

Woodiness within the Spermaceae–Knoxieae alliance (Rubiaceae): retention of the basal woody condition in Rubiaceae or recent innovation?

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- **Background and Aims** The tribe Spermaceae is essentially a herbaceous Rubiaceae lineage, except for some species that can be described as ‘woody’ herbs, small shrubs to treelets, or lianas. Its sister tribe Knoxieae contains a large number of herbaceous taxa, but the number of woody taxa is higher compared to Spermaceae. The occurrence of herbaceous and woody species within the same group raises the question whether the woody taxa are derived from herbaceous taxa (i.e. secondary woodiness), or whether woodiness represents the ancestral state (i.e. primary woodiness). Microscopic observations of wood anatomy are combined with an independent molecular phylogeny to answer this question.
- **Methods** Observations of wood anatomy of 21 woody Spermaceae and eight woody Knoxieae species, most of them included in a multi-gene molecular phylogeny, are carried out using light microscopy.
- **Key Results** Observations of wood anatomy in Spermaceae support the molecular hypothesis that all the woody species examined are secondary derived. Well-known wood anatomical characters that demonstrate this shift from the herbaceous to the woody habit are the typically flat or decreasing length vs. age curves for vessel elements, the abundance of square and upright ray cells, or even the (near-) absence of rays. These so-called paeodomorphic wood features are also present in the Knoxieae genera *Otiophora*, *Otomeria*, *Pentas*, *Pentanisia* and *Phyllopentas*. However, the wood structure of the other Knoxieae genera observed (*Carphalea*, *Dirichletia* and *Triainolepis*) is typical of primarily woody taxa.
- **Conclusions** In Spermaceae, secondary woodiness has evolved numerous times in strikingly different habitats. In Knoxieae, there is a general trend from primary woodiness towards herbaceousness and back to (secondary) woodiness.

Key words: Knoxieae, LM, primary woodiness, Rubiaceae, Rubioideae, secondary woodiness, Spermaceae, wood anatomy.

INTRODUCTION

In its currently accepted circumscription, the tribe Spermaceae is essentially a herbaceous lineage of the family Rubiaceae, representing about 61 genera and 1235 species (Kårehed *et al.*, 2008; Groeninckx *et al.*, 2009a). It includes the former tribes Spermaceae *sensu stricto* and Manettieae, and the *Hedyotis*–*Oldenlandia* group of the former tribe Hedyotideae. Representatives are usually annuals or short-lived perennials found in grasslands or open forests throughout the (sub)tropics. A number of species are adapted to more extreme habitats, such as sand dunes, Kalahari sand plateaus (Fig. 1A), shores (Fig. 1B, C), montane scrublands or rocky areas (Table 1). Many species are entirely herbaceous, but several (short-lived) perennial species – especially those adapted to extreme habitats – produce a limited-to-considerable amount of wood (‘woody’ herbs to small shrubs, or occasionally treelets, Fig. 1D–F) while others develop secondary growth mainly in their underground organs (geoxyllic herbs, Fig. 1A–C; Dessein *et al.*, 2002, 2003). The occurrence of herbaceous and woody species within the same group raises the question whether

the herbaceous taxa are derived from woody lineages, as is the case in the early-diverging lineages of the subfamily Rubioideae (e.g. in *Coccocypselum* and *Cruckshanksia* of the tribe Coussareae *sensu lato*), or if herbaceousness represents the plesiomorphic state. From an evolutionary point of view, the second hypothesis refers to a secondary derived origin of the wood, meaning that these woody species are derived from herbaceous ancestors, which in turn have evolved from (primarily) woody species.

With respect to higher-level phylogenetic relationships of Spermaceae, the tribe is considered as a derived taxon within Rubioideae and there is strong evidence for a sister relationship with Knoxieae (Bremer and Manen, 2000; Dessein, 2003; Robbrecht and Manen, 2006). The Knoxieae as currently accepted are a much smaller tribe than Spermaceae: they include only 17 genera and 128 species, and unite Knoxieae *s.s.*, Triainolepideae and members of the *Pentas* group of the former tribe Hedyotideae (Dessein, 2003; Kårehed and Bremer, 2007). Knoxieae generally differ from Spermaceae in having predominantly 5-merous flowers instead of 4-merous flowers, but exceptions occur in both tribes. As in Spermaceae, herbaceous and woody species are present in Knoxieae, although the percentage of woody

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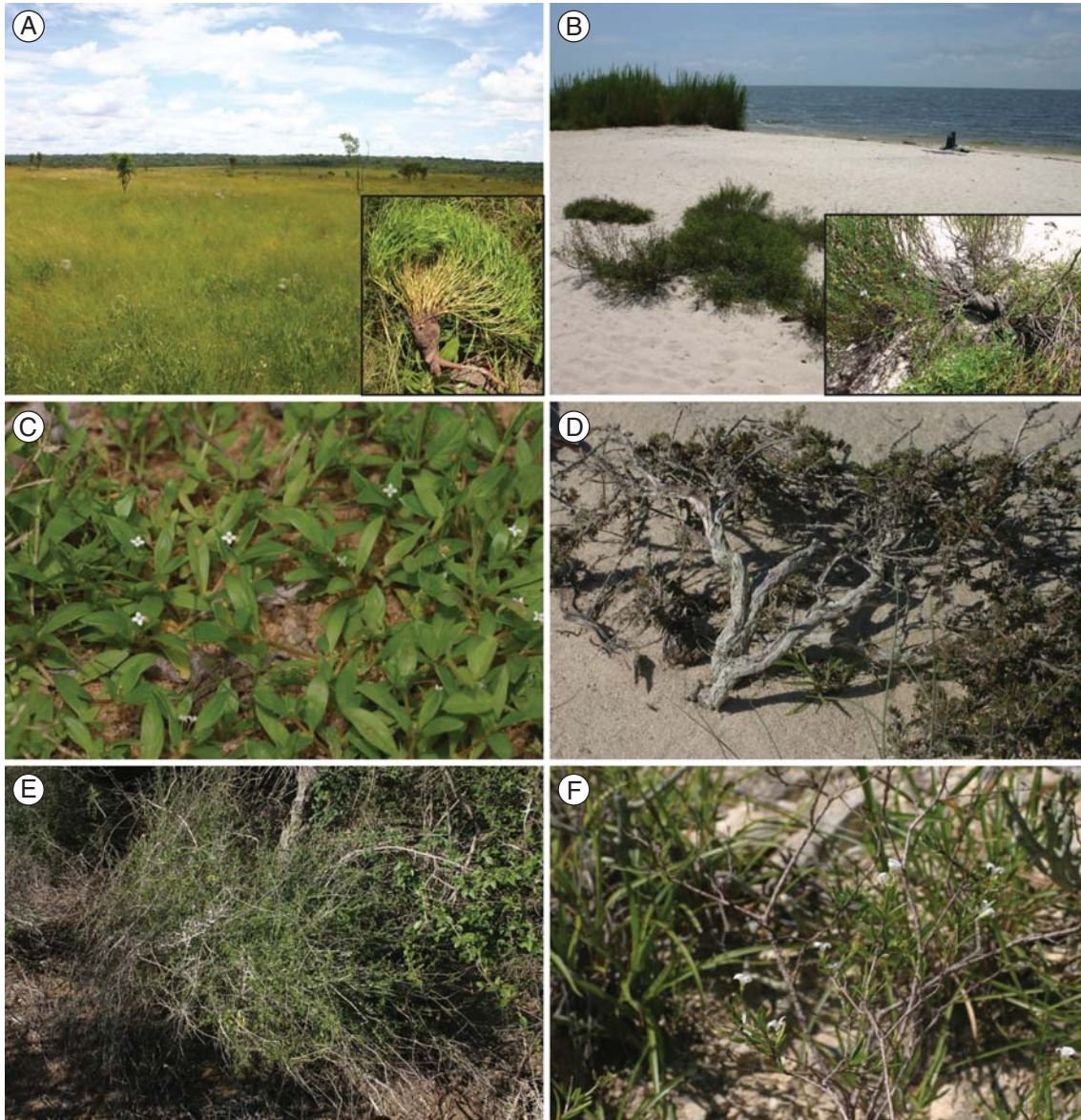


FIG. 1. Examples of the variation in habitats and growth forms of woody Spermacoceae. (A, B) habitats plus growth form; (C–F) growth form. (A) Overview of Kalahari sand plateau (Mwinilunga, Zambia) where *Spermacoce manikensis* grows (geoxylic herb; inset, detail of habit). (B) Shore of Lake Bangweulu (Zambia) with *S. bangweolensis* (inset, detail of habit). (C) *Gomphocalyx herniarioides*, geoxylic herb (south-west Madagascar). (D) *Lathraeocarpa acicularis*, shrub (south-west Madagascar). (E) ‘*Oldenlandia*’ *ambovombensis*, shrub (south Madagascar). (F) ‘*Oldenlandia*’ *humbertii*, shrub (south-west Madagascar).

representatives is clearly higher. For instance, the genera *Carphalea*, *Dirichletia* and *Triainolepis* are entirely woody and also usually taller than most woody Spermacoceae. In most remaining Knoxieae genera, some of the species produce a limited amount of wood and can be described as small (sub-)shrubs, woody herbs or geoxylic herbs (cf. most woody Spermacoceae; Verdcourt, 1976; Table 1).

The intratribal relationships of Spermacoceae have been the subject of controversy. Continuous changes in the delimitation of genera have resulted in a very complex taxonomic history of the tribe. Recent molecular studies (Kårehed *et al.*, 2008; Groeninckx *et al.*, 2009a) have shed new light on the phylogeny of Spermacoceae and relationships between genera are starting to crystallize. The genera of the Spermacoceae *s.s.* form a

monophyletic clade deeply nested among taxa of the *Hedyotis*–*Oldenlandia* group, and are closely related to *Bouvardia* and *Manettia*. *Oldenlandia* is polyphyletic and should be restricted to include only close relatives of its type species *O. corymbosa*. *Hedyotis* is possibly to be restricted to Asia. Many small-to-medium-sized genera (e.g. *Arcytophyllum*, *Conostomium*, *Hedythyrus*, *Houstonia*, *Stenotis*, etc), often reduced to the synonymy of either *Hedyotis* or *Oldenlandia*, should be recognized at generic level. With respect to the Knoxieae, generic rearrangements have been proposed by a molecular study of Kårehed and Bremer (2007). For instance, *Pentas* was shown to be polyphyletic and three new genera were recognized to accommodate its species, *Placopoda* and African members of the genus *Carphalea* should be placed into *Dirichletia*, and the generic circumscription

TABLE 1. *Habit and habitat details of the woody species studied*

Species	Habit	Habitat and distribution
Spermaceae		
<i>Arcytophyllum lavarum</i>	Subshrub	Volcanic mountains at high altitude; Costa Rica to Panama
<i>Arcytophyllum setosum</i>	Shrub	Grassland above timberline; western South America
<i>Arcytophyllum thymifolium</i>	Subshrub or shrub	Grassland above timberline; Colombia to Peru
<i>Diodella sarmentosa</i>	Straggling, scrambling or climbing, often perennial herb	Large ecological amplitude, including evergreen forest, riverine vegetation, bush land, rocky places; widespread in the tropics
<i>Emmeorrhiza umbellata</i>	Climbing, perennial herb	Tropical forest; South America
<i>Gomphocalyx herniarioides</i>	Prostrate or decumbent herb with well-developed, often woody taproot	Sandy soils of thorn forest, dunes and beaches; Madagascar
<i>Hedyotis flavescens</i>	Shrub	Upper montane zone on open waterlogged sites; Sri Lanka
<i>Hedyotis fruticosa</i>	Shrub or small tree to 4 m	Open vegetation, rocky areas on mountain tops; southern India, Sri Lanka, Myanmar
<i>Hedyotis lessertiana</i>	Shrub or small tree to 4 m	Upper montane scrubland; Sri Lanka
<i>'Hedyotis' trichoglossa</i>	Woody herb of suffrutex with slightly woody stems and taproot	Eastern humid forest; Madagascar
<i>Kadua cordata</i>	Subshrub or shrub	Mesic to wet forests; Hawaiian islands
<i>Lathraeocarpa acicularis</i>	Subshrub with woody stems and taproot	Sandy soils in dunes close to the sea; Madagascar
<i>Mitracarpus frigidus</i>	Subshrub or shrub	Scrubland, grassland, also in disturbed areas; tropical America
<i>Nesohedyotis arborea</i>	Tree up to 7 m	Tree fern thicket; St. Helena island
<i>'Oldenlandia' ambovombensis</i>	Shrub	Dry spiny forest on limestone plateau; south-western Madagascar
<i>'Oldenlandia' humbertii</i>	Subshrub	Dry spiny forest on limestone plateau; south-western Madagascar
<i>Spermaceae bangweolensis</i>	Prostrate or suberect subshrub with woody taproot	Sandy soil bordering Lake Bangweulu; Zambia
<i>Spermaceae manikensis</i>	Geoxylic herb with woody taproot and plant base	High plateaus on Kalahari sand; Zambia and D.R. Congo
<i>Spermaceae macrocephala</i>	Subshrub	Savanna areas on river banks; Colombia and Venezuela
<i>Spermaceae occidentalis</i>	Annual or more often perennial spreading or erect woody herb	Open woodland on sandy soil or dune vegetation close to sea; Australia
<i>Spermaceae verticillata</i>	Woody herb or (sub)shrub	Savanna, scrubland and disturbed areas; tropical and subtropical America, introduced elsewhere
Knoxieae		
<i>Carphalea kirondrion</i>	Shrub or tree up to 10 m	Mostly at edges of dry, deciduous forest on laterite or calcareous sand; Madagascar
<i>Dirichletia virgata</i>	Shrub	Forest and scrubland; Socotra, Republic of Yemen
<i>Otiophora rupicola</i>	Subshrub	Rocky grassland at high altitude; Burundi
<i>Otomeria micrantha</i>	Herb, often with woody base	Savanna, clearings in forest, along roadsides; Nigeria to west-central tropical Africa
<i>Pentansia schweinfurthii</i>	Geoxylic herb with woody taproot and plant base	Submontane and lower grassland, also in clearings of woodland; tropical Africa
<i>Pentas zanzibarica</i>	Woody herb or shrub with a woody rootstock	Forest edge, scrubland and grassland; Uganda to Mozambique
<i>Phyllopetas schimperiana</i>	Woody herb or shrub	Forest edge and scrubland, at high altitudes; tropical Africa
<i>Triainolepis polyneura</i>	Shrub	Deciduous and semi-deciduous forest; Madagascar

of *Pentanisia* and *Triainolepis* must be enlarged. The current molecular framework of Spermaceae (*sensu* Kårehed *et al.*, 2008; Groeninckx *et al.*, 2009a) and Knoxieae (Kårehed and Bremer, 2007) forms a good starting point to assess the origin of woodiness within both tribes.

In order to evaluate secondary woodiness of a particular species, independent methods should be applied. If a molecular phylogeny is present, the first and most obvious way is to trace evolutionary shifts from herbaceous to woody taxa (e.g. Böhle *et al.*, 1996; Thiv *et al.*, 1999; Lee *et al.*, 2005). Another option might be to make woody mutants from herbaceous wild-types (Groover, 2005; Melzer *et al.*, 2008). If molecular data are insufficient or unavailable, evidence in the microscopic wood structure – the so-called paedomorphic features – can be used to identify secondary derived shrubs and trees (Carlquist, 1962). Paedomorphic features typically remain in a juvenile ontogenetic state, which can be demonstrated in the wood structure by (juvenile) characters of the primary xylem that are transferred into the (mature) secondary xylem (= wood). Examples are the continuous decrease of vessel element length from the pith towards the cambium, the presence of elongated scalariform intervessel pits in wood (or even wide, gaping intervessel pits resembling helical tracheids in the primary xylem; e.g. fig. 7 in Lens *et al.*, 2005), and the absence of rays and/or the presence of rays with an abundance of square-to-upright ray cells.

In at least some (derived) Rubioideae lineages with mixed herbaceous and woody species, such as Paederieae and Rubieae, earlier publications of wood anatomy favour the secondary woodiness option for at least some species (Koek-Noorman, 1976; Koek-Noorman and Puff, 1983; Carlquist, 1992). Consequently, it would be interesting to investigate if this is also valid in other derived Rubioideae clades, such as Spermaceae and its sister tribe Knoxieae. In order to do this, the wood anatomical variation of Spermaceae and Knoxieae should be studied using a broader sampling than is currently available. The poor knowledge of wood anatomy of both tribes can be illustrated by two large-scale wood anatomical review papers in Rubiaceae: the first one covering the subfamily Rubioideae (Jansen *et al.*, 2001) studied only two young twigs of *Triainolepis* (Knoxieae), while the subsequent wood anatomical survey concerning the entire family (Jansen *et al.*, 2002) dealt with mainly juvenile material of only six Spermaceae species and six Knoxieae species.

The objectives of this study were to present a detailed overview of the wood anatomy of woody representatives of the tribe Spermaceae and Knoxieae, and to compare this overview with a multi-gene molecular phylogeny in order to assess the origin of woodiness in the two tribes. In addition, a comparison is made of the wood structure between Spermaceae and its sister tribe Knoxieae.

MATERIALS AND METHODS

Plant material

Wood samples from 21 Spermaceae species and eight Knoxieae species were collected from the xylaria in Kew (Kw), Tervuren (Tw), Utrecht (Uw) and from the National

Botanic Garden of Belgium (BR; see Table 2 and Appendix). The sampling covers all major Spermaceae and Knoxieae clades as identified in the molecular phylogenies (Kårehed and Bremer, 2007; Kårehed *et al.*, 2008; Groeninckx *et al.*, 2009a).

We tried to investigate as many mature wood samples as possible, but due to the limited wood production in some species, some samples need to be considered as juvenile twigs, for example *Arcytophyllum lavarum*, *A. thymifolium*, *Emmeorhiza umbellata*, *Hedyotis flavescens*, *H. fruticosa*, *H. lessertiana* and ‘*Oldenlandia*’ *humbertii* (all Spermaceae). The Knoxieae species *Carphalea kirondrion*, *Otiophora rupicola* and *Phyllopentas schimperiana* also represent juvenile wood material.

Geoxylic Spermaceae species with woody underground organs are observed in *Gomphocalyx herniarioides* and *Spermaceae manikensis*; of these, *G. herniarioides* has primary xylem in the centre, while the underground parts of *S. manikensis* has a stem anatomy. Species with a similar geoxylic habit in Knoxieae are *Otiophora rupicola* and *Pentanisia schweinfurthii*. *Spermaceae bangweolensis* and *S. occidentalis* have woody underground parts combined with more elaborate woody parts above ground.

‘*Oldenlandia*’ *humbertii* and ‘*Oldenlandia*’ *ambovombensis* represent two undescribed species from Madagascar: both will be described in a new genus (I. Groeninckx, unpubl. data). The Malagasy species ‘*Hedyotis*’ *trichoglossa* also needs to be transferred to a new genus. *Lathraeocarpa acicularis*, a small (sub)shrub also endemic to Madagascar, was previously placed within the monogeneric tribe Lathraeocarpeae (Bremekamp, 1957); however, a recent molecular-morphological study by Groeninckx *et al.* (2009b) strongly supports the inclusion of this Malagasy endemic genus within the tribe Spermaceae, sister to *Phyllohydrax* and *Gomphocalyx*.

Description of wood anatomy and microtechniques

The methodology of wood sectioning and the subsequent steps are described in Lens *et al.* (2005). The wood anatomical terminology follows *IAWA list of microscopic features for hardwood identification* (IAWA Committee, 1989), except for the concept of imperforate tracheary elements with distinctly bordered pits (i.e. fibre-tracheids and tracheids). Because the distinction between these two cell types is very hard to make in Spermaceae due to continuous overlap in pit border size and pit density, we prefer to distinguish between fibre-tracheids and tracheids based on the degree of vessel grouping (Carlquist, 1984). In general, species with a low vessel grouping that grow in mesic conditions possess non-conducting fibre-tracheids (or libriform fibres) in the ground tissue, but when these species occur in dry conditions the imperforate cells are hypothesized to be functionally water-conducting tracheids that take over the water transport from drought-induced embolized vessels (although we acknowledge that more functional work remains to be done to assess the difference in water-conducting capacity between fibre-tracheids and tracheids).

For length vs. age curves, measurements for vessel elements were made using radial sections from the pith towards the cambium. In addition, the length of vessel elements and

TABLE 2. Overview of selected wood anatomical characters within Spermaceae and Knoxieae (Rubiaceae). Species are arranged alphabetically according to the tribal studies of Groeninckx et al. (2009a) and Kårehed and Bremer (2007). Numbers between hyphens are mean values flanked by minimum and maximum values. For specimens of the same taxon, superscript numbers after the species name refer to the order of the specimens as followed in the species list (see Appendix). Species names in bold represent underground woody organs, and juvenile wood samples are marked with an asterisk behind the species names

Species studied	Primarily or secondarily woody	Length vs. age curve	Tendency to (semi-)ringporosity	Solitary vessels	Radial vessel multiples	Tangential vessel multiples	Vessel clusters	Vessel diameter (µm)	Vessel density (mm ⁻¹)	Vessel element length (µm)	Tracheid length (µm)	Tracheids thick-walled	Occasional septate libriform fibres	Dif axial parenchyma	Dia axial parenchyma	Banded axial parenchyma	Scanty axial parenchyma	Rays absent	Rays exclusively uniseriate	Multiseriate ray width (no. cells)	Multiseriate ray height (µm)	Ray cells mostly or all square-to-upright
Spermaceae																						
<i>Arcytophyllum lavarum</i> *	S	Flat	- +	+ +	- -	- -	- -	15-20-30	100-125-160	150-195-250	250-300-350	- -	±	- -	±	- -	±	- +	/ /	/ /	+ /	
<i>Arcytophyllum setosum</i>	S	Decr	- +	+ +	- -	- -	- -	15-20-25	80-100-140	150-290-400	300-370-450	- -	- -	- -	- -	- -	- -	+ C	/ /	/ /	+ /	
<i>Arcytophyllum thymifolium</i> *	S	Flat	- +	+ +	- -	- -	- -	10-16-20	480-540-640	100-160-250	150-200-250	- -	- +	- +	- +	- +	- +	- +	/ /	/ /	+ /	
<i>Diodella sarmentosa</i>	S	Flat	- ±	+ ±	+ ±	+ ±	+ ±	25-60-120	36-48-66	150-300-550	350-465-550	- -	- +	- +	- +	- +	- +	- +	- 2-14	200-2130-8000	+ /	
<i>Emmeorhiza umbellata</i> *	S	Decr	- +	+ +	- -	- -	- -	15-55-120	60-81-85	200-350-500	500-610-700	- +	- +	- +	- +	- +	- +	- +	- +	/ /	+ /	
<i>Gomphocalyx herniarioides</i>	S	Decr	- +	+ ±	- -	- -	- -	15-28-40	135-150-180	100-160-250	250-340-400	- -	- -	- +	- +	- +	- +	- +	- 2-3	300-840-1500	+ /	
<i>Hedyotis flavescens</i> *	S	Decr	- +	+ +	- -	- -	- -	10-23-30	90-110-140	300-650-850	750-910-1150	- +	±	- ±	- ±	- ±	- ±	- ±	- +	/ /	+ /	
<i>Hedyotis fruticosa</i> *	S	Decr	- +	+ ±	- -	- -	- -	25-36-50	80-95-120	250-480-700	600-765-900	- +	±	- ±	- ±	- ±	- ±	- ±	- +	/ /	+ /	
<i>Hedyotis lessertiana</i> *	S	Flat	- +	+ ±	- -	- -	- -	15-25-30	75-45-120	300-660-1000	850-1050-1300	- +	±	- ±	- ±	- ±	- ±	- ±	- +	/ /	+ /	
<i>'Hedyotis' trichoglossa</i>	S	Decr	- +	+ ±	- -	- -	- -	15-21-30	100-120-145	300-540-700	500-665-800	- +	±	- ±	- ±	- ±	- ±	- ±	- +	/ /	+ /	
<i>Kadua cordata</i>	S	Flat	- +	+ ±	- -	- -	- -	20-29-50	140-170-230	350-450-700	500-630-800	- +	±	- ±	- ±	- ±	- ±	- ±	- +	/ /	+ /	
<i>Lathraeocarpa acicularis</i>	S	Decr	+ +	+ ±	+ ±	+ ±	+ ±	10-21-40	200-255-290	100-220-300	400-470-600	±	- ±	- ±	- ±	- ±	- ±	- ±	- +	/ /	+ /	
<i>Mitracarpus frigidus</i>	S	Flat	±	+ ±	±	±	±	15-25-45	140-170-200	250-375-550	550-690-850	- +	±	- ±	- ±	- ±	- ±	- ±	- 2	150-345-650	+ /	
<i>Nesohedyotis arborea</i> ¹	S	Flat	- +	+ +	- -	- -	- -	20-50-80	16-20-25	200-340-500	600-750-900	- -	- -	- +	- ±	- ±	- ±	- ±	- 4-6	300-550-900	± /	
<i>'Oldenlandia' ambovombensis</i> ^{1*}	S	Flat	+ +	+ +	- -	- -	- -	10-19-40	240-265-300	150-300-450	350-485-600	±	- ±	- ±	- ±	- ±	- ±	- ±	- +	/ /	+ /	
<i>'Oldenlandia' ambovombensis</i> ²	S	Flat	+ +	+ +	- -	- -	- -	10-20-40	260-285-320	150-275-350	400-460-550	±	- ±	- ±	- ±	- ±	- ±	- ±	- +	/ /	+ /	
<i>'Oldenlandia' humbertii</i> *	S	Flat	±	+ +	- -	- -	- -	10-19-30	290-325-380	100-200-300	300-405-500	+ -	- -	- -	- +	- +	- +	- +	- +	/ /	+ /	
<i>Spermaceae bangweolensis</i>	S	Flat	- +	+ +	- -	- -	- -	20-30-45	110-125-160	150-320-400	350-500-650	- -	- -	- +	- ±	- ±	- ±	- ±	- 2	200-300-550	+ /	
<i>Spermaceae macrocephala</i>	S	Flat	- +	+ ±	±	±	±	15-26-35	110-140-160	450-690-950	650-840-950	- -	±	- ±	- ±	- ±	- ±	- ±	- 2	600-950-1800	+ /	
<i>Spermaceae manikensis</i>	S	Flat	- +	+ ±	±	±	±	15-23-30	100-115-130	100-300-500	500-605-800	- +	- +	- +	- +	- +	- +	- +	- 2-3	200-400-700	+ /	
<i>Spermaceae occidentalis</i>	S	Flat	- +	+ ±	- -	- -	- -	20-35-50	120-140-160	200-350-450	350-460-550	- -	- -	- +	- +	- +	- +	- +	- 2-3	200-400-700	+ /	
<i>Spermaceae verticillata</i> ¹	S	Decr	- +	+ ±	- ±	- ±	- ±	15-22-30	120-160-190	300-415-550	500-640-800	- +	±	- ±	- ±	- ±	- ±	- ±	- +	/ /	+ /	
<i>Spermaceae verticillata</i> ²	S	Decr	±	+ ±	±	±	±	15-30-45	130-145-170	150-240-500	400-495-600	- +	±	- ±	- ±	- ±	- ±	- ±	- 2	300-440-600	+ /	
Knoxieae																						
<i>Carphalea kirondrone</i> *	P	Norm	±	+ ±	±	- ±	±	25-55-100	60-72-85	300-410-550	600-720-850	- -	- -	- +	±	+ -	- -	- -	- 2-3	300-565-1000	- /	
<i>Dirichletia virgata</i>	P	Norm	±	- ±	- +	- +	- +	20-40-70	60-75-85	150-330-500	850-1045-1200	- -	- -	- +	- +	- +	- +	- +	- 3-4	250-410-600	- /	
<i>Otiophora rupicola</i> *	S	Flat	- +	+ ±	- -	- -	- -	15-20-30	140-160-180	300-450-600	400-575-700	- -	±	- ±	- ±	- ±	- ±	- ±	- 2-3	100-520-1100	+ /	
<i>Otomeria micrantha</i>	S	Flat	- +	+ ±	- -	- -	- -	15-22-30	210-240-280	450-680-1100	700-840-950	- -	- -	- -	- -	- -	- -	- +	- +	/ /	+ /	
<i>Pentania schweinfurtii</i> ¹	S	Decr	- +	+ +	- -	- -	- -	20-29-50	200-215-240	150-285-400	400-525-650	- -	- -	- +	+ -	- -	- -	- -	- 2-3	400-760-1850	+ /	
<i>Pentania schweinfurtii</i> ^{2*}	S	Decr	- +	+ +	- -	- -	- -	15-35-75	100-135-160	150-270-350	300-380-450	- -	- -	- +	+ -	- -	- -	- -	- 2-8	600-2060-4500	+ /	
<i>Pentas zanzibarica</i>	S	Flat	±	+ +	- -	- -	- -	15-30-50	140-180-185	200-390-600	600-725-800	- -	±	- ±	- ±	- ±	- ±	- ±	- 2-4	200-720-1600	+ /	
<i>Phyllopetas schimperiana</i> *	S	Decr	±	+ +	- -	- -	- -	20-30-50	90-105-120	250-450-650	550-680-800	- -	- -	- +	- +	- +	- +	- +	- 2-3	600-760-1500	+ /	
<i>Triainolepis polyneura</i>	P	Norm	±	- +	- +	- +	- +	25-65-130	25-32-38	300-490-650	800-1020-1200	- -	- -	- +	+ -	- -	- -	- -	- 2-3	200-400-600	- /	

Dif, diffuse; Dia, diffuse-in-aggregates; P, primarily woody; S, secondarily woody; Decr, decreasing length vs. age curve; Flat, flat length vs. age curve; Norm, normal length vs. age curve; +, present; ±, sometimes present; -, absent; / indicates not applicable.

fibres from wood splinters taken at various distances between the pith and cambium region – usually 1 mm between each splinter – were measured using maceration slides. In Table 2, the length of vessel elements and fibres were always based on the outer part of the wood sample.

Phylogenetic analysis

The phylogenetic analysis is based on an existing data matrix of *atpB-rbcL*, *rps16*, *petD* and *trnL-trnF* sequences, which includes most of the Spermaceae species studied (Groeninckx *et al.*, 2009a). The matrix was enlarged with own sequences of *Anthospermum* sp., *Coccocypselum decumbens*, *Colletecoema magna*, *Coprosma repens*, *Danais* sp., *Galium mollugo*, *Geophila repens*, *Mycettia malayana*, *Myrmecodia tuberosa*, *Ophiorrhiza mungos*, *Paederia foetida*, *Payera decaryi*, *Psychotria kirkii*, *Rubia fruticosa*, *Saldinia* sp., *Serissa japonica*, *Spermaceae bangweolensis*, *S. manikensis*, *Stelechantha makakana*, *Triainolepis polyneura* and *Trichostachys aurea*. Additional *rps16* and *trnL-trnF* sequences from GenBank were incorporated for *Dirichletia virgata* (accession numbers AM266894, AM266980), *Pentanisia schweinfurthii* (AM266861, AM266949), *Pentas zanzibarica* (AM266892, AM266978) and *Phyllopentas schimperiana* (AM266887, AM266973). No sequences were obtained for the woody Spermaceae *Hedyotis flavescens*, *Spermaceae macrocephala* and *S. occidentalis*, and the woody Knoxieae species *Otomeria micrantha* and *Otiophora rupicola*. The combined matrix includes 133 taxa, of which six basal Rubioideae were designated as the outgroup (*Coccocypselum decumbens*, *Colletecoema magna*, *Ophiorrhiza mungos*, *Saldinia* sp., *Stelechantha makakana* and *Trichostachys aurea*), eight Knoxieae species, 107 representatives of Spermaceae and 12 species from additional rubioid lineages. The dataset comprised 6045 characters, of which 1021 were parsimony informative.

Parsimony analyses were conducted with NONA (Goloboff, 1999) using WinClada ver. 1.0000 as interface (Nixon, 2002). A heuristic search was carried out with 1000 random addition replicates, tree-bisection and reconnection (TBR) branch-swapping holding ten trees per replicate, followed by TBR branch-swapping on all trees resulting from the 1000 replicates. In order to evaluate the relative support of the clades, jackknife (JS) and bootstrap (BS) analyses were executed using 1000 replicates with 100 initial trees holding one tree per random addition, performing TBR to hold 1000 trees and calculating a consensus on each repetition. Frequency values (>65%) were plotted onto the consensus of the most-parsimonious trees.

The evolution of woodiness was investigated by deltran optimization by adding four habit types [type 1 = (sub)shrubs (or occasionally treelets); type 2 = woody herbs to (sub)shrubs; type 3 = geoxylic herbs; type 4 = herbs] onto the strict consensus tree from the parsimony analysis using WinClada ver. 1.0000 (Nixon, 2002). We chose deltran optimization to overcome the problem of restricted sampling in several Rubioideae clades (such as, for example, Knoxieae and Psychotriaceae): the result was an optimization pattern that agreed with what was expected based on the much more

elaborate phylogenetic studies within these clades (Kårehed and Bremer, 2007; Razafimandimbison *et al.*, 2008).

RESULTS

Wood description

The Spermaceae studied are described according to the recent tribal delimitation of Groeninckx *et al.* (2009a) and Kårehed *et al.* (2008; Figs 2–4 and 6A–D). For each genus examined, the numerator presented in the text represents the number of species studied and the denominator includes the total number of species. Numbers without parentheses are ranges of means, while numbers between parentheses represent minimum or maximum values. Descriptions of continuous characters are based on mature wood samples. A summary of selected wood features is shown in Table 2. Because the number of mature wood samples within Knoxieae is rather limited, we decided not to provide a tribal wood description. As an alternative, we have summarized our anatomical observations of Knoxieae in Table 2, added with illustrations (Figs 5 and 6E, F). The species of Spermaceae that were studied and their descriptions are as follows.

Arcytophyllum 3/17, *Diodella* 1/ ~ 10, *Emmeorhiza* 1/1, *Gomphocalyx* 1/1, *Hedyotis* 4/ ~ 115, *Kadua* 2/28, *Lathraeocarpa* 1/2, *Mitracarpus* 1/ ~ 50, *Nesohedyotis* 1/1, *Oldenlandia* 2/ ~ 240, and *Spermaceae* 5/ ~ 275 (Figs 2–4 and 6A–D). Growth ring boundaries usually distinct. Diffuse-porous, but tendency to (semi-)ring-porosity in *Lathraeocarpa acicularis* (Fig. 2C), *Mitracarpus frigidus*, ‘*Oldenlandia*’ *ambovombensis*, ‘*Oldenlandia*’ *humbertii* and *Spermaceae verticillata*. Vessels (16)–20–540–(640) μm^{-2} , mostly solitary and in radial multiples of 2–3 (Fig. 2A, B, E), few additional vessel clusters in species of *Diodella* (Fig. 2D), *Lathraeocarpa* (Fig. 2C) and *Spermaceae*; vessel outline mostly slightly angular, sometimes rounded; perforation plates exclusively simple (Fig. 4D), sometimes a small percentage of scalariform perforations in *Hedyotis flavescens* (10%; 2–5 bars) and *H. fruticosa* (20%; 4–10 bars), or a high percentage of reticulate and irregular perforations in *Spermaceae bangweolensis* (>50%). Intervessel pits alternate (Fig. 4C), pits 3–5–(6) μm in horizontal diameter, vestured; additional (pseudo-)scalariform vestured intervessel pits also present in ‘*Hedyotis*’ *trichoglossa* (Fig. 4D), 10–15 μm in horizontal diameter. Vessel-ray pits similar to intervessel pits in shape and size. Wall sculpturing absent. Tyloses sometimes present in *Gomphocalyx*. Tangential diameter of vessels (10)–16–60–(120) μm , vessel elements (100)–160–690–(1000) μm long. Tracheids generally present, generally thin- or thin-to-thick-walled but thick-walled in *Lathraeocarpa* and *Oldenlandia*; pit borders generally distinctly bordered, (3)–4–5 μm in diameter concentrated in tangential and radial walls; tracheid length (150)–200–1050–(1300) μm . Few septate fibres with simple-to-minutely bordered pits (pit borders 2–3 μm in diameter and concentrated in radial walls) found in *Emmeorhiza*, *Hedyotis*, *Kadua*, *Mitracarpus* and *Spermaceae*; in ‘*Hedyotis*’ *trichoglossa* much more septate fibres are present (Fig. 4C, D). Axial parenchyma often scarce to apparently absent (Fig. 2B), usually a combination of diffuse or diffuse-in-aggregates apotracheal

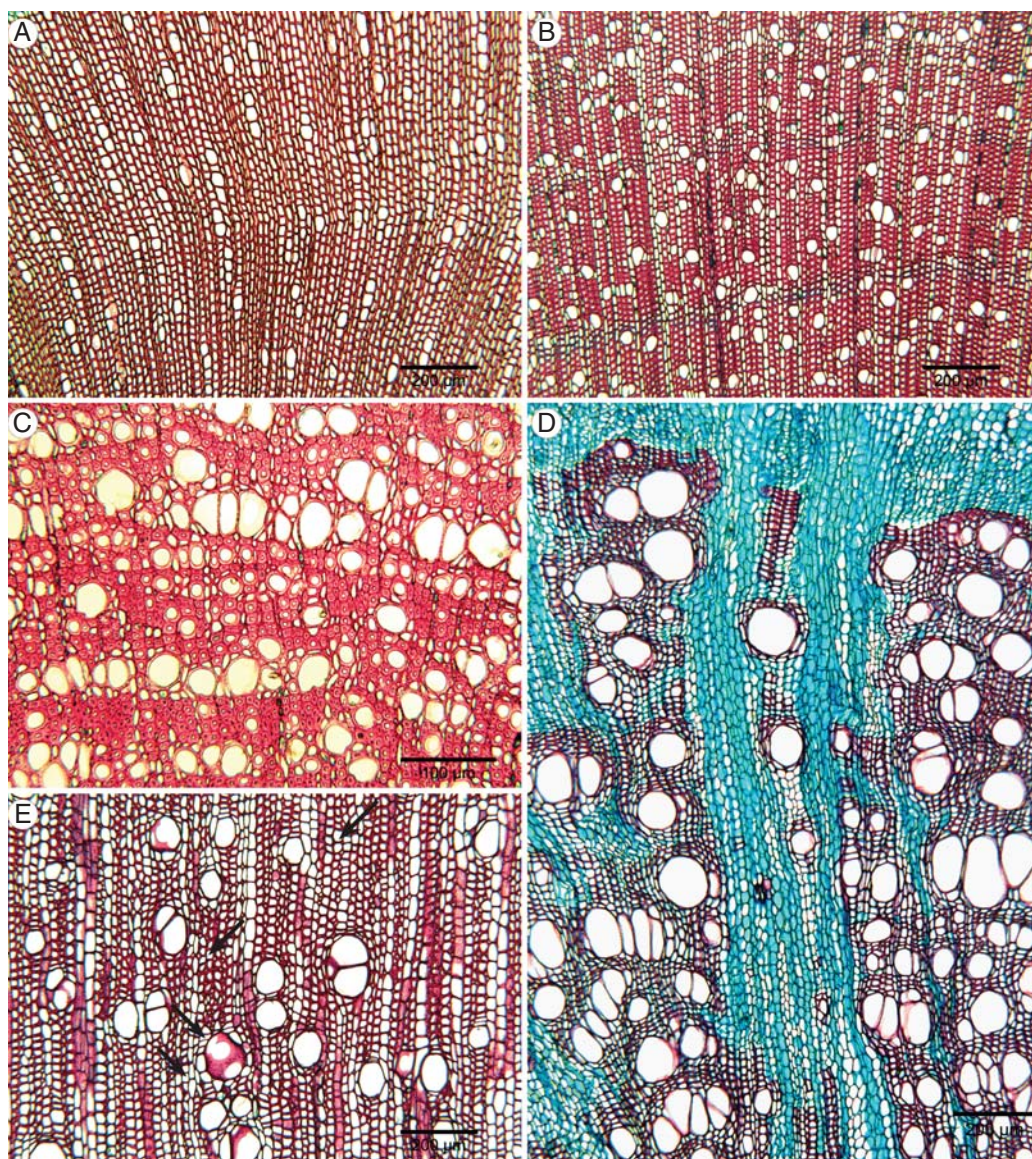


FIG. 2. Transverse light-microscope sections of woody Spermacoceae showing variation in vessel distribution, thickness of tracheids and scarcity of axial parenchyma. (A) *Arcytophyllum setosum*. (B) *Spermacoce macrocephala*. (C) *Lathraeocarpa acicularis*, thick-walled tracheids and vessels in the ground tissue. (D) *Diodella sarmentosa*, climbing species, wood cylinder partly broken up by large unligified rays. (E) *Nesoledyotis arborea*, larger mean vessel diameter and lower vessel density than in most other woody Spermacoceae, axial parenchyma more common (arrows).

parenchyma with scanty paratracheal parenchyma (Fig. 2C), parenchyma not observed in *Arcytophyllum setosum* (Fig. 2A), *Hedyotis lessertiana* and ‘*Hedyotis*’ *trichoglossa*; 2–3–(4) cells per parenchyma strand. Rays present, except in *Arcytophyllum setosum* (Fig. 4A). Exclusively uniseriate rays present in species of *Arcytophyllum*, *Emmeorhiza*, *Hedyotis*, *Kadua*, *Oldenlandia* and *Spermacoce* (Fig. 4B); 0–25 rays mm^{-1} , (50)–120–1950–(3300) μm long, consisting of upright cells (Fig. 4F). If present, multiseriate rays generally 2–3-seriate, 4–6-seriate in *Nesoledyotis arborea* and 2–14-seriate in *Diodella sarmentosa*, (150)–300–2130–(8000) μm high, (0)–2–5 rays mm^{-1} , consisting of mainly upright or square ray cells (Fig. 4F), but an even distribution of procumbent, square and upright ray cells in *Nesoledyotis arborea* (Fig. 4E); sheath cells present in *N. arborea*.

No dark amorphous contents in ray cells. No crystals or silica bodies observed.

Phylogenetic analysis

The phylogenetic analysis of the molecular data resulted in 8519 most-parsimonious trees with length 3412 (CI = 0.49; RI = 0.75). The strict consensus tree with length 3511 (CI = 0.47; RI = 0.74) is shown in Figure 7. The tribe Spermacoceae *s.l.* was well supported, but the sister relationship with Knoxieae was not found.

Within Spermacoceae *s.l.*, a polytomy representing four major lineages was present: (1) the herbaceous genera *Dentella*–*Pentodon*; (2) a predominantly herbaceous lineage comprising *Kohautia* and the *Pentanopsis* clade (including

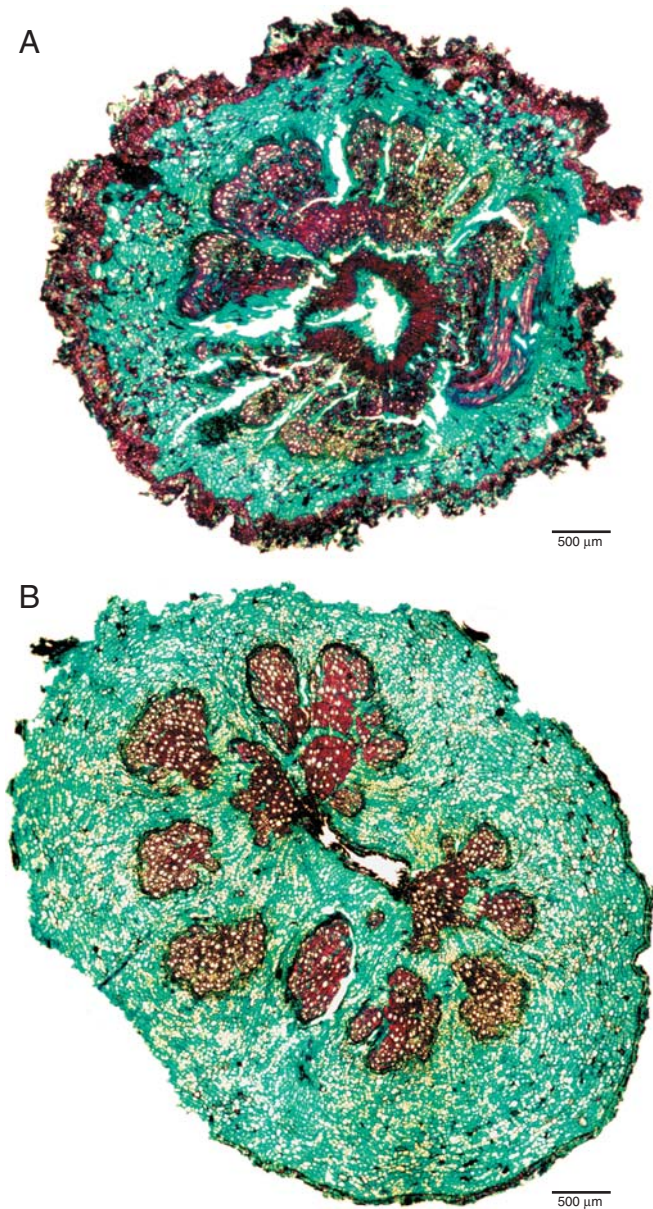


FIG. 3. Transverse light-microscope sections of the underground stem of *Spermaceae manikensis* showing an initial wood cylinder that is more dramatically dispersed by intense divisions of the surrounding parenchyma tissue with age. (A) Part of young stem, and (B) part of older stem.

the woody *Lathraeocarpa* and *Gomphocalyx*); (3) a lineage including herbaceous *Agathisanthemum* species that are sister to the woody herbs and (sub)shrubs of the *Hedyotis* s.s. clade (including the type species *H. fruticosa* and *H. lessertiana*); and (4) a large clade representing the remaining herbaceous and woody species of Spermaceae. Within this fourth clade, relationships were not fully resolved, but it is clear that the woody species are scattered over different clades, such as the *Kadua* clade, the *Arcytophyllum*–*Houstonia* clade and the *Spermaceae* s.s. clade. The relationships within Knoxieae cannot be discussed in detail because of the limited sampling. Nevertheless, some results of the Kärehed and Bremer (2007) study were also found in

our analysis: *Carphalea* sister to *Triainolepis*, and the relationship between the genera *Pentas*, *Batopedina* and *Pentanisia*.

DISCUSSION

Diversity of wood anatomy within Spermaceae

A typical feature in Spermaceae wood is the flat or decreasing length vs. age curve for vessel elements (Fig. 6A–D). Although we are aware that the maximal stem diameter in some of our samples might not be wide enough to present definitive length vs. age curves (e.g. Fig. 6B), we feel confident that the results are representative for the species. This is predominantly based on the ideas of Bailey (1920), who demonstrated that the vessel element length remains almost constant with age in species having so-called ‘derived’ vessel elements, i.e. very short vessel elements (<350 µm) combined with exclusively simple perforations. Furthermore, Carlquist (1962) demonstrated that nearly all secondarily woody species with very short vessel elements had a flat length vs. age curve. Based on the maceration slides observed, length vs. age curves for fibre-tracheids/tracheids are similar but show less variation in length than vessel elements. For example, average length values of fibre-tracheids/tracheids from the pith towards the cambium range from 560–495 µm in *Spermaceae verticillata* (cf. Fig. 6A), from 500–460 µm in ‘*Oldenlandia*’ *ambovombensis* (cf. Fig. 6C) and from 925–690 µm in *Mitracarpus frigidus* (cf. Fig. 6D).

Most Spermaceae species observed had a similar wood anatomy (Figs 2 and 4; Table 2). Besides the paedomorphic length vs. age curves, the tribe could also be identified by the following features: exclusively simple perforations, alternate and vested intervessel pits, mainly solitary vessels in combination with short radial multiples, narrow vessels (usually 20–40 µm in tangential width), high vessel densities (often more than 100 mm⁻²), relatively short vessel elements and imperforate tracheary elements with distinctly bordered pits (often between 150–500 µm and 350–800 µm, respectively), scarce apo- and paratracheal axial parenchyma, and a predominance of uniseriate rays including nearly exclusively square-to-upright cells. Furthermore, about half of the species studied had few septate libriform fibres, which most likely act as a rapidly increased photosynthetic storage capacity in woods with few (or nearly no) axial parenchyma cells (so-called tracheid dimorphism, *sensu* Carlquist, 1988). The evolution of fibre-tracheids towards septate libriform fibres was most pronounced in the underground wood sample of ‘*Hedyotis*’ *trichoglossa*, in which many tracheids in the ground tissue have been replaced during evolution by septate libriform fibres (Fig. 4C, D).

The variation in wood anatomy described above corresponds to the rubiaceaceous wood type I (Koek-Noorman, 1977; Jansen *et al.*, 2002), in which the type of imperforate element in the ground tissue is the main characterizing feature (I, tracheids/fibre-tracheids with distinctly bordered pits vs. II, libriform fibres with simple-to-minutely bordered pits). Furthermore, the fibre character in Rubiaceae was found to be highly correlated with other wood characters, such as vessel grouping (I, mainly solitary vs. II, mostly in short multiples), axial parenchyma distribution (I, diffuse,

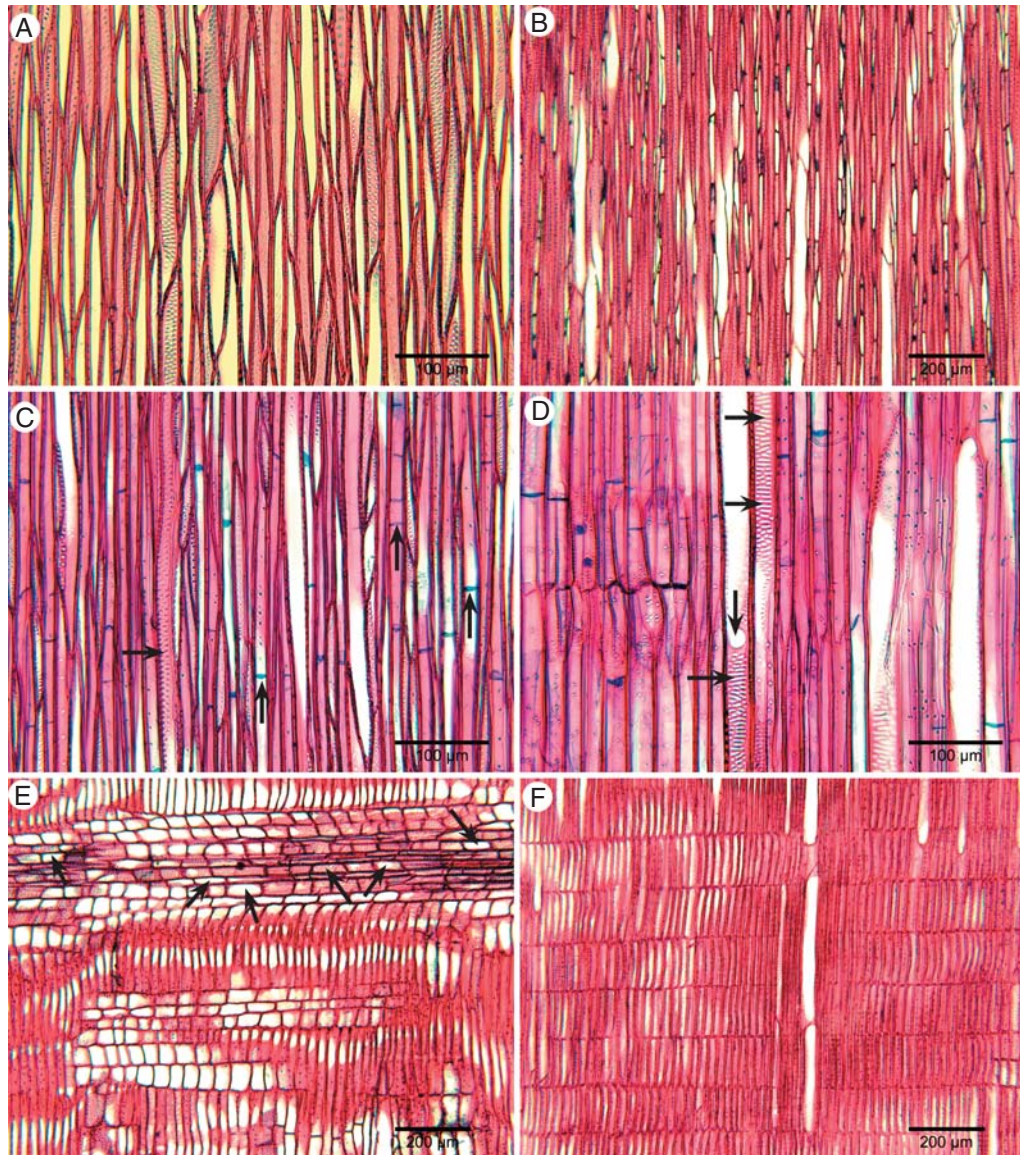


FIG. 4. Tangential and radial longitudinal light-microscope sections of woody Spermacoceae showing intervessel pits (C, D) and ray characters. (A–C) Tangential sections; (D–F) radial sections. (A) *Arcytophyllum setosum*, rayless wood. (B) *Spermacoce macrocephala*, abundance of uniseriate rays with upright cells. (C) *Hedyotis trichoglossa*, intervessel pits alternate (horizontal arrow), ground tissue mostly consisting of septate libriform fibres (vertical arrows), intervessel pitting alternate. (D) *Hedyotis trichoglossa*, simple perforation (vertical arrow), wide scalariform intervessel pitting (horizontal arrows). (E) *Nesoledyotis arborea*, upper ray with many procumbent body ray cells (arrows). (F) *Kadua cordata*, ray consisting of upright cells.

diffuse-in-aggregates or banded vs. II, absent or scanty paratracheal) and ray width (I, narrow with long upright uniseriate margins vs. II, wider rays with short uniseriate margins). With regard to Spermacoceae, the combination of type I fibres/tracheids with the axial parenchyma distribution (scarce apotracheal and paratracheal parenchyma) and ray structure (no difference between body and marginal ray cells) is unusual within Rubiaceae, and can be at least partly attributed to the secondary origin of the wood structure (see following sections, below).

Some Spermacoceae species deserve special attention because of their distinctive anatomy. The first one is *Diodella sarmentosa*, the only straggling-to-climbing species with mature wood in our study, which can be distinguished

by its wide and mainly unligified rays (2–14-seriate) that partly break up the wood cylinder (Fig. 2D). This division of the wood cylinder is much more prominent in the underground wood material of the geoxylic herb *Spermacoce manikensis* (Fig. 3). Although the wood cylinder is more-or-less intact in the youngest part of the underground structure (Fig. 3A), it dramatically breaks down into several small ‘lignified islands’ that float in a sea of dividing parenchyma cells (Fig. 3B; see divided xylem cylinder according to the cambial variant types of Carlquist, 2001). A similar, but less pronounced cambial variant is encountered in the underground structure of *S. occidentalis*, in which larger wood portions are starting to get dispersed by parenchyma divisions. The abundant presence of parenchymatous tissue in these two species

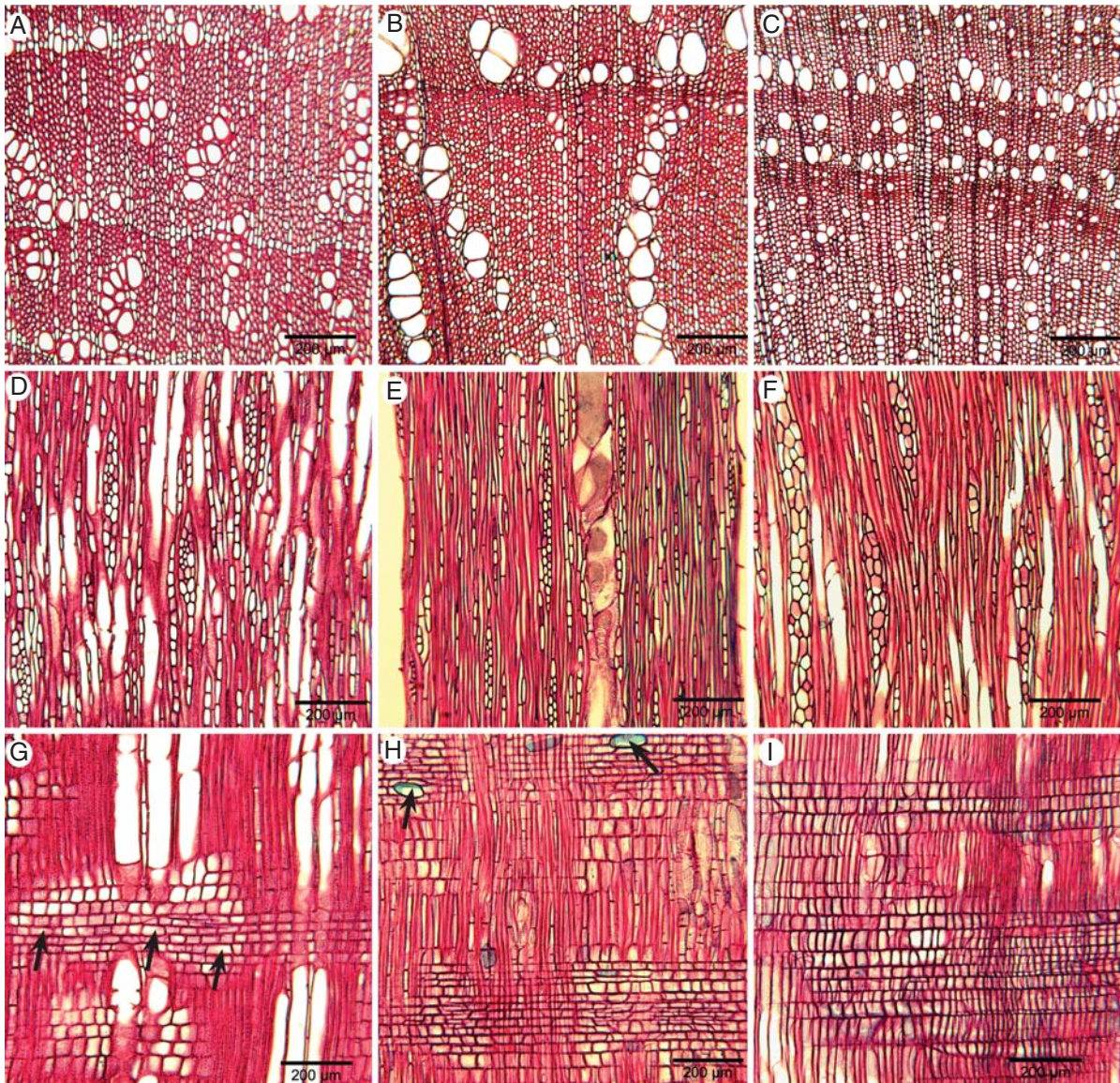


FIG. 5. Transverse (A–C), tangential (D–F) and radial (G–I) light-microscope sections of woody Knoxieae showing vessel distribution (A–C), ray width (D–F) and ray composition (G–I) of primarily woody representatives (A, B, D, E, G, H) and secondarily woody members (C, F, I). (A, D, G) *Dirichletia virgata*; (B, E, H) *Triainolepis polyneura*; (C, F, I) *Pentas zanzibarica*. (A) Tendency to dendritic vessel pattern; (B) pronounced radial vessel multiples ranging into dendritic pattern; (C) semi-ring porous wood, vessels mainly solitary and in short radial multiples; (D) body ray cells small and rounded; (E) body ray cells small and rounded; (F) rays consisting of mainly upright ray cells; (G) body ray cells procumbent (arrows); (H) body ray cells procumbent, enlarged ray cells containing raphides (arrows); and (I) multiseriata ray with mainly upright cells.

is probably related to an increased water-storage capacity in order to cope with long periods of drought (Table 1).

Variation in wood anatomy within Knoxieae

From a wood-anatomy point of view, the variation within Knoxieae is more complex than in Spermacoceae (Figs 5 and 6E, F). Primarily based on differences in the length vs. age curve and the cellular composition of rays, the anatomical diversity of wood in Knoxieae can be divided into two groups: (1) *Otomeria*, *Pentanisia*, *Pentas* (Fig. 5C, F, I), and probably *Otiophora* and *Phyllopentas*; and (2) *Carphealea*, *Dirichletia* (Fig. 5A, D, G) and *Triainolepis* (Fig. 5B, E, H). The first group of genera is nearly identical to the Spermacoceae

species studied: flat or decreasing length vs. age curves for vessel elements (Fig. 6F, and to a lesser extent also for fibre-tracheids/tracheids), exclusively simple perforations, alternate intervessel pits, mainly solitary vessels in combination with radial vessel multiples, narrow vessels (usually 20–50 μm in tangential width), high vessel densities (often $>100 \text{ mm}^{-2}$), relatively short vessel elements and imperforate tracheary elements with distinctly bordered pits (often between 200–600 μm and 450–800 μm , respectively), and a predominance of upright-to-square ray cells in uni- and multiseriata rays (Fig. 5F, I). More mature material of *Otiophora* and *Phyllopentas* needs to be carefully studied in order to investigate specific characters more in detail, such as length vs. age curves and cellular ray composition, before

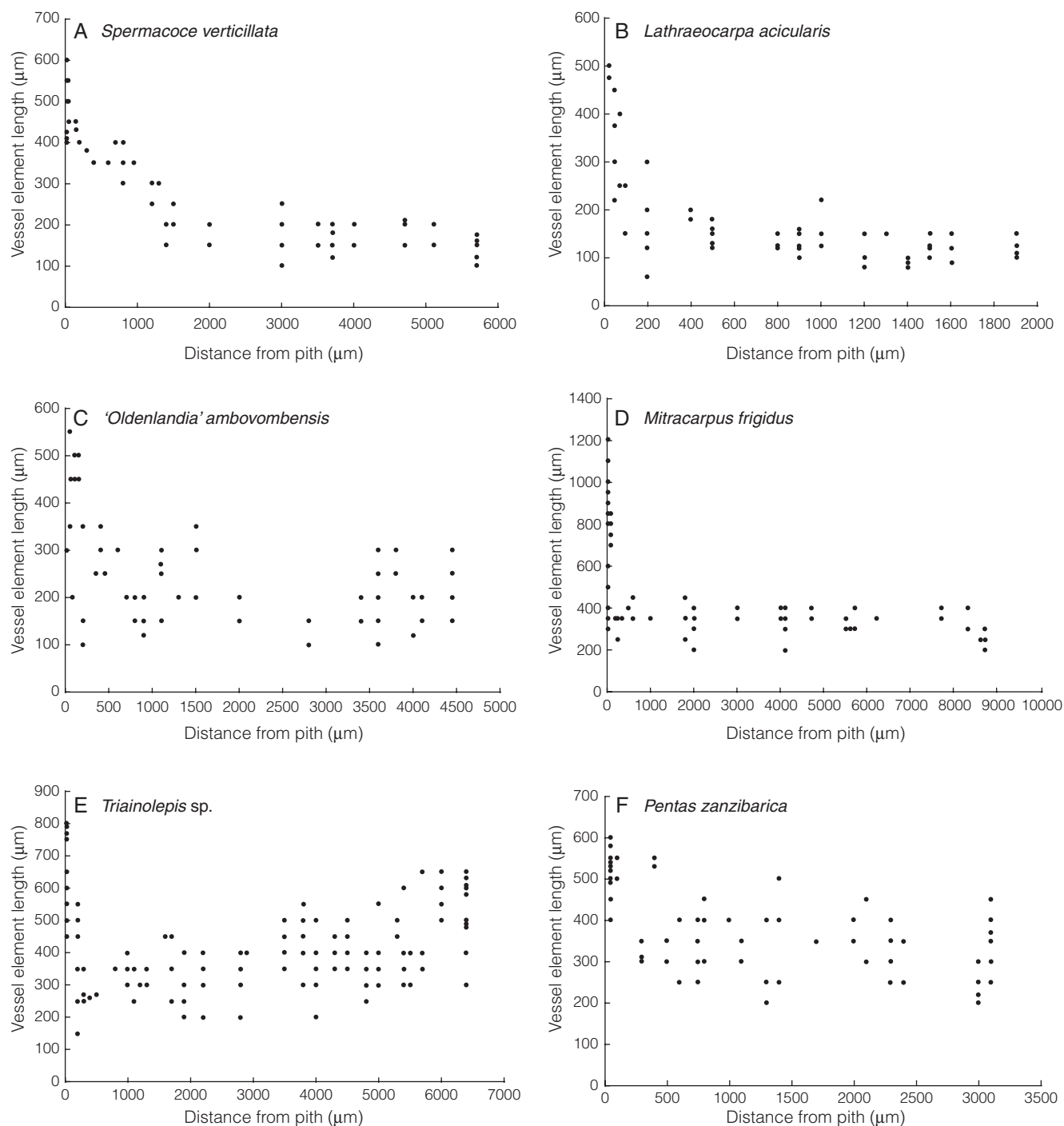


FIG. 6. Length vs. age curves for vessel elements in Spermacoceae (A–D) and Knoxieae (E–F). Vessel element length strongly decreases in the primary xylem and the first-formed wood (close to the pith), followed by a subsequent slight decrease (decreasing curve, A, B) or subsequent stabilization (flat curve, C, D, F) or slight increase (normal curve, E) towards the cambium. (A–D, F) Secondarily woody species; (E) primarily woody species.

we can more confidently place these two genera in the first group.

The second group of species can be distinguished from the first group by the presence of normal length vs. age curves (Fig. 6E) and the predominance of procumbent body ray cells (Fig. 5D, E, G, H). Among the three species of the

second group, *Dirichletia virgata* and *Triainolepis polyneura* are remarkable in having prominent radial vessel multiples and/or large vessel clusters (even a strong tendency to a dendritic vessel pattern in *D. virgata*; Fig. 5A), wider vessels (up to 130 µm in tangential width), and much longer fibres (800–1200 µm; Table 2). In addition, the juvenile sample of

Carphalea kiron-dron also showed a tendency to produce more pronounced vessel multiples and wider vessels. The difference in vessel width and fibre length between both groups can be related to differences in habit: species of the second group are all shrubs to trees, which are taller than the woody members of the first group (smaller shrubs to woody herbs or geoxylic herbs; Verdcourt, 1976). In addition, the pronounced vessel multiples in *Dirichletia* and *Triainolepis* provide support for their adaptation to relatively dry (semi-) deciduous forests (Carlquist, 1984; Table 1).

When the diversity of wood anatomy of Knoxieae is compared with the remaining Rubiaceae, it is evident that the anatomical characters of the three species of the second group (i.e. *Triainolepis polyneura*, *Dirichletia virgata* and *Carphalea kiron-dron*) fit better within the rubiaceous wood type I due to their narrow rays with long uniseriate ends (Fig. 5D, E). However, the pronounced vessel multiples, especially in *Triainolepis* and *Dirichletia* (Fig. 5A–B), strikingly contradict the general occurrence of solitary vessels in type I Rubiaceae (Jansen *et al.*, 2002).

Are Spermaceae and Knoxieae primarily woody or secondarily woody?

With respect to the wood anatomy of Spermaceae, the presence of flat or decreasing length vs. age curves for vessel elements (and to a lesser extent also fibre-tracheids/tracheids) in all the species observed (Fig. 6A–D) and the occurrence of mainly square-to-upright ray cells in nearly all the species observed [Fig. 4B–D, F; rays absent in *Arcytophyllum setosum* (Figs 2A and 4A); deviating ray cell morphology in *Nesohedyotis arborea*] provide strong arguments for secondary woodiness (Carlquist, 1962, 1992). Our study also provides new evidence that these typical paedomorphic features are also valid in underground woody organs of geoxylic herbs. In addition, the presence of wide scalariform intervessel pits in combination with the normal alternate vessel pits in '*Hedyotis trichoglossa*' provides further support for the general validity of paedomorphic features in underground woody structures (Fig. 4C, D). Nevertheless, there are some wood features that deviate from the typical paedomorphic wood pattern. For instance, rays with a significant percentage of procumbent body ray cells were observed throughout the large wood sample of the only arborescent member within the tribe Spermaceae that we investigated, *Nesohedyotis arborea* (trees up to 7 m tall). Apparently, the expanded ontogeny of wood cells in this species has changed many body ray cells into the matured procumbent shape (Fig. 4E). Nevertheless, according to the overview study of Carlquist (1962), it is the gradual decrease in vessel element length towards the outer stem parts that is the decisive factor to infer secondary woodiness in a given species [although the pith region was not included in our large sample (70 mm in diameter), we did measure, on average, a 150- μ m decrease in vessel element length between the youngest part and the cambium zone]. Other wood features in Spermaceae that are unusual in paedomorphic species were the low level of parenchymatization throughout the tribe (Fig. 2A–C; except in the underground structure of *Spermaceae manikensis*, Fig. 3) and the presence of thick-walled fibres/tracheids in the genera *Oldenlandia* and *Lathraeocarpa* (Carlquist 1962, Fig. 2C).

According to our observations of wood anatomy, the origin of woodiness in Knoxieae is more complex. All taxa observed of the first group (*Otiophora*, *Otomeria*, *Pentanisia*, *Pentas* and *Phyllopentas*) are hypothesized to be secondarily woody based on the presence of paedomorphic length vs. age curves for vessel elements (and to a lesser extent also fibre-tracheids/tracheids) and the predominance of square-to-upright ray cells (Figs 5C, F, I and 6F). In contrast, the three genera observed in the second group (*Carphalea*, *Dirichletia* and *Triainolepis*) can be distinguished from the first group by wood features that are typical of 'normal' primarily woody dicots: (1) length vs. age curves indicate a strong initial decrease of vessel element length in the primary xylem and in the first-formed wood, followed by an increase and subsequent stabilization (Fig. 6E), and (2) rays are composed of procumbent body ray cells and square-to-upright rows of marginal ray cells (Fig. 5D, E, G, H).

Figure 7 demonstrates that the general assumption of a primarily woody Rubioideae ancestor is plausible: early-diverging Rubioideae clades include many woody shrubs or (small) trees having non-paedomorphic rays with procumbent body ray cells and upright marginal ray cells, such as for example *Stelechantha* (Urophyllaeae; Jansen *et al.*, 2001), *Lasianthus* (Lasiantheae, represented by *Saldinia* and *Trichostachys* in Fig. 7; Jansen *et al.*, 2001) and *Psychotria* (Psychotrieae; Jansen *et al.*, 1997). Moreover, our study in Knoxieae reveals that at least some primarily woody species remain present in deeply nested Rubioideae lineages. With respect to Knoxieae, the molecular phylogeny of Kårehed and Bremer (2007) shows that our three primarily woody species are all placed in early-diverging branches within the tribe, which would indicate that the ancestor of Knoxieae was probably primarily woody (Fig. 7). This is further corroborated by the fact that all species of *Carphalea*, *Dirichletia* and *Triainolepis* are clearly woody (shrubs to small trees), although the earliest diverging Knoxieae lineage, *Chamaepentas*, consists of species that are considered to be woody herbs (Verdcourt, 1976). In contrast, all the secondarily woody species studied belong to later-diverging Knoxieae branches (Kårehed and Bremer, 2007), in which entirely herbaceous or herbaceous-like shrubby species are common (Verdcourt, 1976). Within Spermaceae *s.l.*, Fig. 7 demonstrates that woodiness has developed multiple times in all major essentially herbaceous lineages, and therefore current molecular evidence suggests that the ancestor of Spermaceae was herbaceous. As a result, woody members are best interpreted as secondarily woody.

Consequently, the present molecular phylogenies seem to support our wood anatomical hypothesis that points to primary as well as secondary woodiness in Knoxieae on the one hand, and to exclusively secondary woodiness within Spermaceae on the other. However, it should be kept in mind that several important nodes within the molecular phylogeny of Rubioideae as a whole and Spermaceae–Knoxieae in particular are not fully resolved.

Ecology of secondarily woody species

Why do some species that belong to essentially herbaceous clades develop a woody habit? First of all it should be noted that probably a majority of Spermaceae species are not

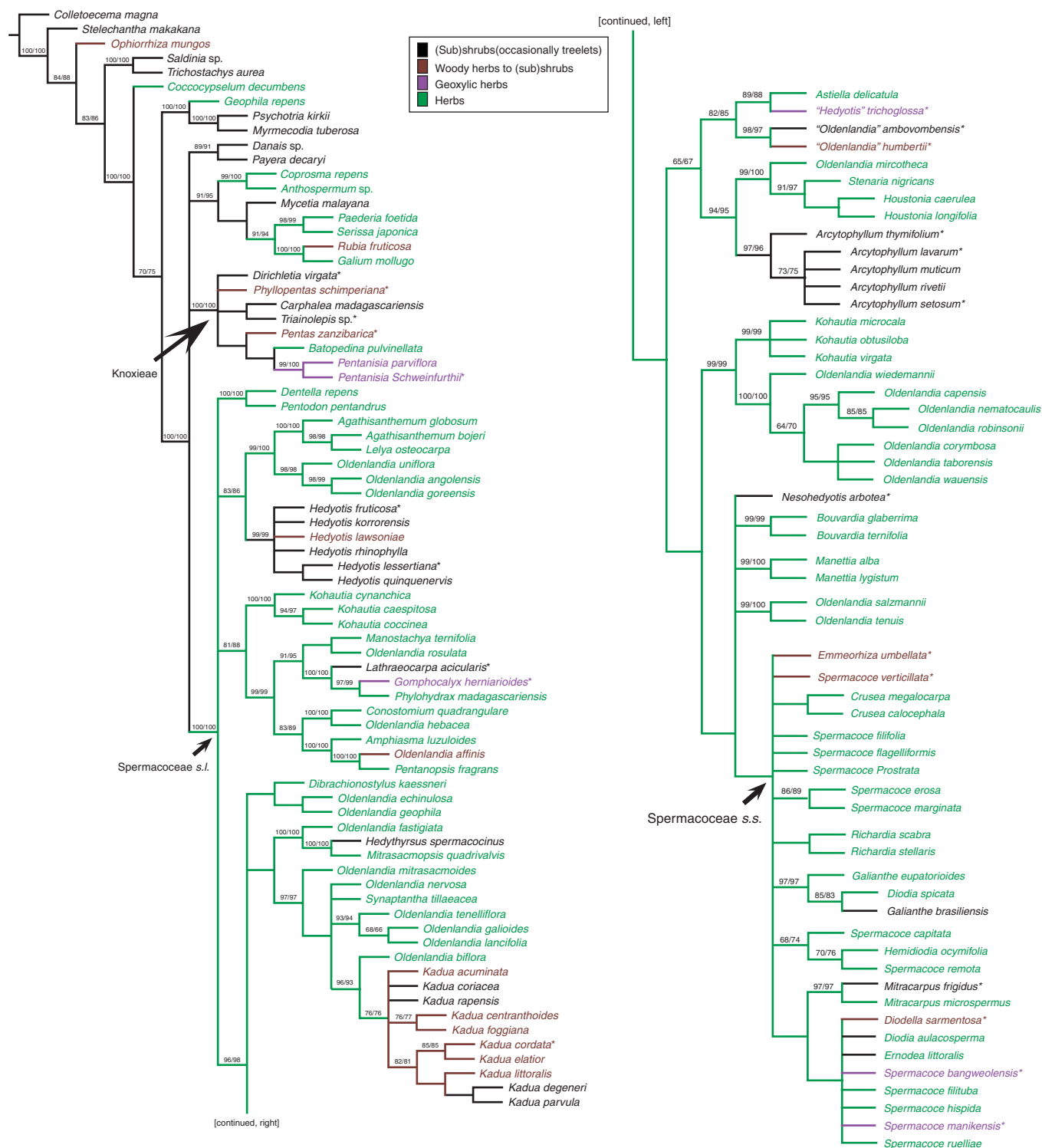


FIG. 7. Strict consensus tree based on maximum-parsimony analysis of *atpB-rbcL*, *rps16*, *petD* and *trnL-trnF* showing bootstrap/jackknife support values above the branches. The colour of the species' names indicates their habit: black = (sub)shrubs (or occasionally treelets); brown = woody herbs to (sub)shrubs; purple = geoxylic herbs; green = herbs. Asterisks indicate the species of woody Spermaceae and Knoxieae taxa that were investigated in this study. This tree supports our wood anatomical hypothesis: the common ancestor of Knoxieae is primarily woody, while the common ancestor of Spermaceae is herbaceous.

annuals but short-lived perennials. In addition, some essentially annual species become short-lived perennials when growth conditions (ideal temperature and water availability) allow the species to grow longer. The wood production in

most of these species, however, is limited. Geoxylic herbs, sometimes developing considerable amounts of secondary derived wood (Fig. 1A, detail), probably evolved to withstand longer periods of drought. This could, for example, be true for

Spermaceae manikensis growing on the nutrient poor Kalahari sands, which are inundated during the rainy season but are completely dry for the rest of the year. In this species there is also evidence that the massive amount of parenchyma tissue in the underground organs plays a role in water storage (Fig. 3B). Another evolutionary advantage of geoxyllic herbs is their ability to survive under periodic fire conditions, which has been clearly demonstrated for example in some *Otiophora* species (Knoxieae; Puff, 1981). Other secondarily woody Spermaceae–Knoxieae can be generally described as woody herbs, subshrubs or shrubs, and some grow in habitats that are dry for at least part of the year (Table 1); in particular, the woody Spermaceae growing in Madagascar undergo severe drought stress (Table 1). Consequently, it appears that – at least for some Spermaceae and Knoxieae species – recurring water stress goes hand-in-hand with wood production.

From the point of view of wood anatomy, there are some well-known features that can be correlated with dry habitats, such as exclusively simple vessel perforations, vested inter-vessel pits, high vessel densities (often more than 100 mm^{-2}), narrow vessels (often between 20–40 μm), short vessel elements (often below 350 μm , although this is also induced by the secondary woodiness), and the presence of water-conducting tracheids in the ground tissue that acts as a subsidiary water transport system in case the vessels become embolized (Carlquist, 1966, 1984; Baas *et al.*, 1983; Carlquist and Hoekman, 1985; Baas, 1986; Choat *et al.*, 2004). The species observed that have the most pronounced xeromorphic wood features were *Arcytophyllum thymifolium*, *Lathraeocarpa acicularis*, ‘*Oldenlandia*’ *ambovombensis* and ‘*Oldenlandia*’ *humbertii*. Furthermore, the latter three species were the only ones having thick-walled (narrow) vessels and thick-walled fibre-tracheids/tracheids in their ground tissue, which were linked in order to support against vessel implosion by negative pressures in the xylem during drought (Hacke *et al.*, 2001; Jacobsen *et al.*, 2005). Not surprisingly, these three species are found in the markedly dry south-western part of Madagascar, characterized by a short rain season and a long dry season (Jury, 2003; Table 1).

Although the link between drought resistance and wood production is also observed in other angiosperms, such as some additional Rubiaceae (*Rubia* and *Crucianella*; Koek-Noorman, 1976), Brassicaceae (Carlquist, 1971), and some Asteraceae (*Argyroxiphium* and *Dubautia*; Carlquist, 2003), it is certainly not the most typical habitat for secondarily woody species to occur. Indeed, Table 1 shows that most non-Malagasy Spermaceae/Knoxieae species studied have developed a secondarily woody habitat in areas that do not experience severe drought stress (cf. Carlquist, 1974). Examples are oceanic islands such as Hawaii (*Kadua cordata*) and St. Helena (*Nesohedyotis arborea*), and various high-elevation tropical montane areas (*Arcytophyllum*, *Hedyotis*). Likewise, when we look for a worldwide distribution of secondarily woody species throughout angiosperms, it is evident that environments with uniform annual temperatures and rainfall (for instance, on oceanic islands and tropical mountains) seem perfectly suitable for secondary woodiness to occur (Carlquist, 1974).

The degree of woodiness within most species studied (woody herbs or shrubs) appears to be related to the

temperature and amount of rainfall. For example, the particular environmental conditions on St. Helena (15–20°C mean annual temperature, approx. 1000 mm annual precipitation) favour the arborescent habit of *Nesohedyotis arborescens*, and might explain why this species is the only truly arborescent Spermaceae in our study. This aberrant habit type also accounts for the wider and fewer vessels of *N. arborescens* (Fig. 2E) compared to the other smaller woody species studied, although vessel element length is comparable (Table 2).

The numerous independent evolutionary shifts from herbaceousness to secondary woodiness in various flowering plant families and in diverse habitats must go hand-in-hand with a flexible genetic background mechanism that allows these frequent shifts. Preliminary evidence for this plastic genetic basis is provided by Melzer *et al.* (2008), who demonstrated that the inactivation of only two genes in the herbaceous *Arabidopsis thaliana* resulted in a perennial-like, shrubby mutant phenotype.

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APPENDIX

List of taxa investigated in this study with reference to their locality, vouchers and stem diameter. Nomenclature of species' names follow Govaerts *et al.* (2006) and Kårehed and Bremer (2007). Institutional wood collections used in this study are abbreviated according to Index Xylariorum (Stern, 1988). Species names in bold refer to underground wood material.

SPERMACEAE: *Arcytophyllum lavarum* K.Schum: Costa Rica, J. Jangoux 1394 (BR), 3 mm; *Arcytophyllum setosum* (Ruiz & Pav.) Schldtl.: Ecuador, P. M. Jørgensen *et al.* 2378 (BR), 6 mm; *Arcytophyllum thymifolium* (Ruiz & Pav.) Standl.: Ecuador, F. Billiet and B. Jadin 6580 (BR), 3 mm; *Diodella sarmentosa* (Sw.) Bacigalupo & E.L.Cabral ex Borhidi: Cameroon, S. Dessein & B. Sonké 1423 (BR), 8 mm; *Emmeorhiza umbellata* (Spreng.) K.Schum: origin and collector unknown (BR-S.P.802 367), 3.5 mm; *Gompholalixherniarioides* Baker: Madagascar, P. De Block 2217 (BR), 4 mm; *Hedyotis flavescens* Thwaites: Sri Lanka, Fosberg 58004 (BR), 5 mm; *Hedyotis fruticosa* L.: Sri Lanka, D. D. Tirvanganum 663 (BR), 4 mm; *Hedyotis lessertiana* Arn.: Sri Lanka, P. L. Comanor 959 (BR), 4 mm; '*Hedyotis*' *trichoglossa* Baker (unplaced taxon): Madagascar, H. Humbert 17722 (BR), 7 mm; *Kadua cordata* Cham. & Schldtl.: Hawaii Islands (USA), Stern, W.L. 2996 (Uw 18607), 7 mm; *Lathraeocarpa acicularis* Bremek.: Madagascar, P. De Block 2316 (BR), 6 mm; *Mitracarpus*

frigidus (Willd. ex Roem. & Schult.) K.Schum.: National Botanic Garden of Belgium, F. Van Caekenberghe 2 (BR living collection), 19 mm; *Nesohedyotis arborea* (Roxb.) Bremek.: UK (St. Helena), J. C. Melliss s.n. (Kw 11160), mature (about 70 mm); '*Oldenlandia*' *ambovombensis* sp. nov. ined.: Madagascar, P. De Block 2328 (BR), 4 mm, 11 mm; '*Oldenlandia*' *humbertii* sp. nov. ined.: Madagascar, P. De Block 2294 (BR), 3 mm; *Spermacoce bangweolensis* (R.E.Fr.) Verdc.: Zambia, Dessein *et al.* 550 (BR), 20 mm; *Spermacoce manikensis* Dessein: Zambia, Dessein *et al.* 976 (BR), 16 mm; *Spermacoce macrocephala* (Standl. & Steyerl.) Govaerts: Venezuela, B. Maguire *et al.* 41668 (Tw 36238), 7 mm; *Spermacoce occidentalis* Hardwood: Australia, Harwood 1541 (BR), 10 mm; *Spermacoce verticillata* L.: South America, Cavalcanti *et al.* 358 (Uw 33690), 10 mm; *Spermacoce verticillata* L.: Cultivated in the

National Botanic Garden of Belgium, F. Van Caekenberghe 104 (BR), 11 mm;

KNOXIEAE: *Carphalea kirondrion* Baill. subsp. *gayi* (Homolle) Puff: Madagascar, A. M. Homolle 1415 (BR), 6 mm; *Dirichletia virgata* (Balf.f.) Kårehed & B.Bremer: Yemen (Socotra Archipelago), Schweinfurth 43 (Uw 25955), mature; *Otiophorarupicola* Verdc., Burundi, M. Reekmans 178 (BR), 7 mm; *Otomeria micrantha* K.Schum.: DR Congo, J. Louis 3389 (BR), 5 mm; *Pentania schweinfurthii* Hiern: DR Congo, G. F. de Witte 07197 (BR), 7 mm; *Pentania schweinfurthii* Hiern: DR Congo, G. F. de Witte 02767 (BR), 5 mm; *Pentas zanzibarica* Vatke var. *rubra* Verdc.: DR Congo, Lebrun 3691 (BR), 8 mm; *Phyllopentas schimperiana* (Vatke) Kårehed & B.Bremer: Ethiopia, C. Puff 810920–1/2 (Uw 27081), 8 mm; *Triainolepis polyneura* Bremek.: Madagascar, P. De Block 593 (BR), 16 mm.