

## VESSEL GROUPING PATTERNS IN SUBFAMILIES APOCYNIOIDEAE AND PERIPLOCOIDEAE CONFIRM PHYLOGENETIC VALUE OF WOOD STRUCTURE WITHIN APOCYNACEAE<sup>1</sup>

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This study contributes to our understanding of the phylogenetic significance and major evolutionary trends in the wood of the dogbane family (Apocynaceae), one of the largest and economically most important angiosperm families. Based on LM and SEM observations of 56 Apocynoideae species—representing all currently recognized tribes—and eight Periplocoideae, we found striking differences in vessel grouping patterns (radial multiples vs. large clusters) between the mainly nonclimbing apocynoid tribes (Wrightieae, Malouetieae, Nerieae) and the climbing lineages (remaining Apocynoideae and Periplocoideae). The presence of large vessel clusters in combination with fibers in the ground tissue characterizing the climbing Apocynoideae and Periplocoideae clearly contrasts with the climbing anatomy of the rauvolfioids (solitary vessels plus tracheids in ground tissue), supporting the view that (1) the climbing habit has evolved more than once in Apocynaceae, (2) the three nonclimbing apocynoid tribes are basal compared to the climbing apocynoids, and (3) Periplocoideae belong to the crown clade. The wood anatomy within the nonclimbing and climbing lineages is rather homogeneous, although a combination of specific characters (e.g. presence of septate fibers, axial parenchyma distribution, abundance of uniseriate compared to multiseriate rays, and presence and location of prismatic crystals) may be used to identify several tribes.

**Key words:** Apocynaceae; Apocynoideae; APSA clade; climbing vs. nonclimbing anatomy; Periplocoideae; systematic wood anatomy; tribal classification.

The dogbane family is one of the largest families of angiosperms, with an estimated 375 genera and 5100 species (Endress, 2004; Endress et al., 2007), and is broadly distributed mainly in tropical and subtropical regions of the world. Apocynaceae are especially rich in bioactive secondary compounds and have long been used in folk medicine to treat a wide range of ailments, including cancer, malaria, diarrhea, diabetes, and skin diseases (Schultes, 1979; Van Beck et al., 1984; Schultes and Raffauf, 1990; Neuwinger, 1994; Middleton, 2007). A number of genera are employed in modern medicine for a diverse array of uses such as controlling tumor growth in treating cancer (Balandrin et al., 1985; Moza, 2005), as antiplasmodial agents in parasitic infections (Zirihhi et al., 2005), as muscle relaxants during surgery (Bisset, 1992) and as an appetite suppressant in controlling obesity (van Heerden, 2008). Because related taxa often possess similar bioactive properties, it is expedient to have a better understanding of the generic affinities in several apocynaceous lineages, including the subfamilies Apocynoideae and Periplocoideae. Consequently, one of the

major objectives of the present work is to search for phylogenetically informative wood anatomical characters that can help us to identify clades that are mainly defined molecularly.

The pantropical Apocynoideae sensu Livshultz et al. (2007) are a paraphyletic subfamily harboring a broad array of different flower types and comprise about 860 species distributed among 81 genera and eight tribes, representing about 1/5–1/6 of the species diversity within the family (Endress et al., 2007; Endress and Hansen, 2007). Subfamily Periplocoideae, on the other hand, are much smaller, including about 190 species and 33 genera restricted to the Old World, and have always been considered to be a natural group because of their very homogeneous floral structure (Nilsson et al., 1993; Stevens, 2001 onward; Endress et al., 2007; Venter, 2009).

Both subfamilies can generally be distinguished by their growth form and habitat preference. For instance, the bulk of the Apocynoideae have a climbing habit, from robust lianas climbing 40 m or higher up into the canopy (e.g., *Alafia*, *Motandra*, *Oncinotis*) or festooning the branches of trees at forest margins (e.g., *Peltastes*) to slender scramblers bending over shrubs and rocks in more open habitats (e.g., some species of *Parsonsia*). The nonclimbing Apocynoideae are often small understory trees or shrubs growing in tropical lowland forests. In both subfamilies, some species can occasionally grow as lianas as well as erect life forms (e.g., *Cryptostegia*, *Mandevilla*). Whereas most Apocynoideae grow in humid tropical lowland forests, some of them occupy drier scrub vegetations (e.g., certain species of *Aganosma*, *Amphineurion*, *Holarrhena*, *Parsonsia*, *Peltastes*, *Spirolobium*, and *Urceola*). Periplocoideae, in contrast, are mainly smaller climbers (or occasionally epiphytes) restricted to the (sub)tropics of the Old World and inhabit mostly tropical evergreen or seasonal forests and

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savannas, with a number of erect or straggling shrubs extending into grasslands, Mediterranean regions and (semi)desert areas (e.g., *Ectadium*, *Periploca*, and *Raphionacme*) (Endress and Bruyns, 2000; Venter and Verhoeven, 2001; Ionta and Judd, 2007; Middleton, 2007; Venter, 2009). About one-third of the periplocoid genera have large semisubterranean tubers, the great majority of which are restricted to arid and semiarid habitats in Africa (Meve and Liede, 2004). These water-storing tubers aid the plants during times of water shortage and can reach sizable proportions and masses up to 100 kg (Venter et al., 1990, 2006; Klackenberg, 1999).

Apocynaceae s.l. are one of the five families nested within Gentianales, where they stand out for the presence of latex (Stevens, 2001 onward; Middleton, 2007; Hagel et al., 2008). However, the taxonomic position of Apocynaceae within the order remains unclear, and insights about the higher-level intrafamilial relationships have changed dramatically over the years (Struwe et al., 1994; Endress et al., 1996; Sennblad and Bremer, 1996, 2002; Backlund et al., 2000; Endress and Bruyns, 2000; Potgieter and Albert, 2001; Bremer et al., 2002; Livshultz et al., 2007; Simões et al., 2007; Fig. 1). For instance, the current subfamilies Apocynoideae and Periplocoideae were formerly placed into two different but closely related families, i.e., Apocynaceae s.s. (also including Rauvolfioideae) and the former Asclepiadaceae (also including the currently recognized subfamilies Asclepiadoideae and Secamonoideae), respectively. Within Apocynaceae s.s., Apocynoideae were and still are believed to be “derived” compared to Rauvolfioideae (cf. Fig. 1), and can be distinguished by their dextrorsely contorted corolla lobes in bud, specialized anthers adnate to the style head forming a gynostegium, and usually dry follicles with comose seeds (Endress and Bruyns, 2000). With respect to Asclepiadaceae, periplocoids were elevated for the first time to the family level by Schlechter (1905) based mainly on the lack of lignified guide-rails on the anthers, the spoon-like structure of the translators and the erroneous assumption that periplocoid pollen is never gathered into pollinia (Verhoeven and Venter, 1998). The distinct pollination mechanism combined with erroneously interpreted morphological characters led Wanntorp (1988) to conclude that the most recent common ancestor of Periplocoideae was to be found in basal Rauvolfioideae, rather than in the derived clades of the group. In addition, despite the observation of a gynostegium in all members of the Periplocoideae investigated (Nilsson et al., 1993), some authors continued to maintain that it was lacking and used this supposed absence as support for the recognition of the group as a separate family (Swarupnanandan et al., 1996). Recognition of Asclepiadaceae or Periplocaceae as separate families has been rejected, however, because the evolution in flower morphology represents an overall trend of increasing complexity, beginning with the Rauvolfioideae culminating in the highly derived asclepiad condition (Endress and Bruyns, 2000). Recent molecular work has supported the idea of recognizing only one broadly circumscribed family, Apocynaceae s.l., and changed our ideas about higher-level relationships. As currently delimited, Apocynoideae as well as Rauvolfioideae are paraphyletic, and the monophyletic Periplocoideae are nested within Apocynoideae, making the former Asclepiadaceae polyphyletic (Fig. 1; Livshultz et al., 2007; Simões et al., 2007).

Phylogenetic relationships within certain lineages of Apocynoideae and Periplocoideae remain unresolved, however, and morphological synapomorphies are still lacking for a number of recently identified molecular-based subclades (Ionta and Judd,

2007; Livshultz et al., 2007). Livshultz and coworkers (2007) shed new light on the controversial relationships between and within Apocynoideae and Periplocoideae using a phylogenetic analysis based on more than 1600 informative characters from plastid DNA in combination with 16 morphological characters (Fig. 1). The resulting phylogeny rejects all traditional Apocynoideae tribes sensu Pichon (1950) and Leeuwenberg (1994) and the former Periplocoideae classification of Venter and Verhoeven (1997). In their current circumscription, the paraphyletic Apocynoideae comprise eight tribes (Fig. 1; Endress et al., 2007), whereas relationships within Periplocoideae are still too insufficiently known to identify the major evolutionary lines (Ionta and Judd, 2007). With respect to the family classification of Apocynaceae, subfamily Rauvolfioideae forms a basal grade, with Carisseae being sister to the APSA clade (Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae) (Livshultz et al., 2007). Within the APSA clade, three largely nonclimbing Apocynoideae tribes (Wrightieae, Nerieae, and Malouetieae) diverge first, followed by the well-supported crown clade, which is composed mainly of species with a dependent (climbing or straggling) growth form. In this crown clade, Periplocoideae are sister (although without bootstrap support) to a large group including subclades that correspond remarkably well with geographical regions: predominantly Asian apocynoids (tribe Apocyneae), predominantly neotropical apocynoids (tribes Odontadenieae, Echiteae, and Mesechiteae), and three African apocynoid genera (Baisseeae), which consistently come out as sister to a clade comprising the subfamilies Secamonoideae-Asclepiadoideae (Fig. 1; Potgieter and Albert, 2001; Livshultz et al., 2007; Lahaye et al., 2007).

The current study is a sequel to a previous paper describing the microscopic wood structure of Rauvolfioideae (Lens et al., 2008). An update of the anatomical variation in Apocynoideae (81 genera) and Periplocoideae (33 genera, of which most of them are perennial “woody” herbs) is urgently needed: wood anatomical descriptions are often incomplete, and the number of genera described in the literature is rather limited (in total 17 Apocynoideae and 3 Periplocoideae; e.g., Pearson and Brown, 1932; Chalk et al., 1933; Record and Hess, 1943; Metcalfe and Chalk, 1950; Ingle and Dadswell, 1953; Détienne and Jacquet, 1983; Schweingruber, 1990; Neumann et al., 2001; InsideWood website [InsideWood Working Group, 2004 onward]; Baas et al., 2007). We have found no wood anatomical data in the literature for 19 apocynoid and two periplocoid genera included in this study, indicating that the present work adds considerably to our wood anatomical knowledge within Apocynaceae.

The goals of this investigation are not merely to fill the gaps in the wood anatomical knowledge of Apocynaceae. We also strive to (1) look for potential phylogenetic wood characters and their evolutionary patterns within Apocynaceae (cf. Lens et al., 2007ab, 2008), (2) contribute to the renewed interest in Apocynaceae systematics, (3) search for characters that can offer anatomical understanding for the predominantly molecular-based classification at the tribal level, and (4) analyze adaptive xylem evolution in these subfamilies, which are highly diverse in habit and ecology. Because Apocynaceae belong to the top 10 angiosperm families in terms of size, we assembled an extensive collection of wood samples (about 250 spp.) from various xylaria. Consequently, we have chosen to split our Apocynaceae treatment into four separate anatomical studies, one focusing on the subfamily Rauvolfioideae based on 50 of 84 genera (Lens et al., 2008), the current study dealing with Apocynoideae and Periplocoideae (including 41 of 108 gen-

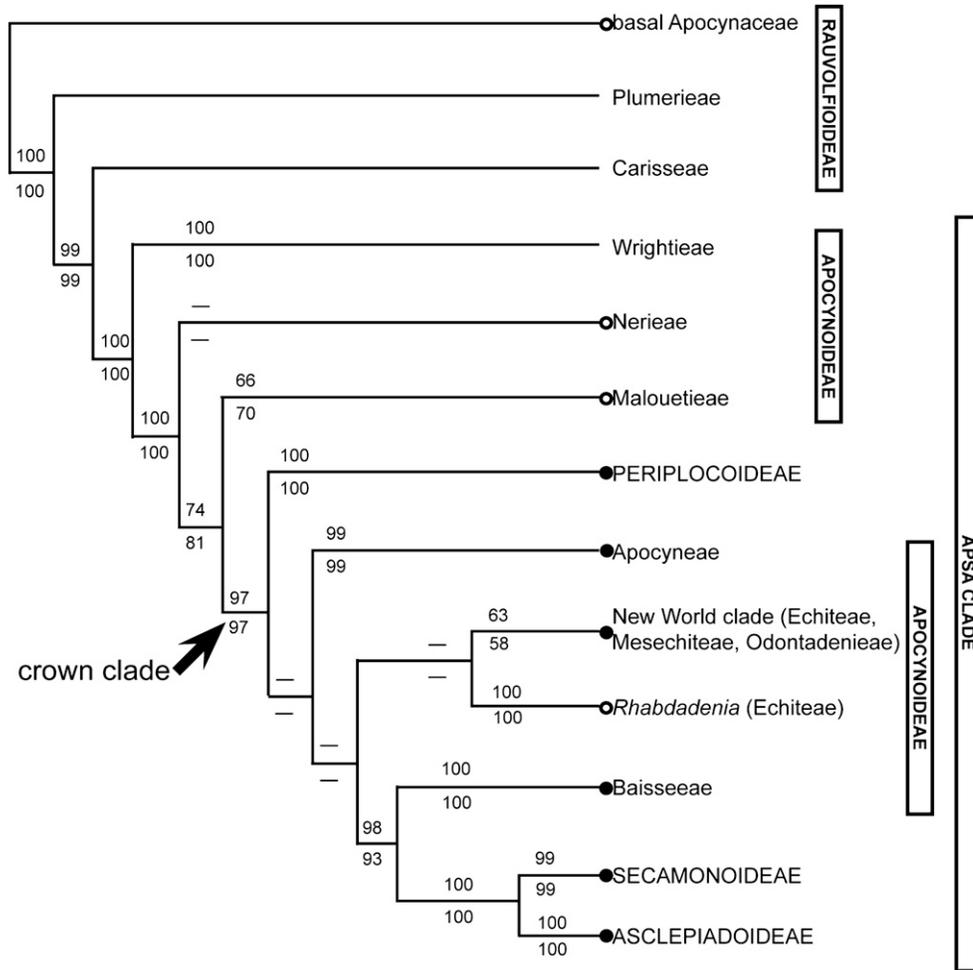


Fig. 1. Simplified phylogenetic tree of Apocynaceae based on one of the 144 most parsimonious trees retrieved in the analysis of Livshultz et al. (2007) using four chloroplast markers (*trnL* intron/*trnL-trnF* spacer, *matK/3'* *trnK* intron, *rpl16* intron and *rps16* intron) combined with 16 morphological characters. Bootstrap values are indicated above (molecular data only) and below branches (molecules plus morphology); dashes represent bootstrap values below 50. Tribes and subfamilies with mainly climbing taxa are marked with a filled circle; taxa with a mix of climbing and nonclimbing species have an open circle. This tree is a simplified representation of Fig. 1A–D in Livshultz et al. (2007).

era), a manuscript in preparation on Secamonoideae-Asclepiadoideae (F. Lens, M. E. Endress, U. Meve [University of Bayreuth, Germany], and E. Smets, unpublished manuscript), and finally, a family overview including phylogenetic analyses using wood anatomical and molecular data.

## MATERIALS AND METHODS

In total, 60 apocynoid wood specimens belonging to 56 species and 36 genera from all major clades as delimited by Livshultz et al. (2007; including two genera of Wrightieae, four genera of Nerieae, six genera of Malouetieae, 11 genera of Apocynaceae, five genera of Echiteae, two genera of Mesechiteae, two genera of Odontadenieae, three genera of Baisseeae, and *Galactophora*, genus incertae sedis within Apocynoideae), and eight periplocoid wood samples representing eight species and five genera were investigated using LM and SEM (Appendices S1, S2, see Supplemental Data with the online version of this article). Most samples are represented by mature sapwood, except those indicated by an asterisk in Appendix S2. 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 slide preparation is described in Lens et al. (2005). The wood anatomical terminology largely follows the "IAWA list of microscopic features for hardwood identification" (IAWA Com-

mittee, 1989). We refer readers who are not familiar with wood anatomical terms to the Material and Methods section of our first Apocynaceae paper (Lens et al., 2008). Our interpretation of some of the characters described in the standardized IAWA list was adjusted to some extent: (1) Vascentric tracheids are considered here as long and slender cells (without an irregular shape) nearby vessels, having abundant large bordered pits (4–6  $\mu\text{m}$  in horizontal diameter) resembling pits in lateral vessel walls, and differing from the ground tissue fibers in the size and density of their pits (cf. Carlquist, 1985a). (2) We consider imperforate tracheary elements with clearly bordered pits in the ground tissue as (true) tracheids when vessels are mainly solitary (cf. Carlquist, 1984; in few cases where similar cells co-occur with pronounced vessel multiples, we chose the name tracheid-like fiber or tracheid-like cell). (3) The total density of rays was split into the density of uniseriate and multiseriate rays separately because this division is more informative in Apocynaceae. The degree of vessel grouping was quantified using the vessel grouping index of Carlquist (2001), which is measured by counting the number of vessels in 25 groups (solitary vessels are also considered as one group) and dividing the total number by 25. The range of the mean values of quantitative wood characters, such as vessel element length, number of axial parenchyma cells per strand and height of multiseriate rays, was determined for all species within a certain subclade to assess the phylogenetic significance of these characters (Table 1). Statistical differences between means were calculated at the 0.1% level using the online *Independent Groups T-Test for Means* calculator (Dimension Research, Chicago, USA; <http://www.dimensionresearch.com/resources/calculators/ttest.html>).

## RESULTS

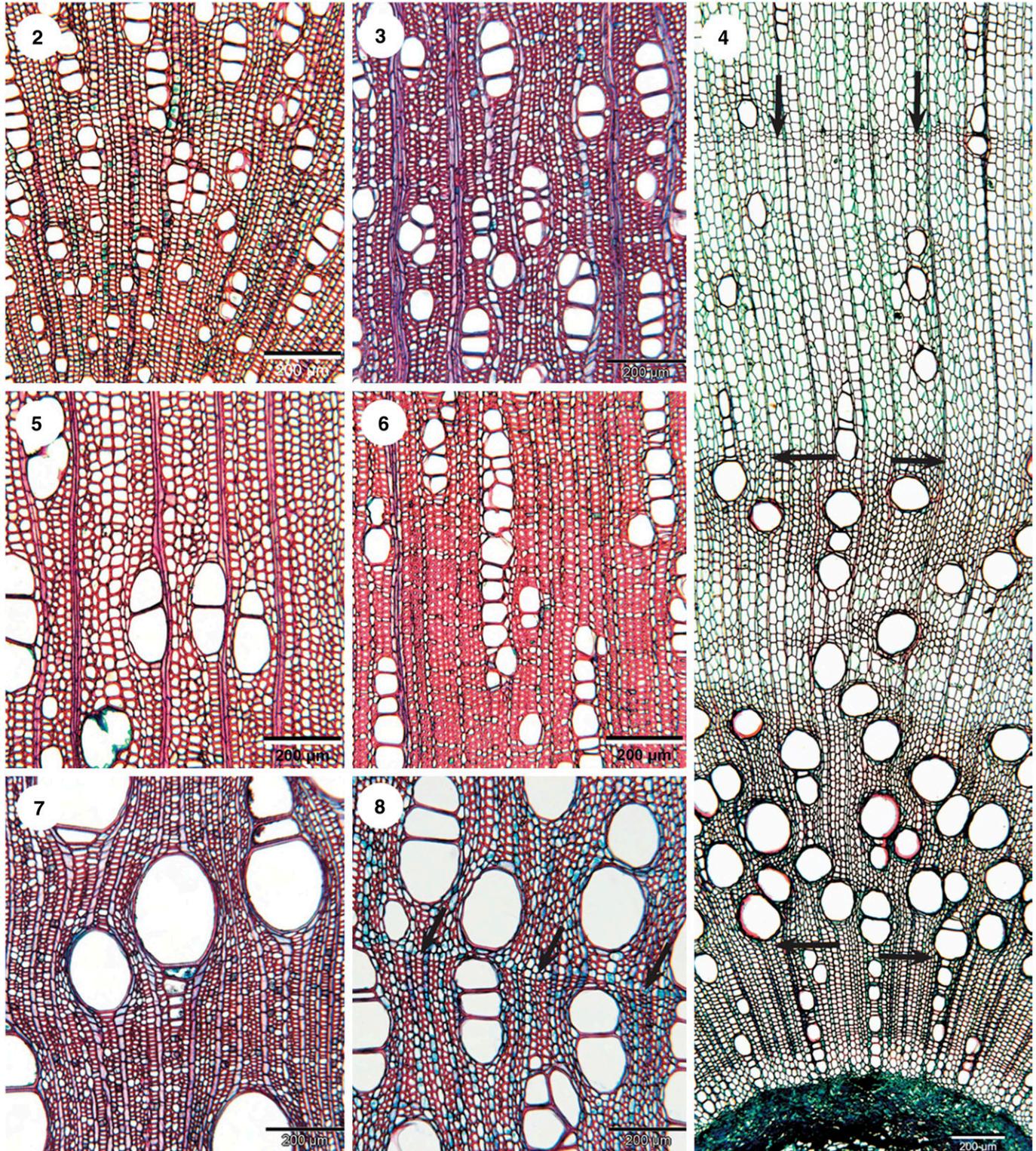
The Apocynoideae and Periplocoideae material studied is described separately. Numbers without parentheses are ranges of means, while numbers between parentheses represent minimum or maximum values. Measurements of juvenile stems and the root wood specimen of *Holarrhena curtisii* are not taken into account in the descriptions. A summary of the results is shown in Table 1 and online Appendix S2. As illustrated in Fig. 1 and Appendix S2, most species of the tribes Wrightieae (Figs. 2, 3), Nerieae and Malouetieae (Figs. 5, 6, 20), as well as most of the included species of Periplocoideae (Figs. 30–36) are erect trees or shrubs, whereas the great majority of species in the other tribes are climbing.

**Apocynoideae (Figs. 2–29)**—Growth ring boundaries distinct (Figs. 4, 8, 12) or indistinct (Fig. 11); no growth ring boundaries observed in the genera *Alafia* (Fig. 7), *Beaumontia*, *Peltastes*, and *Pleioceras* (Fig. 2). Wood diffuse-porous. Vessels (3)–6–100–(70)/mm<sup>2</sup>; vessel grouping in radial multiples in the nonclimbing tribes Malouetieae (Figs. 5, 6), Wrightieae (Figs. 2, 3), in the mixed nonclimbing/climbing tribe Nerieae (Figs. 7, 8), and in the later-formed wood of *Rhabdadenia* (Fig. 4); vessels largely in clusters in the climbing tribes Apocyneae (Figs. 11, 12), Baisseeae (Fig. 10), Echiteae (except *Rhabdadenia biflora*, Fig. 4), Mesechiteae (except in *Mandevilla rugellosa*, Fig. 9) and Odontadenieae; zones of vessels alternating with zones of fibers and rays in *Amphineurion marginata*, *Anodendron candolleianum* (Fig. 12) and *Macropharynx spectabilis*; vessel outline generally rounded to elliptical (Figs. 2–14), although sometimes angular in some nonclimbing species (Figs. 4, 6); perforation plates exclusively simple (Fig. 15). Intervessel pits alternate, pits 3–8 µm in horizontal diameter, vestured (Figs. 16, 17). Vessel-ray pits similar to intervessel pits in size and shape throughout the ray cell. Wall sculpturing absent. Tyloses occasionally present in *Alafia*, *Epigynum*, *Forsteronia*, *Funtumia*, *Holarrhena*, *Malouetia*, *Oncinotis*, *Peltastes*, *Pleioceras*, *Strophanthus*, *Urceola*, *Vallaris*,

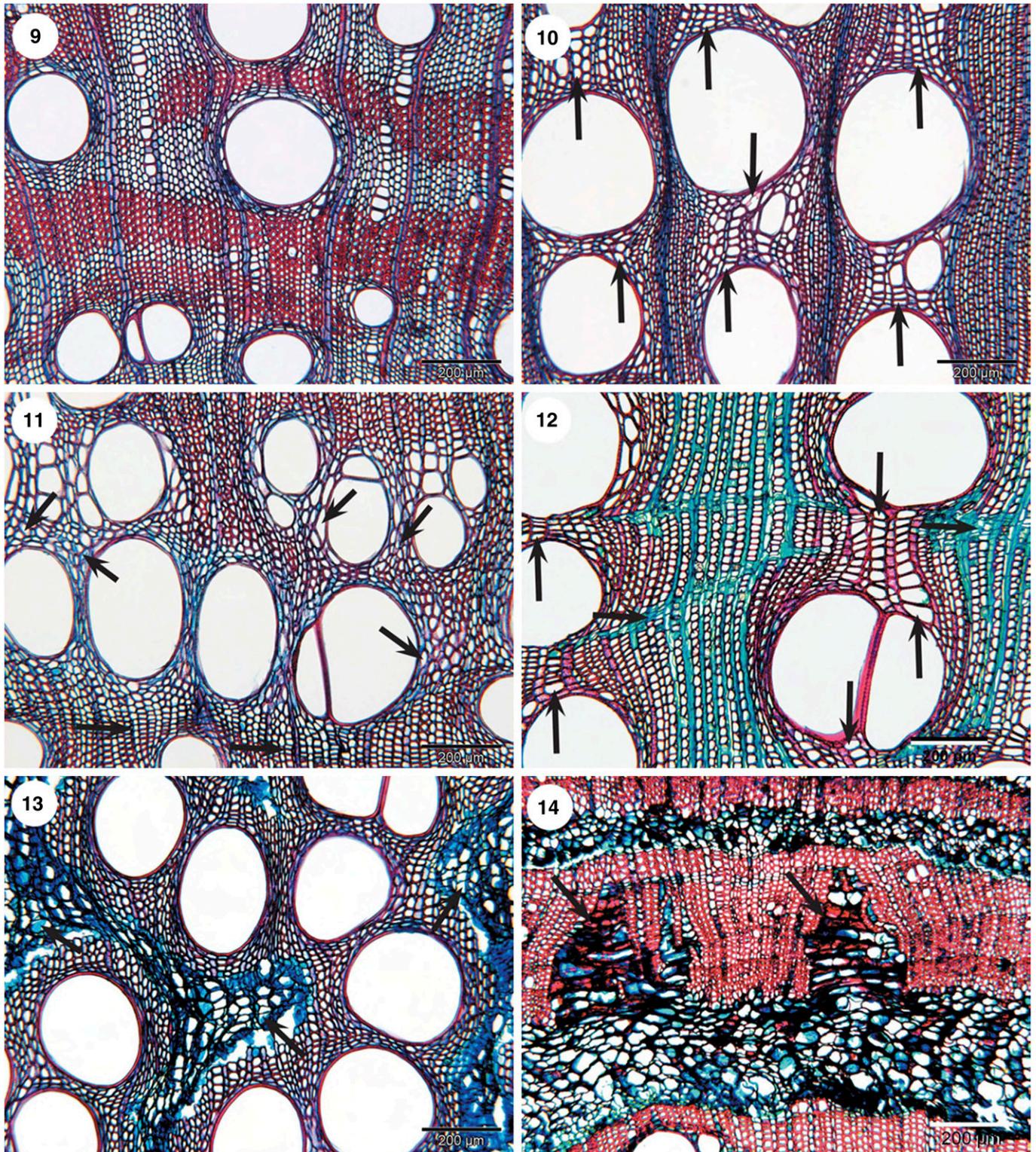
and *Wrightia*. Tangential diameter of vessels (10)–30–260–(470) µm, two vessel size classes in nearly all climbing species (Figs. 7, 9–12) present as many narrow vessels in combination with few wide ones; vessel elements (100)–270–850–(1300) µm long. Tracheids absent in the nonclimbing tribes Malouetieae (except in *Carruthersia*) and Wrightieae, and in the mixed climbing/nonclimbing Nerieae and Rhabdadenia; few vascentric tracheids present in the vessel clusters of the climbing tribes Apocyneae, Baisseeae, Echiteae, and Mesechiteae; tracheid length (300)–500–700–(950) µm. Fibers usually with rather reduced pit borders, 3–4 µm in horizontal diameter, concentrated in radial walls typically present in most tribes (although pits larger and more abundant in Apocyneae and Echiteae); true libriform fibers with simple to minutely bordered pits, 2–3 µm in horizontal diameter, present in *Rhabdadenia* (nonseptate, Figs. 4, 25), in the climbing Odontadenieae (often septate) and some species of the climbing Mesechiteae (occasionally septate, Fig. 26), and in the climbing *Strophanthus* (nonseptate), fiber length (500)–580–1750–(1950) µm; nonseptate fiber-tracheids with distinctly bordered pits in radial and tangential walls, pits 4–6 µm in horizontal diameter, common in the climbing tribes Apocyneae and Echiteae (Fig. 24), fiber-tracheid length (500)–675–1070–(1300) µm; fibers mainly thin-walled or thin- to thick-walled (Figs. 2, 3, 5–13). Axial parenchyma mainly diffuse-in-aggregates to narrowly banded (usually 1-seriate) in the nonclimbing tribes Wrightieae (Figs. 2, 3), Malouetieae (Figs. 5, 6) and the mixed climbing/nonclimbing tribe Nerieae (Figs. 7, 8), a mixture of diffuse or diffuse-in-aggregates apotracheal parenchyma and scanty paratracheal parenchyma common in the climbing tribes Apocyneae (Figs. 11, 12) and Baisseeae (Fig. 10), mainly scanty paratracheal parenchyma common in the climbing tribes Mesechiteae and Odontadenieae and in *Rhabdadenia*, axial parenchyma distribution more variable in the climbing Echiteae, atypical axial parenchyma types in *Mandevilla rugellosa* (aliform plus confluent paratracheal, Fig. 9); banded marginal axial parenchyma, 1–3–(7)-seriate, present in the climbing tribe Baisseeae (sometimes partly nonlignified) and most genera of the climbing

TABLE 1. Wood anatomical comparison of subfamily Periplocoideae (PERI) and the tribes of subfamily Apocynoideae sensu Endress et al. (2007); Wrigh = tribe Wrightieae, Neri = tribe Nerieae, Malou = tribe Malouetieae, Apoc = Apocynoideae, Echi = tribe Echiteae, Mese = tribe Mesechiteae, Odon = tribe Odontadenieae, Bais = tribe Baisseeae. UR = uniseriate rays, MR multiserial rays, + = always or predominantly present, ± = sometimes present, – = absent or very infrequent

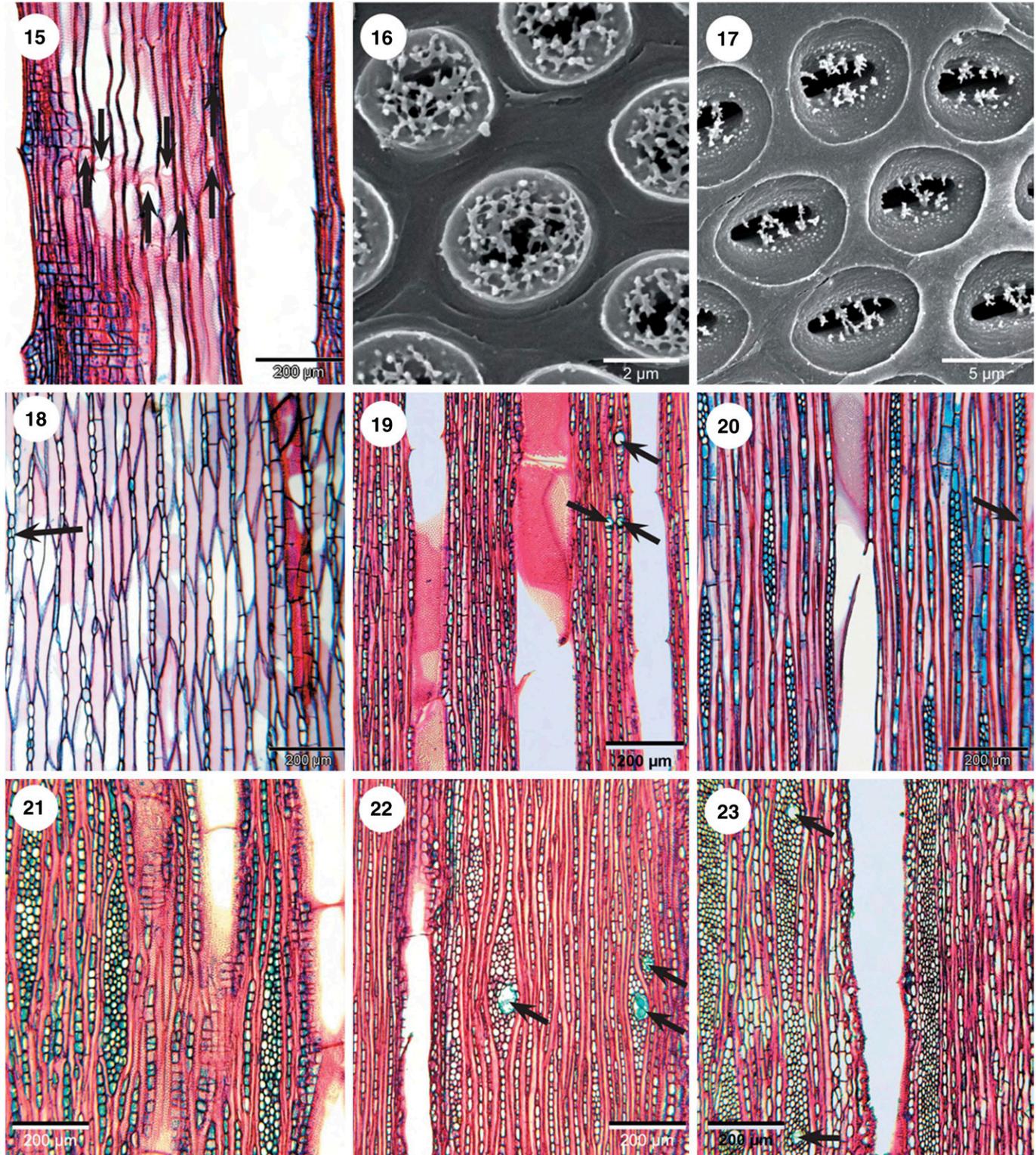
Character	PERI	Wrigh	Neri	Malou	Apoc	Echi	Mese	Odon	Bais
Radial vessel multiples abundant	–	+	+	+	–	–	–	–	–
Vessel clusters abundant	+	–	–	–	+	+	+	+	+
Vessel grouping index	3–14	3	2–3	2–4	10–20	7–15	5–20	10–40	10–25
Range of mean vessel element lengths (µm)	200–500	300–500	400–700	400–900	300–600	200–600	300–600	300–600	300–600
Vascentric tracheids present	+	–	–	–	+	+	+	+	+
Fibers with distinctly bordered pits	+	–	–	–	+	±	–	–	–
Fibers with reduced pit borders	+	+	+	+	–	+	+	+	+
Septate fibers	–	–	–	–	–	–	±	+	–
Axial parenchyma mainly apotracheal	–	+	+	+	–	–	–	±	–
Axial parenchyma apo- and paratracheal	+	–	–	–	+	–	–	–	+
Axial parenchyma mainly paratracheal	–	–	–	–	–	+	+	±	–
Mean range of axial parenchyma cells/strand	2–5	4–8	4–8	3–8	3–8	3–7	4–8	3–6	4–8
UR more frequent than MR	+	–	+	–	+	+	+	+	+
UR equally common as MR	–	+	–	+	–	–	–	–	–
Multiserial ray height (µm)	300–900	300–600	300–1200	300–1000	500–1500	200–800	300–1500	400–900	400–800
Crystals in rays	±	+	–	–	±	±	+	–	±
Crystals in axial parenchyma	+	–	±	+	+	±	+	±	+
Laticifers in rays	–	–	±	–	+	+	+	–	+
Interxylary phloem	–	–	–	–	–	±	–	–	–



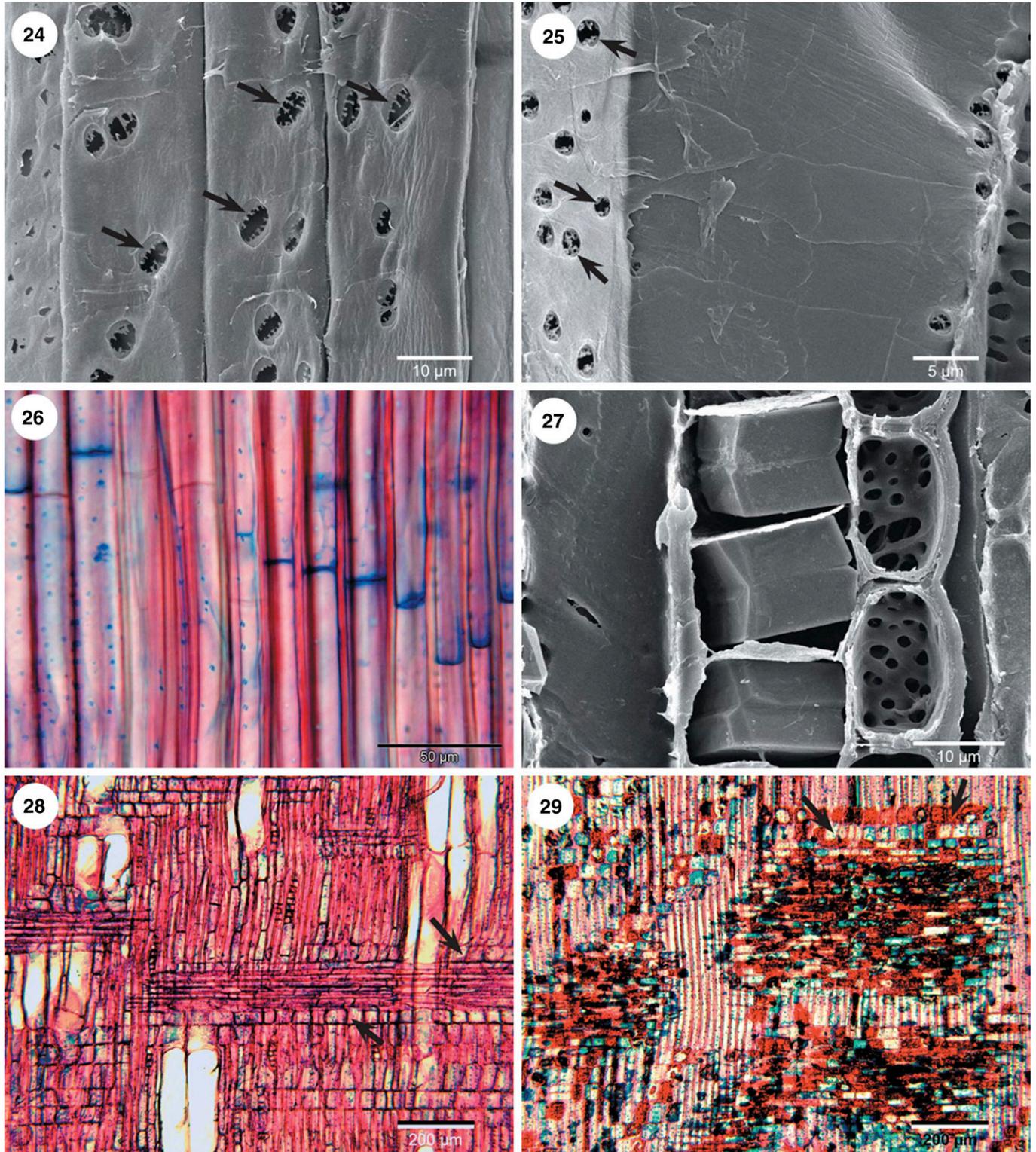
Figs. 2–8. Transverse LM sections of the predominantly nonclimbing tribes Wrightieae, Malouetieae, and Nerieae and the genus *Rhabdadenia*, illustrating the variation in vessel and axial parenchyma distribution. Climbers are represented by Figs. 7 and 8. **2.** *Pleioceras gillettii* (Wrightieae): TS, vessels in radial multiples. **3.** *Wrightia pubescens* (Wrightieae): TS, vessels in radial multiples, diffuse-in-aggregates axial parenchyma. **4.** *Rhabdadenia biflora* (Echiteae): TS, horizontal arrows point to transition zones between erect habit in the first-formed wood, followed by lianescent habit in subsequently formed wood and erect habit in last-formed wood, growth ring boundaries in later-formed wood (vertical arrows). **5.** *Funtumia africana* (Malouetieae): TS, vessels in short radial multiples, diffuse-in-aggregates axial parenchyma. **6.** *Malouetia peruviana* (Malouetieae): TS, vessels in long radial multiples, narrowly banded axial parenchyma. **7.** *Alafia multiflora* (Nerieae): TS, narrow and wide vessels in radial multiples, axial parenchyma diffuse-in-aggregates to narrowly banded. **8.** *Strophanthus hispidus* (Nerieae): TS, vessels mainly in radial multiples, marginal banded axial parenchyma (arrows), axial parenchyma diffuse-in-aggregates to narrowly banded.



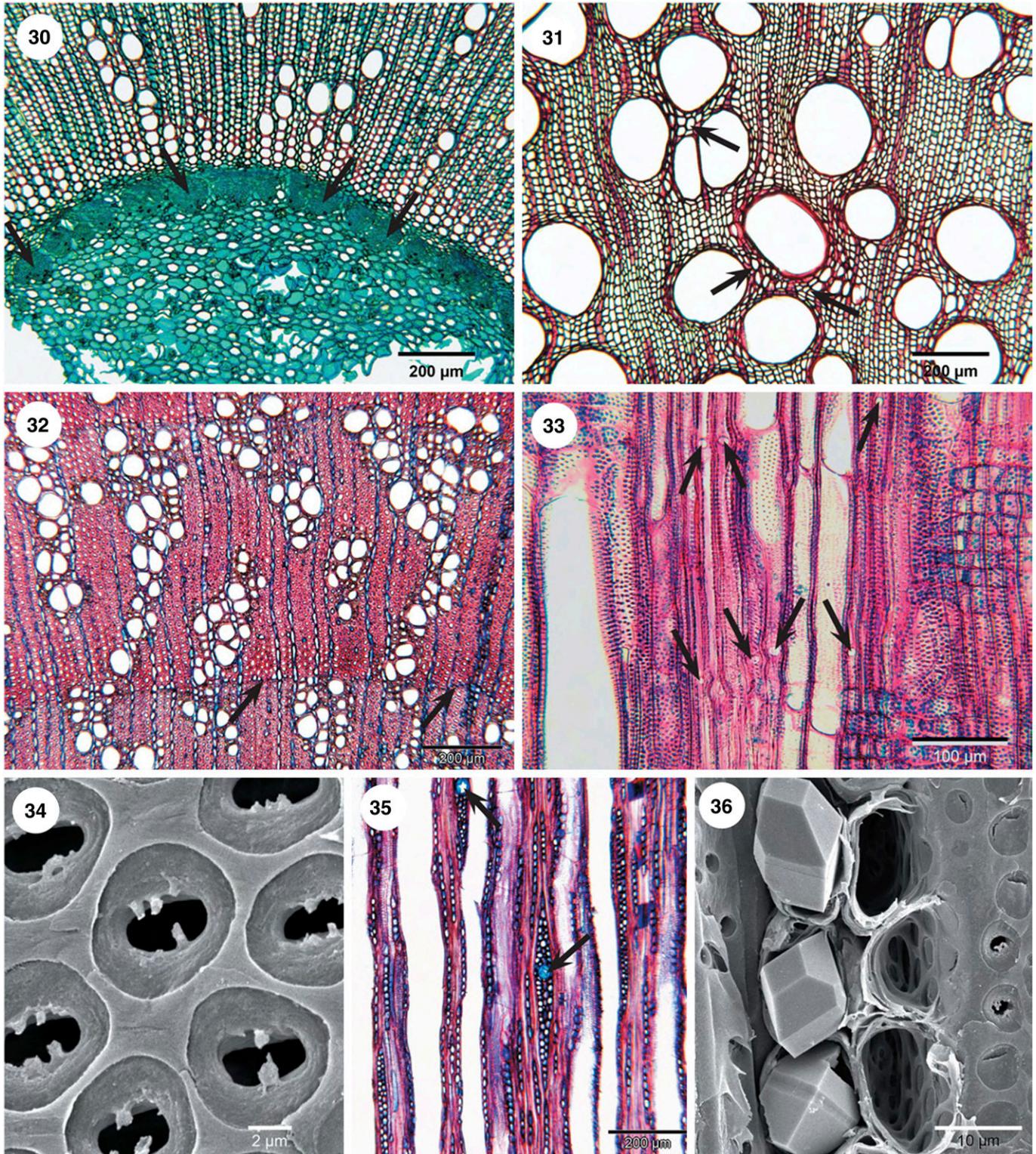
Figs. 9–14. Transverse LM sections showing vessel and axial parenchyma distribution, included phloem and successive cambia in the climbing tribes Apocynaceae, Baisseeae, Echiteae, Mesechiteae, and Odontadenieae. All figures represent climbers. **9.** *Mandevilla rugellosa* (Mesechiteae): TS, wide solitary vessels co-occurring with narrow vessels in radial multiples, abundant axial parenchyma aliform and in wide bands. **10.** *Oncinotis gracilis* (Baisseeae): TS, extensive vessel clusters including few wide and many narrow vessels (arrows), axial parenchyma diffuse-in-aggregates and scanty paratracheal. **11.** *Chonemorpha fragrans* (Apocynaceae): TS, extensive vessel clusters including few wide and many narrow vessels (arrows), axial parenchyma diffuse-in-aggregates and scanty paratracheal. **12.** *Anodendron candolleianum* (Apocynaceae): TS, extensive vessel clusters including few wide and many narrow vessels (vertical arrows), banded marginal axial parenchyma (nonlignified, horizontal arrows). **13.** *Parsonsia buruensis* (Echiteae): TS, vessels mainly solitary, interxylary phloem (arrows). **14.** *Odontadenia verrucosa* (Odontadenieae): TS, successive cambial activity showing subsequent xylem and phloem cylinders; parenchymatous dilatation wedges sometimes present in the wood cylinder (arrows).



Figs. 15–23. Tangential and radial sections (LM) and tangential longitudinal wood surfaces (SEM) and showing simple vessel perforations, vestured pits, and ray characters. Climbers are represented by Figs. 15, 17, 19, 21–23. **15.** *Oncinotis gracilis* (Baisseeae): RLS, many narrow vessels with simple perforations (arrows) in between larger vessels. **16.** *Kibatalia macrophylla* (Malouetieae): TLS, vestures filling most of the pit chamber and outer pit aperture. **17.** *Macropharynx spectabilis* (Echiteae): TLS, vestures highly branched but less abundant. **18.** *Rhabdadenia biflora* (Echiteae): TLS, rays predominantly uniseriate, occasionally biseriate (arrow). **19.** *Alafia lucida* (Nerieae): TLS, rays predominantly uniseriate, sometimes including laticifers (arrows). **20.** *Malouetia quadricasarum* (Malouetieae): TLS, multiseriate rays with long uniseriate ends sometimes interconnecting with other rays (arrow). **21.** *Beaumontia grandiflora* (Apocynae): TLS, uniseriate rays co-occurring with multiseriate ones. **22.** *Baissea gracillima* (Baisseeae): TLS, uniseriate rays co-occurring with wider multiseriate rays including laticifers (arrows). **23.** *Urceola lucida* (Apocynae): TLS, uniseriate rays and tall multiseriate rays including laticifers (arrows).



Figs. 24–29. Wood anatomical sections (LM; Figs. 26, 28, 29) and longitudinal surfaces (SEM; Figs. 24, 25, 27) of Apocynoideae showing multiseri-ate ray composition, crystal occurrence, laticifers and intraxylary phloem. Climbers are represented by Figs. 24, 26, 27, and 29. **24.** *Macropharynx spectabilis* (Echiteae): RLS, fiber-tracheids with distinctly bordered pits (arrows). **25.** *Rhabdadenia biflora* (Echiteae): TLS, libriform fiber with vestured pits (arrows) showing small rudimentary pit borders in radial walls. **26.** *Mandevilla rugellosa* (Mesechiteae): RLS, septate fibers. **27.** *Alafia multiflora* (Nerieae): TLS, prismatic crystals in chambered axial parenchyma strand. **28.** *Holarrhena pubescens* (Malouetieae): RLS, multiseri-ate rays with procumbent body ray cells and few rows of square to upright marginal ray cells (arrows). **29.** *Urceola brachysepala* (Apocyneae): RLS, multiseri-ate rays with procumbent body ray cells and few rows of square to upright marginal ray cells (arrows).



Figs. 30–36. Wood anatomical diversity of Periplocoideae based on LM pictures of transverse sections (TS), tangential longitudinal sections (TLS) and radial longitudinal sections (RLS), combined with tangential longitudinal SEM surfaces. Climbers are represented by Figs. 31 and 34. **30.** *Cryptostegia grandiflora*: TS, intraxylary phloem (arrows). **31.** *Tacazzea pedicellata*: TS, few wide vessels often forming clusters with narrow vessels (arrows). **32.** *Periploca laevigata*: TS, growth ring boundary (arrows), vessels arranged in flame-like dendritic pattern. **33.** *Pentopetia grevei*: RLS, many narrow vessels with simple perforations (arrows) in between wider vessels. **34.** *Tacazzea pedicellata*: TLS, poorly developed vestures observed from the outer pit aperture. **35.** *Pentopetia grevei*: TLS, uniseriate rays combined with multiseriate rays including laticifers (arrows). **36.** *Pentopetia grevei*: TLS, prismatic crystals in ray cells.

Apocynae (*Aganosma*, *Anodendron* [nonlignified, Fig. 12], *Chonemorpha*, *Micrechites* [nonlignified], *Parameria*, *Urceola* [partly nonlignified], and some other climbing genera such as *Carruthersia*, *Forsteronia* (partly nonlignified), *Isonema*, *Macropharynx* (partly nonlignified), *Odontadenia* and *Strophantus* (Fig. 8); in (2)–4–8–(12) celled strands. Rays 1–4–(7)-seriate. Uniseriate rays more abundant than multiseriate rays (5–15 vs. 1–5 rays/mm) in the climbing tribes (Figs. 21–23), in the mixed climbing/nonclimbing Nerieae (10–15 vs. 1–5 rays/mm; Fig. 19) and *Rhabdadenia* (7–15 vs. 0–2 rays/mm; Fig. 18); uniseriate rays equally common in the nonclimbing Wrightieae and Malouetieae (4–9 rays/mm, Fig. 27); height (50)–150–1250–(2500)  $\mu\text{m}$ ; uniseriate rays generally consisting of upright cells. Multiseriate rays generally 2–4-seriate (Figs. 20, 21); 4–7-seriate in some climbing species of *Aganosma*, *Baissea* (Fig. 22), *Carruthersia*, *Chonemorpha*, *Micrechites*, and *Urceola* (Fig. 23); multiseriate ray height (100)–220–2700–(4800)  $\mu\text{m}$  high; typically less than 1000  $\mu\text{m}$  in most tribes (Figs. 20–22), although more variation in the climbing tribes Apocynae (Fig. 23) and Mesechiteae; multiseriate ray density (0)–2–5–(11) rays/mm; consisting of procumbent body ray cells and mostly 1–2–(4) or up to 15 rows of predominantly upright marginal ray cells (Figs. 28, 29); sometimes multiseriate rays fused in *Carruthersia*, *Funtumia*, *Kibatalia*, *Malouetia* (Fig. 20), *Mandevilla*, *Strophantus*, and *Wrightia*; sheath cells absent; rays partly nonlignified in *Anodendron candolleianum*, *Macropharynx spectabilis*, and *Peltastes peltatus*. Dark amorphous contents generally absent, but sometimes observed in *Alafia*, *Odontadenia*, *Rhabdadenia*, and *Urceola* (Fig. 29). Prismatic crystals in procumbent and marginal (often chambered) ray cells common in Wrightieae and Mesechiteae, and occasionally in Apocynae and Baisseeae; prismatic crystals typically present in chambered axial parenchyma cells of the tribes Apocynae, Baisseeae, Malouetieae, Mesechiteae, and Odontadenieae and occasionally also in Nerieae (Fig. 27); silica bodies absent; laticifers common in the climbing tribes Apocynae (Fig. 23), Echiteae, Mesechiteae, Baisseeae (Fig. 22), in some climbing genera of Nerieae (*Alafia* [Fig. 19] and *Strophantus*), and in *Rhabdadenia*; intraxylary phloem observed in all wood samples with pith tissue, interxylary (included) phloem observed in two genera of Echiteae (*Parsonsia* [Fig. 13] and *Peltastes*). Successive cambia present in the two *Odontadenia* species studied (Fig. 14).

Peculiar rootwood features of *Holarrhena curtisii* compared to stemwood of the remaining Apocynoideae species are very thin-walled fibers and the absence of multiseriate rays.

Statistical differences between anatomical measurements of climbing and nonclimbing Apocynoideae are often significant at the 0.01% level, such as vessel diameter (128  $\mu\text{m} \pm 42$  vs. 63  $\mu\text{m} \pm 24$ , respectively;  $t = 5.434$ ,  $df = 48$ ,  $P < 0.0001$ ), vessel density (24/mm<sup>2</sup>  $\pm 11$  vs. 39/mm<sup>2</sup>  $\pm 22$ , respectively;  $t = 3.2158$ ,  $df = 48$ ,  $P = 0.0023$ ), vessel element length (462  $\mu\text{m} \pm 82$  vs. 585  $\mu\text{m} \pm 166$ , respectively;  $t = 3.5117$ ,  $df = 48$ ,  $P = 0.001$ ), fiber length (897  $\mu\text{m} \pm 143$  vs. 1229  $\mu\text{m} \pm 328$ , respectively;  $t = 5.0223$ ,  $df = 48$ ,  $P < 0.0001$ ), and multiseriate ray height (811  $\mu\text{m} \pm 435$  vs. 472  $\mu\text{m} \pm 199$ , respectively;  $t = 2.7911$ ,  $df = 48$ ,  $P = 0.0075$ ). Statistical differences between Apocynoideae and Rauvolfioideae of the same habit type are only present for vessel element length (460  $\mu\text{m}$  vs. 570  $\mu\text{m}$  for climbers;  $t = 3.7984$ ,  $df = 56$ ,  $P = 0.0004$ ; and 585  $\mu\text{m}$  vs. 780  $\mu\text{m}$  for nonclimbers;  $t = 2.7161$ ,  $df = 81$ ,  $P = 0.0081$ ; respectively), and fiber length (900  $\mu\text{m}$  vs. 1115  $\mu\text{m}$  for climbers;  $t = 4.2003$ ,  $df = 56$ ,  $P = 0.0001$ ; and 1230  $\mu\text{m}$  vs. 1600  $\mu\text{m}$  for nonclimbers;  $t = 2.915$ ,  $df = 81$ ,  $P = 0.0046$ ; respectively).

**Periplocoideae (Figs. 30–36)**—Growth ring boundaries usually indistinct or distinct (Fig. 32). Wood generally diffuse-porous, but ring-porous in *Periploca graeca*. Vessels (5)–7–86–(100)/mm<sup>2</sup>; vessels often grouped in clusters (Figs. 31–33) co-occurring with fewer solitary vessels, tendency to form dendritic vessel patterns in *Periploca* (Fig. 32) and *Pentopetia*, tangential multiples rare in some species, vessels typically solitary in *Cryptolepis apiculata* and *Periploca nigrescens*; vessel outline generally rounded to elliptical (Figs. 30–32); perforation plates exclusively simple (Fig. 33). Intervessel pits alternate, pits 4–8  $\mu\text{m}$  in horizontal diameter, up to 10  $\mu\text{m}$  in *Pentopetia grevei*, vestured (Fig. 34). Vessel-ray pits similar to intervessel pits in size and shape throughout the ray cell. Wall sculpturing absent. Tyloses absent. Tangential diameter of vessels (15)–40–240–(410)  $\mu\text{m}$ , two vessel size classes in the climbing species of *Periploca* and *Tacazzea* (Fig. 31) present as few narrow vessels in combination with many wide ones; vessel elements (100)–225–440–(650)  $\mu\text{m}$  long. Vasicentric tracheids associated with vessel clusters; tracheid length (250)–350–500–(700)  $\mu\text{m}$ . Nonseptate fiber-tracheids with distinctly bordered pits, 4–6  $\mu\text{m}$  in horizontal diameter, in radial and tangential walls of *Cryptolepis* and *Periploca*, fiber-tracheid length (400)–500–850–(1000)  $\mu\text{m}$ ; fibers with rather reduced pit borders, 3–4  $\mu\text{m}$  in horizontal diameter, concentrated in radial walls of *Cryptostegia* (nonseptate), *Pentopetia* (nonseptate), and *Tacazzea* (usually septate), fiber length (400)–540–825–(1000)  $\mu\text{m}$ ; fibers thin-walled (Figs. 30, 31) or thin- to thick-walled (Fig. 32). Axial parenchyma scarce, usually a combination of diffuse apotracheal and scanty paratracheal parenchyma; banded lignified marginal axial parenchyma present in *Periploca graeca*, *P. laevigata*, *Pentopetia grevei* (1–3 cells wide), banded unlignified marginal axial parenchyma present in *Tacazzea pedicellata* (1–6 cells wide); in 2–5-celled strands. Rays 1–4–(5)-seriate. Uniseriate rays more abundant than multiseriate rays (5–15 vs. 0–3 rays/mm; Fig. 35); height (50)–100–540–(950)  $\mu\text{m}$ ; uniseriate rays generally consisting of upright cells. Multiseriate rays 2–4–(5)-seriate (Fig. 35); multiseriate ray height (100)–170–900–(1400)  $\mu\text{m}$  high; multiseriate ray density generally low (1–4 rays/mm); consisting of procumbent or mixed procumbent/square body ray cells and 1–2–(4) rows of predominantly upright marginal ray cells; sheath cells absent; rays partly nonlignified in *Periploca nigrescens* and *Tacazzea pedicellata*. Dark amorphous contents present in rays of *Cryptolepis apiculata*. Prismatic crystals common in (sometimes) chambered body ray cells of *Cryptolepis*, *Periploca* (not in *P. graeca*) and *Pentopetia* (Fig. 36); prismatic crystals generally present in chambered axial parenchyma cells (except in *Cryptolepis apiculata* and *Periploca graeca*); silica bodies absent; laticifers only observed in rays of *Cryptostegia grandiflora* and *Pentopetia grevei* (Fig. 35); intraxylary phloem observed in all wood samples with pith tissue (Fig. 30), interxylary phloem not observed.

## DISCUSSION

**Diagnostic wood features at the tribal level**—As described by Lens et al. (2008), there are several wood features that are uniform throughout Apocynaceae, such as simple vessel perforations, alternate vestured intervessel pits, and vessel-ray pits that are similar in shape and size to the intervessel pits. On the other hand, the combination of vessel grouping, vessel element length, fiber type, tracheid presence, axial parenchyma distribution, uniseriate ray frequency, multiseriate ray fusion, and laticifer occurrence allowed identification of most Rauvolfioideae tribes, indicating that the wood structure of Apocynaceae is

phylogenetically relevant at the tribal level. Table 1 confirms that most of these features are also phylogenetically informative in Apocynoideae and Periplocoideae. Especially the diagnostic presence of radial vessel multiples (on average 2–3 vessels, sometimes up to 10 vessels) in the nonclimbing apocynoid tribes Wrightieae (Fig. 3) and Malouetieae (Figs. 5, 6), and in the mixed climbing/nonclimbing Nerieae (Figs. 7, 8) is remarkable and provides a clear contrast compared to the abundance of large vessel clusters in the climbing apocynoid members of the crown clade (on average often more than 10 narrow vessels grouped with few wide vessels in one cluster in combination with vasicentric tracheids; occasionally up to more than 50 vessels per cluster; Figs. 10–12; Appendix S2, see Supplemental Data with the online version of this article). This striking divergence in vessel grouping between the predominantly nonclimbing and climbing groups is not entirely due to differences in habit, because all climbing Nerieae species investigated have abundant radial vessel multiples and only occasionally form vessel clusters (Figs. 7, 8; Table 1). Interestingly, the basal apocynoid tribes occur in the same tropical lowland regions as the crown clade apocynoids, making explanations about the functional significance of various vessel grouping patterns in this lineage difficult. Most likely, the two vessel strategies have evolved independently in the same environment to cope with conductivity and safety constraints of the hydraulic mechanism. Nevertheless, the current study supports our previous hypothesis that vessel grouping patterns are taxonomically important throughout the entire family (cf. Lens et al., 2008).

In the predominantly nonclimbing tribes Wrightieae, Malouetieae, and Nerieae, the ratio of uniseriate/multiseriate ray frequency and the location of crystals can provisionally be used to identify the three tribes. In Nerieae, the number of uniseriate rays (10–15 rays/mm) exceeds by far the number of multiseriate ones (1–5 rays/mm; Fig. 19) compared to the more or less equal abundance of uniseriate and multiseriate rays in Wrightieae and Malouetieae (3–10 rays/mm; Fig. 20; Table 1). Furthermore, nearly all species of Malouetieae studied typically have prismatic crystals only in chambered axial parenchyma cells (crystals absent in *Kibatalia arborea* and *Malouetia quadricasurum*), whereas the species of Wrightieae investigated all have prismatic crystals in rays. In Nerieae, crystal occurrence and location is more variable: crystals are usually absent, but they are observed in axial parenchyma cells only (*Isonema* and *Nerium*) or in axial parenchyma and rays *Alafia multiflora* (Fig. 27).

The wood anatomy of Periplocoideae shares many similarities with the other derived Apocynaceae lineages of the crown clade. In addition to the uniform wood characters throughout Apocynaceae and the typical wood features of the crown clade (abundance of vessel clusters in association with vasicentric tracheids and the tendency toward paratracheal axial parenchyma), there is also a common evolutionary trend toward ground tissue fibers with reduced pit borders concentrated in radial walls (still considered as fiber tracheids, but “approaching” the libriform fiber condition in the Baileyan sense, although tracheid-like fibers common in Apocyneae). Based on our rather limited periplocoid sampling, it is difficult to find diagnostic wood characters to distinguish Periplocoideae from Apocynoideae. Two characters that could be informative in this regard are the reduced number of axial parenchyma cells per strand (fewer than five cells per strand in periplocoids vs. 4–8 cells per strand in Apocynoideae; Table 1) and the occurrence

of laticifers in rays (generally absent in periplocoids vs. present in Apocynoideae). Based on the current (predominantly molecular) phylogenies, periplocoids are a strongly supported lineage within the crown clade, which is fully justified based on its wood anatomy, although their exact position within the crown clade remains unresolved. Two periplocoid wood features, which have been shown to be phylogenetically informative at the family level (Lens et al., 2008; F. Lens, personal observation), point to a close relationship with the subfamilies Secamonoideae and Asclepiadoideae, i.e., the low number of axial parenchyma cells per strand (often 2–3 or up to 5) and the strong reduction in vessel element length (usually on average between 200–500  $\mu\text{m}$ ). However, the resemblance in vessel element length could be the result of parallel evolution, as it is well known that vessel elements are shorter in plants extending into arid regions (such as periplocoids, Secamonoideae and Asclepiadoideae) than in plants growing in wetlands (Carlquist and Hoekman, 1985; Dickison, 2000). In addition, the generally smaller stature as shrubs of these drought tolerant species compared to the much taller tropical lowland Apocynaceae contributes further to this possibly parallel trend (Carlquist, 1966; Baas and Schweingruber, 1987).

With the exception of the pantropical tribe Echiteae, the secondary xylem of the climbing Apocynoideae tribes Apocyneae (predominantly Asian), Baisseeae (African), and Mesechiteae and Odontadenieae (both restricted to the neotropics) is rather homogeneous, although the wood of *Odontadenia* is peculiar because of its successive cambia (Fig. 14). This wood anatomical uniformity is due not only to the diagnostic wood features of the crown clade, but also to the common presence of prismatic crystals in axial parenchyma and the occurrence of laticifers in rays (Figs. 22, 23). Nevertheless, the two neotropical tribes (Mesechiteae and Odontadenieae) can be generally identified based on the occurrence of mainly paratracheal axial parenchyma (vs. a combination of apo- and paratracheal parenchyma in Apocyneae and Baisseeae) and septate fibers (Fig. 26; except in some Mesechiteae species studied, vs. absent in Apocyneae and Baisseeae). More genera of Odontadenieae need to be studied before one can comment more knowledgeably on its uncertain relationships with Mesechiteae or Echiteae as hypothesized by Livshultz et al. (2007). In Apocyneae (and in some Echiteae), the abundance of tracheid-like fibers with distinctly bordered pits in tangential and radial walls (often 4–6  $\mu\text{m}$  in horizontal diameter) in the ground tissue of most species studied is remarkable in a group characterized by pronounced vessel multiples (Carlquist, 1984). The presence of tracheid-like cells can be interpreted in two ways: (1) it may reflect the common condition in the basal subfamily Rauvolfioideae, and therefore these cells could be considered as a plesiomorphic character, or (2) the tracheid-like cells in the ground tissue of these climbing lineages have outcompeted the normal fiber tracheids, and should therefore be considered as secondarily derived. According to Carlquist (1985b), the second option might be the more plausible based on his observations that tracheids are much more abundant in climbing species than in their nonclimbing relatives to provide a safety background mechanism for the wide, and thus more vulnerable, vessels of climbers. At this point, the presence of abundant tracheid-like fibers in the ground tissue of Apocyneae and fiber tracheids having fewer and smaller pits in Baisseeae seems to be the only anatomical difference that could provisionally support the segregation of Baisseeae from Apocyneae as indicated by molecular data (Livshultz et al., 2007).

**Echiteae**—The wood anatomical diversity within Echiteae as circumscribed by Endress et al. (2007) is unexpectedly high (Table 1; online Appendix S2), which questions the monophyletic origin of the group (cf. Livshultz et al., 2007). For instance, *Rhabdadenia biflora* does not have vessel clusters, *Pentalinon* has prismatic crystals in rays and axial parenchyma but lacks laticifers, and *Parsonsia* and *Peltastes* are the only two Apocynoideae genera observed with interxylary phloem (Fig. 13). Because *Parsonsia* and *Peltastes* do not seem to be particularly closely related (Livshultz et al., 2007), it is possible that a more extensive sampling within the New World clade and especially Echiteae would reveal additional genera with interxylary phloem. The presence of interxylary phloem in Apocynaceae is not restricted to Apocynoideae, but has also been observed in the asclepiad genera *Asclepias* (Asclepiadeae), *Gymnema* (Marsdenieae), and *Leptadenia* (Ceropegieae) (Carlquist, 1989; F. Lens, personal observation).

The neotropical species *Rhabdadenia biflora* is peculiar in several aspects. As demonstrated by the horizontal arrows in Fig. 4, the earliest formed wood is typical of an erect growth form, but then abruptly, the anatomy changes to that of a lianescent growth form and back to that of an erect plant. This strange pattern probably corresponds to the variable habit described by Nowicke (1970) for the given species (liana to erect subshrub). Another noteworthy wood character is the presence of extremely thin-walled, parenchyma-like fibers (Fig. 18) with small pits only in radial walls (Fig. 25). These exceptionally thin-walled fibers have also been observed in the very light basal stemwood and rootwood of some Malaysian *Alstonia* species from swamp forests (Alstonieae, Rauvolfioideae; P. Baas, personal observation; Ingle and Dadswell 1953), which are known as driftwood species that can travel for very long distances via water. Other striking resemblances between *R. biflora* and the *Alstonia* swamp forest species are very short vessel elements (200–400  $\mu\text{m}$ ), and mainly uniseriate and notably short rays (100–400  $\mu\text{m}$  in length). *Rhabdadenia biflora* occurs along canals and in other marshy habitats in Florida and can tolerate high levels of salinity. Its preferred habitat, however, is *Rhizophora* mangroves where it ranges from Florida to the Caribbean and northwestern South America (Alvarez-León, 2003; Menezes et al., 2008). The widespread occurrence of *R. biflora* in mangroves, combined with its extremely light basal stemwood including parenchyma-like ground tissue fibers, points to a parallel evolutionary trend in various angiosperm families occurring in swamp forests (Berry and Wiedenhoef, 2004; P. Baas, personal observation).

**Odontadenia**—To the best of our knowledge, our two samples of Odontadenieae represent the first report of successive cambia in Apocynaceae (Fig. 14). Since our sample of *Secodontia*, which is placed as sister to *Odontadenia* in the Livshultz et al. (2007) analysis, does not show successive cambia, this unusual character might be a synapomorphy for the genus. However, a better sampling of the entire tribe Odontadenieae, including genera such *Stipecoma*, *Thyrsanthella* (Endress et al., 2007) and *Pinochia* (Endress and Hansen, 2007), is required to support this assumption.

**Galactophora**—The taxonomic position of this genus remains obscure. Erected by Woodson in 1932, it was included by Pichon (1950) in his tribe Parsonsieae, whereas Leeuwenberg (1994) placed it in Echiteae, both of which have been shown to be polyphyletic (Livshultz et al., 2007). In 2000,

Endress and Bruyns transferred *Galactophora* to the tribe Mesechiteae, based on the presence of five ribs at the base of the style-head. In a more detailed phylogenetic study of Mesechiteae by Simões et al. (2004), cpDNA rejected inclusion of *Galactophora* in Mesechiteae, and this was further supported by the very weak attachment of the anthers with the ribs of the style-head (vs. firmly postgenitally fused in Mesechiteae). The Livshultz et al. (2007) analysis included the genus again in the Malouetieae clade (although with low support), but because of its uncertain affinities, Endress et al. (2007) elected to treat *Galactophora* as a genus incertae sedis within Apocynoideae. With respect to its habit and morphology, *Galactophora* is rather unusual within Apocynoideae: it is described as a woody herb or erect shrub up to 50 cm growing in periodically flooded white sand savannas, and its sticky glandular hairs on the stems, leaves and inflorescences are unique in the family (Morales, 2005). The *Galactophora* sample that we have observed was narrower (3 mm in diameter) than the bulk of our remaining material, thus hampering comparison. Nonetheless, the uniform presence of radial vessel multiples in combination with few solitary vessels in *Galactophora* suggests a position within one of the three basal Apocynoideae lineages (including among others Malouetieae), rather than within the more derived lineages.

**Climbers vs. nonclimbers**—As illustrated by Baas et al. (2007) and Lens et al. (2008), differences between the wood anatomy of climbers (representative Figs. 7–14, 21–23, 31) and nonclimbers (representative Figs. 2, 3, 5, 6, 18–20, 32) deserve special attention in Apocynaceae. Baas et al. (2007) suggested a distinction in vessel grouping between the erect species (vessels in multiples common) and climbers (predominantly solitary vessels), but this generalization was refined after a more detailed study of Rauvolfioideae (Lens et al., 2008), in which solitary vessels were confirmed as the main type in rauvolfioid climbers, but the vessel distribution of nonclimbing rauvolfioids varied much more than previously recognized (exclusively solitary or abundant radial multiples). The current study illustrates that the situation is even more complex, especially with regard to the climbing species of the APSA clade. Whereas the climbing members of Nerieae have radial vessel multiples (Figs. 7, 8), the lianescent anatomy of Apocynoideae and Periplacoideae (and also Secamonoideae and Asclepiadoideae) is characterized by the presence of large vessel clusters and vascentric tracheids (Figs. 10–12; occasionally an extremely high vessel grouping index of over 50). The striking difference in vessel grouping patterns between the climbing taxa of rauvolfioids and crown clade members is further stressed by an obvious distinction in the type of imperforate tracheary cells present in the ground tissue: climbing rauvolfioids are characterized by cells with abundant distinctly bordered pits in tangential and radial walls (tracheids sensu Carlquist, 1984), while the climbing crown clade taxa have cells with fewer and smaller bordered pits in their ground tissue (fiber tracheids according to IAWA Committee, 1989; but often tracheid-like cells in ground tissue of Apocynaceae) in combination with vascentric tracheids. Consequently, there is strong anatomical support for an independent origin of the climbing habit in the two groups.

A functional explanation for the strikingly different anatomical strategy in both climbing groups remains difficult to achieve, but the variation in tracheid distribution probably reflects two independent ways to protect the vulnerable, wide vessels of lianas by acting as a subsidiary water transport system in case

many wide vessels embolize (Carlquist, 1985b). Both groups typically occur in the same humid tropical lowland forests throughout the world and can therefore be assumed to face similar hydraulic demands and (rather low levels of) drought stresses. A similar type of pronounced vessel clustering is commonly found in representatives of many other angiosperm families with a lianescent habit (Carlquist, 1989, 2001), but the co-occurrence of climbing species with exclusively solitary vessels or large vessel groupings within one family is remarkable. In the APSA clade, the presence of extensive vessel clusters is not restricted to climbers: they even tend to form flame-like dendritic patterns in some periplocoide shrubs that are adapted to dry regions, such as *Periploca laevigata* (Fig. 4; coastal sand/gravel areas on Canary Islands) and *Pentopetia grevei* (dry savanna or scrub forests in South and West Madagascar) (online Appendix S2).

In addition to the general qualitative differences in the wood between climbers and nonclimbers in Apocynoideae (vessel clusters vs. radial multiples; tendency to form paratracheal parenchyma vs. only apotracheal parenchyma; presence of vascentric tracheids and laticifers vs. absence), there are also several quantitative differences, all statistically significant at the 0.01% level, which correspond to what has been observed in Rauvolfoideae. In Apocynoideae, climbing species have wider vessels than nonclimbing species (on average 130  $\mu\text{m}$  vs. 65  $\mu\text{m}$ ), which is a well-known correlation throughout the angiosperms (Carlquist, 1985b, 1989; Bamber and ter Welle, 1994). The lower vessel density in climbers compared to nonclimbers (25/mm<sup>2</sup> vs. 40/mm<sup>2</sup>) might be an underestimation of the true value due to the many narrow vessels, which are sometimes very difficult to observe in transverse sections. Vessel elements and fibers are also significantly shorter in climbers than in nonclimbers (460  $\mu\text{m}$  vs. 585  $\mu\text{m}$  and 900  $\mu\text{m}$  vs. 1230  $\mu\text{m}$ , respectively). Finally, climbing species have higher multi-seriate rays compared to nonclimbing taxa (810  $\mu\text{m}$  vs. 470  $\mu\text{m}$ ), although this was not the case in Rauvolfoideae. The widest rays in Apocynoideae (4–7 seriate) all belong to climbing taxa (Figs. 21–23), but ray width in climbing apocynoids is too variable to make generalizations about ray width differences between climbing and nonclimbing species.

When the quantitative wood characters of climbing and nonclimbing apocynoids are compared with the same habit groups in rauvolfoids, similar values of vessel diameter (on average 130–140  $\mu\text{m}$  in climbers and 65  $\mu\text{m}$  in nonclimbers) and vessel density (on average 19–24/mm<sup>2</sup> in climbers and 40/mm<sup>2</sup> in nonclimbers) are reported. The same applies to the multiserial ray height (on average 810  $\mu\text{m}$  in climbers and 470–720  $\mu\text{m}$  in nonclimbers) and width (generally 2–4 seriate, with wider rays in some climbing taxa). However, the length of vessel elements and fibers differs significantly at the 0.01% level between apocynoids and rauvolfoids of the same habit type: climbing as well as nonclimbing apocynoids have considerably shorter vessel elements than their rauvolfoide counterparts (460  $\mu\text{m}$  vs. 570  $\mu\text{m}$  for climbers, and 585  $\mu\text{m}$  vs. 780  $\mu\text{m}$  for nonclimbers, respectively); the same is true for fiber length (900  $\mu\text{m}$  vs. 1115  $\mu\text{m}$  for climbers and 1230  $\mu\text{m}$  vs. 1600  $\mu\text{m}$  for nonclimbers, respectively). Consequently, on the one hand, the general length reduction of vessel elements and fibers within a specific clade is dependent on the climbing habit, as demonstrated in the rauvolfoide tribe Willughbeieae (Lens et al., 2008) and in the subfamily Apocynoideae (this study). On the other hand, there is also a significant evolutionary trend of habit-independent length reduction toward the later-diverging lineages of Apocynaceae,

as evidenced by climbers and nonclimbers of Apocynoideae compared to those of the same habit types in the early-diverging Rauvolfoideae.

**General evolutionary wood trends within Apocynaceae s.l.**—As discussed by Lens et al. (2008), the wood of Apocynaceae exhibits several evolutionary trends that become evident when the early-diverging rauvolfoide lineages are compared with the later-diverging APSA clade members. One of the most conspicuous wood trends is the decreasing vessel element length (on average 700–1000  $\mu\text{m}$  in basal Rauvolfoideae vs. 200–500  $\mu\text{m}$  in Periplocoideae, Secamonoideae, and Asclepiadoideae), illustrating that the well-known Baileyan trend from long to short vessel elements (Bailey and Tupper, 1918) has undergone much more parallel evolution in various angiosperm families than initially recognized (cf. Baas and Wheeler, 1996; Lens et al., 2007b). Vessel grouping also displays a marked evolutionary trend from exclusively solitary vessels or radial vessel multiples in Rauvolfoideae toward large vessel clusters in the more derived lineages of the APSA clade. Thus, the presence of radial vessel multiples in Wrightieae, Nerieae, and Malouetieae is best interpreted as a plesiomorphy that provides additional morphological support for their “basal” position in the APSA clade (cf. Livshultz et al., 2007). Furthermore, the large vessel clusters—sometimes even forming a flame-like dendritic pattern—together with several other wood features justify the taxonomic position of Periplocoideae within the derived crown clade. The great variation of vessel grouping patterns is accompanied by the type of imperforate tracheary cells in the ground tissue: (conductive) tracheids coevolve with solitary vessels, while (nonconductive) fibers are strongly linked with vessel multiples and clusters (Carlquist, 1984). The reduction of vessel element length and the evolution toward pronounced vessel clusters within Apocynaceae go also hand in hand with vascentric tracheid abundance, a high frequency of paratracheal parenchyma, and a decrease in number of cells per axial parenchyma strand (6–12 in basal Rauvolfoideae vs. 2–5 in Periplocoideae, Secamonoideae, and Asclepiadoideae). Most of these major evolutionary trends are linked with—or probably even caused by—a habitat shift toward drier regions and/or an abundance of the climbing habit in the more derived Apocynaceae (Baas et al., 1983; Swarupanandan et al., 1996; Carlquist, 1989, 2001; Dickison, 2000; Venter and Verhoeven, 2001; Verhoeven et al., 2003; Middleton, 2007; Wheeler et al., 2007).

In conclusion, the differences in vessel distribution, vascentric tracheid occurrence and axial parenchyma distribution between the mainly nonclimbing apocynoid tribes (Wrightieae, Malouetieae, Nerieae) and the climbing apocynoids and periplocoide (and by extension also the entire climbing crown clade) confirm the phylogenetic significance of wood characters within Apocynaceae. Furthermore, a combination of additional wood characters may provisionally be used to define several higher-level taxonomic entities within Apocynoideae-Periplocoideae, although the microscopic wood structure within the nonclimbing and climbing taxa is rather uniform. The typical occurrence of large vessel clusters in the climbing apocynoids and periplocoide (and remaining crown clade members) and co-occurring fibers in the ground tissue is remarkable, because this is in contrast with the typical anatomy of climbing rauvolfoids showing solitary vessels and tracheids in the ground tissue. This strikingly different climbing anatomy illustrates that the climbing habit in Apocynaceae must have been originated more than

once, which is verified by molecular phylogenies (Livshultz et al., 2007; Simões et al., 2007). Moreover, the observed vessel grouping pattern supports the basal position of Wrightieae, Malouetieae, and Nerieae within the APSA clade and provides further evidence for the current taxonomic placement of Periplocoideae within the crown 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 Endress et al. (2007). Abbreviations of institutional wood collections: 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. 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 Endress et al. (2007).

**Aganosma cymosa** (Roxb.) G.Don; Sri Lanka (Kurunagele); *Kostermans* 24937; L; 22 mm; Apocynaceae. **Alafia landolphioides** (A.DC.) Benth. & Hook.f. ex K.Schum.\*; Cameroon (Mount F  b  ); *Breteler* 2727; WAG; 13 mm; Nerieae. **Alafia lucida** Stapf; Cameroon (Doum  ); *Breteler* 1857; WAG; 19 mm; Nerieae. **Alafia multiflora** (Stapf) Stapf; Cameroon (Mvila Dep., near Ebom); *Elad & Parren* 398; WAG; 54 mm; Nerieae. **Amphineurion marginata** (A.DC.) D.J.Middleton\*; Philippines (Palawan, St. Paul’s Bay); *Ridsdale SMHI 1554*; L; 16 mm; Apocynaceae. **Amphineurion marginata** (A.DC.) D.J.Middleton; USA (Miami, Fairchild Tropical Garden); *Ewers FG X-1-480*; L; 28 mm; Apocynaceae. **Anodendron candolleianum** Wight; Malesia; *Koloniaal Museum Haarlem 2137*; L; 32 mm; Apocynaceae (APO). **Anodendron paniculatum** A.DC.\*; Thailand; *Maxwell 90-413*; L; 10 mm; Apocynaceae. **Baissea gracillima** (K.Schum.) Hua; Cameroon; *de Kruif 896*; WAG; 27 mm; Baisseeae. **Baissea leonensis** Benth.; Ivory Coast; *de Koning 6890*; WAG; 15 mm; Baisseeae. **Baissea welwitschii** (Baill.) Stapf ex Hiern; Ivory Coast (Abidjan); *Jongkind 4097*; WAG; 11 mm; Baisseeae. **Beaumontia grandiflora** Wall.; USA (Miami, Fairchild Tropical Garden); *Ewers FW X-2-393B*; L; 27 mm; Apocynaceae. **Carruthersia scandens** (Seem.) Seem.; Fiji Islands; *origin and collector unknown*; Kw 24834; 20 mm; Malouetieae. **Chonemorpha fragrans** (Moon) Alston; USA (Miami, Fairchild Tropical Garden); *Ewers FG 70116*; L; 35 mm; Apocynaceae. **Cryptolepis apiculata** K.Schum. ex Engl.; Tanzania; *Holst s.n.*; Kw 23014; 25 mm; Periplocoideae. **Cryptostegia grandiflora** R.Br.; Cuba (Cienfuegos, Horpuitas); *Dechamps R. et al. 12536A*; Tw 50022; 10 mm; Periplocoideae. **Epigynum ridleyi** King & Gamble; India; *Ridsdale PBU 491*; L; 11 mm; Apocynaceae. **Forsteronia gracilis** (Benth.) M  ll.Arg.\*; Surinam; *Maguire et al. 24799*; MADw 12116; 8 mm; Mesechiteae. **Forsteronia guyanensis** M  ll.Arg.\*; Surinam; *Leeuwenberg 1980*; WAG; 25 mm; Mesechiteae. **Funtumia africana** (Benth.) Stapf; Democratic Republic of Congo; *de Briey 181*; L 0369511; mature; Malouetieae. **Funtumia africana** (Benth.) Stapf; Uganda; *Dentzman 1671*; MADw 10183; mature; Malouetieae. **Galactophora pumila** Monach.; Venezuela; *Wurdack & Adderley 42773*; MADw 22413; 3 mm; Malouetieae. **Holarrhena curtisii** King & Gamble; Thailand (Songkla); *Tongseedam 16*; L; 28 mm (root); Malouetieae. **Holarrhena pubescens** (Buch.-Ham.) Wall. ex G.Don; Bangladesh; *Majumder & Islam 60*; L; MADw 24505; mature; Malouetieae. **Holarrhena pubescens** (Buch.-Ham.) Wall. ex G.Don; Thailand (Erawan National Park); *van Beusekom & Geesink 3881*; L; 53 mm; Malouetieae. **Isonema smeathmannii** Roem. & Schult.\*; Ivory Coast; *de Koning 6904*; WAG; 15 mm; Nerieae. **Kibatalia arborea** (Blume) G.Don; Philippines (Palawan, Puerto Princesa); *Podzorski SMHI 2170*; L; 97 mm; Malouetieae. **Kibatalia arborea** (Blume) G.Don; *origin and collector unknown*; WAG; mature; Malouetieae. **Kibatalia macrophylla** (Pierre ex Hua) Woodson; Thailand (Chiang Mai); *collector and number unknown*; L0369526; 19 mm; Malouetieae. **Macropharynx spectabilis** (Stadelm.) Woodson; Bolivia; *Nee 41809*; MADw 46939; 13 mm; Echiteae. **Malouetia peruviana** Woodson; Peru (Loreto); *Mathias & Taylor 5442*; L; 95 mm; Malouetieae. **Malouetia quadricasarium** Woodson; Colombia; *Cuatrecasas 17522*; L; mature; Malouetieae. **Mandevilla rugellosa** (Rich.) L.Allorge; Guyana; *Jansen-Jacobs et al. 3568*; Uw 34801; Mesechiteae. **Mascarenhasia arborescens** A.DC.; USA

(Miami Fairchild Tropical Garden); *Curtis FG FG4376B*; L; 46 mm; Malouetieae. **Micrechites rhombifolius** Markgr.; Indonesia (NW Buru); *Van Balgooy 4900*; L; 20 mm; Apocynaceae. **Micrechites serpyllifolius** (Blume) Kosterm.; Indonesia (Sumatra, Lampung prov.); *Jacobs 8490*; L; 39 mm; Apocynaceae. **Micrechites warianus** (Schltr.) D.J.Middleton; New Guinea (SE of Lae); *Jacobs 9687*; L; 46 mm; Apocynaceae. **Motandra guineensis** (Thonn.) A.DC.; Ghana (Ashanti); *Jongkind 3925*; WAG; 27 mm; Baisseeae. **Nerium oleander** L.; The Netherlands (Botanical Garden of Utrecht); *collector and number unknown*; UN 398; 27 mm; Nerieae. **Odontadenia puncticulosa** (Rich.) Pulle; Brazil; *Krukoff 8090*; MADw 14002; mature; Odontadenieae. **Odontadenia verrucosa** (Willd.) ex Roem. & Schult. K.Schum. ex Markgr.; Brazil (Amazonas, Manaus, Reserva Ducke); *Simois et al. 05/2008*; 8 mm; Odontadenieae. **Oncinotis glabrata** (Baill.) Stapf ex Hiern\*; Cameroon (Lomi  ); *Breteler 1270*; WAG; 12 mm; Baisseeae. **Oncinotis gracilis** Stapf; Ghana (Ashanti); *Jongkind & Abbiw 1985*; WAG; 21 mm; Baisseeae. **Papuechites aambe** (Warb.) Markgr.; Papua New Guinea (near Kutubu); *Jacobs 9238*; L; 17 mm; Apocynaceae. **Parameria laevigata** (Juss.) Moldenke; Philippines (Palawan, Puerto Princesa); *Ridsdale SMHI 156*; L; 17 mm; Apocynaceae. **Parsonsia buruensis** (Teijsm. & Binn.) Boerl.; Indonesia (NW Buru, SE of Bara); *van Balgooy 5079*; L; 30 mm; Echiteae. **Pelastotes pelatus** (Vell.) Woodson; Brazil (Paran  ); *Lindeman & Horreus de Haas 2945*; Uw 13958a; Echiteae. **Pentalinon luteum** (L.) B.F.Hansen & Wunderlin\*; USA; *Stern & Brizicki 210*; MADw 18233; 7 mm; Echiteae; **Pentopetia grevei** (Baill.) Venter; Madagascar; *collector and number unknown*; Kw 13192; 30 mm; Periplocoideae. **Periploca graeca** L.\*; Greece (Serre); *Schweingruber 13-6-1982*; L; 12 mm; Periplocoideae. **Periploca laevigata** Ait.; Spain (Carbonara); *Schweingruber 27-4-1983*; L; 14 mm; Periplocoideae. **Periploca nigrescens** Afzel.; D. R. Congo (East Kasai); *Sapin 35*; Tw 41506; 17 mm; Periplocoideae. **Pleioceras gillettii** Stapf\*; Democratic Republic of Congo (Yangambi); *Louis 6092*; K; 5 mm; Wrightieae. **Rhabdadenia biflora** (Jacq.) M  ll.Arg.; Surinam; *Lindeman & Heyde 468*; Uw 23146; 24 mm; Echiteae. **Secodontia duckei** Markr.; Brazil (Flora da Reserva Ducke, Amazonas); *Costa & Assun  o 385*; K; 9 mm; Odontadenieae. **Strophanthus caudatus** (L.) Kurz; Philippines (Palawan, St. Paul’s Bay); *Podzorski SMHI 2028*; L; 54 mm; Nerieae. **Strophanthus perakensis** Scortechnin ex King & Gamble\*; Thailand (Chiang Mai, Muang); *Maxwell 92-146*; L; 9 mm; Nerieae. **Strophanthus hispidus** DC.; *Botanical Garden Basel 425/H*; L; 36 mm; Nerieae. **Strophanthus singaporianus** (Wall. ex G.Don) Gilg; Philippines (Palawan, Narra); *Ridsdale SMHI 1715*; L; 50 mm; Nerieae. **Tacazzea apiculata** Oliv.; Kenya; *Bally 906*; Kw 23019; 9 mm; Periplocoideae. **Tacazzea pedicellata** K.Schum.; D. R. Congo; *Louis 106*; Tw 32810; 16 mm; Periplocoideae. **Urceola brachysepala** Hook.f.\*; Indonesia (Kalimantan Tengah); *Ridsdale PBU 182*; L; 14 mm; Apocynaceae. **Urceola brachysepala** Hook.f.; Indonesia (Sumatra); *Meijer 6808*; L; 40 mm; Apocynaceae. **Urceola laevis** (Elmer) Merr.; Indonesia (Palawan, Taytay); *Ridsdale SMHI 312*; L; 22 mm; Apocynaceae. **Urceola lucida** (Wall. ex G.Don) Benth. ex Kurz; Indonesia; *Krukoff 4382*; MADw 27145; Apocynaceae. **Vallaris glabra** (L.) Kuntze; origin unknown; *collector and number unknown*, Koloniaal Museum Haarlem 1507-6; L; 33 mm; Apocynaceae. **Wrightia antidysenterica** (L.) R.Br.\*; Sri Lanka (Galle, District Hiniduma); *Nooteboom 3181*; L; 6 mm; Wrightieae. **Wrightia coccinea** (Roxb.) Sims; Thailand (E of Mae Sod); *Geesink 5547*; L; 52 mm; Wrightieae. **Wrightia pubescens** R.Br.; origin unknown *collector and number unknown*; L 0085278; mature; Wrightieae.