



Growth Form Evolution in Piperales and Its Relevance for Understanding Angiosperm Diversification: An Integrative Approach Combining Plant Architecture, Anatomy, and Biomechanics

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GROWTH FORM EVOLUTION IN PIPERALES AND ITS RELEVANCE FOR UNDERSTANDING ANGIOSPERM DIVERSIFICATION: AN INTEGRATIVE APPROACH COMBINING PLANT ARCHITECTURE, ANATOMY, AND BIOMECHANICS

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A striking feature of early angiosperm lineages is the variety of life forms and growth forms, which ranges from herbs, aquatic herbs, climbers, and epiphytes to woody shrubs and trees. This morphological and anatomical diversity is arguably one of the factors explaining how angiosperms dominate many ecosystems worldwide. However, just how such a wide spectrum of growth forms has evolved in angiosperms remains unclear. In this review, we investigate patterns of growth form diversification in Piperales, an early-diverging lineage (with stem age estimated at 201–128 Myr ago) and the most morphologically diverse clade among magnoliids. We outline patterns of growth form diversity and architecture as well as the biomechanical significance of developmental characters, such as the organization, loss, and gain of woodiness. Asaroideae and Saururaceae are terrestrial as well as semiaquatic to aquatic herbaceous perennials bearing rhizomes. The Aristolochioideae and Piperaceae show higher levels of growth form diversity and biomechanical organization, with complex patterns of increasing or decreasing woodiness and architectural organization. The climbing habit has probably evolved independently in the Aristolochiaceae and Piperaceae, while mechanically unstable shrubs and, less frequently, treelets have evolved several times within these two most species-rich clades. A key developmental character underlying diversity in most Piperales—with the exception of the herbaceous *Saruma* (Asaroideae)—is the conserved development of the wood cylinder, in which fusiform initials are limited to fascicular cambial initials. The resulting large fraction of raylike tissue in the stem—a highly characteristic feature of woody species in the Piperales—potentially introduced mechanical constraints on the diversification of self-supporting architectures. This was possibly circumvented by the architectural development of repeated, large-diameter meristems in some shrublike habits via sympodial growth. Patterns of growth form evolution within Piperales potentially mirror some of the overall trends observed among early-diverging angiosperms as a whole as well as angiosperms in general. These include profound changes in life form and growth form linked to large-scale transitions in woodiness, diversity of mechanical organization, and shifts in architectural development.

Keywords: architecture, anatomy, biomechanics, evolution, growth form, Piperales.

Online enhancement: appendix.

Introduction

Extant angiosperms dominate many terrestrial ecosystems on Earth, and their diversity of life forms and growth forms contributes to a large part of the physical complexity in ecosystems. Diverse life and growth forms, such as herbs, shrubs, trees, vines, lianas, terrestrial plants, aquatics, and epiphytes, need to adapt to and survive under very different mechanical constraints and at the same time meet other life history re-

quirements, such as light capture, water and nutrient uptake, and reproduction.

To understand the angiosperm radiation and rise to dominance in many ecosystems, efforts have to be made to shed light on how such a spectrum of growth forms and habitat has evolved. Enormous progress has been made in clarifying angiosperm evolutionary history and unequivocally establishing the first branches of extant angiosperm diversity (Soltis et al. 2008). Many studies have established strong support for sister-group relationships of Amborellales followed by Nymphaeales and Austrobaileyales—the ANITA grade—to all other extant angiosperms (Qiu et al. 1999; Jansen et al. 2007; Moore et al. 2007). Following the diversification of the ANITA grade,

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the remaining angiosperms—or mesangiosperms—included magnoliids, Chloranthaceae, monocots, Ceratophyllaceae, and eudicots. Well-resolved phylogenies for these early-diverging lineages have been extensively used in recent years as a framework to infer the habit and ecology of the earliest angiosperms (Feild et al. 2004, 2009, 2011*b*; Feild and Arens 2005, 2007; Coiffard et al. 2006). However, difficulties in identifying the sister group of angiosperms and the paucity of fossils leave the picture of the earliest angiosperms still unclear. This is reflected in the variety of ecological scenarios linked to ancestral angiosperms (Feild et al. 2004; Coiffard et al. 2007; Feild and Arens 2007; Friis and Crane 2007).

Whatever the ecological conditions and putative habit of the ancestral angiosperm, the early-diverging lineages of extant angiosperms show a diversity of forms ranging from woody trees and shrubs (e.g., Amborellales, Austrobaileyales, Chloranthales), woody vines (Austrobaileyales, Magnoliales, Laurales, Piperales), and herbs with limited woody development (Chloranthales, Piperales) to herbs lacking wood and often linked to aquatic life forms (Nymphaeales; Feild and Arens 2005; Spicer and Groover 2010; fig. 1). The diversity of forms among these lineages is a convincing demonstration that ancestral angiosperm growth habits and life histories may have involved one to several potential habitats.

In addition to identifying the habitats and growth forms of ancestral angiosperms, a further difficulty exists concerning the functional significance of morphological and anatomical

traits of the vegetative body plan that enabled high diversity and competitive physiologies. Exciting recent discoveries have focused on the comparative functional biology of angiosperms, particularly with respect to water transport, appearance of vessels, and ecophysiology (Hacke et al. 2007; Sperry et al. 2007; Hudson et al. 2010; Feild et al. 2011*a*). These have shown that vessel evolution per se was not necessarily associated with greater stem hydraulic efficiency or safety. Instead, small increases in the hydraulic efficiency of primitive vessels over tracheids enabled other functional specializations in the wood, as proposed in the heteroxylly hypothesis (Sperry et al. 2006). Simple perforation plates as well as the length and frequency of vessels in wood probably represented the critical adaptation for producing large hydraulic gains during angiosperm evolution (Sperry et al. 2007). Finally, at the risk of simplification, the separation of functions within the stem—with vessels for water transport, a matrix of fibers for mechanical support, and varying amounts of axial parenchyma for storage—is suggested to have played a crucial role in angiosperm diversification.

Recent findings based on extant angiosperms and the fossil record include further evidence that angiosperm success was potentially linked with ecophysiological functional advantages based on leaf vein density, stomatal geometry, and photosynthetic capacity (Boyce et al. 2009; Brodrribb and Feild 2010; Feild et al. 2011*a*, 2011*b*). However, adaptation of early angiosperm ecophysiology did not initially promote

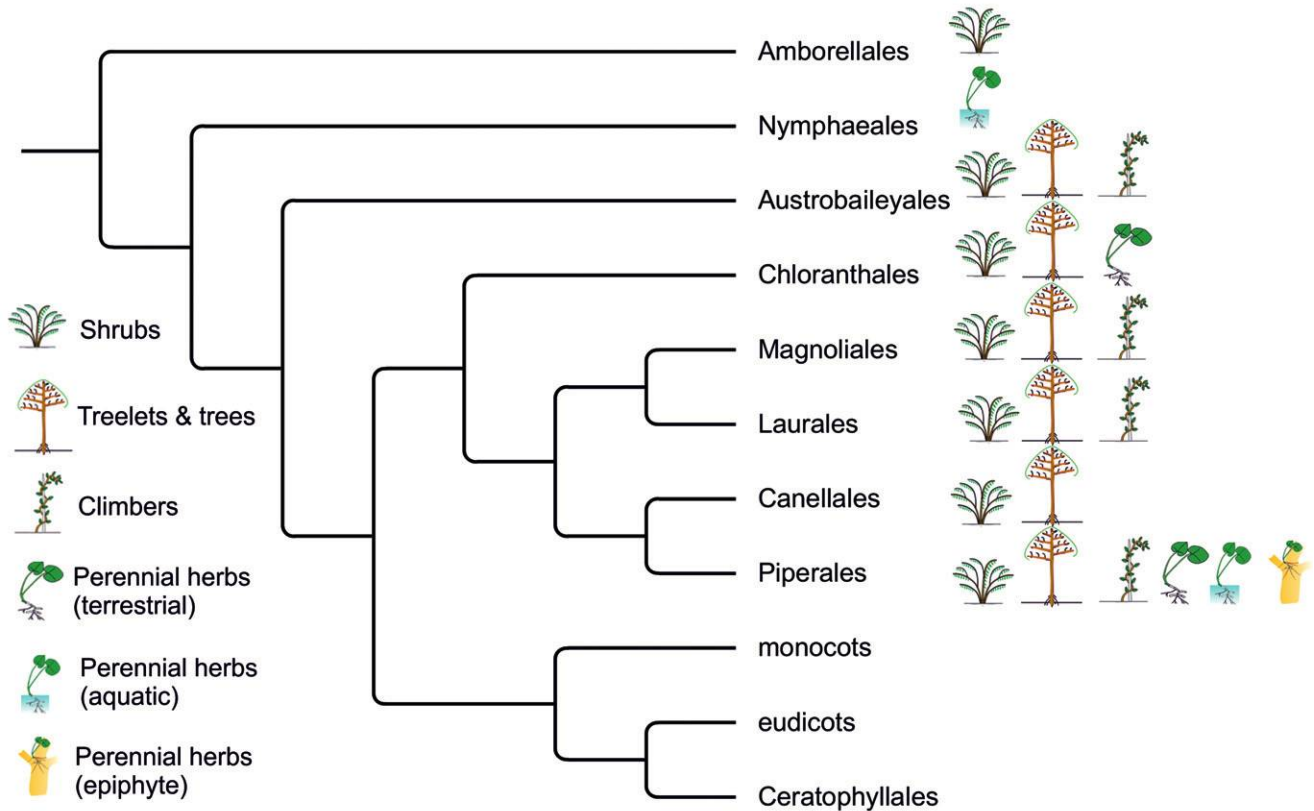


Fig. 1 Summary topology of the current picture of early-diverging lineages of angiosperms from Stevens (2001 onward) illustrating prevailing growth and life form occurrences. Information is compiled from different sources (Stevens 2001 onward; Heywood et al. 2007; Mabberley 2009).

massive diversification and ecological dominance. Instead, innovations in the leaves and wood were co-opted gradually for new roles during the rise to dominance of angiosperms in a wide range of habitats.

Comparative functional studies of extant angiosperm lineages therefore have a lot to offer regarding how we can advance our understanding of early angiosperm diversification. Moreover, it is becoming increasingly clear that studies focusing on further aspects of plant function are likely to provide a more complete picture. From our point of view, comparative studies based on phylogenetic analyses that focus on functional attributes of growth form and plant architecture will provide information relevant to understanding the angiosperm radiation and rise to dominance. Trees, shrubs, vines, aquatics, and epiphytes, to cite a few, show complex arrays of integrated traits linking hydraulics, mechanics, and plant architecture, which are particularly relevant for understanding the growth form diversity of angiosperms.

The Piperales as a model for deciphering diversification of growth forms among angiosperms. Mesangiosperms have been shown to diversify rapidly, within a few million years, and consequently represent the “big bang” of angiosperm evolution (Soltis et al. 2008). Magnoliids are sister to a remarkable diversity of monocots and eudicots and thus are also the closest outgroup in comparative analysis of form, genetics, genomics, and ecophysiology (Jansen et al. 2007; Moore et al. 2007). The Piperales, which comprises more than 4300 species, is morphologically the most diverse group within the magnoliids (fig. 1). The group embraces virtually all growth and life forms known in vascular plants, including geophytes, epiphytes, aquatics, parasites, annuals, perennials, herbs, succulents, lianas, shrubs, and trees. This diversity of form is arguably symptomatic for the success of the Piperales in many ecosystems. Piperales is a relatively early-diverging lineage, with stem age estimated at 201–128 Myr ago (for relaxed and constrained penalized likelihood; Magallón and Castillo 2009). Crown age estimation also places the Piperales among early-diversifying clades, at 120–175 Myr ago (for relaxed and constrained penalized likelihood; Magallón and Castillo 2009). Thus, this early-diverging lineage underwent tremendous growth and life form diversification along with all their respective adaptations. Taxonomic relationships in the order have been progressively untangled over recent years (Nickrent et al. 2002; Neinhuis et al. 2005; Wanke et al. 2006, 2007a; Jaramillo et al. 2008; Samain et al. 2009b, 2010), and current phylogenetic trees provide well-supported phylogenies from the family to infrageneric levels. The objective of this article is to shed light on some of the key functional and architectural traits that underlie the evolutionary transitions within the Piperales and to discuss these within the context of early angiosperm diversification. We present an overview of growth form evolution within the Piperales in which we combine recent field observations of representative species of the main clades along with the key changes in anatomical traits, biomechanics, and plant architecture.

Material and Methods

Observations of growth forms within Piperales and sampling for anatomy, biomechanics, and architectural studies

are based on plants studied and collected in the field (Mexico, French Guyana, Colombia, China, and India) as well as from greenhouse and garden collections in Dresden and Gent (appendix, available in the online edition of the *International Journal of Plant Sciences*). Growth forms were observed in the field for a range of species representing the Piperales and for a range of developmental stages. Stem segments were pruned along the main axes and branches and submitted to biomechanical tests and anatomical analysis. Biomechanical tests were carried out in the field or in the greenhouse on freshly collected material. Stems were measured using standard three-point bending tests following standard protocols for measuring Young's elastic modulus in bending, which are detailed elsewhere (e.g., Lahaye et al. 2005; Rowe et al. 2006). These values provide a means of comparing the stiffness (Young's modulus, E) of the stem tissues for stem segments across different developmental stages, clades, and growth forms.

Segments of tested stems were prepared for anatomical observation using standard techniques. Macroanatomical sections were prepared using a vibratome (Microm HM 650 V; Thermo Scientific) or a sliding wood microtome. Sections were stained using a variety of methods for distinguishing lignified from nonlignified tissues depending on the wide range of materials examined, including carmine green, phloroglucinol-HCl, and toluidine blue.

The architectural analysis, a field approach, followed the concepts and methods established by Hallé and Oldeman (Hallé and Oldeman 1970; Hallé et al. 1978) and developed by Edelin (Edelin 1984, 1991) and Barthélémy and colleagues (Barthélémy et al. 1989, 1991; Barthélémy and Caraglio 2007). The method consists of four main steps: (a) selection of plant individuals at various developmental stages, from seedling to adult (fertile) to senescent individuals; (b) identification of axis categories, mode of branching, and pattern of development of meristems; (c) comparisons of architectures of the different stages to determine the species' developmental pattern; and (d) identification of repetitions of elementary developmental patterns within the organism—a general process termed “reiteration.” Reiteration is a widespread morphogenetic process by which plants duplicate their own architecture spontaneously during their development as well as following injury (Oldeman 1974).

For analyzing the ancestral character states within Piperales, we conducted a parsimony reconstruction with Mesquite 2.75 (Maddison and Maddison 2011). This method finds the ancestral state, which minimizes the number of steps of character changes based on the given tree and observed character distribution. The topology used was obtained from recent phylogenetic studies (Wanke et al. 2007a, 2007b). Character states were treated as “unordered,” allowing free transformation of one state of a character to any other state. Reconstructed nodes with equivocal states are shown in gray.

Terminology. In this article, we use the term “growth form” as a synonym of the equally frequently used “growth habit.” Both of these terms refer to the ultimate form of a plant as expressed in its physiognomy by an overall appearance (e.g., herb, shrub, tree, climber). Different growth forms have different life histories and can develop highly contrast-

ing mechanical organizations reflecting physical and environmental factors (Speck and Rowe 1999). Growth form, which refers to the ultimate expression of the organism, is therefore not to be confused with architecture, which is a dynamic process involving a precise differentiation sequence (Hallé et al. 1978).

We adopt a broad definition of the term “herbaceous” (Eiten 1991; Mori et al. 2002) for this study of Piperales. For example, we use this term to refer to plants that (a) show little or no development of secondary cambial vascular tissues in the aerial part of the stem, (b) do not produce a cork cambium, and (c) may develop an underground rhizomelike stem with or without secondary vascular cambial tissues.

Results

Patterns of Growth Form Diversification

Asaroideae. The small subfamily Asaroideae contains two genera with an exclusively temperate distribution: the monospecific *Saruma*, which is endemic to central China, and *Asarum*, which comprises ~85 species from temperate areas of North America, Europe, and Asia (Kelly 1998). *Saruma henryi* is an erected perennial, rhizomatous herb that reaches up to ~50 cm in height. All species of *Asarum* are small, perennial, terrestrial rhizomatous herbs (Kelly 1998).

Aristolochioideae + Lactoris. This clade contains ~550 species with a mostly tropical and subtropical distribution (Wanke et al. 2006). Growth forms include small herbs, herbaceous to woody climbers, and small to large shrubs. A wider diversification of growth forms may have arguably occurred in *Aristolochia*, since both *Lactoris* and *Thottea* contain shrublike species exclusively (fig. 2).

Lactoris fernandeziana is a small, woody shrub reaching ~1.5 m in height (Hallé et al. 2007) that belongs to the monospecific family Lactoridaceae (González and Rudall 2001). The sister group relationship of this species with the Aristolochioideae is well supported by a recent molecular study (Wanke et al. 2007a).

The genus *Thottea* includes ~35 species of shrubs (up to 2–3 m in height) or subshrubs (less than a meter in height) distributed from Southeast Asia to India (Hou 1981; Oel-schlägel et al. 2011). The genus *Aristolochia* contains ~500 species, with the majority being herbaceous or woody twiners. *Aristolochia* is divided into three monophyletic subgenera, *Isotrema*, *Pararistolochia*, and *Aristolochia* (Wanke et al. 2006). The shrub habit in *Aristolochia* occurs only in the subgenus *Isotrema* and is restricted to a few understory species in forests of Central America (S. T. Wagner, S. Isnard, N. P. Rowe, M.-S. Samain, C. Neinhuis, and S. Wanke, unpublished manuscript). The subgenus *Pararistolochia* is represented by more than 20 species from Africa and Australasia, all being climbers. Finally, the subgenus *Aristolochia*, which has a worldwide distribution, contains herbaceous species and climbers.

Hydnoraceae. Hydnoraceae are distinctive plants, being referred to as the “strangest plants in the world” (Musselman and Visser 1986). Hydnoraceae is a subterranean holoparasitic lineage found in semiarid regions whose phylogenetic relationships among Piperales are still unclear (Nickrent et al.

2002). The family contains two genera (*Hydnora*, *Proso-panche*); all species are leafless, achlorophyllous, root-parasitic herbs with flowers that are frequently semisubterranean, developing from the rooting body. The vegetative body has been found to consist of a rhizome (Tennakoon et al. 2007). The peculiar subterranean habit and a highly modified morphology as parasitic plants bear little resemblance with other herbs, shrubs, or climbers found elsewhere in the Piperales. The lineage has therefore not been included in our study.

Saururaceae. The small family Saururaceae consists of four genera containing six species, with a north temperate (Asia, North America) distribution. The family includes terrestrial rhizomatous herbs (*Gymnotheca*) as well as semiaquatic rhizomatous herbs (*Saururus*, *Houttuynia*, *Anemopsis*).

Piperaceae. The Piperaceae has a pantropical distribution and is a highly diverse clade in terms of species richness (~3700 species) and growth form diversity, specifically in comparison to its sister group Saururaceae, which has only 6 herbaceous species. The large majority of species occur in the genera *Piper* and *Peperomia*, both of which are in the top 10 of the most species-rich genera of angiosperms (Frodin 2004); both genera are found in tropical regions throughout the world (Smith et al. 2008). A relatively ancient stem age in the late Cretaceous has been proposed as the estimated diverging time for *Piper* and *Peperomia*. The major diversification in both lineages may, however, have occurred much later in the Tertiary (Smith et al. 2008). This would suggest that in both lineages a long period of stasis was followed by a relatively recent diversification.

This species richness in *Piper* and *Peperomia* contrasts with the species-poor sister lineages *Manekia* and *Zippelia* (7 species in total). The genus *Verhuellia*, with two accepted species formerly considered as synonyms of or at least closely related to *Peperomia*, has recently been discovered as a new branch that is sister to all other Piperaceae (Wanke et al. 2007b; Samain et al. 2010; fig. 2). *Verhuellia* is a delicate lithophytic creeping herb from Hispaniola and Cuba. Its new phylogenetic position has thus raised the question of ancestral character states within the Piperaceae in terms of growth form (Wanke et al. 2007b). *Zippelia begoniifolia* (a monospecific genus) is a large herb from South Asia, while the genus *Manekia* (6 species) contains root climbers from Central America, South America, and the Caribbean.

Piper s.l. includes ~2000 species, with the greatest diversity of species occurring in the Neotropics (1300 species), followed by southern Asia (600 species) and the South Pacific (100 species; Quijano-Abril et al. 2006; Jaramillo et al. 2008). Current analyses support three major clades that correspond to these major biogeographic regions (Jaramillo and Manos 2001; Jaramillo et al. 2008).

Despite the range of growth forms observed in *Piper* (fig. 2), most species grow as shrubs or treelets in the understory of wet forests. In all clades of Neotropical *Piper*, most species grow as small to medium-sized shrubs or treelets, while herbs or climbing species occur scarcely in some clades (Jaramillo et al. 2008). In certain clades, some species are described as “climbing shrubs” or “sarmentose shrubs” (Jaramillo et al. 2008); some truly climbing species (stem twiners) nevertheless occur in the Neotropics and can be common locally (fig. 10A). The most common climbing habit in *Piper* corresponds

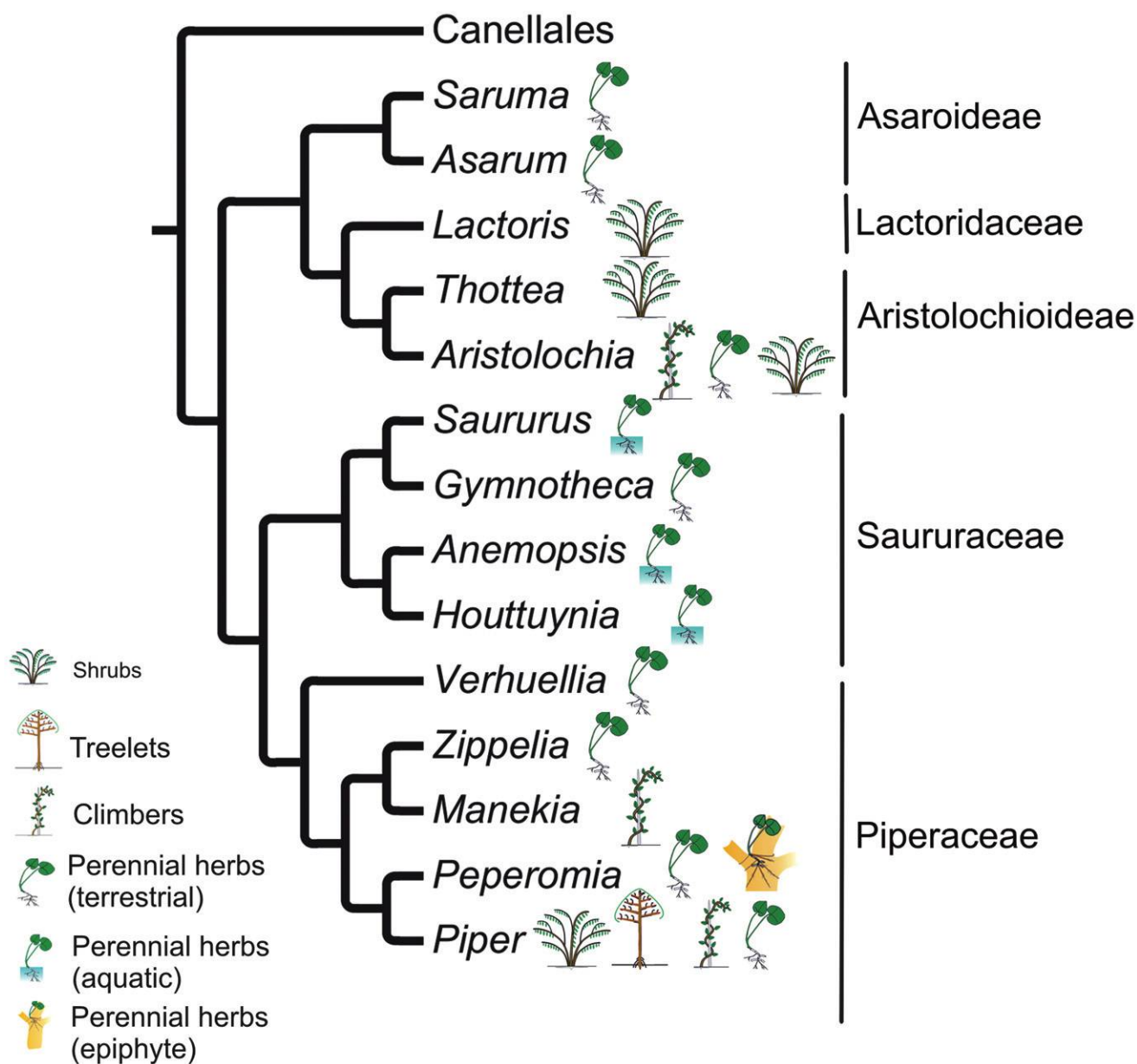


Fig. 2 Phylogeny of Piperales (modified from Wanke et al. 2007a, 2007b) illustrating the distribution of growth and life forms. Because of the sister-group relationship of *Lactoris* and Aristolochioideae, the Aristolochiaceae family is not monophyletic, and the two monophyletic subfamilies, Asaroideae and Aristolochioideae, are treated separately in this article. Herbaceous perennials, including terrestrial and aquatic life forms, occur iteratively in the phylogeny. Asaroideae and Saururaceae are exclusively herbaceous. The epiphytic habit evolved only in *Peperomia*. The shrub habit characterizes most of the woody clades, while the treelet habit occurs only in the genus *Piper*. The climbing habit includes vines (without significant wood in the stem) and lianas (woody climbers) and evolved in *Manekia*, *Piper*, and *Aristolochia*.

to plants that ascend their host trees by using adventitious roots produced from thickened nodes of the stem. This type of climber is frequent in Asian *Piper* (Jaramillo and Manos 2001).

Contrary to its sister clade *Piper*, the genus *Peperomia* lacks a woody habit and is characterized by small herbs. The genus consists of ~1600 species, showing a large radiation of small terrestrial and epiphytic herbaceous perennials (Samain et al. 2009b, 2011; Mathieu et al. 2011; Symmank et al.

2011). *Peperomia* has several species that are known to be widespread, and, contrary to *Piper*, a clear biogeographical pattern is not observed (Smith et al. 2008).

Within *Peperomia*, current topologies (Samain et al. 2009a, 2009b) indicate that the first successive lineages within the genus are terrestrial, forming a grade relative to clades that contain epiphytic lineages with multiple reversals back to either the terrestrial life form (geophytic to lithophytic) or species that can grow as both terrestrial and

epiphytic plants. There is thus only one major shift to epiphytism that can be localized at a specific node in the phylogenetic tree. The clades with several life forms are also more species rich. Diversification of reproductive biology as indicated by the diversity of fruit morphology as well as the differences in fruit adhesion mechanisms may have been particularly important in the group (Martínez-Colín et al. 2006; Samain et al. 2009b).

Summary of Growth Form Diversification in the Piperales

This overview of growth form occurrence in Piperales indicates that herbaceous perennials are widespread and present in all of the main clades (fig. 2). Despite the frequent occurrence of this habit, it often occurs in few species or in species-poor clades (e.g., Saururaceae, *Verhuellia*, *Zippelia*, Asaroideae). One of the most striking characteristics of the Piperales is the dominance of one growth form in species-rich clades—namely, climbers in *Aristolochia*, shrubs, and treelets in *Piper* and small-body epiphytes or terrestrial herbs in *Peperomia*. Woody, self-supporting habits represented by shrubs and treelets have probably evolved independently in Piperaceae and in Aristolochioideae + *Lactoris* and have led to an asymmetry in the type of growth form and ecological dominance in each clade. The climbing habit evolved and diversified at least twice independently in the Piperales—within the Aristolochioideae as twiners and within the Piperaceae essentially as root climbers.

Architectural Analysis of the Piperales: A Tribute to R. E. Holttum's "Variations on a Theme"

Secondary growth and specialization of wood tissues such as vessels and fibers, although indubitably important in the diversification of plant forms, may not be the only hierarchical developmental level underlying growth form diversification. Monocots (~60,100 species; Stevens 2001 onward), for example, have explored a large diversity of growth forms and life forms in the absence of secondary growth, including arboreal forms with modified primary thickening meristems (Holttum 1955; Tomlinson 1973). Much of the structural diversity in monocots arguably can be attributed to reorganization of an existing growth plan—what the botanist Holttum referred to among monocotyledons as “variations of detail within the sympodial pattern” (Holttum 1955, p. 412). Development of the bifacial cambium may certainly allow flexibility in growth form evolution, particularly in terms of mechanical and hydraulic functional traits (Rowe and Speck 2005), as well as in plant construction (Carlquist 2009). However, diversity in plant form may also be brought about by variations in existing organization. It is therefore likely that understanding growth form diversification among angiosperms requires consideration of their organization at different levels, including changes in positioning, timing, and organization of the meristems.

Architectural analysis is an approach that emerged some 40 years ago and aims to understand the overall morphological and developmental organization of plants (Edelin 1984; Barthélémy and Caraglio 2007). It was initially used by Hallé and colleagues and was applied to tropical trees (Hallé et al.

1978). Nevertheless, this method is applicable to all organisms with a modular construction (Tomlinson 1987). Architecture is defined as the morphological expression of the genetic program of a plant during the ontogenetic phases (Hallé et al. 1978), and it results from the activity of all meristems (aboveground and belowground). Architectural studies have much potential interest for the developmental and evolutionary biologist. They allow a rigorous analysis of plant development, which can lead to a better comprehension of the morphological diversity and development process that underline changes in growth habit among clades.

In this study, we appraised recent data from ongoing projects as well as information compiled from the literature. We characterized the main architectural traits of different growth forms, which are representative of the Piperales. We aimed to define precisely the architectural variation found among herbaceous perennials and shrubs within different clades and document the differing patterns of meristem development and their organization. The approach provides a relatively detailed method of comparing growth forms over traditional descriptive approaches for understanding plant diversification.

Architectural Development in the Asaroideae

All species have sympodial growth (i.e., each stem results from a series of meristems) with terminal inflorescences (fig. 3). *Saruma henryi* is an erect, herbaceous plant in which each sympodial growth unit (i.e., module) consists of two leaves and a terminal flower (González 1999a, 1999b; fig. 3A, 3B). Herbaceous stems are produced from a dense rhizome (fig. 3C). Species of *Asarum* are rhizomatous, creeping herbs in which each module has only two to four scale leaves followed by one to three fully developed leaves, terminating in a flower (González 1999a, 1999b; fig. 3D, 3E); these creeping growth forms develop roots at the nodes. The main difference between the two genera concerns the position of the meristems, which are always semisubterranean in *Asarum* but aerial in *Saruma*, in which the herbaceous stem grows vertically by stacks of sympodial units.

Architectural Development in Aristolochioideae and Lactoris

Despite the richness of climbing species in *Aristolochia*, their architecture remains largely unstudied, to our knowledge. Generally speaking, the architecture of lianas has received little attention (Cremers 1973, 1974; Hallé et al. 1978), probably because of their complexity and difficulty of access in the canopy. In a study of inflorescence structure in Aristolochiaceae, González (1999a, 1999b) showed that inflorescences are lateral in many *Aristolochia* except for some Australian species of *Pararistolochia*, in which inflorescences are always terminal. Although González (1999a, 1999b) assigned inflorescence type to either monopodial or sympodial growth, we will see that sympodial growth in Piperales might incorporate differing degrees of meristem life span and cannot be inferred only from inflorescence position. Further studies of climbers in *Aristolochia* will be carried out in the future to complete our picture of growth habit in the Piperales.

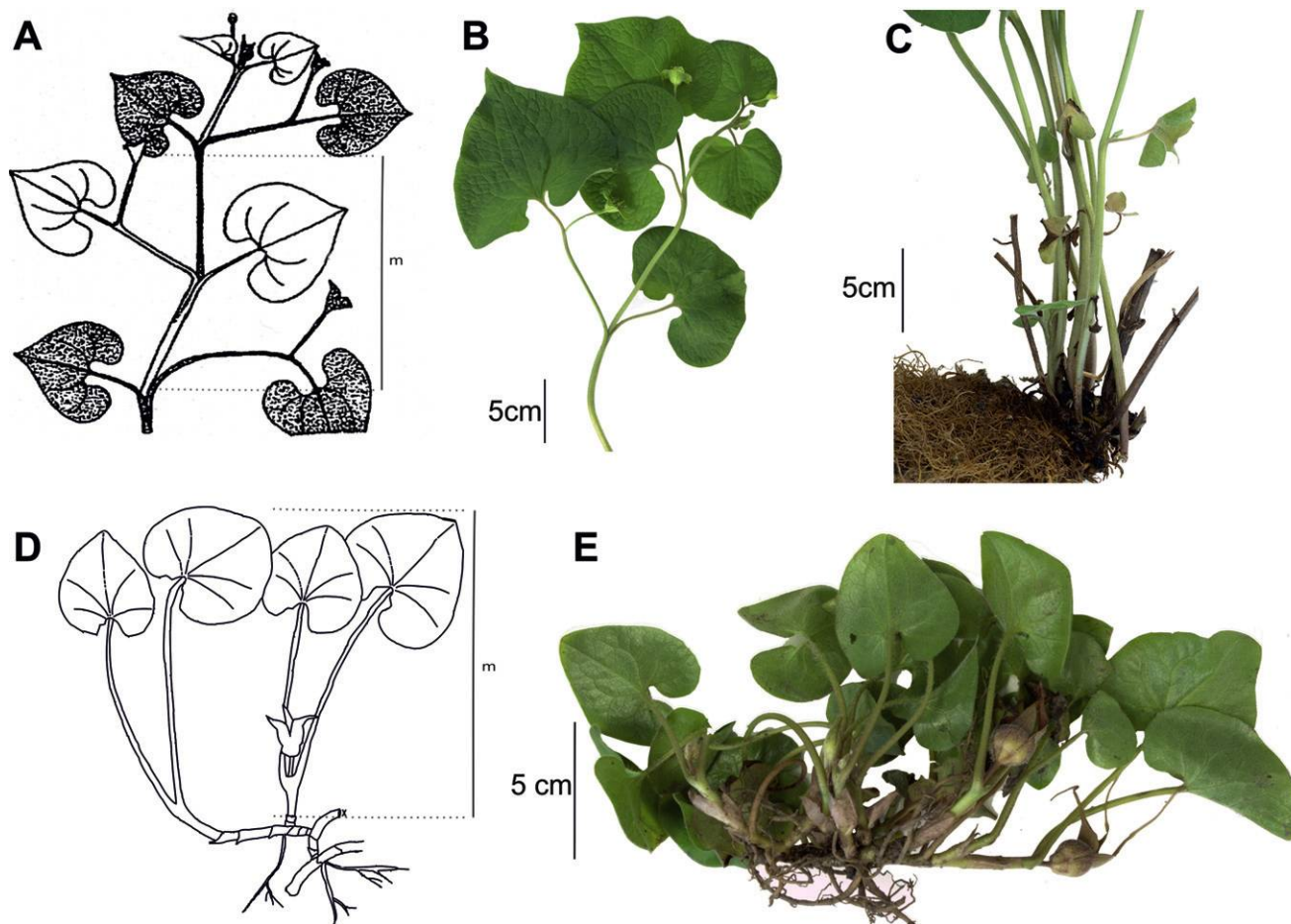


Fig. 3 Homogeneity of sympodial growth in the herbaceous perennial Asaroideae. In both genera, the stem is composed of one to several modules, which consist of few leaves and a terminal flower. *A–C, Saruma henryi*. An aerial erected stem composed of several modules (*A, B*) is produced from an underground rhizome with a dense network of adventitious roots (*C*). *D, E, Asarum*. These hemicyptophyte plants have a sympodial rhizome and short modules with only two leaves and a terminal flower. *D, Asarum canadense*. *E, Asarum caudatum*. m = module. *A* is from González (1999a, 1999b) and is reproduced with permission of the author.

Because shrublike habits evolved in all three genera (*Lactoris*, *Aristolochia*, and *Thottea*) within the Aristolochioideae + *Lactoris* clade (fig. 2), we aimed to characterize and compare the main architectural traits of this growth form within this clade. The following account first describes the architecture of *Aristolochia arborea* (fig. 4), providing a basis for exploring patterns of architectural variation within the clade. In *A. arborea*, the juvenile plant consists of an unbranched stem ~50 cm in height (fig. 4A). The apical meristem produces an initially orthotropic part, followed by a distal plagiotropic part. The architecture of this species is built up of what are termed “mixed axes” (labeled “ma” in fig. 4B, 4C). These consist of (1) a vertical proximal part with a spiral phyllotaxy representing the stem and (2) a horizontal distal part with a distichous phyllotaxy representing the branches. Superposition of such mixed axes at the level of transition builds the architecture of the plant, with the stem being made up of successive proximal vertical parts and the branches of horizontal distal parts (fig. 4B). In such plant development, single meristems produce both stem and branch axis.

Adult plants (~1 m in height) rapidly reach sexual maturity, and flowers develop at the base of the unique stem (fig. 4B, fl). Crown formation occurs when the plant is 2.5–4 m in height (fig. 4C); at this point, the stem, which is about half the total height, is cylindrical and slender (~5 cm in diameter) and is built up by a superposition of similar axes with a mixed structure. The crown (fig. 4D) is built up of long, stacked epitonic (developing from the upper position of parent axis) branches emerging from the region of maximum curvature. These lateral branches may develop partial reiterations (fig. 4D, pr; i.e., representing only a part of the mixed axes, with a reduced orthotropic part) and rarely bear a third order of short branches at the extremity (fig. 4C, A3). During crown formation, one or more similar stems can develop from the collar zone (the interface zone between the stem and root systems), and these correspond to basal reiterations (fig. 4D, tr).

Other shrublike species of *Aristolochia* (subgenus *Iso-trema*) from Mexico as well as species of *Thottea* from the Western Ghats (J. Prosperi et al., unpublished data) exhibit a similar developmental pattern based on a stack of mixed

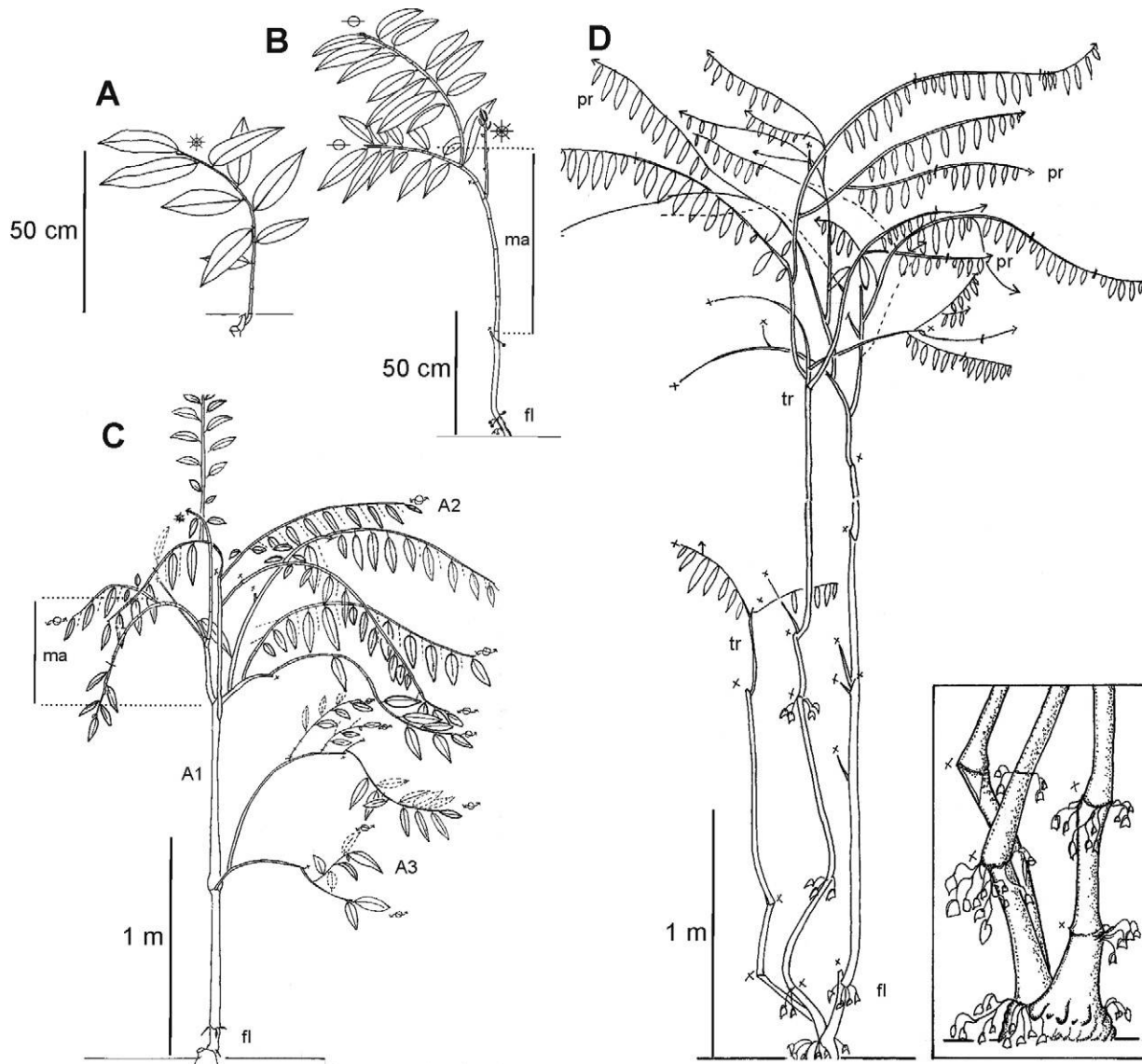


Fig. 4 Architectural development in *Aristolochia arborea* from forest understory habit in Tabasco (Mexico). *A*, Juvenile composed of only one mixed axis (i.e., the elementary module). *B*, Adult individual. The sympodial trunk is built up by stacks of several modules; flowering (fl) occurs at the base of the trunk. *C*, Formation of the crown from stacks of mixed axes, with each mixed axis having a reduced orthotropic growth. *D*, Mature stage with basal total reiterations (tr) and starting point of mechanically unstable habit. Apical plagiotropic branches tend to lean and drape over the surrounding vegetation (*inset*: basal inflorescences). A1, A2, A3 = ramification order, fl = flowers, ma = mixed axis, pr = partial reiteration, tr = total reiteration.

axes (figs. 5, 6). In both *Thottea* and *Aristolochia*, a shift from small subshrubs (less than 30 cm in height) to larger shrubs (up to 5–6 m) is observed among species and corresponds to (a) changes in the number of sympodial units forming the stem, (b) the life span of the apical meristem, (c) the degree of secondary growth, and (d) the frequency of reiteration and inflorescence position (strictly basal to crown; figs. 5, 6).

While such architecture in *Thottea* and *Aristolochia* has been previously assigned to Troll's model (Hallé et al. 1978; Hallé 2004), this mode of development is probably better assigned to Mangenot's model. In both the Troll and the

Mangenot models, the architecture is built up of a continuous superposition of axes with a vertical proximal part and a plagiotropic distal part. The close relationship between these models is indicated by the complete plagiotropy of axes in species conforming strictly to the Troll model, where the proximal part becomes erected most often secondarily, after leaf fall. However, in all *Aristolochia* and *Thottea* species studied the apical meristem always has a vertical growth; the proximal part of axes is thus primarily orthotropic and never becomes erected secondarily. The accurate description of processes leading to similar architecture might increase our understanding of meristematic and cambial processes. In the

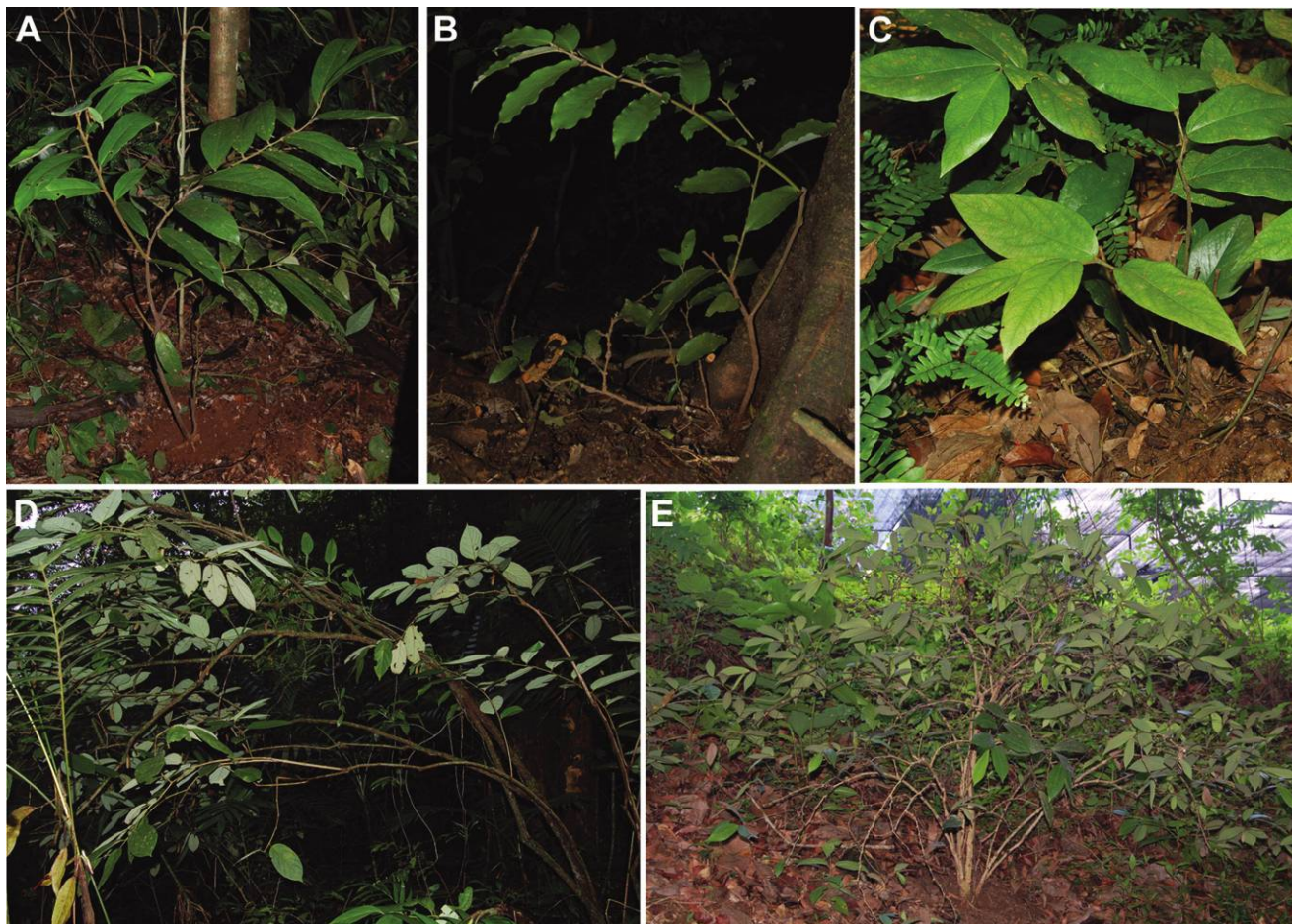


Fig. 5 Shrublike habit in Aristolochioideae. *A*, Juvenile stage of *Aristolochia arborea* from Tabasco (Mexico). Mixed axes (i.e., module) combining orthotropic and plagiotropic growth start to build a small sympodial trunk. *B*, Subshrub of *Aristolochia kalebii* from Chiapas (Mexico). The small “trunk” is also built up of stacks of sympodial relays. Apical branches have lost orthotropy and show only plagiotropic growth, forming an assimilation plateau. The habit of this species is unstable; this subshrub is leaning against a tree. *C*, Subshrub habit in *Thottea abrahamii* from Kerala (Western Ghats, India). The plant does not exceed 30 cm in height, and stems bear few apical leaves on each assimilation plateau. *D*, Adult stage of *Aristolochia tricaudata* from Oaxaca (Mexico). The plant, ~5 m in height, exhibits a mechanically unstable habit. The plant sprouts abundantly from the base. *E*, *Thottea siliquosa* from Kerala (Western Ghats, India). Frequent basal reiterations produce a tufted shrub habit. Stems are not fully self-supporting and bend over.

Troll model reaction wood is involved in the architecture and induces a reorientation of the basal part of shoots, which becomes secondarily erected, while in the Mangenot model orthotropy is an intrinsic character of the growing meristem (Hallé et al. 1978; Tomlinson 1987).

The architecture of *Aristolochia* and *Thottea* represents a variation of sympodial growth, since there is not a strict substitution of the terminal meristem. In strictly sympodial plants, branches usually develop after the death, abscission, abortion, or transformation of the apex (Barthélémy and Caraglio 2007). But there are actually several developmental modes of sympodial branching. In *Terminalia* (Combretaceae), for instance, plagiotropic branches are composed of successive short modules with an indeterminate growth known as “plagiotropic by apposition” (Hallé et al. 1978).

In all Aristolochioideae species studied, the new mixed axis develops when the meristem of the parent axes is still growing. The habit is nevertheless referred to as sympodial

here, since the trunk is built up of several meristems, with each apical meristem having a finite duration.

Lactoris fernandeziana has been described as a profusely branched subshrub with sympodial growth (González and Rudall 2001). In this species, flowers are terminal or lateral, and up to four branching orders can be observed (F. Hallé, personal communication). Despite referring to the Troll model in a brief description of the species, Hallé et al. (2007) described upright axes that bend over apically, each forming an assimilation plateau; complete plagiotropy seems not to occur in *Lactoris*. Aging stems bend over and bear branches with a proximal vertical part and a distal horizontal part (fig. 6C). This species might therefore also be built up following the Mangenot model. In *Lactoris*, basal reiterations are also frequent and produce a multistemmed shrub habit (Hallé et al. 2007).

None of these woody species in the Aristolochioideae and *Lactoris* produce truly large-sized body plans and tree-sized

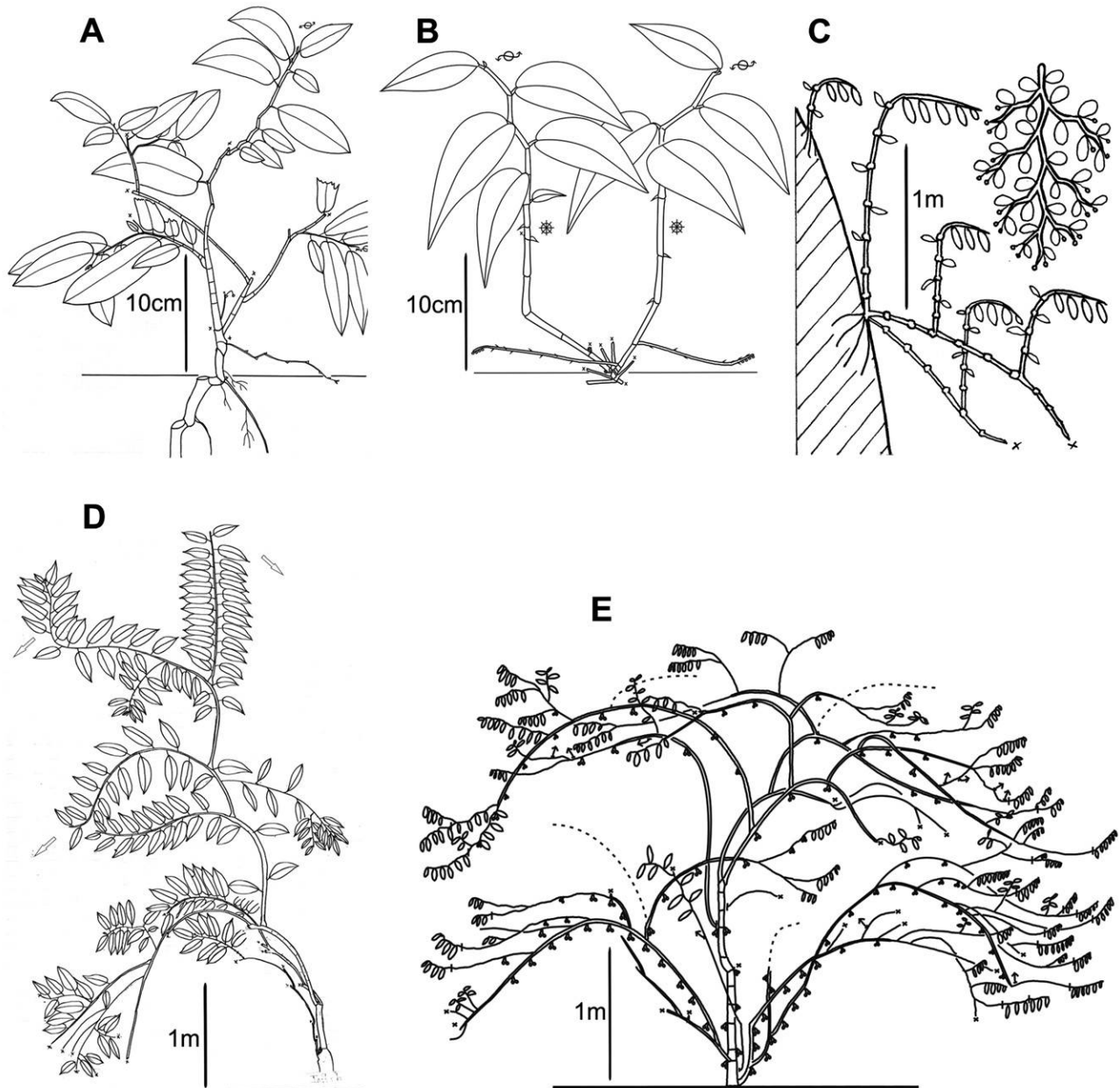


Fig. 6 Homogeneity of shrub construction in Aristolochioideae + *Lactoris*. A, B, Small subshrubs of *Aristolochia kalebii* (A) and *Thottea abrahamii* (B), both composed of mixed axes with short life spans, apical plagiotropy, and an assimilation plateau with few leaves. *Thottea abrahamii* (B) has a unique mixed axis bearing few apical leaves; both species have basal inflorescences. C–E, Variations in size and number of mixed axes as well as frequency of basal reiterations produce different growth habits in mature shrubs. All of these species have a tendency toward unstable mechanical habits. C, *Lactoris fernandeziana*, a small unstable shrub (from Hallé et al. 2007; reproduced with permission of the author). D, *Aristolochia arborea* growth habit of large individual with a tendency to lean against the surrounding vegetation. Apical branches lean into neighboring vegetation (arrows). E, *Thottea siliquosa*, a densely branched shrub with numerous basal and apical reiterations.

dimensions. The largest shrubs that we observed in the field tend to develop flexible and curved axes (figs. 5, 6). In the large-bodied species of *A. arborea*, the largest individuals observed actually showed habits that are mechanically unstable (not fully self-supporting) although rarely exceeding 6 m in height. In such plants, long and flexible apical

axes drape over and lean on the surrounding vegetation (fig. 6D).

In summary, most species of Aristolochioideae + *Lactoris* are built up of stacks of mixed axes and reiterations, as described for *A. arborea* (fig. 4). Order of branching does not exceed three in Aristolochioideae, while it may reach four in *Lactoris*.

The position and frequency of reiterations (basal to apical) determine crown structure as well as tufted growth habits.

Architectural Development in Saururaceae

All Saururaceae species have sympodial growth with terminal inflorescences and adventitious roots that are linked with herbaceous rhizomatous growth habits (fig. 7). *Saururus*, *Houttuynia*, and *Gymnotheca* are all herbaceous and have upright sympodial stems developing from various types

of sympodial rhizome (fig. 7A, 7B, 7D). *Anemopsis* consists of a short, thick underground stem with very short internodes, bearing a rosette of leaves (fig. 7C). In *Saururus cernuus* and *Houttuynia cordata*, branching is continuous, and inflorescences are terminal and opposed to leaves (Blanc and Andraos 1983; fig. 7D). An immediate growth relay develops in the axil of the last leaf below the inflorescence and continues the growth of the upright stem (Blanc and Andraos 1983). Sympodial branching and a terminal inflorescence are also observed in *Gymnotheca* (fig. 7B).

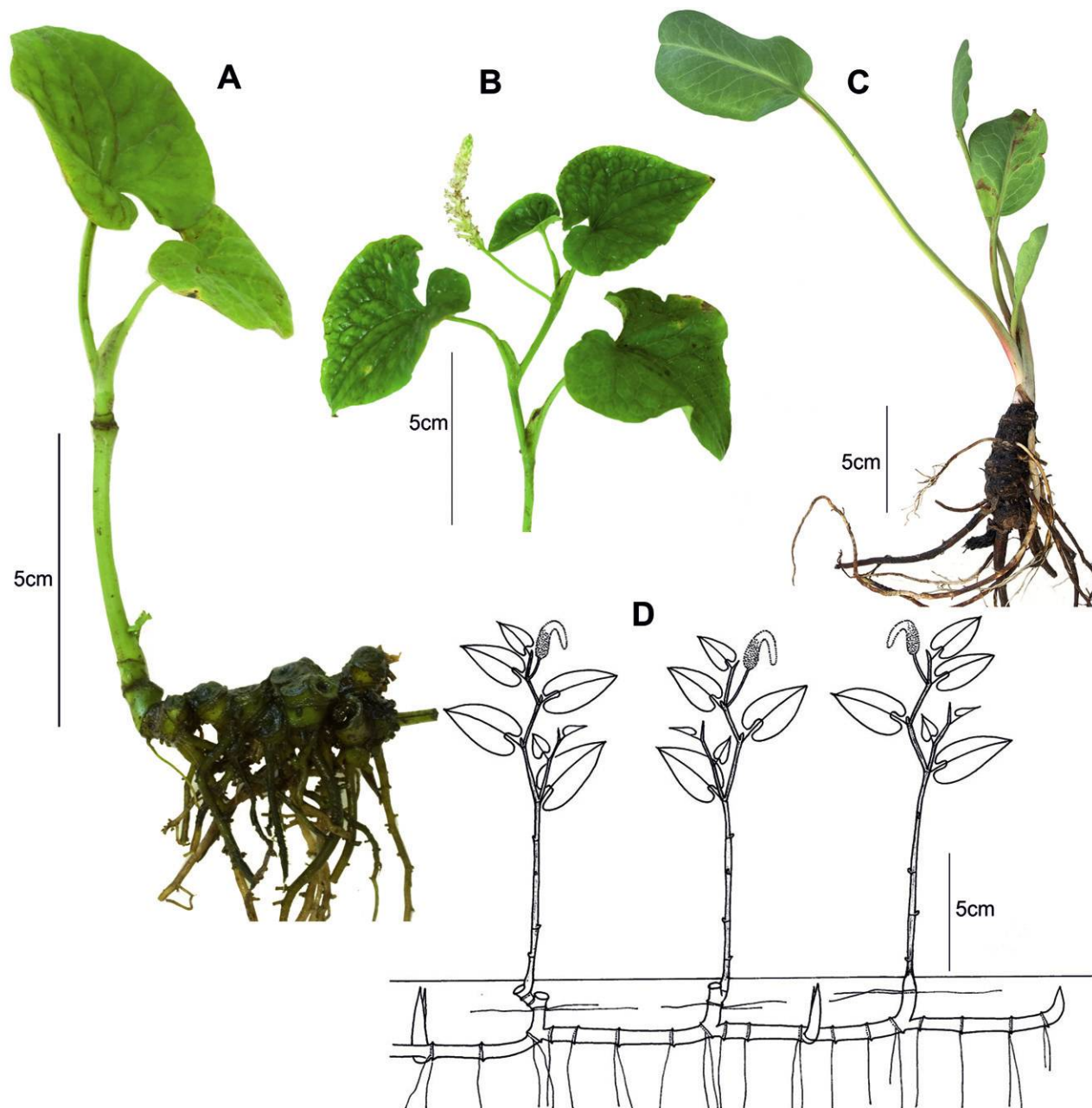


Fig. 7 Sympodial growth and rhizome organization in the herbaceous perennial Saururaceae. A, B, *Gymnotheca chinensis* with sympodial herbaceous stem and sympodial rhizome. C, Growth habit of *Anemopsis californica* with thick underground stem producing a rosette of leaves. D, Rhizome and sympodial herbaceous stem of *Saururus cernuus* (from F. Hallé, unpublished data; reproduced with permission of the author).

Architectural Development in Piperaceae

Piperaceae, the sister clade of Saururaceae, exhibit the largest diversity of growth forms in the Piperales (figs. 2, 8, 9, 10) and include the evolution of monopodial growth.

Verhuellia lunaria is a minute, herbaceous, saxicolous creeping perennial with monopodial growth, whorled leaves, and flowers arranged in solitary lateral spikes (Samain et al. 2010; fig. 8A). The monotypic *Zippelia begoniifolia* is a perennial herb with erect stems (fig. 8B), which can reach ~40–80 cm in height. Adventitious roots form at basal nodes; inflorescences are terminal and opposed to leaves. The stem consists of a short orthotropic monopodial part composed of few internodes, followed by a plagiotropic part with a sympodial growth (fig. 8B). The first terminal inflorescence, giving rise to the sympodial growth, follows the initiation of plagiotropy. A sympodial branch results from a linear succession of short modules with one internode and a terminal inflorescence opposed to the leaf.

Manekia, consisting of root-climbing species, grow as monopodial stems (Blanc and Andraos 1983); infertile individuals of *Manekia sydowii* climb up to 10–15 m on supporting trees, where they branch actively via multiple reiterations in their crown (figs. 9A, 9C, 12B). In *M. obtusa* and *M. incurva*, lateral branches are limited by the production of a terminal inflorescence (Blanc and Andraos 1983).

Despite the diversity of growth forms found in *Piper* (figs. 2, 10, 11), they have been shown to be remarkably uniform in their construction, with most species built up following the architectural model of Petit (Hallé et al. 1978; Blanc and

Andraos 1983; fig. 12A). The main orthotropic stem is monopodial and produces lateral, plagiotropic branches, which are sympodial. Plagiotropic branches (second branching order) result from successive hypotonic branching (development of branches on a basal position of the parent axis) of short modules with a terminal inflorescence (fig. 12A), giving rise to horizontal branched systems named “plagiotropic by substitution” (Hallé et al. 1978). Each module is composed of a short internode (hardly perceptible and called “subnull”) bearing a prophyll and a fully developed internode bearing a leaf and a terminal inflorescence (Blanc and Andraos 1983). These lateral branches may bear up to two branching orders, which are built in a similar way as lateral branches. Thus, *Piper* species may have up to four branching orders, which differ from species of Aristolochioideae studied (see above), in which only third-order branching has been observed.

Variation of growth form in *Piper* is mostly due to the duration of the growth of the main monopodial stem and the frequency and position of reiteration (Blanc and Andraos 1983; fig. 12C). Species with a long period of monopodial growth develop branches with a short life span, which are lost by natural pruning; this mode of growth characterizes treelet species. Species with shorter monopodial growth develop branches with a longer life span that continue their growth throughout the life of the plant; this mode of growth is found in shrubs and more densely branched species from the understory. Dwarf habits in some *Piper* species have developed via reduction in the size and number of modules without a change in the basic architectural model (Jaramillo

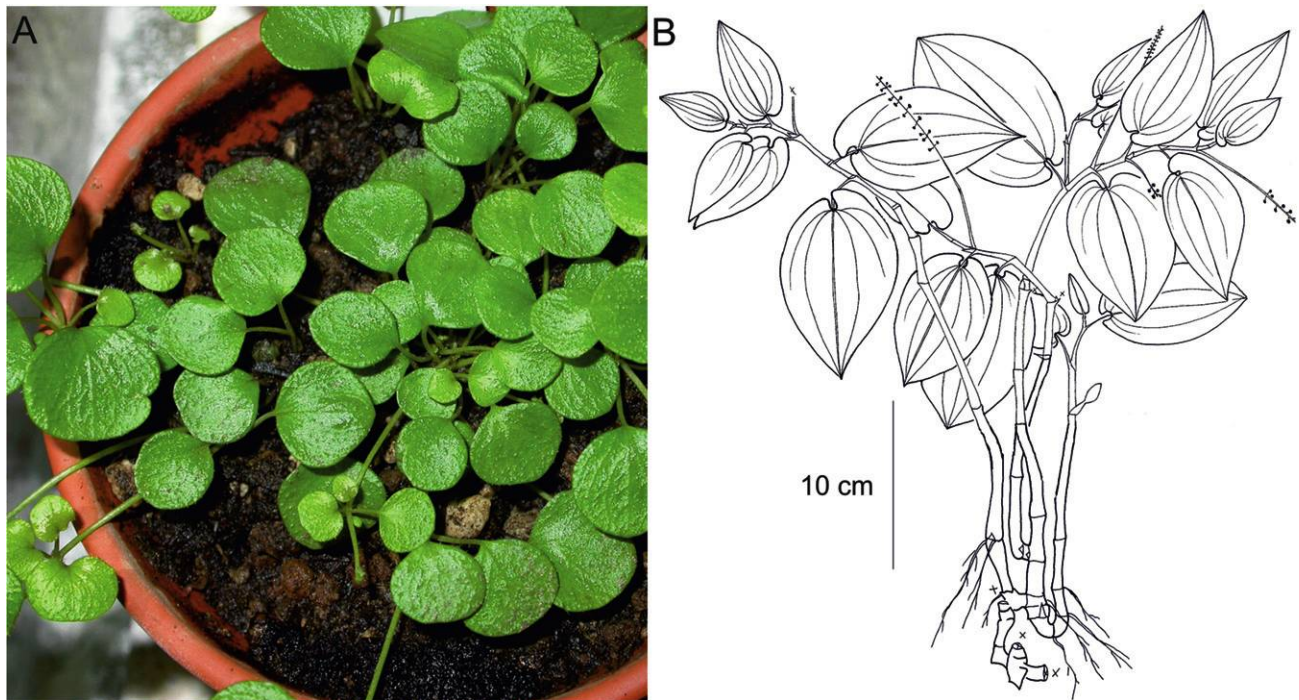


Fig. 8 Creeping and herbaceous habit in Piperaceae. *A*, *Verhuellia lunaria*, a delicate creeping herb. *B*, Monotypic perennial herb *Zippelia begoniifolia* from Yunnan (China). Visible are erect stems with terminal inflorescences opposed to leaves. The stem consists of a short orthotropic monopodial part, followed by a plagiotropic part with a sympodial growth.

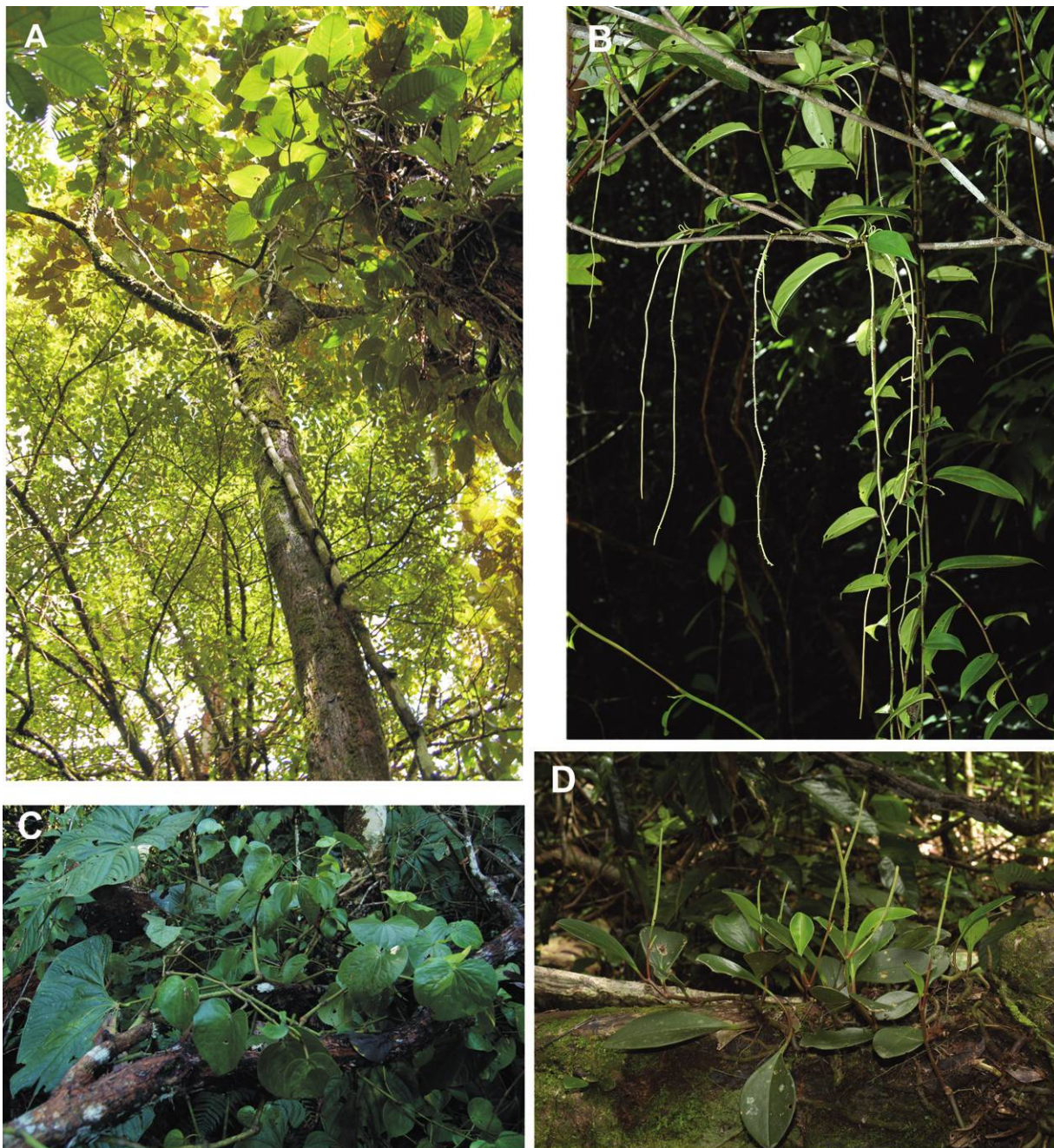


Fig. 9 Climbing and epiphytic habit in Piperaceae. *A, C*, Large climbing stem of the root climber *Manekia sydownii* from Antioquia (Colombia). The species branches actively in the canopy through dense reiterations, observed here in a tree-fall gap (*C*). *B*, Epiphytic pendulous herb of *Peperomia macrostachya*, rooted in ant nests perched in trees ~3–5 m from the ground in forest margin. *D*, Epiphytic herbs of *Peperomia magnoliifolia*. Individuals commonly grow on fallen branches in forest understory. Stems are upright to creeping or even climbing and attach via adventitious rootlets.

and Callejas-Posada 2004). Climbing species of *Piper* are characterized by a main stem adhering to the host tree by adventitious roots (fig. 10*B*) or more seldom by stem winding (fig. 10*A*). The main difference in architecture between root-climbing species and other growth forms lies in the indeterminate development of the monopodial stem and the later development of lateral branches, which occur several meters above ground level and sometimes only in the canopy.

In *Peperomia*, a large diversity of small-bodied growth habits have been documented (fig. 9*B, 9D*), linked to changes in stem orientation, growth dynamic, phyllotaxy, position, and arrangement of inflorescences, among others (Blanc and Andraos 1984; Samain et al. 2009*b*). In some species, the internodes seem to be extremely reduced so that the leaves virtually appear in whorls (Kwiatkowska 1999). Most of the epiphytic species possess adventitious roots.

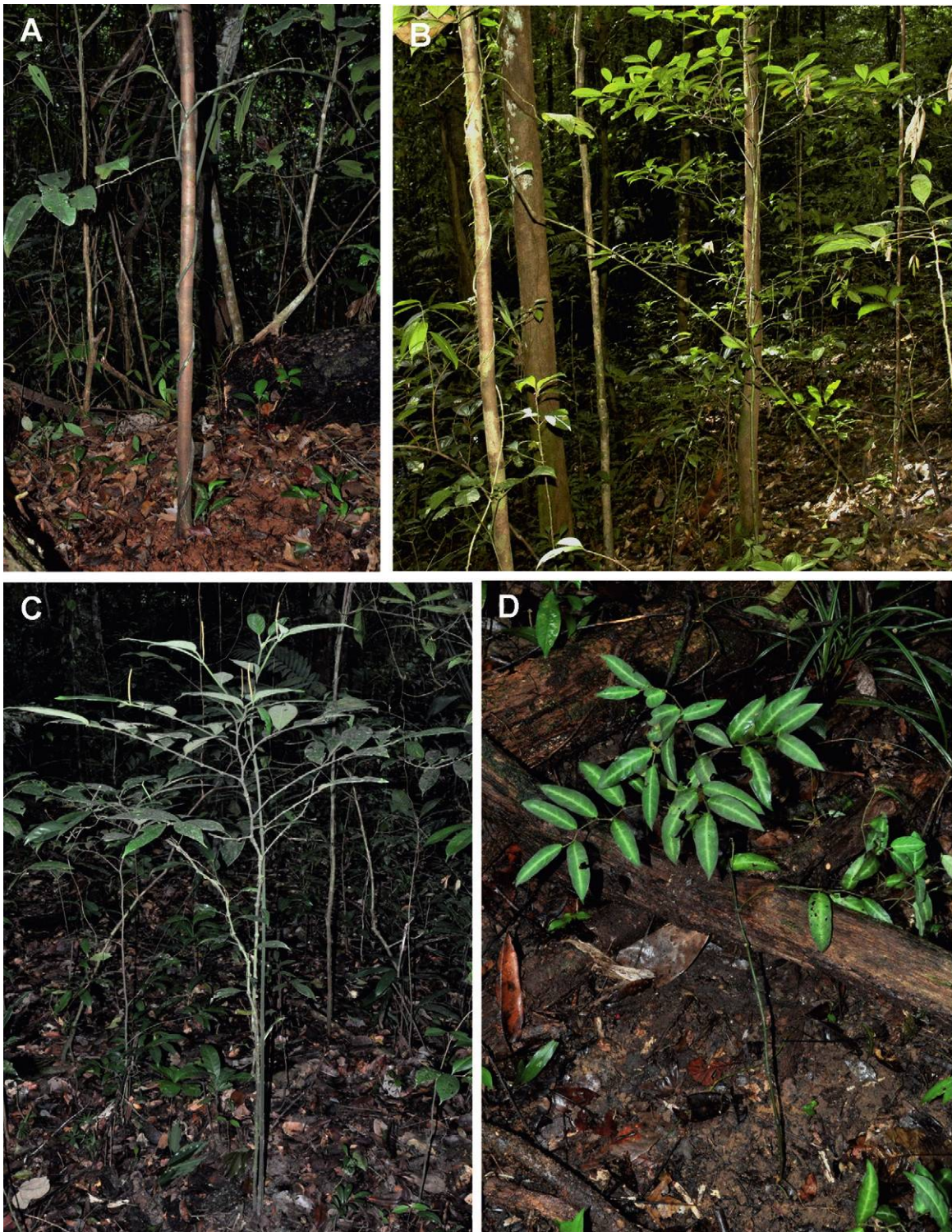


Fig. 10 Growth forms of *Piper* from understory habitats in tropical French Guyana. *A*, Climbing species of *Piper hostmannianum*. Individual stems wind loosely around the host supports and branch in the understory forest and forest margin. Stems reach up to 7–8 m in height in this area. *B*, Scandent or leaning shrub of *Piper bartlingianum*. Individual stems lean against surrounding vegetation in forest understory; they do not attach by twining or winding stems but lodge against surrounding plants via their branches. Stems reach 7–8 m in height in this area. *C*, Self-supporting shrub of *Piper alatabaccum*. In this area plants reach 2–3 m in height and remain self-supporting in dark understory habitats. Individuals develop several upright stems from the base. *D*, Self-supporting to leaning “dwarf” shrub of *Piper consanguineum*. The species produces small woody stems, which reach heights of up to 30–40 cm. Plants may be upright and self-supporting or unstable and leaning to creeping on surrounding vegetation close to the forest floor.

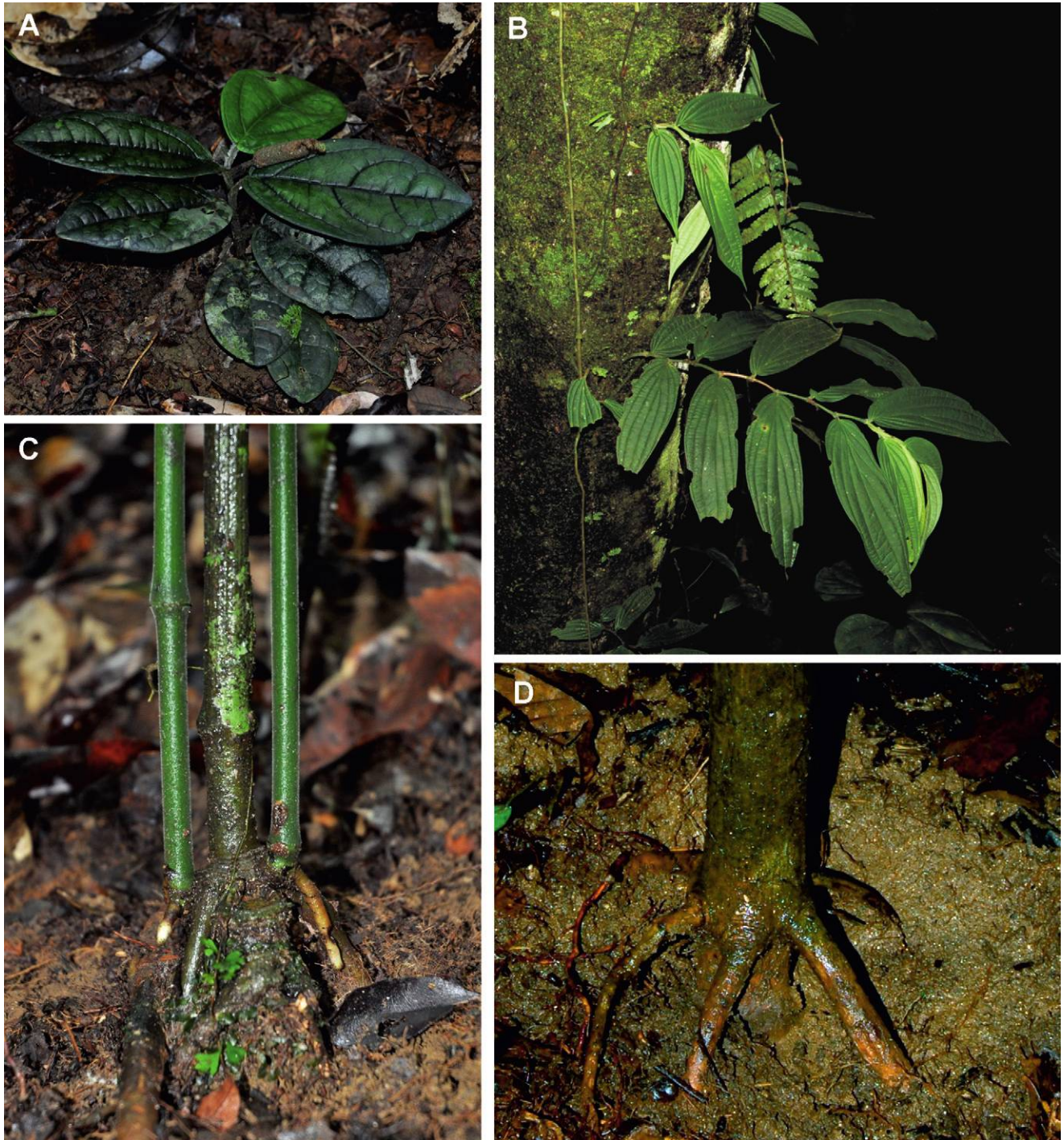


Fig. 11 Growth forms of *Piper*. *A*, Upright to creeping herb of *Piper humistratum* from understory habitat in French Guiana. Individuals produce fleshy, nonwoody short stems that grow in clusters on the forest floor reaching several centimeters in height. *B*, Small root climber of *Piper* species from understory forest in Colombia (Antioquia). The growth is sympodial, and the apical portion of the climbing stem ends with an assimilation plateau bearing inflorescences. A relay developed in the zone of curvature, on the supporting trunk. *C*, Base of multistemmed shrub of *Piper bartlingianum*. Typical of many shrublike and climbing woody *Piper* species, several stems are produced from the woody stem base. *D*, Base of a single-stemmed treelet of *Piper nudibracteatum* from understory forest in Colombia (Antioquia). Inhibition of basal sprouting in some large individuals gives rise to a treelet habit.

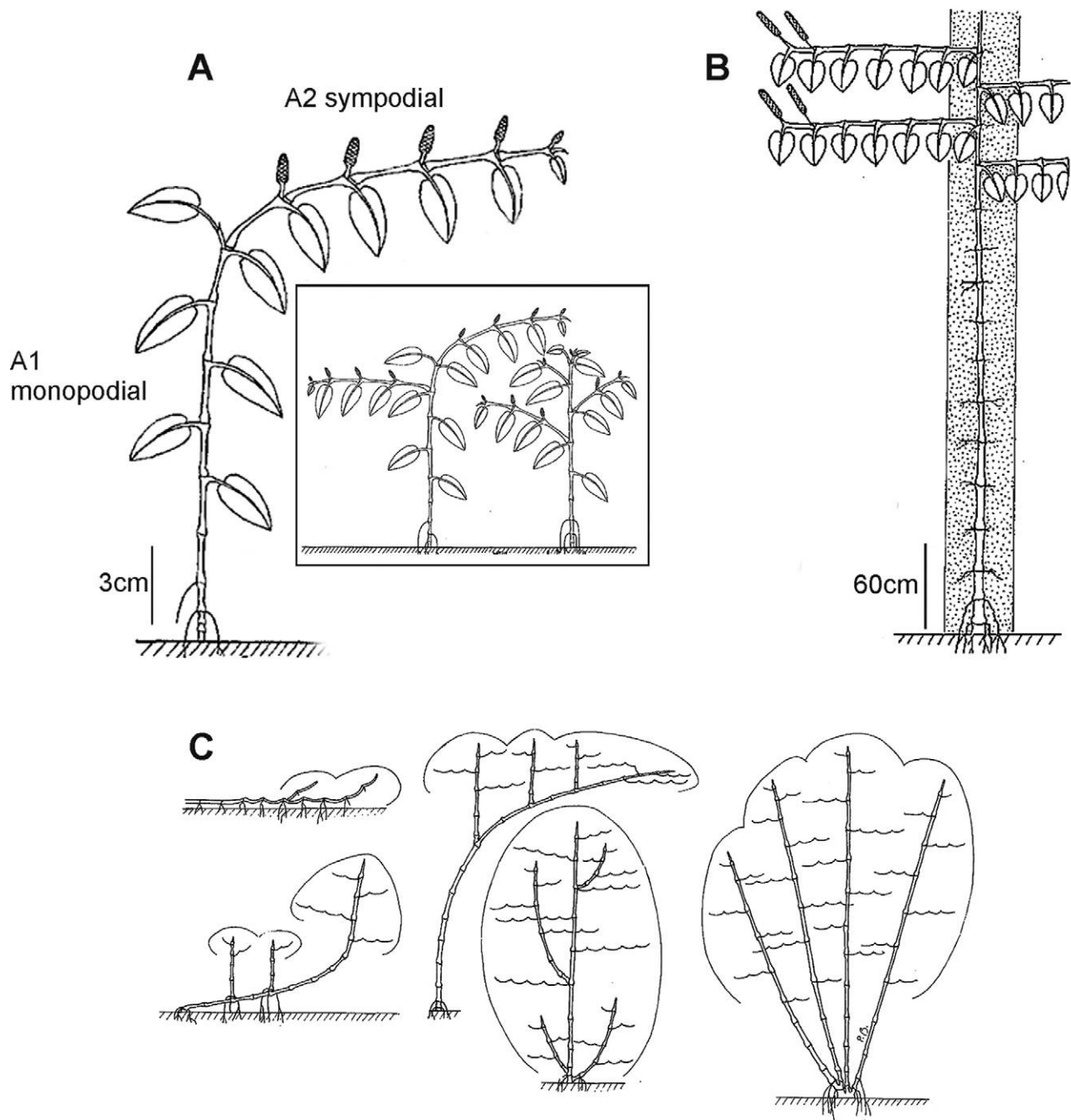


Fig. 12 Architecture of shrubs, treelets, and climbers in Piperaceae (from Blanc and Andraos 1983; reproduced with permission of the authors). A, Growth architecture (Petit model) of an unbranched plant with a typical monopodial trunk (A1). A new meristem marks the development of a sympodial branch (A2), which bears a terminal inflorescence (plagiotropic by substitution; see text). Variation exists in the position and timing of branching for the same species, depending on habitat conditions (*inset*). B, Growth habit in the root climber *Manekia obtusa*. The climbing stem is monopodial and produces branches several meters above soil level. Lateral branches are limited by the production of a terminal inflorescence, as in *Piper*. C, Different types of reiteration in *Piper*, underlying a wide range of growth habits. The position and frequency of reiterations is one of the key architectural features of *Piper* that contributes to the organization of widely differing growth forms.

Sympodial growth is widespread and is probably the most common architectural development in *Peperomia*; sympodial branches develop just below the flowering zone. Few *Peperomia* species, however, have a monopodial stem with

lateral inflorescences (Blanc and Andraos 1984). The diversity of morphological traits and habits in *Peperomia* has recently been shown to have resulted from parallel evolution (Samain et al. 2009b).

Sympodial Growth and Basal Sprouting in the Piperales

Sympodial growth is one of the main architectural features of the Piperales; it is found in all clades, although with variable expressions in the different clades. For example, inflorescences are terminal in Asaroideae and Saururaceae but are either terminal, lateral, or cauliflorous in Aristolochioideae, *Lactoris*, and Piperaceae. Basal sprouting is the other widespread architectural feature linked to growth habit variation within the Piperales. In the small-bodied Asaroideae and Saururaceae, basal sprouting is linked to sympodial development of the rhizome with determinate aerial meristems, as marked by the terminal inflorescence. In their sister clades (*Lactoris* + Aristolochioideae and Piperaceae, respectively), many species exhibit a longer life span of aboveground stems linked to woody habits, sometimes forming a small trunk by sympodial growth. Sprouting develops from basal meristems at the collar zone, which give rise to the multistemmed shrub habit observed in *Thottea*, *Aristolochia*, and *Piper*. Species that develop only one or two stems simultaneously tend to have larger trunks, suggesting that suppression or reduction of sprouting made way for the development of larger trunks and the treelet habit, as observed in *Piper* (fig. 10D).

The development of basal meristems appears to be an opportunistic mechanism providing architectural plasticity in shrubs or treelets. The adaptive appearance of branches in some *Piper* and *Peperomia* species has prompted some authors to describe them as adaptive reiterations and not as an additional branch order (Blanc and Andraos 1983, 1984). In shrublike species of *Aristolochia*, the whole plant is built up of reiterations of simple elementary units (i.e., mixed axes). This would suggest that reiteration is a central part of the architecture in Piperales and may have played a key role in their growth form diversification.

Anatomy of Piperales

Vascular Cambium

While some angiosperm taxa truly lack a secondary vascular cambium (e.g., monocots, Nymphaeales; Metcalfe and Chalk 1979; Carlquist and Schneider 2009; Carlquist et al. 2009), many angiosperms described as herbaceous do in fact show cambial activity, which may be limited to vascular bundles of the primary body. Although the term “herbaceous” is convenient for categorizing some growth habits and life histories of plants, it does not adequately describe the degree of cambial activity and the amount of woodiness found in many angiosperm lineages; this may vary significantly whether they are termed herbs or not (Eiten 1991; Carlquist 2009).

The following overview of the anatomy of Piperales demonstrates the limits and gradients of vascular cambium activity and degrees of woodiness among species; most of the herbaceous forms in the Piperales have at least limited secondary growth even if confined to the vascular bundles.

In Asaroideae, the vascular cambium is active in both intra- and interfascicular areas (within and between vascular bundles, respectively), albeit with a limited accumulation of secondary xylem. In *Asarum canadense*, vascular bundles are arranged in a circle at the periphery of the pith, and only

a little secondary growth has been observed in the underground stem (rhizome; not shown). In *Saruma henryi*, the cambium forms a continuous ring producing a small quantity of secondary xylem in aerial stems but is rayless (fig. 13A).

In *Aristolochia* and *Thottea* species studied, secondary growth either can be limited or can represent a large proportion of the stem in the shrubs and lianas (fig. 13B–13D, 13F, 13G). Vascular bundles form a single ring in these two genera, but some climbers in *Aristolochia* show a bilateral symmetry linked with a distinctive kind of growth strain and cell collapse indicated by a “Y-shaped” compression zone of cells in the pith (fig. 13F). Pith compression has been shown to result from internal growth stresses in *Aristolochia* (Busch et al. 2010). Among the herbs there is less secondary growth, but the vascular cambium is nevertheless active (fig. 13D). The shrubby species *Lactoris* also produces large quantities of secondary tissues (Carlquist 1990).

In Saururaceae, vascular bundles are isolated in ground tissue (fig. 13E); cambial activity is mainly restricted to collateral bundles, as observed from the clear radial alignment of a few cells in files in secondary xylem and phloem (e.g., *Saururus cernuus*, *Gymnotheca chinensis*; fig. 13E). Secondary growth can be insignificant in other species (e.g., *Houttuynia cordata*; Carlquist 1995, 2009).

The most outstanding anatomical character of the Piperaceae is the nature and arrangement of the vascular bundles in their stems. All Piperales are characterized by a primary stem with distinct vascular bundles. *Manekia*, *Piper*, *Peperomia*, and *Zippelia* have a polystelic structure, comprising several rings of collateral bundles (Debray 1885; Wu and Raven 1999; fig. 14E–14H). In most *Piper* species, as in *Zippelia*, two types of collateral bundles can be distinguished (fig. 14F, 14G): (1) peripheral bundles, which form a ring at the stem periphery and are interconnected by a continuous ring of sclerenchyma located below the xylem, and (2) medullary bundles, which are arranged in a smaller circumference at the stem center. Peripheral bundles can undergo significant cambial activity in *Piper*, while the activity of medullary bundles is limited (fig. 13H; Debray 1885; Ravindran and Remashree 1998). Additional collateral bundles (more than two rings) are found in *Manekia* (Wu and Raven 1999). *Manekia sydowii* possesses one cycle of vascular bundles embedded in an undulated sclerenchyma ring and three inner cycles of vascular bundles (fig. 14F). Intensive secondary growth occurs only in the second cycle of vascular bundles (fig. 13I). *Verhuellia lunaria* has a rudimentary anatomy with a single vascular bundle located in the center of a ground parenchyma (fig. 14D). The bundle is composed of five to nine protoxylem tracheids with spiral thickenings surrounded by protophloem; thus, secondary growth is lacking in this species.

Comparative anatomical studies in *Peperomia* (Souza et al. 2004) and our own observations suggest a uniform tissue arrangement in *Peperomia* stems. The epidermis is uniseriate in most species and covered by a cuticle of varying thickness. A continuous ring of collenchyma (fig. 15A) of variable thickness is composed of cells of various sizes and cell wall thickenings among species. Collenchyma cells represent the only mechanical tissue in *Peperomia* stems (L. Frenzke, unpublished data). In all species there is a more or less well-defined peripheral ring of vascular bundles surrounding an irregu-

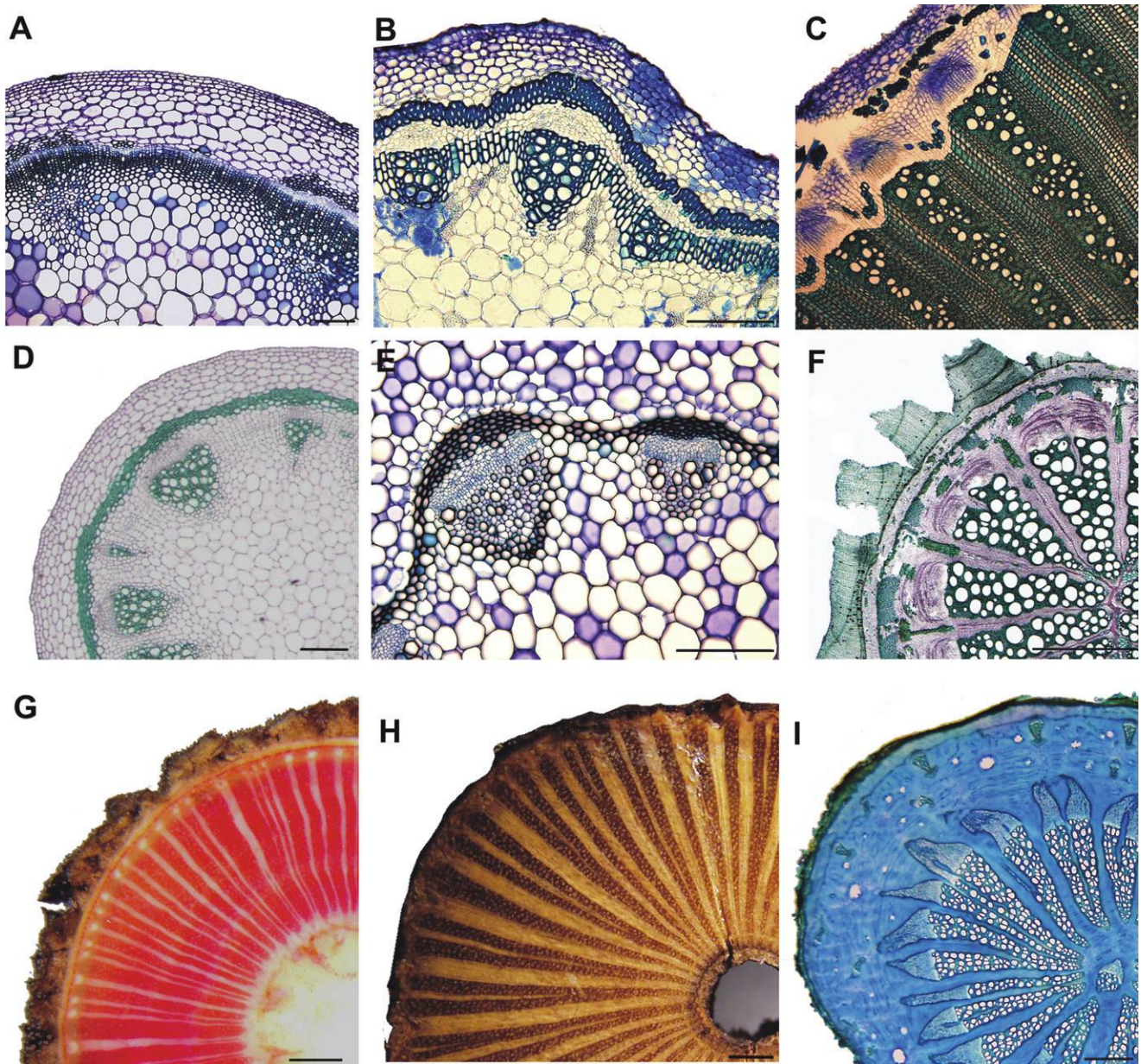


Fig. 13 Secondary growth in Piperales. *A*, The vascular cambium in the herb *Saruma henryi* is active in both fascicular and interfascicular areas. *B*, The subshrub *Thottea tomentosa* exhibits limited secondary growth, with wide rays and a thick ring of peripheral fibers. *C*, The larger shrub *Thottea siliquosa* shows significant wood development with wide rays. *D*, The small herb *Aristolochia serpentaria* produces a limited amount of secondary growth. *E*, In the herbaceous species *Saururus chinensis*, cambial activity is restricted to vascular bundles and is only slightly active. *F*, Lianas of *Aristolochia ovalifolia* develop significant secondary growth. Climbing species in *Aristolochia* are characterized by wide rays, a wide cortex, and thick bark. *G*, *H*, In shrub and treelet species of *Aristolochia* (*G*, *A. arborea*) and *Piper* (*H*, *P. gorgonillense*), wood is characterized by wide lignified rays and smaller diameter vessels. In *Piper*, the internal ring of the vascular bundle has been lost with the pith during secondary growth. *I*, In the root climber *Manekia sydowii*, internal and peripheral bundles undergo little secondary growth and are still present in mature stems. Secondary growth has occurred in the “median” ring of vascular bundles. This climber is also characterized by wide, unlignified rays; large vessels; and a thick cortex. Scale bars show 200 μ m (*A–E*) or 2 mm (*F–I*).

larly arranged central group of bundles (Yuncker and Gray 1934; fig. 14*H*). The scattered arrangement of the vascular bundles in *Peperomia* stems resembles the polystelic arrangement commonly found in monocots. The vascular bundles are embedded in the parenchymatous ground tissue, which comprises the main part of the cross sectional stem area.

Cambium, restricted to vascular bundles, develops briefly in *Peperomia* and may have been completely lost in many species. In a study of Hawaiian species (Yuncker and Gray 1934), cambium was lacking in all the species considered, which we can confirm for other non-Hawaiian species as well (fig. 15*B*).

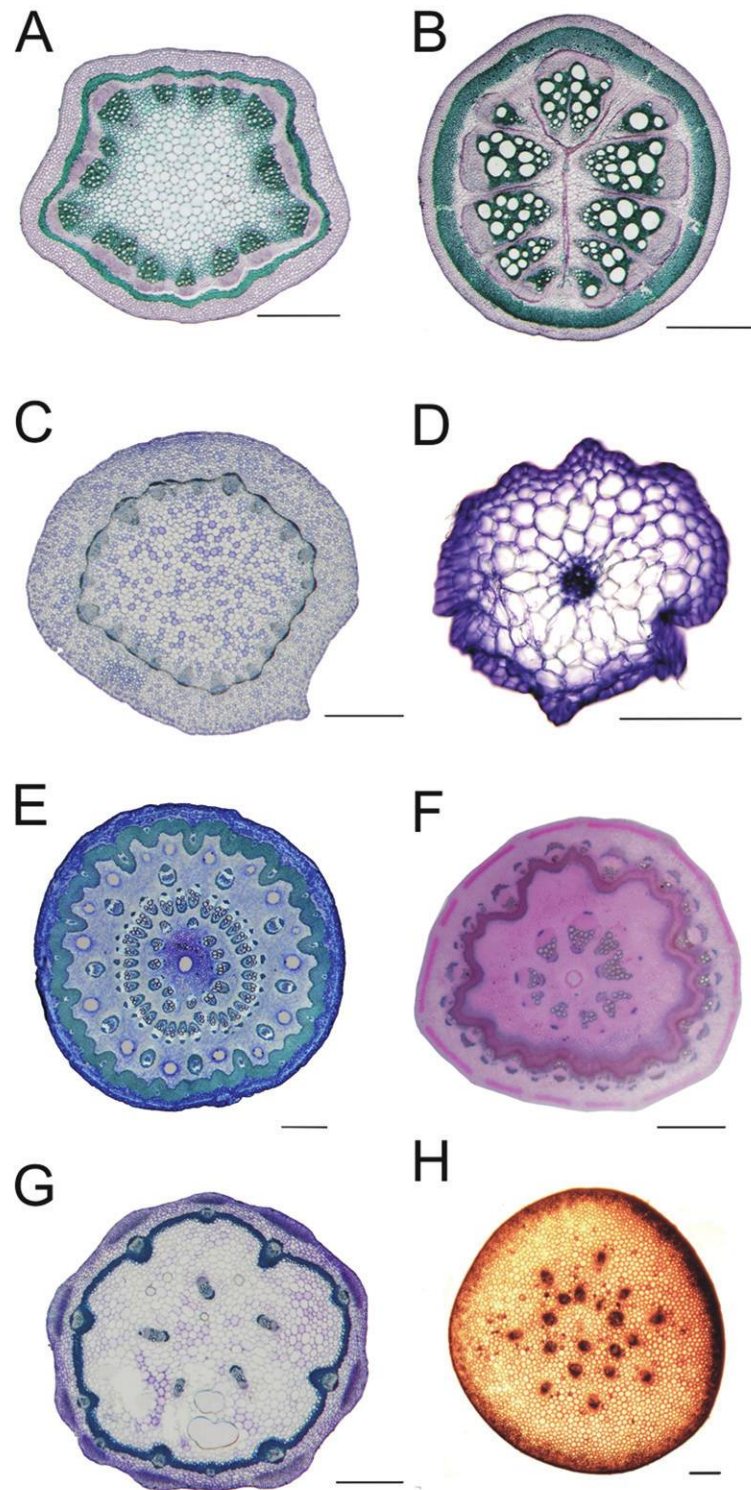


Fig. 14 Distribution of vascular bundles and the hypodermal layer in stems of some Piperales. A–C, Formation of a single ring by vascular bundles in Aristolochioideae (A, *Thottea siliquosa*, shrub; B, *Aristolochia gigantea*, liana), as in Saururaceae (C, *Gymnotheca chinensis*). D, Reduction of the primary vascular system in *Verbuellia lunaria* to one single central bundle. E–H, Polystelic organization of the stem in Piperaceae (E, *Manekia sydowii*; F, *Piper* species; G, *Zippelia begoniifolia*; H, *Peperomia pecuniifolia*). Mechanical tissue (hypodermal layer) is present as an entire ring of thick-walled cells toward the outside of the stem cross section in all species except for the protostelic *Verbuellia lunaria*. Scale bars show 500 μm (A), 1 mm (B, C, E–G), or 200 μm (D, H).

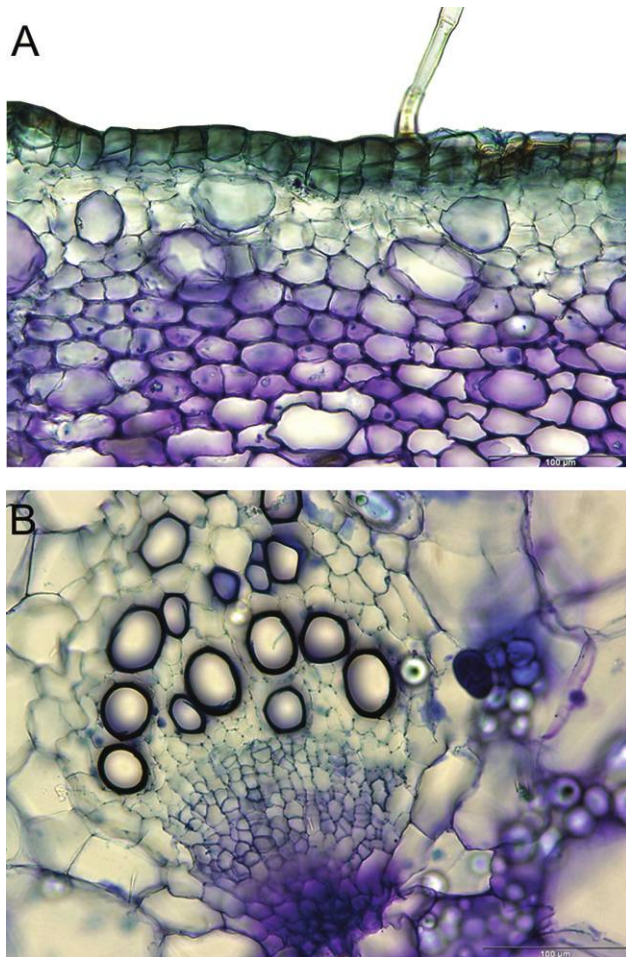


Fig. 15 *Peperomia incana*. A, Thick-walled mechanical tissue (i.e., collenchyma). This tissue forms a continuous ring at the stem periphery. B, Peripheral vascular bundle with only protoxylem and metaxylem elements. Secondary growth is lacking in this species.

Continuous Rays from Primary to Secondary Development

The most noticeable anatomical feature of woody Piperales are the tall, wide, multiseriate rays that persist during ontogeny and represent a relatively large volume of the wood cylinder. This results in a strict alternation of fascicular areas separated by wide rays, which characterized all of the woody species in *Aristolochia*, *Thottea*, *Piper*, *Manekia* (fig. 13F–13I), and *Lactoris* (Carlquist 1990). The interfascicular cambium produces secondary ray tissue, similar to the primary rays of juvenile stems, but is incapable of producing fusiform initials (i.e., meristematic cells that give rise to longitudinally aligned cells [e.g., fibers and vessels]). The only exception to this cambium functioning has been observed in the herbaceous species *S. henryi*, which lacks vascular rays and produces a complete and uniform cylinder of secondary xylem and phloem (fig. 13A; Dickison 1996). A limited amount of secondary xylem is, however, produced at the base of the aerial stem in this herbaceous species. Thus, in all woody Piperales the anatomical organization of mature stems resembles the collateral organization of juvenile stems, although active

secondary growth has occurred. The complete loss of intrafascicular cambium in *Houttuynia* and the general reduction of fascicular and interfascicular cambia in Saururaceae as a whole have led to the idea that Saururaceae may serve as a model for how monocotyledons lost cambial activity (Carlquist 1995, 2009).

A number of anatomical features found in Piperales are noticeably original among magnoliids; this includes (1) a recurrent reduced cambial activity in the different clades (Saururaceae, Asaroideae, Piperaceae), (2) a peculiar type of woodiness with large rays resulting from a potential inability of interfascicular cambium to produce fusiform initials (*Aristolochia*, *Thottea*, *Lactoris*, *Piper*, and *Manekia*), (3) the lack of cambial activity in many species of *Peperomia* and in *Verhuellia*, and (4) polycyclic distribution of vascular bundles in Piperaceae.

Anatomy and Growth Form Diversification

Anatomy within the Piperales is relatively homogeneous in comparison to the anatomical diversity of many eudicot groups (Spicer and Groover 2010). All species of the order share a similar anatomy of secondary vascular tissues involving extension of wide rays between fascicular areas. In many angiosperms, vascular bundles are also separated by wide primary rays, but the onset of secondary growth often results in their subdivision into smaller rays. In some groups, as in the Piperales, interfascicular cambium continues to produce ray tissue without subdividing the rays to any marked degree. Such lack of changes from primary to secondary structure has been proposed as a form of heterochrony (Carlquist 2009).

Large rays are a common characteristic of lianas and occur in many eudicots but are not always a result of extension of the primary rays. In the Ranunculaceae, woodiness of aerial stems is probably secondary in the family (Judd et al. 2002) and occurs principally in climbing species, where large rays also extend into the phloem and remain little changed during secondary growth (Isnard et al. 2003). In Piperales, such wide rays may have been preadapted for the evolution of the climbing habit in *Aristolochia*, *Piper*, and *Manekia*. Variations around this basic organization do occur, although in different groups. Ray lignification occurs in shrubs and treelets in *Piper*, *Thottea*, and *Aristolochia*. Climbing species in *Aristolochia*, *Manekia*, and *Piper*, however, have wide and unligified rays. The phylogenetic patterns of ray lignification have recently been investigated in *Aristolochia*, with the results suggesting that lignification is linked with the shrub habit in the group (S. T. Wagner, S. Isnard, N. P. Rowe, M.-S. Samain, C. Neinhuis, and S. Wanke, unpublished manuscript). A surprising discovery was the lignification of rays observed in climbing species belonging to *Aristolochia* subgenus *Isotrema*, in contrast to the wide unligified rays characterizing both of the other subgenera of *Aristolochia* (*Aristolochia* and *Pararistolochia*). In the Piperales, such variation in stem anatomy may be linked with different life histories, climbing preferences, body size, length of self-supporting phases of growth, speed of attachment, and stem twining.

This survey of anatomy in the Piperales suggests that an herbaceous organization is prevalent, with the result that fusiform initials are limited to fascicular areas and, consequently, a large part of the stem cross section taken up with ray tissue. This would have potentially far-reaching effects on the mechanical stability and general mechanical properties of the stem. In the following section, we investigate the mechanical architectures within herbs, shrubs, climbers, and epiphytes among the Piperales and explore how patterns of architectural development and anatomy are linked with the biomechanical properties and diversity of growth forms within the order.

Mechanical Properties

Differences in the mechanical properties of different plant growth forms are an essential part of their organization, diversity, and adaptation to different niches (Niklas 1992, 1997, 2000). Quantitative measurements of stem stiffness allow detailed comparisons of how different species maintain mechanically self-supporting shrublike or treelike growth forms and how such forms differ from climbing species, herbs, and epiphytes (Speck and Rowe 1999). Such studies are of particular interest for investigating evolutionary diversifications of growth forms, especially when linked to observations of developmental traits that produce different degrees of stiffness and flexibility in different growth forms (Rowe and Speck 2005). Biomechanical studies have been applied for analyzing diversification of forms of both living (Civeyrel and Rowe 2001; Isnard et al. 2003; Speck et al. 2003; Lahaye et al. 2005) and anatomically preserved extinct groups from early land plants (Speck and Vogellehner 1988; Speck and Rowe 1994; Bateman et al. 1998) to the diversification of growth habits in fossil seed plants (Rowe et al. 1993; Speck and Rowe 1994; Masselter et al. 2007, 2009).

Piperales develop a wide range of mechanical stiffness in relation to the diversity of growth habits characterizing the group (fig. 16). Values of Young's modulus are plotted against stem diameter for representative shrubs, climbers, epiphytes, and herbs. The resulting scatterplots provide a means of comparing how stiffness develops among different growth forms from young (small diameter) to older (larger diameters) stages of development and for representative growth forms selected from each clade.

Terrestrial Herbs

Species within the Asaroideae comprise terrestrial herbs. The mechanical architecture of the upright herb, *Saruma henryi*, shows values of Young's modulus ranging from 400 to 1300 MPa for stem diameters between 2 and 6 mm (fig. 16A).

Species within the Saururaceae also comprise mostly terrestrial herbs. Both species measured (*Gymnotheca chinensis* and *Houttuynia cordata*) have an organization similar to that of the herbaceous perennial *S. henryi* (Asaroideae) and produce aerial stems of a similar diameter. They show slightly wider-ranging values of Young's modulus, from ~100 MPa to a maximum of 2000 MPa among stems with diameters of

1–6 mm (fig. 16A). This pattern of mechanical development is consistent with aerial herbaceous growth with little or no increase in the primary stem diameter and where changes in stiffness are limited to maturation and lignification of primary tissues within the primary body (fig. 13E). Species within the Saururaceae as well as *S. henryi* develop herbaceous aerial stems from underground rhizomelike stems. The mechanical organization is representative of a relatively fast-growing "light" architecture making little or no investment in secondary tissues and is characterized by the development of "young" primary mechanical tissues. Anatomical observations suggest that the main mechanical element of such stems is provided by the ring of hypodermal fibers in the outer part of the cortex.

Shrubs

Woody, shrublike growth habits are present within clades of both Aristolochioideae + *Lactoris* and Piperaceae. Species of *Thottea*, *Aristolochia*, and *Piper* studied develop woody stems of 30–40 mm in diameter. Stem stiffness in all shrublike species of Aristolochioideae (*Thottea* and *Aristolochia*) reaches a maximum of ~5000–6000 MPa for stems of ~5–7 mm in diameter (fig. 16B). Stem stiffness in treelets of *Piper* reaches a higher maximum, of ~8000 MPa, with a steady increase in larger stems up to 15 mm in diameter. Above this, values of Young's modulus remain relatively constant in all species and might even decline slightly in some species. Woody shrubs and treelets clearly develop at least twice the stiffness of the terrestrial herbs for the same diameter of ~5 mm. Maximum values of Young's modulus correspond to a point in development when the cylinder of wood has developed significantly but while the outer ring of hypodermal tissue is still intact at the periphery of the stem, prior to its rupture from increasing secondary growth.

The leveling off of stem stiffness observed in the shrubs and treelets (fig. 16B) is consistent with previous patterns of mechanical development observed in woody shrubs, where the relative proportion of stiffening tissues in the stem cross section ceases to increase with increasing diameter (Speck and Rowe 1999). Such phenomena can be due to a number of developmental traits from ultrastructural properties of the cell walls to the amount and distribution of mechanical tissues, such as wood and fibers. However, the pattern of stiffness observed in the Piperales is consistent with the large and relatively constant fraction of ray tissue in the wood cylinder, even in large-diameter stems.

The relatively early plateau in Young's modulus is consistent with the fact that shrubs and treelets in the Piperales are often unstable and have a tendency to lean against the surrounding vegetation (figs. 5D, 6B, 10B). In Aristolochioideae, truly self-supporting large-bodied trees are absent, and shrubs have a tendency toward unstable leaning growth forms. The survey of growth forms, especially observations from naturally growing populations, supports previous hypotheses that large-bodied, mechanically stable growth forms are precluded within some groups of the Piperales by a number of developmental, anatomical, and mechanical constraints within the clade (Speck et al. 1997, 2003).

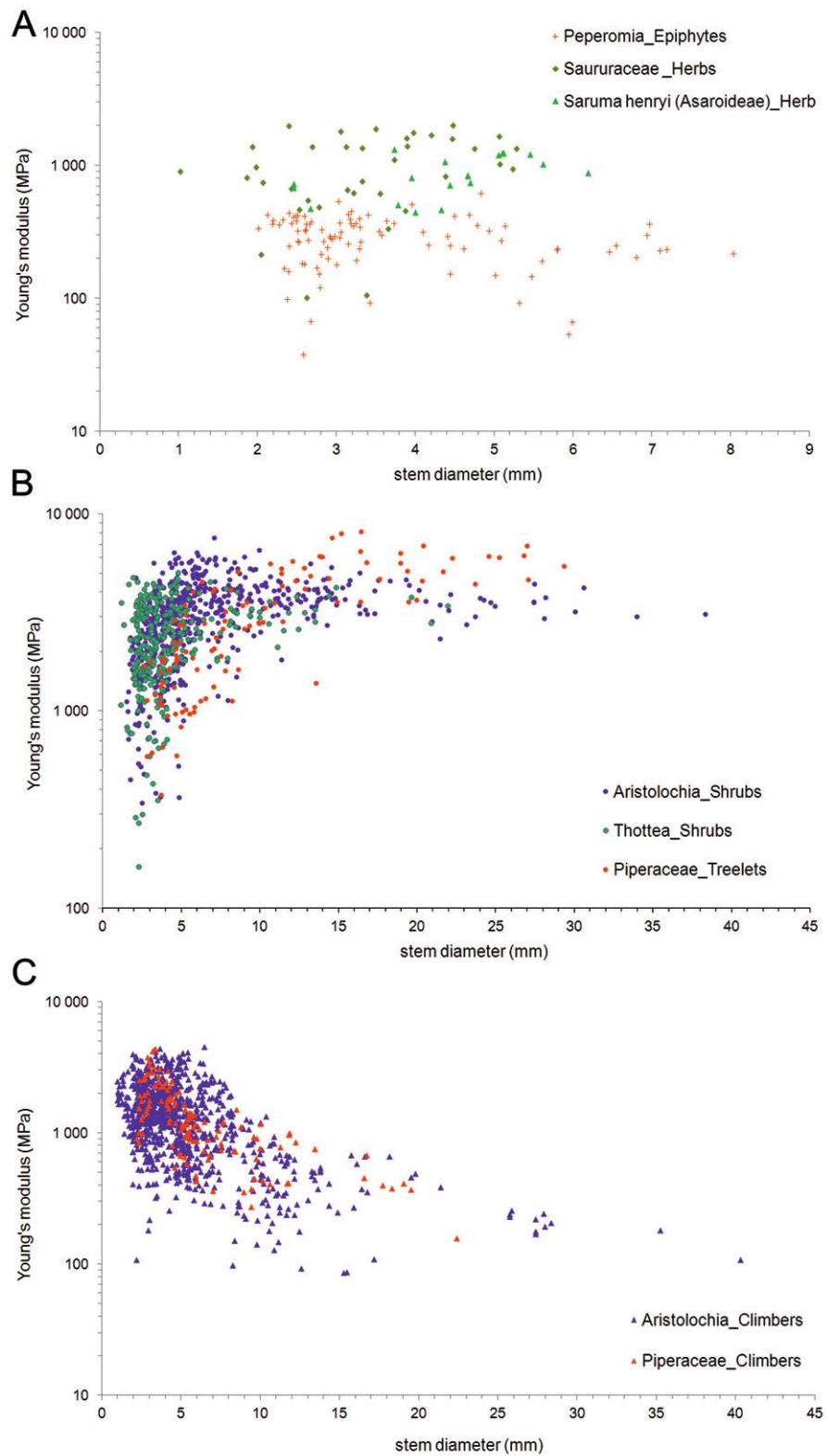


Fig. 16 Changes in stem stiffness (Young's modulus) with increasing stem diameter in representative growth forms of Piperales. *A*, Herbs in Asaroideae, Saururaceae, and epiphytic *Peperomia*. *B*, Shrubs and treelets in Aristolochioideae and *Piper*. *C*, Climbers in Aristolochioideae and Piperaceae.

Values of stem stiffness have a tendency to level off with increasing stem diameter among woody shrubs of Piperaceae. Shrubs of *Piper*, however, appear to develop higher-bending moduli than *Thottea* or *Aristolochia*. Treelet species of *Piper* studied (*P. gorgonillense* and *P. nudibracteatum*) reach up to 6 m in length and lean against surrounding vegetation in forest understory. *Piper* species also exist as treelets of up to 10 m in height, suggesting that constraints on self-supporting habit within the Aristolochioideae and Piperaceae may differ. Most Neotropical species of *Piper* are relatively small-bodied shrubs and treelets from the forest understory (figs. 10, 11), which also show a tendency toward unstable leaning habits.

Climbers

The climbing habit is common in the Aristolochioideae, where it appears to have evolved at least twice (Speck et al. 2003; S. T. Wagner, S. Isnard, N. P. Rowe, M.-S. Samain, C. Neinhuis, and S. Wanke, unpublished manuscript). Climbers are also common in the Piperaceae, in the genus *Manekia*, and in Asian species of *Piper*. The distinction between truly climbing and merely leaning or not fully self-supporting plants is an important, although sometimes difficult, distinction to make in the different clades. Woody climbers within *Aristolochia* are nearly always strongly attached via twining of the main stem and branches. Woody climbers within the genus *Piper* and *Manekia* are mostly recorded as root climbers. Some species of *Piper* in the Neotropics, however, climb via winding (defined here as coiling of the main stem around a host stem but not tightly as in twining stems; fig. 10A).

The species of climbers sampled here developed stems of up to 20 and 40 mm in diameter; they showed an initial increase in stiffness up to 4000 MPa in narrow stems (up to ~5 mm) of young individual plants or in “searcher” stems of mature climbing plants. Maximum values of bending stiffness for the climbing species therefore reach slightly lower values than for woody shrub species but nevertheless reach higher values than for herbs of the Saururaceae or Asaroideae. Maximum stiffness is associated with the development of a thick dense ring of fiber tissue in the outer primary cortex. Our survey of climbers within *Aristolochia* has demonstrated that self-repair of the outer hypodermal fiber tissue via division, intrusion, and lignification of parenchyma cells (Masselter and Speck 2008) is found in all the climbing species observed (fig. 13F).

Following the peak in stiffness, all climbing species observed show a marked reduction of Young’s modulus with a further increase in stem diameter (fig. 16C). Large-diameter climbing stems exhibit Young’s modulus values down to ~200–100 MPa. Reduction in stem stiffness is initially linked to fragmentation of the outer fiber tissue resulting from expansion of secondary xylem and then an increasing fraction of wood with a high parenchyma component as well as a large fraction of wide-diameter vessels (fig. 13F). Since these compliant tissues occupy increasingly large proportions of the stem cross section, stems of climbers become increasingly flexible. Changes in values of Young’s modulus show at least an order of magnitude of difference between shrubs and lianas. These are closely linked with specific differences in

stem anatomy and represent fundamental differences in structural organization between unstable shrubs and lianas within the Piperaceae and Aristolochioideae.

Epiphytes

Epiphytic species within the genus *Peperomia* showed the lowest values of stem stiffness, ranging from below 100 MPa to ~500 MPa for stems up to 7–8 mm in diameter (fig. 16A). Bending stiffness was measured for a range of epiphytes, including recumbent, erect, and pendulous forms. The highest values of stem stiffness never approached the maximum values observed among terrestrial herbs of Saururaceae and Asaroideae or shrubs and climbers of the Aristolochiaceae and Piperaceae. Comparison of prostrate, erect, and pendulous species indicated only subtle variation in terms of stem mechanical properties among these small-bodied growth forms (L. Frenzke, unpublished data). Pendent stems of *Peperomia pecuniifolia* developed marginally higher values of Young’s modulus than upright terrestrial forms, but these pendulous stems were nevertheless more slender than upright forms. The principal mechanical tissue consisted of a subepidermal layer of relatively thin-walled collenchyma and parenchyma, which was unligified (fig. 15A). Vascular bundles were scattered throughout the primary body and were not present in peripheral bands in all species; thus, they were not geometrically optimized for creating bending stiffness within the stem.

Discussion

Shrubs, Unstable Shrubs, Scandent Shrubs, and Climbers

Within the Piperales, we found a highly homogeneous anatomical organization with distinct vascular bundles and large rays as well as a uniform basic plant construction based on sympodial growth and a succession of equivalent structural units. Despite this phylogenetically conserved anatomy and architecture, the survey of growth forms and biomechanics indicates high degrees of mechanical specialization, particularly among woody shrubs, treelets, and climbers.

True climbers characterized by stem twining, winding, or root-climbing modes of attachment within the Aristolochioideae and Piperaceae show highly reduced values of Young’s modulus in old stages of growth that approach the lowest recorded in general comparisons of climbing plants (Rowe et al. 2006). Fine-tuned changes in the stem anatomy of shrubs and treelets, ray lignification, fraction of parenchyma in wood (S. T. Wagner, S. Isnard, N. P. Rowe, M.-S. Samain, C. Neinhuis, and S. Wanke, unpublished manuscript), and tracheid structure (Carlquist 1993) are all likely to influence stem biomechanics. The difference between unstable shrub-like growth forms and truly climbing plants within the Piperales is apparent from the values of stem stiffness. Many species within the Aristolochioideae and Piperaceae are, however, represented as “unstable” (S. T. Wagner, S. Isnard, N. P. Rowe, M.-S. Samain, C. Neinhuis, and S. Wanke, unpublished manuscript), “sarmentose,” or “lianescent” shrubs (Jaramillo et al. 2008) or as treelets that lean against

surrounding vegetation (fig. 6B; Blanc and Andraos 1983; Mori et al. 2002).

There is thus a range of unstable growth forms that retain a relatively stiff stem that differ in mechanical properties from truly climbing forms that develop high compliancy and flexibility. Such differences probably have wide implications for overall body size, stem length, niche, and habitat preference as well as ecophysiology within the Piperales. Observations of shrub and climber diversification within the Piperales may therefore be relevant for interpreting the significance of growth form diversity and origins in other lineages sister to the diversification of eudicots. *Amborella* shows evidence of unstable shrublike growth, with long and flexible shoots exhibiting a pendent branched construction (Feild et al. 2009). This has been interpreted as a prior specialization of vesselless wood in angiosperms toward hydraulics rather than mechanics (Feild et al. 2009). Growth forms reported for other woody angiosperm lineages sister to the diversification of eudicots, such as Austrobaileyales, are diverse and include (a) shrubs to small trees in *Illicium* and *Trimenia*, (b) scrambling shrubs that eventually become climbers without any kind of morphological specialization (leaning on supporting vegetation) in *Kadsura* and *Schisandra* (Carlquist 1999), and (c) true twining lianas in *Austrobaileya scandens* (Feild et al. 2003). Anecdotal reports of lianas in other lineages include the Chloranthaceae, where a liana (*Chloranthus* species) with small branches modified into hooks has been described from Sarawak (Blanc 1986; PB84-600 MNHM), and vesselless Winteraceae, including *Tasmannia cordata*, which climbs by “grappling onto the stems of other plants with stiff cordate leaves and wiry branches” (Feild et al. 2012, p. 235). Stems of the latter have been shown to develop high values of Young’s modulus (Feild et al. 2012). Species within the Winteraceae also include short treelets, which have been reported to produce leaning trunks (Hudson et al. 2010).

The presence of unstable, shrublike treelets and scandent growth forms in many lineages sister to the eudicots suggests that this range of growth forms is common and may have evolved early in angiosperm diversification. Forms that develop relatively stiff stems and not the kind of compliancy associated with truly climbing woody lianas do not appear to be linked to vesselless angiosperms only. These observations are in agreement with some authors who suggest that the evolution of vessels in angiosperm wood may have required early angiosperms to survive a phase of mechanic and hydraulic instability (Sperry et al. 2006). Extant angiosperms, belonging to early-diverging lineages, might thus reveal some clues as to how some of the complex functioning in wood organization may have originated and how different kinds of woodiness might have been linked to different kinds of growth habit.

The appearance of highly flexible twining and winding stems in Piperales and other angiosperm lineages could be viewed as more derived climbing behavior compared with scramblers or mechanically unstable shrubs. More specialized climbing forms may well have opened up a new range of potential niches involving longer, more flexible, truly canopy-climbing stems with effective attachment organs, necessitating an increasingly diverse range of ecophysiological tolerances. Such derived climbing forms within the Piperales demon-

strates that these growth habits are not confined to eudicots, in which lianoid forms can show extremely derived cambial variants along with specialized mechanical and hydraulic properties (Carlquist 1991; Gentry 1991). The report of arguably simpler, leaning, or partly scandent forms among early-diverging lineages to some extent resonates with the pioneering observations of Schenk, who proposed that “primitive” flowering plant climbers showed simple and less complex modes of attachment and less complex cambial variant organization compared with more “advanced” forms (Schenk 1892, 1893). Further studies should focus on lianoid diversity among early angiosperm lineages along with their mechanical, hydraulic, and ecophysiological characteristics. For example, *Austrobaileya* appears to show a complex climbing life history involving twining stems as well as stolonlike creeping stems that occupy the understory vegetation in different ways (Feild et al. 2003). Finally, wood anatomy similar to that observed in Piperales has also been reported in *Kadsura*, *Schisandra*, and *Austrobaileya* (Carlquist 1999, 2009), including large vessels and wide rays. However, none of these climbing species exhibit cambial variants, as observed in many groups of eudicots.

Herbaceous and Woody Mechanical Architectures

In addition to the diversity of woody shrubs, treelets, and climbers within the Piperales, another major source of growth form diversity is the appearance of herbaceous growth forms represented by the Asaroideae, Saururaceae, and the genera *Verhuellia*, *Zippelia*, and *Peperomia*. Herbaceous life forms in the Piperales appear to involve relatively extreme shifts of structural and organizational traits in at least three levels: (1) tissue properties, (2) tissue organization and development, and (3) architecture.

Herbaceous growth forms within the Piperales rely on the outer hypodermal layer of collenchyma and sclerenchyma cells for stem stiffness (fig. 14), as all species studied produce a limited amount of secondary wood along the main aerial stem. If a significant amount of wood is produced, it is usually confined to the basalmost part of the upright stem and to the rhizome. Hypodermal organizations with rings of stiff tissues may be effective in stems with a large fraction of such tissues (Niklas 1989). Forms with thin hypodermal layers may rely on the turgor pressure from the surrounding cells for mechanical support. It is therefore possible that many of the herbaceous species within Piperales with “light” hypodermal architectures might thus rely on turgor pressure, which could explain the habitat preferences of some clades for aquatic, flooded, or wet environments (e.g., *Saururus*, *Houttuynia*, *Verhuellia*, *Zippelia*, and some *Peperomia* species). Reduction to an herbaceous organization also characterizes the huge diversification of terrestrial and epiphytic species of *Peperomia*. In our study, mechanical construction within *Peperomia* showed a highly conserved organization, varying in terms of the thickness of the ring of un lignified collenchyma tissue and cell wall thickness of constituent cells.

So, how might transitions to herbaceous life forms have affected diversification within the Piperales?

Reduction or disappearance of secondary vascular tissues of the main stem would have had a profound influence on

overall life history, with (a) drastic limitation of mechanical stiffness and, depending on primary tissues, for mechanical support, (b) a significant constraint on hydraulic conductivity.

Within the Piperales, current phylogenies place the herbaceous Asaroideae as sister to Aristolochioideae + *Lactoris* and the herbaceous Saururaceae as sister to the Piperaceae (fig. 2). Although the herbaceous habit is widespread and occurs in both sister clades of the main lineages in Piperales, it is uncertain under the current phylogenetic hypotheses whether the putative ancestral state for the Piperales is herbaceous (fig. 17A). The equivocal ancestral state results from uncertainty of the ancestral state within Asaroideae and Aristolochioideae + *Lactoris*, in which the latter sister groups share a putative woody ancestor. In addition, this equivocal reconstruction might come from outgroup comparisons, which favor a woody habit for the putative ancestor of Piperales.

Our overview of the anatomy of Piperales reveals a continuum of variation in cambial activity involving various degrees of intra- and interfascicular cambium development. For instance, cambial activity is mostly restricted to collateral bundles and is present, although only minutely expressed, between bundles in some species (e.g., Saururaceae, *Zippelia*), while the cambium and cambial derivatives may form a continuous and somewhat broader homogenous ring in other species (e.g., *Saruma*). Increasing degrees of cambial activity was observed within some genera, such as *Thottea*, ranging from a relatively reduced intervacular cambial activity in the small subshrub species (fig. 13B) to extensive secondary growth in larger shrubs (fig. 13C). Further studies might integrate such trends into hypotheses based on ancestral state reconstruction and thus provide a more robust anatomical framework to characterize various degrees of cambial activity (e.g., intra- and interfascicular cambium, numbers and nature of cells produced by secondary growth).

Sherwin Carlquist (1993) put forward the hypothesis that anatomical organization in Aristolochiaceae (*Aristolochia* + *Thottea*) may have been derived from an herbaceous or “minimally woody” ancestor. Despite the lack of unequivocal phylogenetic evidence of ancestral herbaceousness in Piperales, several anatomical characters found in woody species from different clades do indeed evoke a putative ancestral herbaceous organization. First, many of the shrubs and climbers rely on the ring of hypodermal tissue for mechanical stiffness during early development; this dependence is underlined by the fact that all of the woody species analyzed also showed evidence of hypodermal repair, which ensures the mechanical integrity of the tissues despite expansion of the wood cylinder (Masselter and Speck 2008). Second, the inability of the interfascicular cambium to produce fusiform initials (i.e., meristematic cells that give rise to longitudinally aligned cells [e.g., fibers and vessels]) results in wide rays extending into secondary xylem. Interestingly, the herbaceous *S. henryi* is suggested to be the most ancestral species of Aristolochiaceae based on floral morphology (Dickison 1992) and is the only member of Piperales that lacks xylem rays and axial parenchyma and exhibits a complete cylinder of secondary xylem, although limited to only a few cambium-derived cells.

Whatever the exact putative ancestral organization in the Piperales, our survey of the anatomy of Piperales suggests that the restriction of fusiform initials to fascicular cambial initials—which is probably linked to some characteristics related to an herbaceous organization—was generally retained and potentially influenced the anatomical and functional development of the entire group. The organization of the wood cylinder in all Piperales may thus represent an “unbreakable trait” (Bateman 1994; Bateman and DiMichele 1994) with wide, parenchymatous, raylike tissue derived from interfascicular cambial initials (S. T. Wagner, S. Isnard, N. P. Rowe, M.-S. Samain, C. Neinhuis, and S. Wanke, unpublished man-

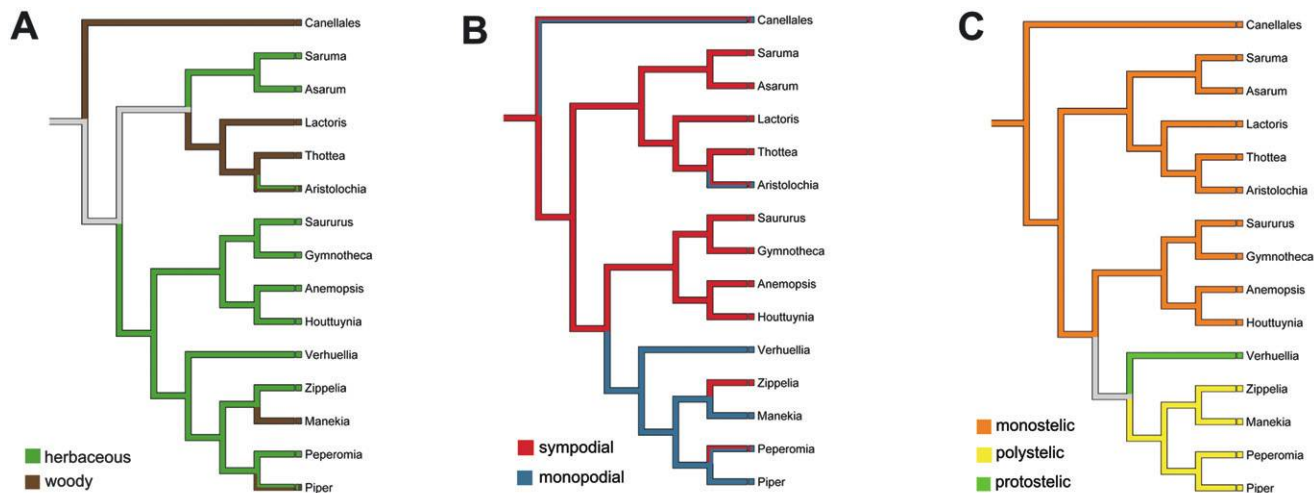


Fig. 17 Evolution of anatomical and architecture character among Piperales inferred from parsimony reconstruction, with Canellales used as the outgroup. Inferred ancestral traits illustrate the habit (A), branching process of the main stem (B), and distribution of vascular bundles in the stem (C). This last character illustrates the conducting system forming a single cylinder (monostelic), multiple cylinders (polystelic), or a unique central cylinder of protoxylem surrounded by phloem (protostelic).

uscript). This organization explains the limited development of Young's modulus in many of the shrubs as well as the widespread occurrence of woody climbers with inherently highly flexible stems. A limited number of truly arborescent species in Piperales might also be explained by this constrained anatomical organization.

If constraints on cambium development have canalized growth form diversification in the Piperales, their effects have varied between different clades. Growth forms within Aristolochioideae + *Lactoris* show a radiation similar to that of many climbing plants with flexible stems and mechanically unstable shrubs. *Peperomia* shows a radiation into small-body-sized herbs, and *Piper* contains mainly shrubs, treelets, and climbers. The appearance of treelets in *Piper* is possibly linked with the appearance of monopodial growth of the main stem.

On the basis of a parsimony reconstruction (fig. 17A), the herbaceous habit is proposed to be ancestral in Piperaceae, involving secondary evolution of woodiness independently in *Manekia* and *Piper*. In the Aristolochioideae + *Lactoris* and Asaroideae, the herbaceous habit arose either primarily or secondarily. A particular type of vascular bundle organization has evolved in Piperaceae. Most species have a polystelic stem organization, although the number, origin, and disposition of vascular bundles is variable and can vary from a single central vascular bundle (protostelic organization) up to several rings of vascular bundles. Character-state reconstruction performed using parsimony suggests that a single ring of vascular bundles is shared by the common ancestor of Saururaceae and Piperaceae (fig. 17C). However, the origin of a polystelic organization in Piperaceae remains equivocal, because the protostelic *Verhuellia* is sister to all other Piperaceae. The similarity between external and internal bundles in *Peperomia* was commented on as early as the 19th century by Debray (1885), who proposed that vascular bundles of *Peperomia* might be homologous to the medullary vascular bundles of *Piper* species. The loss of peripheral vascular bundles where intensive secondary growth occurs in *Piper* could lead to stems with scattered medullary bundles with only limited or no secondary growth, as observed in *Peperomia*. Such hypotheses would explain why the cambium develops only minutely or has been lost entirely in many *Peperomia* species. A detailed study of the developmental origin of peripheral and medullary bundles is needed to test the homology between the outer and inner vascular system and shed light on the evolution of polystelic and protostelic structures in Piperaceae. The laxness of the nature and distribution of vascular bundles in Piperaceae and the absence of cambial activity following Debray's observations provide a compelling developmental framework for understanding the loss of vascular cambium in the monocotyledons and the similar relatively unordered organization of vascular bundles in many taxa.

Some taxa, such as the diminutive *Verhuellia*, as well as all species of *Peperomia* that we have observed have undergone a complete loss of secondary xylem and secondary phloem, which is certainly linked with the evolution of small-bodied, slender, terrestrial herbs and epiphytes. Interestingly, the genus *Peperomia* underwent a remarkable diversification, so while loss of secondary growth profoundly limited large-bodied growth form diversity, it arguably facilitated large-

scale diversification of small-bodied species into new niches (Samain 2008; Samain et al. 2009b). Growth form and life form diversification within these small-bodied plants is probably closely linked with a fine-tuned adjustment of mechanical properties and anatomical organization in diverse species of *Peperomia* (L. Frenzke, unpublished data) as well as architectural and ecophysiological modifications between epiphytic, terrestrial, geophytic, upright, prostrate, and pendulous life forms and growth forms.

Sympodial Architecture and Relevance of Piperales in Understanding Early Angiosperm Diversification

In contrast to its sister group Canellales and other members of magnoliids, which are dominated by woody growth forms (including large trees), Piperales lack arborescent species and show significant radiations of herbaceous species linked to a sympodial architecture (fig. 17C).

Sympodial architecture has been proposed as a putative ancestral trait in angiosperms, possibly coupled with a functional limitation resulting from a reduced cambium (Blanc 1986; Carlquist 2009; see also Holtum [1955] for monocots). In Piperales, sympodial growth is shown to be an ancestral state (fig. 17C), which was historically and developmentally linked with the evolution of other traits—mechanical, anatomical, hydraulic, and ecophysiological—that underlie the radiation into different growth habits.

First, the widespread occurrence of the Mangenot model in shrubs of Aristolochioideae + *Lactoris*, characterized by a stack of sympodial modules, may have allowed some species to at least partly bypass the mechanical limitations of a single tapered wood cylinder composed of a large proportion of raylike parenchyma. The development seen in the Mangenot model arguably ensures a higher level of mechanical stability by developing a main stem composed of larger-diameter basal parts of each module, while the more distal flexible part of each module acted as lateral branches.

Second, basal sprouting in woody Piperales in forest understory habitats might also be an efficient way of surviving mechanical failure of the main stem by producing additional stems following damage from branch and debris fall—an important source of mortality in understory environments. Interestingly, a variation of sympodial growth (compatible with that described for Piperales) by sympodial establishment through basal emission of axes of increasing vigor occurs in understory species of *Thottea* from Malaysia as well as in many species of *Piper* and in herbs and trees of Chloranthaceae (Blanc 1986). The phylogenetic position of these taxa in the angiosperm phylogeny prompted Blanc (1986) to suggest that angiosperms were originally understory shrubs. Blanc (1986) also reported that this type of sympodial growth is frequently found in other forest understory taxa. The “damp, dark, and disturbed” hypothesis similarly proposed an understory origin of early angiosperms that exhibited lower xylem conductive capacities and lower water use efficiency of leaves, compared with most extant angiosperms (Feild et al. 2001, 2003, 2004, 2009, 2011b; Feild and Arens 2007).

Amborella, the Austrobaileyales, and some Chloranthaceae also show evidence of abundant sprouting in multitemmed shrubs, treelets, or climbers (Feild et al. 2004; Feild and

Arens 2007). Further architectural studies among early-diverging angiosperms are needed to shed light on the potential links between sympodial architectures and “dark and disturbed” habitats and their connection with some of the principal developmental characters underling the plant’s overall origination, such as secondary growth and stem biomechanics and hydraulics.

Sympodial development has given way to monopodial growth in many of the lineages belonging to the grade sister to eudicots (Magnoliaceae, Lauraceae, Canellaceae, Winteraceae, Chloranthaceae, and Austrobaileyales) and is related to the tree habit (Blanc and Andraos 1983; Blanc 1986; Carlquist 2009). In Piperales, monopodial growth has evolved independently in only a few lineages in the Piperaceae and Aristolochioideae (fig. 17B). Our survey indicates that the appearance of indeterminate monopodial growth of the main stem is linked with the emergence of treelets, which in *Piper* may grow while leaning on surrounding vegetation. Apart from *Piper*, monopodial growth occurs only in small herbaceous or climbing species of *Aristolochia* or *Manekia*.

Shifts toward Herbaceous Organization and “Resetting” the Capacity for Morphological Diversification

This broad survey of growth form, architecture, anatomy, and biomechanics within the Piperales demonstrates that shifts in overall growth form between herbs, woody shrubs, climbers, and epiphytes is a major characteristic of the diversity within the group.

Further studies should test the hypothesis that transitions—particularly to herbaceous or climbing growth forms—placed specific structural and functional constraints on derived growth forms. A recurring transition within the Piperales is the reduction in body size and/or simplification of the body plan toward an herbaceous organization. Modification, redundancy, reduction of wood development, or even the disappearance of wood development strongly influenced growth form, architecture, and mechanical organization. Shifts toward an herbaceous organization and the subsequent canalization of developmental and mechanical architectures have been suggested for the Ranunculaceae among early-diverging eudicots (Isnard et al. 2003). In this study, it was hypothesized that secondary xylem development within the Ranunculaceae was irreversibly modified among ancestral perennial herbs toward soft, parenchyma-rich, rhizomelike stems. This suggested that subsequent radiation of the group constrained self-supporting growth forms while being preadapted (Gould and Vrba 1982) for flexible aerial stems adapted for the climbing habit.

Within Piperales, shifts toward herbaceous or climbing organizations arguably represent a “resetting” of the Piperales bauplan to a simplified organization that then underwent subsequent diversification. Such transition may have produced yet more novel and ecologically “successful” growth forms, such as highly flexible lianas in *Aristolochia*—in contrast to leaning or weakly scandent shrubs—and diverse terrestrial to epiphytic herbs in *Peperomia*. The phenomenon is comparable to the evolutionary “developmental ratchet” (Vermeij 1987; Levinton 1988; Bateman and DiMichele 1994), which is “reset at a lower level, leaving a combination

of adaptation and contingency to define a new evolutionary trajectory for the lineage should it survive the establishment bottleneck” (Bateman and DiMichele 1994, p. 83). That shifts toward herbaceous growth forms might invigorate subsequent diversification was proposed for the Paleozoic diversification of now mostly extinct rhizomorphic lycopsids (Bateman 1994).

From a wider perspective, the shifts in growth form we observed in the Piperales toward herbaceous organizations are widespread among early-diverging lineages, such as Nymphaeales and some Chloranthales and Ceratophyllales, and monumentally so with the evolution of the monocotyledons. Our study demonstrated that “reinvention” and “reconfiguration” following major shifts in growth form can involve both (a) small-scale modification, such as self-repair of hypodermal tissue or wood ray lignification, and (b) higher-order rearrangement of architectural units, such as shifts in the size, position, and longevity of meristems (strict to lax sympodial growth and monopodial growth of the main stem). A compelling task for future studies is to investigate how these different levels influence changes in plant form across and within clades of early angiosperms and to what extent Holtum’s “architectural variations on a theme” may influence or be influenced by developmental shifts at lower hierarchical levels, such as tissue-level mechanics and hydraulics.

Further studies should also attempt to infer potential shifts between woody, herbaceous, and climbing growth forms from the fossil record of early angiosperms. Such tools are now available for modeling both mechanical (Speck and Rowe 1999) and hydraulic (Wilson et al. 2008) functioning of fossils based on even fragmentary material, given the appropriate level of cellular preservation. Extinct angiosperm clades may show potentially similar levels of growth form shift and subsequent “reinvention” and “reconfiguration.” Functional studies may therefore significantly aid us in the task of identifying the ecological factors and corresponding life history traits underlying the early diversification of angiosperms. Our survey of growth forms and their traits within the Piperales suggests that it is possibly an understanding of the shifts of form and function across related clades as well as the traits that make up such shifts that will potentially offer the best insight into the life histories and habitats of early angiosperms in addition to plausible hypotheses of their functional biology and success.

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