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Author(s): Santiago Trueba, Nick P. Rowe, Christoph Neinhuis, Stefan Wanke, Sarah T. Wagner, Sandrine Isnard

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STEM ANATOMY AND THE EVOLUTION OF WOODINESS IN PIPERALES

Santiago Trueba,^{1,*}† Nick P. Rowe,‡ Christoph Neinhuis,† Stefan Wanke,† Sarah T. Wagner,† and Sandrine Isnard*†

*Institut de Recherche pour le Développement, Unité Mixte de Recherche, Botanique et Bioinformatique de l'Architecture des Plantes, Laboratoire de Botanique et d'Écologie Végétale Appliquées, BPA5, 98800 Noumea, New Caledonia; †Institut für Botanik, Technische Universität Dresden, D-01062 Dresden, Germany; and ‡Université Montpellier 2, Unité Mixte de Recherche, Botanique et Bioinformatique de l'Architecture des Plantes, Montpellier, France, and Centre National de la Recherche Scientifique, Unité Mixte de Recherche, Botanique et Bioinformatique de l'Architecture des Plantes, Montpellier, France

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Premise of research. Piperales displays a wide diversity of growth forms that appears to be linked with differences in cambial activity and subsequent derived wood production. To date, no overall synthesis of the evolution of woodiness in Piperales has been done and few studies have proposed an ancestral habit (woody/herbaceous). We provide anatomical data of all lineages within Piperales and reconstruct ancestral character states, focusing on the origin of woodiness within the order and on the ecological significance of key anatomical features.

Methodology. Stem anatomical observations with special emphasis on wood anatomical features were performed on 28 species of the Piperales; by combining previously published studies with original data, we conducted phylogenetic reconstructions of cambial activity and vessel element perforation plates to assess the origin of woodiness and vessel evolution in Piperales.

Pivotal results. Different patterns of cambial activity are observed in Piperales, from active secondary growth in both intra- and interfascicular areas in *Aristolochia*, *Thottea* (Aristolochiaceae), *Saruma* (Asaraceae), *Manekia*, and *Piper* (Piperaceae) to cambial activity mainly restricted to fascicular areas in Saururaceae and a complete lack of secondary growth in *Verhuellia*. Vessels in Piperaceae, Aristolochiaceae, and Asaraceae present simple perforation plates, while those of Saururaceae are mostly scalariform. A stem endodermis bearing a Casparian band—an atypical feature in aerial stems—is reported for all genera within the Piperaceae and for *Saururus* and *Houttuynia* in the Saururaceae.

Conclusions. The common ancestor of the order likely had an active cambium and woody habit, including vessel elements with simple perforation plates. All woody species share several wood features, including wide and tall rays, suggesting a single origin of wood in the order. The high diversity of growth forms observed in Piperales is linked to frequent shifts in cambial activity and changes in habit-related features within the different lineages.

Keywords: Piperales, protracted juvenilism, stem endodermis, vessel element perforation plates, wood evolution, woodiness.

Online enhancements: supplementary tables.

Introduction

Variations in the degree of cambial activity and the subsequent production of secondary tissues are key elements behind the diversity of forms that we observe in extant angiosperms (Rowe and Speck 2005; Carlquist 2009; Spicer and Groover 2010). Flowering plants present a broad array of growth forms ranging from massive trees with a high produc-

tion of wood; woody herbs with a limited secondary growth production, frequently localized at the base of the stems; herbs with a very reduced cambial activity limited to the fascicular areas; and genuine herbs that present a complete loss of the cambial activity with stems that are exclusively formed by primary tissues (Spicer and Groover 2010; Lens et al. 2012a; Rowe and Paul-Victor 2012). This diversity in the degrees of woodiness has arguably enabled flowering plants to explore new and diverse ecological strategies (Rowe and Speck 2005; Rowe and Paul-Victor 2012; Carlquist 2013).

The transitions from woody to herbaceous growth forms and vice versa seem to have occurred many times within the different lineages of angiosperms, leading to complex patterns

¹ Author for correspondence; e-mail: santiago.trueba-sanchez@ird.fr.

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of woodiness across the flowering plant phylogeny. An increasing number of studies demonstrate that secondary growth can be highly variable and represents a labile process in plants (Kim et al. 2004; Dulin and Kirchoff 2010; Lens et al. 2012a, 2013). Distributions of stem anatomical traits within phylogenetic hypotheses can provide meaningful insights about the evolution of plant's anatomical features (Olson et al. 2003; Lens et al. 2009; Pace et al. 2009; Wagner et al. 2012). Moreover, we are gaining knowledge about the distribution of woodiness among angiosperms, and the genetic mechanisms behind the secondary growth are being progressively untangled (Oh et al. 2003; Ko et al. 2004; Groover 2005; Melzer et al. 2008; Spicer and Groover 2010; Lens et al. 2012b). In this context, the study of wood structure and the variations in the degrees of woodiness within the main angiosperm groups is of great importance in understanding how these modifications have occurred and influenced the evolution of plant forms.

Current phylogenetic hypotheses strongly support the grade containing Amborellales, Nymphaeales, and Austrobaileyales as the first successive lineages of angiosperms (Zanis et al. 2002; Qiu et al. 2005; Soltis et al. 2008; Moore et al. 2010; Amborella Genome Project 2013), followed by the Chloranthaceae and the magnoliids (Laurales, Magnoliales, Canellales, and Piperales). This topology is suggested to support the idea that early-diverging lineages of angiosperms possessed an active bifacial cambium (Carlquist and Schneider 2001; Feild and Arens 2005; Spicer and Groover 2010). Because of its phylogenetic position and its diversity of growth forms, Piperales has been considered as a key lineage for understanding the early diversification of angiosperms (Carlquist 2009; Isnard et al. 2012).

Piperales is a species-rich clade with approximately 4300 extant species; it includes a wide spectrum of growth habits including herbs, shrubs, treelets, and climbers (fig. 1) living in both terrestrial and semiaquatic environments. A broad survey of growth forms, architecture, anatomy, and biomechanics within Piperales has recently demonstrated that the frequent shift in growth forms is probably a major source of diversity within the group (Isnard et al. 2012). The patterns of growth form evolution within Piperales potentially reflect some overall changes within angiosperms, especially related to transitions in woodiness, diversity of mechanical organizations, and shifts in architectural development.

Among the several works that have treated the anatomy of Piperales, Sherwin Carlquist's studies are key references for describing the anatomy of Aristolochiaceae (Carlquist 1993), *Lactoris* (Carlquist 1990), and Saururaceae (Carlquist et al. 1995; Schneider and Carlquist 2001). Substantial amounts of information on Piperaceae anatomy can be found in the literature since the nineteenth century (Schmitz 1871; Debray 1885; Hoffstadt 1916; Yuncker and Gray 1934; Murty 1959; Ravindran and Remashree 1998; Souza et al. 2004). Datta and Dasgupta (1977) published the only study attempting to outline the anatomy of the entire Piperales order, but this work included only three genera out of 16 and merely considered the general distribution of tissues. More recently, the stem anatomy of the perianth-bearing Piperales has been investigated with a special focus on *Aristolochia* (Wagner et al. 2012, 2014). In addition, Isnard et al. (2012) briefly explored

the stem anatomy of Piperales but did not describe the wood anatomy and the differences in cambial activity within the order.

Woodiness in Piperales has long been argued to be secondarily derived based on observations of wood anatomical features pointing to protracted juvenilism (Carlquist 1993; Carlquist et al. 1995; Spicer and Groover 2010). Protracted juvenilism (or wood paedomorphosis sensu Carlquist 1962) is a form of heterochrony where features of primary xylem are observed in the secondary xylem, which consequently maintains a juvenile appearance (Carlquist 1962, 2009; Dulin and Kirchoff 2010). The presence of protracted juvenilism in wood has been proposed as indicative of secondary woodiness, where woody species are derived from an herbaceous ancestor (Carlquist 2009, 2012). This association results from the frequent observation of protracted juvenilistic features in insular woody species, which are supposed to be derived from an herbaceous ancestor (i.e., secondary woody species). Because protracted juvenilism cannot be strictly associated with secondary woodiness (Lens et al. 2013), the origin of woodiness should be evaluated through comparative anatomy in a phylogenetic context.

The phylogenetic relationships within Piperales have been progressively resolved over recent years, and this provides a well-founded basis from family to generic levels to be used for ancestral character state reconstructions (Jaramillo and Manos 2001; Nickrent et al. 2002; Jaramillo et al. 2004; Neinhuis et al. 2005; Ohi-Toma et al. 2006; Wanke et al. 2006, 2007a, 2007b; Samain et al. 2009; Naumann et al. 2013). This provides the opportunity to explore the variations in cambial activity and wood anatomy in this large order and to reconstruct the putative ancestral cambial activity and wood features of Piperales.

We combine available data from literature with original anatomical descriptions of Piperales representatives, including some poorly known taxa such as *Manekia*, *Verhuellia*, and *Zippelia* (Piperaceae), whose phylogenetic positions have been only recently resolved (Wanke et al. 2007a, 2007b). The main objectives of this study are to (1) compare the cambial activities and anatomical features between all main lineages and (2) address the origin of woodiness in Piperales using ancestral character state reconstructions over a recent molecular phylogenetic hypothesis.

Material and Methods

Plants

Piperales comprises two well-supported clades that can be named perianthless and perianth bearing (Naumann et al. 2013). The first clade consists of Piperaceae (*Piper*, *Peperomia*, *Manekia*, *Zippelia*, and *Verhuellia*) and Saururaceae (*Anemopsis*, *Gymnotheca*, *Houttuynia*, and *Saururus*). The second clade includes Aristolochiaceae (*Aristolochia* and *Thottea*), Asaraceae (*Asarum* and *Saruma*), Lactoridaceae (*Lactoris*), and Hydnoraceae (*Hydnora* and *Prosopanche*). We adopted the most recent molecular phylogenetic hypothesis where all Piperales families are statistically supported as monophyletic (Naumann et al. 2013). Stem samples of 28 species belonging to 11 genera were collected during fieldwork in China, Colombia, India, Mexico, and New Caledonia and from the living



Fig. 1 Illustration of the diversity of growth forms in Piperales. *A*, *Piper hispidinervum* (Piperaceae), treelet with significant wood production, growing in the living collection of the Xishuangbanna Tropical Botanical Garden, China. *B*, *Piper flaviflorum* (Piperaceae), overview of this woody liana showing a thick stem, growing in Yunnan, China. *C*, *Saruma henryi* (Asaraceae), rhizomatous woody herb producing a limited amount of wood restricted to the base of the stem, growing in the greenhouse of the Botanical Garden of Dresden, Germany. *D*, *Thottea iddukiana* (Aristolochiaceae), subshrub presenting several stems with a slight production of wood, growing in Kerala, India. *E*, *Peperomia blanda* (Piperaceae), a strictly herbaceous and small-sized plant, growing on a rocky hillside in Yunnan, China.

collections of the Botanical Gardens of Bonn and Dresden in Germany and of Kerala in India (see app. A for the species list and collection sites). Two to five samples per species were collected on basal portions of the stems corresponding to the main axis of mature plants. This allowed us to ensure that the analysis was done on fully developed stems, since wood development can be restricted to the most basal parts of stems. Plant maturity was assessed through the observation of sexual maturity (flowering and fruiting) or through architectural features as the presence of reiterations and the full development of branches on large and unpruned individuals. After collection, all samples were preserved in 70% ethanol.

Anatomical Descriptions and Microtechnique

Anatomical sections were carried out on all three different planes (transverse, radial, and tangential) for all of the sampled species. Laboratory work was carried out in the Mixed

Research Unit, Botanique et Bioinformatique de l'Architecture des Plantes (UMR AMAP), in Montpellier, France. Before sectioning, samples were immersed for 5 min in a histological clearing agent (Histo-Clear; National Diagnostics, Atlanta, GA). Histological sections were cut using a vibratome (Thermo Scientific Microm HM 650V); the more resistant samples and stems with diameters greater than 2 cm were sectioned using a sliding microtome. Hand-sectioning using a razor blade was also carried out for some samples. In the case of *Zippelia begoniifolia*, we followed the method proposed by Barbosa et al. (2010), using a polystyrene foam solution to reinforce the section. The latter method as well as embedding in agar 6% were used for *Verhuellia lunaria* because of the small diameter and softness of its stems. Stem sections were stained with 0.1% aqueous toluidine blue O for 5–10 min. Once stained, sections were mounted on microscope slides using one of the following mounting media: Eukitt (Kindler, Freiburg, Germany) or Isomount (Labonord, Templemars, France).

Wood macerations were used for observations of vessel element morphologies. Pieces of the outer and most recently produced layers of wood were dissected using a double-edged razor blade. Strips of wood were then chemically macerated by immersion in capped 2-mL vials containing a maceration solution (1:1 by volume, 35% H₂O₂:glacial acetic acid) and placed in an oven at 60°C for 72 h until the wood became translucent. Wood macerates were then stained by immersion in 0.1% aqueous toluidine blue O for 10 min; wood macerates were then shaken in order to loosen vessel elements, filtered, and immersed in distilled water. After settling, a drop of the colored wood macerates was pipetted onto a slide, and digital images of macerated xylem vessel elements were carried out immediately after mounting. Anatomical observations were carried out using a binocular stereo microscope (Olympus SZX9, Tokyo, Japan) and an optical LM (Olympus BX51). The 21 wood anatomical characters used in the study follow the International Association of Wood Anatomists terminology for microscopic features (Wheeler et al. 1989). Twelve additional characters of stem anatomy were used (see table 1 for the complete list of characters). IAWA wood features could not be attributable for species producing a limited amount of wood; vessel element descriptions were based on the metaxylem tracheary elements for these species.

Literature Review

Our sampling includes representatives of all Piperales genera with the exception of *Lactoris*, *Anemopsis*, *Asarum*, and the Hydnoraceae family, for which anatomical data were compiled from literature (see table B1, available online, for review of anatomical features of Piperales species reported in previous works). We also completed our anatomical observations of species-rich genera through an extensive literature survey; this allowed us to cover a wide proportion of infrageneric diversity and to increase the number of species for large genera (see table B2, available online, for a complete list of the proportion of species and infrageneric clades). A high representativeness was obtained for poorly diversified genera. Species representation clearly falls to low percentages in very large genera such as *Aristolochia*, *Peperomia*, and *Piper*, for which it would be nearly impossible to sample all species. Our work, however, includes representatives from most of the major infrageneric clades (table B2).

Data Processing and Character Mapping

We adopted the topology of Piperales from a recent molecular phylogenetic analysis where the internal nodes of the order are well supported (Naumann et al. 2013). The topology at the generic level was obtained from previous phylogenetic studies where the relationships were statistically supported (Wanke et al. 2007a, 2007b). We scored character states at the generic level, and ancestral character state reconstructions were carried out using maximum parsimony (MP) and maximum likelihood (ML) methods using the StochChar module (Maddison and Maddison 2006) of Mesquite 2.7.5 (Maddison and Maddison 2011). ML reconstructions estimate the uncertainty of ancestral state reconstructions and help to quantify the inferred ambiguities (Pagel 1999). We employed ML recon-

structions using a Markov k -state one-parameter model of evolution, assuming one step per change with the cost of gains equal to the cost of losses of a given state for each character; this is designed to estimate transition rates of discrete characters over a phylogeny (Pagel 1994; Jaramillo et al. 2004). The likelihood of each character state is shown in the pie chart at each single node of the tree. ML reconstruction methods do not, however, allow polymorphic states; MP was consequently used to code polymorphisms when two potential character states were known to occur in terminal taxa. We employed an unordered parsimony model with equal gain/loss costs assuming one step per change. We chose *Canella winterana* from the Piperales sister order Canellales (Qiu et al. 2005; APG III 2009; Massoni et al. 2014) as outgroup. The anatomical description of the outgroup was obtained from Feild et al. (2002) and from the InsideWood database (InsideWood 2004–).

Cambial Activity Characterization and Character States Description

Distinguishing the limits between herbaceous and woody species is especially difficult when dealing with intermediate expressions of cambial activity leading to different degrees of wood production as occurring in Piperales (fig. 1; Lens et al. 2012a, 2013; Rowe and Paul-Victor 2012). We used observations of cambial activity location and assessments of wood productivity in order to classify the degrees of woodiness in Piperales. We established five possible states of cambial activity that correspond to herbaceous and woody conditions following the next criteria: (1) absent = no cambial activity; (2) restricted to fascicular areas = cambial activity exclusively located at the intrafascicular areas; (3) woody herb = cambial activity in both intra- and interfascicular areas, producing a complete wood cylinder of determinate thickness (less than 20 cell layers) and restricted to the base of the main stem; (4) slightly woody = cambial activity in both intra- and interfascicular areas, producing a complete wood cylinder of determinate thickness (less than 20 cell layers) extended along the main stem; (5) truly woody = cambial activity in both intra- and interfascicular areas, forming a complete wood cylinder with a significant and indeterminate production of wood (more than 20 cell layers).

The threshold of 20 cell layers of secondary tissues produced by cambium was established after observing that small-sized species of Piperales corresponding to poorly lignified lianas and subshrub forms had a limited and determinate wood production that never exceeded ca. 20 cell layers. On the other hand, all of the observed species exceeding 20 cell layers corresponded to small trees, shrubs, or lianas with a high and indeterminate production of wood. During literature survey, if the location and productivity of cambial activity were not explicitly reported, we used the descriptions of growth habit to code character states. For instance, *Anemopsis* and *Asarum* were coded as woody herbs according to our literature review (table B1). Secondary xylem in *Asarum canadense* and *Asarum cardiophyllum* have been declared as forming a wood cylinder restricted to the base of the aerial stem (Wagner et al. 2014), thus corresponding to the woody herb state. Carlquist et al. (1995) reported that secondary growth is obvious in *Anemopsis californica* in both fascicular and interfascicular areas; as

secondary growth of this species is exclusively located to the rhizomes, it can be coded as a woody herb.

MP character optimization in cambial activity reconstruction allowed us to code two possible states for a given genus. This alternative coding concerned *Aristolochia*, *Thottea*, *Peperomia*, and *Piper*, where various degrees of cambial activity might occur. Coding of *Aristolochia* was supported by a recent evolutionary analysis on the growth forms of the genus (Wagner et al. 2014), proposing a climbing or a shrub ancestral habit for *Aristolochia* and rejecting the possibility of an herbaceous ancestry. In terms of cambial activity, these growth forms correspond to truly woody (shrub and liana) or slightly woody (vine) states. *Piper* and *Thottea* were alternatively coded as truly woody or slightly woody, corresponding to our own observations (table 1) and literature survey (table B1). Despite a low specific representativeness, our work includes species of all major clades and growth forms within these genera (table B2), providing a good level of confidence. Cambial activity in *Peperomia* was coded as absent or restricted to fascicular areas, as our literature survey and our anatomical observations suggested both possibilities in this large genus for which anatomy is known to be homogeneous. Finally, we performed ML ancestral character state reconstruction on vessel element perforation plates, a feature of major interest in the understanding of the evolution of wood structure. Perforation plates were coded with two possible states (simple or scalariform); *Verhuellia*'s perforation plates were coded as inapplicable because this species lacks metaxylem vessel elements.

Results

Tissue Distribution and Cambial Activity

Saururaceae. All species present a cambial activity restricted to the fascicular areas and producing only a few cell layers. Vascular bundles are organized in a single ring. Tangential alignments of cells are exclusively restricted to the vascular bundles, and the fascicular cambium produces a negligible amount of secondary tissues (fig. 2A, 2B). Interfascicular cambium is never active (no radial or tangential divisions were observed; fig. 2A, 2B). Aerenchyma is present in both the medullar and the cortical areas of *Saururus chinensis* (fig. 2A, stars), while in *Gymnotheca chinensis* it was exclusively observed in the cortical area (fig. 2B, star); aerenchyma is absent in *Houttuynia cordata*. In *G. chinensis* and *H. cordata*, a complete ring of sclerenchyma fibers is present outside the vascular bundles with a width of one to three cell layers (fig. 2B). A few fibers are present at the adaxial and abaxial surfaces of the bundles in *S. chinensis* but do not form a complete ring (fig. 2A).

Aristolochiaceae. In *Aristolochia* and *Thottea*, many species can develop a large amount of secondary xylem forming large wood cylinders (fig. 2C, 2D). Despite active secondary growth, the fascicular cambium rarely produces rays, and the interfascicular cambium never produces fusiform initials. Vessel elements and fibers are consequently absent in the interfascicular area, and we observe exclusively wide multiserial rays composed of secondary parenchyma cells (fig. 2C, 2D). Ray cells are lignified in all *Thottea* species studied (fig. 2C; table 1); ray lignification was variable between *Aristolochia* species (table 1). Growth rings were observed in

Thottea duchartrei and *Thottea sivarajanii* (table 1). A continuous ring of pericyclic fibers was observed in *Aristolochia* and *Thottea* (fig. 2C, 2D). This ring of fibers undergoes fragmentation and subsequent repair via parenchyma intrusion and lignification during secondary growth.

Asaraceae. *Saruma henryi* shows little and determinate production of wood regarding the rest of the woody representatives in the perianth-bearing Piperales. Interfascicular cambium does not produce radial initials (fig. 2E), consequently presenting a rayless wood. Raylessness in *S. henryi* is a unique feature regarding the remaining woody species of the perianth-bearing Piperales. *Saruma henryi* does not present the continuous ring of fibers commonly observed in the sister group Aristolochiaceae; mechanical support is then provided by sclerenchyma bundle caps and an external ring of three to five cell layers of collenchyma (fig. 2E).

Piperaceae. All Piperaceae except *Verhuellia lunaria* presented a polycyclic arrangement of vascular bundles, which is a characteristic of the family. Vascular bundles are organized in two or more concentric rings in *Piper*, *Manekia*, and *Zippelia* (fig. 2G–2I; table 1). In species undergoing secondary growth, secondary thickening is restricted to peripheral bundles (fig. 2H). In *Peperomia*, vascular bundles are surrounded by ground parenchyma and are scattered throughout the stem section without an obvious concentric distribution.

Similar to Aristolochiaceae and Asaraceae, the interfascicular cambium of woody Piperaceae produces exclusively wide secondary rays composed of secondary parenchyma (fig. 2F, 2H). Rays are entirely lignified in mature wood except in climbing species where ray lignification is partial or absent. The climber *Manekia sydowii* produces a large amount of wood, and secondary rays remain completely unlignified (table 1). We recorded a procambial inversion of the most internal medullary bundles of *M. sydowii*, where primary xylem developed centrifugally while primary phloem developed centripetally (fig. 2I). *Peperomia blanda* and *Peperomia incana* show only a slight cambial activity, where secondary production is very limited and is absent in most of the observed vascular bundles (not shown). Cambial activity is also very limited in *Zippelia begoniifolia*, and the fascicular cambium produces only a tiny amount of secondary tissues (fig. 2G, inset, black arrow). Cell alignments were observed in some sections between the bundles of *Zippelia*, but the interfascicular cambium can be considered inactive as it only presents a few divisions of cells and the observed alignments seem to be the product of tangential procambial divisions; therefore, no wood cylinder is present. Cambial activity is completely absent in *V. lunaria*, and the stem comprises only one exclusively medullary bundle, which is embedded in ground parenchyma (fig. 6B). This is composed of five to nine protoxylem elements and a small amount of primary phloem. A complete ring of sclerenchyma fibers was observed in *Manekia*, *Piper*, and *Zippelia*. The ring of fibers is internal to the peripheral bundles and presents an undulating pattern (figs. 2G, 2H, 5A). Fibers are present only as externally oriented caps on the vascular bundles of *Peperomia*, and they are completely absent in *Verhuellia* (table 1).

Wood Features of Piperales

Rays. All of the woody species of Piperales present very wide and tall multiserial rays (figs. 2C, 2D, 2F, 2H, 3A; ta-

Table 1
Stem Anatomical Characters of Piperales

	Aristolochiaceae											Asaraceae	
	<i>Aristolochia arborea</i>	<i>Aristolochia impudica</i>	<i>Thottea abrahamii</i>	<i>Thottea barberi</i>	<i>Thottea dingboui</i>	<i>Thottea dichartrei</i>	<i>Thottea iddukiana</i>	<i>Thottea pommidiana</i>	<i>Thottea siliquosa</i>	<i>Thottea sivarajami</i>	<i>Thottea tomentosa</i>	<i>Saruma henryi</i>	
General anatomy:													
No. cycles of vascular bundles	1	1	1	1	1	1	1	1	1	1	1	1	
Medullary bundles	-	-	-	-	-	-	-	-	-	-	-	-	
One exclusive medullary bundle	-	-	-	-	-	-	-	-	-	-	-	-	
Presence of mucilage canals	-	-	-	-	-	-	-	-	-	-	-	-	
Active phellogene, bark production	+	+	-	+	-	+	+	+	+	+	-	-	
Stem endodermis with a Casparian band	-	-	-	-	-	-	-	-	-	-	-	-	
Sclerenchyma ring geometry	C	C	C	C	C	C	C	C	C	C	U	D	
Width of the sclerenchyma ring (mean no. cells)	4	4	5	2	3	2	2	4	3	3	3	NA	
Aerenchyma	-	-	-	-	-	-	-	-	-	-	-	-	
Calcium oxalate	D	D	-	-	-	-	-	-	-	-	-	-	
Cambial activity	5	4	4	5	4	5	4	5	5	5	4	3	
Cell layers produced by cambium	>20	>20	<20	>20	<20	>20	<20	>20	>20	>20	<20	<20	
Wood anatomy:													
Growth rings boundaries distinct (1)	-	-	-	-	-	-	-	-	-	-	-	-	
Vessels in diagonal and/or radial pattern (7)	+	+	+	+	+	+	+	+	+	+	+	+	
Vessels exclusively solitary (90% or more; 9)	+	+	-	+	-	+	+	+	-	-	+	+	
Vessels in radial multiples (10)	-	-	-	-	-	-	-	-	-	-	-	-	
Vessel clusters common (11)	-	-	+	-	+	-	+	+	+	+	+	-	
Simple perforation plates (13)	+	+	+	+	+	+	+	+	+	+	+	+	
Scalariform perforation plates (14)	-	-	-	-	-	-	-	-	-	-	-	-	
Intervessel pits scalariform (20)	-	-	-	-	-	-	-	-	-	-	-	-	
Intervessel pits opposite (21)	-	-	-	-	-	-	-	-	-	-	-	-	
Intervessel pits alternate (22)	+	+	+	+	+	+	+	+	+	+	+	+	
Axial parenchyma absent or extremely rare (75)	-	-	NA	-	NA	-	-	-	-	-	NA	+	
Axial parenchyma diffuse (76)	+	-	NA	+	NA	-	+	-	-	NA	NA	-	
Axial parenchyma diffuse in aggregates (77)	+	+	NA	+	NA	+	+	+	+	+	NA	-	
Axial parenchyma scanty paratracheal (78)	+	+	NA	+	NA	+	+	+	+	+	NA	-	
Axial parenchyma vasicentric (79)	-	-	NA	-	NA	-	-	-	-	-	NA	-	
Axial parenchyma in narrow bands (86)	+	+	NA	+	NA	+	+	+	+	+	NA	-	
Ray width, commonly 4- to 10-seriate (98)	-	+	-	+	-	-	-	-	-	-	-	-	
Ray width, commonly >10-seriate (99)	-	-	+	-	+	-	+	+	+	+	+	+	
Wood rayless (117)	-	-	-	-	-	-	-	-	-	-	-	-	
All ray cells upright and/or square (105)	-	-	-	-	-	-	-	-	-	-	-	-	
Ray lignification	E	E	E	E	E	E	E	E	E	E	E	E	

Table 1 (Continued)
Stem Anatomical Characters of Piperales

	Piperaceae										Saururaceae				
	<i>Manekia sydownii</i>	<i>Peperomia blanda</i>	<i>Peperomia incana</i>	<i>Peperomia comptonii</i>	<i>Piper flaviflorum</i>	<i>Piper gorgonillense</i>	<i>Piper hispidinerum</i>	<i>Piper insectifugum</i>	<i>Piper midbracteatum</i>	<i>Piper sarmentosum</i> sp.	<i>Piper lunaria</i>	<i>Zippelia begoniifolia</i>	<i>Gymnotheca chinensis</i>	<i>Houttuynia cordata</i>	<i>Saururus chinensis</i>
General anatomy:															
No. cycles of vascular bundles	>2	>2	>2	2	2	2	>2	2	2	2	2	1	1	1	1
Medullary bundles	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
One exclusive medullary bundle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Presence of mucilage canals	P, C	-	P	P, C	P, C	-	P, C	-	-	-	-	-	-	-	-
Active phellogene, bark production	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+
Stem endodermis with a Casparian band	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+
Sclerenchyma ring	C	D	D	U	U	U	U	U	U	U	U	C	C	D	NA
Width of the sclerenchyma ring (mean no. cells)	4	NA	NA	7	3	4	5	8	4	6	4	4	4	3	3
Aerenchyma	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+
Calcium oxalate	D, P, R	-	D, R	-	-	R	-	P, R	-	-	R	R	R	D	-
Cambial activity	5	1	2	5	5	5	5	5	4	5	2	2	2	2	2
Cell layers produced by cambium	>20	-	R	>20	>20	>20	>20	>20	>20	>20	>20	R	R	R	R
Wood anatomy:															
Growth rings	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Boundaries	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Distinct	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vessels in diagonal and/or radial pattern (7)	+	NA	NA	+	+	+	+	+	+	+	+	NA	NA	NA	NA
Vessels exclusively solitary (90% or more; 9)	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA
Vessels in radial multiples (10)	-	NA	NA	+	+	-	-	-	-	-	-	NA	NA	NA	NA
Vessel clusters common (11)	+	NA	NA	+	+	+	-	+	-	-	-	NA	NA	NA	NA
Simple perforation plates (13)	+	NA	NA	+	+	+	+	+	+	+	+	-	-	-	-
Scalariform perforation plates (14)	-	NA	NA	-	-	-	-	-	-	-	-	+	+	+	+
Intervessel pits scalariform (20)	+	NA	NA	+	-	+	-	+	+	+	+	+	+	+	+
Intervessel pits opposite (21)	-	NA	NA	-	-	-	-	-	-	-	-	-	-	-	-
Intervessel pits alternate (22)	-	NA	NA	-	+	-	+	-	-	-	-	-	-	-	-

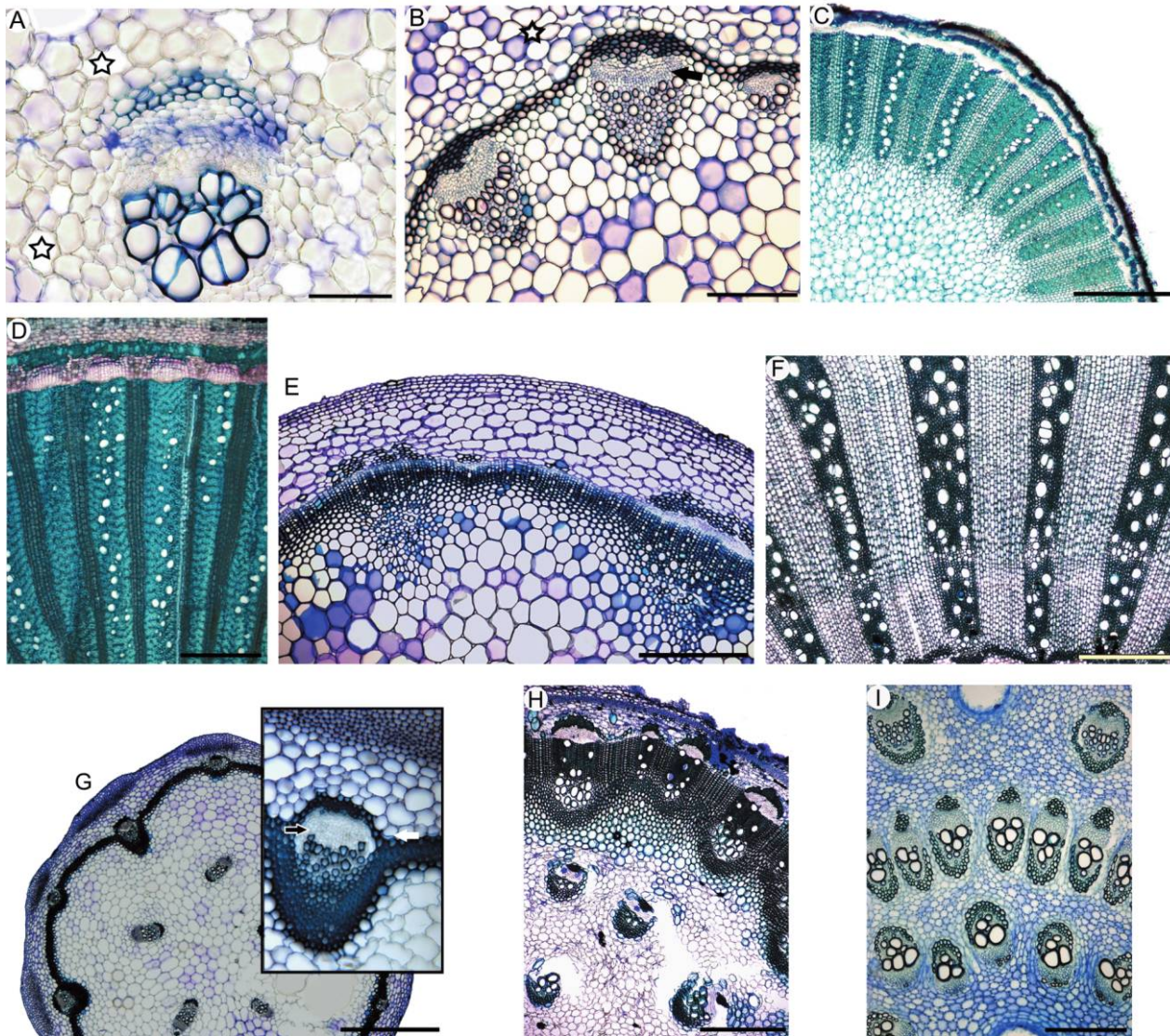


Fig. 2 Variation in cambial activities and tissues distribution in Piperales species observed in transverse sections. *A*, *Saururus chinensis*, vascular bundle with intrafascicular cambium producing a limited number of cells; absence of interfascicular cambial activity; note the presence of aerenchyma (stars). Scale bar = 100 μm . *B*, *Gymnotheca chinensis*, vascular bundles surrounded by a continuous band of fibers; limited cambial activity is restricted to intrafascicular areas (arrow); absence of interfascicular cambial activity; cortical aerenchyma (star). Scale bar = 200 μm . *C*, *Thottea barberi*, full cambial activity in both inter- and intrafascicular areas; interfascicular cambium produces exclusively raylike secondary parenchyma; vessels are arranged in a radial pattern. Scale bar = 500 μm . *D*, *Aristolochia impudica*; vessels are predominantly solitary and arranged in a radial pattern; wide secondary rays; a ring of fibers surrounding the vascular system is still present. Scale bar = 500 μm . *E*, *Saruma henryi*; vessels are arranged in a radial pattern; vessels are not much wider than the surrounding fibers. Scale bar = 200 μm . *F*, *Piper gorgonillense*, wide secondary rays produced by the interfascicular cambium; vessels solitary or in clusters; presence of a growth ring. Scale bar = 500 μm . *G*, *Zippelia begoniifolia*, presents two cycles of vascular bundles; only the peripheral bundles present a slight cambial activity (inset, black arrow); note the presence of a Casparian band (inset, white arrow). Scale bar = 1000 μm (inset = 100 μm). *H*, *Piper nudibracteatum*; secondary production is limited to the peripheral vascular bundles while the medullary bundles remain inactive; notice the wide secondary rays formed by parenchyma cells. Scale bar = 500 μm . *I*, *Manekia sydowii*; three rings of vascular bundles are visible; the innermost bundles present a procambial inversion with primary xylem developing externally; parts of the central and peripheral mucilage canals are visible at the bottom and the top. Scale bar = 500 μm .

ble 1). Most of the species present rays of more than 10 cells wide (fig. 3A); only *Thottea barberi* and *Piper* sp. present rays with a width less than 10 cells but that are nevertheless considered as multiseriate. As mentioned above, *S. henryi* is the only observed species with rayless wood (fig. 2E). We observed

a few short lignified biseriate rays in some sections of *S. henryi*; however, they become indistinguishable from the rest of the lignified tissues by the late stages of development. Ray composition is the same for all species with predominantly upright cells and a few square cells (fig. 3B–3D). For most of the ex-

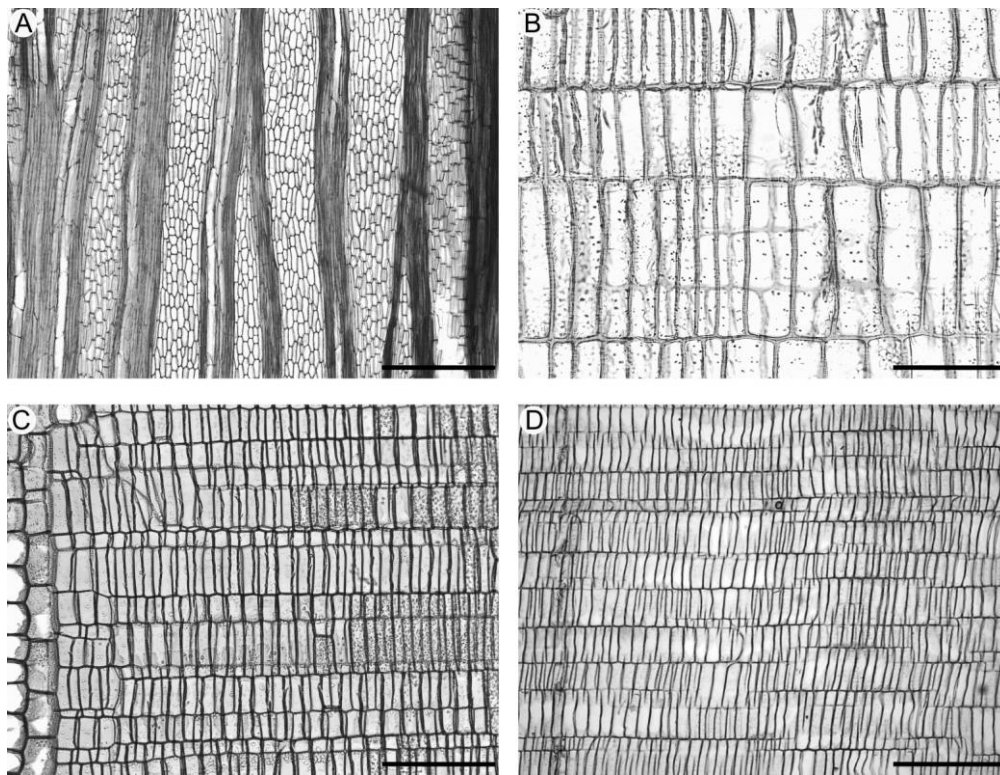


Fig. 3 Ray features of Piperales. A, *Thottea iddukiana*, tangential plane; rays are 10–15-seriate. Scale bar = 500 μm . B, *Thottea sivarajanii*, radial plane; upright cells of ray tissue with lignified walls. Scale bar = 100 μm . C, *Thottea siliquosa*, radial plane; rays are composed of mixed upright and square cells. Scale bar = 200 μm . D, *Piper gorgonillense*, radial plane; upright and square cells of ray tissue. Scale bar = 300 μm .

aminated species, ray cells become lignified; ray lignification is absent or partial in most climbing species.

Vessel elements: perforation plates and lateral wall pitting. All species within Aristolochiaceae, Asaraceae, and Piperaceae present simple perforation plates (fig. 4A–4J; table 1). The metaxylem tracheary elements of Saururaceae presented scalariform perforation plates (fig. 4K–4M). Vessels of *Aristolochia* and *Thottea* generally present alternate pitting, and only some species of *Thottea* bear vessel elements with opposite to alternate pits (fig. 4A–4D; table 1). We observed mostly alternate pitting in *S. henryi*, but a few scalariform pits were also observed in some vessel elements (fig. 4E; table 1). Most Piperaceae species present scalariform pitting (fig. 4F–4J). Finally, Saururaceae metaxylem vessel elements also present scalariform pitting (fig. 4K–4M), and only some vessels in *H. cordata* and *S. chinensis* bear opposite pits.

Vessel elements: vessel arrangement and grouping. All the woody Piperales species present a radial distribution of vessels with linear or diagonal arrangements (fig. 2C–2F; table 1). Solitary vessels were predominantly observed in *Aristolochia*, *Thottea*, and *Piper* (figs. 2D, 5B, 5C; table 1); only *Thottea pommudiana* and *Thottea siliquosa* exhibit radially distributed clusters of more than four vessels (fig. 5A). Vessel clusters were frequently observed in both *Thottea* and the Piperaceae representatives (table 1). Vessel grouping was very ambiguous, as in some species we observed grouped and solitary vessels with an almost equal ratio; *Piper gorgonillense*, for example, presents both solitary and grouped vessels (fig. 2F).

Axial parenchyma. Axial parenchyma is one of the wood characters that differs between woody species of Aristolochiaceae and Piperaceae. In *Aristolochia* and *Thottea*, apotracheal axial parenchyma is sometimes diffuse to diffuse in aggregates, and a predominance of banded parenchyma forming narrow tangential bands was observed in both genera (fig. 5A, 5B, black arrows). Conversely, *Piper* and *Manekia* have mostly paratracheal vasicentric axial parenchyma (fig. 5C, arrows). Absence of axial parenchyma was observed in *Saruma*.

Degrees of Woodiness in Piperales

Piperales species exhibit several degrees of cambial activity, from species with significant wood production to species completely lacking secondary growth (fig. 2). These shifts in cambial activity arise repeatedly within the different lineages of Piperales. Many *Piper* and *Manekia* species, along with *Lactoris fernandeziana*, produce a large wood cylinder along the main axis (fig. 2F; tables 1, B1). Similarly, most of the species within *Aristolochia* and *Thottea* are truly woody plants with significant secondary growth (fig. 2C, 2D; tables 1, B1). Some shifts toward a decrease of woodiness are observed within these groups; some *Thottea* representatives produce narrow wood cylinders and are considered as slightly woody shrubs (table 1). *Aristolochia serpentaria* presents a few cell divisions in the interfascicular areas, and its wood cylinder is restricted to the base corresponding to a woody herb typology (table B1). The monospecific *Saruma* and its related genus

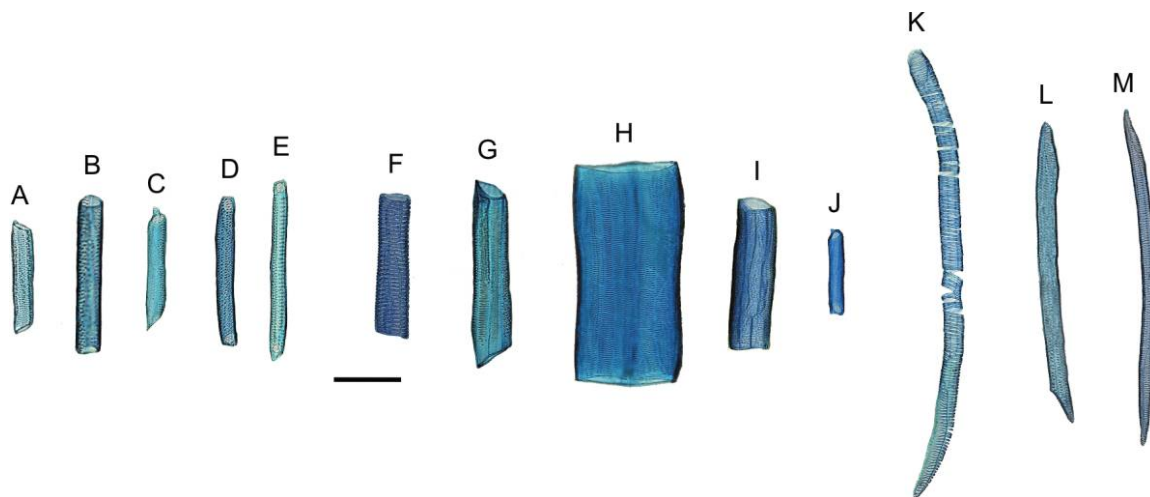


Fig. 4 Morphological diversity of xylem vessel elements of Piperales representatives. Simple perforation plates in Aristolochiaceae (A–D), Asaraceae (E), and Piperaceae (F–J); scalariform perforation plates in Saururaceae (K–M). A, *Thottea dinghoui*. B, *Thottea ponnudiana*. C, *Thottea duchartrei*. D, *Thottea barberi*. E, *Saruma henryi*. F, *Manekia sydowii*. G, *Piper nudibracteatum*. H, *Piper insectifugum*. I, *Piper hispidinervum*. J, *Piper sarmentosum*. K, *Saururus chinensis*. L, *Houttuynia cordata*. M, *Gymnotheca chinensis*. Scale bar = 200 μm .

Asarum (Asaraceae) both present cambial activity in the interfascicular and fascicular areas, but they produce a narrow wood cylinder (fig. 2E) restricted to the rhizome or to the base of its stems and therefore can also be considered as woody herbs (tables 1, B1). In extreme cases of reduction of cambial functioning, species such as *Verhuellia* and some *Peperomia* species completely lack cambial activity (tables 1, B1). *Peperomia* and *Zippelia* (Piperaceae) never form a wood cylinder; when secondary growth is present, it is restricted to a few tangential divisions in the fascicular areas. In Saururaceae, only a few tangential divisions may occur in the intrafascicular areas in *Gymnotheca*, *Houttuynia*, and *Saururus* (fig. 2B), while *Anemopsis* presents secondary growth in both intra- and interfascicular areas, forming a narrow wood cylinder at the base of

the stem (table B1). Finally, the holoparasitic genera *Hydnora* and *Prosopanche* (Hydnoraceae) also exhibit a reduction of cambial activity with a restriction to the fascicular areas (table B1).

Presence of an Endodermis with Casparian Bands in Aerial Stems of Piperales

Stem endodermis with a Casparian band was observed in all genera within Piperaceae as well as in *H. cordata* and *S. chinensis* (Saururaceae). The endodermis was present as a layer of cells of procambial origin, generally located at the limits of the vascular system and the cortical area. The endodermis shows a typical Casparian band, staining with a dark blue indi-

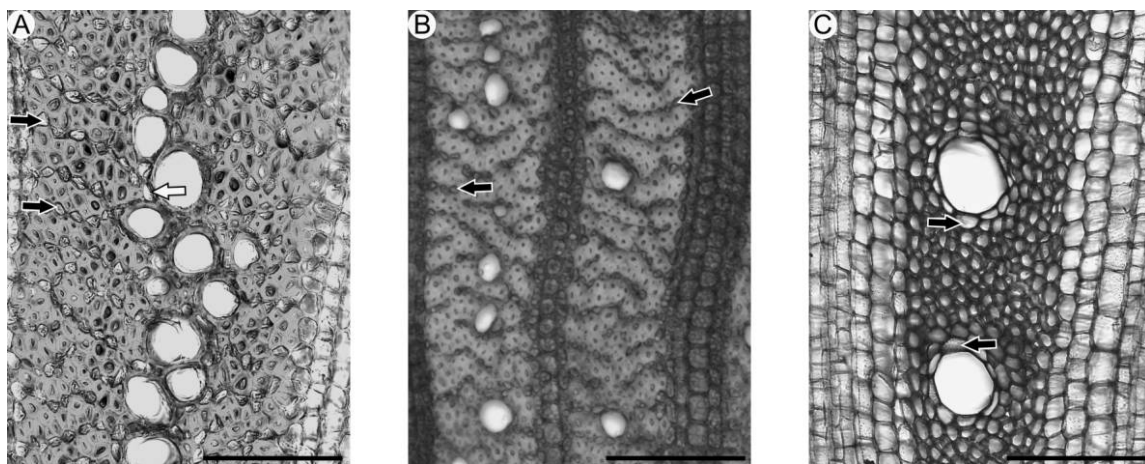


Fig. 5 Axial parenchyma in Piperales woody species observed in transverse sections. A, *Thottea siliquosa*, apotracheal axial parenchyma in tangential narrow bands (black arrows); occasional parenchyma cells are associated with the vessels (white arrow). Scale bar = 100 μm . B, *Aristolochia impudica*, apotracheal axial parenchyma in tangential narrow bands (black arrows). Scale bar = 200 μm . C, *Piper hispidinervum*, paratracheal vasicentric axial parenchyma forming a narrow sheath around the vessels (black arrows). Scale bar = 200 μm .

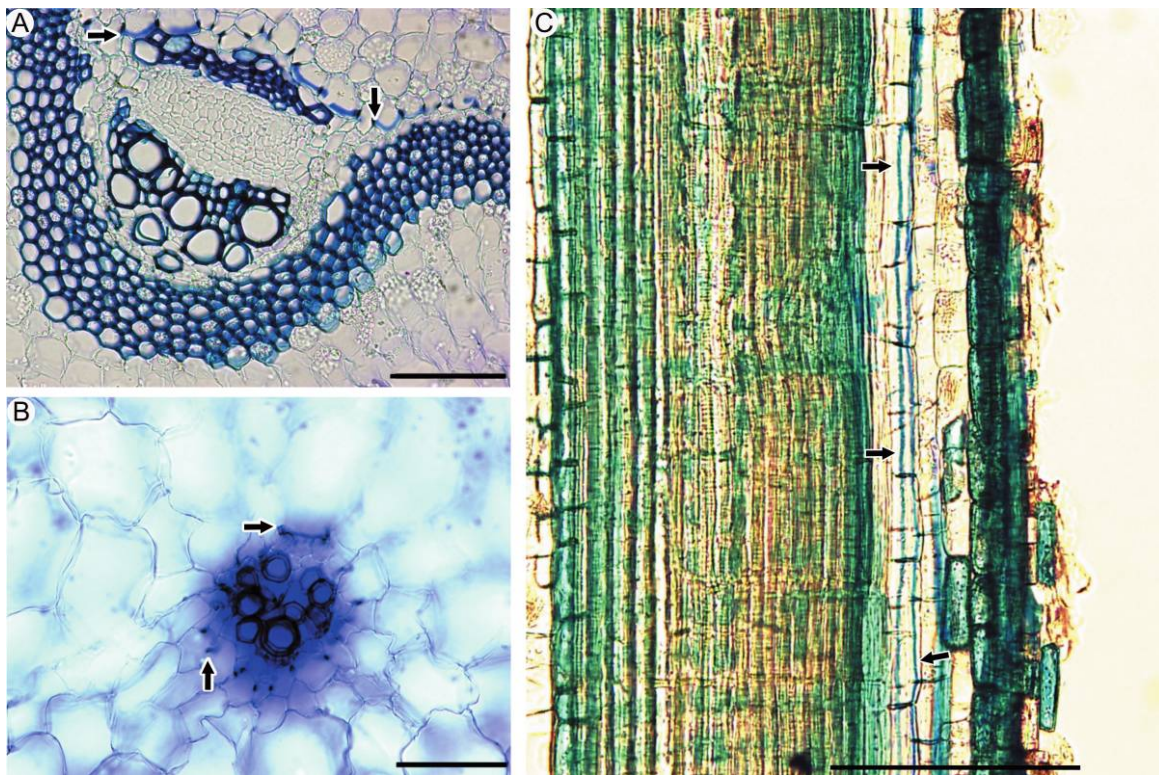


Fig. 6 Stem endodermis with a Casparian band in Piperaceae. *A*, *Piper sarmentosum*, transverse view of a young stem; note the endodermis with a Casparian band colored in dark blue and visible as a single layer of cells external to the vascular bundles ring (arrows). Scale bar = 100 μm . *B*, *Verhuellia lunaria*, transverse section showing the central and single vascular bundle composed exclusively of helical protoxylem elements; note the endodermis surrounding the vascular bundle and presenting a Casparian band on its radial walls (arrows). Scale bar = 50 μm . *C*, *Piper* sp. radial view of the Casparian band (arrows) that contains suberin and lignin, occurring on the radial and tangential anticlinal walls of cells. Scale bar = 200 μm .

cating the presence of suberin and/or lignin, observed in the radial and tangential walls of the endodermic cells (fig. 6). Stem endodermis was external to the sclerenchyma cap of the peripheral bundles and observed around the entire circumference of the stem in *M. sydowii*, *Piper flaviflorum*, *Piper sarmentosum*, *Piper* sp., and *Z. begoniifolia* (fig. 2G, inset, white arrow; fig. 6A, 6C, arrows). Stem endodermis was present surrounding some vascular bundles in *Peperomia* species and around the single bundle of *V. lunaria* (fig. 6B). In *H. cordata*, a ring of rectangular shaped cells contiguous to the peripheral ring of sclerenchyma was observed; some of these cells presented a marked Casparian band. In *S. chinensis*, a Casparian band was observed as a continual layer external to the vascular bundles. This character was not observed in *G. chinensis*.

Anatomical Character States Reconstructions

Maximum parsimony reconstruction of five states using polymorphisms in large genera presenting different cambial activities supports a truly woody ancestral state for the Piperales (fig. 7A, node 2), with a continuous cambium producing a complete wood cylinder of significant development. Cambial activity reconstruction is also univocal for the ancestor of the perianth-bearing Piperales (fig. 7A, node 3), where cambial ac-

tivity is also reconstructed as truly woody (fig. 2C, 2D, 2F). The restriction of cambial activity to fascicular areas (fig. 2A, 2B) was consistently reconstructed as ancestral for the Saururaceae (fig. 7A, node 5), while the ancestral state of the cambial activity remains ambiguous for Piperaceae (fig. 7A, node 6). MP suggests that woody herbs presenting a wood cylinder restricted to basal parts of the stem evolved at least two times within Piperales, in the Saururaceae (*Anemopsis*) and the Asaraceae. Complete loss of vascular cambium has probably evolved independently in *Verhuellia* and in some *Peperomia* representatives.

While all Aristolochiaceae, Asaraceae, and Piperaceae present simple perforation plates (fig. 4A–4J), scalariform perforation plates occur in all Saururaceae (fig. 4K–4M) with the exception of *Anemopsis* (table B1). ML reconstruction indicates that the presence of scalariform perforation plates is most probably a plesiomorphic feature of Saururaceae (0.61 proportional likelihood [pl] value; fig. 7B, node 5), thus suggesting that simple plates have evolved secondarily in *Anemopsis*. ML reconstruction strongly supports the hypothesis of simple perforation plates as an ancestral state for the perianth-bearing Piperales (0.95 pl; fig. 7B, node 3) as well as for the internal node within Piperaceae excluding the protostelic *Verhuellia* (0.94 pl; fig. 7A, node 7). The simple perforation plate is highly

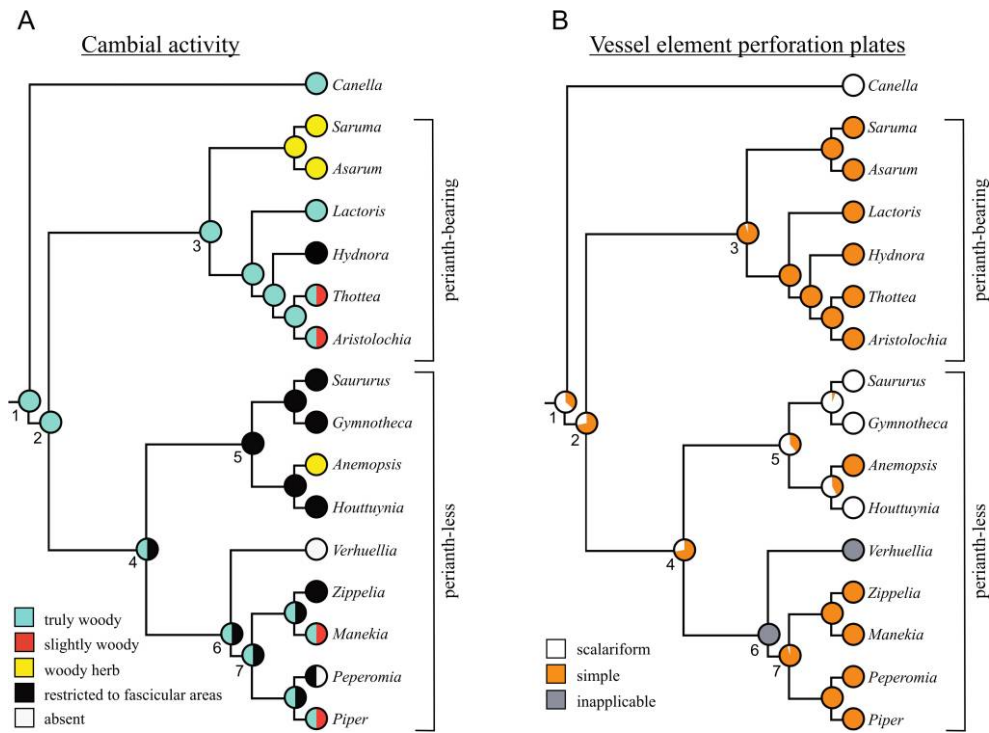


Fig. 7 Ancestral character state reconstructions of Piperales. *A*, Maximum parsimony (MP) reconstruction of the cambial activity in Piperales; MP method is used to code polymorphisms when two potential character states are known to occur. *B*, Maximum likelihood (ML) reconstruction of the vessel element perforation plates; pie charts indicate ML probabilities for each character state at each internal nodes. Descriptions of character states and the employed reconstruction methods are provided in “Material and Methods.” Nodes are numbered for easier reference to the text.

supported in the common ancestor of Piperales (0.71 pl; fig. 7*B*, node 2).

Discussion

Primary Woodiness in Piperales

Uniformity of wood features in Piperales. A striking feature of Piperales is the characteristic and highly conserved kind of wood anatomy. Specific combinations of wood features distinguish woody Piperales from many other groups of angiosperms, including vessels in radial patterns, vessel elements with simple perforation plates, vessel-to-vessel pitting alternate to scalariform, vessel-to-axial parenchyma pitting mainly scalariform, and wide and tall rays composed exclusively of upright and squared cells and storied wood. Among the wood features mentioned previously, the wide and tall rays composed of secondary parenchyma, which change little over time, and the upright and squared nature of these ray cells constitute the main trademark of Piperales wood anatomy. As mentioned by Carlquist (1993, p. 355), the wood of the Aristolochiaceae, *Lactoris*, and Piperaceae is “amazingly similar.” Even the highly specialized holoparasite *Hydnora* presents piperalean anatomical features such as simple perforation plates and scalariform to alternate vessel pitting (Tennakoon et al. 2007) despite some divergences due to their

holoparasitic habit and their highly derived subterranean morphology (Wagner et al. 2014).

The uniformity of numerous wood features throughout Piperales constitutes a strong argument supporting a unique origin of wood formation in the order. Some combinations of features might distinguish woody lineages within Piperales; these include (a) exclusively vascentric axial parenchyma in Piperaceae and Lactoridaceae versus the paratracheal axial parenchyma in combination with predominantly diffuse in aggregates or narrowly banded axial parenchyma in *Aristolochia* and *Thottea* (fig. 5; table 1; Carlquist 1993) and (b) alternate intervessel pitting in *Aristolochia* and *Thottea* versus scalariform intervessel pitting mostly observed in Piperaceae (table 1). However, these features are not strictly fixed in each lineage. For instance, we observed predominantly alternate pitting in *Piper flaviflorum* and *Piper hispidum* and only scanty paratracheal axial parenchyma in several *Aristolochia* and *Thottea* species (fig. 5; table 1). We suggest that these relatively minor anatomical divergences might thus reflect specific habitat or growth form adaptations rather than independent origins of wood formation in the different lineages.

A truly woody putative ancestor of Piperales. Despite a wide diversity of cambium functioning in extant Piperales, our ancestral character state reconstruction univocally supports a strictly woody common ancestor for Piperales. The uni-

form wood organization of Piperales supports this view and suggests that herbaceousness is secondarily derived in the different lineages. Reduction of woodiness in woody herbs and strictly herbaceous habits might result from a reduction of cambial activity and its limitation to the fascicular areas. Canellales, the sister group to Piperales, is widely represented by woody species, suggesting that full cambial activity and a woody habit were already present in the common ancestor of Canellales + Piperales (fig. 7A, node 1). At a large phylogenetic scale, magnoliids have been suggested to be evolved from a woody ancestor, while Piperales have been alternatively coded as herbaceous or woody (Kim et al. 2004; Feild and Arens 2005). Our work supports the view that the herbaceous habit arose several times independently within magnoliids.

Herbaceousness as a Secondary Condition in Extant Piperales

Loss of secondary growth in Piperaceae, a rearrangement of polycyclic vascular bundles? *Verhuellia* and *Peperomia* (Piperaceae) present a secondarily herbaceous condition involving the complete loss of the bifacial cambium (fig. 7A). Stem anatomy of *Verhuellia lunaria* is strikingly rudimentary and reduced to a primary vascular system with a single central vascular bundle composed of five to nine annular and helically thickened protoxylem tracheary elements surrounded by protophloem (fig. 6B). In *Peperomia*, the scattered vascular bundles are composed of proto- and metaxylem tracheary elements. Piperaceae evolved several derived anatomical features, the most striking being the polycyclic or scattered arrangement of their vascular bundles (fig. 2G–I; Isnard et al. 2012). Debray (1885) proposed that the vascular bundles of *Peperomia*, undergoing little or no secondary growth, might be homologous to the medullary vascular bundles of *Piper* species, which also have little or no secondary growth. Intensive secondary growth indeed occurs preferentially in the peripheral vascular bundles of *Piper* (fig. 2H); consequently, the loss of the peripheral cycle of vascular bundles could lead to stems with scattered medullary bundles with limited or no secondary growth, as observed in *Peperomia*. The protostelic organization of *Verhuellia* might represent an extreme rearrangement of the vascular system where only one single medullary bundle is produced. In fact, very few dicotyledonous species have completely lost secondary growth; the pervasiveness of the genetic ability to produce secondary xylem among dicotyledonous plants suggests a significant genetic stability of the vascular cambium. In Piperaceae, the rearrangement/loss of vascular bundles is a source of variation in habits, which might differ from any irreversibly suppressed cambial activity since the loss of secondary growth could result from the loss of the peripheral vascular bundles.

Anatomical Particularities in Piperales

Stem endodermis in Piperaceae + Saururaceae. One of the main clade-specific anatomical features is the presence of a stem endodermis in the aerial stems of Piperaceae and Saururaceae. A stem endodermis bearing Casparian bands has been previously reported in several genera including *Piper*,

Peperomia, and *Saururus* (Bond 1931; Carlquist et al. 1995; Lersten 1997; Souza et al. 2009). This study extended the presence of a stem endodermis to other genera within Piperaceae and Saururaceae (*Manekia*, *Houttuynia*, *Verhuellia*, and *Zippelia*; fig. 6), indicating that the Casparian bands in aerial stems are present in all genera of Piperaceae and in several genera of Saururaceae. To our knowledge, stem endodermis has, however, never been reported for other Piperales lineages, and the occurrence of this feature suggests that stem endodermis was present in the common ancestor of Piperaceae + Saururaceae. Stem endodermis occurs sporadically in angiosperm phylogeny (Lersten 1997) and has been attributed to several functions associated with water or oxygen conservation and pathogen protection by acting as a barrier (Enstone et al. 2003; Meyer and Peterson 2011). In Saururaceae, stem endodermis is found in aquatic to semiaquatic species (*Saururus* and *Houttuynia*). In Piperaceae, stem endodermis is found in epiphytic and terrestrial species producing adventitious roots, suggesting a water storage function. Some *Piper* species developed additional water-related adaptations such as mucilage canals, and Saururaceae possesses aerenchyma, which points to the importance of water-related adaptations in these lineages.

Scalariform perforation plates in the vessel elements of the Saururaceae. The scalariform perforation plates of Saururaceae (except *Anemopsis*; Carlquist et al. 1995; Schneider and Carlquist 2001) are one of the main diverging xylem features in Piperales. The vessel elements of Saururaceae have been proposed to be relictuals based on their primitive nature according to the Bailey scheme of wood evolution (Bailey and Tupper 1918) and on the assumption of an unchanged history of life in mesic habitats (Carlquist et al. 1995). The occurrence of scalariform plates in Saururaceae could be explained as the result of limited secondary growth, lack of hydrolysis of the pit membrane in the end walls, and retention of primary xylem features. Such relictual condition of scalariform plates in Saururaceae would imply at least three independent evolutionary lines of simple plates within Piperales (fig. 7B); despite not being the most likely, this evolutionary scenario is still plausible, as simple perforation plates, which provide an enhanced hydraulic conductance (Christman and Sperry 2010), are found in genera presenting large-bodied and climbing forms (*Aristolochia*, *Thottea*, and *Piper*; fig. 1A, 1B), which demand an efficient water conductance.

Reversal from simple to scalariform perforation plates (i.e., after complete loss of the structure) in Saururaceae, however, is supported by our character reconstruction (fig. 7B, node 5). Previous works have suggested possible reversals in perforation plate morphology in a large clade of Ericales and in the genus *Meryta* (Lens et al. 2007; Oskolski and Jansen 2009). These previously documented reversals from simple to scalariform perforation plates together with the observed widespread occurrence of scalariform perforation plates along all major groups of angiosperms (Olson 2014) challenge the vision of irreversibility of the Bailey trend in perforation plate morphology and suggest that parallelisms and reversals resulting in scalariform perforation plates may be more common than previously thought. Further work on the ecophysiology of the Saururaceae and other angiosperms presenting this potential character reversal would be needed in order to understand

which conditions are driving the reversal from simple to scalariform perforation plates.

*Protracted Juvenilism in Wood in Relation to
Piperale Growth Habits*

Several pedomorphic wood features (in the Carlquistian sense) have been previously reported in Piperales; these include the flat length-on-age curve recorded for *Macropiper excelsum* (Carlquist 1962), the rayless wood of *Saruma henryi* (Dickison 1996), and the multiseriate rays composed by upright cells that correspond to the pedomorphic ray type II according to Carlquist (2009). This last pedomorphic condition, characterizing the woody Piperales, involves a slower rate of horizontal subdivision of cambial initials resulting in vertically longer ray cells. Additionally, subdivision of rays that commonly occur through intrusive growth of fusiform cambial initials does not occur in Piperales, leading to rays that remain largely unaltered in secondary xylem. In typically woody angiosperms, the primary rays are usually wide and composed of upright cells that become progressively procumbent, subdivided, and replaced by fiber or vessel elements in the secondary xylem (Carlquist 2009, 2013).

In a recent article, Lens et al. (2013) provide an extended list of primarily woody taxa that actually exhibit protracted juvenilism in wood, such as the rays composed by upright cells. It was proposed that protracted juvenilism in wood may be related to specific growth forms such as small-sized shrubs, rosette trees, or succulent stems. The presence of juvenilistic rays in Piperales may be linked to the sympodial growth form, which is widely represented in the order and has been recently reconstructed as ancestral for Piperales (Isnard et al. 2012). A relationship between the sympodial construction observed in early-diverging angiosperms (including Aristolochiaceae and Piperaceae) and the wide and tall rays commonly observed in their wood was already suggested by Carlquist (2009). Functional reasons can explain the widespread occurrence of upright cells in Piperales rays. Many *Aristolochia*, *Thottea*, and *Piper* species present liana and cane-like habits with several elongated stems growing from the base of the plant (Isnard et al. 2012; fig. 1B, 1D). Radial transport of photosynthates in these relatively narrow stems may not be an important constraint for the plant, promoting the development of upright cells that enhance a vertical conduction (Carlquist 2012), which plays a more important role in these growth forms.

Mabberley (1974) proposed that the flat age-on-length curves obtained by Carlquist (1962) for the vessel elements of *M. excelsum* are the result of the geometry of its stems. Indeed, Mabberley (1974) argued that *M. excelsum* presents wide piths that become wider at the higher portions of the stem, considerably increasing stem radius. A greater radius may increase the number of cambial initials for anticlinal divisions and therefore diminish the intrusive growth from cambial initials from a lower level; as a consequence of this, vessel element length remains constant (Dulin and Kirchoff 2010). In addition, as reported by Lens et al. (2013), the study of Bailey (1923) presented flat length-on-age curves for vessel elements of primarily woody species with storied cambia. We have observed storied wood structure in *Aristolochia* and

Piper representatives (not shown), and it is reported by Carlquist (1993) as a common feature in Piperales; this condition may also explain the reported curve for *M. excelsum* by Carlquist (1962).

Raylessness is also considered as a criterion of wood juvenilism (Carlquist 2009) pointing to secondary woodiness; the rayless wood observed in *Saruma henryi* appears to be an original feature regarding the rest of Piperales and can also be linked to a particular growth form. The rayless condition of *Saruma* has already been reported by Dickison (1996), who observed multiseriate and tall rays in the vicinity of the cambium, suggesting that the upright ray cells become transformed into more elongated and lignified cells (Dickison 1996). Based on this report, we can interpret the cambium in *Saruma* as not devoid of piperalean rays but instead a derived organization where wide and tall rays undergo further specialization into fiber-like elements. Raylessness in the woody herb *Saruma* might represent a stem mechanical adaptation where the fiber elements provide additional structural support for the stem, which undergoes limited secondary growth restricted to the basal parts of the stems (Dickison 1996; Carlquist 2001).

*Piperale Anatomy, a Major Source of
Growth Forms Diversification*

A significant challenge in Piperales, as in angiosperms in general, is to identify a putative herbaceous/woody ancestor, as the state observed today may not be the herbaceous/woody state of yesterday's ancestors. Therefore, this work focused on cambial activities and wood anatomical comparisons to identify the putative form of a common ancestor of Piperales. Our data show that woodiness is actually homogeneous in Piperales, while herbaceousness can show diverse anatomical organizations, from the single vascular bundle of *Verhuellia* and the scattered bundles of *Peperomia* to the single cycle of bundles with very limited production observed in Saururaceae and Hydnoraceae, suggesting that herbaceousness is most probably derived in the different lineages. This hypothesis is supported by a recent article proposing that the herbaceous habit in *Aristolochia* and in *Asarum* + *Saruma* evolved independently (Wagner et al. 2014).

Large self-supporting forms (fig. 1A) are, however, confined to few species within Piperales where ray lignification could have reduced flexibility of the wood cylinder (Isnard et al. 2012). Since the climbing growth form has been reconstructed as derived in *Aristolochia* (Wagner et al. 2014), the wide and tall rays that seem to be a synapomorphy of Piperales wood could be an exaptation for the climbing form. Wide rays in lianas are indeed widely known to promote stem flexibility and are largely considered as an adaptation to the climbing habit (Putz and Holbrook 1991; Rowe et al. 2004; Isnard and Silk 2009). The evolution of the climbing habit in angiosperms might have promoted diversification (Gianoli 2004), while the evolution of herbaceousness from woodiness is argued to correlate with an increase in the diversification rate and to be implicated as a direct cause of species richness in angiosperm families (Dodd et al. 1999). The lability of the cambial activity, allowing recurrent shifts in habit from woodi-

ness to herbaceousness, and the piperalean wood construction, promoting highly variable growth forms, have therefore been key elements in the diversification of species and habitat preferences in Piperales.

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Appendix A

Voucher Information and Localities for Wood Samples

The following information is given for the taxa investigated in each family: taxon, authority, collection locality, and voucher (institution). Voucher specimens collected by Carolina Granados (CG), David Bruy (DB), Sandrine Isnard (SI), and Santiago Trueba-Sánchez (STS) were deposited in the Dresden Herbarium of Germany (DR); the French Institute of Pondicherry, India (HIFP); the Mexico National Herbarium (MEXU); the University of Antioquia, Medellín, Colombia (HUA); the Xishuangbanna Botanical Garden Herbarium (HITBC); and the IRD Nouméa Herbarium (NOU). Wood specimens were collected from basal segments of the main aerial stem; all samples are preserved in 70% ethanol in the UMR-AMAP collection in Montpellier, France. An asterisk indicates climbing species being described by Ricardo Callejas (University of Antioquia).

Aristolochiaceae. *Aristolochia arborea* Linden, cultivated at the living collection of Bonn Botanical Garden in Germany (BG Bonn); *Aristolochia impudica* J.F. Ortega, Mexico, CG-486 (DR, MEXU); *Thottea abrahamii* Dan, P.J. Mathew, Unnithan & Pushp., India, SI-15 (DR, HIFP); *Thottea barberi* (Gamble) Ding Hou, India, SI-09 (DR); *Thottea dinghousi* Swarupan., India, SI-07 (DR); *Thottea duchartrei* Sivar., A. Babu & Balach., India, SI-13 (DR, HIFP); *Thottea iddukiana* Pandur. & V.J. Nair, India, SI-08 (DR); *Thottea ponnudiana* Sivar., India, SI-06 (DR); *Thottea siliquosa* (Lam.) Ding Hou, India, SI-16 (DR); *Thottea sivarajanii* E.S.S. Kumar, A.E.S. Khan & Binu, India, SI-14 (DR); *Thottea tomentosa* (Blume) Ding Hou, India, SI-20 (DR).
Asaraceae. *Saruma henryi* Oliv., cultivated at the living collection of Dresden Botanical Garden in Germany (BG Dresden).
Piperaceae. *Manekia sydowii* (Trel.) T. Arias, Callejas & Bornst., Colombia, SI-42 (HUA); *Peperomia blanda* (Jacq.) Kunth, China, STS-322 (HITBC); *Peperomia incana* (Haw.) A. Dietr., cultivated at BG Dresden; *Piper comptonii* S. Moore, New Caledonia, STS-380, STS-382, STS-383 (NOU); *Piper flaviflorum* C. DC., China, STS-321 (HITBC); *Piper gorgonillense* Trel. & Yunck., Colombia; *Piper hispidinervum* C. DC., China, STS-315 (HITBC); *Piper insectifugum* C. DC. ex Seem., New Caledonia, DB-59, DB-60 (NOU); *Piper nudibracteatum* C. DC., Colombia; *Piper sarmentosum* Roxb., China, STS-311 (HITBC); *Piper* sp. *, Colombia, SI-37 (HUA); *Verhuellia lunaria* (Desv. ex Ham.) C. DC., cultivated at BG Bonn; *Zippelia begoniifolia* Blume, China.
Saururaceae. *Gymnotheca chinensis* Decne., cultivated at BG Bonn; *Houttuynia cordata* Thunb., cultivated at BG Bonn; *Saururus chinensis* (Lour.) Baill., cultivated at BG Bonn.

Literature Cited

- Amborella Genome Project 2013 The *Amborella* genome and the evolution of flowering plants. *Science* 342:1241089.
- APG (Angiosperm Phylogeny Group) III 2009 An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* 161:105–121.
- Bailey IW 1923 The cambium and its derivative tissues. IV. The increase in girth of the cambium. *Am J Bot* 10:499–509.
- Bailey IW, WW Tupper 1918 Size variation in tracheary cells. I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms and angiosperms. *Proc Am Acad Arts Sci* 54:149–204.
- Barbosa ACF, MR Pace, L Witovisk, V Angyalossy 2010 A new method to obtain good anatomical slides of heterogeneous plant parts. *IAWA J* 31:373–383.
- Bond G 1931 The stem endodermis in the genus *Piper*. *Trans R Soc Edinb* 56:695–724.
- Carlquist S 1962 A theory of pedomorphosis in dicotyledonous woods. *Phytomorphology* 12:30–45.
- 1990 Wood anatomy and relationships of Lactoridaceae. *Am J Bot* 77:1498–1504.
- 1993 Wood and bark anatomy of Aristolochiaceae: systematic and habitual correlations. *IAWA J* 14:341–357.
- 2001 Comparative wood anatomy. Springer, Berlin.
- 2009 Xylem heterochrony: an unappreciated key to angiosperm origin and diversifications. *Bot J Linn Soc* 161:26–65.
- 2012 How wood evolves: a new synthesis. *Botany* 90:901–940.
- 2013 More woodiness/less woodiness: evolutionary avenues, ontogenetic mechanisms. *Int J Plant Sci* 174:964–991.
- Carlquist S, K Dauer, SY Nishimura 1995 Wood and stem anatomy of Saururaceae with reference to ecology, phylogeny and origin of the monocotyledons. *IAWA J* 16:133–150.

- Carlquist S, E Schneider 2001 Vegetative anatomy of the New Caledonian endemic *Amborella trichopoda*: relationships with the Illiales and implications for vessel origin. *Pac Sci* 55:305–312.
- Christman MA, JS Sperry 2010 Single-vessel flow measurements indicate scalariform perforation plates confer higher flow resistance than previously estimated. *Plant Cell Environ* 33:431–443.
- Datta PC, A Dasgupta 1977 Comparison of vegetative anatomy of Piperales. I. Juvenile xylem of twigs. *Acta Biol Acad Sci Hung* 28: 81–96.
- Debray F 1885 Etude comparative des caractères anatomiques et du parcours des faisceaux fibro-vasculaires des Pipéracées. PhD diss. Faculté des Sciences de Paris, Paris.
- Dickison WC 1996 Stem and leaf anatomy of *Saruma henryi* Oliv., including observations on raylessness in the Aristolochiaceae. *Bull Torrey Bot Club* 123:261–267.
- Dodd ME, J Silvertown, MW Chase 1999 Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. *Evolution* 53:732–744.
- Dulin M, B Kirchoff 2010 Paedomorphosis, secondary woodiness, and insular woodiness in plants. *Bot Rev* 76:405–490.
- Enstone D, C Peterson, F Ma 2003 Root endodermis and exodermis: structure, function, and responses to the environment. *J Plant Growth Regul* 21:335–351.
- Feild TS, NC Arens 2005 Form, function and environments of the early angiosperms: merging extant phylogeny and ecophysiology with fossils. *New Phytol* 166:383–408.
- Feild TS, T Brodribb, NM Holbrook 2002 Hardly a relict: freezing and the evolution of vesselless wood in Winteraceae. *Evolution* 56: 464–478.
- Gianoli E 2004 Evolution of a climbing habit promotes diversification in flowering plants. *Proc R Soc B Biol* 271:2011–2015.
- Groover AT 2005 What genes make a tree a tree? *Trends Plant Sci* 10:210–214.
- Hoffstadt RE 1916 The vascular anatomy of *Piper methysticum*. *Bot Gaz* 62:115–132.
- InsideWood 2004– InsideWood database. <http://insidewood.lib.ncsu.edu/search>. Accessed March 2014.
- Isnard S, J Prosperi, S Wanke, S Wagner, M-S Samain, S Trueba, L Frenzke, C Neinhuis, NP Rowe 2012 Growth form evolution in Piperales and its relevance for understanding the angiosperm diversification—an integrative approach combining plant architecture, anatomy and biomechanics. *Int J Plant Sci* 173:610–639.
- Isnard S, WK Silk 2009 Moving with climbing plants from Charles Darwin’s time into the 21st century. *Am J Bot* 96:1205–1221.
- Jaramillo MA, PS Manos 2001 Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). *Am J Bot* 88:706–716.
- Jaramillo MA, PS Manos, EA Zimmer 2004 Phylogenetic relationships of the perianthless Piperales: reconstructing the evolution of floral development. *Int J Plant Sci* 165:403–416.
- Kim S, DE Soltis, PS Soltis, MJ Zanis, Y Suh 2004 Phylogenetic relationships among early diverging eudicots based on four genes: were the eudicots ancestrally woody? *Mol Phylogenet Evol* 31: 16–30.
- Ko J-H, K-H Han, S Park, J Yang 2004 Plant body weight-induced secondary growth in *Arabidopsis* and its transcription phenotype revealed by whole-transcriptome profiling. *Plant Physiol* 135:1069–1083.
- Lens F, N Davin, E Smets, M del Arco 2013 Insular woodiness on the Canary Islands: a remarkable case of convergent evolution. *Int J Plant Sci* 174:992–1013.
- Lens F, S Eeckhout, R Zwartjes, E Smets, SB Janssens 2012a The multiple fuzzy origins of woodiness within Balsaminaceae using an integrated approach: where do we draw the line? *Ann Bot* 109: 783–799.
- Lens F, ME Endress, P Baas, S Jansen, E Smets 2009 Vessel grouping patterns in subfamilies Apocynoideae and Periplocoideae confirm phylogenetic value of wood structure within Apocynaceae. *Am J Bot* 96:2168–2183.
- Lens F, J Schönenberger, P Baas, S Jansen, E Smets 2007 The role of wood anatomy in phylogeny reconstruction of Ericales. *Cladistics* 23:229–254.
- Lens F, E Smets, S Melzer 2012b Stem anatomy supports *Arabidopsis thaliana* as a model for insular woodiness. *New Phytol* 193: 12–17.
- Lersten N 1997 Occurrence of endodermis with a Casparian strip in stem and leaf. *Bot Rev* 63:265–272.
- Mabberley DJ 1974 Pachycauly, vessel-elements, islands and the evolution of arborescence in “herbaceous” families. *New Phytol* 73: 977–984.
- Maddison WP, DR Maddison 2006 StochChar: a package of Mesquite modules for stochastic models of character evolution. Version 1.1. <http://mesquiteproject.org/>.
- 2011 Mesquite: a modular system for evolutionary analysis. Version 2.75. <http://mesquiteproject.org>.
- Massoni J, F Forest, H Sauquet 2014 Increased sampling of both genes and taxa improves resolution of phylogenetic relationships within Magnoliidae, a large and early-diverging clade of angiosperms. *Mol Phylogenet Evol* 70:84–93.
- Melzer S, F Lens, J Gennen, S Vanneste, A Rohde, T Beeckman 2008 Flowering-time genes modulate meristem determinacy and growth form in *Arabidopsis thaliana*. *Nat Genet* 40:1489–1492.
- Meyer CJ, CA Peterson 2011 Casparian bands occur in the periderm of *Pelargonium hortorum* stem and root. *Ann Bot* 107:591–598.
- Moore MJ, PS Soltis, CD Bell, JG Burleigh, DE Soltis 2010 Phylogenetic analysis of 83 plastid genes further resolves the early diversification of eudicots. *Proc Natl Acad Sci USA* 107:4623–4628.
- Murty YS 1959 Studies in the order Piperales. IV. A contribution to the study of vegetative anatomy of three species of *Piper*. *Proc Natl Inst Sci India* 25:31–88.
- Naumann J, K Salomo, JP Der, EK Wafula, JF Bolin, E Maass, L Frenzke, et al 2013 Single-copy nuclear genes place haustorial Hydnoraceae within Piperales and reveal a Cretaceous origin of multiple parasitic angiosperm lineages. *PLoS ONE* 8:e79204.
- Neinhuis C, S Wanke, KW Hilu, K Müller, T Borsch 2005 Phylogeny of Aristolochiaceae based on parsimony, likelihood, and Bayesian analyses of trnL-trnF sequences. *Plant Syst Evol* 250:7–26.
- Nickrent DL, A Blarer, Y-L Qiu, DE Soltis, PS Soltis, M Zanis 2002 Molecular data place Hydnoraceae with Aristolochiaceae. *Am J Bot* 89:1809–1817.
- Oh S, S Park, K-H Han 2003 Transcriptional regulation of secondary growth in *Arabidopsis thaliana*. *J Exp Bot* 54:2709–2722.
- Ohi-Toma T, T Sugawara, H Murata, S Wanke, C Neinhuis, J Murata 2006 Molecular phylogeny of *Aristolochia* sensu lato (Aristolochiaceae) based on sequences of *rbcL*, *matK*, and *phyA* genes, with special reference to differentiation of chromosome numbers. *Syst Bot* 31:481–492.
- Olson ME 2014 Xylem hydraulic evolution, I. W. Bailey, and Nardini & Jansen (2013): pattern and process. *New Phytol* 203:7–11.
- Olson ME, JF Gaskin, F Ghahremani-Nejad 2003 Stem anatomy is congruent with molecular phylogenies placing *Hypericopsis persica* in *Frankenia* (Frankeniaceae): comments on vascentric tracheids. *Taxon* 52:525–532.
- Oskolski A, S Jansen 2009 Distribution of scalariform and simple perforation plates within the vessel network in secondary xylem of Araliaceae and its implications for wood evolution. *Plant Syst Evol* 278:43–51.
- Pace MR, LG Lohmann, V Angyalossy 2009 The rise and evolution of the cambial variant in Bignoniaceae (Bignoniaceae). *Evol Dev* 11: 465–479.
- Pagel M 1994 Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc R Soc B Biol* 255:37–45.

- 1999 The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Syst Biol* 48:612–622.
- Putz FE, N Holbrook 1991 Biomechanical studies of vines. Pages 73–97 in FE Putz, HA Mooney, eds. *The biology of vines*. Cambridge University Press, Cambridge.
- Qiu YL, O Dombrowska, J Lee, L Li, BA Whitlock, F Bernasconi-Quadroni, JS Rest, et al 2005 Phylogenetic analyses of basal angiosperms based on nine plastid, mitochondrial, and nuclear genes. *Int J Plant Sci* 166:815–842.
- Ravindran PN, AB Remashree 1998 Anatomy of *Piper colubrinum* Link. *J Spices Aromat Crops* 7:111–123.
- Rowe N, S Isnard, T Speck 2004 Diversity of mechanical architectures in climbing plants: an evolutionary perspective. *J Plant Growth Regul* 23:108–128.
- Rowe N, C Paul-Victor 2012 Herbs and secondary woodiness—keeping up the cambial habit. *New Phytol* 193:3–5.
- Rowe N, T Speck 2005 Plant growth forms: an ecological and evolutionary perspective. *New Phytol* 166:61–72.
- Samain M-S, L Vanderschaeve, P Chaerle, P Goetghebeur, C Neinhuis, S Wanke 2009 Is morphology telling the truth about the evolution of the species rich genus *Peperomia* (Piperaceae)? *Plant Syst Evol* 278:1–21.
- Schmitz F 1871 Fibrovasalsystem im Blütenkolben der Piperaceen. PhD diss. University of Bonn.
- Schneider EL, S Carlquist 2001 SEM studies on vessel elements of Saururaceae. *IAWA J* 22:183–192.
- Soltis DE, CD Bell, S Kim, PS Soltis 2008 Origin and early evolution of angiosperms. *Ann N Y Acad Sci* 1133:3–25.
- Souza LA, IS Moscheta, KSM Mourão, ALM Albiero, MDC Iwazaki, JHG Oliveira, SMD Rosa 2009 Vegetative propagation in Piperaceae species. *Braz Arch Biol Technol* 52:1357–1361.
- Souza LA, IS Moscheta, JHG Oliveira 2004 Comparative morphology and anatomy of the leaf and stem of *Peperomia dahlstedtii* C.DC., *Otonia martiana* Miq. and *Piper diospyrifolium* Kunth (Piperaceae). *Gayana Bot* 61:6–17.
- Spicer R, A Groover 2010 Evolution of development of vascular cambia and secondary growth. *New Phytol* 186:577–592.
- Tennakoon KU, JF Bolin, LJ Musselman, E Maass 2007 Structural attributes of the hypogeous holoparasite *Hydnora triceps* Drège & Meyer (Hydnoraceae). *Am J Bot* 94:1439–1449.
- Wagner ST, L Hesse, S Isnard, MS Samain, JF Bolin, E Maass, C Neinhuis, NP Rowe, S Wanke 2014 Major trends in stem anatomy and growth forms in the perianth-bearing Piperales, with special focus on *Aristolochia*. *Ann Bot* 113:1139–1154.
- Wagner ST, S Isnard, NP Rowe, M-S Samain, C Neinhuis, S Wanke 2012 Escaping the lianoid habit: evolution of shrub-like growth forms in *Aristolochia* subgenus *Isotrema* (Aristolochiaceae). *Am J Bot* 99:1609–1629.
- Wanke S, F González, C Neinhuis 2006 Systematics of pipevines: combining morphological and fast-evolving molecular characters to investigate the relationships within subfamily Aristolochioideae (Aristolochiaceae). *Int J Plant Sci* 167:1215–1227.
- Wanke S, MA Jaramillo, T Borsch, M-S Samain, D Quandt, C Neinhuis 2007a Evolution of Piperales—*matK* gene and *trnK* intron sequence data reveal lineage specific resolution contrast. *Mol Phylogenet Evol* 42:477–497.
- Wanke S, L Vanderschaeve, G Mathieu, C Neinhuis, P Goetghebeur, MS Samain 2007b From forgotten taxon to a missing link? the position of the genus *Verhuellia* (Piperaceae) revealed by molecules. *Ann Bot* 99:1231–1238.
- Wheeler EA, P Baas, PE Gasson 1989 IAWA list of microscopic features for hardwood identification. *IAWA Bull* 10:219–332.
- Yuncker TG, WD Gray 1934 Anatomy of Hawaiian *Peperomias*. 10. Bernice P Bishop Museum, Honolulu, HI.
- Zanis MJ, DE Soltis, PS Soltis, S Mathews, MJ Donoghue 2002 The root of the angiosperms revisited. *Proc Natl Acad Sci USA* 99: 6848–6853.