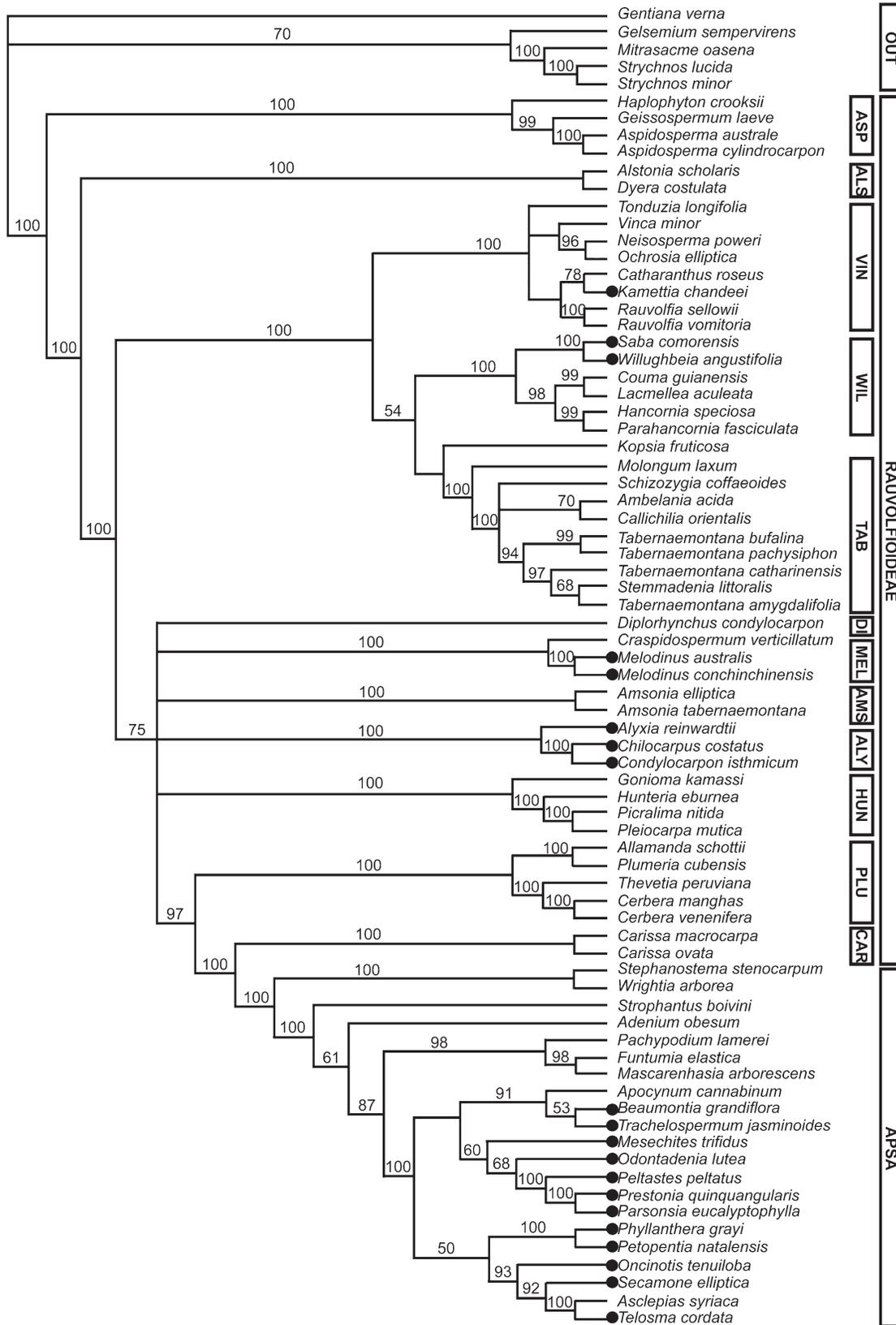


Fig. 1. Strict consensus of the 28 most parsimonious trees generated by five molecular data sets (*matK*, *rbcl*, *r116* intron, *rps16* intron, and *3' trnK* intron) combined. Climbing taxa are indicated with a black circle. Bootstrap values greater than 50% are indicated above the branches. The clades identified are abbreviated as follows: OUT = outgroup; ASP = Aspidospermeae clade; ALS = Alstonieae clade; VIN = Vinceae clade; WIL = Willughbeieae clade;

and porate ectoapertures having thickened margins (Endress et al., 2007a), there are few distinguishing morphological characters useful at the tribal level. Due to the monotonously similar, small, whitish flowers found in many genera, traditional classifications of Rauvolfioideae were based almost exclusively on fruit and seed characters (Schumann, 1895; Pichon, 1948a, b, 1949; Leeuwenberg, 1994). The study of Potgieter and Albert (2001) demonstrated, however, that fruit and seed characters are strongly homoplasious, meaning that most traditional tribes in Rauvolfioideae were not monophyletic.

Simões and coworkers (2007) contributed toward the unraveling of the complex subfamilial relationships in Rauvolfioideae using a phylogenetic analysis based on more than 1500 informative characters from the plastid DNA in combination with 41 morphological characters (Fig. 1). The results still showed that three of nine tribes recognized at that time (Endress and Bruyns, 2000) were polyphyletic, which led to a revised classification with 10 tribes (Endress et al., 2007b). Compared to the rest of the family, Rauvolfioideae form a basal grade (Fig. 1), supporting its presumably “primitive” features based on morphological observations (Endress and Bruyns, 2000). The tribe Aspidospermeae is the earliest branching clade, followed by Alstonieae. The next clade is formed by a group including Vinceae, which are sister to Willughbeieae and Tabernaemontaneae. In the remaining Rauvolfioideae, the relationships between the tribes Alyxieae, Hunterieae, and Melodineae, as well as the taxonomic position of the genera *Amsonia* and *Diplorhynchus* remain unresolved, although good support is found for the tribes Plumerieae and Carisseae, which contain the most derived taxa in the Rauvolfioideae and are placed at the base of the higher Apocynaceae, represented by the subfamilies Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae (the APSA clade; Fig. 1; Livshultz et al., 2007).

The current study provides an overview of the wood anatomy of Rauvolfioideae, incorporating a significant amount of new data, thereby filling in a number of gaps left by previously published anatomical studies (Record and Hess [1943], 23 genera; Metcalfe and Chalk [1950], 34 genera; Ingle and Dadswell [1953], 8 genera; Woodson et al. [1957], root wood of 24 *Rauvolfia* species; Détienne et al. [1982], 8 genera; Détienne and Jacquet [1983], 12 genera; Sidiyasa [1998], 44 *Alstonia* specimens representing 13 species; Baas et al. [2007], 30 genera with species descriptions on the InsideWood website [IWG, 2004 onward]). We have found no literature data on wood anatomical descriptions of 13 genera included in our study, indicating that this work adds considerably to our wood anatomical knowledge within Apocynaceae.

Four major considerations triggered the initiation of the current study: (1) the lack of a thorough wood anatomical study at the family level, (2) the evaluation of potentially useful phylogenetic wood characters and their evolutionary significance within Apocynaceae (cf. Lens et al., 2007b) (3), the renewed interest in Apocynaceae systematics, and (4) the search for meaningful non-DNA characters that can help circumscribe the Rauvolfioideae tribes with morphologically similar flowers and/or fruits and seeds. Because of the large number of species within the family (over 4500 spp.) and our extensive sampling (about 250 spp.), we have chosen to split our Apocynaceae

treatment into three extensive studies: one focusing on the paraphyletic subfamily Rauvolfioideae based on 50 of 84 genera (the current study), a second one dealing with the subfamilies Apocynoideae-Periplocoideae (F. Lens, M.E. Endress, and E. Smets, unpublished manuscript), and a third one treating the wood anatomy of Secamonoideae-Asclepiadoideae (F. Lens, M. E. Endress, U. Meve [University of Bayreuth, Germany] and E. Smets, unpublished manuscript). Information from these three studies will allow us to combine the wood anatomical features with available molecular data in future phylogenetic analyses at the family level.

MATERIALS AND METHODS

In total, 103 wood specimens of Rauvolfioideae belonging to 91 species and 50 genera, including members of all 10 tribes as delimited by Simões et al. (2007), were investigated using LM and SEM (Appendix 1; S1, S2 with Supplemental Data in online version of this article). We found no wood descriptions in the literature for several of the genera included in this study: *Callichilia*, *Chilocarpus*, *Cyclocotyla*, *Dictyophleba*, *Kamettia*, *Leuconotis*, *Melodinus*, *Orthopichonia*, *Pleiocarpa*, *Saba*, *Stephanostegia*, *Vahadenia* and *Willughbeia*. Most samples are represented by mature sapwood, except for the juvenile twigs of *Alyxia subalpina*, *A. sulana*, *Callichilia subsessilis*, *Carissa* sp., *Chilocarpus torulosus*, *Kamettia caryophyllata*, *Landolphia gummifera*, *Melodinus forbesii*, *Orthopichonia cirrhosa*, one (Jongkind 4737) of the two samples of *Saba comorensis* investigated, *Tabernaemontana eglandulosa*, *T. siphilitica*, *Thevetia ahouai*, *Vahadenia laurentii*, and *Vallesia glabra* (Appendices 1 and online S1). In general, wood of stem samples less than 20 mm in diameter is considered to be juvenile in Apocynaceae.

The methodology of wood sectioning and the subsequent steps are described in Lens et al. (2005). The wood anatomical terminology follows the “IAWA list of microscopic features for hardwood identification” (IAWA Committee, 1989). The phylogenetic significance of quantitative wood characters, such as vessel element length, abundance of uniseriate rays, number of cells per axial parenchyma strand, and total height of multiseriate rays, was based on the range of mean values for all species studied within a tribe (Table 1).

To make this paper more understandable for a broad audience, we briefly explain the most important wood anatomical characters relevant to this study. *Vessel grouping* is observed using a transverse section and has three major states in Apocynaceae: exclusively solitary (more than 95% of the vessels do not touch each other), predominantly in radial multiples (vessels form groups that are orientated radially, i.e., parallel to the direction of the rays), and predominantly in clusters (vessels grouped without any orientation). *Vessel elements* are perforated xylem cells that make up *vessels* (= multicellular tubes). The *axial parenchyma* distribution, also determined using transverse sections, is variable within Apocynaceae but there are two major types: apotracheal (parenchyma cells not in association with vessels) and paratracheal (parenchyma cells adjacent to vessels). We define *tracheids* as long, imperforate cells with more than one row of distinctly bordered pits in tangential and radial walls (usually between 5–8 μm in horizontal diameter), or with only one row of very large conspicuously bordered pits (more than 8 μm in horizontal diameter). *Fibers* have a similar shape to tracheids, but have fewer pits and usually less distinct pit borders; two fiber types can be distinguished within Rauvolfioideae: *fiber-tracheids* with one row of distinctly bordered pits occurring in both radial and tangential walls (pit borders usually 3–6 μm in horizontal diameter in Rauvolfioideae), and (septate or nonseptate) *librifform fibers* with simple to minutely bordered pits more common in the radial than the tangential walls (pits 2–3 μm in horizontal diameter).

RESULTS

In our descriptions of the studied material, tribal names correspond to those on the phylogenetic tree in Fig. 1. Numbers without

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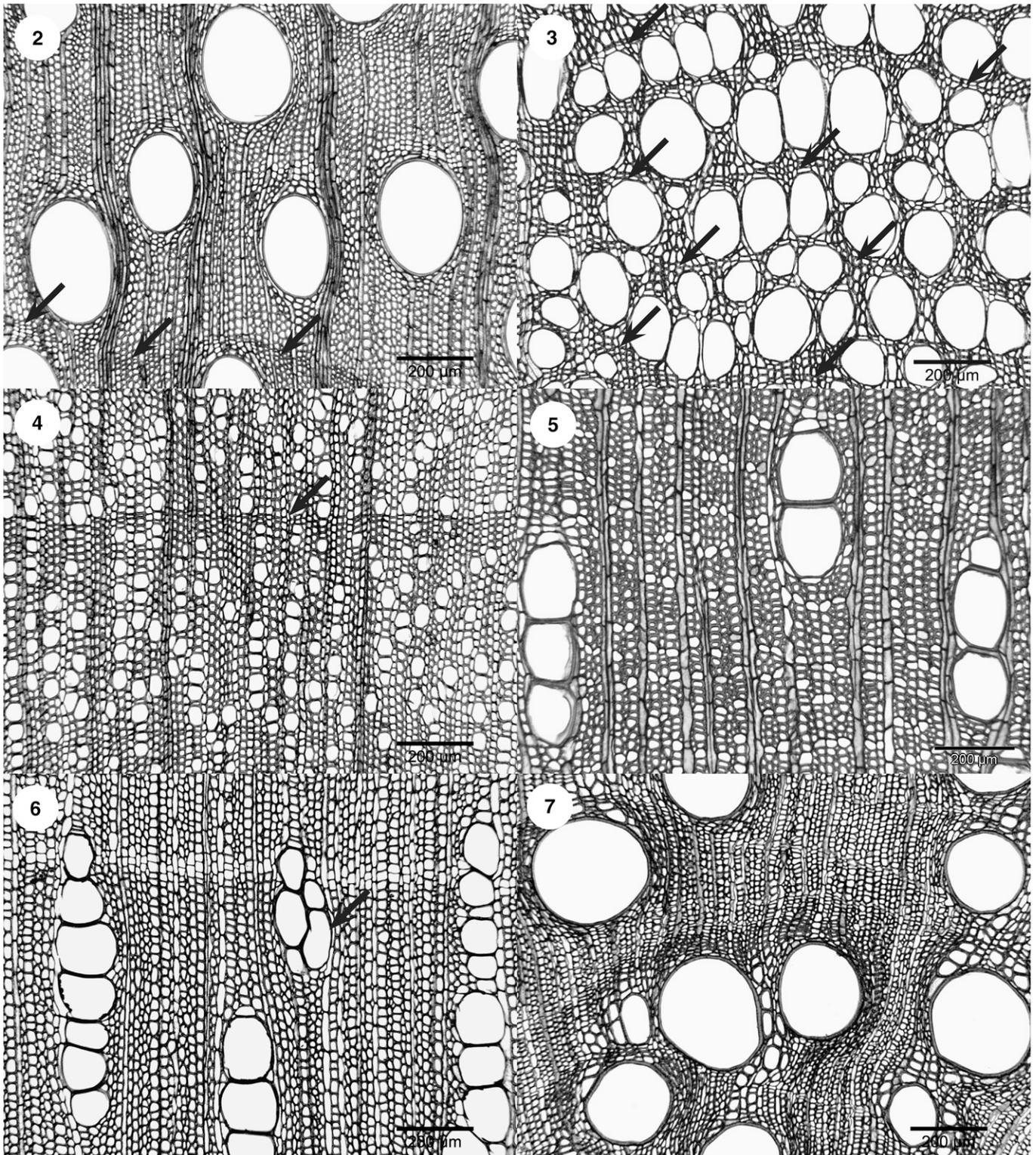
TAB = Tabernaemontaneae s.l. clade; DI = *Diplorhynchus*; MEL = Melodineae clade; AMS = *Amsonia* clade; ALY = Alyxieae clade; HUN = Hunterieae clade; PLU = Plumerieae clade; CAR = Carisseae clade; APSA = Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae. This figure was first published in Simões et al. (2007) and is reproduced here with permission from *Annals of the Missouri Botanical Garden*.

TABLE 1. Wood anatomical comparison among the tribes of the subfamily Rauvolfioideae sensu Simões et al. (2007). Tribe abbreviations are defined in Fig. 1. + = always or predominantly present, ± = sometimes present, — = absent or very infrequent.

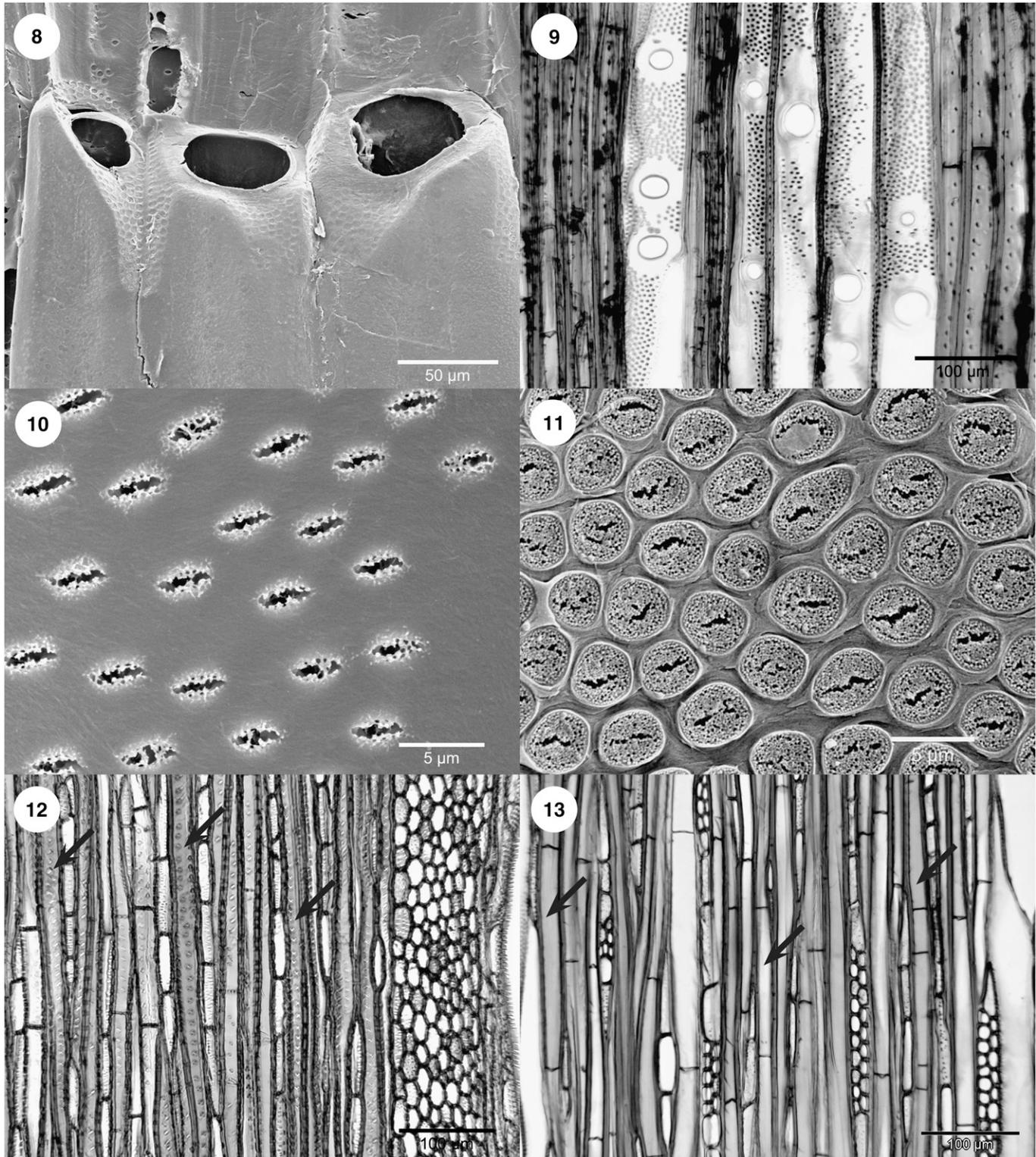
Diagnostic wood features at tribal level	ASP	ALS	VIN	WIL	TAB	MEL	ALY	HUN	PLU	CAR
Solitary vessels abundant	+	—	+	+	—	+	+	+	—	+
Radial multiples abundant	—	+	±	±	+	—	—	—	+	—
Vessels typically solitary and in radial multiples	—	—	—	—	+	±	—	—	—	—
Range of mean vessel element lengths (µm)	700–900	800–1000	700–1000	500–700	800–1100	500–700	600–850	500–700	400–650	450–650
Fibers with distinctly bordered pits	+	+	+	+	+	+	+	+	±	+
Fibers with simple to minutely bordered pits	—	—	—	—	+	—	—	—	±	—
Septate fibers	+	—	—	—	—	—	—	+	—	—
Thick- to very thick-walled fibers	—	—	—	—	—	—	—	—	—	—
Tracheids present	—	—	—	—	—	—	—	—	—	—
Axial parenchyma mainly apotracheal	—	—	—	—	—	—	—	—	—	—
Axial parenchyma apo- and paratracheal	+	+	+	±	±	±	+	+	+	+
Axial parenchyma mainly paratracheal	—	—	—	±	—	±	—	—	±	—
Axial parenchyma scarce to absent	±	—	—	±	—	—	—	—	—	—
Axial parenchyma scarce to absent	—	—	—	—	+	—	—	—	—	—
Mean range of cells per axial parenchyma strand	7–12	6–10	5–8	5–8	5–8	5–9	4–8	4–7	4–7	5–8
Uniseriate rays scarce to absent	+	+	—	—	±	—	—	—	—	—
Multiseriate ray (MR) height (µm)	300–500	400–700	700–900	400–600	700–1500	400–600	500–1500	300–500	200–500	200–450
MR often fused via their long uniseriate ray margins	—	—	+	—	+	±	±	—	—	—
Crystals in rays	±	—	+	—	+	+	—	—	—	—
Crystals in axial parenchyma	+	±	+	±	—	±	±	—	—	+
Latificers in rays	—	±	—	+	—	—	±	—	—	—

parentheses are ranges of means, while numbers within parentheses represent minimum or maximum values. A summary of the results is shown in Appendix S2 (see Supplemental Data with the online version of this article) and Table 1. The following genera represent climbers: *Kametia* (Fig. 7; Vinceae), *Cyclocotyla* (Figs. 17, 25), *Dictyophleba* (Figs. 19, 28), *Landolphia*, *Leuconotis*, *Orthopichonia* (Fig. 31), *Saba* (Fig. 2), *Vahadenia*, *Willughbeia* (all eight Willughbeieae), *Melodinus* (Melodineae), *Alyxia* (Fig. 3), *Chilocarpus* (Figs. 12, 24, 34), *Condylocarpon* (all three Alyxieae), and *Allamanda* (Plumerieae).

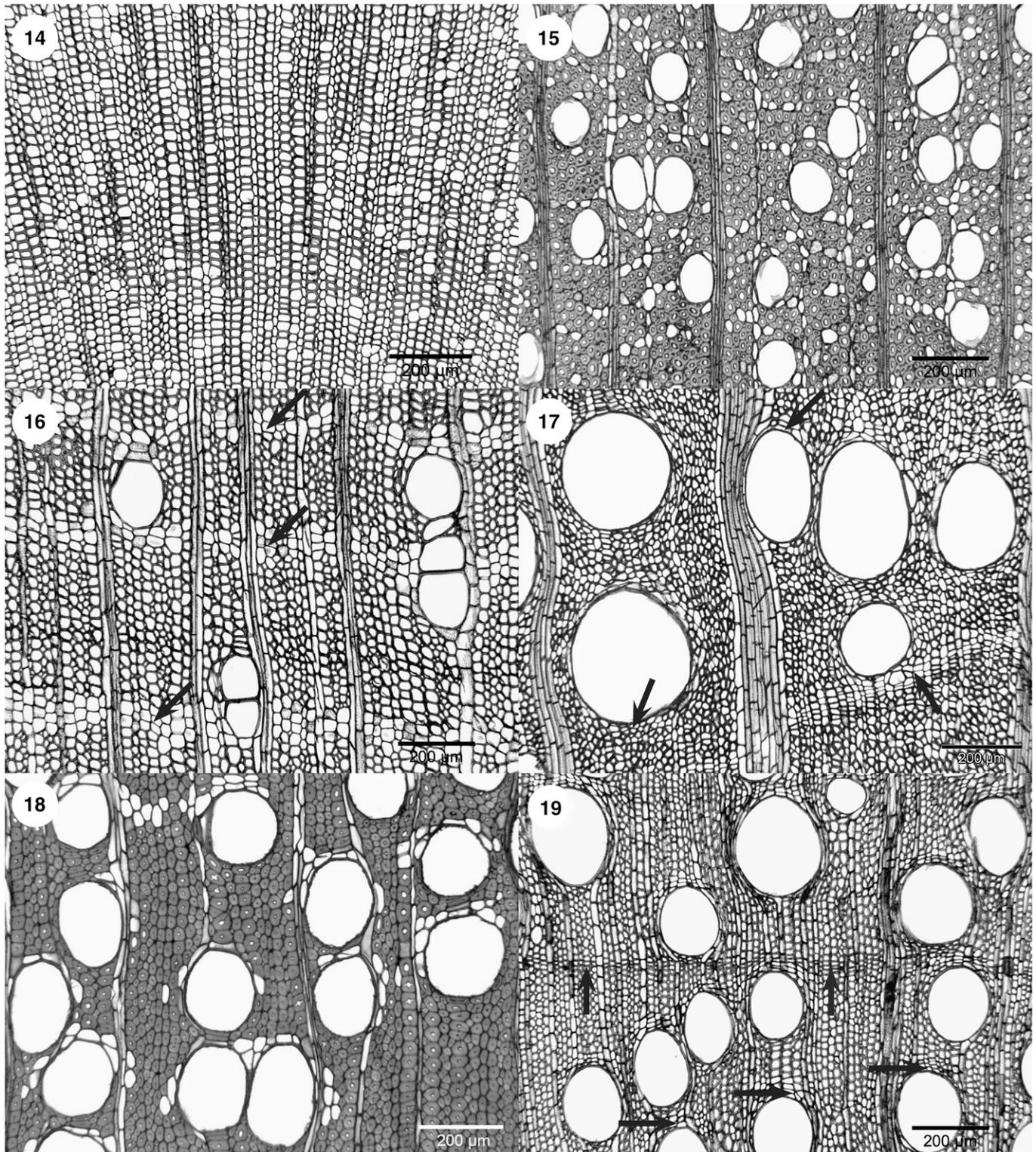
Wood description of Rauvolfioideae (Figs. 2–34)—Growth ring boundaries usually indistinct (Figs. 2–3), although distinct in species of *Callichilia* (Fig. 4), *Chilocarpus*, *Dictyophleba* (Fig. 19), *Diplorhynchus*, *Geissospermum*, *Landolphia*, *Orthopichonia*, *Plumeria*, *Saba*, and *Tabernaemontana*; no growth ring boundaries observed in the genera *Alstonia*, *Cerberiopsis*, *Couma*, *Dyera*, *Hancornia*, *Lepiniopsis*, *Macoubea*, and *Microplumeria*. Wood diffuse-porous in most genera, but with a tendency to (semi)ring-porosity in the climbing genera *Alyxia* (Fig. 3) and *Chilocarpus*. Vessels (1–)4–150(–190)/mm²; vessel grouping predominantly solitary (Figs. 2, 15, 17–19) in the nonclimbing tribes Aspidospermeae, Hunterieae, Carisseae, in the nonclimbing representatives of Vinceae (although *Kopsia* species with abundant radial vessel multiples), and also in the climbing species of Alyxieae (Fig. 3), Willughbeieae and Melodineae; vessel grouping mixed solitary and in radial multiples in the nonclimbing tribe Tabernaemontaneae (Fig. 4); vessel grouping typically in radial multiples of 2–4(–6) vessels in the nonclimbing tribe Alstonieae (Fig. 16) and in the nonclimbing genera of Willughbeieae (Fig. 5), and radial multiples of 2–6(–8) vessels in the nonclimbing genera of Plumerieae (Fig. 6); vessel outline generally rounded to elliptical (Figs. 2, 3, 5–7), although sometimes angular (Fig. 4); perforation plates exclusively simple (Figs. 8, 9), sporadically double simple perforations in *Tabernaemontana* (Fig. 9). Intervessel pits alternate, pits 3–8 µm in horizontal diameter, vestured (Figs. 10–11). Vessel-ray pits similar to intervessel pits in size and shape throughout the ray cell. Wall sculpturing absent. Tyloses occasionally present in *Ambelania*, *Aspidosperma*, *Cyclocotyla*, *Dictyophleba*, *Hunteria*, *Kopsia*, *Leuconotis*, *Melodinus*, *Vahadenia*, and *Willughbeia*. Tangential diameter of vessels (15–)25–230(–430) µm, two vessel size classes in nearly all climbing species present as few narrow vessels in combination with many wide ones, many narrow vessels grouped with few wide ones in vessel clusters of *Kametia caryophyllata* (Fig. 7); vessel elements (100–)270–1380(–1850) µm long. Tracheids mainly absent, but present as the main imperforate cell type in the ground tissue in the mainly climbing genera of Alyxieae (*Alyxia*, *Chilocarpus* (Fig. 12), *Condylocarpon* and *Pteralyxia*), Melodineae (*Melodinus*), and some Willughbeieae (*Cyclocotyla* and *Leuconotis*); in the other climbing genera of Willughbeieae and in *Carissa* vascentric tracheids co-occur with tracheid-like cells or imperfect vessel elements having only one perforation; tracheid length (350–)600–1400(–1800) µm. Nonseptate fibers with distinctly bordered pits in radial and tangential walls (= fiber tracheids) common in all tribes (except for Tabernaemontaneae), fiber tracheid length (500–)580–2980(–3500) µm; septate fibers with simple to minutely bordered pits concentrated in radial walls (= libriform fibers) typically present in most genera of Tabernaemontaneae (*Callichilia*, *Stemmadenia*, *Tabernaemontana* [Fig. 13], *Voacanga*), (occasionally septate) libriform fibers present in species of



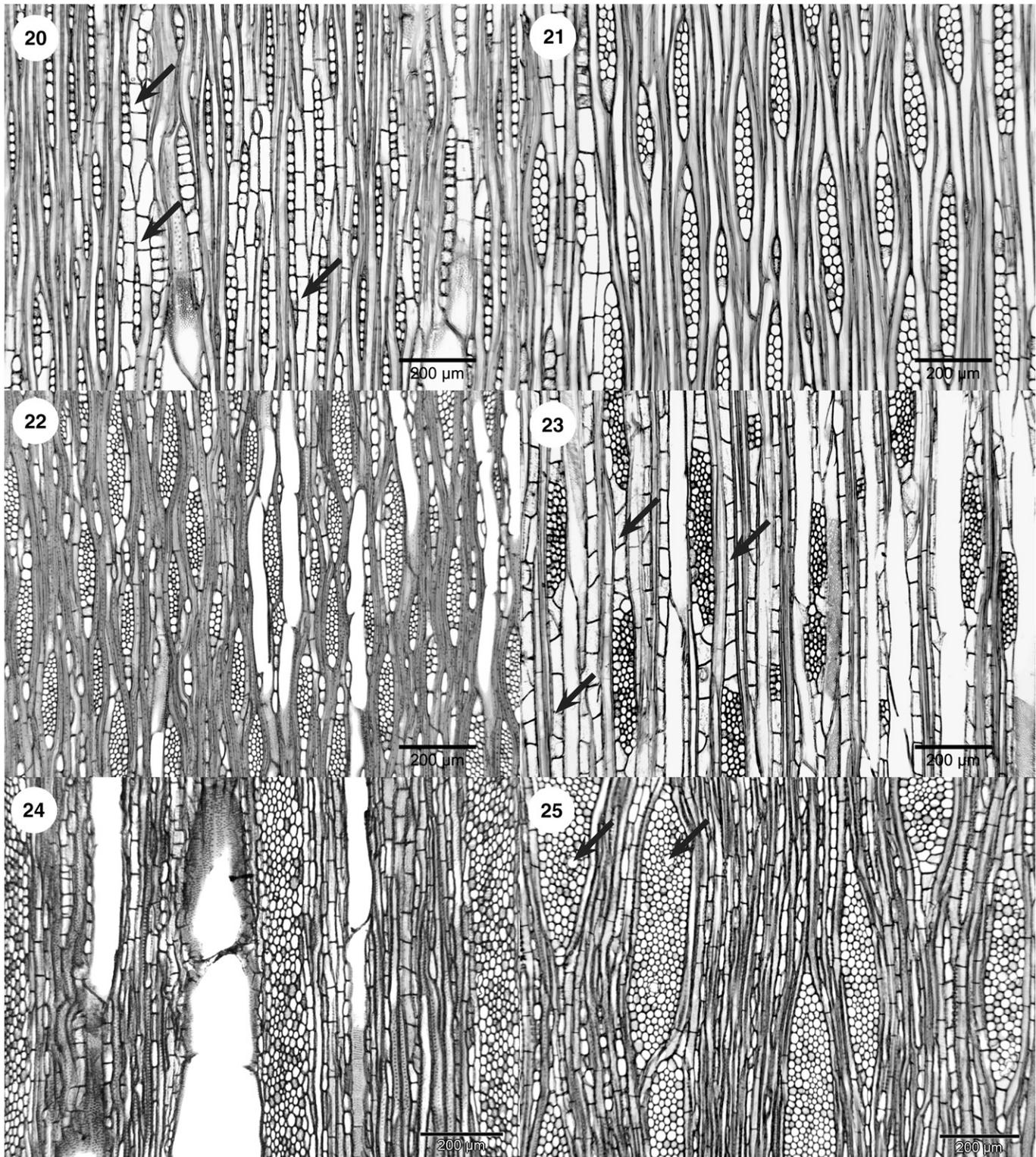
Figs. 2–7. LM images of transverse sections (TS) showing the diversity in vessel grouping patterns of Rauvolfioideae. Climbers are represented by Figs. 2 and 7. 2. *Saba comorensis* (WIL): TS, solitary vessels, growth ring boundary (arrows). 3. *Alyxia scabrida* (ALY): TS, tendency to (semi)ring-porosity, growth ring boundaries indistinct (arrows), vessels usually solitary or in short tangential multiples. 4. *Callichilia barteri* (TAB): TS, vessels solitary and in short radial multiples, growth ring boundary (arrow). 5. *Couma macrocarpa* (WIL): TS, vessels predominantly in short radial multiples. 6. *Cerbera floribunda* (PLU): TS, vessels in long radial multiples, with occasional vessel clusters (arrow). 7. *Kamettia caryophyllata* (VIN): TS, wide and narrow vessels grouped in pronounced vessel clusters.



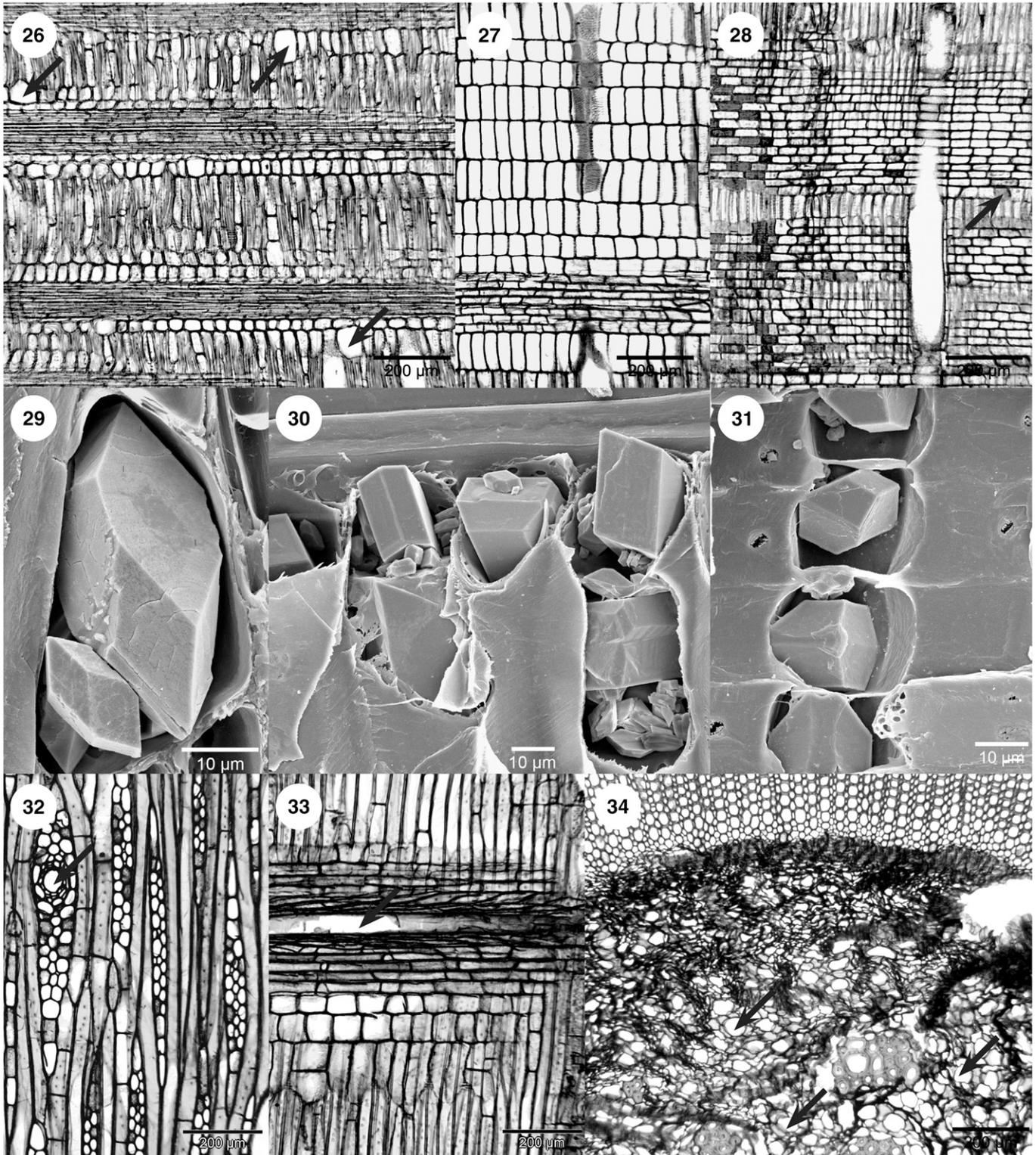
Figs. 8–13. Radial and tangential longitudinal SEM surfaces (Figs. 8, 10–11) and LM sections (9, 12–13) of the Rauvolfioideae wood structure showing vessel perforations, intervessel pitting, and imperforate tracheary elements. Climbers are represented by Fig. 12. **8.** *Thevetia peruviana* (PLU): RLS, simple vessel perforations. **9.** *Tabernaemontana attenuata* (TAB): RLS, double or triple simple perforations. **10.** *Aspidosperma cylindrocarpon* (ASP): TLS, vestured intervessel pits observed from the inside wall of vessels. **11.** *Stemmadenia tomentosa* (TAB): TLS, vestured intervessel pits observed from the outside wall of vessels. **12.** *Chilocarpus suaveolens* (ALY): TLS, ground tissue consisting of tracheids with conspicuously bordered pits. **13.** *Tabernaemontana siphilitica* (TAB): TLS, septate libriform fibers (arrows) with almost no pits in the tangential walls.



Figs. 14–19. Transverse LM sections illustrating the variation in axial parenchyma distribution of Rauvolfioideae. Climbers are represented by Figs. 17 and 19. **14.** *Tabernaemontana panamensis* (TAB): TS, axial parenchyma scarce to absent. **15.** *Microplumeria anomala* (ASP): TS, axial parenchyma apotracheal: diffuse-in-aggregates with tendency to form short bands. **16.** *Alstonia scholaris* (ALS): TS, banded apotracheal axial parenchyma (arrows). **17.** *Cyclocotyla congolensis* (WIL): TS, axial parenchyma in narrow apotracheal bands in combination with scanty paratracheal parenchyma (arrows pointing downward) and banded marginal parenchyma (arrow pointing upwards). **18.** *Aspidosperma album* (ASP): TS, axial parenchyma unilateral paratracheal. **19.** *Dictyophleba ochracea* (WIL): TS, distinct growth rings (vertical arrows), axial parenchyma unilateral paratracheal (horizontal arrows).



Figs. 20–25. Tangential longitudinal wood sections (LM) showing width, height, and density of rays. Climbers are represented by Figs. 24–25. **20.** *Himatanihus articulatus* (PLU): TLS, very narrow and low multiserial rays, axial parenchyma strands well visible (arrows). **21.** *Alstonia scholaris* (ALS): TLS, low 3-seriate rays, uniseriate rays scarce. **22.** *Gonioma kamassi* (HUN): TLS, low multiserial rays. **23.** *Voacanga globosa* (TAB): TLS, fused multiserial rays interconnected by their long uniseriate ray margins, which sometimes fuse multiserial rays (arrows). **24.** *Chilocarpus suaveolens* (ALY): TLS, wide and high multiserial rays. **25.** *Cyclocotyla congolensis* (WIL): TLS, wide multiserial rays with laticifers (arrows).



Figs. 26–34. Wood anatomical sections (LM; Figs. 26–28, 32–34) and longitudinal surfaces (SEM; Figs. 29–31) of Rauvolfioideae showing multiserial ray composition, crystal occurrence, laticifers, and intraxylary phloem. Climbers are represented by Figs. 28, 31, and 34. **26.** *Pleiocarpa pycnantha* (HUN): RLS, procumbent body ray cells and 1–2 square to upright marginal ray cells, arrows point to oil/mucilage cells. **27.** *Ambelania acida* (TAB): RLS, procumbent body ray cells and over 10 rows of upright marginal ray cells. **28.** *Dictyophleba ochracea* (WIL): RLS, homogeneous rays consisting of procumbent body cells and mainly square marginal ray cells (arrow). **29.** *Stemmadenia tomentosa* (TAB): TLS, two elongate crystals in ray cell. **30.** *Tabernaemontana macrocarpa* (TAB): RLS, large prismatic crystals co-occurring with microcrystals of varying shapes in the same ray cell. **31.** *Orthopichonia seretii* (WIL): RLS, prismatic crystals in axial parenchyma. **32.** *Dyera costulata* (ALS): TLS, laticifer in multiserial ray (arrow). **33.** *Dyera costulata* (ALS): RLS, laticifer in multiserial ray (arrow). **34.** *Chilocarpus torulosus* (ALY): TS, intraxylary phloem in the pith region (arrows).

Cerberiopsis, *Plumeria*, and *Thevetia* (all nonclimbing Plumerieae), libriform fiber length (650–)865–2300(–3300) μm ; fibers mainly thin-walled or thin- to thick-walled (Figs. 2–7), but very thin-walled in the nonclimbing species of *Alstonia* (Fig. 16), *Cerbera* (Fig. 6), *Dyera*, and *Macoubea*, and thick- to very thick-walled in most Aspidospermeae (Figs. 15, 18), Hunterieae, and in *Plumeria rubra*. Axial parenchyma predominantly diffuse-in-aggregates (Fig. 15) to narrowly banded (usually 1- or 2-seriate, sometimes up to 4-seriate in *Aspidosperma*, *Geissospermum*, and *Alstonia*) in the nonclimbing species of the following tribes: Aspidospermeae (also unilateral paratracheal parenchyma in some species of *Aspidosperma*, Fig. 18), Alstonieae (strong tendency to form bands, Fig. 16), Vinceae, Willughbeieae, Melodineae, Hunterieae, Plumerieae (also tendency to paratracheal parenchyma in several species), and Carisseae; a mixture of diffuse apo- and scanty paratracheal parenchyma typically present in the climbing representatives of Alyxieae and Melodineae, and in the nonclimbing *Diplorhynchus*; a mixture of diffuse-in-aggregates to banded apotracheal parenchyma in combination with scanty or unilateral paratracheal parenchyma present in the climbing genera of Willughbeieae (Figs. 17, 19); axial parenchyma scarce to absent in many genera of the nonclimbing tribe Tabernaemontaneae (Fig. 14); although banded apotracheal parenchyma in *Ambelania*, *Macoubea*, and *Mucoa*; banded marginal axial parenchyma present in *Allamanda*, *Cerbera*, *Chilocarpus*, *Condylocarpon*, *Cyclocotyla* (Fig. 17), *Lacmellea*, *Landolphia*, *Saba*, and *Thevetia*; axial parenchyma nonlignified in *Kametia caryophyllata*; in (3–)4–8(–16)-celled strands. Uniseriate rays scarce to absent (0–2 uniseriate rays/mm) in the species of the nonclimbing tribes Aspidospermeae and Alstonieae (Fig. 21) and in some Tabernaemontaneae (*Callichilia*, *Tabernaemontana*, *Voacanga*; Fig. 23) and in *Diplorhynchus* and *Plumeria rubra*; uniseriate rays less common than multiseriate rays (2–5 rays/mm) in most Hunterieae and Tabernaemontaneae; uniseriate rays equally common or more abundant in the other tribes (5–15 rays/mm); height (50–)150–500(–2000) μm ; uniseriate rays generally consisting of square to upright cells, although square to procumbent in *Aspidosperma*, *Cerberiopsis*, *Couma*, *Dictyophleba*, *Diplorhynchus*, *Geissospermum*, *Himantanthus*, *Landolphia*, *Lacmellea*, *Parahancornia*, *Pteralyxia*, *Orthopichonia*, and *Plumeria*. Multiseriate rays generally 2–4-seriate (Figs. 20, 21); 4–6-seriate in the climbing genera *Dictyophleba*, *Landolphia*, *Leuconotis*, *Saba*, and in the nonclimbing *Gonioma* (Fig. 22), *Ochrosia*, *Stemmadenia*, and *Voacanga* (Fig. 23); up to 10-seriate in the climbing *Chilocarpus* (Fig. 24) and *Condylocarpon*, up to 14-seriate in the climbing *Cyclocotyla* (Fig. 25); multiseriate ray height (100–)180–3160(>11000) μm high; typically less than 500 μm in the nonclimbing tribes Aspidospermeae (Fig. 22), Hunterieae, Carisseae, and in the nonclimbing members of Plumerieae (Fig. 20), commonly between 400–700 μm in the nonclimbing tribe Alstonieae (Fig. 21), and in the (non)climbing representatives of Willughbeieae and Melodineae, often more than 700 μm in the nonclimbing tribe Tabernaemontaneae (Fig. 23), nonclimbing members of Vinceae, and in the predominantly climbing tribe Alyxieae (Fig. 24); multiseriate ray density (0–)3–7(–11) rays/mm; consisting of procumbent body ray cells and 1–4(–20) rows of predominantly upright marginal ray cells (Figs. 26, 27), but square marginal ray cells often in *Cerberiopsis*, *Dictyophleba* (Fig. 28), *Hancornia*, *Landolphia*, *Leuconotis*, *Orthopichonia*, *Parahancornia*, and *Vahadenia*; multiseriate rays often fused with long uniseriate ends in most Vinceae and Tabernaemontaneae (Fig. 23), and in some Melodineae and Alyxieae; sheath cells some-

times present in *Gonioma*, *Melodinus*, *Pleiocarpa*, *Rauvolfia*, and *Voacanga* (Fig. 23); distended ray cells suggesting oil or mucilage cells in species of *Melodinus*, *Ochrosia*, *Pleiocarpa* (Fig. 26), *Stemmadenia*, *Tabernaemontana*, and *Voacanga*; rays nonlignified in *Kametia caryophyllata*. Dark amorphous contents present in rays of *Cerberiopsis*, *Couma*, *Dictyophleba*, *Lacmellea*, *Landolphia*, *Orthopichonia*, *Picalima*, *Vahadenia*, and *Willughbeia*. Prismatic crystals in procumbent and marginal (often chambered) ray cells frequent in Vinceae and Melodineae, and less common throughout rays of Aspidospermeae and Tabernaemontaneae (Fig. 30); microcrystals co-occurring in the same ray cells in *Rauvolfia sumatrana*, *Hancornia speciosa*, and *Tabernaemontana macrocarpa* (Fig. 30), prismatic and elongate crystals in upright ray cells of *Stemmadenia tomentosa* (Fig. 29), elongate crystals and microcrystals in upright ray cells of *Voacanga chalitiana*; prismatic crystals, microcrystals, and elongate crystals observed in septate fibers of *Tabernaemontana columbiensis*, *Callichilia subsessilis*, and *Stemmadenia tomentosa*; prismatic crystals typically present in chambered axial parenchyma cells of the tribes Aspidospermeae, Vinceae, and Carisseae, and in some species of Alstonieae, Willughbeieae (Fig. 31), Melodineae, and Alyxieae; microcrystals co-occurring in the same axial parenchyma cells of *Alstonia scholaris*, *Kametia caryophyllata*, and *Mucoa duckei*; silica bodies absent; laticifers common in all climbing genera of Willughbeieae plus the nonclimbing *Parahancornia*, and less frequent in the nonclimbing tribe Alstonieae (*Dyera*; Figs. 32, 33), *Diplorhynchus*, and *Vallesia*, and in the climbing genera of Alyxieae (*Alyxia*, *Chilocarpus*, *Condylocarpon*) and the climbing *Kametia*; intraxylary phloem observed in wood samples with pith tissue (Fig. 34), interxylary (included) phloem not observed.

DISCUSSION

Diagnostic wood features at the tribal level—Although some wood features remain constant within Rauvolfioideae (simple vessel perforations, alternate vested intervessel pits, vessel-ray pits that are similar in shape and size to the intervessel pits), our results show that several other wood characters vary conspicuously throughout the subfamily. Many of these variable features, however, are more or less uniform at the tribal level (Table 1), and include (1) vessel grouping (two main types: exclusively solitary [Fig. 2] or predominantly in radial vessel multiples [Fig. 5]), (2) vessel element length (two main types: on average below or above 700 μm), (3) fiber type (two main types: fiber tracheids with distinctly bordered pits or libriform fibers with simple to minutely bordered pits), (4) the presence of tracheids (present/absent; Fig. 12), (5) the distribution of axial parenchyma (four main types: mainly apotracheal, mainly paratracheal, a combination of both, or scarce to absent; Figs. 14–19), (6) the scarcity of uniseriate rays (two main types: 0–2/mm or more abundant; Figs. 20–25), (7) the presence of long uniseriate ray margins that connect multiseriate rays (present/absent; Fig. 23), and (8) the occurrence of laticifers in rays (present/absent; Figs. 32, 33). In addition to these diagnostic features, more variable characters at the tribal level can sometimes also be used to distinguish between tribes. Examples are the mean range of cells per axial parenchyma strand (two main types: mean range 4–8 or 7–12), the height of multiseriate rays (two main types: on average below or above 500 μm ; Figs. 20–25), and the occurrence and location of prismatic crystals (four main types: in rays, in axial parenchyma, a combination of both,

or absent; Figs. 29–31). As explained in the following paragraphs, a combination of all these diagnostic wood characters allows the identification of most tribes (Table 1), which is a major step forward in finding meaningful morphological support for the revised Rauvolfioideae classification.

From a wood anatomical point of view, most species observed of Tabernaemontaneae s.l. (according to the broad circumscription of Endress and Bruyns [2000]) can be clearly distinguished from the remaining rauvolfioids due to their septate fibers with simple pits (Fig. 13) and their loss of axial parenchyma (Figs. 4, 14), a combination of characters that is also present in *Stenosolen* (Record and Hess, 1943) and in many (or even all?) generic synonyms that are now included in *Tabernaemontana*, such as *Anartia* (Record and Hess, 1943; present study), *Bonafousia* (Record and Hess, 1943; present study), *Conopharyngia* (Metcalf and Chalk, 1950; present study), *Ervatmia* (Ingle and Dadswell, 1953; present study), *Hazunta* (present study), *Pagiantha* (present study), *Peschiera* (Record and Hess, 1943; present study), and *Rejoua* (Ingle and Dadswell, 1953). Moreover, when calcium oxalate crystals are present, different types (prismatic, elongate and/or microcrystals; Figs. 29–30) can be observed in the marginal ray cells and sometimes in the septate fibers. In contrast, we have observed three atypical Tabernaemontaneae genera, i.e., *Ambelania*, *Macoubea*, and *Mucoa*, with distinctly bordered fiber pits, diffuse-in-aggregates to banded axial parenchyma, and calcium oxalate crystals only in the axial parenchyma cells (no crystals in *Ambelania*). These obvious differences agree with a preliminary molecular study of Simões et al. (2006), who found two major monophyletic clades within Tabernaemontaneae s.l.: the small neotropical Ambelanieae clade (including among others *Ambelania*, *Macoubea*, and *Mucoa*) characterized by indehiscent fruits, and the larger pantropical Tabernaemontaneae s.s. clade comprising nine genera, all — with perhaps one or two exceptions — with dehiscent fruits. Although the presence of septate fibers with simple pits and the lack of axial parenchyma in Tabernaemontaneae s.s. represent a unique combination of wood characters within Rauvolfioideae (and even within the entire family), three additional features demonstrate that the genera *Ambelania*, *Macoubea*, and *Mucoa* can be linked with Tabernaemontaneae s.s., i.e., (1) long vessel elements (on average >1000 µm), (2) high proportion of fused multiseriate rays via their long uniseriate ray margins (Fig. 23), and (3) abundance of multiseriate rays compared to uniseriate ones (Fig. 23). Therefore, wood anatomy supports a relationship between the Ambelanieae clade and Tabernaemontaneae s.s., which is also corroborated by chemical, floral, and molecular evidence (Endress et al., 1996; Sennblad and Bremer, 1996; Endress and Bruyns, 2000; Simões et al., 2007). On the other hand, their similar habits and environmental preferences (almost all trees occurring in tropical lowland forests) have not prevented the development of markedly different wood anatomical characters, providing support for a possible resurrection of the previously defined Ambelanieae tribe (plus *Macoubea*) and the traditional Tabernaemontaneae s.s. (cf. Leeuwenberg, 1994).

Two tribes can be distinguished from the remaining Rauvolfioideae based on the abundance of radial vessel multiples, i.e., Alstonieae (Fig. 16) and Plumerieae, although the multiples in Plumerieae tend to be much longer (up to 10 vessels; Fig. 6) and have a tendency to form vessel clusters (Fig. 6; Appendix S2, see Supplemental Data with the online version of this article). Nevertheless, frequent radial vessel multiples are not restricted to these two tribes: they are also observed in the genera *Kopsia* (Vinceae), in *Couma* (Fig. 5), *Hancornia*, *Lacmellea*,

and *Parahancornia* (all four nonclimbing Willughbeieae), and in *Ambelania*, *Macoubea*, and *Mucoa* (Ambelanieae clade of Tabernaemontaneae s.l.), demonstrating the homoplasious nature of vessel grouping within Rauvolfioideae (Fig. 1). Despite their rather similar vessel grouping pattern, Alstonieae can be easily distinguished from Plumerieae because of their longer vessel elements (on average 800–1000 µm vs. 400–650 µm, respectively), fiber type (distinctly bordered pits vs. reduced pit borders), axial parenchyma distribution (exclusively apotracheal vs. tendency to form paratracheal parenchyma), mean range of cells per axial parenchyma strand (6–10 vs. 4–7), and uniseriate ray abundance (0–2/mm vs. 4–10/mm). Therefore, the wood structure rejects a close relationship between the two tribes (cf. Simões et al., 2007; Fig. 1). Within Alstonieae, the comprehensive revision of the genus *Alstonia* (Sidiyasa, 1998) revealed a surprisingly high number of phylogenetically informative characters for distinguishing between section *Alstonia* on the one hand (light *Alstonia*), and sections *Monuraspermum* and *Dissuraspermum* on the other (heavy *Alstonia*). Examples are vessel density, intervessel pit size, fiber thickness, axial parenchyma distribution, and the presence/absence of laticifers. Based on our sampling in the genus *Aspidosperma* (Aspidospermeae), another rauvolfioid genus with a remarkable variation in axial parenchyma distribution, the distinction between the apotracheal vs. paratracheal type is not in agreement with the most recent intrageneric classification, which divided *Aspidosperma* into a *Nobilia* and *Excelsa* alliance (Potgieter, 1999).

Four tribes are entirely characterized by exclusively solitary vessels: Aspidospermeae (Figs. 15, 18), Alyxieae (except for the genus *Lepiniopsis*, which has an equal proportion of solitary vessels and radial vessel multiples), Hunterieae, and Carisseae. Within this group of four tribes, Aspidospermeae (except for *Microplumeria* and *Vallesia*) and Hunterieae can be identified by thick- to very thick-walled fibers (Figs. 15, 18), although obvious differences in the mean range of vessel element length (700–900 µm vs. 500–700 µm, respectively), the mean range of cells per axial parenchyma strand (7–12 vs. 4–7), the scarcity of uniseriate rays (0–2/mm vs. more), and the presence of crystals (nearly always present in the axial parenchyma and often in rays vs. nearly always absent) reveal that Aspidospermeae and Hunterieae are not closely related (cf. Simões et al., 2007). The two other tribes, Alyxieae and Carisseae, resemble each other more, but they can be distinguished from each other based on the axial parenchyma distribution (apo- and paratracheal vs. exclusively apotracheal), and the multiseriate ray height (500–1500 vs. 200–450 µm). Among the four tribes with exclusively solitary vessels, Hunterieae and Carisseae resemble each other the most: only the thick-walled fibers in Hunterieae and the presence of prismatic crystals in the axial parenchyma of Carisseae provide distinctive characters. Alyxieae is the most easily recognizable because of its tracheids being the main imperforate cell type in the ground tissue (Fig. 12), apo- as well as paratracheal parenchyma, and high multiseriate rays (500–1500 vs. <500 µm; Fig. 12), while most Aspidospermeae can be identified by the presence of more than seven cells per axial parenchyma strand.

In addition to the four tribes mentioned in the previous paragraph, most of the species observed in Vinceae, Willughbeieae (Figs. 2, 17, 19), and perhaps also Melodineae have exclusively solitary vessels, although these three tribes contain at least one genus that is characterized by abundant radial vessel multiples (Fig. 5; online supplemental Appendix S2.). Among these three tribes, Vinceae can be identified by its long vessel elements (700–1000 µm vs. 500–700 µm in the two other tribes), the

absence of tracheids (vs. the general presence in the other two tribes), and its long multiseriate rays (700–900 μm vs. 400–600 μm in the two other tribes). Within Vinceae, the presumably isolated position of *Kopsia* as sister to the rest (Simões et al., 2007) is supported by its abundant radial vessel multiples and relatively short vessel elements (mean range 500–600 μm). A diagnostic character of Willughbeieae is the tendency to form homogenous rays (i.e., procumbent to square cells common in uni- and multiseriate rays; Fig. 28). Although the Willughbeieae sampling in Simões et al. (2007) is limited, two well-supported major lineages are found: a New World clade consisting of erect trees (*Couma-Lacmellea-Hancornia-Parahancornia*) and an Old World clade consisting of lianas (*Saba-Willughbeia*) (Fig. 1). Both clades can be easily recognized by their vessel grouping (abundant radial vessel multiples (Fig. 5) vs. exclusively solitary (Figs. 2, 17, 19), respectively). In addition, most rays in the erect clade lack laticifers and tracheids (vs. present in the lianescent clade). Consequently, the occurrence of exclusively solitary vessels, presence of tracheids and laticifers in rays in the remaining Willughbeieae genera observed (*Cyclocotyla* (Fig. 17), *Dictyophleba* (Fig. 19), *Landolphia*, *Leuconotis*, *Orthopichonia*, and *Vahadenia*), which are all lianas confined to the Old World, point to a close relationship with *Saba* and *Willughbeia*. Because of our limited sampling in Melodineae, no combination of distinctive wood characters could be elucidated for the tribe.

Diplorhynchus—With respect to the unplaced monotypic African genus *Diplorhynchus*, the wood anatomy of the two specimens studied provides some evidence for assigning this genus to a tribe. Relevant characters are: exclusively solitary vessels, relatively short vessel elements (mean range 500–600 μm), nonseptate fibers with distinctly bordered pits, tracheids, apotracheal in combination with paratracheal parenchyma, number of cells per axial parenchyma strand (mean range 5–9), scarce uniseriate rays (0–3/mm), intermediate multiseriate ray height (450–600 μm), prismatic crystals in rays, and laticifers. Based on these features, the genus *Diplorhynchus* resembles most members of the tribes Alyxieae, Melodineae and Willughbeieae (Table 1). Taking into account the results of the molecular analysis of Simões et al. (2007), a position in Willughbeieae is not supported. Rather, Alyxieae or Melodineae seem to be the best candidates. A possible inclusion of *Diplorhynchus* in Alyxieae is not favored because Alyxieae are characterized by a unique type of pollen with large porate apertures, which is a synapomorphy for the tribe and found nowhere else in the entire family, and also by the lack of indole alkaloids (Van Der Ham et al., 2001; Endress et al., 2007a). Melodineae, in contrast, have pollen that is colpate or with the small pores typical for the family and contain indole alkaloids. Thus, a position in Melodineae is considered to be the better option. In particular, *Diplorhynchus* shows some morphological similarity to *Pycnobotrya* (Melodineae), another monotypic genus from West and Central Africa. The two genera share the following characters: copious white to yellow latex, terminal many-flowered inflorescences, anthers with sterile appendages at the base and apex, carpels with only four ovules in two series, fruit a pair of stout follicles, and compressed seeds with a diaphanous wing (Simões et al., 2007). The two genera were also considered to be closely related by Markgraf (1947) and Pichon (1950). Although additional data are needed to support this idea, we propose an interim position in Melodineae for *Diplorhynchus* based on the information currently available.

Wood anatomy vs. habit—Differences between the wood anatomy of climbers (representative Figs. 2–3, 7, 12, 17, 19, 24–25) and nonclimbers (representative Figs. 4–6, 8–9, 13–16, 18, 20–23) deserve special attention in Apocynaceae (Baas et al., 2007). Baas and coauthors proposed a divergence in vessel grouping between the erect species (vessels in multiples common) and climbers (predominantly solitary vessels). Within the tribe Willughbeieae, this distinction in vessel grouping is fully supported (Figs. 5 vs. 2, 17, 19), but an examination at the subfamily level suggests a more complex correlation. With respect to the climbers, the proposed generalization can be justified: nearly all climbing species have a high abundance of solitary vessels (Figs. 2, 17, 19), except for the atypical rauvolfioid climbers *Allamanda cathartica* with long radial vessel multiples and *Kamettia caryophyllata* (Fig. 7) with vessel clusters. On the other hand, much more variation in the vessel grouping of nonclimbing rauvolfioids is observed: radial multiples are abundant in Alstonieae, Tabernaemontaneae (Figs. 4, 14), Plumerieae (Fig. 6), the erect genera of Willughbeieae (Fig. 5), *Kopsia*, *Lepiniopsis*, and *Stephanostegia*, whereas other erect taxa have exclusively solitary vessels (Aspidospermeae (Figs. 15, 18), Carisseae, Hunterieae, Vinceae (except for *Kopsia*), *Diplorhynchus*, and *Pteralyxia*). The pronounced vessel clusters in the climbing species of *Kamettia caryophyllata* (Fig. 7) are strikingly different from all other climbing rauvolfioids investigated and resemble much more the typical climbing anatomy of later diverging Apocynaceae tribes, such as Apocyneae, Baisseeae, and Echiteae (F. Lens, personal observation).

Vessel diameter and vessel density are more congruent with the two habit types in Rauvolfioideae: climbers have wider and fewer vessels than nonclimbing taxa (on average 140 μm vs. 65 μm and 20/mm² vs. 40/mm², respectively), which is a general wood anatomical correlation throughout the angiosperms. In addition, rauvolfioid climbers generally have tracheids and abundant paratracheal parenchyma, two features that are known to be common in lianescent taxa (cf. Carlquist, 1989), while these two features have a much more restricted occurrence in the erect species studied. In addition, rauvolfioid climbers have a slight vessel dimorphism pattern (much more pronounced in apocynoid climbers): few narrow vessels (20–30 μm) co-occur with many wide vessels (over 150 μm). The length of vessel elements and fibers also differs between climbers and nonclimbers (570 μm vs. 780 μm and 1120 μm vs. 1620 μm , respectively). Multiseriate ray width and height (810 μm vs. 710 μm) are not correlated with habit at the subfamily level.

Considering only the tribe Willughbeieae, which has the highest diversity of climbing taxa, climbing and nonclimbing species have similar anatomical differences as compared to the entire subfamily. Besides the distinctive wood features that have already been mentioned between climbing-nonclimbing Willughbeieae (vessel grouping, tracheid occurrence and laticifer occurrence), we found that the climbing Willughbeieae species have on average wider vessels (140 μm vs. 100 μm) and shorter vessel elements and fibers (570 μm vs. 780 μm and 1120 μm vs. 1620 μm , respectively). The difference in vessel diameter between lianescent and erect species is well known, but the length difference of vessel elements and fibers may vary throughout the angiosperms depending on the family (Carlquist, 1989). Furthermore, vessel densities in both groups of Willughbeieae are nearly identical (15 vs. 16/mm²). Ray width is more complex: all erect Willughbeieae species observed have narrow rays (2–3-seriate), whereas climbing species exhibit a wide range of variation from 2- or 3-seriate rays in some species and 3–6(–15)-seriate

in others. Likewise, multiseriate ray height is variable in the climbing species studied (average values of species range from 300–1430 μm ; general mean 600 μm), while the erect species have more uniform multiseriate ray heights ranging from 300–550 μm (general mean 415 μm).

Some rauvolfioid species have the ability to grow as erect trees or scandent shrubs, depending on the presence of a substrate or host to climb on. Although this hybrid tree-liana growth form is rare within the subfamily, two species in this study have this growth form: *Diplorhynchus condylocarpon* (Stapf, 1902; Plazier, 1980; considered as nonclimbing in online Appendix S2) and *Allamanda catharactica* (Sakane, 1981; Morales, 2005; considered as climbing in Appendix S2). With respect to the monotypic *Diplorhynchus*, the two specimens studied have a mixture of wood characters typical of the erect rauvolfioid syndrome (relatively narrow vessels) and the climbing syndrome (tracheids, apotracheal as well as paratracheal axial parenchyma, and laticifers). The wood anatomy of *Allamanda catharactica* has no obvious signs of a typical climbing rauvolfioid habit and is similar to the nonclimbing Plumerieae species observed.

Within Rauvolfioideae, understory trees reaching 10–15 m occur frequently, whereas tall trees over 30 m are found mainly in the genera *Alstonia*, *Aspidosperma*, and *Dyera*. In our sampling, tall trees are represented by *Alstonia scholaris* (Fig. 16; up to 60 m), *A. spectabilis* (up to 30 m), *Aspidosperma album* (Fig. 18; up to 40 m), *A. carapanauba* (up to 38 m), *A. cylindrocarpon* (up to 30 m), *A. megalocarpon* (up to 35 m), *A. discolor* (up to 30 m), *Dyera costulata* (up to 80 m), and *D. polyphylla* (up to 60 m). Although it is unknown whether our mature wood samples are derived from the main trunk or from side branches, some trends can be observed: the tall trees have wider and fewer vessels compared to the smaller trees (on average 86 μm vs. 65 μm and 23 vs. 40/mm², respectively) (cf. Baas and Schweingruber, 1987; Carlquist, 2001; Lens et al., 2007a). Another known trend in tall trees vs. understory trees is the occurrence of longer vessel elements and fibers (860 μm vs. 775 μm and 1910 μm vs. 1600 μm , respectively). The difference in multiseriate ray height between tall and understory trees is more pronounced (440 μm vs. 710 μm), but this distinction might be correlated with the systematic position of the tall trees (all placed in Aspidospermeae and Alstonieae) rather than with the habit.

Wood anatomy vs. habitat—The remarkably short vessel elements and fibers of *Vallesia* as compared to the remaining Aspidospermeae species observed (on average 300 μm vs. 810 μm and 830 μm vs. 2010 μm , respectively), and the presence of vasicentric tracheids (vs. absent in the remaining genera observed) is most likely related to differences in habitat (cf. Carlquist and Hoekman, 1985): some species of *Vallesia* (including the one used here) are adapted to more xerophytic regions and/or higher elevations in subtropical regions, whereas *Geissospermum*, *Microplumeria*, and the majority of *Aspidosperma* species are native to more humid lowland tropical habitats. Although *Vallesia* seems to be strongly nested in the tribe Aspidospermeae based on *rbcl* (Sennblad and Bremer, 1996) and *trnL-F* (Potgieter and Albert, 2001) sequences, wood anatomical evidence shows some additional deviating features in the genus, which cannot be explained by differences in habitat, such as fibers with more reduced pit borders (3–4 μm vs. 5–7 μm in horizontal width for the remaining Aspidospermeae), fewer cells per axial parenchyma strand (4–7 vs. 7–12), and laticifer occurrence (present vs. absent). Especially noteworthy is the low number of cells per axial parenchyma strand in a genus of the early diverging Aspidosper-

meae tribe because this feature would suggest a much more derived phylogenetic position within Apocynaceae based on our extensive sampling (see next section). However, we feel confident that our specimen is correctly identified because our description corresponds to the one found in the InsideWood database (IWG, 2004 onward). Unfortunately, there is no information available about the microscopic wood anatomy of the small erect species of *Haplophyton* and *Strepeliopsis*, the two closest relatives of *Vallesia* according to Potgieter and Albert (2001).

A number of species in *Alyxia* (though none of those included in this study) and *Carissa* also typically grow in drier scrub forests (Endress and Bruyns, 2000; Middleton, 2007). Thus, the mean shorter vessel element length in the two *Carissa* species observed (on average 500–550 μm) is presumably related to its preference for drier habitats (cf. Carlquist and Hoekman, 1985), although the phylogenetic signal of vessel element length within the entire family could also have played a role (see next section). In addition, *Carissa* has vasicentric tracheids, which has been shown to be correlated with drier environments (Carlquist and Hoekman, 1985).

General evolutionary wood trends within Apocynaceae s.l.—When the wood anatomy of Rauvolfioideae is compared with the rest of the Apocynaceae s.l., some characters stand out. Besides its phylogenetic significance, most major evolutionary trends within the wood of the family are correlated with (1) the shift of many APSA species toward drier regions (Swarupandan et al., 1996; Venter and Verhoeven, 2001; Verhoeven et al., 2003; Middleton, 2007), and/or (2) the abundance of climbers in the more derived Apocynaceae (Fig. 1). These major trends can be summarized as follows: (1) decreasing vessel element length, (2) more pronounced vessel grouping, (3) tracheid abundance, and (4) high frequency of paratracheal parenchyma (cf. Baas et al., 1983; Carlquist, 1989, 2001; Dickison, 2000). In addition, (5) the number of cells per axial parenchyma strand steadily decreases toward the more derived Apocynaceae.

(1) Vessel element length strikingly follows the generally accepted wood trends sensu Bailey and Tupper (1918): long vessel elements (mean >700 μm) are abundant in the early diverging Rauvolfioideae tribes Aspidospermeae, Alstonieae, Vinceae, and Tabernaemontaneae, while the later diverging rauvolfioids (Plumerieae and Carisseae) have much shorter vessel elements (mean 400–650 μm ; Table 1). In this regard, the proposed phylogenetic position of the tribe Hunterieae, which is placed with low support as sister to the Plumerieae-Carisseae-APSA clade (Simões et al., 2007), is supported by its relatively short vessel elements (mean 500–700 μm) as compared to the remaining Rauvolfioideae. The Bailey trend in Apocynaceae becomes even more pronounced when the APSA clade is taken into account: most APSA species have vessel elements of less than 500 μm with mean minimum ranges of 200–300 μm in several Asclepiadoideae (F. Lens, personal observation). The evolutionary decrease in length of water-conducting cells from cycads to angiosperms, which has long been considered to be unidirectional, is one of the key factors in the Bailey trends (Bailey and Tupper, 1918). Nowadays, this trend remains valid at a high taxonomic level, but the current study gives further support for the growing awareness—triggered by progress in ecological wood anatomy and the increasing robustness of independently generated molecular phylogenies—that the evolution of long to short vessel elements has undergone much more parallel evolution in various angiosperm families than previously realized (Baas and Wheeler, 1996; Lens et al., 2007b).

(2) Whereas about half of the rauvolfoid species studied have exclusively solitary vessels and the other half have abundant radial multiples, the APSA clade almost entirely lacks exclusively solitary vessels. Moreover, outside of Rauvolfioideae, abundant radial multiples are only present in the three small apocynoid tribes Wrightieae (all trees), Nerieae (mostly lianas), and Malouetieae (mostly trees), all nested at the base of the APSA clade (Livshultz et al., 2007). The more derived Apocynaceae (APSA clade except the three tribes just mentioned) share the synapomorphic presence of a dependent growth form (i.e., climbers and scramblers; Livshultz et al., 2007) and are characterized by prominent vessel clusters (cf. Fig. 7), which sometimes tend to be arranged into a flame-like dendritic pattern (F. Lens, personal observation). Consequently, the vessel grouping in Wrightieae, Nerieae, and Malouetieae provides additional morphological support for their “basal” position in the APSA clade (cf. Livshultz et al., 2007), and this is further corroborated by the (3) lack of tracheids and (4) paratracheal parenchyma.

(3) As mentioned in the Results, tracheids are usually absent and confined to only a few tribes of Rauvolfioideae. The same is true for the three “basal” APSA tribes, in which tracheids are scarce. On the other hand, tracheids in the higher Apocynaceae are generally present as the main cell type in the ground tissue (cf. in Alyxieae, Fig. 12) or they can be concentrated in the neighborhood of the vessel clusters (i.e., vasicentric tracheids) containing few wide plus many narrower vessels, which is a common situation in many climbers. The physiological significance of these narrow vessels (20–30 µm) and vasicentric tracheids is believed to provide the plant with a safe subsidiary water transport mechanism that can take over the sap stream in case the wide vessels become embolized from drought-induced cavitation (Carlquist, 1989; Choat et al., 2007).

(4) The current study has demonstrated that paratracheal parenchyma is strongly linked with climbing taxa in Rauvolfioideae (Figs. 2, 19), although it also is found in a few erect genera, such as some species of *Alstonia* and *Aspidosperma* (Fig. 18) and in *Diplorhynchus*, *Cerberiopsis*, and *Thevetia*. Despite the presence of many climbing taxa in Nerieae, paratracheal parenchyma is almost completely absent in the three “basal” APSA tribes, Wrightieae, Nerieae, and Malouetieae, whereas it occurs frequently in the APSA clades.

(5) The mean number of cells per axial parenchyma strand, which is not believed to be related to environmental conditions, also has a clear evolutionary trend in Apocynaceae s.l. The so-called primitive tribes Aspidospermeae and Alstonieae have by far the most cells per strand (often more than seven, sometimes up to 12 and more), whereas Periplocoideae, Secamonoideae, and Asclepiadoideae have nearly always fewer than five cells per strand (often only two to three).

In conclusion, the paraphyletic subfamily Rauvolfioideae is highly diverse in its wood anatomy. When this diversity is compared with recent molecular phylogenies, a combination of several meaningful wood characters prove to be diagnostic for most morphologically poorly defined tribes, despite considerable homoplasy caused by the scattered occurrence of the climbing habit, which has evolved independently in several clades. Diagnostic wood features are vessel grouping, vessel element length, fiber type, distribution of axial parenchyma, abundance of uniseriate rays, the presence/absence of tracheids, fused multiseriate rays and laticifers in rays, and to a lesser extent also the mean range of cells per axial parenchyma strand, height of multiseriate rays, and the occurrence and location of prismatic crystals. The

two major vessel grouping patterns, i.e., exclusively solitary vessels vs. abundant radial vessel multiples, prove to be phylogenetically useful in defining major evolutionary lines within the tribes Tabernaemontaneae s.l., Vinceae, and Willughbeieae. When the entire family is taken into consideration, a phylogenetic trend is found toward shorter vessel elements, more pronounced vessel grouping, higher tracheid abundance, more paratracheal parenchyma, and fewer cells per axial parenchyma strand in the later diverging APSA clades. These trends also corroborate the “basal” position of Wrightieae, Nerieae, and Malouetieae within the APSA clade.

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APPENDIX 1. List of taxa investigated in this study with reference to their locality, voucher information, and the tribal classification sensu Simões et al. (2007).

Abbreviations of institutional wood collections: CTFw = Centre Technique Forestier Tropical wood collection; K = Royal Botanic Gardens, Kew; L = National Herbarium of the Netherlands – Leiden University Branch, MADw = Madison wood collection; Tw = Tervuren wood collection; WAG = National Herbarium of the Netherlands – Wageningen University Branch; WIBw = Department of Forestry Techniques, Wageningen. Wood specimens that were considered to be juvenile are marked with an asterisk. "Mature" means that the wood sample is derived from a trunk or mature branches, although the exact diameter of the wood sample could not be traced.

Taxon; Collection locality; **Voucher;** Institution; Sample diameter; Tribal classification sensu Simões et al. (2007).

- Acokanthera oblongifolia** (Hochst.) Codd; Spain (Canary Islands, Tenerife); Schweingruber 23; L; 25 mm; Carisseeae. **Acokanthera oblongifolia** (Hochst.) Codd; The Netherlands (Hortus Botanicus Leiden); Botanic Laboratory 66005; L; 35 mm; Carisseeae. **Allamanda cathartica** L.; Indonesia; *Flora of Malaya* 2357; L; 23 mm; Plumerieae. **Alstonia scholaris** (L.) R.Br.; Philippines (Quézon); *Rajo s.n.*; L, MADw 37849; mature; Alstonieae. **Alstonia spectabilis** R.Br.; New Guinea; *Darbyshire* 883; L; mature; Alstonieae. **Alyxia concatenata** (F.Blanco) Merr.; Philippines; *Philippine Bureau of Forestry Museum Plank* 361; MADw 7721; mature; Alyxieae. **Alyxia scabrida** Markgr.; East New Guinea; *Jacobs* 9073; L; 29 mm; Alyxieae. **Alyxia subalpina** Markgr.*; East New Guinea; *Kalkman* 4718; L; 18 mm; Alyxieae. **Alyxia sulana** Markgr.*; Indonesia (Sulawesi); *de Vogel* 6091; L; 17 mm; Alyxieae. **Ambelania acida** Aubl.; Surinam; *Stahel* 265; L; mature; Tabernaemontaneae. **Aspidosperma album** Pichon; Surinam; *Stahel* 77; L; mature; Aspidospermeae. **Aspidosperma carapanauba** Pichon; Brazil; *Krukoff* 563; MADw 19123; mature; Aspidospermeae. **Aspidosperma cylindrocarpon** Müll.Arg.; Peru (Huanuco); *Lao s.n.*; L, MADw 22279; mature; Aspidospermeae. **Aspidosperma discolor** A.DC.; Surinam; *Stahel* 70; L; mature; Aspidospermeae. **Aspidosperma megalocarpon** Müll.Arg.; Guatemala; *Ortiz s.n.*; L, MADw 23119; mature; Aspidospermeae. **Aspidosperma steyermarkii** Woodson; Peru (Huanuco); *Gutierrez* 95; L, MADw 22409; mature; Aspidospermeae. **Callichilia barteri** (Hook.f.) Stapf; Origin unknown; *van de Laa* 215; WAG; 27 mm; Tabernaemontaneae. **Callichilia subsessilis** (Benth.) Stapf*; Ivory Coast (Forest of Blanco); *Beentje* 581; WAG; 9 mm; Tabernaemontaneae. **Cameraria latifolia** L.; USA (Miami, Fairchild Tropical Garden); *Curtis* 522; L; 115 mm; Plumerieae. **Carissa spinarum** L.; Benin; *Geerling & Bokdam* 2191; WAG; 25 mm; Carisseeae. **Carissa sp.***; India (Pamba Dam); *Ridsdale* 570; L; 15 mm; Carisseeae. **Cerbera floribunda** K.Schum.; USA (New Britain); *Sudo s.n.*; L, TWTw 11590; mature; Plumerieae. **Cerberiopsis candelabra** Pancher & Sebert; New Caledonia (route de Yaté); *Sarlin* 98; L; mature; Plumerieae. **Chilocarpus suaveolens** Blume; Origin unknown; *collector and number unknown*; L 0369491; 22 mm; Alyxieae. **Chilocarpus torulosus** (Boerl.) Markgr.*; Indonesia (Kalimantan Tengah); *collector and number unknown*; L; 18 mm; Alyxieae. **Condylocarpon amazonicum** (Markgr.) Ducke; Brazil; *Krukoff* 8007; MADw 27230; mature; Alyxieae. **Couma guianensis** Aubl.; Surinam; *Stahel* 128a; L; mature; Willughbeieae. **Couma macrocarpa** Barb.Rodr.; Colombia; *Cabrera s.n.*; L, WAG, MADw 37908; mature; Willughbeieae. **Cyclocotyla congolensis** Stapf; Cameroon (Muila Dep., Ebom); *Elad & Parren* 378; WAG; 86 mm; Willughbeieae. **Dictyophleba ochracea** (Hallier f.) Pichon; Cameroon (Mvila Dep., near Ebom); *Parren* 41; WAG; 28 mm; Willughbeieae. **Dictyophleba stipulosa** (Wernham) Pichon; Cameroon (Mvila Dep., near Ebom); *Parren* 59; WAG; 30 mm; Willughbeieae. **Diplorhynchus condylocarpon** (Müll.Arg.) Pichon; Mozambique; *Carvalho* 863; MADw 30765; mature; incertae sedis. **Diplorhynchus condylocarpon** (Müll.Arg.) Pichon; South Africa; *Dentzman* 1767; MADw 9571; mature; incertae sedis. **Dyera costulata** Hook.f.; Borneo; *collector and number unknown*; L 0121593; mature; Alstonieae. **Dyera polyphylla** (Miq.) Steenis; Singapore (Singapore Botanical Gardens, Sepilok); *collector and number unknown*; WIBw 3509; WAG; mature; Alstonieae. **Geissospermum sericeum** Miens; Brazil; *Capucho* 440; MADw 27152; mature; Aspidospermeae. **Gonioma kamassi** E.Mey.; South Africa; *collector and number unknown*; WIBw 824; WAG; mature; Hunterieae. **Hancornia speciosa** Gomes; Brazil; *Irwin et al.* 13317; MADw 36493; 18 mm; Willughbeieae. **Himatanthus articulatus** (Vahl) Woodson; Surinam; *Stahel* 200; L; mature; Plumerieae. **Himatanthus articulatus** (Vahl) Woodson; Surinam; *Stahel* 329; L; mature; Plumerieae. **Himatanthus succuba** (Müll.Arg.) Woodson; Peru; *Arostegui* 64; L, MADw 22085; mature; Plumerieae. **Hunteria eburnea** Pichon; The Netherlands (Botanical Garden Wageningen); *van Veldhuizen* 33; WAG; 46 mm; Hunterieae. **Kamettia caryophyllata** (Roxb.) Nicolson & Suresh*; India (Karin Schola); *Ridsdale* 328; L; 15 mm; Vinceae. **Kopsia arborea** Blume; Sumatra (Lamping prov., Mount Tanggamus); *Jacobs* 8043; L; 42 mm; Vinceae. **Kopsia rosea** D.J.Middleton; Thailand; *Geesink* 5039; L; 56 mm; Vinceae. **Lacmellea edulis** H.Karst.; Colombia; *Cuatrecasas* 15626; L; 65 mm; Willughbeieae. **Lacmellea floribunda** (Poepp. & Endl.) Benth. & Hook.f.; Colombia; *Cuatrecasas* 17201; L; mature; Willughbeieae. **Landolphia gummifera** (Poir.) K.Schum.*; Madagascar; *collector and number unknown*, Koloniaal Museum Haarlem 1507-5; L; 13 mm; Willughbeieae. **Landolphia watsoniana** Romburgh; Origin unknown; *collector and number unknown*, Koloniaal Museum Haarlem 1507-01; L; 25 mm; Willughbeieae. **Lepiniopsis ternatensis** Valetton; Celebes (Sulawesi Selatan); *de Vogel* 6114; L; mature; Alyxieae. **Leuconotis cf. anceps** Jack; Brunei (Meranking, Belait); *Ogata et al.* *Og-B137*; L; 76 mm; Willughbeieae. **Leuconotis griffithii** Hook.f.; Indonesia (Sumatra); *Meijer* 4384; L; 72 mm; Willughbeieae. **Macoubea guianensis** Aubl.; Surinam; *Stahel* 183; L; mature; Tabernaemontaneae. **Macoubea sprucei** (Müll.Arg.) Markgr.; Brazil; *Krukoff* 7924; MADw 27206; mature; Tabernaemontaneae. **Melodinus forbesii** Fawc.*; Papua New Guinea (Mount Bosavi); *Jacobs* 9337; L; 15 mm; Melodineae. **Melodinus orientalis** Blume; Asia; *collector and number unknown*, Koloniaal Museum Haarlem 1507-2; L; 29 mm; Melodineae. **Microplumeria anomala** (Müll.Arg.) Markgr.; Brazil; *Collector and number unknown*; MADw 5374; mature; Aspidospermeae. **Mucosa duckei** (Markgr.) Zarucchi; Brazil; *Zarucchi et al.* 2973; MADw 46502; mature; Tabernaemontaneae. **Ochrosia acuminata** Valetton; Celebes (Sulawesi Selatan); *de Vogel* 6110; L; mature; Vinceae. **Ochrosia glomerata** (Blume) Valetton; Philippines (Palawan, Lake Manguao); *Podzorski SMHI* 752; L; 78 mm; Vinceae. **Orthopichonia cirrhosa** (Radlk.) H.Huber*; Cameroon (Yaoundé); *Breteler et al.* 2467; WAG; 13 mm; Willughbeieae. **Orthopichonia indeniensis** (A.Chev.) H.Huber; Liberia; *Jansen* 1476; WAG; 34 mm; Willughbeieae. **Orthopichonia seretii** (De Wild.) Vonk; Cameroon (Bidou); *van der Burgt & Mbmba* 239; WAG; 28 mm; Willughbeieae. **Parahancornia fasciculata** (Poir.) Benoist; Surinam; *Stahel s.n.*; L; mature; Willughbeieae. **Parahancornia peruviana** Monach.; Peru (Loreto); *Arostegui* 106; L, MADw 22097; mature; Willughbeieae. **Picalima nitida** Th. & H.Dur.; Cameroon; *Letouzey* 5069; MADw 36998; mature; Hunterieae. **Picalima sp.**; Democratic Republic of Congo (N Kasai); *Dechamps s.n.*; L, Tw; mature; Hunterieae. **Pleiocarpa mutica** Benth.; Ivory Coast (Banco); *Forest Service* 35; CTFw 24446; mature; Hunterieae. **Pleiocarpa pycnantha** (K.Schum.) Stapf; Cameroon; *Delcroix* 168; CTFw 24857; mature; Hunterieae. **Plumeria rubra** L.; Mexico (Oaxaca); *Hansen et al.* 1589; L; 58 mm; Plumerieae. **Pteralyxia macrocarpa** (Hillebr.) K.Schum.; USA (Hawaii); *Board of Agriculture and Forestry s.n.*; MADw 37174; mature; Alyxieae. **Rauvolfia moluccana** Markgr.; Indonesia (Maluku Islands, NW Buru); *van Balgooy* 4927; L; mature; Vinceae. **Rauvolfia nitida** Jacq.; Origin unknown; *Eggers Lign. Ind. Occ.* 184; L; 53 mm; Vinceae. **Rauvolfia sumatrana** Jack.; Philippines (Palawan, Puerto Princessa); *Podzorski SMHI* 521; L; 60 mm; Vinceae. **Saba comorensis** (Bojer) Pichon*; Ivory Coast (Bouaflé); *Jongkind* 4737; WAG; 60 mm; Willughbeieae. **Saba comorensis** (Bojer) Pichon; Nigeria (Oyo Prov.); *van Meer* 592; WAG; 8 mm; Willughbeieae. **Stemmadenia tomentosa** Greenm.; Mexico; *Williams* 9499; MADw 27231; mature; Tabernaemontaneae. **Stephanostegia sp.**; Madagascar; *collector and number unknown*; CTFTw, WIBw 876; WAG; mature; Melodineae. **Tabernaemontana attenuata** (Miers) Urb.; Surinam; *Stahel* 303; L; mature; Tabernaemontaneae. **Tabernaemontana aurantiaca** Gaudich.; New Guinea (Madang District); *Hoogland* 4962; L; mature; Tabernaemontaneae. **Tabernaemontana columbiensis** (L.Allorge) Leeuwenb.; Ecuador; *Beck et al.* 2265; MADw 48959; mature; Tabernaemontaneae. **Tabernaemontana cymosa** Jacq.; Venezuela; *Pittier* 12383; MADw 27181; mature; Tabernaemontaneae. **Tabernaemontana cymosa** Jacq.; Brazil; *Krukoff* 5784; MADw 19152; mature; Tabernaemontaneae. **Tabernaemontana durissima** Stapf; Liberia; *Cooper* 242; MADw 27123; mature; Tabernaemontaneae. **Tabernaemontana durissima** Stapf; Democratic Republic of Congo (Kasai); *Dechamps s.n.*;

- L 0369499; mature; Tabernaemontaneae. *Tabernaemontana eglandulosa* **Stapf***; Cameroon (Bertoua); *Breteler 1297*; WAG; 15 mm; Tabernaemontaneae. *Tabernaemontana macrocarpa* **Jack**; Indonesia (N Sumatra, Ketambe); *de Wilde & de Wilde-Duyffes 16961*; L; 95 mm; Tabernaemontaneae. *Tabernaemontana pachysiphon* **Stapf**; Kenya; Federal Forestry Department of Kenya, *Wormald 35*; MADw 40116; mature; Tabernaemontaneae. *Tabernaemontana panamensis* (**Markgr., Boiteau & L.Allorge**) **Leeuwenb.**; Panama (Veraguas); *Nee 11159*; L; 22 mm; Tabernaemontaneae. *Tabernaemontana siphilitica* (**L. f.**) **Leeuwenb.***; Peru (San Martin, Loreto); *Mathias & Taylor 3536*; L; 15 mm; Tabernaemontaneae. *Tabernaemontana* **sp.**; Brazil; *Brazilian Forest Service 3004*; MADw 13175; mature; Tabernaemontaneae. *Tabernaemontana* **sp.**; Madagascar; *Barnett et al. 368*; MADw 44509; 30 mm; Tabernaemontaneae. *Thevetia ahouai* (**L.**) **A.DC.***; Panama (SW slope of Cerro Cabra); *Nee 6655*; L, MADw; 16 mm; Plumerieae.
- Thevetia peruviana* (**Pers.**) **K.Schum**; China; *NTU 488*; MADw 42374; mature; Plumerieae. *Vahadenia laurentii* (**De Wild.**) **Stapf***; Gabon (Oveng); *Louis et al. 526*; WAG; 14 mm; Willughbeieae. *Vallesia glabra* (**Cav.**) **Link***; Bolivia; *Nee 35314*; MADw 44308; 15 mm; Aspidospermeae. *Voacanga chalongiana* **Stapf**; Democratic Republic of Congo (N Kasai); *Dechamps s.n.* (L Tw), mature; Tabernaemontaneae.
- Voacanga globosa* (**Blanco**) **Merr.**; Origin unknown; *collector and number unknown*; L 0085266; mature; Tabernaemontaneae. *Voacanga* **sp.**; Philippines; *Jacobs 7961*; L; 45 mm; Tabernaemontaneae. *Willughbeia angustifolia* (**Miq.**) **Markgr.**; Indonesia (Kalimantan Timur, Wanariset); *van Valkenburg 1161*; L; 30 mm; Willughbeieae. *Willughbeia coriacea* **Wall.**; Thailand (Naratiwat); *Maxwell 87-588*; L; 25 mm; Willughbeieae. *Willughbeia tenuiflora* **Hook.f.**; Indonesia (Sumatra); *Meijer 4214*; L; 40 mm; Willughbeieae.
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