Ecology, forms and functions of the basal angiosperms from New Caledonia

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Docteur

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Spécialité : Écologie, Évolution,
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Présentée par Santiago Trueba-Sánchez

Ecology, forms and functions of the basal angiosperms from New Caledonia

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Ecologie formes et fonctions des angiospermes basales en Nouvelle-Calédonie

Soutenue le 26 avril 2016 devant le jury composé de:

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UNIVERSITÉ MONTPELLIER

Ecology, forms and functions of the basal angiosperms from New Caledonia

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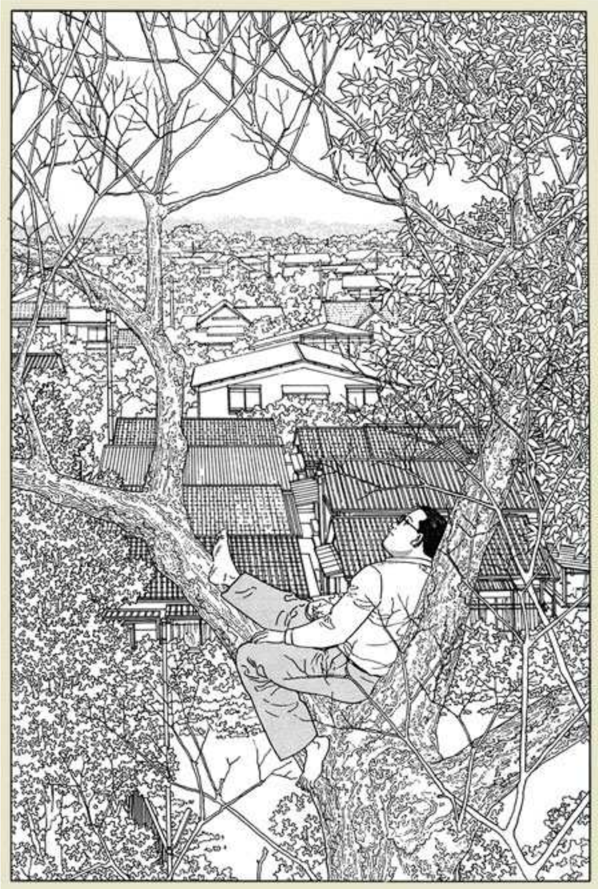


Jiro Taniguchi, The Walking Man (1995)

Que beleza é sentir a natureza
Ter certeza pr'onde vai
E de onde vem
Que beleza é vir da pureza
E sem medo distinguir
O mal e o bem

Tim Maia, Imunização Racional (1974)

A mis padres

Résumé

L’une des remarquables originalités de la flore néo-calédonienne repose sur la présence de nombreuses lignées correspondant aux premières divergences des plantes à fleurs, compte tenu de leurs positions phylogénétiques. Au sein de ces lignées, certaines espèces sont susceptibles de porter des traits morpho-anatomiques ancestraux. Par conséquent, dans un contexte comparatif, l'étude de ces espèces peut fournir des informations cruciales pour comprendre les premières étapes évolutives des angiospermes. Un premier volet de cette thèse vise à étudier des caractéristiques structurelles et fonctionnelles des groupes représentatifs d’angiospermes basales. L'étude d'*Amborella trichopoda*, espèce sœur de toutes les angiospermes, nous a montré une covariation des traits fonctionnels (tige-feuille) et une plasticité morphologique en réponse à des variations de l'environnement lumineux. Cela suggère que ces réponses plastiques étaient déjà présentes chez l'ancêtre commun de toutes les plantes à fleurs. En parallèle, l’étude de l'évolution anatomique des Piperales, ordre le plus riche parmi les angiospermes basales, suggère que leur ancêtre commun aurait possédé un cambium actif. Ces résultats supportent que les premières angiospermes avaient une forme de vie ligneuse et que la structure sympodiale à été acquise dans les premières étapes évolutives des angiospermes.

Les angiospermes basales comptent parmi les lignées d'angiospermes surreprésentées en Nouvelle-Calédonie. Cependant, les mécanismes à l’origine de cette dysharmonie demeurent inexplorés. Un deuxième volet de cette thèse analyse la répartition environnementale des angiospermes basales de l'archipel afin de connaitre leurs préférences en termes d’habitats, ainsi que leurs exigences environnementales. Cette distribution environnementale a également été analysée au regard de leur résistance à la sécheresse. Nous montrons que la plupart des espèces présentent une préférence marquée pour des habitats de forêt humide avec des faibles variations en température. La vulnérabilité hydraulique face à la sécheresse apparait comme un trait majeur qui confine la distribution de ces espèces dans des habitats humides. Ces conditions auraient persisté dans des zones refuges dans l'archipel lors de la dernière période glaciaire, permettant ainsi le maintien de certaines lignées d’angiospermes basales. Une stabilité climatique passée pourrait donc être à l'origine de la surreprésentation de certains groupes d'espèces forestières qui ont disparu dans les régions voisines. La distribution des angiospermes basales néo-calédoniennes, ainsi que leur sensibilité à la sécheresse, supportent l'hypothèse suggérant que les premières angiospermes habitaient des milieux humides et stables.

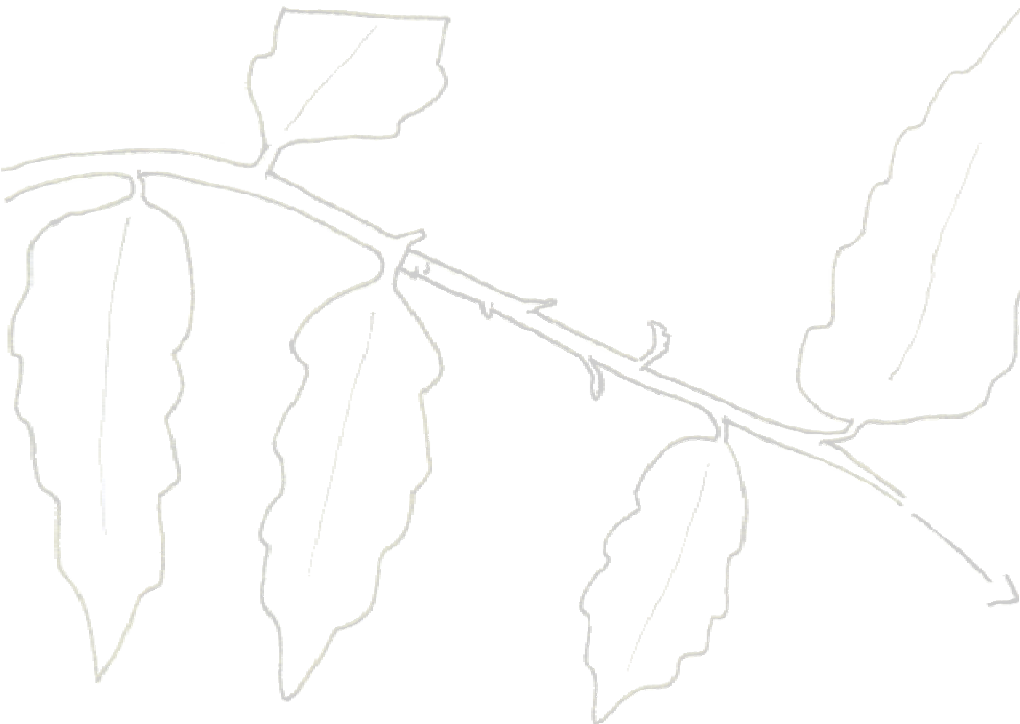
Mots-clés: anatomie du bois, angiospermes basales, biogéographie, écologie fonctionnelle, écophysiologie, évolution, Nouvelle-Calédonie, vulnérabilité à la sécheresse.

Abstract

One of the remarkable characteristics of the New Caledonian flora is the presence of numerous angiosperm lineages recognized as the earliest divergences of the flowering plants, due to their phylogenetic positions. Within these lineages, some species are likely to bear ancestral morpho-anatomical features. Therefore, under a comparative perspective, the study of these species can provide compelling information for understanding the early evolutionary stages of angiosperms. The first part of this thesis aims to study the structural and functional characteristics of representative groups of basal angiosperms. The study of *Amborella trichopoda,* sister species to the remaining flowering plants, shows a covariation of functional traits (stem-leaf) and a morphological plasticity in response to changes in the light environment. This suggests that these plastic responses were already present in the common ancestor of angiosperms. In parallel, the study of the anatomical evolution of Piperales, the most diversified basal angiosperm order, suggests that their common ancestor had an active cambium. These results support the hypothesis that early angiosperms had a woody habit and that sympodial growth may have been acquired early during angiosperms evolution.

Basal angiosperms are among the over-represented angiosperm lineages of New Caledonia. However, the mechanisms underlying this disharmony remain unexplored. A second component of this thesis analyzes the environmental distribution of New Caledonian basal angiosperms to know their habitat preferences and their environmental requirements. Further, we assess the influence of their drought tolerance on their environmental distribution. We show that most species have a preference for rain forest habitats with small variations in temperature. Drought-induced hydraulic vulnerability stands as a major trait that restricts the distribution of these species to humid habitats. These stable conditions seem to have persisted in refugial areas in the archipelago during the last glacial maximum, allowing the persistence of basal angiosperm species. Therefore, a past climatic stability could explain the over-representation of some groups of forest species that may have disappeared in neighboring regions. The distribution of basal angiosperms in New Caledonia, as well as their drought sensitivity, support the hypothesis suggesting that early angiosperms lived in humid and stable environments.

Keywords: basal angiosperms, biogeography, drought vulnerability, ecophysiology, evolution, functional ecology, New Caledonia, wood anatomy.



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AVANT-PROPOS

Cette thèse est le résultat de trois ans de travail au sein du **Laboratoire de Botanique et d'Ecologie Végétale Appliquées**, de l'**Institut de Recherche pour le Développement** de Nouméa, Nouvelle-Calédonie.

Le laboratoire fait partie de l'unité mixte de recherche **Botanique et Modélisation de l'Architecture des Plantes et des Végétations** (UMR AMAP) basée à Montpellier, France.

Cette thèse a été financée par le **Consejo Mexicano de Ciencia y Tecnología** (CONACYT) avec la bourse de thèse numéro 217745.

Ce document est rédigé sous forme de thèse sur publications. L'information compilée dans cette thèse a été partialement publiée ou soumise à des journaux scientifiques internationaux. Une liste de publications est disponible dans la dernière section de ce document.

Afin de répondre à la demande de l'organisme mexicain qui finance cette thèse, ainsi que pour la rendre plus accessible, ce document a été rédigé en anglais. Un résumé détaillé en français est disponible à la fin de ce document de thèse.

FOREWORD

This PhD thesis is the result of three years of work carried out at **Laboratoire de Botanique et d'Ecologie Végétale Appliquées** of the **Institut de Recherche pour le Développement** at Noumea, New Caledonia.

The laboratory is part of the mixed research unit **Botanique et Modélisation de l'Architecture des Plantes** (UMR AMAP) which is based in Montpellier, France.

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This is a thesis by publications. The information contained in this document has been partially published or submitted to international scientific journals. A list of publications is available at the last section of this thesis document.

In order to respond to the demand of the Mexican institution that funded this PhD, and to reach a larger number of readers, most of the content of this thesis is written in English. An extended abstract in French is provided at the end of the document.

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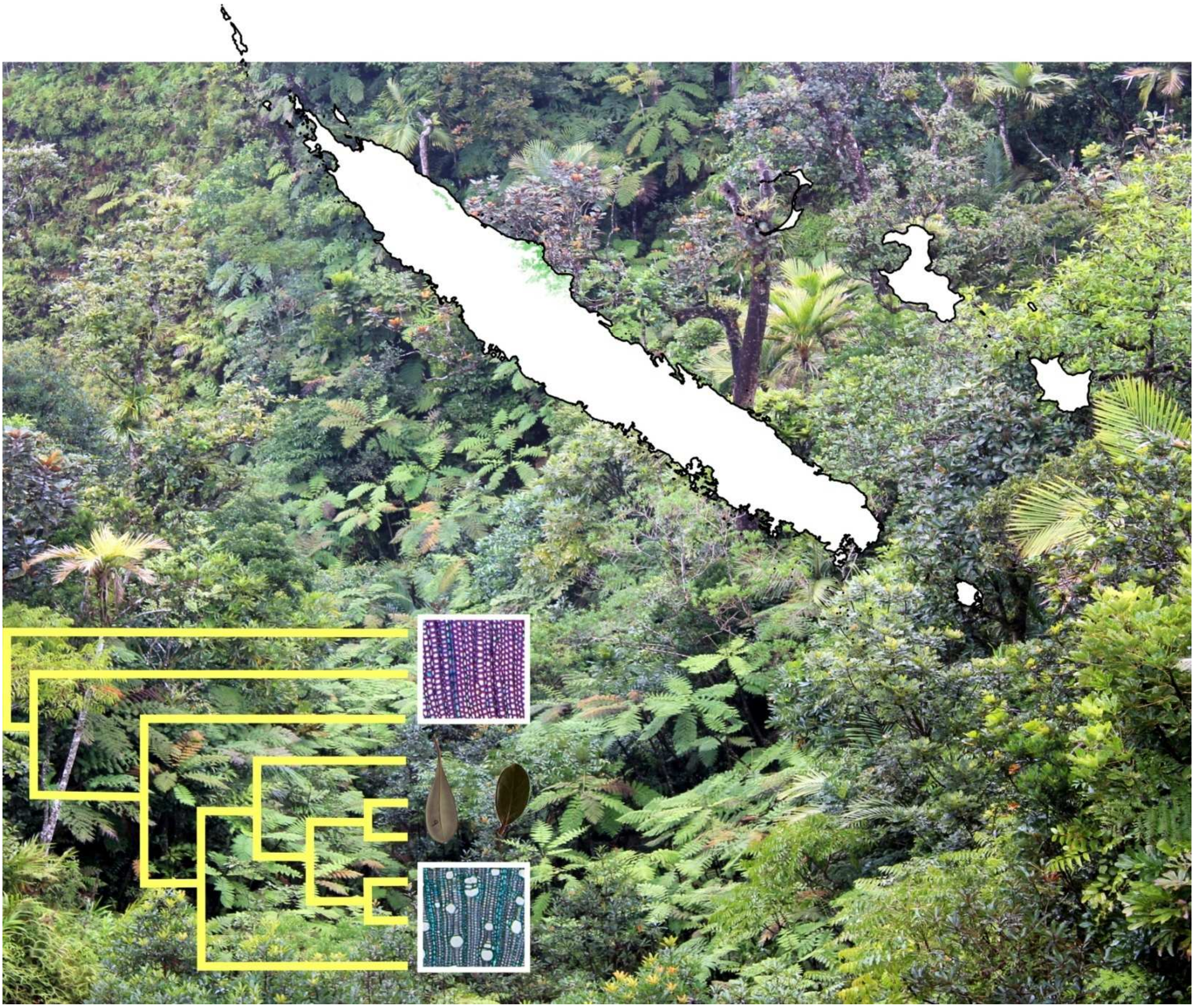
General Introduction



- Fundamentals and rationale
- Context of the study
- Main objectives

1.1. A brief summary of the evolutionary history of vascular plants

The earliest land plants (embryophytes) evolved from charophycean green algal ancestors about 475 million years ago during the Ordovician (Wellman *et al.*, 2003; Steemans *et al.*, 2009; Fiz-Palacios *et al.*, 2011). About 45 Myr later, during the Siluro-Devonian transition, a great radiation of land biotas occurred (Steemans *et al.*, 2009). Plant diversity increased across an ecologically undersaturated and thus low-competition landscape, producing a radiation that has been compared to the Cambrian explosion of marine faunas (Bateman *et al.*, 1998). Pioneer land plants struggled with physical and physiological problems posed by a terrestrial existence. It has been suggested that lignin was first synthesized in order to provide protection against solar ultraviolet radiation and desiccation (Lowry *et al.*, 1980). The synthesis of lignin provided structural rigidity for the first tracheophytes to stand upright, and strengthened the cell walls of their water-conducting tracheary elements to withstand the negative pressure generated during transpiration (Weng and Chapple, 2010). Tracheophytes had a rapid expansion during the Silurian-Devonian transition (Gray, 1993). The diversification of tracheophytes had far-reaching consequences on terrestrial ecosystems (Kenrick and Crane, 1997). The appearance of secondary growth in the mid-Devonian (*c*. 380 Myr ago) favored water conductance and mechanical support, prompting diversification in growth forms (Bateman *et al.*, 1998).

The photosynthetic activity of megaphyll leaves, with branched veins and flat shape, provided the basis of net primary production for the development of land organisms *c*. 360 Myr ago during the Devonian period (Beerling *et al.*, 2001). During the same period *c*. 370 Myr ago, the earliest known modern trees emerged (Meyer-Berthaud *et al.*, 1999). In parallel, the late Devonian was also the period during which the first seed plants (spermatophytes) evolved (Rothwell *et al.*, 1989). During the Permian (*c*. 320 Myr ago), modern gymnosperm trees began to dominate the forest canopy. Through the Carboniferous and Permian (340 - 260 Myr ago) the first extensive forests appeared, and the ecosystem dynamics that control modern vegetation were established (Bateman *et* al., 1998). Gymnosperms had a long ecological dominance through the Triassic and Jurassic (250 -150 Myr). During the late Jurassic *c*. 150 Myr ago the first flowering plants appeared (Sun *et al.*, 1998). The rise of the flowering plants was followed by a major invasion into the gymnosperm niche during the Cretaceous (Bond, 1989), leading to an upset of the composition of the terrestrial vegetation over a relatively short period.

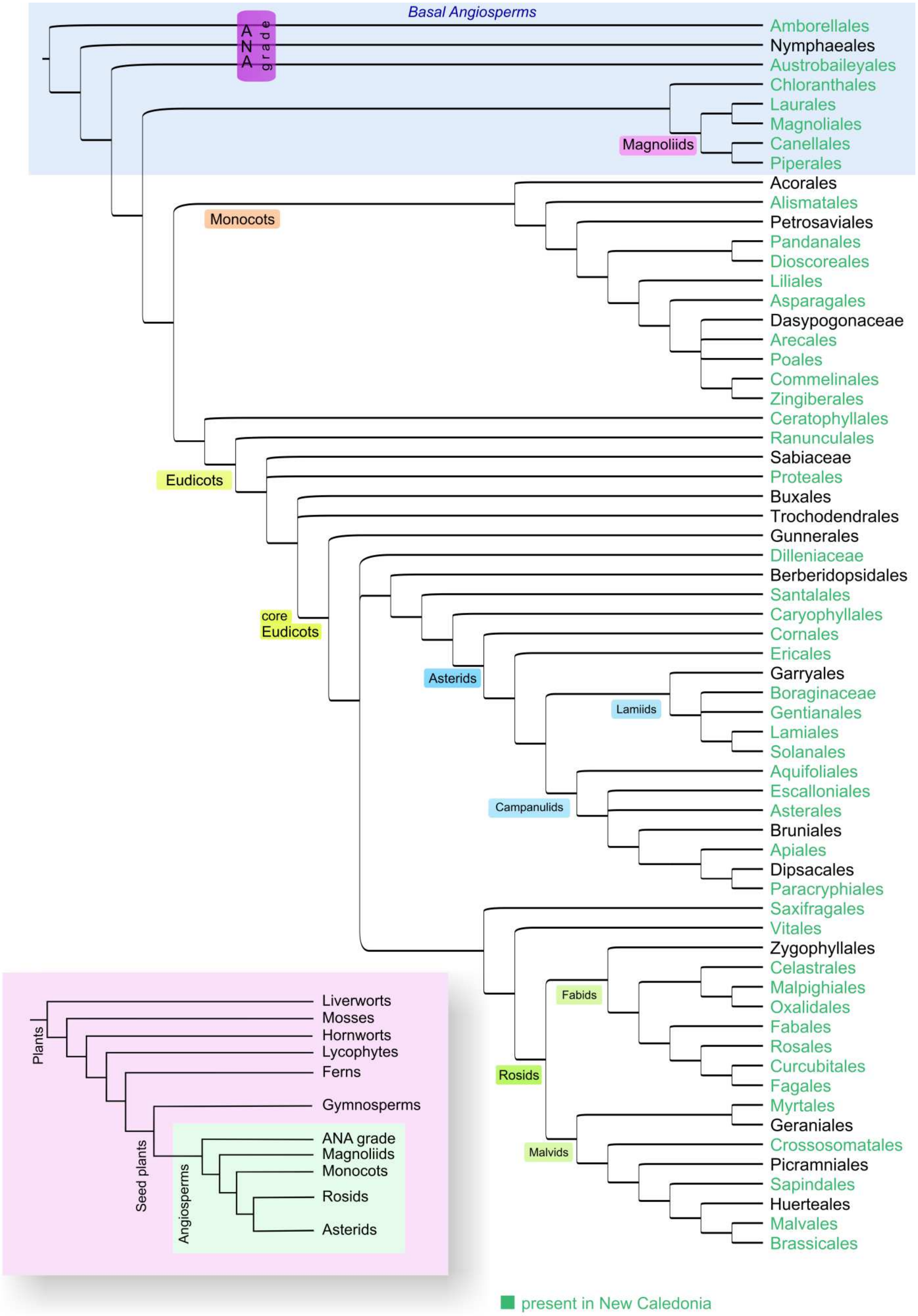


FIGURE 1.1 Angiosperm phylogeny. The flowering plants systematics is based on APG III (2009) relationships. Major clades are indicated by text boxes. Angiosperm orders present in New Caledonia are highlighted in green.

1.2 The flowering plants, evolution and extant lineages

Described by Charles Darwin as an "abominable mystery" and a "perplexing phenomenon", the origin and early evolution of flowering plants (angiosperms) is pivotal in the evolutionary history of terrestrial biota (Leebens-Mack *et al.*, 2005). The fascination of Darwin with the early radiation of angiosperms is legendary, and perhaps no other group of organisms merited Darwin's attention in such dramatic terms (Friedman, 2009). It is not by chance that one of the most outstanding naturalists of all times showed much fascination (and frustration) with the evolutionary history of a particular group of organisms. The origin of angiosperms was followed by a rapid rise of ecological dominance, inducing one of the greatest terrestrial radiations of life (Davies *et al.*, 2004). Since their origin, angiosperms have extraordinarily diversified and they currently dominate the vegetation of most terrestrial ecosystems (Crane *et al.*, 1995). Along with their species richness, angiosperms also exhibit a vast morphological, ecological, and functional versatility, establishing the structural and energetic basis of the great majority of current terrestrial ecosystems (Magallón and Castillo, 2009; Crepet, 2013). Angiosperm richness represents more than that of all other groups of land plants combined. Crepet and Niklas (2009) have estimated that 89.4% of extant embryophyte species are angiosperms, having much more species richness than ferns (3.99%) or gymnosperms (0.29%). Angiosperms are currently represented by *c.* 350 000 extant species, and advances in molecular systematics have provided strong bases for the recognition of major angiosperm clades and the establishment of their relationships (Fig. 1.1) (APG III 2009). Within flowering plants, eudicots (Fig. 1.1) constitute the richest group, containing 73% of extant angiosperm species richness (Magallón *et al.*, 2015). The largest proportion of species richness of eudicots is contained in the large clades Asterids and Rosids (Fig. 1.1), which represent 35.2% and 29.2% of extant angiosperm richness, respectively (Magallón *et al.*, 2015). Monocots, the second largest angiosperm group (Fig. 1.1) has 23.3% of extant species richness (Magallón *et al.*, 2015). Preceding the massive monocots-eudicots clade, "basal angiosperms" are an ensemble of poorly diversified lineages, arising from the first nodes of the angiosperms' phylogeny (Fig. 1.1).

1.3 Basal angiosperms, the earliest diverged lineages of the flowering plants

Amborellales, Nymphaeales, and Austrobaileyales have been identified by several studies (using multiple nuclear, plastid, and mitochondrial genes) as successive sister lineages relative to all other flowering plants, and represent the earliest diverging branches of the angiosperms' phylogeny (Figs. 1.1; 1.2) (Mathews and Donoghue, 1999; Soltis *et al.*, 1999; Qiu *et al.*, 2000; Zanis *et al.*, 2002; Moore *et al.*, 2007). These orders form a paraphyletic group which is usually referred to as the ANA grade (Figs. 1.1; 1.2) (Finet *et al.*, 2010). Within this grade, Amborellales is the earliest lineage, and diverged about 139.4 Myr ago (Fig. 1.2) (Magallón *et al.*, 2015).

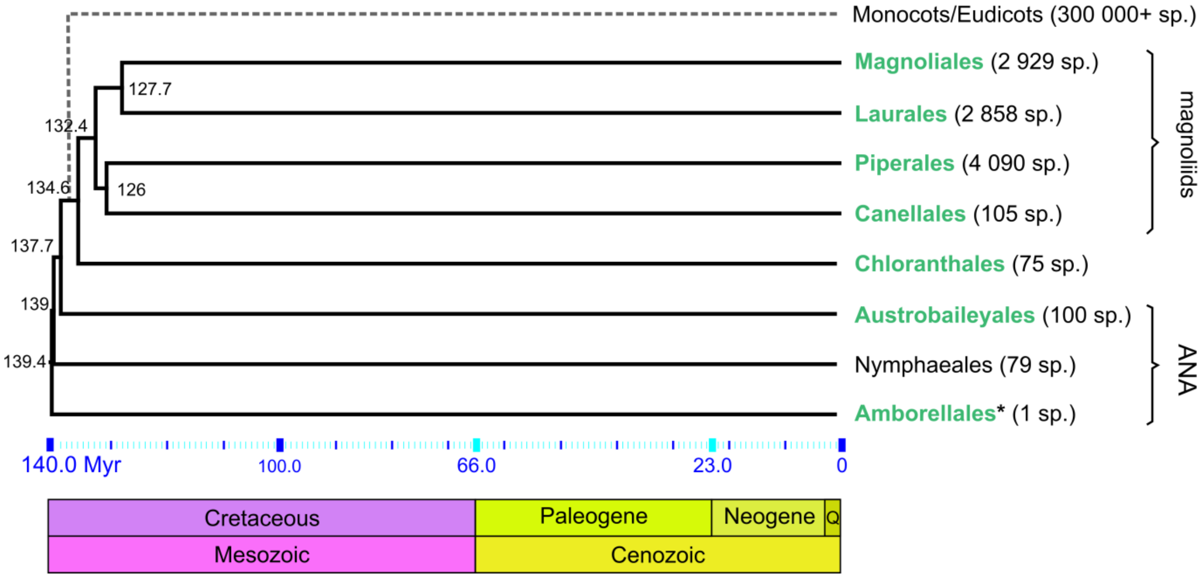


FIGURE 1.2 Basal angiosperm time-tree. Terminals were collapsed to represent orders. Numbers next to nodes indicate median ages for each lineage. The tree was constructed using the R package ape (Paradis *et al.*, 2004) with node ages estimated in Magallón et al. (2015). The number of species within each group was obtained from Stevens (2001-onwards). Basal angiosperm orders present in New Caledonia are highlighted in green. Endemic lineages from New Caledonia are indicated with an asterisk.

Stem ages of all basal angiosperm lineages date back from the early angiosperm radiation during the Early Cretaceous (Fig. 1.2). Diverging immediately after the ANA grade, Chloranthales and magnoliids form another early branching angiosperm clade (Moore *et al.*, 2007; Moore *et al.*, 2010) with an estimated common stem age of *c.* 134.62 Myr (Fig. 1.2) (Magallón *et al.*, 2015). These clades do not form a natural group given that they do not share a direct common ancestor (*i.e.* they are not monophyletic) (Figs. 1.1; 1.2). However, because of their early divergence, the grouping of the ANA grade + magnoliids + Chloranthales are considered as "basal angiosperms" (Amborella-Genome-Project, 2013). This nomenclature will be followed in this work and we will hereafter consider as basal angiosperms all species diverging before the monocots/eudicots node (Figs. 1.1; 1.2). Basal angiosperms have low species richness compared to eudicots and monocots (Fig 1.2). For instance, although Piperales have high species richness (Fig. 1.2), the entire magnoliids clade contains only 3.6% of extant angiosperm species richness (Magallón *et al.*, 2015). The low number of basal angiosperm species is probably linked to their old ages given that the age of a clade is negatively related to its diversification rate in angiosperms (Magallón and Castillo, 2009).

FIGURE 1.3. Global geographical distribution of families in the ANA grade. Cabombaceae and Nymphaeaceae (Nymphaeales) are cosmopolitan and not displayed on the map. Reproduced from Buerki *et al.* (2014).

Five of the seven families in the ANA grade are distributed in tropical East and South-East Asia, Australia and the Pacific islands (Fig. 1.3) (Buerki *et al.*, 2014). Only Cabombaceae and Nymphaeaceae, composed of aquatic plant species, are cosmopolitan. Families within the magnoliid clade have a much larger geographical distribution. However, they are mostly pantropical, and a great majority of their lineages are represented by tropical rainforest species (Liu *et al.*, 2014). Although some species-rich magnoliid families have representatives in temperate regions (*e.g.* Aristolochiaceae, Lauraceae and Magnoliaceae), the greatest species richness of these groups is observed within the tropics, notably in Asian and Australasian tropical rainforests (Morley, 2001). Moreover, some magnoliid families such as Eupomatiaceae, Degeneriaceae, and Himantandraceae have distributions restricted to tropical Australia and the Pacific islands (Stevens, 2001-onwards). Finally, Chloranthales are also largely restricted to tropical and subtropical habitats, distributed predominantly in non-seasonal montane cloud forests with high rainfall (Feild *et al.*, 2003; Feild *et al.*, 2004).

1.4 ‘Basal’ but not ‘primitive’, what does basal mean?

As shown in the previous section, basal angiosperms are the species-poor successive sister lineages of the highly diversified monocots-eudicots clade (Fig. 1.2). Differences in the diversification rates between these two angiosperm groups result in an unbalanced tree (Fig. 1.1). Unbalance in phylogenetic trees frequently leads scientists to adopt a linear and cohesive narrative of evolutionary stories, assuming that the poorly diversified group is more ‘primitive’ (O'Hara, 1992; Crisp and Cook, 2005; Gregory, 2008). However, evolution is not linear, but branched, and evolution does not cohere, but diverges (O'Hara, 1992). Moreover, the tree balance that we observe today may have significantly changed over time (Fig. 1.4). A linear narrative of evolution prevailed for over a century since the ‘great chain of being’ (also known as *scala naturae*) was proposed by Charles Bonnet in 1745 (Rigato and Minelli, 2013). The production of the first tree diagram and the notion of ‘evolutionary tree’ contained in the *The Origin of Species* of Charles Darwin (1859) changed the long-held linear vision of evolution.

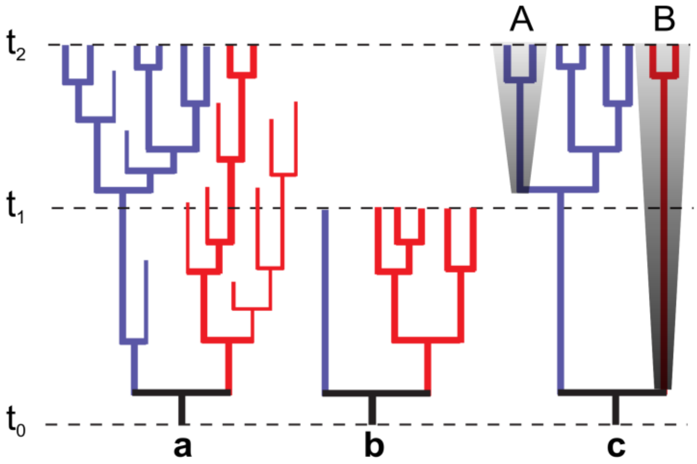


FIGURE 1.4. Hypothetical evolutionary tree showing differences in tree balance through time in two sister groups (blue and red). (a) Both groups originate at the same speciation event at time t_0, and therefore have the same age. All extant and extinct lineages arising within both taxa are shown until the present (t_2). Thin lines indicate lineages that are now extinct. (b) Reversal of tree balance; at time t_1 the red group was more speciose. (c) Current tree where the blue group is more speciose. Although lineages A and B have the same crown age; clade B has greater stem age and diverged earlier from their most recent common ancestor. Redrawn and adapted from Crisp and Cook (2005).

Although the idea of a ‘ladder of progress’ driving evolution has been left behind since the end of the 19th century, many biological studies aiming to reconstruct ancestry confuse present-day descendants and long-dead ancestors because of misinterpretation of phylogenetic trees (Crisp and Cook, 2005; Omland *et al.*, 2008; Rigato and Minelli, 2013). For instance, statements such as "*Amborella* is the most ancient angiosperm" (Goremykin *et al.*, 2003), or "*Amborella* represents the first stage of angiosperm evolution" (Qiu *et al.*, 1999), denote linear narratives in evolutionary stories, and can eventually confuse unwary readers. Therefore, to avoid possible misinterpretations, we would like to underline that all extant angiosperms are contemporary and 'basal' angiosperm does not mean 'primitive' angiosperm. However, a phylogenetic tree can effectively show successive branching over time (case *c* in Fig. 1.4) (Krell and Cranston, 2004). In this sense, by embracing ‘tree thinking’, we consider that basal angiosperms are species belonging to the earliest branching lineages of angiosperms. Even if they are not primitive *per se*, the study of basal angiosperms can provide insightful information for understanding the early stages of flowering plant evolution.

1.5 What can studying basal angiosperms tell us about flowering plant evolution?

Under a comparative perspective, basal angiosperms are indispensable in studies interested in the evolutionary patterns of angiosperm functional and structural attributes. Moreover, it has been suggested that basal angiosperm species bear primitive features.

1.5.1 Basal angiosperms are fundamental in comparative studies

Comparative biology depends on understanding the distribution patterns of organismal characters across taxa. Using a comparative approach we can detect feature that characterize single clades, pointing to potential *synapomorphies*, or characters that arise repeatedly in different evolutionary lineages resulting in evolutionary *convergences* (Losos, 2011). Comparative studies are stronger to the extent that they include more lineages. The more representative lineages are included in a study, the more "evolutionary opportunities” for diverging from a given pattern are included (Harvey and Pagel, 1991). When there are many different ancestral character states, the potential number of independent evolutions of the same feature increases in the dataset, improving statistical confidence in the result. Consequently, for comparative studies it is crucial to include an array of clades as wide as possible. Given that basal angiosperms represent the successive sister lineages of the monocots-eudicots clade (Figs. 1.1; 1.2), they constitute an essential part of the array of flowering plants. Therefore, sister lineages such as *Amborella*, when compared with other key lineages, can provide unique insights into angiosperm ancestral characteristics (Amborella Genome Project, 2013). In the previous section of this chapter we have stressed the risks of using intuitive interpretations of ancestry from phylogenetic trees. However, ancestral features can be inferred from a phylogeny by applying a method that optimizes the distribution of the states of a character over the whole phylogenetic tree (Pagel, 1999). Phylogenetic optimizations help to infer ancestral character states at each node of a tree by using an evolutionary model that provides the best-fit of a character distribution along the tree

(Crisp and Cook, 2005). Examples of case studies using such adequate comparative methods are provided in Box 1.

Box 1. Basal angiosperms in comparative studies to infer evolutionary histories

Tracheids, the ancestral xylem conduits of flowering plants.

The lack of protoxylem vessels in all non-angiosperm lineages, as well as in key basal angiosperm lineages such as Amborellales and Nymphaeales (Fig. 1.5), suggests that the common ancestor of all angiosperms had vesselless wood. This was confirmed by a maximum parsimony phylogenetic optimization performed by Feild and Brodribb (2013), which suggests that tracheids are the most parsimonious ancestral state for the common node of all angiosperms (Fig. 1.5, arrow). These results suggest that angiosperms are the only vascular plants that have acquired xylem vessels in the primary xylem. Furthermore, their results suggest that at least three independent origins of vessel elements with simple perforation plates occurred late during flowering plant evolution (Fig. 5, stars).

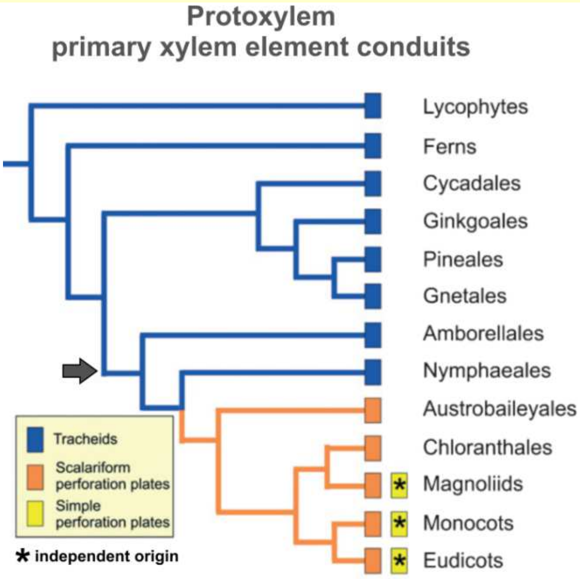


FIGURE 1.5 Summary of the phylogenetic distribution of primary element conduits across vascular plants. The figure represents a maximum parsimony character state reconstruction of primary xylem evolution. Redrawn from Feild and Brodribb (2013).

1.5.2 Plesiomorphic features are evident in basal angiosperms

In a previous section we have underscored that basal angiosperm is not equal to primitive angiosperm. However, while a living species cannot be more primitive in comparison to another living species, the characters they bear can be primitive (i.e. *plesiomorphy*), or derived (i.e. *apomorphy*) (Gregory, 2008). It has been proposed that primitive structural and functional features are observed in basal angiosperm representatives (Box 2). For instance, in a recent synthesis of wood anatomical evolution, Carlquist (2012) states that "*primitive features are evident in earlier branchings of phylogenetic trees*". Moreover, Feild *et al.* (2004) stated that when several lineages are successively sister to a major clade, as is the case in angiosperms, any states that they share can be inferred to be ancestral, even if each of these lineages is apomorphic for other characters. Examples of primitive anatomical and physiological features occurring in basal angiosperms are provided in Box 2.

Box 2. Basal angiosperms bear primitive character states

Xylem conduits and their physiological implications.

The wood anatomy of basal angiosperms is by no means uniform (Metcalfe, 1987). However, it has been proposed that primitive xylem conduits (*i.e.* tracheary elements), such as tracheids or narrow vessels with long scalariform perforation plates (Fig. 1.6A), are frequently evident in basal angiosperm representatives (Carlquist, 2012). It has been shown that xylem conduit anatomy can strongly influence hydraulic conductivity (Christman and Sperry, 2010). Similarly, xylem conduit type seems to be related to leaf vein density (Fig. 1.6B) (Feild and Brodribb, 2013), secondarily affecting associated functions such as leaf gas exchange and photosynthetic capacity. Basal angiosperms have low vein densities as compared to monocots and core eudicots (Fig. 1.6C). Moreover, when considering lineage ages, it has been evidenced that the ANA grade species, Chloranthales, and magnoliids represent early stages of the angiosperm leaf vein escalation that occurred during the Cretaceous (Fig. 1.6C). The lower vein densities of basal angiosperms probably result from their constrained xylem vasculature (Fig. 1.6B). If a feature observed in basal angiosperm species is plesiomorphic, or similar to a trait plesiomorphic in most angiosperms, then studying its properties can reveal information about the functional performance of the traits present in early angiosperms.

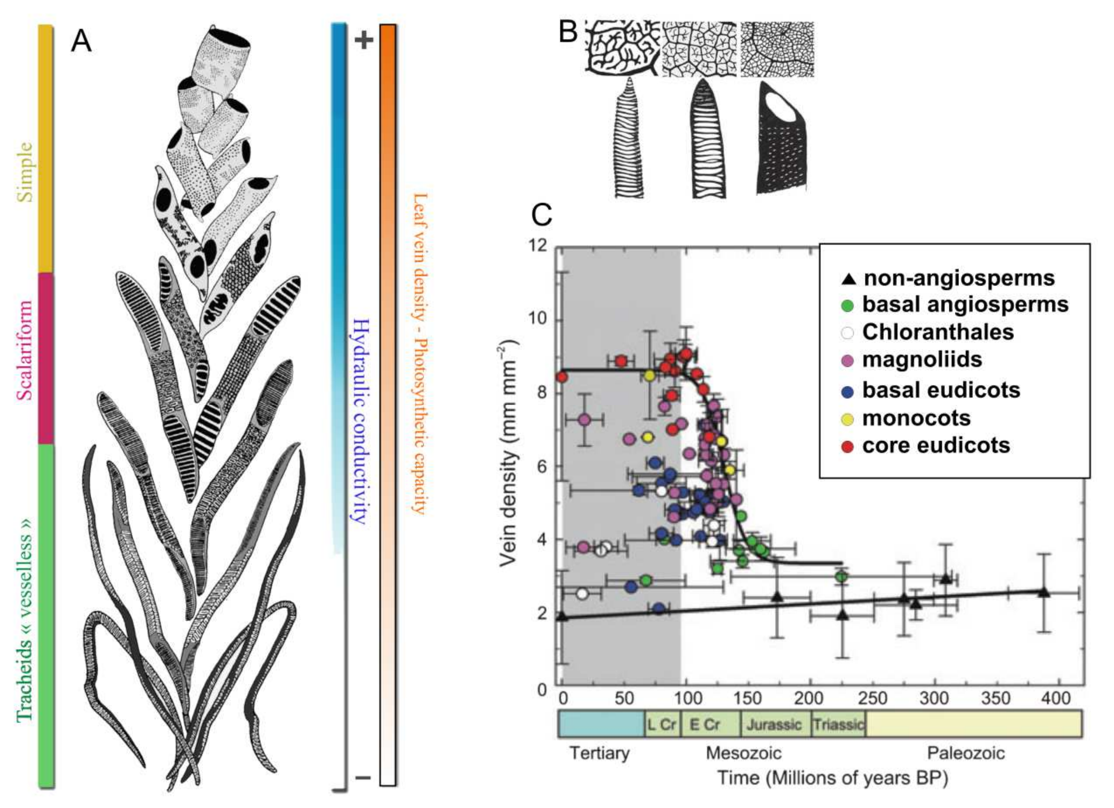


FIGURE 1.6. Anatomical diversity of xylem conduits and associated functions. (A) Morphological gradient from tracheids to vessel elements with scalariform and simple perforation plates; redrawn from Nardini and Jansen (2013). (B) Relationship between xylem conduit morphology and leaf vein density in angiosperms; reproduced from Feild and Brodribb (2013) . (C) Increase of angiosperm leaf vein density over evolutionary time. Non-angiosperms and major groups of angiosperms are included; redrawn from Brodribb and Feild (2010).

1.6 Basal angiosperms as modern analogs of early flowering plants

The early diversification of angiosperms in diverse ecological niches, and their ancestral form and function, are among the aspects of flowering plant evolution that remain very ambiguous due to gaps in the fossil record. Several scenarios of the ancestral structure and ecophysiology of angiosperms have been proposed using extant basal angiosperms as modern analogs. The resolution of the earliest divergences of flowering plants has influenced the formulation of various hypotheses about the ancestral ecophysiology of angiosperms. The traditional representations of early angiosperms proposed by Takhtajan (1969; 1980) and Cronquist (1988) prevailed during a long time. This hypothesis considers woody magnoliids, with large multiparted flowers like those of extant Magnoliales and Winteraceae, as a starting point for angiosperm evolution (Crane *et al.*, 1995). Based on comparison with these living taxa, the first angiosperms would be slowly growing trees with large leaves having low photosynthetic rates (Feild *et al.*, 2004). By analogy with the ecophysiology of extant magnoliid taxa, early angiosperms would have established in wet and low-light habitats (Takhtajan, 1969; Cronquist, 1988). Other authors have proposed alternative scenarios that challenge this traditional vision. For instance, Stebbins (1974) has suggested that early angiosperms were weedy xeric shrubs that lived in disturbed and sun-exposed habitats of tropical semi-arid regions. Later, during the nineties, Taylor and Hickey (1992; 1996) performed a cladistic analysis to reconstruct the ancestral form and ecophysiology of angiosperms. Their analysis placed Chloranthaceae and Piperales at the base of the angiosperm tree. By tracing ancestral character states, they proposed the 'paleoherb' hypothesis, which holds that the protoangiosperm was a diminutive, rhizomatous and perennial herb.

At the beginning of the 21st century, several studies identified the ANA grade as the earliest branching lineages of angiosperms (see section 1.3). Following this updated phylogenetic topology, Sun *et al*. (2002) proposed that early angiosperms were aquatic herbs similar to modern Nymphaeales and *Ceratophyllum*. This hypothesis is supported by lower Cretaceous fossil evidence of herbaceous aquatic angiosperms such as *Archaefructus* (Sun *et al.*, 1998; Sun *et al.*, 2002) and *Montsechia* (Gomez *et al.*, 2015). However, it has been shown that aquatic angiosperms were derived from plants that previously occupied terrestrial environments (Philbrick and Les, 1996). The most recent hypothesis on the ancestral ecophysiology of angiosperms has been developed by Taylor S. Feild and colleagues (Feild *et al.*, 2003; Feild *et al.*, 2004; Feild and Arens, 2005; 2007; Feild *et al.*, 2009) by studying

Amborella, Austrobaileyales and Chloranthales in combination with fossil records to elucidate the ancestral ecophysiology of angiosperms. Considering that most basal angiosperms have low hydraulic capacities, which restrict them to humid localities, and a photosynthetic apparatus adapted to dark environments, their work led them to propose that early angiosperms were woody plants that inhabited wet, dark, and disturbed habitats (Feild *et al.*, 2004; Feild and Arens, 2007).

1.7 New Caledonia, study location

As underscored in a previous section of this chapter, a large proportion of basal angiosperm families are located in South-East Asia, Australia and the Pacific islands (Buerki *et al.*, 2014). The archipelago of New Caledonia stands out among these territories because, despite its small surface, it harbors two ANA grade families (Fig. 1.3), along with representative species from Chloranthales and from all magnoliid orders (Fig. 1.2). Given its isolation and its distinctive biota, the archipelago of New Caledonia is considered to be one of the main Pacific biogeographic regions (Mueller-Dombois and Fosberg, 2013). This intrinsic property, along with its particular richness in basal angiosperms, makes New Caledonia an ideal location for the study of the evolutionary history and the ecological behavior of basal angiosperms.

1.7.1 Geography

New Caledonia is a remote archipelago located in the south-west Pacific just above the tropic of Capricorn (17-23°S, 162-169°E), about 1400 km east of Australia, 2000 km north of New Zealand, and 250 km south-west of Vanuatu (Fig. 1.7A). The archipelago comprises *Grande Terre*, which is the main island with a total area of 16 400 km^2, and other smaller islands (Fig. 1.7B). *Grande Terre* is about 50 km wide and extends over 400 km from south-east to north-west (Fig. 1.7B). The Loyalty Islands are composed of three main islands located *c*. 100 km east of *Grande Terre*. North to south, the Loyalty Islands are *Ouvéa* (132 km^2), *Lifou* (1 207 km^2), and *Maré* (642 km^2). Other islands with smaller surfaces are *Belep*, and *Ile des Pins*, located respectively north and south of *Grande Terre*.

1.7.2 Orography and Geology

Grande Terre has a continuous mountain range that traverses the entire island. The highest peaks of the mountain range are Mt. Panié (1629 m) in the north and Mt. Humboldt

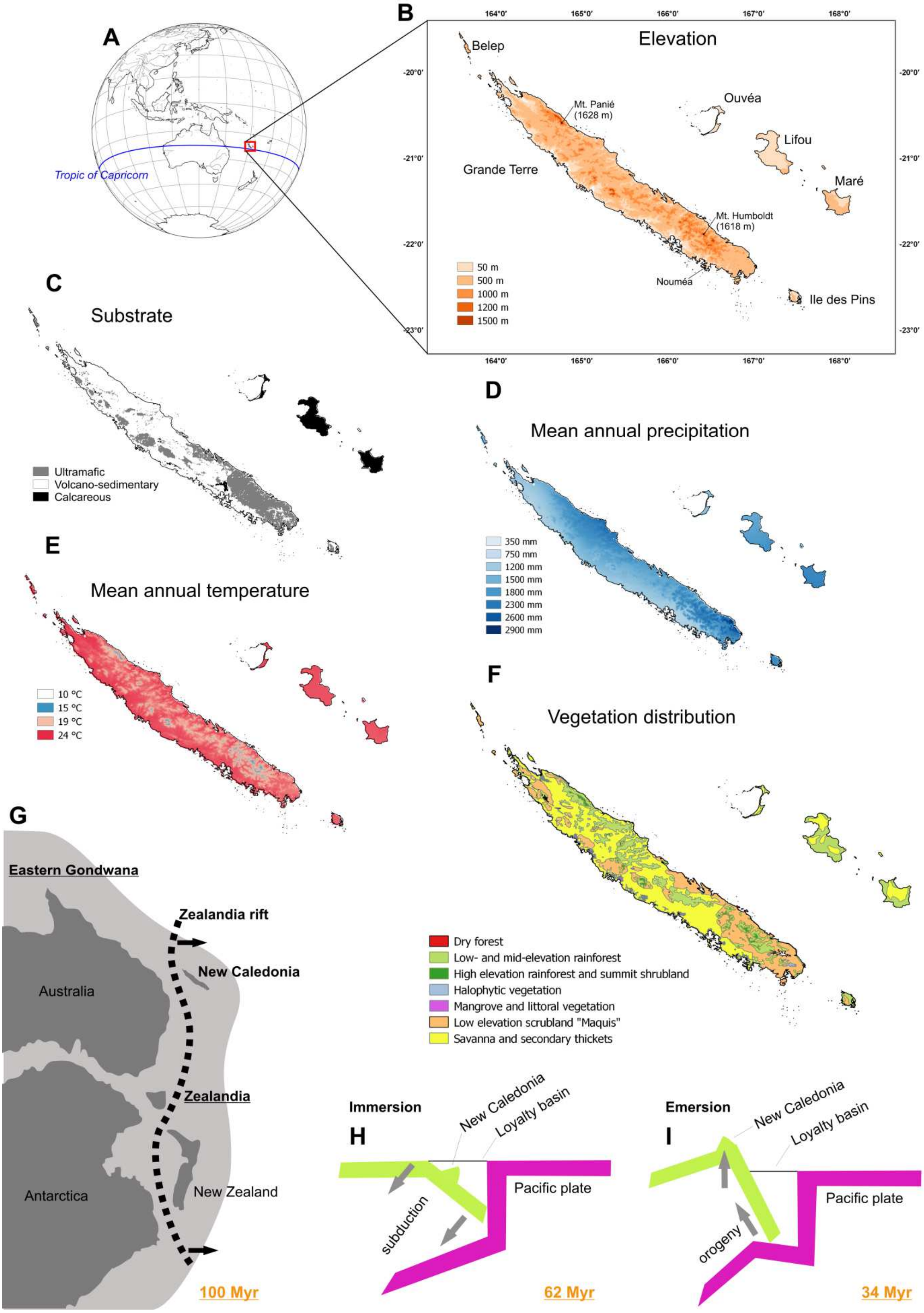


FIGURE 1.7 Geographical, environmental and geological characteristics of New Caledonia. Location of New Caledonia in the South-West Pacific (A); geographic and environmental characteristics (B-F); and tectonic evolution of New Caledonia (G-I).

(1618 m) in the south (Fig. 1.7B) (Bonvallot, 2012). The mountain range is closer to the east coast of *Grande Terre,* constituting a geographical boundary between the steep and mountainous east coast and the large coastal plains of the west coast. Some isolated massifs are also present in the northwestern coast with several peaks exceeding 800 m (Bonvallot, 2012). The highest elevations of the other islands of the New Caledonian archipelago do not exceed 300 m. Three main types of geological substrates occur in New Caledonia (Fig. 1.7C) (Fritsch, 2012): 1) ultramafic substrates (UM) covering the southern third of the *Grande Terre*, and some isolated massifs along the north-west coast; 2) substrates derived from volcano-sedimentary rocks (non-UM) covering two-thirds of the *Grande Terre* and occurring mainly at the northern half of the island; and 3) calcareous substrates, which are the least represented type of substrate, prevailing mainly on the Loyalty Islands.

1.7.3 Climate

New Caledonia has a humid subtropical climate with a pronounced cool and dry season from May to October, and a warm and rainy season from November to April (Maitrepierre, 2012). As a consequence of *Grand Terre*'s topography and the associated rain shadow effect, mean annual precipitation (MAP) ranges from 800 mm yr^{-1} along the western coastal plains to 4500 mm yr^{-1}on the eastern slopes of the mountain range (Fig. 1.7D) (Météo-France, 2007). Mean annual temperature (MAT) in lowland areas is between 27 and 30 °C (Fig. 1.7E), and it is also influenced by topography with an adiabatic lapse rate of *c*. 0.6°C /100 m of elevation (Maitrepierre, 2012). Like several tropical regions of the southern hemisphere, New Caledonia has a south-east trade wind regime. These winds have little annual variability and wind speeds of 10 to 20 knots dominate over the year (Maitrepierre, 2012).

1.7.4 Vegetation types

The combination of elevation, substrate, rainfall, and human-induced disturbances determines the occurrence of different vegetation types in New Caledonia (Jaffré *et al.*, 2012). Vegetation types are commonly classified into rainforest of low- to high-elevations (*i*.e. dense humid evergreen forest), summit shrubland, dry sclerophyll forest, low elevation shrubland (known as "*maquis*"), halophytic vegetation, including mangrove and littoral vegetation, savanna and secondary thickets (Fig. 1.7F) (Jaffré *et al.*, 2012). Rainforest is the richest vegetation of the island with more than 2000 native vascular plant species, and covers *c*. 3800 km^2 of the island (1800 km^2 on non-UM, 1100 km^2 on UM, and 900 km^2 on calcareous substrates) (Birnbaum *et al.*, 2015). A recent study analyzing the distribution of 702 tree species has shown that most rainforest tree species are likely to have a high environmental tolerance given that 56% of the tree species are ubiquist with regard to geological substrate, rainfall and elevation (Birnbaum *et al.*, 2015). However, while substrate-generalists seem to be the most represented, many plant species are restricted to a single substrate. In this sense, New Caledonian tree species can be classified into three edaphic groupings corresponding to: UM specialists, non-UM specialists, and substrate-generalists (Ibanez *et al.*, 2014).

1.7.5 Characteristics of the flora

The flora of New Caledonia is characterized by a high level of species richness with 3371 inventoried species of vascular plants (Morat *et al.*, 2012). Angiosperms account by themselves for 91% of the New Caledonian vascular plants with *c*. 3100 species (Morat *et al.*, 2012). The most diverse families in New Caledonia are Myrtaceae and Rubiaceae, and the three most speciose genera in the archipelago are *Phyllanthus* L. (116 sp.), *Psychotria* L. (81 sp.), and *Syzygium* Gaertn. (70 sp.). Gymnosperms also have a great species richness with 46 endemic species (Jaffré *et al.*, 1994). Indeed, the archipelago comprises a very distinctive and diversified conifer flora, representing the largest assemblage in the Pacific region (De Laubenfels, 1996). The flora of New Caledonia is also very distinctive as reflected by its high endemism levels. For instance, 74.7% of the vascular plant species and 77.8% of the spermatophyte species are endemic to the archipelago (Morat *et al.*, 2012). Endemism is also observed at the supra-specific level with 98 endemic genera (13.7%) and three endemic families (Amborellaceae, Oncothecaceae, and Phellinaceae). Given its high levels of species diversity and endemism, and because of its high rates of habitat loss, New Caledonia is considered to be one of the global hotspots for conservation priorities (Myers *et al.*, 2000).

A third major characteristic of the New Caledonian flora is its disharmony. The flora of New Caledonia is regarded as disharmonic because some groups are either over- or under-represented compared to the floras of neighboring areas (Jaffré, 1980; Pillon *et al.*, 2010). For instance, Cunoniaceae, Rubiaceae, and Sapindaceae are over-represented in terms of species richness compared to the Australian flora; while other families such as Asteraceae, Ericaceae, and Lamiaceae are under-represented (Jaffré, 1980; Barrabé *et al.*, 2014). Because of its isolation, the disharmony of the New Caledonian flora has been generally attributed to different dispersal abilities among plant groups (Carlquist, 1974). However, it has also been hypothesized that the high incidence of ultramafic (UM) geological substrates may be another main explanation for the over-representation of some plant lineages (Pillon *et al.*, 2010). Because of their chemical and physical properties, UM substrates present several important constraints to plant growth (Jaffré, 1980). They have high levels of heavy metals (*e.g.* chrome, cobalt and nickel), low levels of nitrogen, phosphorus and potassium, and very poor water retention (Jaffré, 1980; Jaffré *et al.*, 1994; van der Ent *et al.*, 2015). In order to cope with these constraints, plants have specific adaptations to UM substrates such as sclerophyllous life-form traits or hyperaccumulation of heavy metals (Jaffré *et al.*, 2013). Pillon *et al*. (2010) assessed the two competing hypotheses: a disharmony due to uneven dispersability *versus* resulting from uneven adaptation to UM substrates. The authors concluded that exaptation (i.e. 'features that now enhance fitness but were not built by natural selection for their current role' (Gould and Vrba, 1982)) of plant species to UM substrates seems to be a main mechanism explaining the disharmony observed in the New Caledonian flora. For instance, species of *Phyllanthus* and *Psychotria*, which are among the largest and over-represented genera in New Caledonia, possess well-known nickel hyperaccumulating species (Jaffré, 1980; Reeves, 2003; Pillon *et al.*, 2010; Barrabé *et al.*, 2014).

1.8 Geological and natural history of New Caledonia

According to Cluzel et al. (2001; 2012) and Pelletier (2006) the tectonic evolution of New Caledonia can be synthesized in the following three main stages:

1) Early to Late Cretaceous (120-80 Mya): the New Caledonian basin split from the east Gondwanian margin through a marginal rifting and a subsequent spread (Fig. 1.7G).

2) Paleocene to Eocene (62-50 Mya): the New Caledonian basin immersed, probably entirely (Fig. 1.7H). The New Caledonian landmass was subducted and covered with oceanic crust, the addition of ultramafic terranes occurred during this period.

3) Middle to Late Eocene (50-34 Mya): the New Caledonian basin reemerged by the action of orogenic forces that uplifted the New Caledonian landmass (Fig. 1.7I). Previously accreted ultramafic terranes emerged along with the New Caledonian basin originating the ultramafic soils that we observe today across *Grande Terre*.

For a long time New Caledonia has been described as an intact and unchanged piece of Gondwana. This assumption is linked to the high representation of remarkable relictual endemic taxa such as representatives of basal angiosperms, gymnosperms and Proteaceae (Morat, 1993; Lowry, 1998). However, the shaky geological history of New Caledonia invalidates this hypothesis. The geological history of New Caledonia implies that the local biodiversity is not older than its reemergence *c*. 37 Myr ago (Cluzel *et al.*, 2012) and that the local biota is the result of long- or short-distance dispersal events (Grandcolas *et al.*, 2008; Keppel *et al.*, 2009; Espeland and Murienne, 2011; Pillon, 2012). A second plausible scenario implicates the existence of other intermediate islands, now sunken, during the submersion of the New Caledonian basin (Pelletier, 2006). These islands may have temporarily hosted a part of the New Caledonian biota during the submersion of the main New Caledonian landmass.

By abandoning the ‘Gondwanian museum’ model and adopting a new vision considering the spatio-temporal history of New Caledonia, recent studies have raised the question of whether the local biodiversity is the consequence of recent dispersals and local radiations. Grandcolas *et al*. (2008) summarized several studies that provided evidence of post-emersion local radiations in several animal lineages including insects (Murienne *et al.*, 2005; Murienne *et al.*, 2008), freshwater fishes (Waters *et al.*, 2000), and reptiles (Bauer *et al.*, 2006). Similar scenarios have been proposed in plants in the genera *Codia* (Pillon *et al.*, 2009), *Diospyros* (Paun *et al.*, 2015), *Geisois* (Pillon *et al*., 2014), *Psychotria* (Barrabé *et al.*, 2014) and *Pycnandra* (Swenson *et al*., 2015). However, even if we assume that the New Caledonian flora is the result of post-emergence colonization events and local radiations, there is a lack of explanations for the spatio-temporal distribution and the macroevolutionary mechanisms behind the presence of basal angiosperm lineages with ages older than the reemergence of the archipelago. A plausible explanation is that representatives from these early divergence lineages colonized the island from nearby territories such as Australia, subsequently disappearing from their lands of origin (Pillon, 2008).

1.9 New Caledonia, a land of basal angiosperms

Several authors have highlighted that one of the main characteristics of the New Caledonian flora is the significant representation of ‘relictual’ groups such as gymnosperms and basal angiosperms (Morat *et al.*, 1994; Pillon, 2008; Morat *et al.*, 2012). Basal angiosperms are represented by 109 species distributed in 22 genera and 10 families in New Caledonia (Table 1.1). Four genera are endemic to the archipelago (*Adenodaphne* S. Moore, *Amborella* Baill., *Kibaropsis* Vieill. ex Guillaumin, and *Nemuaron* Baill.). Ninety percent of basal angiosperm species are endemic to the archipelago and only 11 species are considered to be autochthonous. A full list of the orders, families, genera, species, and subspecies of all currently recognized New Caledonian basal angiosperms is provided in Table 1.1

TABLE 1.1 *List of basal angiosperms from New Caledonia*

Amborellales

Amborellaceae (1 genus / 1 species)
Amborella trichopoda Baill.

Austrobaileyales

Trimeniaceae (1 genus / 1 species)
Trimenia neocaledonica Baker f.

Canellales

Winteraceae (1 genus / 19 species)
Zygogynum acsmithii Vink
Zygogynum amplexicaule subsp. *amplexicaule* var. *amplexicaule* (Vieill. Ex P.Parm.) Vink
Zygogynum amplexicaule subsp. *amplexicaule* var. *isoneuron* (Tiegh.) Vink
Zygogynum amplexicaule subsp. *luteum* Vink
Zygogynum baillonii Tiegh.
Zygogynum bicolor Tiegh.
Zygogynum comptonii var. *angustifolium* Vink
Zygogynum comptonii var. *comptonii* (Baker f.) Vink
Zygogynum comptonii var. *taracticum* Vink
Zygogynum crassifolium (Baill.) Vink
Zygogynum cristatum Vink
Zygogynum fraterculum Vink
Zygogynum mackeei subsp. *mackeei* Vink
Zygogynum mackeei subsp. *paniense* Carlquist
Zygogynum oligostigma Vink
Zygogynum pancheri subsp. *arrhantum* Vink
Zygogynum pancheri subsp. *deplanchei* (Tiegh.) Vink
Zygogynum pancheri subsp. *elegans* Vink
Zygogynum pancheri subsp. *pancheri* (Baill.) Vink
Zygogynum pancheri subsp. *rivulare* (Vieill. ex P.Parm.) Vink
Zygogynum pauciflorum (Baker f.) Vink
Zygogynum pomiferum subsp. *balansae* (Tiegh.) Vink
Zygogynum pomiferum subsp. *pomiferum* Baill.
Zygogynum schlechteri (Guillaumin) Vink
Zygogynum stipitatum Baill.
Zygogynum tanyostigma Vink
Zygogynum tieghemii subsp. synchronanthum Vink
Zygogynum tieghemii subsp. *thulium* Vink
Zygogynum tieghemii subsp. *tieghemii* Vink
Zygogynum vieillardii Baill.
Zygogynum vinkii Sampson

Piperales

Piperaceae (2 genera / 13 species)
Peperomia baueriana Miq.*
Peperomia blanda var. *floribunda* (Decne. ex Miq.) H.Huber*
Peperomia caledonica C.DC.
Peperomia insularum Miq.*
Peperomia kanalensis C.DC.
Peperomia leptostachya Hook. & Arn.*
Peperomia lifuana C.DC.
Peperomia sarasinii C.DC.
Peperomia subpallescens C.DC.
Peperomia tetraphylla (G.Forst.) Hook. F & Arn.*
Peperomia urvilleana A.Rich.*
Piper insectifugum C.DC. ex Seem.*
Piper staminodiferum C.DC.

Laurales

Atherospermataceae (1 genus / 1 species)
Nemuaron vieillardii (Baill.) Baill.

Hernandiaceae (2 genera / 3 species)
Gyrocarpus americanus Jacq.*
Hernandia cordigera Vieill.
Hernandia nymphaeifolia (C.Presl) Kubitzki*

Lauraceae (6 genera / 47 species)
Adenodaphne macrophylla Kosterm.
Adenodaphne spathulata Kosterm.
Adenodaphne triplinervia Kosterm.
Adenodaphne uniflora var. *francii* (Däniker) Kosterm.
Adenodaphne uniflora var. *uniflora* (Guillaumin) Kosterm.
Beilschmiedia neocaledonica Kosterm.
Beilschmiedia oreophila Schltr.

Cassytha filiformis L.*
Cryptocarya aristata Kosterm.
Cryptocarya bitriplinervia Kosterm.
Cryptocarya chartacea Kosterm.
Cryptocarya elliptica Schltr.
Cryptocarya gracilis Schltr.
Cryptocarya guillauminii Kosterm.
Cryptocarya leptospermoides Kosterm.
Cryptocarya lifuensis Guillaumin
Cryptocarya longifolia Kosterm.
Cryptocarya mackeei Kosterm.
Cryptocarya macrocarpa Guillaumin
Cryptocarya macrodesme Schltr.
Cryptocarya odorata Guillaumin
Cryptocarya oubatchensis Schltr.
Cryptocarya phyllostemon Kosterm.
Cryptocarya pluricostata Kosterm.
Cryptocarya schmidii Kosterm.
Cryptocarya transversa Kosterm.
Cryptocarya velutinosa Kosterm.
Endiandra baillonii (Pancher & Sebert) Guillaumin
Endiandra lecardii Guillaumin
Endiandra neocaledonica Kosterm.
Endiandra polyneura Schltr.
Endiandra poueboensis Guillaumin
Endiandra sebertii Guillaumin
Litsea deplanchei Guillaumin
Litsea humboldtiana Guillaumin
Litsea imbricata Guillaumin
Litsea lecardii Guillaumin
Litsea longipedunculata Kosterm.
Litsea mackeei Kosterm.
Litsea miana Guillaumin
Litsea neocaledonica S.Moore
Litsea ovalis Kosterm.
Litsea paouensis Guillaumin
Litsea pentaflora Guillaumin
Litsea racemiflora Däniker
Litsea ripidion Guillaumin
Litsea stenophylla Guillaumin
Litsea triflora Guillaumin

Monimiaceae (2 genera /10 species)
Hedycarya aragoensis Jérémie
Hedycarya baudouini Baill.
Hedycarya chrysophylla Perkins
Hedycarya cupulata Baill.
Hedycarya engleriana S.Moore
Hedycarya parvifolia Perkins & Schltr.
Hedycarya perbracteolata Jérémie
Hedycarya rivularis Guillaumin
Hedycarya symplocoides S.Moore
Kibaropsis caledonica (Guillaumin) Jérémie

Magnoliales

Annonaceae (5 genera / 12 species)
Fissistigma punctulatum (Baill.) Merr.
Goniothalamus dumontetii R.M.K.Saunders & Munzinger
Goniothalamus obtusatus (Baill.) R. M. K. Saunders
Huberantha nitidissima (Dunal) Chaowasku*
Meiogyne baillonii (Guillaumin) Heusden
Meiogyne dumetosa (Vieill. ex Guillaumin) Heusden
Meiogyne lecardii (Guillaumin) Heusden
Meiogyne tiebaghiensis (Däniker) Heusden
Xylopia dibaccata Däniker
Xylopia pallescens Baill.
Xylopia pancheri Baill.
Xylopia vieillardii Baill.

Chloranthales

Chloranthaceae (1 genus / 2 species)
Ascarina rubricaulis Solms
Ascarina solmsiana var. *grandifolia* Jérémie
Ascarina solmsiana var. *solmsiana* Schltr.

Notes: Native species whose distribution also extends outside the archipelago are marked with an asterisk. This list of species was extracted from the **FLORICAL** Database (Morat et al. 2012); an online checklist of the flora of New-Caledonia: http://botanique.nc/herbier/florical. **Last update: 27/05/2014**

All basal angiosperm orders, with the sole exception of Nymphaeales, are represented in New Caledonia (Fig. 1.2). Pillon *et al*. (2010) have shown that most of these families are among the over-represented groups of the New Caledonian flora in comparison to the Australian flora. Seven out of ten basal angiosperm families are among the over-represented families in the local flora. Only Atherospermataceae, Chloranthaceae and Hernandiaceae are not over-represented, although they are not considered as under-represented (Appendix S1 in Pillon *et al*., 2010). Because of the occurrence of basal angiosperm lineages, New Caledonia is part of the global region that has been proposed by Buerki *et al*. (2014) as a potential refugium that triggered early angiosperm diversification (Fig. 1.3).

Among the basal angiosperms of the archipelago, the endemic dioecious shrub *Amborella trichopoda* Baill., is perhaps the most notorious because of its prominent phylogenetic position. Since the end of the last century, several studies have strongly supported *Amborella* as the single living species of the sister lineage to all other extant angiosperms (Figs. 1.1; 1.3) (Mathews and Donoghue, 1999; Soltis *et al.*, 1999; Mathews and Donoghue, 2000; Qiu *et al.*, 2000; Soltis *et al.*, 2000; Amborella Genome Project 2013; Poncet *et al.*, 2013), attracting the attention of plant biologists to this discrete species. Given that *A. trichopoda* is the only species of the order Amborellales, this species is the single survivor of a lineage that diverged *c*. 140 Mya (Fig. 1.2) (Magallón *et al.*, 2015). Because of its phylogenetic position, the analysis of *Amborella* from a comparative perspective can contribute to the comprehension of the evolution of flowering plants (Amborella Genome Project, 2013). However, in spite of its importance, several aspects of the biology and ecology of this key species remain to be analyzed.

1.10 Main objectives

The present work aims to increase our understanding of basal angiosperms form, function and ecology by giving a special focus to species from New Caledonia. By describing mechanical, anatomical, and morphological features linked to the variation of growth forms in key basal angiosperm lineages such as *Amborella* and the globally megadiverse Piperales, this thesis aims to provide new perspectives for understanding the ancestral growth form of flowering plants. Using a trait-based approach, we examine variation in functional traits as well as patterns of trait-trait and trait-environment relationships, to understand the fundamental coordination between morphological and functional traits in basal angiosperms.

A summary of the methodological approaches used in this study, along with a list of the traits measured, are provided in figure 1.8 and table 1.2 respectively.

As previously underscored, New Caledonia has a great and unusual diversity of basal angiosperms. However, no study to date has proposed a mechanism to explain the persistence and the diversity of basal angiosperms in the archipelago. By assessing the past and present environmental conditions linked to the distribution of basal angiosperm lineages in New Caledonia, this thesis aims to test different hypotheses that could explain their persistence and local over-representation. Finally, we assess whether drought vulnerability can explain the patterns of distribution of basal angiosperms in the archipelago.

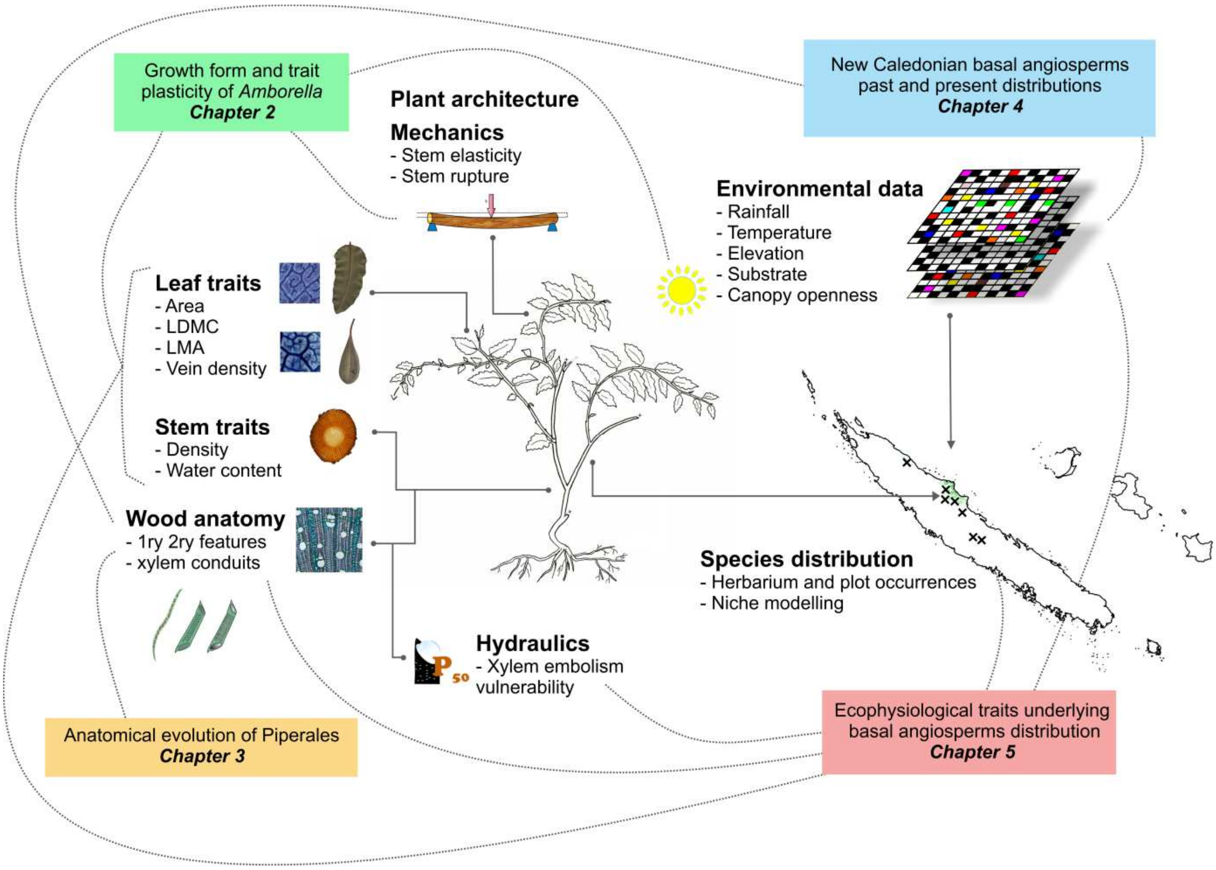


FIGURE 1.8 Overview of the methodological approaches used in each thesis chapter.

TABLE 1.2 *List of functional traits and structural features measured*

Group	Attribute (abbreviation)	Units	References	Chapter
Whole plant	Architecture	categorical	Hallé *et al*., 1978; Barthélémy and Caraglio, 2007	2
Whole plant	Growth form	categorical	Péréz-Harguindeguy *et al*., 2013	2, 3
Stem	Primary and secondary xylem anatomy	categorical	Esau, 1960; Carlquist, 1975; Carlquist, 2001	3
Stem	Wood density (WD)	g/cm^{-3}	Chave *et al*., 2009; Péréz-Harguindeguy *et al*., 2013	5
Stem	Stem water content (SWC)	%	Poorter *et al*., 2010	2
Stem	Stem specific density (SSD)	g/cm^{-3}	Méndez-Alonzo *et al*., 2012	2
Stem	Xylem conduit type	categorical	Carlquist, 1975	3, 4, 5
Stem	Modulus of elasticity (MOE)	*N*/mm2	Rowe and Speck, 2005	2
Stem	Modulus of rupture (MOR)	*N*/mm2	Gere and Timoshenko, 1999	2
Stem	Xylem embolism vulnerability ($P_{12/50/88}$)	MPa	Cochard *et al*., 2013	5
Leaf	Leaf area (LA)	cm^2	Wilson *et al*., 1999; Péréz-Harguindeguy *et al*., 2013	2
Leaf	Leaf mass per area (LMA)	$mg\ mm^{-2}$	Poorter *et al*., 2009; Péréz-Harguindeguy *et al*., 2013	2, 5
Leaf	Leaf dry-matter content (LDMC)	$mg\ g^{-1}$	Wilson *et al*., 1999; Péréz-Harguindeguy *et al*., 2013	2
Leaf	Leaf vein density (VD)	$mm\ mm^2$	Sack and Scoffoni, 2013	5

Note: Traits are arranged into different groups according to organ categories. References of studies containing fundamentals and protocols are provided. Detailed descriptions of trait measurement techniques are included in each chapter.

1.11 Thesis outline

This thesis follows an article-based format. It comprises four chapters that have been published or are in preparation for submission to scientific journals (Fig. 1.8). Each chapter aims to examine specific aspects of basal angiosperms form, function, and ecology. The main objectives of each chapter are as follows:

Chapter 2. Describe the growth form of *Amborella trichopoda*, the only representative of the first diverging lineage of angiosperms, and assess its morphological and functional plasticity in response to environmental variation in canopy openness.

Chapter 3. Investigate the variations in the degree of cambial activity and wood anatomical features underlying the high diversity of forms in Piperales, the largest basal angiosperm order. The evolutionary history of woodiness in Piperales is assessed, and an ancestral form is proposed.

Chapter 4. Outline the current geographical distribution of New Caledonian basal angiosperms, examine their distribution during the last glacial maximum, and propose possible Pleistocene refugia in New Caledonia.

Chapter 5. Investigate whether drought vulnerability is correlated with the environmental distribution of basal angiosperms.

Finally, in **Chapter 6** the main conclusions are summarized and research perspectives for future studies are provided. Moreover, this chapter discusses the contributions of our study to the evolutionary history of flowering plants, the natural history of New Caledonia, and the conservation of its unique flora.

CHAPTER 2

Trait coordination, mechanical behavior, and growth form plasticity of *Amborella trichopoda* under variation in canopy openness[1]

- Architectural and biomechanical properties of *Amborella*
- Morphological and functional responses to light availability
- Whole-plant functional coordination in *Amborella*

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[1] A version of this chapter has been **submitted** for publication. Trueba, S., Isnard, S., Barthélémy, D., Olson, M.E. (2016). *AoB Plants*.

Abstract

Understanding the distribution of traits across the angiosperm phylogeny can help map the nested hierarchy of features that characterize key evolutionary nodes. Finding that *Amborella trichopoda* is sister to the rest of the angiosperms has raised the question of whether it shares certain key functional trait characteristics and plastic responses apparently widespread within the angiosperms at large. With this in mind, we test the hypothesis that local canopy openness induces plastic responses in *Amborella*. We use this variation in morphological and functional traits to estimate the pervasiveness of trait scaling, and leaf and stem economics.

We studied the architecture of *Amborella* and how it varies under different levels of canopy opennesses. We analyzed the coordination of 12 leaf and stem structural and functional traits, and the association of this covariation with differing morphologies.

The *Amborella* habit comprises a series of sympodial branched complexes that vary in size and branching pattern under different levels of canopy openness. Correlations were found between most leaf and stem functional traits. Despite substantial modulation of leaf size and leaf mass per area by light availability, branches in different light environments had similar leaf area-stem size scaling. *Amborella* stems vary from self-supporting mechanical to semi-scandent. Stem mechanics are not affected by local canopy openness.

Changes in stem elongation and leaf size in *Amborella* produce distinct morphologies under different light environments. Stem tissue mechanics do not differ significantly across light environments. The sympodial growth observed in *Amborella* could point to an angiosperm synapomorphy. Our study provides evidence of intraspecific coordination between leaf and stem economic spectra. Variation of traits along these spectra provides *Amborella* axes with adaptive functional strategies under different light environments, and suggests that these plastic responses were likely present in the angiosperm ancestor.

Keywords: Adaptation, allometry, biomechanics, leaf mass per area, leaf dry matter content, modulus of elasticity, phenotypic plasticity, plant architecture, light environment, trait ecology.

2.1 Introduction

Comparative biology is built on an understanding of the patterns of distribution of organismal characters. Those that uniquely characterize single clades are known as synapomorphies. The nested hierarchy of synapomorphies across the tree of life helps to reconstruct the patterns of relationships between taxa (Nixon and Wheeler, 1990). In contrast, characters that arise repeatedly can reflect an array of processes from convergent evolution to shared propensities for evolving similar traits independently, reflecting convergence or parallelism (Harvey and Pagel, 1991; Losos, 2011; Scotland, 2011). To understand the distribution of traits within a group, it is crucial to study as wide an array of lineages as possible. Among the flowering plants, *Amborella trichopoda* Baill. (Amborellaceae), a dioecious woody plant endemic to the moist forests of New Caledonia, has attracted the attention of plant science since the end of the last century, after several phylogenetic studies supported the position of *Amborella* as the single surviving representative of a lineage sister to all other extant angiosperms (Mathews and Donoghue, 1999; Soltis *et al.*, 1999; Mathews and Donoghue, 2000; Qiu *et al.*, 2000; Soltis *et al.*, 2000; Amborella Genome Project 2013; Poncet *et al.*, 2013).

Because of its phylogenetic position, the analysis of *Amborella* traits can provide key elements to understand the evolution of the ecology, function, and structure of flowering plants (Amborella Genome Project 2013). Finding that there are features shared by *Amborella* and other flowering plants, but not the gymnosperms, could point to angiosperm synapomorphies. On the other hand, finding that there are features shared by *Amborella* and the gymnosperms but not the rest of the angiosperms could reveal useful information regarding the early sequence of character evolution within the flowering plants. This study focuses on patterns, potentially synapomorphic as well as homoplasious, that are currently being documented all across the woody plants. Through the description of *Amborella*'s architectural and biomechanical organization, combined with analyses of the coordination of functional leaf and stem traits and their variation under different light environments, we can provide elements for understanding the evolution of growth forms in the flowering plants and how these forms vary developmentally under different light conditions.

One of the longstanding questions in the study of angiosperm structure concerns the habit and growth form of the earliest flowering plants. *Amborella* has a multi-stemmed habit with stems that have been described as scandent (Feild *et al.*, 2001; Feild and Wilson, 2012).

This growth habit is often called "cane-like", and seems to be widespread in the "basal" lineages of angiosperms (*e.g. Aristolochia*, *Eupomatia*, *Illicium*, *Piper*, *Sarcandra*, *Thottea* and *Trimenia*) (Carlquist, 1996; 2001; Feild and Arens, 2005; Carlquist, 2009; Isnard *et al.*, 2012), pointing to a potential angiosperm synapomorphy. The cane-like habit is characterized by a combination of sympodial growth and mechanical laxness, with stems relatively long for their tissue stiffnesses (Feild and Arens, 2007; Carlquist, 2009). The sympodiality and laxity observed in the stems of these cane-like shrubs can be directly assessed by the analysis of their architectural and mechanical properties. Stem mechanical properties are significantly linked to stem anatomical structure and can be used to characterize different growth forms based on the observation of mechanical shifts of structural Young's modulus (*E*) and flexural rigidity (*EI*) during development (Rowe and Speck, 2005; Lahaye *et al.*, 2005). *Amborella* differs from most of the other "basal" cane-like representatives in that it has a vesselless wood, an anatomical feature that involves lower hydraulic conductivity per unit transectional area as compared to species bearing vesselled wood (Feild *et al.*, 2000; Carlquist and Schneider, 2001; Feild *et al.*, 2001; Hacke *et al.*, 2007). Analysis of the stem mechanical properties of *Amborella* can be potentially important in understanding the mechanical organization behind the scandent habit in vesselless plants, as well as in discerning how widespread mechanical plasticity is within vesselless angiosperms.

Independently of the multiple forms expressed by plants, several leading dimensions of trait covariation have been documented (Ackerly and Donoghue, 1998; Enquist, 2002; Niklas and Enquist, 2002; Westoby *et al.*, 2002; Wright *et al.*, 2004; Olson *et al.*, 2009). These apparently highly homoplasious patterns of trait variation appear to span most flowering plant lineages given that they are observed across species and across habitats. One of the best documented of these relationships is the 'leaf size-twig size' spectrum (Ackerly and Donoghue, 1998; Cornelissen, 1999; Westoby *et al.*, 2002; Westoby and Wright, 2003; Sun *et al.*, 2006; Wright *et al.*, 2007; Olson *et al.*, 2009), which includes "Corner's Rules" (Corner, 1949). The leaf size-twig size spectrum includes the tendency for plants with large leaves to have predictably thick twigs made up of tissues with low specific density (Wright *et al.*, 2006; Swenson and Enquist, 2008; Olson *et al.*, 2009). Finding how *Amborella* fits into these global patterns is essential for documenting how widespread these patterns are across the angiosperms. Although these relationships are predictable, the absolute values of functional traits can vary across species reflecting different ecological strategies. Within the context of these strategies, phenotypic plasticity allows individuals to modify developmental trajectories in response to specific environmental cues (Sultan, 2000; Chambel *et al.*, 2005; Pigliucci *et al.*, 2006; Fusco and Minelli, 2010).

Among the environmental variables that influence plant plasticity, light availability is one of the most heterogeneous (Valladares and Niinemets, 2008). Light incidence has a very well documented influence on leaf structure. For instance, light tends to have a negative effect on leaf size (Poorter, 1999; Rozendaal *et al.*, 2006) and a positive effect on specific mass (Abrams and Kubiske, 1990). Other changes induced by light availability include mass allocation (Poorter *et al.*, 2012), and overall plant architecture (Charles-Dominique *et al.*, 2010; 2012). Given that selection seems to favor thicker twigs as leaf size increases, and because light has a documented effect on leaf size, then we can expect that light can indirectly influence stem size. Hence, plants should be able to plastically respond to differing light environments along a given stem-leaf scaling slope, "moving" to different degrees along the leaf size-twig size spectrum. Although the effects of light on leaf traits have been well documented, the effect of environmental variation on other traits such as stem mechanical properties remains poorly explored. A previous work, which assessed the effect of canopy openness on stem mechanics concluded that light has an effect on the bending mechanics of *Croton nuntians*, a liana that exhibits a variety of growth habits during its ontogeny (Gallenmüller *et al.*, 2004). Environmental effects on stem mechanical properties have also been measured by Rosell and Olson (2007) using plant growth rate as an indirect estimation of environmental variation. These authors concluded that environment does not have an effect on stem mechanics. Finally, Zhang *et al*. (2011) showed that wood mechanical parameters are related to mean annual precipitation across a large distributional range. These contradictory conclusions lead future studies to explore the effect of environmental variables on stem mechanics, which is one of the main features behind the development of diverse growth forms.

A previous study has shown that individuals of *Amborella* growing in different light environments exhibit variations in leaf thickness and orientation (Feild *et al.*, 2001). Nevertheless, Feild *et al*. (2001) reported an absence of variation in leaf area-specific hydraulic conductivity, and photosynthetic light use, concluding that *Amborella* has limited developmental flexibility to light flux density. However, no study to date has examined possible plastic responses of *Amborella* in architectural and mechanical organization, as well as in ecologically informative functional traits such as leaf mass per area (LMA), leaf dry matter content (LDMC), stem specific density (SSD), and stem water content (SWC).

Analyzing the influence of light on functional traits and the coordination between these traits can help us to understand to what degree *Amborella* is able to respond to light variability. To the extent that *Amborella* structural variation fits into the currently known spectra of variation across the flowering plants, then this would increase our confidence that the potential for plastic variation along these axes was present in the angiosperm ancestor.

Through our architectural description, we show that *Amborella* growth involves the stacking of sympodial modules. The axes making up these sympodial modules have a pattern of increasing mechanical stiffness with increasing diameter, corresponding to the mechanical profile of a self-supporting plant. Canopy openness influenced leaf size and leaf mass investment, and triggered changes in *Amborella* architecture, varying from a long-branched shrub with pendulous axes under closed canopies to a short-branched self-supporting shrub under open canopies. We use this variation in form to understand how the scandent and self-supporting habits are produced developmentally. Our results show that canopy openness does not seem to affect the mechanical properties of stem tissues. Therefore, the scandent and self-supporting forms in *Amborella* are produced by different stem length-diameter proportionalities without tissue mechanical differentiation. Moreover, our analyses show that across light environments, leaf and stem traits predict one another, following Corner's Rules, with stem tissue density negatively correlated with leaf area. The documentation of this trait coordination in the sister to all other flowering plants reaffirms the pervasiveness of these traits integrations.

2.2 Materials and Methods

2.2.1 Plant material, study sites, and sampling

Amborella trichopoda Baill. is a woody evergreen shrub 6-9 m tall, which grows in the understory of rainforests in the central mountain range of New Caledonia on acidic substrates at 100-900 m elevation (Jérémie, 1982). *Amborella* is dioecious with small (3-5 mm) unisexual flowers that are wind/insect pollinated, and it grows in small, male biased populations with measured densities of 433 individuals per ha (Thien *et al.*, 2003). Our architectural observations were carried out on individuals from a population in the natural reserve of Mount Aoupinié in the northern province of New Caledonia. Mt. Aoupinié has one of the populations of *Amborella* with the highest levels of genetic diversity (Poncet *et al.*, 2013). A forestry road runs east to west along the ridge of Mt. Aoupinié, and the associated clearing has exposed several individuals to a considerable increase in light conditions.

Seedlings and young individuals growing in the greenhouses of the *Institut Agronomique Néo-Calédonien* (IAC) at Saint Louis, Mont Dore, New Caledonia, were also used for architectural observations.

TABLE 2.1 *Structural and functional stem and leaf traits measured.*

Trait	Abbreviation	Units
Stem length	SL	cm
Internode length	IL	cm
Internode diameter	ID	cm
Length-diameter ratio	LDR	-
Number of leaves	NL	-
Leaf area	LA	cm^2
Leaf mass per area	LMA	$g\ m^{-2}$
Leaf dry matter content	LDMC	$mg\ g^{-1}$
Stem specific density	SSD	$g\ cm^{-3}$
Stem water content	SWC	%
Modulus of elasticity	MOE	$N\ mm^{-2}$
Modulus of rupture	MOR	$N\ mm^{-2}$

To evaluate stem and leaf economics within *Amborella*, we measured 9 stem and three leaf structural and functional variables (Table 2.1). Outer canopy branches were collected along a gradient from sun exposed roadside individuals to individuals growing in the shaded forest understory. We sampled 24 peripheral branches bearing all of their distal leaves for allometric analysis as well as for stem and leaf trait measurements. We selected branches bearing fully expanded leaves, avoiding leaves with pathogens or herbivore damage. Additional segments were collected for mechanical and stem trait analyses. Sampled branches were immediately defoliated and wrapped in moist paper, sealed in plastic bags, and stored in the dark for transport.

2.2.2 Measurement of canopy openness

Canopy openness (CO, in %) represents the percentage of open sky at a given point, and is a useful index of the light environment experienced by a given plant (Jennings *et al.*, 1999). CO was measured to assess the effect of light availability on the structural and functional properties of *Amborella*. We used hemispherical photographs to characterize local

CO at each sampled branch (Fig. 2.1). Before collecting each branche, three photographs were taken above the basal, medial, and apical branch sections using a 180° hemispherical lens (Samyang fisheye 8 mm f/3,5. Samyang, South Korea) mounted on a Canon EOS 7D camera body (Canon, Japan). The reported CO for a given branch is the average of the three photographs. Photographs were taken between 1100h and 1300h preferentially on cloudy days. The resulting images were analyzed using gap light analyzer software (Frazer *et al.*, 1999).

Closed canopy
5.74% CO

Open canopy
51.21% CO

FIGURE 2.1 Hemispherical photographs used for canopy openness measurements showing two localities with different values of canopy openness.

2.2.3 Architectural analysis

Plant axes were described morphologically and illustrated following the criteria of Hallé *et al.* (1978), Barthélémy and Caraglio (2007), and Charles-Dominique et al. (2010). The architectural description focused mainly on the aboveground structure. Axes of each observed individual were categorized in terms of: 1) growth process (monopodial or sympodial), 2) growth direction (orthotropic or plagiotropic), 3) branching pattern (whether branches elongate immediately after bud initiation or branches originate from dormant buds with a delayed extension), 4) branch position (whether branches are located at a basal (basitonic), medial (mesotonic) or distal (acrotonic) position on the parent axis), 5) presence and position of inflorescences, and 6) symmetry (whether the leaves and branches are disposed radially or bilaterally). Below, we use the term "module" in describing a structural unit repeated over time and composed of a single dominant axis and its lateral subordinates axes. Our architectural analysis was based on *in situ* observations of individuals at different growth stages, defined *a priori* on the basis of morphological criteria (Charles-Dominique *et al.*, 2010). Some of these criteria included branching and accumulation of relays. "Relay" is used here to denote axes that originate from dormant buds and that grow into new branching systems. Relays accumulate over time, providing a basis for classifying individuals into different stages (Charles-Dominique *et al.*, 2010). Regardless of the environment they are growing, individuals from older stages have more relays than those of earlier stages. The age of each individual was estimated by comparison with field and greenhouse individuals of known age. Through architectural and morphological descriptors we described differences between mature individuals growing in various light environments.

2.2.4 Leaf traits and branch dimensions

We measured leaf area (LA), leaf mass per area (LMA), and leaf dry matter content (LDMC) of all the leaves, petioles included, borne by the 24 branches sampled. This sampling allowed us to determine the total LA for each branch. Leaves were scanned in the field using a portable scanner (CanoScan LiDE 25, Canon, Japan) and fresh mass was immediately measured using an analytical balance. LA was calculated from the scanned images using ImageJ 1.47v. (NIH Image, Bethesda, MD, USA). Leaves were then oven dried at 70°C for 72 h for the LMA and LDMC calculations. LMA was calculated as the ratio of leaf dry mass to LA; LDMC was calculated as leaf dry mass over leaf fresh mass (Pérez-Harguindeguy *et al.*, 2013). Branch measurements included number of leaves (NL), total stem length (SL), internode length (IL), internode diameter (ID), and the ratio of stem length to stem diameter (LDR), which was calculated as SL over ID of the basal most internode. IL and ID measurements were made at each internode of the sampled branches.

2.2.5 Stem mechanics

We measured modulus of elasticity (MOE), also known as Young's modulus, along with modulus of rupture (MOR) and flexural rigidity (*EI*) of stem segments from the same branches sampled for the measurements of leaf traits. To cover the widest possible range of stem thickness given our testing apparatus, we sampled additional stems of wider diameters, which were included in a separate dataset. We measured a total of 100 stem segments with diameters ranging from 1.97 to 22 mm. Segments were tested in three-point bending (Fig. 2.2A) with an Instron InSpec 2200 test machine fitted with 10 kN, 125 kN, or 500 kN load cells (Instron Corporation, Norwood, Massachusetts, USA). Stem segments had length : diameter ratios of 20 : 1 to avoid shear (Lahaye *et al.*, 2005; Méndez-Alonzo *et al.*, 2012). The diameter of the tested segments was calculated as the average of the basal, midpoint, and apical diameters measured with a digital caliper at two perpendicular points. The axial second moment of area (*I*) was calculated by Eq. 1 assuming that the stem cross section is approximated as a solid ellipse:

$$I = \left(\frac{\pi}{4}\right) \cdot (r_1^3 \cdot r_2) \tag{1}$$

where r_1 is the radius of the stem in the direction of the applied force and r_2 is the radius in the perpendicular direction. Stem flexural rigidity (*EI*), represents the resistance of a beam to bending forces in terms of size, geometry, and material properties. It was calculated using Eq. 2:

$$EI = \frac{L^3}{48m} \tag{2}$$

where *L* is the distance between the supports of the testing apparatus and *m* is the slope of the initial elastic portion of the deflection vs. force curve (Fig. 2.2B). For the MOE and *EI* calculations, a force was applied at a speed of 0.25 mm/s, inducing a displacement of 2.5 mm. MOE is an index of the capacity of a material to resist bending assuming that the stem is made of a uniform material. MOE was calculated with Eq. 3:

$$\mathrm{MOE} = \frac{EI}{I} \tag{3}$$

MOR, also known as flexural strength, represents the highest stress experienced by the stem at its moment of rupture. In the MOR tests, load displacement was conducted until reaching maximal force (F_{max}, the maximum load at the moment of breakage or the limit of the elastic phase in absence of breakage) (Fig. 2.2B). F_{max} was calculated with the software IX Instron System (Instron Corporation, Norwood, Massachusetts, USA). F_{max} was used to calculate MOR using Eq. 4:

$$\mathrm{MOR} = \frac{(\mathrm{F_{max}} \times L \times r)}{4I} \tag{4}$$

where *L* is the length between the supports, *r* is the averaged radius, and *I* is the second moment of area (Gere and Timoshenko, 1999; Méndez-Alonzo *et al.*, 2012).

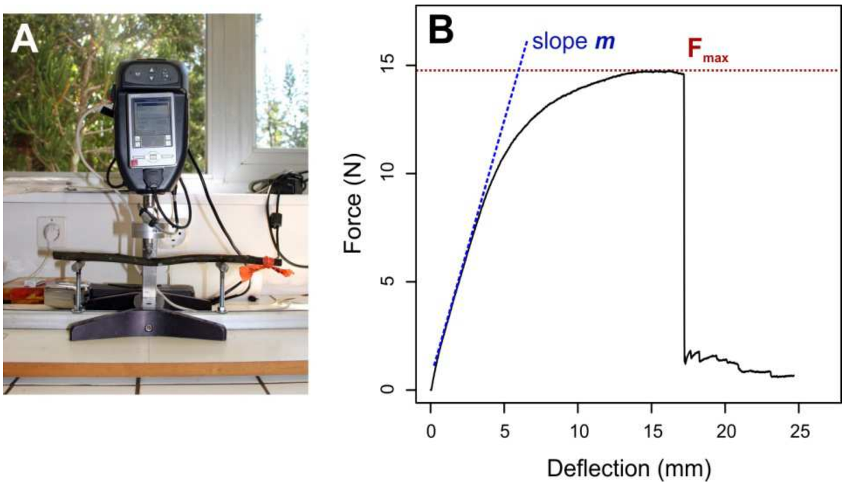


FIGURE 2.2 Three-point bending test performed with an Instron mechanical testing apparatus (A), illustration of a deflection vs. force curve showing the slope of the elastic portion and the maximal force before stem breakage (B).

2.2.6 Stem density and stem water content

We collected stem samples 2.5 to 3 cm long from central sections of the segments tested mechanically. Stem volume was calculated using the water displacement method. We oven-dried stem samples at 70°C for a minimum of 72 h until constant mass. Stem specific density (SSD) was calculated as dry mass/fresh volume (Méndez-Alonzo *et al.*, 2012). Stem water content (SWC), an indicator of water capacitance, was calculated as 100(1 - (dry mass/fresh mass)) (Poorter *et al.*, 2010). To test the hypothesis that branches with greater leaf area have stems with lower tissue density, we used an approach similar to that of Wright et al. (2006) by measuring SSD of the apical-most branch sections, taking exclusively stem segments collected < 350 mm from the branch tip. Apical stem density should reflect the conditions prevailing during the production of the standing crop of leaves, and therefore should correlate well with leaf characteristics.

2.2.7 Data analysis

All of the analyses were conducted in R v.3.1.2 (R Development Core Team, 2014). Functional and structural variables were log-transformed to meet assumptions of normality and homoscedasticity. Data were compiled in two datasets. The first, hereafter referred to as the "branches" dataset, contained arithmetically averaged values of all of the measured traits (Table 2.1) for each of the sampled branches. The second dataset, hereafter referred to as the "biomechanics" dataset, contained values for the stem mechanics traits along with SSD, SWC and CO values of 100 measured segments. The "biomechanics" dataset was used to analyze the variation in mechanical properties on a wider range of stems diameters, allowing us to test predictions regarding mechanical variation during development.

Given that we observed architectural differences under different CO, especially in the terminal branched complexes, we explored whether there were changes in scaling between stem dimensions and total leaf area between light environments. We divided our dataset into two different light environment sites taking 15% CO as a threshold, using a "sun/shade" site categorical variable. This CO threshold was chosen because all of the branches collected in the understory (shade sites) had values of <15% CO while branches sampled in forest clearings along the road (sun sites) had values of > 15% CO. Allometric scaling between stem size (diameter and length) and total leaf area under different CO values was estimated in log-log bivariate relationships using standardized major axis (SMA) regressions using the R package 'smatr' (Warton *et al.*, 2012). We built a model predicting total leaf area based on stem diameter, site, and a stem diameter - site interaction term. A second model predicted total leaf area based on stem length, site, and a stem length - site interaction term. Using these models we estimated the relationship between stem size and total leaf area across CO sites, and we compared scaling slopes of sun branches with those of shade branches via likelihood ratio statistics for common slopes. After finding that there were no slope differences between sites, we fit models without the site interaction term. We then used Wald statistics for equal elevations included in the 'smatr' package to compare intercepts between sites. Similar slopes but different intercepts indicate that stem size differs significantly between sites but foliage-stem scaling is similar. SMA regression was also used to assess the relation between apical SSD and LA. SMA is designed to describe relationships between variables in which the causality of one on the other is likely mutual rather than unidirectionally one variable on the other, making it appropriate for the present situation (Smith, 2009).

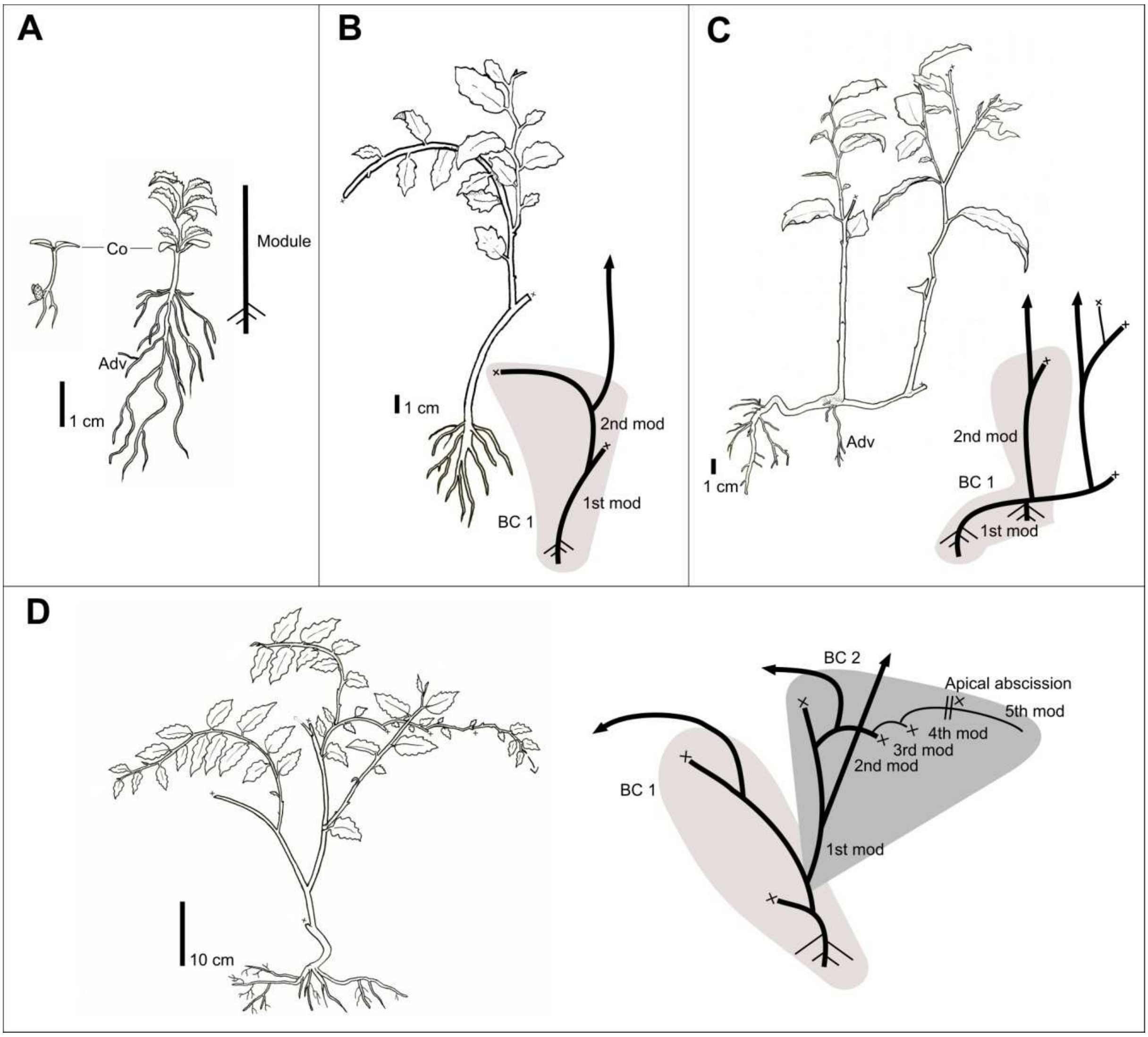
We explored relationships between traits using pairwise Pearson correlations on the "branches" and "biomechanics" datasets. Separately, the effect of CO on the modulation of leaf traits was analyzed with ordinary least squares linear regressions (OLS) on the "branches" dataset. To document changes in stem mechanical properties with ontogeny, we measured the effect of stem diameter on MO and MOR using OLS regressions on the "biomechanics" dataset. To explore a possible joint effect of stem diameter, SSD, and CO on stem mechanical properties, we performed multiple regression analyses on the "biomechanics" dataset. The strength of the contribution of each stem trait and CO was evaluated using semipartial correlations.

2.3 Results

2.3.1 Architectural analysis

Stage 1-small seedlings. *Amborella* seedlings have a tap root and a single orthotropic stem with alternate spiral phyllotaxy (Fig. 2.3A). After about 12 weeks, as observed in the greenhouse, seedlings have a stem 5 cm tall and a large root system (Fig. 2.3A).

Stage 2-young saplings. As the first orthotropic axis elongates, it becomes plagiotropic (i.e. becomes a “mixed” axis, with both orthotropic and plagiotropic sections) becoming pendulous under its own weight (Fig. 2.3B). Phyllotaxy is alternate, oriented spirally in the proximal orthotropic section.



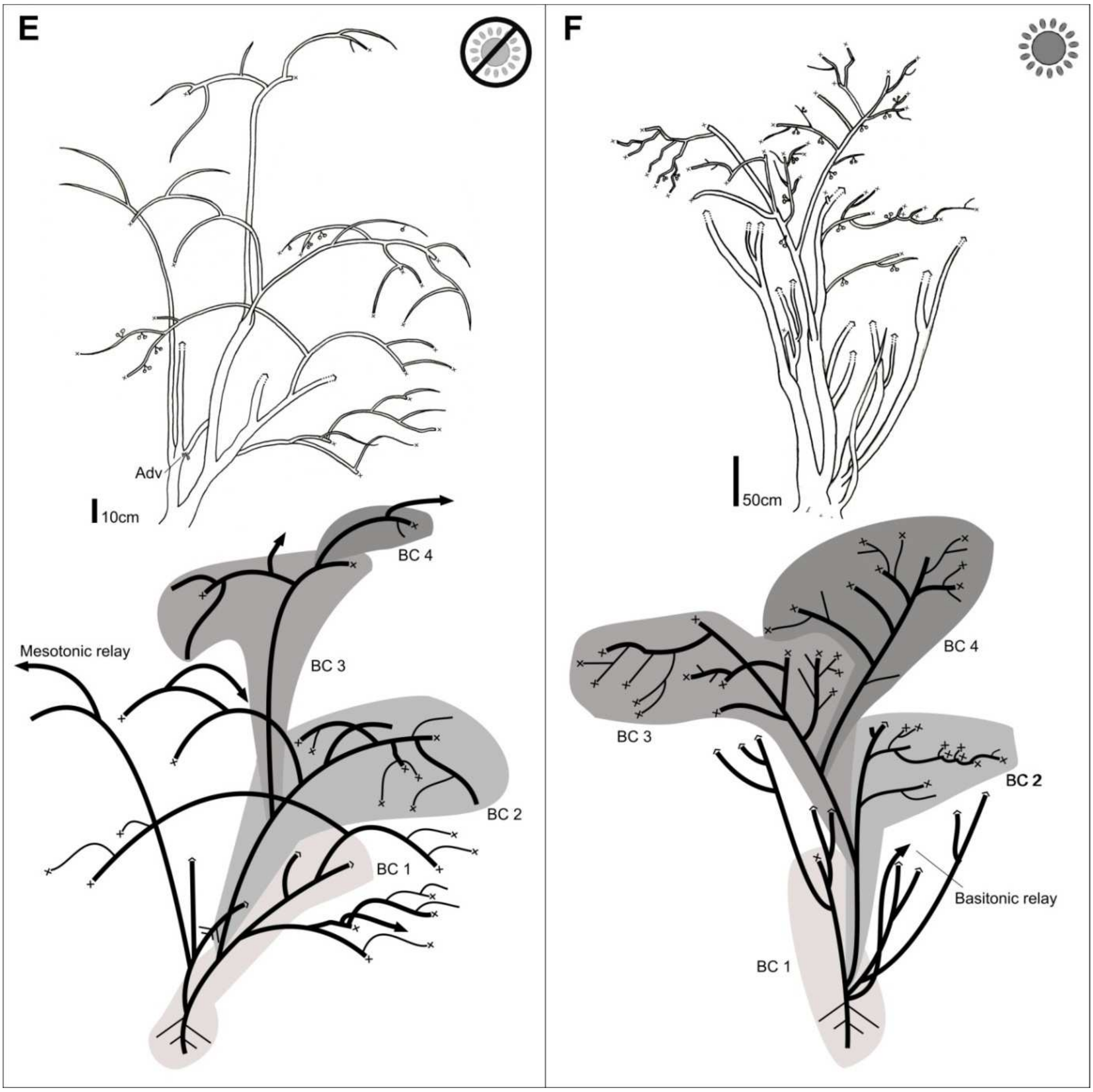


FIGURE 2.3. Illustration of the ontogenetic architectural stages of *Amborella trichopoda*, and architectural variability under closed or open canopies. (A) Seedling and unbranched young plant 6 months after germination (stage 1). (B) 1-year-old plant (stage 2). (C) 1.5-year-old plant with a rooted "pseudo-rhizome" (stage 2). (D) Around 6-year-old plant (stage 3). (E) >10-year-old plant growing under a closed canopy (stage 4). (F) >10 year-old-plant growing under open canopy (stage 4). Only some of the successive branched complexes of stage 4 individuals are represented. Abbreviations: Adv, adventitious root; BC, branched complex; Co, collar zone; mod, architectural module. Thick lines represent structural axes, thin lines represent lateral branches, arrowhead lines represent relays, crosses are dead apices, circles are inflorescences, and gray shadings indicate BCs.

Leaf orientation is bilaterally symmetrical in the distal plagiotropic section. A lateral mixed axis makes up a second architectural module with the same phyllotaxy as the parent axis. Branching is sympodial. In most of the individuals observed, a single branch developed after apical death of the parent axis (Figs. 2.3B,C). Apical death occurs mainly after the bending of axes (Fig. 2.3B). Branching is predominantly mesotonic, and on the upper surface of the bending zone (Fig. 2.3B). The basal diameter of the lateral branch becomes equivalent to that of the section of the parent axis preceding the branching (Fig. 2.4A). As the upper module develops, the distal part of the parent axis withers and decays (Figs. 2.3B,C). Growth of the sympodium stops after reaching about 25 cm, and the set of modules make up a branching system, which will be hereafter referred as a branched complex (BC) (Figs. 2.3B,C). Relay branches of fast growth sprout on the first BC originating from dormant mesotonic buds (Figs. 2.3B,C). In some individuals, the first module comes to lie on the ground, this stem can develop adventitious roots and resprout, then becoming a "pseudo-rhizome" from which several stems develop (Fig. 2.3C).

Stage 3-early maturity. Larger individuals grow continuously with a sympodial branching pattern (Fig. 2.3D). Only some nodes produce lateral branches and there is no obvious regular distribution of branches in tiers (Fig. 2.3D). Branch production seems to be associated with environmental conditions, given that we observed increases in lateral branch production under light patches, and after trauma such as falling branches. All axes are morphologically similar, and we did not observe a hierarchical architectural construction with distinctive axis categories (Fig. 2.3D), unlike as in conifers, which have distinct central and lateral stems. Modules derived from the relay stem originating from the first BC establish a second BC (Fig. 2.3D). At this stage, acrotonic sympodial branching can occur in peripheral branches (Fig. 2.4B). The axillary bud of the terminal leaf activates, producing a new module that maintains the same growth direction as the parent module from which it originates (Figs. 2.3D; 2.4B).

Stage 4-maturity. Individuals at this stage are built by the sequence of over four branched complexes, having thick basal areas and significant height (Figs. 2.3E,F). BCs are formed by a combination of branch-bearing stems and leaf-bearing lateral branches. The sequence of BCs is repeated by mesotonic relays and basitonic relays originating from dormant buds located at the collar zone. As a consequence of the accumulation of iterated complexes, the plant has a multi-stemmed shrub form and a leader stem is not distinguishable (Figs. 2.3E,F). Flowering is lateral, occurring in axial meristems of both stems and lateral branches (Figs. 2.3E,F). No architectural differences were observed between male and female individuals. Adventitious roots were frequently observed above the ground at the stem base (Fig. 2.3E).

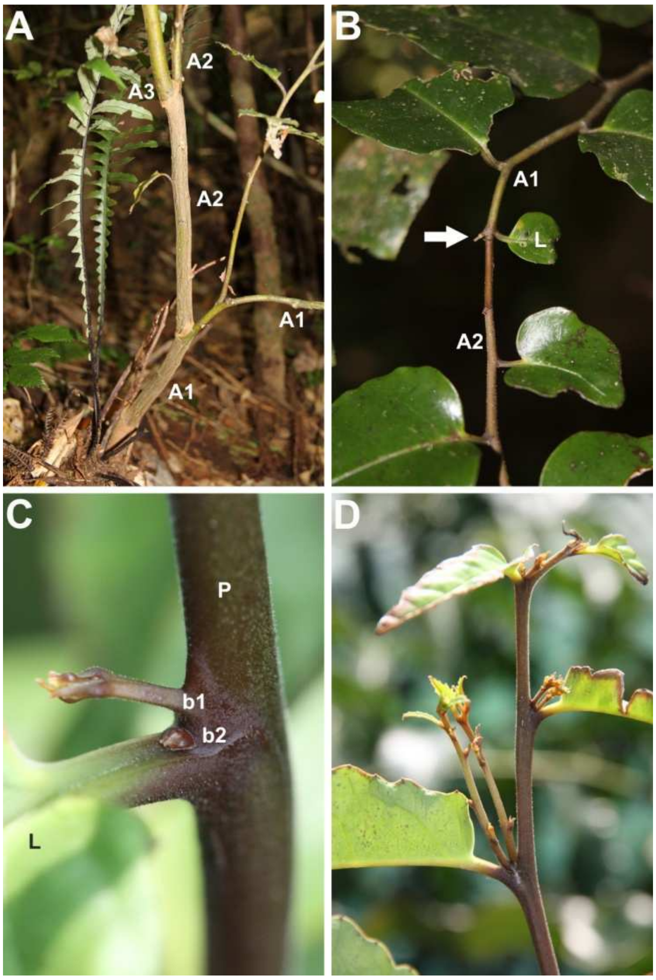


FIGURE 2.4. Illustration of morphological features of *Amborella trichopoda*. (A) Young individual growing in the understory showing sympodiality by stacking of modules. Note the changes in diameter of the parent axes (A1, A2) after the branching of upper modules. (B) Linear sympodial structure after apical death (arrow). A second axis produced by the lateral bud of the more distal leaf continues axis construction keeping the same growth direction. (C) A supernumerary bud is located below an axillary bud showing onset of growth. (D) Under high-light conditions, both axillary buds can activate, producing small axes. Abbreviations: A1, A2, A3, axis orders; b1, axillary bud; b2, supernumerary bud; L, axillary leaf; P, parent axis.

Morphological differences across canopy opennesses. Given a lack of recruitment in sun exposed sites, we observed stages 1 to 3 only in shaded understory conditions. We observed qualitative morphological variation in branched complexes under different canopy opennesses in large (stage 4) individuals. The crowns of plants growing in more shaded environments were made up of very elongate and sparsely branched axes (Fig. 2.3E). Plants in open canopy environments had more lateral branches (Fig. 2.3F). Under a closed canopy, several relays occurred mostly at mesotonic positions, whereas under an open canopy relays were less frequent and were usually basitonic. Under open canopy conditions, inflorescences were more abundant (Fig. 2.3F) and axillary supernumerary meristems activated (Fig. 2.4C). Supernumerary axillary buds produce additional small branches (Fig. 2.4D). The lifetime of the short axes produced by the supernumerary buds seems to be very short, given that we observed a frequent abscission of small branches in the crowns of individuals growing under open canopies (Fig. 2.3F).

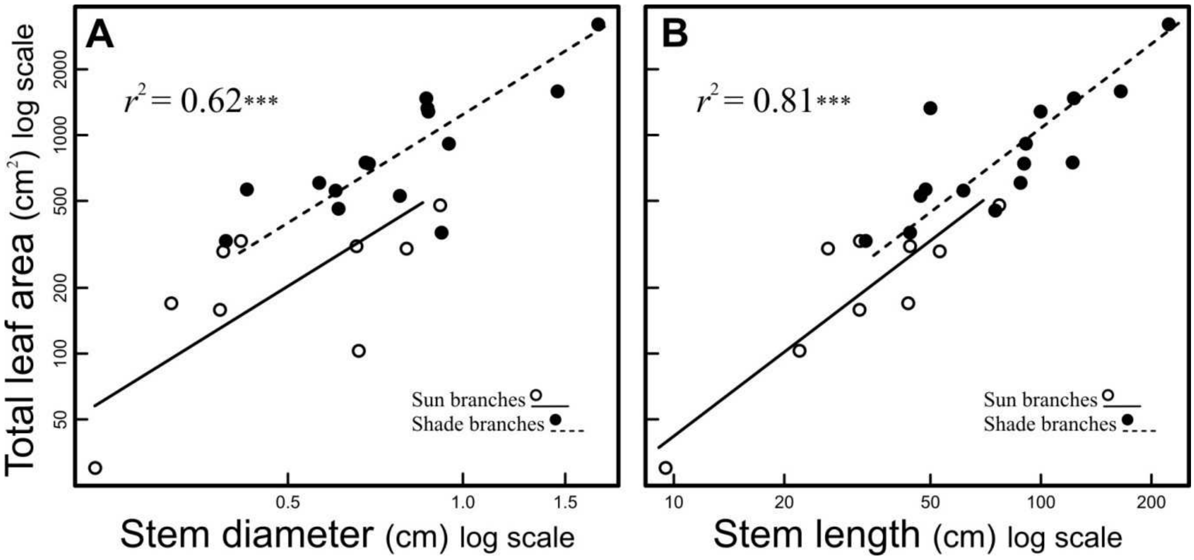


FIGURE 2.5 SMA regressions showing the scaling of total leaf area (sum of areas of all leaves borne by the stem) with stem dimensions of branches growing under open canopy (>15% CO) and closed canopy (<15% CO). (A) Total leaf area and stem diameter allometry. (B) Total leaf area and stem length allometry. *** $P < 0.001$.

2.3.2 Foliage-stem scaling in Amborella

Both stem diameter and length were significantly related to total leaf area (Fig. 2.5). Stem diameter, equivalent to the diameter of the basal most and thickest internode of each branch, predicted 62% of the variation in total leaf area across light environments (Fig. 2.5A). Stem length was also strongly related to total leaf area, explaining 81% of its variation (Fig. 2.5B). When assessing differences in the scaling of stem diameter and total leaf area among sites, the model indicated that both types of sites have similar SMA slopes (Likelihood ratio statistics; $P = 0.68$). SMA slopes ranged from 1.58 (95% CIs 1.10-2.27) for shade branches to 1.82 (95% CIs 0.97-3.41) for sun branches. Assuming homogeneity of slopes, branches from shade exposed sites had an elevation of 3.08 (95% CIs 2.96-3.21), which was not quite significantly higher (Wald's test; P=0.01) than the elevation of sun exposed branches (2.85; 95% CIs 2.39-3.32). Biologically, this result indicates that similar allometric scaling relationships are maintained regardless of the light environment, with sun branches having slightly thinner stems. With regard to stem length, SMA slopes of sun branches (1.39; 95% CIs 0.92-2.10) and shade branches (1.17; 95% CIs 0.83-1.66) were not significantly different (Likelihood ratio statistics; $P = 0.48$). Elevations were also similar (Wald's test; $P = 0.18$)

between sun branches (0.16; 95% CIs -0.74-0.92) and shade branches (0.66; 95% CIs -0.12-1.46). In addition to the leaf area and stem size scaling, total leaf number was also significantly correlated with stem length and diameter (Table 2.2).

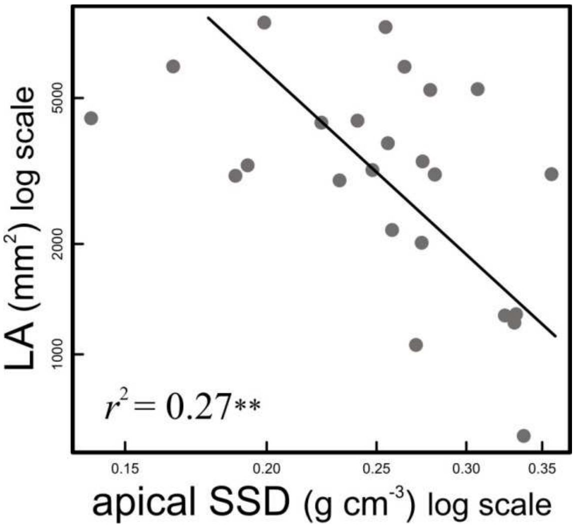


FIGURE 2.6 SMA regression showing the relationship between SSD of apical stem sections and mean LA of the leaves subtended by each stem (**P = 0.008; slope -2.83, 95% CI -4.09 to -1.96).

2.3.3 Coordination of leaf and stem functional traits, and effect of canopy openness

Most stem and leaf traits were significantly correlated (Table 2.2). LDMC and LMA had the strongest relationships with stem traits such as SWC and SSD. LA was strongly correlated with the remaining leaf traits, but it was not significantly related with any of the stem traits (Table 2.2). Stem size traits such as total stem length as well as internode diameter and length were very strongly correlated with leaf traits and stem mechanical traits, but were not correlated with SSD and SWC. SSD was positively correlated with both MOE and MOR. SWC and stem mechanical properties were negatively correlated, indicating that stems with higher water contents had tissues that were both more flexible and less resistant to breakage. Branch averaged values of SSD and LA were not significantly correlated (Table 2.2). However, average LA was negatively correlated with SSD of branch apical segments (Pearson; r = -0.52, P = 0.008) and scaling between both traits was detected (Fig. 2.6), indicating that broad leaved branches of *Amborella* have stem tissues of lower density.

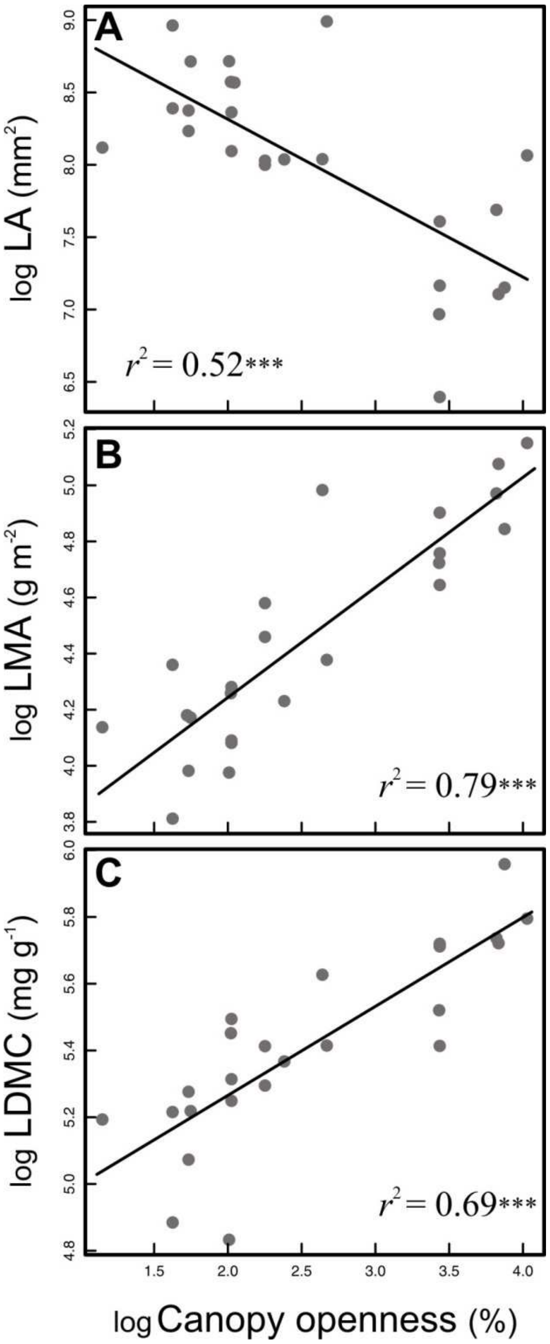


FIGURE 2.7 Influence of canopy openness on leaf traits variation. (A) Decrease of leaf area with increasing canopy openness. (B) Increase of leaf mass per area with increasing canopy openness. (C) Increase of leaf dry matter content with increasing canopy openness (n = 24) *** $P < 0.001$.

CO, which reflects light availability, was significantly correlated with leaf and stem size traits (Table 2.2). SWC was negatively associated with CO (Table 2.2), suggesting lower water contents in sun exposed branches. Canopy openness had a very important effect on leaf trait variation (Fig. 2.7). LA was negatively related to CO (Fig. 2.7A) while CO was strongly positively related to both LMA (Fig. 2.7B) and LDMC (Fig. 2.7C). Sun exposed leaves were smaller than shade leaves but had higher mass per unit of area and higher dry matter content than leaves under closed canopy.

TABLE 2.2 *Pairwise Pearson correlations between stem and leaf traits and canopy openness.*

	SL	IL	ID	LDR	NL	LA	LMA	LDMC	SSD	SWC	MOE	MOR
IL	**0.85*** **											
ID	**0.74*** **	**0.77*** **										
LDR	**0.74*** **	**0.58*** **	0.23									
NL	**0.86*** **	**0.55** **	**0.53** **	**0.62*** **								
LA	**0.77*** **	**0.89*** **	**0.73*** **	0.38	**0.57*** **							
LMA	**-0.49** **	**-0.60*** **	-0.19	**-0.46* **	-0.29	**-0.62*** **						
LDMC	-0.39	**-0.59*** **	-0.25	-0.34	-0.14	**-0.56*** **	**0.89*** **					
SSD	-0.25	-0.36	-0.11	-0.26	-0.06	-0.33	**0.48* **	**0.53* **				
SWC	0.26	0.35	0.10	0.25	0.09	0.39	**-0.67*** **	**-0.69*** **	**-0.85*** **			
MOE	**0.66** **	**0.59*** **	**0.67*** **	0.36	**0.54** **	**0.59** **	-0.12	-0.01	**0.46*** **	**-0.49*** **		
MOR	**0.51* **	**0.44* **	**0.34*** **	0.34	**0.55** **	**0.48* **	-0.07	0.03	**0.56*** **	**-0.59*** **	**0.72*** **	
CO	**-0.49* **	**-0.73*** **	-0.31	-0.35	-0.21	**-0.72*** **	**0.88*** **	**0.85*** **	0.35	**-0.53** **	-0.21	-0.06

Notes: Correlations based on averaged values of 24 sampled axes. Variables were log transformed prior to analysis. See Table 2.1 for traits abbreviations and units. Correlations between MOE, MOR, ID, SWC and SSD were calculated using the "biomechanics" dataset (n = 100). Significant correlations are shown in bold. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

2.3.4 Stem mechanics

Mechanical parameters (MOE and MOR) were significantly predicted by stem diameter (Fig. 2.8). MOE increased with stem diameter (Fig. 2.8A) from 500–2000 N mm^{-2} in stems of 1.98–2.5 mm to 7000–9000 N mm^{-2} in stems with diameters of >10 mm. Because higher values of MOE reflect higher material stiffness, this result indicates that tissues in thicker basal stems are stiffer than those in narrow apical ones. As regards MOR, narrower stems had lower resistance to rupture whereas wider stems were more resistant to rupture (Fig. 2.8B). Flexural rigidity of *Amborella* stems was strongly correlated with diameter (r^2 = 0.97; $P < 0.001$), indicating that higher loads were needed to produce deflection in stems of largest diameters.

Light environment did not have an effect on mechanical parameters, as suggested by the lack of correlation of CO with either MOR orand MOE using branch-averaged values (Table 2.2). However, multiple regressions including the effect SSD and CO (Table 2.3) on stem mechanics suggest slight mechanical variations with CO.

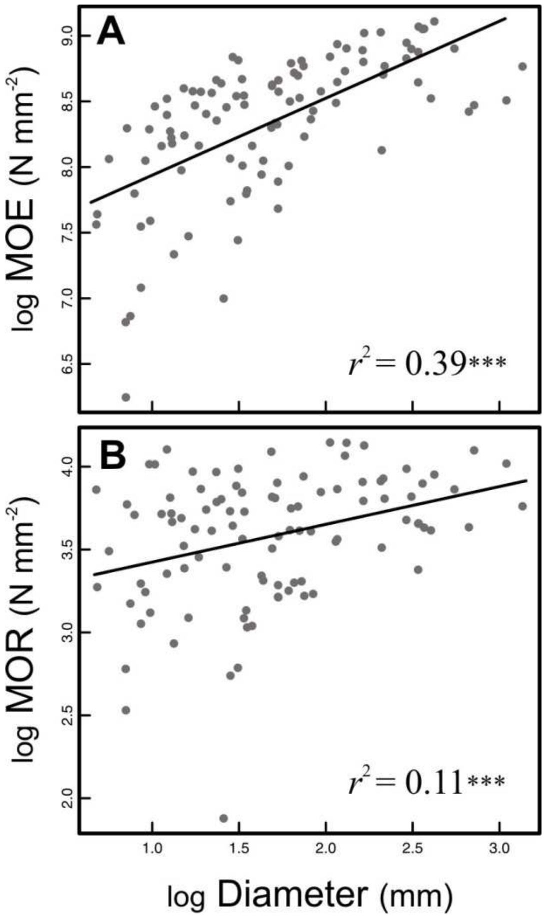


FIGURE 2.8 Relationships between mechanical properties and stem diameter. (A) Increasing of stem stiffness (MOE) with increasing diameter. (B) Increase of stem resistance to breakage (MOR) with increasing diameter. n = 100. *** $P < 0.001$.

When MOR was explained by diameter, SSD, and CO, the coefficient associated with CO was not significant (Table 2.3). As for MOE and EI, the coefficient associated with CO was significant. Despite the significance of these coefficients, CO was the variable that contributed least to the models, as shown by the lower semipartial correlation values when compared to those of the other parameters (Table 2.3). MOE and EI increased with both stem diameter and SSD, with shade axes tending to be stiffer. With regard to MOR, SSD had the highest semipartial correlation explaining more of the total variation in MOR than stem diameter (Table 2.3). The lower association of MOR with stem diameter, as compared to the association between MOE and diameter, was readily observed in the scatter plots (Fig. 2.8). SSD also had a significant effect on MOE, but it had a lower semipartial correlation than stem diameter (Table 2.3), suggesting that SSD plays a major role in stem resistance to rupture and a lesser but nevertheless important effect on stem flexibility.

TABLE 2.3 *Multiple regressions of* Amborella *mechanical properties predicted by canopy openness (CO), stem diameter (D), and stem specific density (SSD).*

response	r^2	Ftest	βD	βSSD	βCO	PD	PSSD	PCO
MOE	0.55	$F_{3,96}$ = 39.56***	0.47***	1.00***	-0.18**	0.47	0.38	0.22
MOR	0.41	$F_{3,96}$ = 39.56***	0.12*	1.02***	-0.07^{ns}	0.18	0.54	0.13
EI	0.98	$F_{3,96}$ = 1717***	4.46***	1.00***	-0.18**	0.91	0.08	0.04

Notes: r^2 = adjusted coefficient of multiple determination. βD = coefficient associated with stem diameter; βSSD = coefficient associated with stem specific density; βCO = coefficient associated with canopy openness. PD, PSSD, and PCO are semipartial correlations indicating the contribution of each predictor. (N= 100). ns = non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

2.4 Discussion

Amborella trichopoda, the sister species to all other flowering plants, varies predictably in functional and structural traits with light environment. Within this variation, leaf and stem economics are coordinated. For example, though axes of *Amborella* varied in LMA and LDMC under variation in canopy openness, they maintained similar foliage-stem scaling. The confirmation of these patterns of covariation in *Amborella*, together with their wide distribution across both the angiosperms and conifers suggests that these coordinated plastic responses were likely part of the basic developmental toolkit of the ancestral angiosperm. Here we discuss some of the patterns of trait coordination that are widespread in plants in the context of the *Amborella* growth form and what these characteristics might indicate regarding angiosperm synapomorphies or symplesiomorphies.

2.4.1 Corner's Rules and *Amborella* growth form plasticity

Across species, plants vary from those with thick twigs bearing large leaves to those with narrow twigs bearing small leaves (Westoby and Wright, 2003). Here we show that this spectrum can be observed among individuals of *Amborella*, which exhibit similar foliage-stem scaling across light environments. It has been proposed that foliage-stem scaling is a consequence of the mechanical and hydraulic requirements of leaves as well as self-shading avoidance trough leaf spacing (Enquist, 2002; Westoby *et al.*, 2002). Moreover, if similar crown areas fix similar amounts of carbon, then carbon limitation requires such a foliage-stem scaling if leaf spacing is greater in larger-leaved species (Olson *et al.*, 2009). In addition to foliage-stem scaling in *Amborella*, we observed an association of low stem specific density with rapid stem extension, high leaf area, and leaf spacing. Across light environments, *Amborella* has narrow stems of high density bearing small leaves with high mass per area or thick branches of low densities bearing wide leaves with low mass per unit area. Our results thus converge with the metabolic mechanism proposed by Olson *et al*. (2009), suggesting that if leaves and stems maintain a metabollically driven proportionality, large-leaved axes with greater leaf spacing require low density tissues and thicker stems as a response of rapid volumetric extension and stem tissue mechanics given carbon limitation.

A second component of Corner's rules implies that species with larger leaves and twigs also tend to have less frequent branching with wider branching angles, whereas species with smaller leaves and twigs have more frequent branching with narrower branching angles (Corner, 1949; Ackerly and Donoghue, 1998; Westoby and Wright, 2003). Our architectural analysis shows that the crowns of *Amborella* individuals have conspicuous morphological differences depending on light environment. Crowns of individuals growing under a closed canopy have sparser branching and few, long lateral branches, whereas crowns of individuals growing under an open canopy show an increase in branching. This greater branching is accompanied by the activation of supernumerary buds leading to a short, densely leaved, and narrow crown. Similar crown morphological responses to light availability have been observed across shade-tolerant angiosperm species (Cornelissen, 1993; Niinemets, 1996). The observed variation in the architecture of *Amborella* individuals under different canopy opennesses suggests that intraspecific architectural plasticity follows Corner's Rules.

2.4.2 Phenotypic plasticity of *Amborella* leaves in response to canopy openness

Numerous studies spanning a wide diversity of plant lineages have highlighted that leaf characteristics can be strongly influenced by local light environment. Here we extend this documentation to *Amborella*, whose leaves vary markedly in size and mass allocation under different canopy opennesses. This variation in leaf traits in different light environments is very likely adaptive (Poorter *et al.*, 2009). For instance, *Amborella* leaves in shade conditions have greater area for a given unit of biomass, increasing the surface available for light interception. Lower LMA in understory *Amborella* leaves reflects a reduction in the cost of leaf construction. It has been shown that lower construction investment is favored in environments with low photosynthetically active radiation (Poorter *et al.*, 2006). In addition to light availability, canopy openness is also positively related to air temperature and negatively related to relative humidity (Pineda-García *et al.*, 2013). Therefore, the reduction of leaf area under open canopy conditions, along with the increases in LDMC and LMA that we observe in *Amborella*, can also be considered as adaptive responses to potential desiccation and water stress (Niinemets *et al.*, 1999).

Several mechanisms can underlie the variation we observed in the absolute values of LMA in response to light availability (Fig. 2.7B). It has been proposed that leaf tissue density is strongly correlated with LMA in woody plants (Castro-Díez *et al.*, 2000; Villar *et al.*, 2013) and leaf tissue density seems to predict LMA better than leaf thickness (Villar *et al.*, 2013). A previous study suggested that *Amborella* leaf epidermal and hypodermal thickness do not change in response to varying light levels (Feild *et al*. 2001). Feild *et al*. (2001) also reported limited adjustments in total leaf thickness, with sun leaves being *ca*. 10% thicker than shade leaves. Our results, however, show a very strong effect of light availability on leaf area and mass investment (LMA and LDMC) (Fig. 2.7). The variability we observe in LMA and LDMC suggests that while *Amborella* leaf thickness is not highly variable, as suggested by Feild *et al*. (2001), light incidence may induce significant shifts in tissue density. Further studies would be needed to detect potential structural changes at the cellular level in both the epidermis and the mesophyll of leaves. This could provide information on the leaf constituents that drive *Amborella* LMA variation under different light environments.

The plastic responses of leaf size and leaf mass allocation to light variability observed in *Amborella* is consistent with numerous reports for angiosperm species in both tropical rainforests and temperate forests, as well as in crops and domesticated plants (Buisson and Lee, 1993; Miyaji *et al.*, 1997; Poorter *et al.*, 2006; Lusk *et al.*, 2008; Matos *et al.*, 2009). Similar leaf responses span both eudicots and monocots (Buisson and Lee, 1993; Laurans *et al.*, 2012; Yang *et al.*, 2014). Analogous variation in leaf traits has also been described at the intraspecific level in *Arabidopsis thaliana* (Pigliucci and Kolodynska, 2002; Poorter *et al.*, 2009). Further, similar LMA increases in response to light have been recorded across gymnosperm species (Abrams and Kubiske, 1990; Bond *et al.*, 1999) and also within individuals (Koch *et al.*, 2004). Our documentation of leaf trait variability as a consequence of habitat openings in *Amborella* highlights the adaptive importance of this phenotypic response, which seems to operate in similar ways across the major lineages of vascular plants.

2.4.3 Trait coordination and tradeoffs, different strategies within a single species

The study of key functional traits and their variation across species can be very informative regarding plant ecological strategies (Westoby and Wright, 2006). For instance, the median LMA in *Amborella* (74.5 $g.m^{-2}$, n = 409) is very close to the 73 $g.m^{-2}$ median reported for tropical rainforest species in general (Poorter *et al.*, 2009). The LMA observed here is thus congruent with the habitat preferences of *Amborella*, whose distribution is restricted to rainforest-type habitats (Poncet *et al.*, 2013; Pouteau *et al.*, 2015). It is now broadly accepted that plant functional traits need to be studied in a 'network' perspective, with multiple traits correlation and tradeoffs assemblages shaping the ecological strategies of species (Poorter *et al.*, 2014). Our results show that coordination of leaf and stem economic traits is present at the intraspecific level in *Amborella*. Hence, *Amborella* traits can coordinately shift as a response to local light environments.

Axes growing under open canopies seem to adopt a resource conservation strategy linked to a slower relative growth rate (RGR). It has been shown that plants with higher LMA have lower RGR, and are favored under high-light environments (Poorter and Van der Werf, 1998). The characteristics of leaves in open canopy environments seem to be coordinated with stem traits that reflect similar conservation strategies. Indeed, mass allocation to leaves and stems seems to be coordinated in *Amborella* as evidenced by the LDMC/LMA - SSD positive correlations. A positive correlation between LDMC and SSD has been observed across angiosperm species (Méndez-Alonzo *et al.*, 2012), suggesting coordinated evolution between these leaf and stem traits. A coordinated increase in mass allocation to stems and leaves may confer a survival advantage by reducing the probability of physical hazards such as wind mechanical stress and herbivore attack (Zimmerman *et al.*, 1994; Poorter *et al.*, 2009). At the other end of the spectrum, plants with low LMA, corresponding here to closed canopy *Amborella* axes, tend to have higher photosynthetic rates per unit leaf mass (Wright and Cannon, 2001) as well as faster resource acquisition and high RGR. Fast stem extension in internodes can minimize self-shading in large-leaved branches (White, 1983). The fast growth of the large-leaved shade axes in Amborella is reflected by their longer internodes and their lower values of SSD. It has been shown that wood density, which greatly contributes to SSD, is negatively related with photosynthetic capacity (Santiago *et al.*, 2004). Accordingly, efficient acquisition of photosynthates likely allows shade branches to have accelerated volumetric expansion, lowering stem tissue densities.

In addition to positively related traits, we also observed negatively related traits that could indicate tradeoffs. Our study suggests inter-organ tradeoffs in *Amborella* such as the negative relationship between SSD of apical branch sections and LA. Apical SSD explained 27% of the variation in leaf size in *Amborella* (Fig. 2.6), which is similar to the relations presented by Wright *et al.* (2007) when relating leaf area and wood density of simple-leaved species. The apical SSD-LA tradeoff in *Amborella* is consistent with similar findings of negative relations of leaf size with both wood density and branch mechanical stiffness across species of different habitats (Pickup *et al.*, 2005; Wright *et al.*, 2006; Wright *et al.*, 2007; Swenson and Enquist, 2008; Olson *et al.*, 2009). To our knowledge, this is the first evidence of this tradeoff at the intraspecific level. Wright *et al*. (2006; 2007) explained this tradeoff via plant hydraulics, suggesting that stems with low wood density enable higher hydraulic conductivity per sapwood area (K_S), allowing higher leaf surface. However, a previous study (Feild *et al.*, 2001) has shown that K_S was not different between sun exposed and understory branches of *Amborella*, which, as we have shown here, tend to have significant variation in LA. If we assume that similar amounts of photosynthates are on average produced per unit crown area among light environments, the apical SSD-LA tradeoff could then be a consequence of the fast primary growth rate of Amborella's shade wide-leaved axes (Olson *et al.*, 2009). A fast primary growth rate can be deduced from the low SSD and wide pith (data not shown) observed in stems from closed canopy environments.

Another tradeoff is likely indicated by the strong negative relation between SWC and SSD. This pattern seems to emerge as a compromise between mechanical strength and water storage (Santiago *et al.*, 2004). Our data were consistent with such a tradeoff in *Amborella*, with the observed negative relationship between both stem mechanical traits (MOR and MOE) and SWC. Respectively, gains of mechanical strength are observed as SSD increases. These results are congruent with studies showing that lower density is associated with lower capacity to resist bending and breakage, but higher xylem water conductivity and storage (Pratt *et al.*, 2007; Onoda *et al.*, 2010; Méndez-Alonzo *et al.*, 2012). Rosell *et al*. (2012) proposed a possible mechanism for this tradeoff, suggesting that higher levels of stem water storage would be associated with greater allocation the cell lumen and less to the cell walls that are largely responsible for stem material mechanical stiffness.

2.4.4 *Amborella* architecture and mechanical properties in the context of the evolution of angiosperm growth form

Flowering plants have evolved into an unparalleled diversity of growth forms and architectures. The architecture of a plant is defined by the nature and arrangement of each of its parts (Barthélémy and Caraglio, 2007). Because most of the axes of *Amborella* are initially orthotropic bending secondarily by gravity, its architecture corresponds to Champagnat's architectural model (Hallé *et al.*, 1978). However, the observed changes in leaf orientation, from radial to bilateral symmetry according to their position on the axis, are features of Mangenot's architectural model (Hallé *et al.*, 1978). *Amborella* may thus represent an intermediate form suggesting an architectural continuum between these models (Barthélémy and Caraglio, 2007). Both models are characterized by the lack of a main trunk and construction based on the superposition of modules. The absence of a main trunk and the similar morphologies of its modules characterize the body of *Amborella*. The lack of a hierarchical organization in the axes of *Amborella* contrasts with the very hierarchical architecture commonly observed in gymnosperms (Grosfeld *et al.*, 1999). These observations suggest that angiosperms may have evolved a less restricted body construction that could have promoted the development of a wider range of growth forms.

A possible example of such novel growth forms includes sympodiality, which is widespread among basal angiosperms. Sympodiality has also been suggested as a retained character in Ranunculales, the eudicot order sister to the rest of the eudicots (APG III, 2009). Because of this phylogenetic pattern, and because of the absence of sympodial growth in gymnosperms, sympodiality has been suggested as a synapomorphy for the angiosperms (Carlquist, 2009). Carlquist (2009) also suggested that sympodiality may have provided angiosperms with numerous competitive advantages, such as rapid spreading over wider lateral areas, securing footholds and tapping new soil resources by the rooting of branches, and escaping hydraulic and mechanical failures by the production of numerous branches that can potentially root. The production of branches from dormant buds confers on *Amborella* the ability to resprout through basitonic and mesotonic relays (Figs. 2.3E,F). Resprouters seem to have an increased ability to persist after disturbance events (Bond and Midgley, 2001). As such, sprouting ability has been suggested to be a key feature of plant strategies (Bond and Midgley, 2001). Collar sprouting, as seen in the basitonic relays observed in *Amborella* (Fig. 2.3F), is generally rare in conifers (Del Tredici, 2001). The sprouting ability and sympodial construction observed in *Amborella* are morphological characteristics observed in other cane-like basal angiosperm groups (Isnard *et al.*, 2012). This suggests that sympodiality, and associated sprouting and rooting ability, which can be considered as competitive morphological attributes, were acquired early during the evolution of the flowering plants.

The cane-like form of *Amborella* and other basal angiosperms is often associated with the presence of scandent stems (Feild and Arens, 2005; 2007). The laxity of these scandent stems should be reflected by the relationship between stem size and stem mechanical properties. Our work provides a first estimation of the stem mechanical properties of

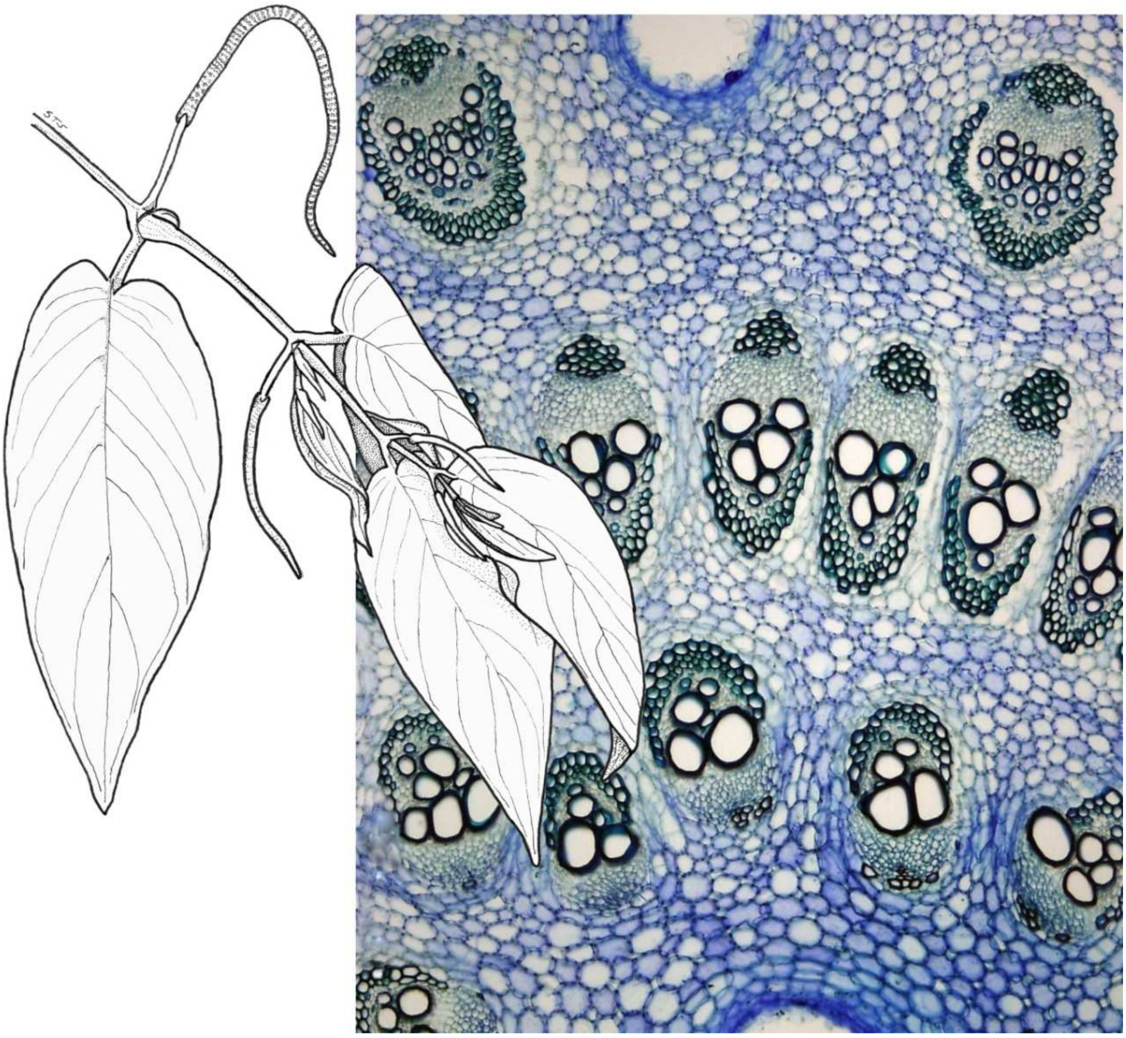
Amborella. We show that its vesselless stems have the mechanical organization of a self-supporting plant, with tissue stiffness increasing with stem diameter (Fig. 2.8A; Table 2.3) (Rowe and Speck, 2005). A previous study analyzing the stem mechanics of cane-like basal angiosperms has shown a similar mechanical organization in shrubs and treelets in *Aristolochia*, *Thottea*, and Piperaceae (Isnard *et al.*, 2012). In spite of the indisputable role of stem tissue mechanical properties in the development of different forms, plant habits are also the result of the interplay of stem mechanics with stem length and stem diameter (Castorena *et al.*, 2015). The mechanical organization of *Amborella* and other cane-like basal angiosperms corresponds to the profile of a self-supporting plant, given that MOE increases with stem diameter, unlike very long lianas, whose flexible tissues do not increase in MOE with increases in stem size. Therefore, the scandent form of *Amborella*, and other cane-like basal angiosperms, should be regarded as the result of increases in stem length without an offset in stem diameter.

Amborella axes can vary morphologically from short axes growing under open canopy (Fig. 2.3F) to long pendulous axes developing under closed canopy (Fig. 2.3E). Given that MOE and stem diameter relationship is similar across canopy opennesses (Table 2.3), local light environment does not seem to have a direct effect on stem tissue mechanical properties. This result converges with the conclusions of Rosell and Olson (2007), who also found a lack of environmental effect on stem tissue mechanical properties. Our findings are however contradictory to the conclusions of Gallenmüller et al (2004), who stated that canopy openness has an influence on stem mechanics. However, *Croton nuntians*, the species considered in their work, exhibits ontogenetic shifts of mechanical patterns between juvenile freestanding and adult climbing individuals. Therefore, canopy openness may influence the ontogenetic development of *C. nuntians* with stem bending as an associated response. It has been suggested that size variations are sufficient to generate functional diversity even in the absence of shifts in stem tissue mechanic properties (Rosell *et al.*, 2012). Our results suggest that light-induced changes in stem length can effectively affect the mechanic behavior of *Amborella* axes, producing habit variations without further shifts in stem tissue mechanical properties. We show here that variation in canopy openness has a significant effect on leaf size and leaf mass investment.

Leaf and stem economics are coordinated in *Amborella* and leaf size shows a strong association with stem size. Moreover, shifts in stem size seem to influence the mechanical behavior and growth habit expressed by *Amborella*. Our study on the sister species to the remaining flowering plants underscores that these coordinated plastic responses in structural and functional traits, which likely provide adaptive functional strategies, were likely already present in the common ancestor of all extant angiosperms.

CHAPTER 3

Evolution of woodiness in Piperales, a mega-diverse basal angiosperm order[1]



- Anatomical organization of Piperales lineages
- Cambial activity and woodiness evolution
- Anatomical variation of vessel element perforation plates

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[1] A version of this chapter has been **published**. Trueba, S., Rowe, N.P., Neinhuis, C., Wanke, S., Wagner, S.T., Isnard, S. (2015). *International Journal of Plant Sciences* 176: 468-485.

Abstract

Piperales, the largest basal angiosperm order with *c*. 4090 species, displays a wide diversity of growth forms. This diversity in growth forms appears to be linked with differences in cambial activity and subsequent derived wood production. To date no overall synthesis of the evolution of woodiness in Piperales has been done and few studies have proposed an ancestral habit (woody/herbaceous). This chapter provide anatomical data of all lineages within Piperales, and reconstruct ancestral character states, focusing on the origin of woodiness within the order and on the ecological significance of key anatomical features.

Stem anatomical observations, with special emphasis on wood anatomical features, were performed on 28 species of the Piperales, including New Caledonian *Piper* species and Piperales representatives from other regions around the world. By combining previously published studies with original data, we conducted phylogenetic reconstructions of cambial activity and vessel element perforation plates to assess the origin of woodiness and vessel evolution in Piperales.

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

The common ancestor of the order likely had an active cambium and woody habit, including vessel elements with simple perforation plates. All Piperales woody species share several wood features including wide and tall rays, suggesting a single origin of wood in the order. The high diversity of growth forms observed in Piperales are linked to frequent shifts in cambial activity and changes in habit-related features within the different lineages. In the local context of New Caledonia, the exceptional climbing and herbaceous forms of Piperaceae species, which are absent in the rest of the New Caledonian basal angiosperms, may underlie the high niche marginality of this family. This suggests that growth form diversity is a promoter of ecological diversification.

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

3.1 Introduction

Variations in the degree of cambial activity and the subsequent production of secondary tissues are key elements behind the diversity of forms that we observe in extant angiosperms (Rowe and Speck 2005; Carlquist 2009; Spicer and Groover 2010). Flowering plants present a broad array of growth forms ranging from massive trees with a high production of wood, woody herbs with a limited secondary growth production—frequently localized at the base of the stems—herbs with a very reduced cambial activity limited to the fascicular areas and genuine herbs which present a complete loss of the cambial activity, with stems that are exclusively formed by primary tissues (Spicer and Groover 2010; Rowe and Paul-Victor 2012; Lens *et al*. 2012*a*). This diversity in the degrees of woodiness has arguably enabled flowering plants to explore new and diverse ecological strategies (Rowe and Speck 2005; Rowe and Paul-Victor 2012; Carlquist 2013).

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

Current phylogenetic hypotheses strongly support the grade containing Amborellales, Nymphaeales and Austrobaileyales as the first successive lineages of angiosperms (Zanis *et al.* 2002; Qiu et al. 2005; Soltis et al. 2008; Moore et al. 2010; Amborella Genome Project 2013), followed by the Chloranthaceae and the magnoliids (Laurales, Magnoliales, Canellales and Piperales). This topology is suggested to support the idea that early diverging lineages of angiosperms possessed an active bifacial cambium (Carlquist and Schneider 2001; Feild and

Arens 2005; Spicer and Groover 2010). Because of its phylogenetic position and its diversity of growth forms, Piperales has been considered as a key lineage for understanding the early diversification of angiosperms (Carlquist 2009; Isnard *et al*. 2012).

Piperales is a pantropical species-rich clade with approximately 4300 extant species; it includes a wide spectrum of growth habits including herbs, shrubs, treelets and climbers (Fig. 3.1), living in both terrestrial and semi-aquatic environments. In the archipelago of New Caledonia, Piperales are represented by 15 species (Table 1.1) belonging to the genera *Peperomia* (herbs), and *Piper* (woody lianas; Fig. 3.1B). A broad survey of growth forms, architecture, anatomy, and biomechanics within Piperales has recently demonstrated that the frequent shift in growth forms is probably a major source of diversity within the group (Isnard *et al*. 2012). The patterns of growth form evolution within Piperales potentially reflect some overall changes within angiosperms, especially related to transitions in woodiness, diversity of mechanical organizations and shifts in architectural development.

Among the several works that have treated the anatomy of Piperales, Sherwin Carlquist's studies are key references, for describing the anatomy of Aristolochiaceae (Carlquist 1993), *Lactoris* (Carlquist 1990) and Saururaceae (Carlquist *et al*. 1995; Schneider and Carlquist 2001). Substantial amounts of information on Piperaceae anatomy can be found in the literature since the 19th century (Schmitz 1871; Debray 1885; Hoffstadt 1916; Yuncker and Gray 1934; Murty 1959; Ravindran and Remashree 1998; Souza *et al*. 2004). Datta and Dasgupta (1977) published the only study attempting to outline the anatomy of the entire Piperales order, but this work included only three genera out of 16 and merely considered the general distribution of tissues. More recently, the stem anatomy of the perianth-bearing Piperales has been investigated with a special focus on *Aristolochia* (Wagner *et al*. 2012; 2014). In addition, Isnard et al. (2012) briefly explored the stem anatomy of Piperales, but did not describe the wood anatomy and the differences in cambial activity within the order.

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

FIGURE 3.1 Illustration of the diversity of growth forms in Piperales. (A) *Piper hispidinervum* (Piperaceae), treelet with significant wood production, growing in the living collection of the Xishuangbanna Tropical Botanical Garden, China. (B) *Piper insectifugum* (Piperaceae), overview of this woody liana, growing in the humid forest of Mt. Aoupinié, New Caledonia. (C) *Saruma henryi* (Asaraceae), rhizomatous woody herb producing a limited amount of wood restricted to the base of the stem, growing in the greenhouse of the Botanical Garden of Dresden, Germany. (D) *Thottea iddukiana* (Aristolochiaceae), subshrub presenting several stems with a slight production of wood, growing in Kerala, India. (E) *Peperomia blanda* (Piperaceae), a strictly herbaceous and small-sized plant, growing on a rocky hillside in Yunnan, China.

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

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

3.2 Material and Methods

3.2.1 Plant material

Piperales comprise two well-supported clades that can be named perianth-less and perianth-bearing (Naumann *et al*. 2013). The first clade consists of Piperaceae (*Piper*, *Peperomia*, *Manekia*, *Zippelia* and *Verhuellia*) and Saururaceae (*Anemopsis*, *Gymnotheca*, *Houttuynia* and *Saururus*). The second clade includes Aristolochiaceae (*Aristolochia* and *Thottea*), Asaraceae (*Asarum* and *Saruma*), Lactoridaceae (*Lactoris*) and Hydnoraceae (*Hydnora* and *Prosopanche*). We adopted the most recent molecular phylogenetic hypothesis where all Piperales families are statistically supported as monophyletic (Naumann *et al*. 2013). Stem samples of 28 species belonging to 11 genera were collected during field work in China, Colombia, India, Mexico and New Caledonia, and from the living collections of the Botanical Gardens of Bonn and Dresden in Germany and Kerala in India (see Appendix A1 for the species list and collection sites). 2 to 5 samples per species were collected on basal portions of the stems corresponding to the main axis of mature plants. This allowed us to ensure that the analysis was done on fully developed stems since wood development can be restricted to the most basal parts of stems. Plant maturity was assessed through the observation of sexual maturity (flowering and fruiting) or through architectural features as the presence of reiterations and the full development of branches on large and unpruned individuals. After collection, all samples were preserved in 70% ethanol.

3.2.2 Anatomical descriptions and microtecnique

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

Wood macerations were used for observations of vessel element morphologies. Pieces of the outer and most recently produced layers of wood were dissected using a double-edged razor blade. Strips of wood were then chemically macerated by immersion in capped 2 ml vials containing a maceration solution (1 : 1 by volume, 35% H_2O_2 : glacial acetic acid) and placed in an oven at 60 °C for 72 h until the wood became translucent. Wood macerates were then stained by immersion in 0.1% aqueous Toluidine Blue O for 10 minutes; wood macerates were then shaken in order to loosen vessel elements, filtered and immersed in distilled water. After settling, a drop of the colored wood macerates was pipetted onto a slide and digital images of macerated xylem vessel elements were carried out immediately after mounting. Anatomical observations were carried using a binocular stereo microscope (Olympus SZX9. Japan) and optical light microscope (Olympus BX51. Japan). The 21 wood anatomical characters used in the study follow the IAWA terminology for microscopic features (Wheeler *et al*. 1989). Twelve additional characters of stem anatomy were used (see Table 3.1 for the complete list of characters). IAWA wood features could not be attributable for species producing a limited amount of wood, vessel element descriptions were based on the metaxylem tracheary elements for these species.

3.2.3 Literature review

Our sampling includes representatives of all Piperales genera, with the exception of *Lactoris*, *Anemopsis*, *Asarum* and the Hydnoraceae family, for which anatomical data were compiled from literature (Appendix A2). We also completed our anatomical observations of species-rich genera though an extensive literature survey; this allowed us to cover a wide proportion of infrageneric diversity and to increase the number of species for large genera (see Appendix A3 for a complete list of the proportion of species and infrageneric clades). A high representativeness was obtained for poorly diversified genera. Species representation clearly falls to low percentages in very large genera such as *Aristolochia*, *Peperomia* and *Piper* for which it would be nearly impossible to sample all species. Our work, however, includes representatives from most of the major infrageneric clades (Appendix A3).

3.2.4 Data processing and character mapping

We adopted the topology of Piperales from a recent molecular phylogenetic analysis where the internal nodes of the order are well supported (Naumann *et al*. 2013). The topology at the generic level was obtained from previous phylogenetic studies where the relationships were statistically supported (Wanke *et al*. 2007*a;* 2007*b*). We scored character states at the generic level and ancestral character state reconstructions were carried out using maximum parsimony (MP) and maximum likelihood (ML) methods using the StochChar module (Maddison and Maddison 2006) of Mesquite 2.75 (Maddison and Maddison 2011). ML reconstructions estimate the uncertainty of ancestral state reconstructions and help to quantify the inferred ambiguities (Pagel 1999). We employed ML reconstructions using a Markov *k*-state 1-parameter model of evolution, assuming one step per change with the cost of gains equal to the cost of losses of a given state for each character, this is designed to estimate transition rates of discrete characters over a phylogeny (Pagel 1994; Jaramillo *et al*. 2004). The likelihood of each character state is shown in the pie chart at each single node of the tree. ML reconstruction methods do not however allow polymorphic states; MP was consequently used to code polymorphisms when two potential character states were known to occur in terminal taxa, we employed an unordered parsimony model with equal gain/loss costs assuming one step per change. We chose *Canella winterana*, from the Piperales sister order Canellales (Qiu *et al*. 2005; APG-III 2009; Massoni *et al*. 2014) as outgroup. The anatomical description of the outgroup was obtained from Feild *et al*. (2002) and from the InsideWood data base (InsideWood 2004-onwards).

3.2.5 Cambial activity characterization and character states description

Distinguishing the limits between herbaceous and woody species is especially difficult when dealing with intermediate expressions of cambial activity leading to different degrees of wood production as occurring in Piperales (Fig. 3.1) (Rowe and Paul-Victor 2012; Lens *et al*. 2012*a;* Lens *et al*. 2013). We used observations of cambial activity location and assessments of wood productivity in order to classify the degrees of woodiness in Piperales. We established 5 possible states of cambial activity which correspond to herbaceous and woody conditions following the next criteria: 1) "Absent" = No cambial activity. 2) "Restricted to fascicular areas" = Cambial activity exclusively located at the intrafascicular areas. 3) "Woody herb" = Cambial activity in both intra- and interfascicular areas, producing a complete wood cylinder of determinate thickness (less than 20 cell layers) and restricted to the base of the main stem. 4) "Slightly woody" = Cambial activity in both intra- and interfascicular areas, producing a complete wood cylinder of determinate thickness (less than 20 cell layers) extended along the main stem. 5) "Truly woody" = Cambial activity in both intra- and interfascicular areas, forming a complete wood cylinder with a significant and indeterminate production of wood (more than 20 cell layers).

The threshold of 20 cell layers of secondary tissues produced by cambium was established after observing that small-sized species of Piperales corresponding to poorly lignified lianas and subshrub forms had a limited and determinate wood production that never exceed *ca.* 20 cell layers. On the other hand, all of the observed species exceeding 20 cell layers corresponded to small trees, shrubs or lianas with a high and indeterminate production of wood. During literature survey, if the location and productivity of cambial activity were not explicitly reported, we used the descriptions of growth habit to code character states. For instance, *Anemopsis* and *Asarum* were coded as "woody herbs" according to our literature review (Appendix A2). Secondary xylem in *Asarum canadense* and *A. cardiophyllum* have been declared as forming a wood cylinder restricted to the base of the aerial stem (Wagner *et al*. 2014) thus corresponding to the woody herb state. Carlquist *et al.* (1995) reported that secondary growth is obvious in *Anemopsis californica* in both fascicular and interfascicular areas, as secondary growth of this species is exclusively located to the rhizomes it can be coded as a woody herb.

MP character optimization in cambial activity reconstruction allowed us to code two possible states for a given genus. This alternative coding concerned *Aristolochia*, *Thottea*, *Peperomia* and *Piper*, where various degrees of cambial activity might occur. Coding of

Aristolochia was supported by a recent evolutionary analysis on the growth forms of the genus (Wagner et al. 2014), proposing a climbing or a shrub ancestral habit for *Aristolochia* and rejecting the possibility of an herbaceous ancestry. In terms of cambial activity these growth forms correspond to “truly woody” (shrub and liana) or “slightly woody” (vine) states. *Piper* and *Thottea* were alternatively coded as "truly woody" or "slightly woody" corresponding to our own observations (Table 3.1) and literature survey (Appendix A2). Despite of a low specific representativeness, our work include species of all major clades and growth forms within these genera (Appendix A3), providing a good level of confidence. Cambial activity in *Peperomia* was coded as "absent" or "restricted to fascicular areas" as our literature survey and our anatomical observations suggested both possibilities in this large genus, for which anatomy is known to be homogeneous. Finally, we performed ML ancestral character state reconstruction on vessel element perforation plates, a feature of major interest in the understanding of the evolution of wood structure. Perforation plates were coded with two possible states (“simple” or “scalariform”), *Verhuellia*'s perforation plates were coded as "inapplicable" because this species lacks metaxylem vessel elements.

3.3 Results

3.3.1 Tissue distribution and cambial activity

Saururaceae. All species present a cambial activity restricted to the fascicular areas and producing only a few cell layers. Vascular bundles are organized in a single ring. Tangential alignments of cells are exclusively restricted to the vascular bundles and the fascicular cambium produces a negligible amount of secondary tissues (Figs. 3.2A,B). Interfascicular cambium is never active (no radial or tangential divisions were observed) (Figs. 3.2A,B). Aerenchyma is present in both, the medullar and cortical areas of *Saururus chinensis* (Fig. 3.2A, stars), while in *Gymnotheca chinensis* it was exclusively observed in the cortical area (Fig. 3.2B, star), aerenchyma is absent in *Houttuynia cordata*. In *G. chinensis* and *H. cordata* a complete ring of sclerenchyma fibers is present outside the vascular bundles with a width of 1-3 cell layers (Fig. 3.2B). A few fibers are present at the adaxial and abaxial surfaces of the bundles in *S. chinensis*, but not forming a complete ring (Fig. 3.2A).

TABLE 3.1. *Stem anatomical characters of Piperales*

	ARISTOLOCHIACEAE											ASARACEAE	PIPERACEAE													SAURURACEAE		
	Aristolochia arborea	*Aristolochia impudica*	*Thottea abrahamii*	*Thottea barberi*	*Thottea dinghoui*	*Thottea duchartrei*	*Thottea iddukiana*	*Thottea ponmudiana*	*Thottea siliquosa*	*Thottea sivarajanii*	*Thottea tomentosa*	*Saruma henryi*	*Manekia sydowii*	*Peperomia blanda*	*Peperomia incana*	*Piper componii*	*Piper flaviflorum*	*Piper gorgonillense*	*Piper hispidinervum*	*Piper insectifugum*	*Piper nudibracteatum*	*Piper sarmentosum*	*Piper sp.*	*Verhuellia lunaria*	*Zippelia begoniifolia*	*Gymnotheca chinensis*	*Houttuynia cordata*	*Saururus chinensis*
GENERAL ANATOMY																												
Number of cycles of vascular bundles	1	1	1	1	1	1	1	1	1	1	1	1	> 2	> 2	> 2	2	2	2	> 2	2	> 2	2	2	-	2	1	1	1
Medullary bundles	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
1 exclusive medullary bundle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Presence of mucilage canals	-	-	-	-	-	-	-	-	-	-	-	-	P, C	-	P	P, C	P, C	-	-	P, C	-	-	-	-	-	-	-	-
Active phellogene, bark production	+	+	-	+	-	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Stem endodermis with a Casparian band	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+
Sclerenchyma ring geometry	C	C	C	C	C	C	C	C	C	C	U	D	C	D	D	U	U	U	U	U	U	U	U	-	U	C	C	D
Width of the sclerenchyma ring (mean no. of cells)	4	4	5	2	3	2	2	4	3	3	3	NA	4	NA	NA	7	3	4	5	8	4	6	4	NA	4	4	3	NA
Aerenchyma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
Calcium oxalate	D	D	-	-	-	-	-	-	-	-	-	-	D,P,R	-	D,R	-	-	R	-	-	P,R	-	R	-	R	R	D	-
Cambial activity	5	5	4	5	4	5	4	5	5	5	4	3	5	1	2	5	5	5	5	5	5	4	5	1	2	2	2	2
Cell layers produced by cambium	>20	>20	<20	>20	<20	>20	<20	>20	>20	>20	<20	<20	>20	-	R	>20	>20	>20	>20	>20	>20	<20	>20	-	<20	R	R	R
WOOD ANATOMY																												
Growth rings boundaries distinct (1)	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Vessels in diagonal and / or radial pattern (7)	+	+	+	+	+	+	+	+	+	+	+	+	+	NA	NA	+	+	+	+	+	+	+	+	NA	NA	NA	NA	NA
Vessels exclusively solitary (90% or more) (9)	+	+	-	+	-	+	+	-	-	-	-	+	-	NA	NA	-	-	-	+	-	+	+	+	NA	NA	NA	NA	NA
Vessels in radial multiples (10)	-	-	-	-	-	-	-	+	+	-	-	-	-	NA	NA	+	+	-	-	-	-	-	-	NA	NA	NA	NA	NA
Vessel clusters common (11)	-	-	+	-	+	-	+	+	+	+	+	-	+	NA	NA	+	+	+	-	+	-	-	-	NA	NA	NA	NA	NA
Simple perforation plates (13)	+	+	+	+	+	+	+	+	+	+	+	+	+	NA	NA	+	+	+	+	+	+	+	+	NA	+	-	-	-
Scalariform perforation plates (14)	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-	-	-	-	-	-	-	NA	-	+	+	+
Intervessel pits scalariform (20)	-	-	-	-	-	-	-	-	-	-	-	+	+	NA	NA	+	-	+	-	+	+	+	+	NA	+	+	+	+
Intervessel pits opposite (21)	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-	-	-	-	-	-	-	NA	-	-	+	+
Intervessel pits alternate (22)	+	+	+	+	+	+	+	+	+	+	+	+	-	NA	NA	-	+	-	+	-	-	-	-	NA	-	-	-	-
Axial parenchyma absent or extremely rare (75)	-	-	NA	-	NA	-	-	-	-	-	NA	+	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA	NA
Axial parenchyma diffuse (76)	+	-	NA	+	NA	-	+	-	-	-	NA	-	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA	NA
Axial parenchyma diffuse-in-aggregates (77)	+	+	NA	+	NA	+	+	+	+	+	NA	-	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA	NA
Axial parenchyma scanty paratracheal (78)	+	+	NA	+	NA	+	+	+	+	+	NA	-	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA	NA
Axial parenchyma vasicentric (79)	-	-	NA	-	NA	-	-	-	-	-	NA	-	+	NA	NA	+	+	+	+	+	+	+	+	NA	NA	NA	NA	NA
Axial parenchyma in narrow bands (86)	+	+	NA	+	NA	+	+	+	+	+	NA	-	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA	NA
Ray width – commonly 4- to 10- seriate (98)	-	+	-	+	-	-	-	-	-	-	-	-	-	NA	NA	-	-	-	-	-	-	-	+	NA	NA	NA	NA	NA
Ray width – commonly > 10-seriate (99)	-	-	+	-	+	+	+	+	+	+	+	-	+	NA	NA	+	+	+	+	+	+	+	-	NA	NA	NA	NA	NA
Wood rayless (117)	-	-	-	-	-	-	-	-	-	-	-	+	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA	NA
All ray cells upright and / or square (105)	-	-	-	-	+	+	+	+	+	+	+	-	+	NA	NA	+	+	+	+	+	+	+	+	NA	NA	NA	NA	NA
Ray lignification	E	E	E	E	E	E	E	E	E	E	E	-	-	NA	NA	-	I	E	E	I	E	E	I	NA	NA	NA	NA	NA

←**TABLE 3.1** *Notes*. Analyzed characters for the general anatomy (primary body) and wood anatomy of Piperales, indicating the presence (+) or absence (-) of each character for each species. NA = not applicable. **Presence of canals**: *P*, Peripheral canals, several canals occurring at the perimeter of the stem as seen in transverse section. *C*, Central canal, a single canal occurring in the center of the stem. **Sclerenchyma ring geometry**: *C*, Circular continuous band of sclerenchyma fibers seen in transverse section. *D*, Discontinuous bands of sclerenchyma, fibers occurring as islands or caps contiguous to vascular bundles. *U*, Undulating and continuous band of sclerenchyma. **Calcium oxalate**: *D*, Druses, *P*, Prismatic crystals. *R*, Raphides. **Cambial activity**: 1, Absent. 2, Restricted to fascicular areas. 3, Woody herb. 4, Slightly woody. 5, Truly woody. **Cell layers produced by cambium**: *>20*, more than 20 cell layers. *<20*, less than 20 cell layers. *R*, few cell layers restricted to fascicular areas. **Ray lignification**: *E*, Complete lignification over the entire width of the stem. *I*, Incomplete or partial ray lignification. Numbers between brackets correspond to the IAWA wood feature numbers.

Aristolochiaceae. In *Aristolochia* and *Thottea*, many species can develop a high amount of secondary xylem forming large wood cylinders (Figs. 3.2C,D). Despite active secondary growth, the fascicular cambium rarely produces rays and the interfascicular cambium never produces fusiform initials. Vessel elements and fibers are consequently absent in the interfascicular area and we observe exclusively wide multiseriate rays composed of secondary parenchyma cells (Figs. 3.2C,D). Ray cells are lignified in all *Thottea* species studied (Fig. 3.2C; Table 3.1), ray lignification was variable between *Aristolochia* species (Table 3.1). Growth rings were observed in *T. duchartrei* and *T. sivarajanii* (Table 3.1). A continuous ring of pericyclic fibers was observed in *Aristolochia* and *Thottea* (Figs. 3.2C,D). This ring of fibers undergoes fragmentation and subsequent repair via parenchyma intrusion and lignification during secondary growth.

Asaraceae. *Saruma henryi* shows little and determinate production of wood regarding the rest of the woody representatives in the perianth-bearing Piperales. Interfascicular cambium does not produce radial initials (Fig. 3.2E), consequently presenting a rayless wood. Raylessness in *S. henryi* is a unique feature regarding the remaining woody species of the perianth-bearing Piperales. *S. henryi* does not present the continuous ring of fibers commonly observed in the sister group Aristolochiaceae, mechanical support is then provided by sclerenchyma bundle caps and an external ring of 3-5 cell layers of collenchyma (Fig. 3.2E).

Piperaceae. All Piperaceae, except *Verhuellia lunaria*, presented a polycyclic arrangement of vascular bundles, which is a characteristic of the family. Vascular bundles are organized in two or more concentric rings in *Piper*, *Manekia* and *Zippelia* (Figs. 3.2G-I; Table 3.1). In species undergoing secondary growth, secondary thickening is restricted to peripheral bundles (Fig. 3.2H).

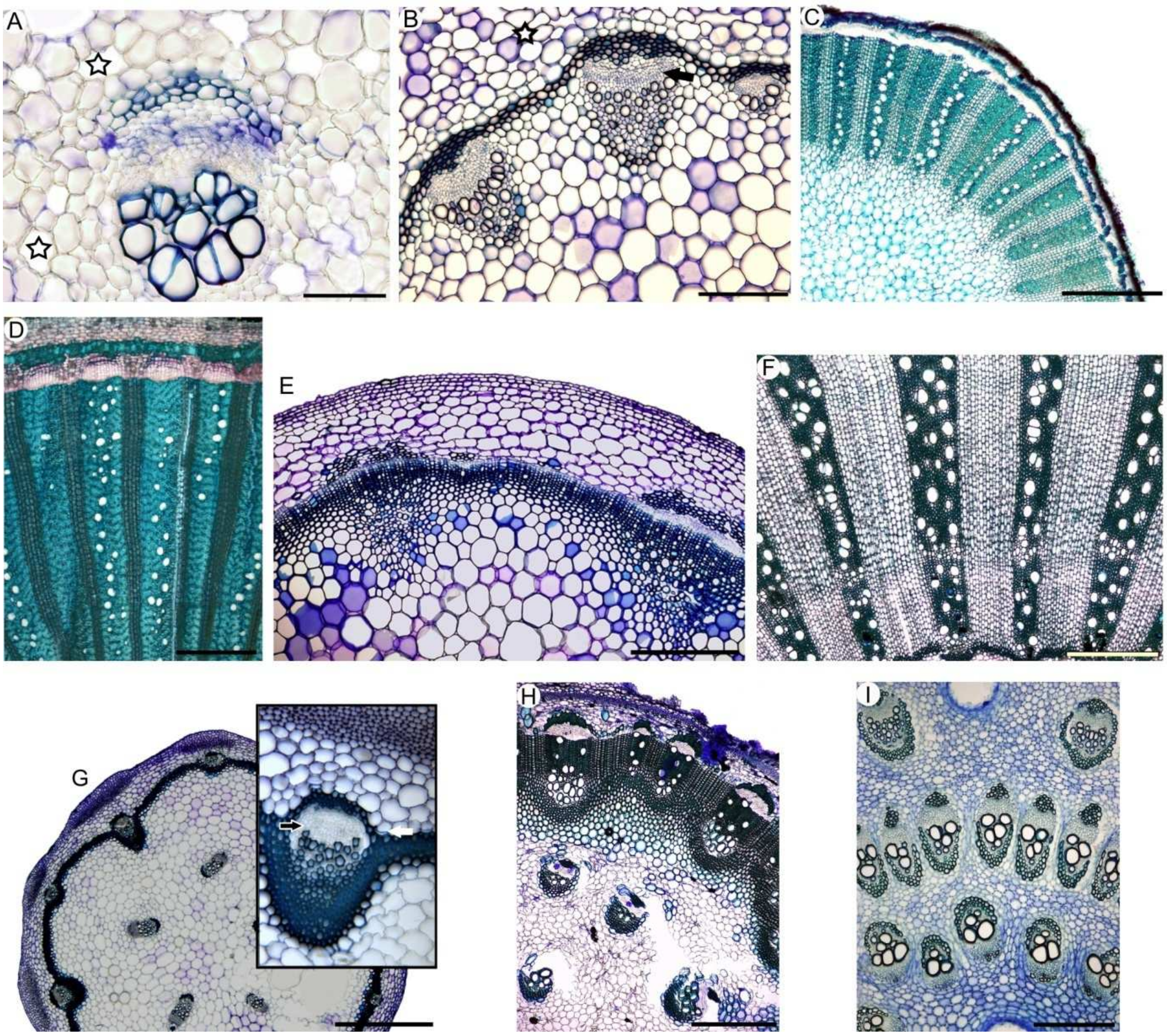


FIGURE 3.2 Variation in cambial activities and tissues distribution in Piperales species observed in transverse sections. (A) *Saururus chinensis*, vascular bundle with intrafascicular cambium producing a limited number of cells, absence of interfascicular cambial activity, note the presence of aerenchyma (indicated by stars), scale bar: 100 µm. (B) *Gymnotheca chinensis*, vascular bundles surrounded by a continuous band of fibers; limited cambial activity is restricted to intrafascicular areas (arrow), absence of interfascicular cambial activity, cortical aerenchyma is indicated by a star, scale bar: 200 µm. (C) *Thottea barberi*, full cambial activity in both inter- and intrafascicular areas, interfascicular cambium produces exclusively ray-like secondary parenchyma and vessels are arranged in a radial pattern, scale bar: 500 µm. (D) *Aristolochia impudica*, vessels are predominantly solitary and arranged in a radial pattern, wide secondary rays, a ring of fibers surrounding the vascular system is still present, scale bar: 500 µm. (E) *Saruma henryi*, vessels are arranged in a radial pattern, vessels are not much wider than the surrounding fibers, scale bar: 200 µm. (F) *Piper gorgonillense*, wide secondary rays produced by the interfascicular cambium, vessels solitary or in clusters and presence of a growth ring, scale bar: 500 µm. (G) *Zippelia begoniifolia*, presents two cycles of vascular bundles, only the peripheral bundles present a slight cambial activity (inset, black arrow), note the presence of a Casparian band (inset, white arrow), scale bar: 1000 µm (100 µm for the inset). (H) *Piper nudibracteatum*, secondary production is limited to the peripheral vascular bundles while the medullary bundles remain inactive, notice the wide secondary rays formed by parenchyma cells, scale bar: 500 µm. (I) *Manekia sydowii*, three rings of vascular bundles are visible, the intern-most bundles present a procambial inversion with primary xylem developing externally, parts of the central and peripheral mucilage canals are visible at the bottom and the top, scale bar: 500 µm.

In *Peperomia*, vascular bundles are surrounded by ground parenchyma and are scattered throughout the stem section without an obvious concentric distribution. Similar to Aristolochiaceae and Asaraceae, the interfascicular cambium of woody Piperaceae produces exclusively wide secondary rays composed of secondary parenchyma (Figs. 3.2F,H).

Rays are entirely lignified in mature wood, except in climbing species where ray lignification is partial or absent. The climber *Manekia sydowii* produces a high amount of wood and secondary rays remain completely unlignified (Table 3.1). We recorded a procambial inversion of the most internal medullary bundles of *M. sydowii*, where primary xylem developed centrifugally while primary phloem developed centripetally (Fig. 3.2I). *Peperomia blanda* and *P. incana* show only a slight cambial activity, where secondary production is very limited and is absent in most of the observed vascular bundles (not shown). Cambial activity is also very limited in *Zippelia begoniifolia*, and the fascicular cambium produces only a tiny amount of secondary tissues (Fig. 3.2G inset, black arrow). Cell alignments were observed in some sections between the bundles of *Zippelia*, but the interfascicular cambium can be considered inactive as it only presents a few divisions of cells and the observed alignments seem to be the product of tangential procambial divisions, therefore no wood cylinder is present. Cambial activity is completely absent in *Verhuellia lunaria*, and the stem comprises only one exclusively medullary bundle, which is embedded in ground parenchyma (Fig. 3.6B). This is composed of 5-9 protoxylem elements and a small amount of primary phloem. A complete ring of sclerenchyma fibers was observed in *Manekia*, *Piper* and *Zippelia*. The ring of fibers is internal to the peripheral bundles and presents an undulating pattern (Figs. 3.2G,H; 3.5A). Fibers are present only as externally-oriented caps on the vascular bundles of *Peperomia* and they are completely absent in *Verhuellia* (Table 3.1).

3.3.2 Wood features of Piperales

Rays. All of the woody species of Piperales present very wide and tall multiseriate rays (Figs. 3.2C,D,F,H; 3.3A; Table 3.1). Most of the species present rays of more than 10 cells wide (Fig. 3.3A), only *Thottea barberi* and *Piper sp.* present rays with a width less than 10 cells but which are nevertheless considered as multiseriate. As mentioned above, *Saruma henryi* is the only observed species with rayless wood (Fig. 3.2E). We observed a few short lignified bi-seriate rays in some sections of *Saruma henryi*, however, they become indistinguishable from the rest of the lignified tissues by the late stages of development. Ray composition is the same for all species with predominantly upright cells and a few square cells (Figs. 3.3B-D). For most of the examined species, ray cells become lignified; ray-lignification is absent or partial in most climbing species.

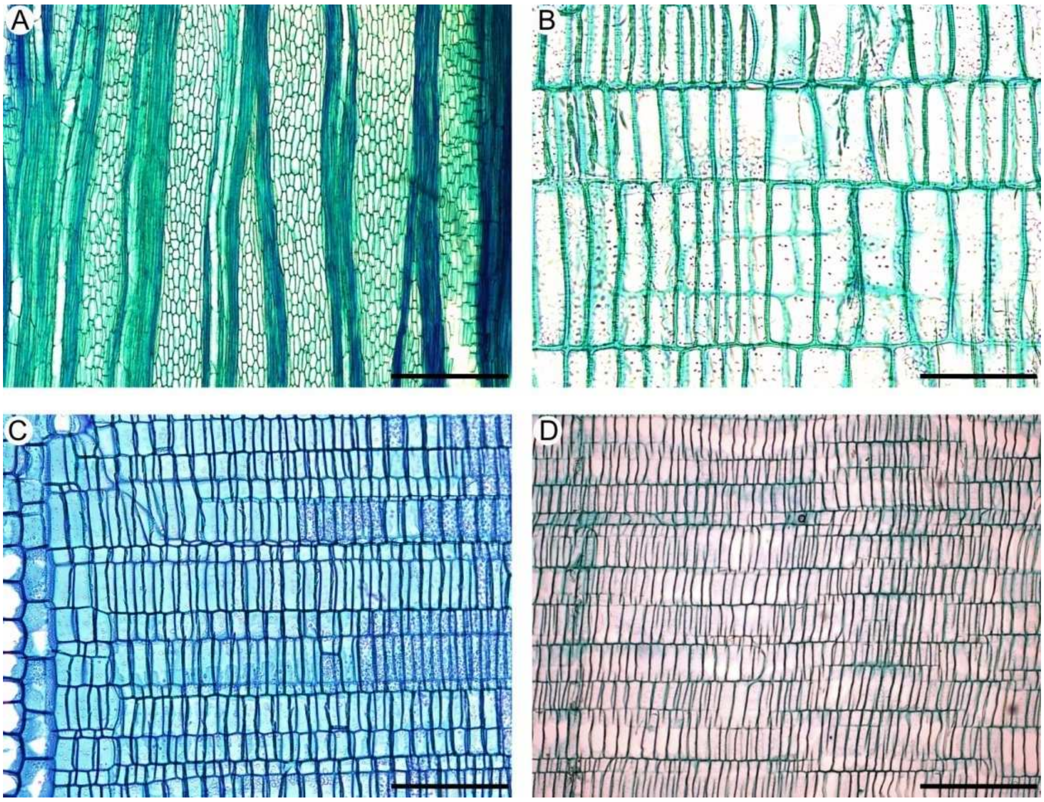


FIGURE 3.3 Ray features of Piperales. (A) *Thottea iddukiana*, tangential plane, rays are 10 to 15 seriate, scale bar: 500 µm. (B) *Thottea sivarajanii*, radial plane, upright cells of ray tissue with lignified walls, scale bar: 100 µm. (C) *Thottea siliquosa*, radial plane, rays are composed of mixed upright and square cells, scale bar: 200 µm. (D) *Piper gorgonillense*, radial plane, upright and square cells of ray tissue, scale bar: 300 µm.

Vessel elements. Perforation plates and lateral wall pitting: All species within Aristolochiaceae, Asaraceae and Piperaceae present simple perforation plates (Figs. 3.4A-J; Table 3.1). The metaxylem tracheary elements of Saururaceae presented scalariform perforation plates (Figs. 3.4K-M). Vessels of *Aristolochia* and *Thottea* generally present alternate pitting and only some species of *Thottea* bear vessel elements with opposite to alternate pits (Figs. 3.4A-D; Table 3.1). We observed mostly alternate pitting in *Saruma henryi* but a few scalariform pits were also observed in some vessel elements (Fig. 3.4E; Table 3.1). Most of Piperaceae species present scalariform pitting (Figs. 3.4F-J). Finally, Saururaceae metaxylem vessel elements also present scalariform pitting (Figs. 3.4K-M) and only some vessels in *Houttuynia cordata* and *Saururus chinensis* bear opposite pits.

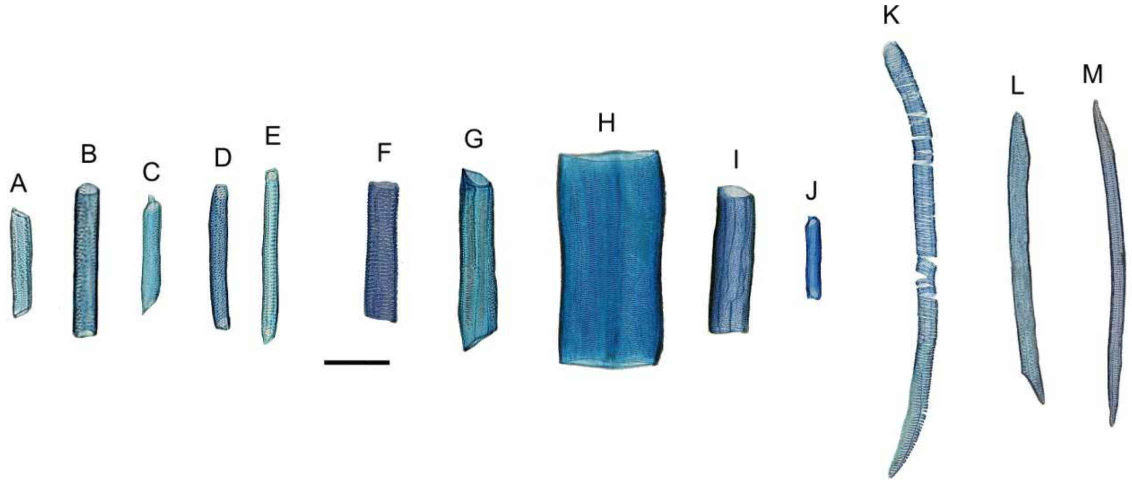


FIGURE 3.4. Morphological diversity of xylem vessel elements of Piperales representatives. Simple perforation plates in Aristolochiaceae (A-D), Asaraceae (E) and Piperaceae (F-J), scalariform perforation plates in Saururaceae (K-M). A, *Thottea dinghoui*. B, *Thottea ponmudiana*. C, *Thottea duchartrei*. D, *Thottea barberi*. E, *Saruma henryi*. F, *Manekia sydowii*. G, *Piper nudibracteatum*. H, *Piper insectifugum*. I, *Piper hispidinervum*. J, *Piper sarmentosum*. K, *Saururus chinensis*. L, *Houttuynia cordata*. M, *Gymnotheca chinensis*. Scale bar: 200 µm.

Vessel arrangement and grouping: All the woody Piperales species present a radial distribution of vessels, with linear or diagonal arrangements (Figs. 3.2C-F; Table 3.1). Solitary vessels were predominantly observed in *Aristolochia*, *Thottea* and *Piper* (Figs. 3.2D; 3.5B,C; Table 3.1), only *Thottea ponmudiana* and *T. siliquosa* exhibit radially distributed clusters of >4 vessels (Fig. 3.5A), vessel clusters were frequently observed in both *Thottea* and the Piperaceae representatives (Table 3.1). Vessel grouping was very ambiguous as in some species we observed grouped and solitary vessels with an almost equal ratio; *Piper gorgonillense* for example, presents both solitary and grouped vessels (Fig. 3.2F).

Axial parenchyma. Axial parenchyma is one of the wood characters that differ between woody species of Aristolochiaceae and Piperaceae. In *Aristolochia* and *Thottea*, apotracheal axial parenchyma is sometimes diffuse to diffuse-in-aggregates and a predominance of banded parenchyma forming narrow tangential bands was observed in both genera (Figs. 3.5A,B, black arrows). Conversely *Piper* and *Manekia* have mostly paratracheal vasicentric axial parenchyma (Fig. 3.5C, arrows). Absence of axial parenchyma was observed in *Saruma*.

3.3.3 Degrees of woodiness in Piperales

Piperales species exhibit several degrees of cambial activity, from species with significant wood production to species completely lacking secondary growth (Fig. 3.2). These shifts in cambial activity arise repeatedly within the different lineages of Piperales. Many

Piper and *Manekia* species along with *Lactoris fernandeziana*, produce a large wood cylinder along the main axis (Fig. 3.2F; Table 3.1; Appendix A2). Similarly, most of the species within *Aristolochia* and *Thottea* are truly woody plants with significant secondary growth (Figs. 3.2C,D; Table 3.1; Appendix A2).

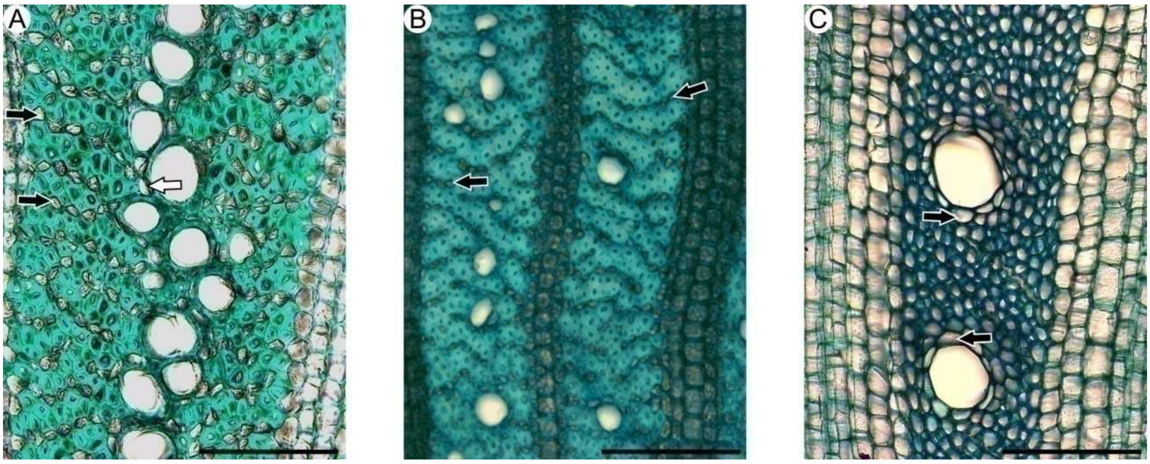
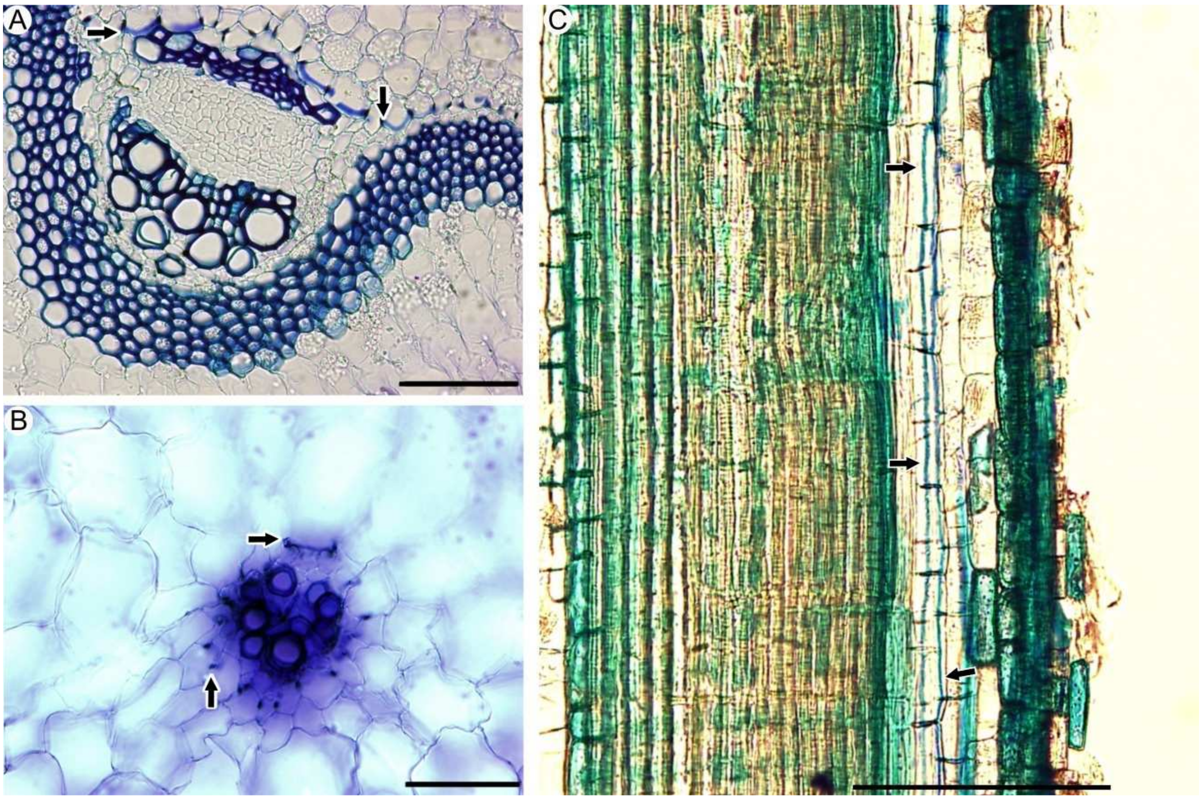


FIGURE 3.5 Axial parenchyma in Piperales woody species observed in transverse sections. (A) *Thottea siliquosa*, apotracheal axial parenchyma in tangential narrow bands (black arrows), occasional parenchyma cells are associated with the vessels (white arrow), scale bar: 100 µm. (B) *Aristolochia impudica*, apotracheal axial parenchyma in tangential narrow bands (black arrows), scale bar: 200 µm. (C) *Piper hispidinervum*, paratracheal vasicentric axial parenchyma forming a narrow sheath around the vessels (arrows), scale bar: 200 µm.

Some shifts towards a decrease of woodiness are observed within these groups, some *Thottea* representatives produce narrow wood cylinders and are considered as slightly woody shrubs (Table 3.1). *Aristolochia serpentaria* present a few cell divisions in the interfascicular areas and its wood cylinder is restricted to the base corresponding to a woody herb typology (Appendix A2). The monospecific *Saruma* and its related genus *Asarum* (Asaraceae), both present cambial activity in the interfascicular and fascicular areas, but they produce a narrow wood cylinder (Fig. 3.2E) restricted to the rhizome or to the base of its stems and therefore can be also considered as woody herbs (Table 3.1, Appendix A2). In extreme cases of reduction of cambial functioning, species such as *Verhuellia* and some *Peperomia* species completely lack cambial activity (Table 3.1, Appendix A2). *Peperomia* and *Zippelia* (Piperaceae) never form a wood cylinder; when secondary growth is present, it is restricted to a few tangential divisions in the fascicular areas. In Saururaceae, only a few tangential divisions may occur in the intrafascicular areas in *Gymnotheca*, *Houttuynia* and *Saururus* (Fig. 3.2B) while *Anemopsis* present secondary growth in both intra- and interfascicular areas forming a narrow wood cylinder at the base of the stem (Appendix A2). Finally, the holoparasitic genera *Hydnora* and *Prosopanche* (Hydnoraceae) also exhibit a reduction of cambial activity with a restriction to the fascicular areas (Appendix A2).

3.3.4 Presence of an endodermis with Casparian bands in aerial stems of Piperales

Stem endodermis with a Casparian band was observed in all genera within Piperaceae as well as in *Houttuynia cordata* and *Saururus chinensis* (Saururaceae). The endodermis was present as a layer of cells of procambial origin, generally located at the limits of the vascular system and the cortical area. The endodermis shows a typical Casparian band, staining with a dark blue indicating the presence of suberin and/or lignin, observed in the radial and tangential walls of the endodermic cells (Fig. 3.6). Stem endodermis was external to the sclerenchyma cap of the peripheral bundles and observed around the entire circumference of the stem in *Manekia sydowii*, *Piper flaviflorum*, *P. sarmentosum*, *P. sp*. and *Zippelia begoniifolia* (Fig. 3.2G inset, white arrow; Figs. 3.6A,C, arrows). Stem endodermis was present surrounding some vascular bundles in *Peperomia* species and around the single bundle of *Verhuellia lunaria* (Fig. 3.6B). In *Houttuynia cordata*, a ring of rectangular-shaped cells contiguous to the peripheral ring of sclerenchyma was observed; some of these cells presented a marked Casparian band. In *Saururus chinensis* a Casparian band was observed as a continual layer external to the vascular bundles. This character was not observed in *Gymnotheca chinensis*.



←**FIGURE 3.6** Stem endodermis with a Casparian band in Piperaceae. (A) *Piper sarmentosum*, transverse view of a young stem, note the endodermis with a Casparian band colored in dark blue and visible as a single layer of cells external to the vascular bundles ring (arrows), scale bar: 100 µm. (B) *Verhuellia lunaria*, transverse section showing the central and single vascular bundle composed exclusively of helical protoxylem elements, note the endodermis surrounding the vascular bundle and presenting a Casparian band on its radial walls (arrows), scale bar: 50 µm. (C) *Piper sp*. radial view of the Casparian band (arrowed) that contains suberin and lignin, occurring on the radial and tangential anticlinal walls of cells, scale bar: 200 µm.

3.3.5 Anatomical character state reconstructions

Maximum parsimony reconstruction of five states, using polymorphisms in large genera presenting different cambial activities, supports a “truly woody” ancestral state for the Piperales (Fig. 3.7A, node 2) with a continuous cambium producing a complete wood cylinder of significant development. Cambial activity reconstruction is also univocal for the ancestor of the perianth-bearing Piperales (Fig. 3.7A, node 3) where cambial activity is also reconstructed as “truly woody” (Figs. 3.2C-F). The restriction of cambial activity to fascicular areas (Figs. 3.2A,B) was consistently reconstructed as ancestral for the Saururaceae (Fig. 3.7A, node 5), while the ancestral state of the cambial activity remains ambiguous for Piperaceae (Fig. 3.7A, node 6). MP suggest that "woody herbs" presenting a wood cylinder restricted to basal parts of the stem evolved at least two times within Piperales, in the Saururaceae (*Anemopsis*) and the Asaraceae. Complete loss of vascular cambium has probably evolved independently in *Verhuellia* and in some *Peperomia* representatives.

While all Aristolochiaceae, Asaraceae and Piperaceae present simple perforation plates (Figs. 3.4A-J), scalariform perforation plates occur in all Saururaceae (Figs. 3.4K-M), with the exception of *Anemopsis* (Appendix A2). ML reconstruction indicates that the presence of scalariform perforation plates is most probably a plesiomorphic feature of Saururaceae (0.61 proportional likelihood value (pl)) (Fig. 3.7B, node 5), thus suggesting that simple plates have evolved secondarily in *Anemopsis*. ML reconstruction strongly supports the hypothesis of simple perforation plates as an ancestral state for the perianth-bearing Piperales (0.95 pl) (Fig. 3.7B, node 3) as well as for the internal node within Piperaceae excluding the protostelic *Verhuellia* (0.94 pl) (Fig. 3.7A, node 7). The simple perforation plate is highly supported in the common ancestor of Piperales (0.71 pl) (Fig. 3.7B, node 2).

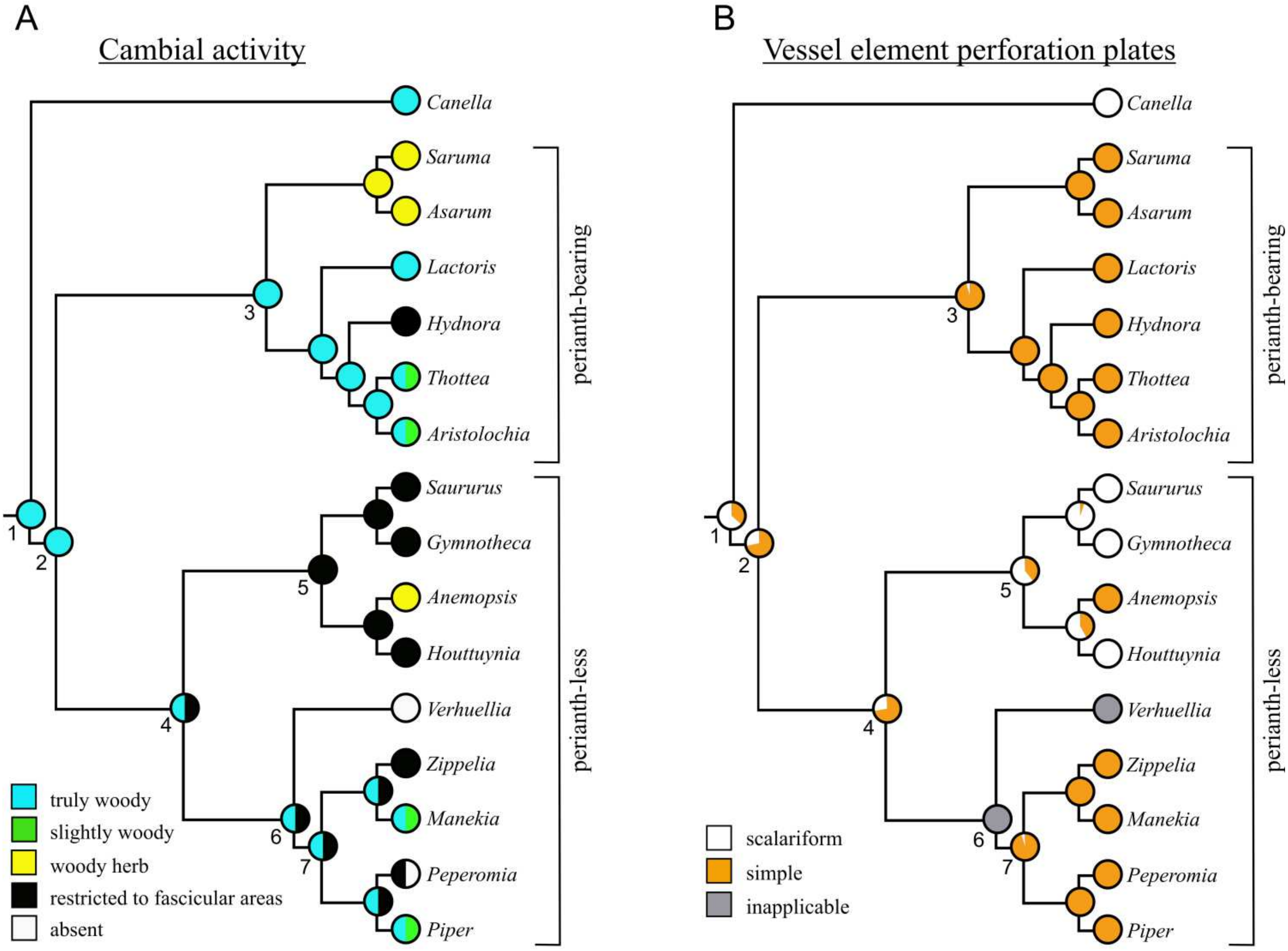


FIGURE 3.7 Ancestral character state reconstructions of Piperales. (A) maximum parsimony (MP) reconstruction of the cambial activity in Piperales, MP method is used to code polymorphisms when two potential character states are known to occur. (B) maximum likelihood (ML) reconstruction of the vessel element perforation plates, pie charts indicate ML probabilities for each character state at each internal nodes. Descriptions of character states and the employed reconstruction methods are provided in Material and Methods section. Nodes are numbered for easier reference to the text.

3.4 Discussion

3.4.1 Primary woodiness in Piperales

Uniformity of wood features in Piperales. A striking feature of Piperales is the characteristic and highly conserved kind of wood anatomy. Specific combinations of wood features distinguish woody Piperales from many other groups of angiosperms, including: vessels in radial patterns, vessel elements with simple perforation plates, vessel-to-vessel pitting alternate to scalariform, vessel to axial parenchyma pitting mainly scalariform, wide and tall rays composed exclusively of upright and squared cells and storied wood. Among the wood features mentioned before, the wide and tall rays composed of secondary parenchyma, which change little over time, and the upright and squared nature of these ray cells, constitute the main "trademark" of Piperales wood anatomy. As mentioned by Carlquist (1993) the wood of the Aristolochiaceae, *Lactoris* and Piperaceae is "amazingly similar". Even the highly specialized holoparasite *Hydnora* presents "piperalean" anatomical features such as simple perforation plates and scalariform to alternate vessel pitting (Tennakoon *et al*. 2007), despite some divergences due to their holoparasitic habit and their highly derived sub-terranean morphology (Wagner *et al*. 2014).

The uniformity of numerous wood features throughout Piperales constitutes a strong argument supporting a unique origin of wood formation in the order. Some combinations of features might distinguish woody lineages within Piperales; these include (a) exclusively vasicentric axial parenchyma in Piperaceae and Lactoridaceae versus the paratracheal axial parenchyma in combination with predominantly diffuse-in-aggregates or narrowly banded axial parenchyma in *Aristolochia* and *Thottea* (Fig. 3.5; Table 3.1)(Carlquist 1993), (b) alternate intervessel pitting in *Aristolochia* and *Thottea* versus scalariform intervessel pitting mostly observed in Piperaceae (Table 3.1). However, these features are not strictly fixed in each lineage. For instance, we observed predominantly alternate pitting in *Piper flaviflorum* and *P. hispidinervum*, and only scanty paratracheal axial parenchyma in several *Aristolochia* and *Thottea* species (Fig. 3.5; Table 3.1). We suggest that these relatively minor anatomical divergences might thus reflect specific habitat or growth form adaptations rather than independent origins of wood formation in the different lineages.

A truly woody putative ancestor of Piperales. Despite a wide diversity of cambium functioning in extant Piperales, our ancestral character state reconstruction univocally supports a strictly woody common ancestor for Piperales. The uniform wood organization of Piperales supports this view and suggests that herbaceousness is secondarily derived in the different lineages. Reduction of woodiness in woody herbs and strictly herbaceous habits might result from a reduction of cambial activity and its limitation to the fascicular areas. Canellales the sister group to Piperales is widely represented by woody species, suggesting that full cambial activity and a woody habit were already present in Canellales + Piperales common ancestor (Fig. 3.7A, node 1). At a large phylogenetic scale, magnoliids have been suggested to be evolved from a woody ancestor, while Piperales have been alternatively coded as herbaceous or woody (Kim *et al*. 2004; Feild and Arens 2005). Our work supports the view that the herbaceous habit arose several times independently within magnoliids.

3.4.2 Herbaceousness as a derived condition in extant Piperales

Loss of secondary growth in Piperaceae, a rearrangement of the polycyclic vascular bundles. *Verhuellia* and *Peperomia* (Piperaceae) present a derived herbaceous condition, involving the complete loss of the bifacial cambium (Fig. 3.7A). Stem anatomy of *Verhuellia lunaria* is strikingly rudimentary and reduced to a primary vascular system with a single central vascular bundle composed of five to nine annular and helically thickened protoxylem tracheary elements surrounded by protophloem (Fig. 3.6B). In *Peperomia* the scattered vascular bundles are composed of proto- and metaxylem tracheary elements. Piperaceae evolved several derived anatomical features, the most striking being the polycyclic or scattered arrangement of their vascular bundles (Figs. 3.2G-I) (Isnard *et al*. 2012). Debray (1885) proposed that the vascular bundles of *Peperomia*, undergoing little or no secondary growth, might be homologous to the medullary vascular bundles of *Piper* species, which also have little or no secondary growth. Intensive secondary growth indeed occurs preferentially in the peripheral vascular bundles of *Piper* (Fig. 3.2H); consequently the loss of the peripheral cycle of vascular bundles could lead to stems with scattered medullary bundles with limited or no secondary growth, as observed in *Peperomia*. The protostelic organization of *Verhuellia* might represent an extreme rearrangement of the vascular system, where only one single medullary bundle is produced. In fact, very few dicotyledonous species have completely lost secondary growth; the pervasiveness of the genetic ability to produce secondary xylem among dicotyledonous plants suggests a significant genetic stability of the vascular cambium. In Piperaceae the rearrangement/loss of vascular bundles is a source of variation in habits, which might differ from any irreversibly suppressed cambial activity since the loss of secondary growth could result from the loss of the peripheral vascular bundles.

3.4.3 Anatomical particularities in Piperales

Stem endodermis in Piperaceae + Saururaceae. One of the main clade-specific anatomical features is the presence of a stem endodermis in the aerial stems of Piperaceae and Saururaceae. A stem endodermis bearing Casparian bands has been previously reported in several genera including *Piper*, *Peperomia* and *Saururus* (Bond 1931; Carlquist *et al*. 1995; Lersten 1997; Souza *et al*. 2009). The present study extended the presence of a stem endodermis to other genera within Piperaceae and Saururaceae (*Manekia, Houttuynia, Verhuellia* and *Zippelia*) (Fig. 3.6), indicating that the Casparian bands in aerial stems are present in all genera of Piperaceae, and in several genera of Saururaceae. To our knowledge, stem endodermis has, however, never been reported for other Piperales lineages, and the occurrence of this feature suggests that stem endodermis was present in the common ancestor of Piperaceae + Saururaceae. Stem endodermis occurs sporadically in angiosperm phylogeny (Lersten 1997) and has been attributed to several functions associated with water or oxygen conservation and pathogen protection by acting as a barrier (Enstone *et al*. 2003; Meyer and Peterson 2011). In Saururaceae, stem endodermis is found in aquatic to semi-aquatic species (*Saururus* and *Houttuynia*). In Piperaceae, stem endodermis is found in epiphytic and terrestrial species producing adventitious roots, suggesting a water storage function. Some *Piper* species developed additional water-related adaptations such as mucilage canals, and Saururaceae possesses aerenchyma, which points to the importance of water-related adaptations in these lineages.

Scalariform perforation plates in the vessel elements of the Saururaceae. The scalariform perforation plates of Saururaceae (except *Anemopsis* (Carlquist *et al*. 1995; Schneider and Carlquist 2001)) is one of the main diverging xylem features in Piperales. The vessel elements of Saururaceae have been proposed to be “relictuals” based on their primitive nature, according to the Baileyan scheme of wood evolution (Bailey and Tupper 1918), and on the assumption of an unchanged history of life in mesic habitats (Carlquist *et al*. 1995). The occurrence of scalariform plates in Saururaceae could be explained as the result of limited secondary growth, lack of hydrolysis of the pit membrane in the end walls and retention of primary xylem features. Such relictual condition of scalariform plates in Saururaceae would imply at least three independent evolutionary lines of simple plates within Piperales (Fig. 3.7B), despite not being the most likely, this evolutionary scenario is still plausible as simple perforation plates, which provide an enhanced hydraulic conductance (Christman and Sperry 2007), are found in genera presenting large-bodied and climbing forms (*Aristolochia*, *Thottea* and *Piper*) (Figs. 3.1A,B) which demand an efficient water conductance.

Reversal from simple to scalariform perforation plates (*i.e.* after complete loss of the structure) in Saururaceae is however supported by our character reconstruction (Fig. 3.7B, node 5). Previous works have suggested possible reversals in perforation plates morphology in a large clade of Ericales and in the genus *Meryta* (Lens *et al*. 2007; Oskolski and Jansen 2009). These previously documented reversals from simple to scalariform perforation plates together with the observed widespread occurrence of scalariform perforation plates along all major groups of angiosperms (Olson 2014), challenge the vision of irreversibility of the

Baileyan trend in perforation plates morphology and suggest that parallelisms and reversals resulting in scalariform perforation plates may be more common than previously thought. Further work on the ecophysiology of the Saururaceae and other angiosperms presenting this potential character reversal would be needed in order to understand which are conditions driving the reversal from simple to scalariform perforation plates.

3.4.4 Protracted juvenilism in wood in relation to Piperales growth habits

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

In a recent work, Lens *et al*. (2013) provide an extended list of primarily woody taxa which actually exhibit protracted juvenilism in wood such as the rays composed by upright cells. It was proposed that protracted juvenilism in wood may be related to specific growth forms such as small-sized shrubs, rosette trees or succulent stems. The presence of juvenilistic rays in Piperales may be linked to the sympodial growth form which is widely represented in the order and has been recently reconstructed as ancestral for Piperales (Isnard *et al*. 2012). A relationship between the sympodial construction observed in early-diverging angiosperms (including Aristolochiaceae and Piperaceae) and the wide and tall rays commonly observed in their wood was already suggested by Carlquist (2009). Functional reasons can explain the widespread occurrence of upright cells in Piperales rays. Many *Aristolochia*, *Thottea* and *Piper* species present liana and cane-like habits with several elongated stems growing from the base of the plant (Isnard *et al*. 2012)(Figs. 3.1B,D). Radial transport of photosynthates in these relatively narrow stems may not be an important constraint for the plant, promoting the development of upright cells that enhance a vertical conduction (Carlquist 2012) which plays a more important role in these growth forms.

Mabberley (1974) proposed that the flat age-on-length curves obtained by Carlquist (1962) for the vessel elements of *Macropiper excelsum* are the result of the geometry of its stems. Indeed, Mabberley (1974) argued that *M. excelsum* presents wide piths which become wider at the higher portions of the stem, considerably increasing stem radius. A greater radius may increase the number of cambial initials for anticlinal divisions and therefore diminishing the intrusive growth from cambial initials from a lower level, as a consequence of this, vessel element length remains constant (Dulin and Kirchoff 2010). In addition, as reported by Lens et al. (2013), the study of Bailey (1923) presented flat length-on-age curves for vessel elements of primarily woody species with storied cambia. We have observed storied wood structure in *Aristolochia* and *Piper* representatives (not shown) and it is reported by Carlquist (1993) as a common feature in Piperales, this condition may also explain the reported curve for *M. excelsum* by Carlquist (1962).

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

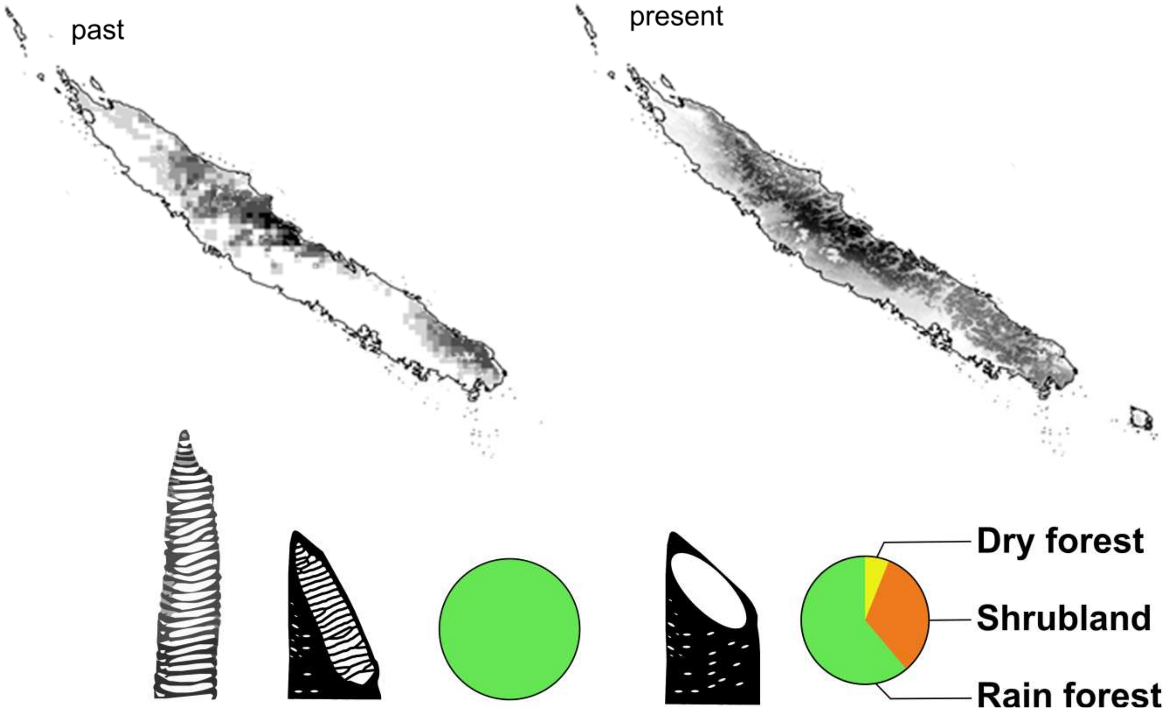
3.4.5 Piperales anatomy, a major source of growth form diversification

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

Large self-supporting forms (Fig. 3.1A) are however confined to few species within Piperales, where ray lignification could have reduced flexibility of the wood cylinder (Isnard *et al*. 2012). Since the climbing growth form has been reconstructed as derived in *Aristolochia* (Wagner *et al*. 2014), the wide and tall rays which seems to be a synapomorphy of Piperales wood, could be an exaptation for the climbing form. Wide rays in lianas are indeed widely known to promote stem flexibility and are largely considered as an adaptation to the climbing habit (Putz and Holbrook, 1991; Rowe *et al*. 2004, Isnard and Silk 2009). The evolution of the climbing habit in angiosperms might have promoted diversification (Gianoli 2004), while the evolution of herbaceousness from woodiness is argued to correlate with an increase in the diversification rate and to be implicated as a direct cause of species richness in angiosperm families (Dodd *et al*. 1999). In the local context of New Caledonia, Piperaceae is the only basal angiosperm family showing liana and herbaceous growth forms. Indeed, with the exception of the notable herbaceous parasite *Cassytha* (Lauraceae), all of the remaining New Caledonian basal angiosperm families are represented by tree and shrub forms. Piperaceae is the basal angiosperm family that shows the highest niche marginality in New Caledonia (Chapter 4) (Pouteau *et al*. 2015). Most likely, the growth forms of Piperales, which diverge from the rest of the local basal angiosperms, may have promoted the marginalization of this family. The lability of the cambial activity, allowing recurrent shifts in habit from woodiness to herbaceousness and the “piperalean” wood construction, promoting highly variable growth forms, have therefore been key elements in the diversifications of species and habitat preferences in Piperales.

CHAPTER 4

Habitat preferences of New Caledonian basal angiosperms, past and present species distribution[1]



- Environmental distribution of basal angiosperms
- Mechanisms behind basal angiosperms over-representation in New Caledonia
- Xylem vascularization and habitat preference

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[1] A version of this chapter has been **published**. Pouteau, R., Trueba, S., Feild, T.F., Isnard, S. (2015). *Journal of Biogeography*. 42: 2062-2077

Abstract

Basal angiosperm lineages are over-represented in New Caledonia. However, the mechanisms responsible for such a distribution remain unclear. This thesis chapter explores two key hypotheses: (1) the diversity of basal angiosperms reflects adaptation to ultramafic substrates, which act as ecological filters for plant colonists; and (2) the diversity stems from wet climatic conditions that have persisted in New Caledonia during the late Quaternary while Australia and some nearby islands likely experienced widespread extinction events. Given that basal angiosperms present a high anatomical diversity of xylem conduits, we estimated if the presence of different xylem conduit types is likely to promote ecological differentiation.

We used species distribution models to determine the environmental correlates for 60 basal angiosperm species. Environmental variables used to characterise habitats included vegetation, substrate, and climate variables. We then tested whether the variety of xylem conduit structures borne by New Caledonian basal angiosperms, which is expected to affect plant hydraulic capacity, was correlated with habitat preference. Finally, we analysed species prevalence on different substrates and projected habitat size and distribution to the last glacial maximum (LGM).

We found a clear habitat preference among basal angiosperms for rainforest habitats located on non-ultramafic substrates, with the exception of taxa bearing true vessels with simple perforation plates which harboured a wider habitat breadth. We also show that these rainforest habitats experienced a range reduction and an eastward shift during the LGM forming two refugial areas located on the warm and rainy east coast of Grande Terre. Prevalence of basal angiosperms in habitats characterised by low evaporative demand appears to be related to xylem hydraulic limitations.

The great representation of basal angiosperm species in New Caledonia can be explained by the persistence of rainforests in the island despite global Quaternary fluctuations that affected floras in the region. This study offers a new model to explain why certain angiosperm families are disharmonically represented in New Caledonia.

Key-words: climatic refugia; geographic information system; niche modelling; palaeodistribution; plant-climate interactions, relictual angiosperms; xylem anatomy.

4.1 Introduction

Basal angiosperms are frequently considered as relict species because of their early divergence times, and because of their low diversification compared to the eudicots-monocots group (see section 1.3). Relict species can be considered as the extant remains of a larger and more widespread group of organisms in which most taxa are now extinct (Fig. 1.4). These relicts have often been incorrectly viewed as indicating the location of the ancient centre of diversity of a group (Ladiges and Cantrill, 2007; Jones *et al.*, 2009). Such a simplistic consideration is inherently flawed since it ignores dispersal and extinction events that shape the biogeographic evolution of most lineages. Although relicts can hardly serve as evidence for permanence in the geographical area they currently occupy, the habitat distribution of extant species can help to elucidate how they have survived in a recent past (Grandcolas *et al.*, 2014). Moreover, under the perspective of niche conservatism, we can expect that modern basal angiosperms occupy an environment similar to the habitat of their common ancestor (Losos, 2008).

New Caledonia harbours an impressive richness in species belonging to early branching lineages such as the flightless bird *Rhynochetos jubatus*, gymnosperm species, and the basal angiosperm *Amborella trichopoda*, which is the sister group to all other extant angiosperms (Morat, 1993; Grandcolas *et al.*, 2008). Basal angiosperms provide a good model to understand ancestral ecology because angiosperms form the largest group of relict taxa in New Caledonia. As mentioned in section 1.9 of the first chapter of this thesis, basal angiosperms include 109 species, 22 genera and 10 families belonging to the ANA grade, Chloranthales and magnoliids (Morat *et al.*, 2012). Analyses of floristic affinities (Morat, 1993) as well as recent phylogenies (Swenson *et al.*, 2014; Thomas *et al.*, 2014) have identified Australia as the most likely origin for a substantial proportion of the New Caledonian flora. However, seven of the 10 basal angiosperm families present in New Caledonia have been recognised as significantly over-represented compared to the flora of Australia (see section 1.7).

Disharmony refers to the non-random representation of species among colonists of oceanic islands as compared with the source mainland (Carlquist, 1974). A common explanation for disharmony on island floras is dispersal limitation. In New Caledonia, a recent work found that dispersal might not be the main process that explains the disharmony of the flora because plant families with low amounts of endemism (used as a surrogate for high dispersal capacities) tended to be under-represented (Pillon *et al.*, 2010). Thus, the disharmony was suggested to stem from particular ecological conditions occurring in New Caledonia.

The distribution of plant species in New Caledonia has often been hypothesised as constrained by New Caledonia's unusual ultramafic substrates (Morat, 1993). Ultramafic rocks possess low amounts of essential plant nutrients and are rich in toxic heavy metals (Jaffré *et al.*, 1987; van der Ent *et al.*, 2015). Preadaptation of immigrating lineages to ultramafic substrates has long been considered to have driven the flora disharmony in New Caledonia because these lineages would be able to establish and radiate on an unusual substrate (Jaffré *et al.*, 1987; Pillon *et al.*, 2010). Examples of such ultramafic pre-adapted lineages include conifers and some angiosperms, for instance the locally diverse Cunoniaceae (Jaffré, 1995; Pillon *et al.*, 2009; Pillon *et al.*, 2010). However, it is not clear whether basal angiosperms also possess a distributional preference for ultramafic substrates (Jaffré, 1995; Pillon, 2008).

More broadly, the richness in basal angiosperm lineages such as ANA grade families and magnoliids in Asia-Australasia (Fig. 1.3) has been hypothesised as arising from the maintenance of stable climatic conditions suitable for their persistence or diversification in the region (Morley, 2001; Buerki *et al.*, 2014). Rapid and extensive climatic shifts, however, affected the Australian flora during the Pleistocene and likely drove the extinction of many lineages, especially in the rainforest flora (Byrne *et al.*, 2011). Thus, another possible explanation of the over-representation of basal angiosperms in New Caledonia is that climatic stability during this period favoured the persistence of basal angiosperms while other nearby regions became relatively depauperate.

Feild *et al*. (2004; 2009) have demonstrated that basal angiosperm distributions are significantly hemmed in by drought. In these studies, only members of the ANA grade as well as Chloranthales were considered. These taxa possess vascular systems that are highly vulnerable to drought-induced xylem cavitation and their leaves wilt at relatively mild leaf water potentials (Feild *et al.*, 2009). In addition, the anatomy of their xylem conduits confer high hydraulic resistances in their leaves and stem xylem systems (Feild and Wilson, 2012). In concert, these physiological functions have been hypothesised to limit species to humid forest habitats where peak transpirational demands are low.

The putative drought intolerance of ANA and Chloranthales would be mechanistically linked to their xylem structure and function (Sperry *et al.*, 2007; Feild and Wilson, 2012).

Indeed, vesselless basal angiosperms, such as *Amborella*, occur preferentially in wet, low evaporative demand rainforest habitats because their low xylem hydraulic capacities do not impose lethal leaf water stress versus derived vessel-bearing angiosperms bearing hydraulically streamlined vessels with simple perforation plates (Sperry *et al.*, 2007). The xylem conduits found in ANA grade and Chloranthales species suggest that maintance of stable, wet conditions in New Caledonia may explain basal angiosperm disharmony in New Caledonia. However, to our knowledge, while a similar distributional pattern has been proposed (Morley, 2001), no study has addressed the question of whether magnoliids might possess similar drought-induced limitations on their habitat distributions. This chapter aims to analyse the influence of xylem conduit type, which influences xylem hydraulic function, on the distribution of basal angiosperms.

Although functional traits could provide the basis to predict a species' habitat, the trait-based approach is inherently limited due to the considerable co-variation and trade-offs among traits that shape plant life-histories (Vuorisalo and Mutikainen, 1999). In contrast, species distribution models (SDMs) assess habitat characteristics directly by statistically correlating the known spatial distribution of species with a number of GIS (geographic information system) environmental variables. In this paper, we determined environmental correlates of 60 basal angiosperms native to New Caledonia through SDM to complement the trait-based hypothesis of relict angiosperms as adapted to wet forests. We address the questions of (i) whether relict angiosperms share ecological requirements; and (ii) whether the over-representation of relict angiosperm families in New Caledonia is linked to the presence of ultramafic substrates versus the persistence of suitable climatic conditions on the archipelago during the Pleistocene. Further, we assess the influence of xylem vasculature on the distribution of plant species.

4.2 Materials and methods

4.2.1. New Caledonia

New Caledonia (Fig. 1.7) has a tropical climate with annual mean temperature in lowland areas between 27°C and 30°C from November to March and between 20°C and 23°C from June to August. Annual precipitation ranges from 300 mm to 4,200 mm with greater precipitation on the windward east coast (Fig. 1.7). Three main types of substrates occur: (i) ultramafic substrates covering the southern third of Grande Terre and isolated ultramafic massifs along the west coast; (ii) substrates derived from volcano-sedimentary rocks roughly covering the northern two thirds of Grande Terre; and (iii) calcareous substrates prevailing on the Loyalty Islands (Fig. 1.7C) (Fritsch, 2012). The landscape is a mosaic composed for half of secondary vegetation, one quarter of low- to mid-elevation shrublands or '*maquis*' found on ultramafic substrates below 800 m, another quarter of low- to mid-elevation rainforests, 1% of montane rainforests and shrublands found above 800 m, with a few relictual patches of dry sclerophyll forests scattered along the west coast, and wetlands including the *c.* 50 km² marshes of the Plaine des Lacs (Fig. 1.7F) (Jaffré, 1993; Jaffré *et al.*, 2012). A detail of the main peaks of the central mountain range is presented in figure 4.1. A full description of the geography and environment of New Caledonia is provided in Chapter 1 (section 1.7).

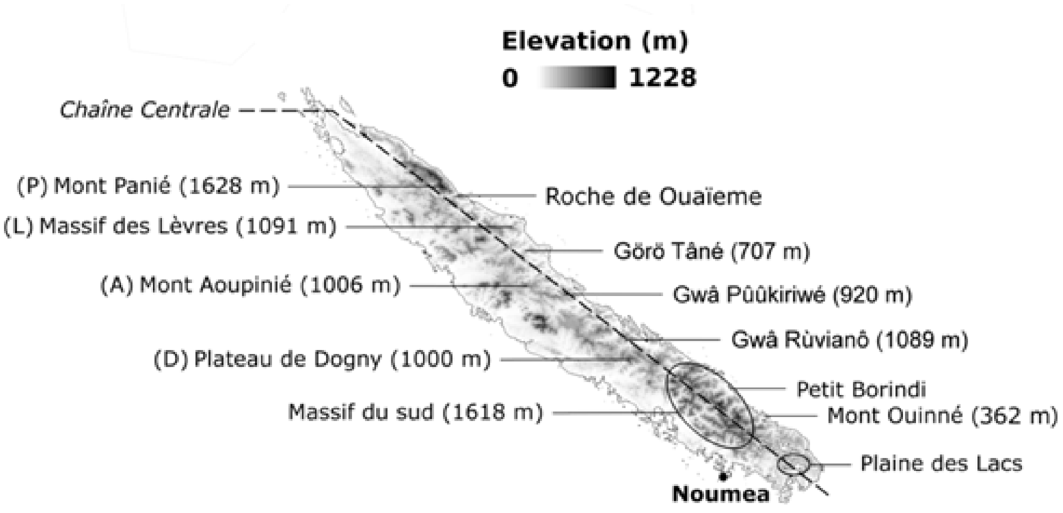

FIGURE 4.1 Elevation and toponyms cited in the text. The main peaks of *Grande Terre*'s mountain range are indicated. Other geographical and environmental features of New Caledonia are presented in the section 1.7.

4.2.2 New Caledonian basal angiosperms; species representation, anatomical data

We gathered distributional data from locality information on specimens from the Herbarium of the IRD Centre of Noumea (NOU) using the Virot database (http://herbier-noumea.plantnet-project.org) and from forest inventories of the NC-PIPPN plot network (Ibanez *et al.*, 2014). Among all basal angiosperm taxa native to New Caledonia (Morat *et al.*, 2012), we selected those with at least 10 occurrence records separated by more than one kilometre to avoid duplication of taxa in the same grid cell. Florical (Morat *et al.*, 2012) served as taxonomic reference base. Taxa with questionable taxonomic limits (J. Munzinger, pers. comm.) and specimens whose identification was uncertain were removed from the sampling. Subspecies and varieties were treated separately as the distinction with different species was sometimes blurred and infraspecific taxa often occupy different habitats. Using these procedures, we sampled 60 taxa belonging to 18 genera and 10 families for which 1,851 occurrence records were compiled. Our sampling captured all basal angiosperm families native to New Caledonia, between 50% and 100% of genera within each family and between 27% and 100% of species per family (Table 4.1). It included the two ANA grade taxa present in the archipelago, *Amborella trichopoda* (Amborellaceae, Amobrellales) and *Trimenia neocaledonica* (Trimeniaceae, Austrobaileyales), two Chloranthaceae (*Ascarina solmsiana* var. *solmsiana* and *A. rubricaulis*), as well as 56 magnoliids (Table 4.1).

Since xylem conduit structure affects plant hydraulic capacity (Sperry *et al.*, 2007; Christman and Sperry, 2010), variation in xylem conduit structure is hypothesized to represent an important trait bearing on variation in species habitat occurrence. To test whether conduit structure was correlated with environmental preference, we conducted a literature survey of xylem tracheary element types possessed across basal angiosperms of New Caledonia (Appendix A4). Taxa were coded as to whether they bear tracheids, vessel elements with scalariform perforation plates, or vessel elements with simple perforation plates (Fig. 4.2). These three categories binned three major hydraulic designs in angiosperms (Feild and Wilson, 2012). Tracheid-based xylem conducts low amounts of water, while xylem with vessels bearing simple perforation plates often possesses high water conduction capacity. Perforation plates exhibit broad structural diversity in angiosperms (Fig. 1.6A). Nevertheless, two broad categories are recognized: 1) simple perforation plates that are a wide and single apical pore; and 2) scalariform perforation plates that consist of a ladder-like array of pits varying in number, widths, and bar number (Feild and Wilson, 2012). Xylem with vessels bearing scalariform perforation plates possess hydraulic capacities generally lower than xylem systems with simple perforation plates vessels (Christman and Sperry, 2010). Moreover, some angiosperm scalariform plated vessels possess hydraulic capacities within the range of tracheid-based xylem (Feild & Wilson, 2012).

4.2.3 Environmental variables

Current climate data were downloaded from the WorldClim database (Hijmans *et al.*, 2005). The 30 arc-second resolution (*c.* one kilometre) of the WorldClim data matched well with the spatial accuracy of the oldest plant occurrence records. Although WorldClim data are derived from global climate databases, they were preferred to local data at similar resolution

TABLE 4.1 *Summary of the 60 basal angiosperm taxa and corresponding modelled distribution area (in km^2) and model performance.*

Biogeographical status: F = Endemic family; G = Endemic genus; E = Endemic species; I = Native non-endemic species. Habit: T = Tree; S = Shrub; E = Epiphyte; L = Liana. Preferred substrate type (model output): V = Volcano-sedimentary substrates only; U = Ultramafic substrates only; S = Several types of substrate. Preferred vegetation type (model output): RF = Rainforest; M = Shrubland; D = Dry forest.

Family	Taxon	Status	Habit	Substr.	Veget.	# of occurrences	Current distr. area	CCSM distr. area	MIROC distr. area	AUC BIOCLIM	AUC DOMAIN	AUC SVM	AUC Ensemble
Amborellaceae	*Amborella trichopoda*	F	ST	V	RF	56	3439	1175	75	0.94	0.93	0.91	0.91
Annonaceae	*Goniothalamus obtusatus*	E	T	V	RF	24	4861	575	300	0.71	0.84	0.81	0.85
Annonaceae	*Hubera nitidissima*	E	T	V	D	32	10640	5875	1925	0.47	0.46	0.67	0.74
Annonaceae	*Meiogyne baillonii*	E	T	U	RF	28	6748	2050	800	0.56	0.52	0.85	0.79
Annonaceae	*Meiogyne lecardii*	E	T	V	RF	13	458	300	75	0.78	0.95	0.97	0.94
Annonaceae	*Meiogyne tiebaghiensis*	E	T	V	D	53	11830	2400	850	0.61	0.56	0.76	0.64
Annonaceae	*Xylopia dibaccata*	E	ST	S	M	14	2912	50	50	0.63	0.74	0.89	0.72
Annonaceae	*Xylopia pancheri*	E	S	U	M	56	3818	925	225	0.77	0.73	0.73	0.80
Annonaceae	*Xylopia vieillardii*	E	T	V	RF	58	3628	1375	450	0.75	0.90	0.83	0.89
Atherospermataceae	*Nemuaron vieillardii*	G	T	V	RF	45	5248	2600	800	0.69	0.80	0.80	0.91
Chloranthaceae	*Ascarina rubricaulis*	E	T	S	RF	36	3392	2075	925	0.89	0.79	0.87	0.95
Chloranthaceae	*Ascarina solmsiana* var. *solmsiana*	E	T	S	RF	28	2978	1775	600	0.88	0.99	0.95	0.97
Hernandiaceae	*Hernandia cordigera*	E	T	U	RF	49	7704	2075	950	0.67	0.82	0.77	0.86
Lauraceae	*Beilschmiedia oreophila*	E	ST	S	M	16	1854	1225	675	0.97	0.91	0.95	0.85
Lauraceae	*Cryptocarya aristata*	E	T	V	RF	25	3836	1475	425	0.96	0.99	0.90	0.93
Lauraceae	*Cryptocarya elliptica*	E	T	V	RF	45	3930	750	300	0.85	0.86	0.90	0.88
Lauraceae	*Cryptocarya guillauminii*	E	ST	U	RF	51	5431	700	275	0.96	0.91	0.94	0.84
Lauraceae	*Cryptocarya leptospermoides*	E	T	S	M	12	3900	2025	725	0.65	0.92	0.87	0.85
Lauraceae	*Cryptocarya lifuensis*	E	T	S	D	11	696	25	25	0.80	0.92	0.74	0.97
Lauraceae	*Cryptocarya longifolia*	E	T	U	M	44	6530	3875	1800	0.80	0.81	0.71	0.84
Lauraceae	*Cryptocarya macrodesme*	E	T	V	RF	19	2396	1125	475	0.89	0.93	0.91	0.92
Lauraceae	*Cryptocarya odorata*	E	T	U	RF	43	4321	1150	525	0.56	0.63	0.61	0.81
Lauraceae	*Cryptocarya oubatchensis*	E	T	S	RF	44	4610	3225	1525	0.83	0.89	0.87	0.92
Lauraceae	*Cryptocarya phyllostemon*	E	T	U	RF	14	688	300	125	0.83	0.97	0.98	0.90
Lauraceae	*Cryptocarya pluricostata*	E	T	S	RF	11	2345	600	75	0.76	0.76	0.86	0.74
Lauraceae	*Cryptocarya transversa*	E	T	U	RF	19	2072	150	125	0.83	0.92	0.93	0.82
Lauraceae	*Cryptocarya velutinosa*	E	T	V	RF	15	3279	450	250	0.80	0.89	0.83	0.92
Lauraceae	*Endiandra baillonii*	E	T	U	M	39	1190	475	250	0.84	0.87	0.91	0.96
Lauraceae	*Endiandra sebertii*	E	ST	S	RF	21	6278	3000	1225	0.59	0.71	0.80	0.87
Lauraceae	*Litsea deplanchei*	E	ST	U	M	39	778	125	75	0.78	0.78	0.80	0.80
Lauraceae	*Litsea ripidion*	E	S	U	M	12	3516	3225	1400	0.98	0.99	0.94	0.98
Monimiaceae	*Hedycarya chrysophylla*	E	S	V	RF	40	4571	3275	1400	0.81	0.92	0.87	0.89
Monimiaceae	*Hedycarya cupulata*	E	S	V	RF	138	4518	1975	900	0.78	0.88	0.78	0.89

TABLE 4.1 (*Continued*)

Family	**Taxon**	**Status**	**Habit**	**Substr.**	**Veget.**	**# of occurrences**	**Current distr. area**	**CCSM distr. area**	**MIROC distr. area**	**AUC BIOCLIM**	**AUC DOMAIN**	**AUC SVM**	**AUC Ensemble**
Monimiaceae	*Hedycarya chrysophylla*	E	S	V	RF	40	4571	3275	1400	0.81	0.92	0.87	0.89
Monimiaceae	*Hedycarya cupulata*	E	S	V	RF	138	4518	1975	900	0.78	0.88	0.78	0.89
Monimiaceae	*Hedycarya engleriana*	E	ST	V	RF	58	7644	4325	1650	0.80	0.86	0.88	0.94
Monimiaceae	*Hedycarya parvifolia*	E	T	S	RF	108	1899	3175	1600	0.74	0.77	0.74	0.89
Monimiaceae	*Kibaropsis caledonica*	G	T	V	RF	49	3679	325	125	0.85	0.93	0.92	0.96
Piperaceae	*Peperomia baueriana*	I	E	V	RF	10	4717	1275	600	0.87	0.68	0.90	0.66
Piperaceae	*Peperomia urvilleana*	I	E	V	RF	17	7628	625	275	0.64	0.61	0.89	0.80
Piperaceae	*Piper comptonii*	E	L	S	RF	17	13961	2700	825	0.51	0.70	0.79	0.72
Piperaceae	*Piper insectifugum*	I	L	S	D	57	1185	2875	1575	0.51	0.58	0.69	0.58
Trimeniaceae	*Trimenia neocaledonica*	E	T	V	RF	22	456	175	125	0.83	0.94	0.95	0.96
Winteraceae	*Zygogynum acsmithii*	E	T	V	RF	10	2408	1925	650	0.99	0.97	0.99	0.88
Winteraceae	*Zygogynum amplexicaule* subsp. *amplexicaule* var. *amplexicaule*	E	S	V	RF	11	1658	1725	600	0.67	0.84	0.93	0.95
Winteraceae	*Zygogynum amplexicaule* subsp. *amplexicaule* var. *isoneuron*	E	ST	U	RF	22	863	850	225	0.87	0.93	0.96	0.95
Winteraceae	*Zygogynum amplexicaule* subsp. *luteum*	E	S	S	RF	24	912	225	125	0.86	0.97	0.94	0.95
Winteraceae	*Zygogynum baillonii*	E	T	V	M	28	127	275	150	0.84	0.97	0.97	0.84
Winteraceae	*Zygogynum bicolor*	E	T	S	RF	11	1259	1325	475	0.98	0.99	0.92	0.99
Winteraceae	*Zygogynum comptonii* var. *comptonii*	E	S	U	RF	20	1809	1525	300	0.84	0.89	0.93	0.97
Winteraceae	*Zygogynum comptonii* var. *taracticum*	E	S	S	RF	16	2282	2625	975	0.77	0.67	0.94	0.89
Winteraceae	*Zygogynum crassifolium*	E	T	V	M	37	2277	750	325	0.80	0.84	0.88	0.88
Winteraceae	*Zygogynum pancheri* subsp. *arrhantum*	E	T	S	RF	16	1427	475	275	0.72	0.86	0.92	0.92
Winteraceae	*Zygogynum pancheri* subsp. *elegans*	E	ST	U	RF	15	2388	900	275	0.75	0.85	0.91	0.85
Winteraceae	*Zygogynum pancheri* subsp. *pancheri*	E	T	S	RF	41	820	500	250	0.84	0.85	0.89	0.91
Winteraceae	*Zygogynum pancheri* subsp. *rivulare*	E	S	S	RF	16	417	1025	150	0.75	0.96	0.92	0.98
Winteraceae	*Zygogynum pauciflorum*	E	T	S	RF	10	608	150	150	1.00	0.91	0.99	0.92
Winteraceae	*Zygogynum schlechteri*	E	S	V	RF	14	3738	500	125	0.83	0.98	0.97	0.99
Winteraceae	*Zygogynum stipitatum*	E	T	V	RF	36	915	725	350	0.86	0.91	0.89	0.92
Winteraceae	*Zygogynum tieghemii* subsp. *tieghemii*	E	T	V	RF	14	2866	225	75	0.87	0.98	0.96	0.94
Winteraceae	*Zygogynum vieillardii*	E	T	U	M	12	1522	1900	425	0.92	0.94	0.89	0.91
Winteraceae	*Zygogynum vinkii*	E	T	U	RF	10	6278	3000	1225	0.76	0.86	0.89	0.98

but without information on climate seasonality or extrema, which are better predictors of species distribution (Zimmermann *et al.*, 2009). With the publication of WorldClim, Hijmans *et al.* (2005) expressed reservations about the reliability of the data for remote islands with limited meteorological stations. We found, however, that WorldClim data fairly well mirrored the orographic control of climate in New Caledonia and the asymmetrical rainfall and temperature patterns between the west and east coasts resulting from the rain shadow effect (Météo-France, 2007). Mean annual precipitation matched well the local grid of Météo-France interpolated through the AURELHY model using records from 1991 to 2000 ($r^2 = 0.69$; P-value < 0.05) (Météo-France, 2007).

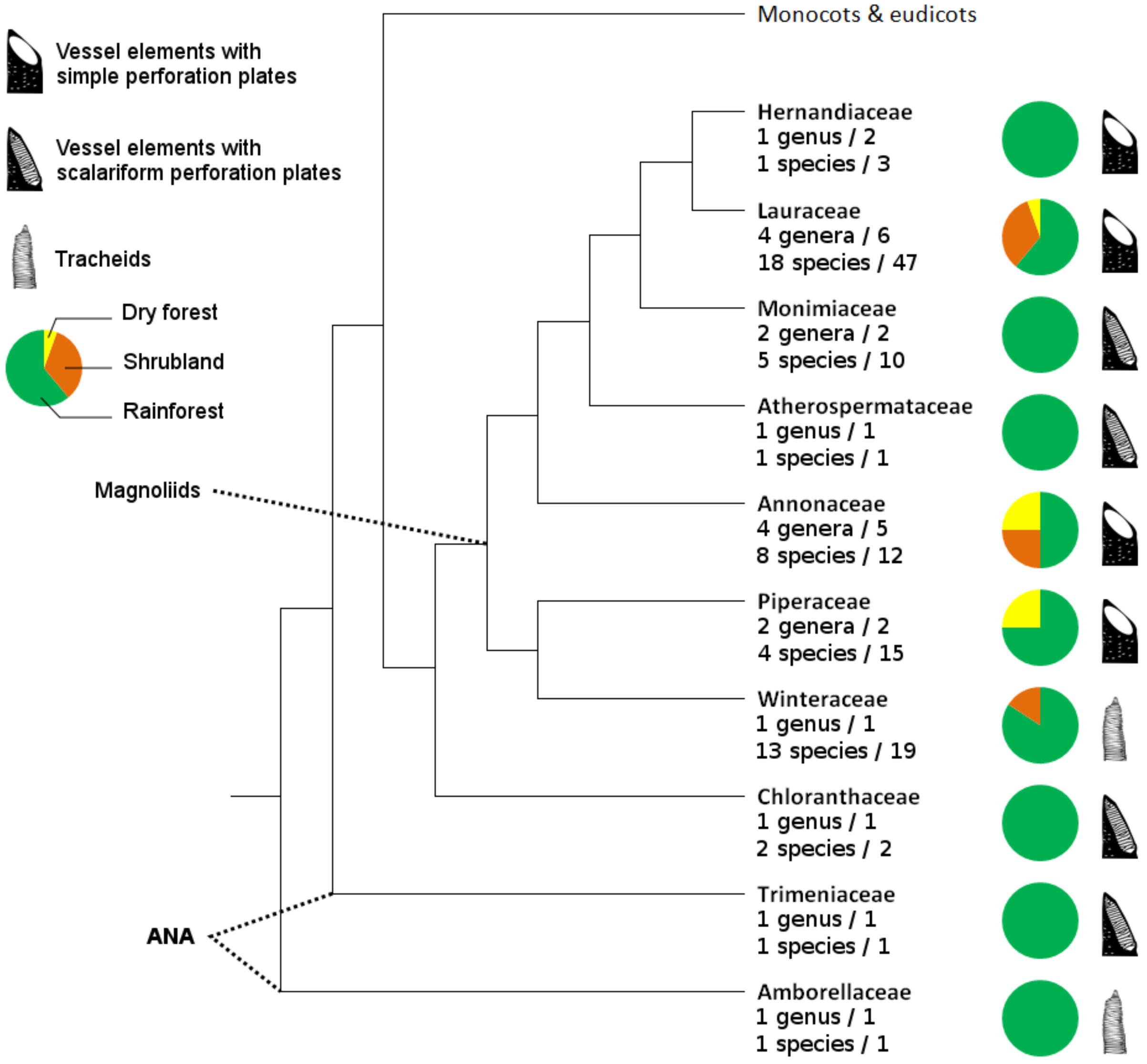

FIGURE 4.2 Representativeness of basal angiosperm taxa sampled in this study with regard to the total basal angiosperm flora of New Caledonia. Pie charts denote vegetation type preferences of species within each family (Table 4.1). Icons indicate dominant xylem conduit structure (see Appendix A4).

We addressed variable multi-collinearity by examining cross-correlations (using Spearman's *r*). For variables with correlations of $r > 0.8$, we retained only the variable that decreased model accuracy the most when omitted from the 60 full models. Such a selection method identified four climate variables: isothermality (BIO3), temperature mean diurnal range (BIO4), precipitation of the driest month (BIO14) and precipitation seasonality (BIO15). Vegetation and substrate shapefiles published in the Atlas of New Caledonia were added to climate data after being rasterized and scaled to 30 arc-seconds (Fritsch, 2012; Jaffré *et al.*, 2012).

TABLE 4.2 *Habitat distribution of narrow-range endemic basal angiosperm species* sensu Wulff *et al*. (2013).

	Rainforest & mountain shrubland					**Low- to mid-elevation shrubland**
	Northern refugial area		Southern refugial area		Outside refugial areas	
	CCSM	MIROC	CCSM	MIROC		
Lauraceae						
Adenodaphne macrophylla	X	X				
Adenodaphne triplinervia						X
Beilschmiedia neocaledonica	X					
Cryptocarya bitriplinervia					X	
Cryptocarya schmidii						X
Endiandra poueboensis	X					
Litsea humboldtiana						X
Litsea imbricata						X
Litsea mackeei						X
Litsea ovalis						X
Litsea paouensis	X	X				
Litsea pentaflora						X
Litsea racemiflora						X
Litsea stenophylla						X
Monimiaceae						
Hedycarya aragoensis	X	X				
Winteraceae						
Zygogynum cristatum	X					
Zygogynum fraterculum	X					
Zygogynum oligostigma	X					
Zygogynum tanyostigma	X					

Notes: Crosses indicate whether narrow-range endemics occur in rainforest and mountain shrubland or in low- to mid- elevation shrubland and, in the former case, whether or not they overlap with last glacial maximum refugial areas we inferred. Species that have not been included in the SDMs (*Zygogynum acsmithii* and *Z. pauciflorum* (Winteraceae)) have not been included in the analyses.

We projected habitat size and distribution to the last glacial maximum (LGM) (*c*. 21 000 yr ago) to test whether species persisted on *Grande Terre* during the Pleistocene climate changes. Historical LGM distributions were estimated using climate simulations of the two coupled global circulation models CCSM (Collins *et al.*, 2006) and MIROC (Hasumi and

Emori, 2004) provided by WorldClim at 2.5 arc-minute resolution. SDMs were re-run with the four most informative climate variables and the substrate map (assumed to be identical to present) upscaled to 2.5 arc-minute resolution. Vegetation was removed from past projection analyses as no historical vegetation map was available and past vegetation pattern was likely to have been shaped by climate and soil in the absence of human impacts. LGM sea level was considered the same as today since the high amplitude and rapid rises/retreats of the lagoon during the Pleistocene temporarily formed a calcareous coastal plain (Pelletier, 2006). Such a habitat is likely to be unsuitable for most of basal angiosperms because of salinity-induced water stress (Feild *et al*., 2009). Another common approach to infer Pleistocene refugia consists in identifying centres of endemism (Pintaud *et al.*, 2001; Weber *et al.*, 2014). To test for putative LGM refugia inferred through SDM, we tested whether refugia overlapped with occurrences of narrow-range endemic basal angiosperms. According to the analysis of Wulff *et al*. (2013), there are 21 narrow-range endemic species which belong to the families Lauraceae (14 species), Monimiaceae (1) and Winteraceae (6). We only included 19 of them in these analyses and did not consider two species (*Zygogynum acsmithii* and *Z. pauciflorum*) which satisfied the conditions to be included in the SDMs (Table 4.2).

4.2.4 Species distribution modelling

We fitted three presence-only SDMs (*i.e.* without absence or pseudo-absence): BIOCLIM, DOMAIN and one-class support vector machines (SVM). BIOCLIM is a climate envelope procedure that computes the suitability of a site by comparing the values of environmental variables at any site to the percentile distribution of the values at sites of known occurrence (Busby, 1986). We used the 5-95% percentile limits as core bioclimate. The DOMAIN algorithm uses a range-standardised point-to-point similarity metric to assign a classification value to any site based on its proximity in environmental space to the most similar occurrence (Carpenter *et al.*, 1993). We set the similarity threshold at 99%. SVM is a machine-learning method that contours the volume occupied by a species in a high dimensional hyperspace (Drake *et al.*, 2006). The shape of the volume fitted by SVM is controlled by a kernel function, γ, and a regularisation parameter, C. We used the one-class SVM implementation provided in the *e1071* package (Meyer *et al.*, 2014) developed under the R software (R Development Core Team, 2014). The 'tune' function searched for the optimal γ and C in the range $[2^{-10}, 2^{-9}, \ldots, 2^{10}]$ after 10-fold cross-validation to prevent overfitting.

Model accuracy was assessed with the area under the curve (AUC) of the receiver operating characteristic (ROC) (Fielding and Bell, 1997). We converted continuous maps to presence/absence maps by selecting for each species a threshold where sensitivity was equal to specificity (Liu *et al.*, 2005). For each species again, the three binary outputs were assembled into a single ensemble model by majority voting (a pixel was labelled as to the class that was chosen by at least two separate models) (Marmion *et al.*, 2009).

4.2.5 Habitat overlap and marginality

To explore whether basal angiosperms share a common environment, we introduced a habitat overlap index (*HO*) as the proportion of relict angiosperms expected in each site according to the individual projection of their fitted habitat. *HO* was implemented by stacking binary maps so that they expressed the local potential richness of basal angiosperms over each pixel of the combined map (Guisan *et al.*, 1999), which was then converted into a proportion by dividing that value by the total number of basal angiosperms. To deal with unbalanced numbers of taxa within genera and genera within families, *HO* was estimated at three taxonomic levels: (i) family, (ii) genus and (iii) species and infra-specific levels (hereafter referred to as the species level). At the genus and family levels, *HO* was computed by merging taxon-based SDMs rather than by building new SDMs from merged taxon occurrences because it was easier to manage unbalanced occurrences and it seemed to be more consistent with the species-specific concept of habitat.

HO was analysed within each vegetation and substrate type and we built a regression model to understand how *HO* varies along environmental gradients on the 23,586 pixels of 30 arc-second resolution covering the archipelago. Environmental variables included the four selected WorldClim variables as well as mean annual rainfall (BIO12) and elevation (SRTM digital elevation model upscaled to 30 arc-seconds). Environmental correlates of *HO* were explored using boosted regression trees (BRTs), a boosting procedure that combines large numbers of relatively simple tree models to optimise predictive performance (Elith *et al.*, 2008). BRT models were built using the R package *dismo* (Elith and Leathwick, 2015). A bag fraction of 0.5 was used, which means that, at each step of the boosting procedure, 50% of the data in the training set were drawn at random without replacement. The learning rate searching for the contribution of each tree to the model was set at 0.01. The maximal number of trees for optimal prediction was limited to 2,000. The tree complexity, referring to the maximal number of nodes in an individual tree, was set to 9.

We also developed a habitat marginality index (*HM*) for each taxon to separate basal angiosperms as to whether they co-occur in a shared environment or occupy a separate habitat. The *HM* index was calculated as follows:

$$HM = 1 - \left(\sum_{i=0}^{p} \frac{HOi}{p}\right)$$

Where p is the number of pixels where a taxon is predicted to be present. A value of 100% reflects a marginal taxon whose suitable habitat is altogether different from the other taxa, while a value of 0% denotes a gregarious taxon whose suitable habitat overlaps that of all its relatives. The main difference between *HM* and other habitat marginality metrics proposed in previous works (Hirzel *et al.*, 2002) is that marginality, as expressed by *HM*, is quantified relatively to the habitats of other species rather than to background environmental conditions available across the study area.

4.3 Results

4.3.1 Modelling assessment

Although SDMs were based on occurrence records derived from opportunistic sampling schemes prone to spatial autocorrelation (*e.g.*, near Noumea, at sites of recognised botanical interest, along walking tracks), we found poor matching between current *HO* pattern (Fig. 4.3) and density of points used as SDM input (Appendix A5). Such a result suggests that our modelling approach provides a good generalisation. The ensemble model also outperformed all individual SDMs in predicting basal angiosperm distribution (mean AUC = 0.877), the difference with BIOCLIM being significant (0.790; pairwise comparison *t*-test with Bonferroni *post-hoc* correction; *P*-value < 0.05) but not with DOMAIN (0.845; *P*-value = 0.54) or SVM (0.875; *P*-value = 1.00) (Table 4.1).

4.3.2 Testing for a shared habitat of New Caledonian basal angiosperms

Similar geographic patterns of basal angiosperm diversity were found at the three taxonomic ranks considered (Fig. 4.3). Occupation of Grande Terre was asymmetrical with regard to a gradient of windwardness (diversity was lower along the drier west coast than the wetter east coast). The area of highest basal angiosperm richness was broadly rhomboidal, oriented northwest-to-southeast whose northwestern angle corresponds to Mont Panié (1,628 m a.s.l.) followed in a clockwise direction by the Massif des Lèvres (1,091 m), Plateau de Dogny (1,000 m) and Mont Aoupinié (1,006 m), where *HO* became maximal (Fig. 4.3A’).

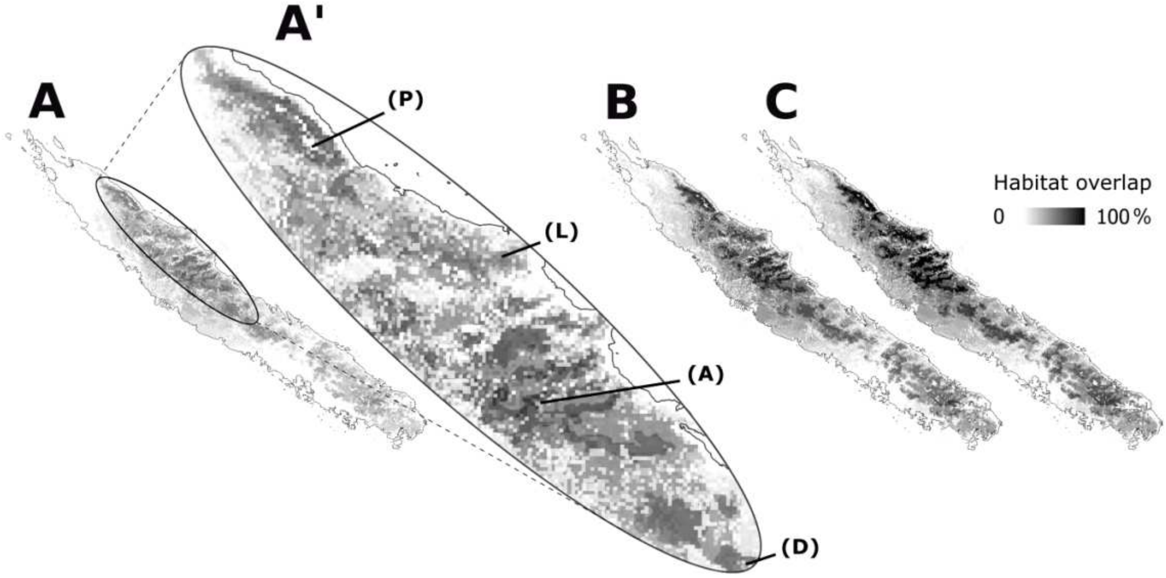

FIGURE 4.3 Distribution of the richness (as expressed by the habitat overlap index) in basal angiosperm species (A and A'), genera (B) and families (C) under current climate. In figure A': P=Mont Panié, L=Massif des Lèvres, A=Mont Aoupinié, D=Plateau de Dogny.

At all taxonomic levels, rainforests harboured a higher proportion of basal angiosperms than any other vegetation type (Fig. 4.4). Sites of highest species richness (*HO* = 62%) were located in low- to mid-elevation rainforests and sites containing the greatest proportion of genera (95%) and families (100%) were almost equally represented in low- to mid-elevation rainforests and in montane rainforests and shrublands. On average, low- to mid-elevation rainforests possessed a greater proportion of basal angiosperm species (38%) as compared to montane rainforests and shrublands (24%; pairwise *t*-test with Bonferroni correction; *P*-value < 0.05). However, the proportion of genera and families was similar in both vegetation types (*P*-value = 0.18 and 0.37, respectively). Low- to mid-elevation shrublands comprised few sites rich in basal angiosperms but on average most of shrubland habitats remained relatively poor. Other vegetation types were more marginally inhabited by basal angiosperms (Fig. 4.4).

Basal angiosperms occurred preferentially in rainforests, with only a small difference between lowland and mountain sites. In addition, elevation did not markedly influence *HO* on BRT plots, although basal angiosperms were slightly more abundant above 1,000 m (Fig. 4.5), especially on Mont Aoupinié and Mont Panié (Fig. 4.3). Suitable habitats converged toward annual precipitation ranging from 1,500 mm to 2,300 mm with a more pronounced abundance of basal angiosperms around 2,000 mm annual precipitation.

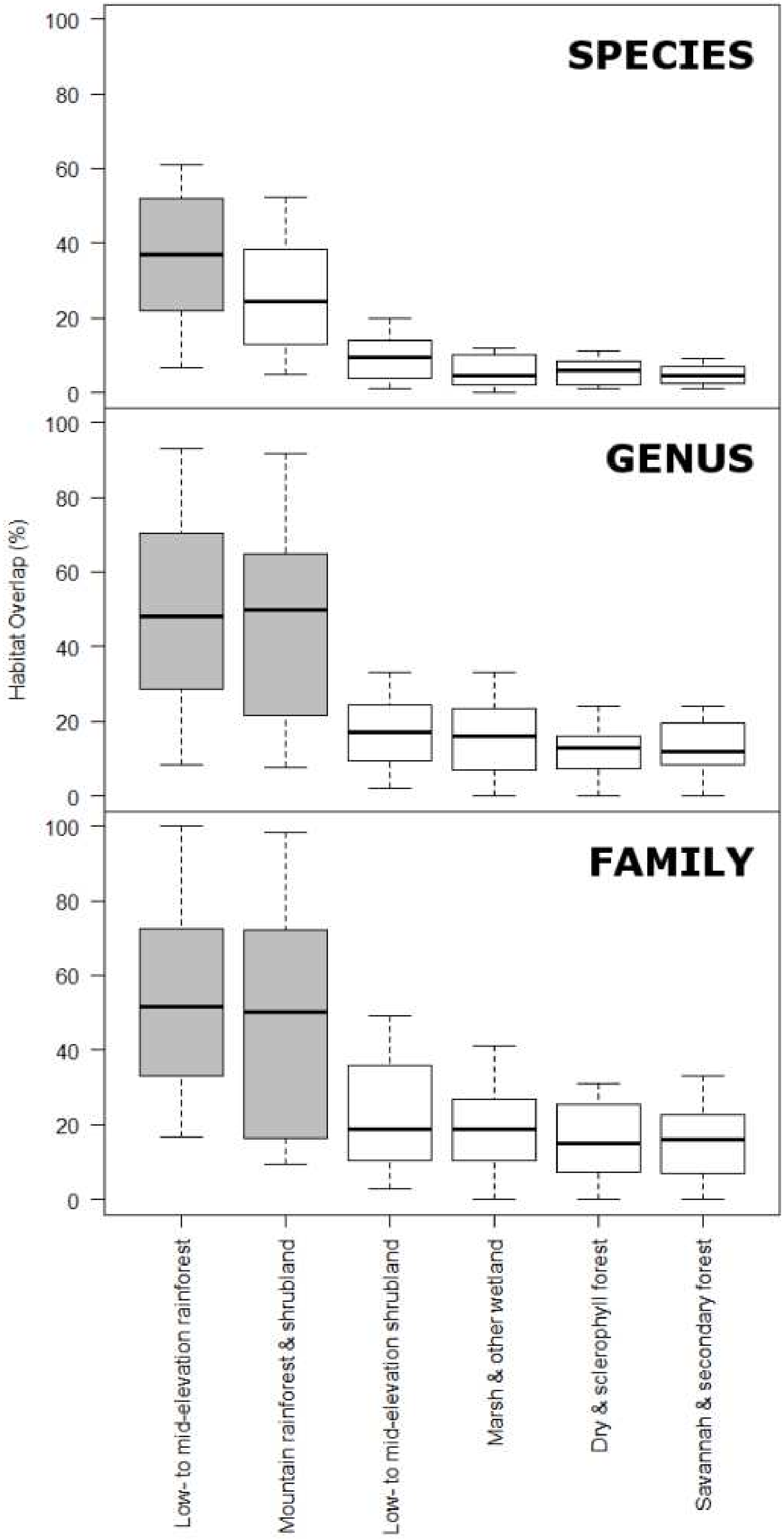

FIGURE 4.4 Distribution of habitat overlap within each vegetation type over the entire territory of New Caledonia (N=23,586 pixels). Shaded boxes show significantly highest habitat overlap at a 5% threshold. Vegetation types are based on Jaffré *et al.* (2012).

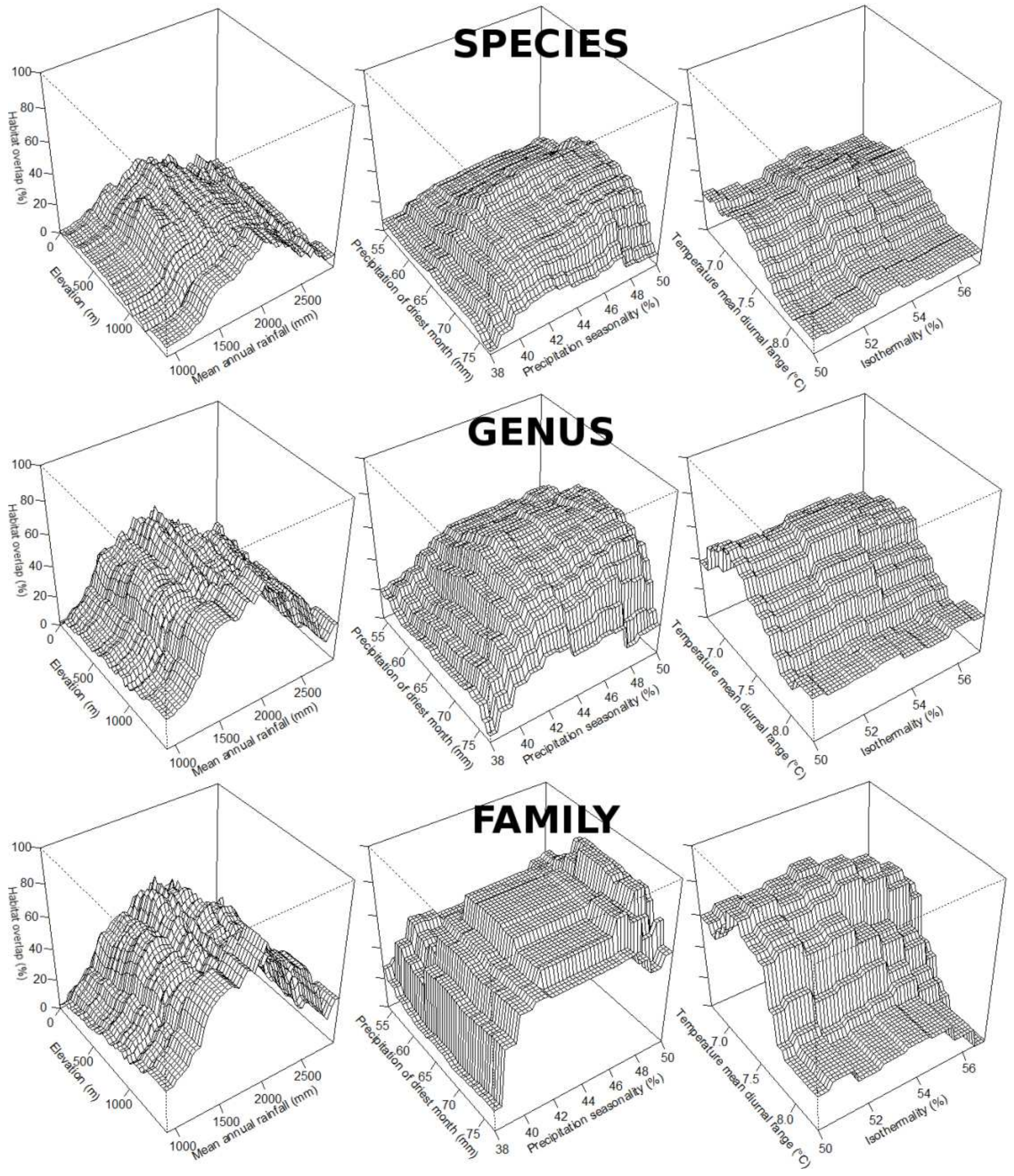

FIGURE 4.5 Joint partial dependence plots of the interactions between habitat overlap and environmental variables fitted with boosted regression trees. Environmental variables are scaled according to their full range in New Caledonia.

SDMs predicted a habitat preference for areas where rainfall was slightly above 60 mm per month during the dry season and where the rainy season was marked as implied by the increased *HO* when precipitation seasonality was between 42% and 50%. In parallel with sensitivity to rainfall regime, basal angiosperm habitats also varied with diurnal variation in temperature. Relict angiosperms did not occur in areas exhibiting variation of diurnal temperature above 7.5°C. However, we found no clear sensitivity of *HO* to diurnal temperature range when expressed as a ratio of the annual range (isothermality).

In relation to edaphic variables, the richest sites in basal angiosperms occurred predominantly on volcano-sedimentary substrates. We observed that 25 species (including both members of the ANA grade), seven genera and four families occurred only on volcano-sedimentary substrates; 16 species, two genera and one family occurred exclusively on ultramafic substrates, no species occurred exclusively on calcareous substrates, and 19 species, nine genera and five families occurred on two or more substrates (Table 4.1). Overall, basal angiosperm species occurred more frequently on volcano-sedimentary substrates (mean *HO* = 20%) than on ultramafic (14%; pairwise *t*-test with Bonferroni correction; *P*-value < 0.05) or calcareous substrates (3%; *P*-value < 0.05) but at supra-specific levels the difference in mean *HO* between volcano-sedimentary and ultramafic substrates gradually decreased and was not statistically significant (*P*-value = 0.72 at the genus level and *P*-value = 1.00 at the family level).

4.3.3 Similarities and differences in habitat

Habitat marginality (*HM*) expresses the degree to which the environment that a basal angiosperm inhabits differs from the most-occupied habitats, i.e. mesic to moist rainforests with slight precipitation and temperature changes and primarily on volcano-sedimentary substrates. The *HM* index was not affected by the number of species per genus (F-test; *P*-value = 0.49; N = 18), the number of species per family (*P*-value = 0.23; N = 10) or the number of genera per family (*P*-value = 0.17; N = 10). Species whose suitable habitat deviated the most from these most-occupied environments were members of the family Lauraceae, including: *Cryptocarya lifuensis* (*HM* = 94%) which is found in dry to mesic forests on sedimentary and calcareous substrates; *Litsea deplanchei* (78%), widespread in shrublands along the west coast of Grande Terre; and *Cryptocarya leptospermoides* (77%), exhibiting a patchy distribution within low-elevation dry to mesic forests and shrublands in northwestern Grande Terre (Fig. 4.6; distribution maps are presented in Appendix A6).

The family Piperaceae was ecologically more marginal (71%) than the locally diverse family Lauraceae (68%). Hernandiaceae were ranked third (67.4%) followed very closely by Annonaceae (66.9%). In contrast, the least marginal species, *Zygogynum pauciflorum* (51%), *Cryptocarya macrodesme* (51%) and *Zygogynum vinkii* (52%), as well as all taxa within the least marginal families, Trimeniaceae (54%), Monimiaceae (56%) and Amborellaceae (59%), were restricted to most-occupied habitats, with the exception of *Hedycarya parvifolia* (Monimiaceae), also found in '*maquis*' vegetation (Fig. 4.6).

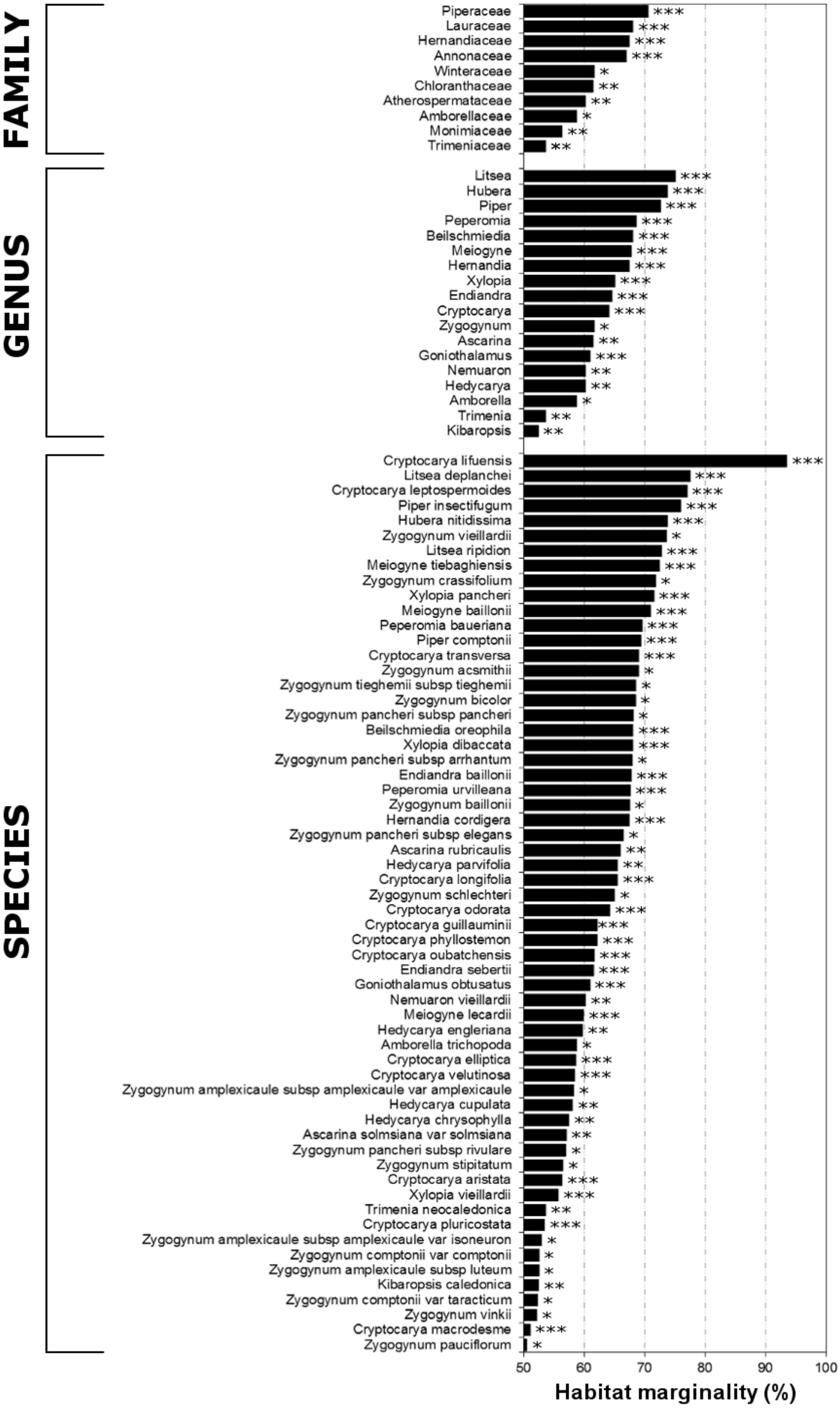

FIGURE 4.6 Habitat marginality of New Caledonian basal angiosperms. Asterisks indicate whether they bear tracheids (*), vessel elements with scalariform perforation plates (**) or vessel elements with simple perforation plates (***) as indicated in Appendix A4.

The four top-ranked families for marginality (Annonaceae, Hernandiaceae, Lauraceae and Piperaceae) represented the only basal angiosperm taxa in New Caledonia bearing simple perforation plates in their vessels (Fig. 4.6; Appendix A4). In contrast, vesselless Amborellaceae and Winteraceae, as well as taxa with scalariform perforation plates (Atherospermataceae, Chloranthaceae, Monimiaceae and Trimeniaceae) were on average the least marginal and tended to be restricted to similar mesic to moist habitats.

2.3.4 Distribution of New Caledonian basal angiosperms at the last glacial maximum

Across New Caledonia, the CCSM and MIROC global circulation models predicted a decrease of annual mean temperature of 1.9°C and 2.1°C and a decrease of annual precipitation of 197 mm and 372 mm, respectively. Under these scenarios, basal angiosperm habitats shifted eastward during the LGM. Mean habitat area also reduced by 44% (CCSM model) to 78% (MIROC model) (Fig. 4.7; Table 4.1). These contractions predicted the formation of two potential refugial areas on the east coast of Grande Terre. Using the CCSM scenario, the northeastern refugium ranged from Mont Panié in the north to Gwâ Rùvianô (1,089 m) in the south, excluding Massif des Lèvres, and the highest species *HO* reached 68% on Mont Aoupinié (Fig. 4.7A). The MIROC scenario yielded a smaller northeastern refugium, located more easterly and ranging from Roche de Ouaïeme in the north to Gwâ Pûûkiriwé (920 m) in the south, but including Massif des Lèvres (Fig. 4.7B). The highest species *HO* was 53% on Görö Tânê (707 m). The southeastern refugium ranged from Petit Borindi in the north to the western Plaine des Lacs in the south according to CCSM, while MIROC again predicted a smaller refugium with Mont Ouinné (362 m) as its northern limit (Fig. 4.7). Overall, these refugial areas overlapped with the restricted distribution of all narrow-range endemic basal angiosperms that currently occur in rainforests (and not in '*maquis*' vegetation) with the only exception of *Cryptocarya bitriplinervia* (Lauraceae) (Table 4.2).

2.4 Discussion

2.4.1 Habitat characteristics of New Caledonian basal angiosperms

We identified a range of rainforest locations along the east coast of Grande Terre, including Mont Aoupinié, Mont Panié, Plateau de Dogny and the Massif des Lèvres, as areas with the most co-occurring basal angiosperm taxa. These sites, hosting up to 62% of basal angiosperm taxa included in the SDMs and almost all genera and families, are characterised by stable wet tropical climates that experience moisture-laden south-east trade winds throughout the year.

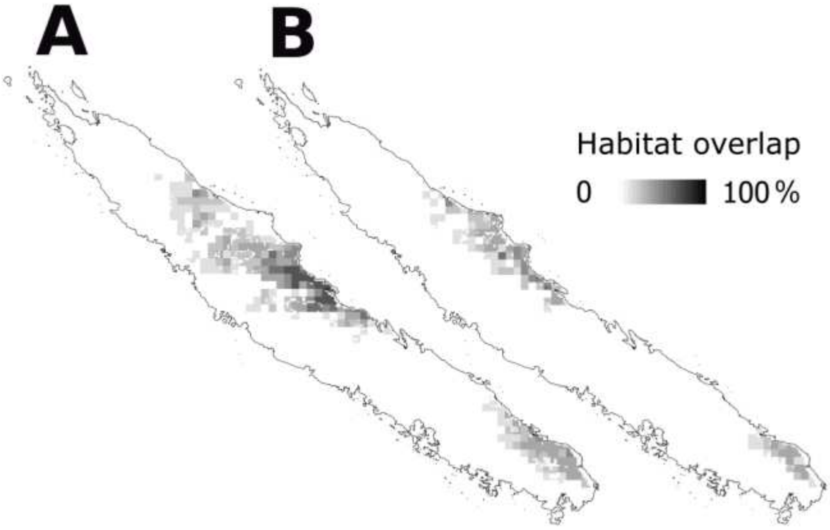

FIGURE 4.7 Distribution of the richness (as expressed by the habitat overlap index) in basal angiosperm species under the last glacial maximum (LGM) climate according to the global circulation models CCSM (A) and MIROC (B).

One could argue that our recovered pattern, with the highest *HO* found in rainforests, does not support the hypothesis that basal angiosperms are adapted to climatically stable rainforests but simply reflects the pattern that rainforests harbour the most species in New Caledonia (Table 4.3). While the maximal *HO* of basal angiosperm species equals the proportion of all New Caledonian vascular plant species found in rainforests (62%) (Morat *et al.*, 2012), the maximal *HO* of basal angiosperm species found in other vegetation types is smaller than expected by chance, which tends to indicate a preference of basal angiosperms for humid environments (Table 4.3). For instance, only one fifth of basal angiosperm taxa occur in low- to mid-elevation shrublands, while this vegetation type hosts a third of the New Caledonian vascular flora (Morat *et al.*, 2012). There are also considerable environmental variations among New Caledonian rainforests. The broad, simplified vegetation categories used in Morat *et al.* (2012) are unlikely to capture more than a single axis of a plant's niche and preclude testing whether species co-occur or occupy different habitats within the same vegetation category. For example, *Zygogynum acsmithii*, *Z. pauciflorum* and *Z. schlechteri* are classified as rainforest specialists by Morat *et al.* (2012) yet their distributions never overlap. Thus, the *HO* index probably underestimates the actual proportion of basal angiosperms present in the rainforest category. A clearer preference of basal angiosperms for rainforests appears to occur at supra-specific ranks since 95% of the genera and 100% of the families co-occur in the same rainforest sites while only 59% of the genera and 70% of the families within the total native vascular flora are found in rainforests (Morat *et al*., 2012).

TABLE 4.3 *Maximal habitat overlap (HO) of basal angiosperms found in the different vegetation types in comparison to the total vascular flora of New Caledonia.*

Taxonomic level	Rainforest	Low- to mid-elev. shrubland	Marsh & other wetland	Dry & sclerophyll forest
Maximal *HO* of basal angiosperms (%) / Proportion of the total vascular flora (%)				
Families	100/70	44/54	41/30	33/41
Genera	95/59	32/39	34/18	24/27
Species	62/62	20/34	20/34	11/10
Over-representation rate				
Families	1.42	0.81	1.36	0.81
Genera	1.60	0.83	1.94	0.91
Species	1.00	0.59	2.30	1.07

Note: secondary forests and anthropogenic grasslands were not presented as they were merged in Jaffré et al. (2012) and divided into two formations in Morat *et al.* (2012) which precludes a comparison.

Similar to Winteraceae and *Amborella*, other basal angiosperm lineages, such as Trimeniaceae and Chloranthaceae, possess functionally and structurally tracheid-like ‘basal vessels’, that are highly obstructive to hydraulic flow *versus* other perforation plate types occurring across most derived angiosperm lineages (Sperry *et al*., 2007; Carlquist, 2012; Feild & Wilson, 2012). These taxa always co-occur with vesselless angiosperms in New Caledonia. Interestingly, we found a similar distributional association for magnoliids bearing vessels with scalariform perforation plates. Overall, the high basal angiosperm diversity that occurs in New Caledonian humid rainforests, where up to 95% of the genera and 100% of the families co-occur, suggests that neither the presence nor absence of vessels is selected for or against in such habitats. In contrast, taxa bearing vessels with simple perforation plates displayed distributions that extended to dry and mesic forests or open shrublands (Table 4.1; Fig. 4.2). Relict angiosperms, and especially those possessing drought sensitive xylem hydraulic characteristics (Feild & Wilson, 2012), appear so constrained by vegetation and climatic conditions that their abundance on *Grande Terre* attests to the prolonged climatic stability in the region since the time they reached New Caledonia. Such a conclusion is strengthened by dated phylogenetic trees of other rainforest-specialist lineages in the families Nothofagaceae, Proteaceae and Arecaceae suggesting that rainforests have probably been continuously present in New Caledonia for the last 7 My (Pillon, 2012).

2.4.2 Reasons for basal angiosperm over-representation in New Caledonia

Because ultramafic substrates represent an ecologically challenging environment for many plants, such substrates have been posited as a barrier to taxa immigrating to New Caledonia from nearby non-ultramafic regions (Jaffré *et al.*, 1987; Pelletier, 2006). Although only covering a third of Grande Terre today, ultramafic substrates have been shown to host an equivalent number of species and more endemics than volcano-sedimentary substrates, which cover almost two thirds of the island (Jaffré, 1993). However, we found no evidence that such a hypothesis is supported for the majority of basal angiosperms. New Caledonian basal angiosperms occur primarily on volcano-sedimentary substrates. Rainforests, their preferential habitats, are two times more prevalent on volcano-sedimentary substrates than on ultramafics (Fritsch, 2012; Jaffré *et al.*, 2012). Thus, we suspect that instead of reflecting an affinity for a particular substrate, the substrate distribution of basal angiosperms signals a predominant preference for rainforest habitats where accumulated soil organic matter buffers the ecological effects of underlying bedrock. In contrast, the drought-feeble xylem hydraulic systems of most basal angiosperms may be more constraining in more open and drier ultramafic shrublands. The hypothesis that basal angiosperms would be better adapted to ultramafic substrates than derived angiosperm colonists should be rejected. Our study therefore provides evidence that ultramafics are not the only driver of disharmony in the New Caledonian flora. Our recovered pattern is clearly distinct from the view drawn from gymnosperm and some other angiosperm lineages which are more diverse in the south of Grande Terre on ultramafic substrates (Jaffré *et al.*, 2010; Pillon *et al.*, 2010).

CCSM and MIROC climate simulations appeared to model well the LGM distribution of basal angiosperms in New Caledonia. We conclude this because the modelled refugia of basal angiosperms overlaid with a number of Pleistocene rainforest refugia previously proposed. Indeed, inferences based on the current distribution of New Caledonian palm micro-endemism suggested four Pleistocene rainforest refugia on the east half of Grande Terre (Pintaud *et al.*, 2001). More recently, LGM projections of *Amborella* distribution supported by genetic evidence revealed that Pleistocene rainforest refugia overlapped with those hypothesized from palm chorology (Poncet *et al.*, 2013). Moreover, our results are supported by palynological records showing a continuous presence of rainforest on the Plaine des Lacs since *c*. 90,000 BP (Stevenson and Hope, 2005).

The distribution of narrow-range endemics belonging to basal angiosperm lineages also supports the location of refugia that we inferred through SDM. In New Caledonia, narrow-range endemism has widely been interpreted as a result of substrate specialisation in the past. In Nattier *et al*. (2013), all species in the grasshopper genus *Caledonula* occur in the southern half of Grande Terre in association with reduced seasonality (BIO 15 < 42%). In our study, most basal angiosperms have poor substrate specialisation and are found in the north-east of the island in association with substantial seasonality (BIO 15 > 42-50%). The confrontation of both studies using two different biological models highlights the importance of climate seasonality as another probable driver of local endemism in New Caledonia. Interestingly, most of narrow-range endemics also remain restricted to rainforests, with the exception of taxa bearing vessels with simple perforation plates (Lauraceae) that exhibit wider habitat breadth. Environmental requirements of basal angiosperms that we identified from the most prevalent half of species occurring in New Caledonia thus appear to apply to *c*. 75% of New Caledonian basal angiosperm taxa.

Our study suggests that Pleistocene rainforest refugia might have influenced the current over-representation of basal angiosperms in New Caledonia by buffering them from recent major climate-driven extinction events that more strongly affected Australia and some nearby South Pacific islands (Byrne *et al.*, 2011). Several other regions of the Pacific where basal angiosperms are also abundant are also recognised for the persistence of rainforests during the late Quaternary through glacial refugia such as in south-east Asia, New Guinea and Queensland (Bowler *et al.*, 1976; Wurster *et al.*, 2010; Byrne *et al.*, 2011).

CHAPTER 5

Drought vulnerability as a major driver of the distribution of basal angiosperms in New Caledonia[1]

- Evaluation of basal angiosperms trait-environment relations
- Drought sensitivity of basal angiosperms
- Effect of xylem conduit type on drought resistance

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[1] A version of this chapter is in **preparation** for submission to *New Phytologist*. Trueba, S., Pouteau, R., Lens, F., Feild, T.S., Isnard, Olson, M.E., Delzon, S. (2016). *in preparation*

Abstract

Basal angiosperm species in New Caledonia are mainly distributed in rainforest habitats. Moreover, these habitats may have acted as potential past refugia for basal angiosperm species from the archipelago. A main hypothesis is that basal New Caledonian basal angiosperm species are restricted to rainforest habitats because of a high vulnerability to drought-induced hydraulic failure. In the current context of climate change, increases in drought-induced tree mortality are being observed in tropical rainforests worldwide, and are also likely to affect the geographical distribution of tropical vegetation. However, the mechanisms underlying the drought vulnerability and environmental distribution of tropical species have been little studied.

We measured vulnerability to xylem embolism (P_{50}) of 13 tropical basal angiosperm rainforest species endemic to New Caledonia with different xylem conduit morphologies. We examined the relation between P_{50} and a range of habitat variables, along with other commonly measured leaf and xylem functional traits.

Selected basal angiosperm species had P_{50} values ranging between -4.03 and -2.00 MPa with most species falling in a narrow resistivity range above - 2.7 MPa. Embolism vulnerability was significantly correlated with elevation, MAT, and percentage of species occurrences in rainforest geographic areas. Xylem conduit type did not explain variation in P_{50}. Commonly used functional traits such as wood density and leaf traits were not related to P_{50}.

Xylem embolism vulnerability behaves as a physiological trait closely associated with the distribution of basal angiosperm species in rainforests. Our study suggests that ecological differentiation in P_{50} is decoupled with wood density in rainforest species, meaning that evolutionary paths between hydraulic safety and wood density are not forced to coordinate in wet habitats. Finally, we underscore the high conservation risk of basal angiosperm species from montane rainforest localities in New Caledonia.

Keywords: basal angiosperms, drought resistance, embolism vulnerability, functional traits, leaf vein density, rainforest, vesselless angiosperms, wood density, xylem conduit.

5.1 Introduction

The previous chapter has shown that basal angiosperms in New Caledonia have a preference for rainforest habitats. Moreover, rainforests appear to have acted as refugia for basal angiosperm diversity in the archipelago (Pouteau *et al.*, 2015). A major hypothesis is that drought sensitivity may restrict most basal angiosperm species to humid and stable habitats. However, the persistence of this particular habitat may be threatened by changes in climate. Climate projections predict changes in rainfall regimes and soil moisture, forecasting more severe and widespread droughts in many areas (Dai, 2013). Global changes in rainfall, combined with increased temperature, are likely to cause tree mortality and biogeographic shifts in vegetation in many parts of the world (Adams *et al.*, 2009; Allen *et al.*, 2010). Moreover, strong shifts in rainfall are expected to affect forest areas of tropical regions (Chadwick *et al.*, 2015). Given that an increase in tropical rainforest tree mortality due to water stress has already been observed (Phillips *et al.*, 2010) and because most species are operating within a narrow hydraulic safety margin (Choat *et al.*, 2012), the survival and distribution of tropical rainforest species clearly seem threatened by drought.

A major goal in plant ecology is to understand the links between functional traits and species distribution (Violle & Jiang, 2009). However, while the distribution of plant species along environmental gradients has been well documented, the plant traits and physiological mechanisms driving distributions of tropical species are poorly known (Engelbrecht *et al.*, 2007). The identification of plant traits underlying the distribution of species along environmental gradients can be very important in the selection of highly informative key ecological traits (Westoby & Wright, 2006). Detecting relevant plant traits and their interactions with environmental variables is especially relevant for understanding the likely fate of current vegetation types in the context of global climate change (Breshears *et al.*, 2005; Allen *et al.*, 2015). Therefore, analyzing key ecophysiological traits related to drought vulnerability and plant water use is essential in understanding current and projected future distributional patterns of plant species in tropical rainforests.

According to the tension-cohesion theory, water transport through xylem is initiated by surface tension during leaf transpiration and the integrity of the water column is maintained by cohesion between the water molecules and adhesion between the water column and xylem conduit walls (Tyree, 1997; Tyree & Zimmermann, 2002). Water movement is prone to dysfunction because significant decreases in xylem pressure can be caused during a drought event. Subatmospheric xylem pressures can disrupt the cohesion between the water molecules, producing gas bubbles by cavitation (Tyree & Sperry, 1989). Cavitation may result in large embolisms inside the xylem conduits, consequently reducing water flow. According to the air-seeding hypothesis, such embolisms can propagate from a non-functional conduit to a functional conduit through the lateral intervessel pits (Tyree & Zimmermann, 2002; Cochard *et al.*, 2009; Delzon *et al.*, 2010). Plant drought resistance can be assessed by measuring vulnerability to xylem embolism, and P_{50}, the negative pressure at which 50% of hydraulic conductivity is lost, is a commonly used parameter in ecophysiological research. It has been shown that vulnerability to embolism (hydraulic failure) is strongly related to drought-induced mortality in gymnosperm and woody angiosperm species in both temperate and tropical forests (Brodribb & Cochard, 2009; Urli *et al.*, 2013; Barigah *et al.*, 2013; Rowland *et al.*, 2015).

Given the strong selective force exerted by water stress on vegetation (Brodribb *et al.*, 2014), the distribution of plant species along environmental gradients can be expected to be strongly influenced by their vulnerability to xylem embolisms (Pockman & Sperry, 2000). Global meta analyses have shown that embolism vulnerability (P_{50}) is related to climate variables such as mean annual precipitation (MAP) and mean annual temperature (MAT) (Maherali *et al.*, 2004; Choat *et al.*, 2012). These studies have shown that angiosperms tend to have less negative P_{50} values than gymnosperms, and are thus more vulnerable to xylem embolism. Moreover, within the angiosperms, tropical evergreen taxa native to high rainfall areas are among the most vulnerable species. Because of the relation between embolism resistance and habitat occupation, it has been suggested that xylem embolism vulnerability can be useful for distinguishing plant adaptive strategies (Lens *et al.*, 2013; Anderegg, 2015). However, tropical rainforest angiosperms, occurring in high rainfall habitats with MAP above 2000 mm are currently poorly studied. Among the rainforests of the world, the ecology of wet rainforests of high-elevation islands is among the least documented in spite of their high percentage of endemic species and the vulnerability of their floras (Loope & Giambelluca, 1998; Kier *et al.*, 2009; Harter *et al.*, 2015). In this study, we analyze embolism vulnerability of basal angiosperm rainforest species and its relation to environmental distribution in New Caledonia, a megadiverse and highly endemic oceanic archipelago.

Because of its high levels of species richness and endemism, New Caledonia is recognized as a global biodiversity hotspot (Myers *et al.*, 2000). Previous work has shown that biodiversity hotspots are highly vulnerable to climate change, reinforcing their status as global conservation priorities (Malcolm *et al.*, 2006; Bellard *et al.*, 2014). A characteristic feature of the New Caledonian flora is an over-representation of basal angiosperms (Pillon, 2012). We have demonstrated that *Amborella trichopoda* Baill. (Amborellaceae) along with some 60 other basal angiosperm species endemic to New Caledonia, have a high environmental niche overlap in habitats with low evaporative demand characterized by moderate diurnal variations in temperature (≤ 7°C) and MAP greater than *c.* 2000 mm year^{-1} (Pouteau *et al.*, 2015). However, the ecophysiological mechanisms behind this habitat distribution, and in particular the potential role of vulnerability to drought-induced xylem embolism, have not been investigated.

Interestingly, these species have a broad diversity of xylem conduit anatomies that range from vesselless, tracheid-only wood, to vessel-bearing woods with scalariform and simple perforation plates (Fig. 5.1). The wood of some of these taxa (vesselless wood or woods with narrow vessels bearing long scalariform perforation plates) is thought to resemble conditions primitive with respect to the majority of the angiosperms, which have short, wide vessel elements with simple perforation plates (Hacke *et al.*, 2007; Carlquist, 2012; Olson, 2012). It has been hypothesized that the evolution of vessels may have provided angiosperms with increased hydraulic efficiency compared to wood comprising only tracheids (Carlquist, 1975; Sperry, 2003). However, a possible developmental trade-off during early vessel evolution may have increased vulnerability to drought-induced xylem embolism (Sperry, 2003). Sperry *et al.* (2007) assessed this hypothesis and showed that vulnerability to embolism was higher in species with vessel elements with scalariform perforation plates than in vesselless angiosperms, suggesting that early vessel evolution may have been limited to wet habitats due to the risk of hydraulic failure in drier environments.

Beyond xylem conduit structure, wood and leaf traits have also been suggested as being linked with resistance to xylem embolism or drought tolerance. Wood structural investment, as quantified in part by wood density (WD), has been suggested to be a predictor of drought tolerance, given that some studies found WD to be negatively related to P_{50} (Hacke *et al.*, 2001; Chave *et al.*, 2009; Delzon *et al.*, 2010; Markesteijn *et al.*, 2011). Leaf dry mass per unit leaf area (LMA) has been suggested as another key trait reflecting leaf and whole plant function (Poorter *et al.*, 2009). It has also been suggested that drought tolerance increases with LMA across species (Poorter *et al.*, 2009). Therefore, a reasonable prediction might be that LMA should be negatively related to embolism vulnerability in rainforest species. Another leaf trait, leaf vein density (VD), has also been suggested to be related to species geographic distribution (Blonder & Enquist, 2014). Moreover, it has been shown that higher values of VD are observed in species growing in sites of higher evaporative demand (Sack & Scoffoni, 2013). As a consequence, a negative relationship between VD and P_{50} can be expected. Our work aims to: i) test the relationship between xylem vulnerability to embolism and distribution of basal angiosperm rainforest species along environmental gradients; ii) test for differences in xylem function between vesselless and vessel-bearing species; and iii) assess the association of commonly used functional traits such as wood density, leaf mass per area and leaf vein density with xylem embolism vulnerability in rainforest species. Explaining the environmental distribution of insular rainforest species by their trait values could help to identify the main organismal attributes that allow ecological differentiation by drought resistance in this rich and vulnerable biome.

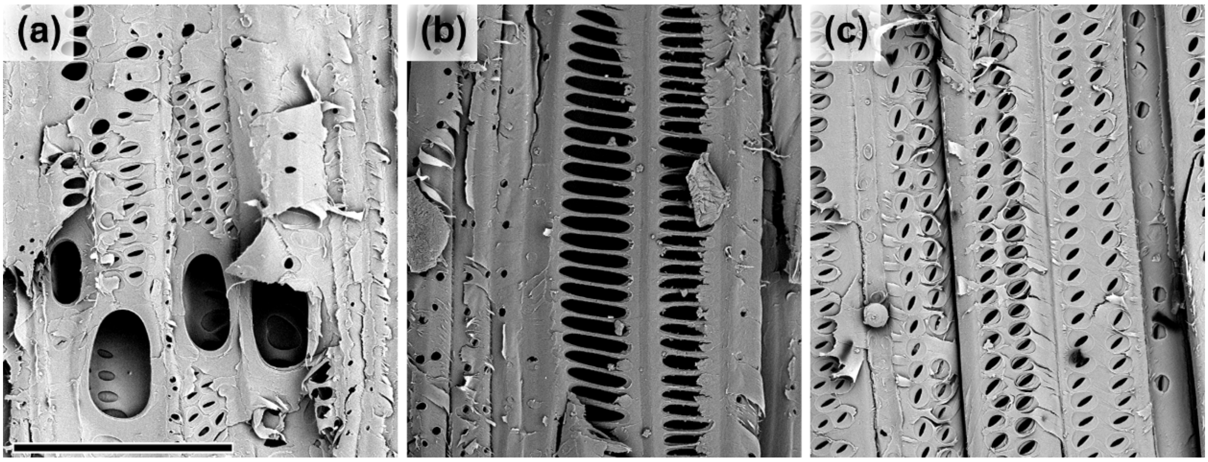


FIGURE 5.1 Illustration of basal angiosperm xylem conduit morphologies as seen in radial view with scanning electron microscopy. (A) Vessel elements with simple perforation plates in *Cryptocarya aristata*. (B) Vessel elements with scalariform perforation plates in *Hedycarya cupulata*. (C) Tracheids with many, distinctly bordered pits in *Zygogynum crassifolium*. Scale bar, 80 µm for a and b, 100 µm for c.

5.2 Materials and Methods

5.2.1 Study site, plant material, and sampling

New Caledonia is an archipelago located north of the Tropic of Capricorn in the southwest Pacific Ocean (Fig. 5.2 inset). The main island (*Grande Terre*, 16 000 km^2) has a central mountain range that runs along the entire island. The highest points are Mt. Panié (1628 m) in the north (Fig. 5.2) and Mt. Humboldt (1618 m) in the south. New Caledonia has a tropical climate with a marked dry season from June to November. As a consequence of *Grand Terre*'s topography and the resulting rain shadow effect, MAP ranges from 800 mm yr^{-1} along the western coastal plains to 4500 mm yr^{-1}on the eastern slopes of the mountain chain (Météo-France, 2007) (Fig. 5.2). Mean annual temperature in lowland areas is between 27 and 30 °C but varies along the elevational gradient with an environmental lapse rate of *c.* 0.6°C /100 m elevation (Maitrepierre, 2012). *Grande Terre* is mainly covered by substrates derived from volcano-sedimentary rocks but the southern third of the island and some isolated massifs of the northwestern coast have substrates derived from ultramafic rocks (Fritsch, 2012). A combination of climate, substrate, and human-induced disturbance determines the presence of different vegetation types in New Caledonia. Terrestrial vegetation types are commonly classified into low-elevation scrubland (known as *maquis*), savanna, rainforest, summit shrubland, and dry sclerophyll forest. Rainforest, the most species-rich vegetation type, with more than 2,000 native vascular plant species, now covers *c.* 3,800 km^2 on the main island (Birnbaum *et al.*, 2015) (Fig. 5.2). The diversity of habitats in New Caledonia, along with the extensive cover of rainforest, provide an ideal context for testing changes of plant traits over environmental gradients.

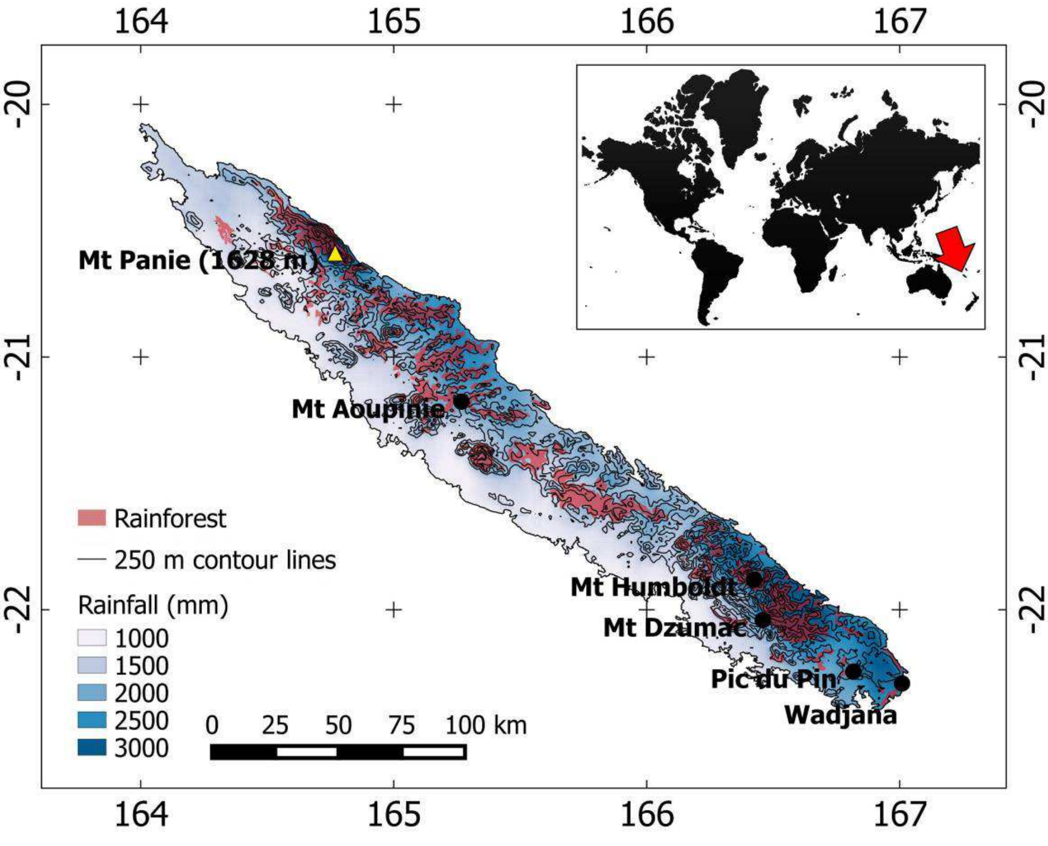


FIGURE 5.2 Map of New Caledonia with rainforest, elevation, and rainfall distributions along the main island. Sampled rainforest localities are indicated in the map. See figure 1.7 for a detailed representation of each environmental variable.

TABLE 5.1 *Xylem embolism vulnerability parameters, and functional traits of 13 insular basal angiosperm rainforest species.*

Species	**Family**	**Conduit type**	P_{12} (MPa)	P_{50} (MPa)	P_{88} (MPa)	**Slope** (% MPa^{-1})	**WD** (g cm^{-3})	**VD** (mm mm^{-2})	**LMA** (g m^{-2})
Amborella trichopoda	Amborellaceae	tracheid	-2.3	-2.7	-3.0	177.2	0.508	3.7	93.1
Ascarina rubricaulis	Chloranthaceae	vessel (scal)	-1.6	-2.2	-2.9	97.5	0.472	4.7	106.7
Cryptocarya aristata	Lauraceae	vessel (sim)	-0.8	-2.0	-3.2	43.9	0.542	8.6	174.9
Hedycarya cupulata	Monimiaceae	vessel (scal)	-1.5	-3.2	-4.9	31.1	0.540	5.4	76.2
Hedycarya parvifolia	Monimiaceae	vessel (scal)	-2.0	-3.1	-4.1	48.8	0.590	6.3	112.0
Kibaropsis caledonica	Monimiaceae	vessel (scal)	-1.6	-2.4	-3.3	65.8	0.682	5.8	117.2
Nemuaron viellardii	Atherospermataceae	vessel (scal)	-1.4	-2.3	-3.2	61.8	0.629	5.3	173.1
Paracryphia alticola	Paracryphiaceae	vessel (scal)	-0.8	-2.1	-3.4	40.2	0.630	2.3	86.0
Quintinia major	Paracryphiaceae	vessel (scal)	-0.9	-2.5	-4.0	33.3	0.652	7.5	214.5
Zygogynum acsmithii	Winteraceae	tracheid	-2.4	-2.7	-3.0	173.0	0.583	4.8	142.9
Zygogynum crassifolium	Winteraceae	tracheid	-3.6	-4.0	-4.5	125.8	0.674	5.8	309.3
Zygogynum stipitatum	Winteraceae	tracheid	-2.2	-2.4	-2.7	214.0	0.455	4.8	85.7
Zygogynum thieghemii	Winteraceae	tracheid	-1.9	-2.2	-2.5	168.2	0.608	5.0	216.3

Note: "scal" means that the vessels consist of elements with scalariform perforation plates; "sim" refer to vessel elements with simple perforation plates.

We studied xylem embolism vulnerability of 13 woody rainforest species endemic to New Caledonia (Table 5.1). Species were selected to represent a diversity of xylem conduit anatomies (Fig. 5.1). Additionally, we based our sampling on the results presented in Chapter 4 to represent New Caledonian basal angiosperm species with different levels of habitat marginality (*i.e.* occupation of distinct habitats). Our sampling also included two rainforest phylogenetically basal eudicots with long scalariform perforation plates (Paracryphiaceae; Table 5.1), a condition though to be primitive with respect to the simple perforation plates found in most angiosperms. The diversity of xylem conduit morphologies spanned by our sampling enabled us to test possible differences in embolism vulnerability between co-occurring vessel-bearing and vesselless angiosperms. Individuals were collected at five rainforest locations of *Grande Terre*: Mt. Aoupinié, Mt. Dzumac, Mt. Humboldt, Pic du Pin, and Wadjana (Fig. 5.2). Maximum vessel lengths were assessed on five branches per species by injecting air at 2-bars and cutting the distal end of the water-immersed stem section until the air bubbles emerged. This procedure allowed us to select species with suitable vessel lengths to avoid a potential open-vessel artifact (Martin-StPaul *et al.*, 2014).

5.2.2 Measurements of embolism vulnerability

We collected 15 sun exposed branches per species. Branches were immediately defoliated and wrapped in moist paper, sealed in plastic bags and stored in the dark for transport. Prior to taking measurements, branches were debarked and cut to a standard length of 27 cm. Xylem embolism resistance was measured using a Cavitron, a centrifugation-based apparratus that lowers the negative pressure in xylem segments while simultaneously measuring hydraulic conductance (Cochard, 2002; Cochard *et al.*, 2005). We followed Delzon *et al*. (2010) for the test procedure. The percentage loss of conductance (PLC) of the stems was measured in 0.5 MPa pressure steps using the software Cavisoft v4.0, which calculates PLC with the following equation:

$$PLC\ = 100\ \times \left(1 - \frac{K}{K_{max}}\right)$$

where K is the stem conductance at a given pressure and K_{max} (m^2 MPa^{-1} s^{-1}) is the maximum conductance of the stem, calculated under xylem pressures close to zero. Using the increase in PLC with decreasing pressure, we can produce vulnerability curves (VC) for each branch (Fig. 5.3). VCs were fit with a sigmoid function (Pammenter & Van der Willigen, 1998) using the next equation:

$$PLC = \frac{100}{\left[1 + \exp\left(\frac{S}{25} \times (P - P_{50})\right)\right]}$$

Where S (% MPa^{-1}) is the slope of the vulnerability curve at the inflexion point and P_{50} (MPa) is the xylem pressure inducing 50% loss of conductance. The slope of the vulnerability curve (S) is a good indicator of the speed at which embolisms affect the stem (Delzon *et al.*, 2010). Additionally, we used our VCs to calculate P_{12} and P_{88}, which are respectively the 12% and 88% loss of conductance. P_{12} and P_{88} are physiologically significant indexes given that they are thought to respectively reflect the initial air-entry tension producing embolisms, and the irreversible point of no return (Urli *et al.*, 2013).

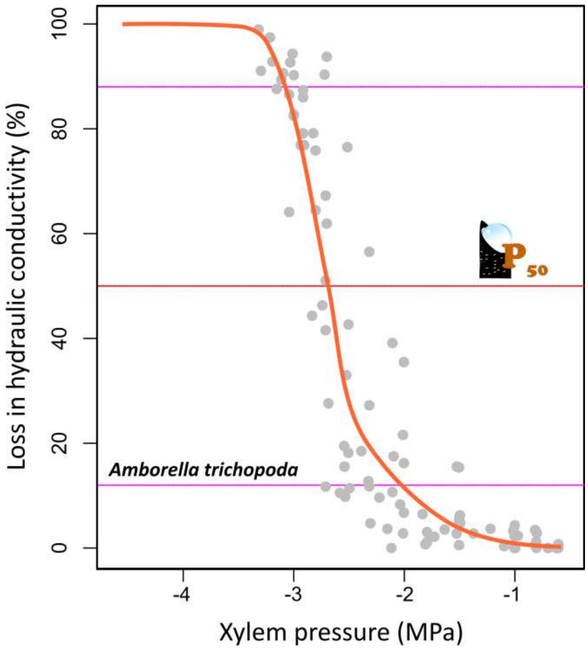


FIGURE 5.3 Illustration of a vulnerability curve (VC). VC of *Amborella trichopoda* showing decreasing hydraulic conductivity with decreasing xylem pressure. P_{12}, P_{50}, and P_{88} are indicated with horizontal lines. VCs were produced for 13 species, mean values are available in Table 5.1.

5.2.3 Measurements of stem and leaf functional traits

Wood density (WD, g cm^{-3}) was calculated using 4 cm long wood segments, from five branches sampled for embolism resistance measurements. Wood volume was calculated using the water displacement method. We oven-dried wood samples at 70°C for a minimum of 72 h until constant mass. WD was calculated as dry mass over fresh volume. We measured leaf mass per area (LMA, g m^{-2}) on 15 leaves, petioles included, borne by the branches used for embolism vulnerability measurements. Leaves were scanned using a portable scanner

(CanoScan LiDE 25, Canon, Japan). Leaf area was calculated from the scanned images using ImageJ 1.47v. (NIH Image, Bethesda, MD, USA). Leaves were then oven-dried at 70°C for 72 h, and weighted for LMA calculations. LMA was calculated as the leaf dry mass over leaf area. Leaf vein density (VD in mm mm^{-2}), also known as leaf vein length per unit leaf area, was measured on five additional leaves. Sections of leaf tissue (*c*. 2 cm^2) were cut from the middle third of the lamina. Leaf sections were cleared in 5% NaOH and rinsed with distilled water. Clearing time varied from 20 to 72 hours depending on the species. After clearing, leaf veins were stained using 0.1% aqueous toluidine blue for 5-10 min, and mounted in a glycerol solution. We imaged the mounted sections at 5x using a light microscope (Leica DM5000B; Leica Microsystems, Wetzlar, Germany). Vein lengths on digital images were measured using ImageJ 1.47v.

5.2.4 Species environmental distribution

Species distribution was obtained from occurrence records in two datasets: (1) the New Caledonian Plant Inventory and Permanent Plot Network (NC-PIPPN) made up of 201 plots measuring 20 m x 20 m (Ibanez *et al.*, 2014), and 8 additional plots measuring 100 m x 100 m distributed across rainforests of *Grande Terre*; and (2) voucher specimens of the Herbarium of the IRD Center of Noumea (NOU). The mean number of occurrences per species was 71. The species with the fewest number of collections was *Zygogynum acsmithii* (Winteraceae) with 14 occurrences and the most collected species was *Hedycarya cupulata* (Monimiaceae) with 171 occurrences. When several occurrences were located within a distance of 500 m, we kept a single occurrence positioned at the centroid to avoid overweighting locations that have been oversampled. For each location, five environmental metrics were computed: (1) MAP to test whether the association between P_{50} and rainfall observed at a global scale (Maherali *et al.*, 2004; Choat *et al.*, 2012) also applies at the island-wide scale; (2) MAT to test whether the association between P_{50} and temperature observed at a global scale (Maherali *et al.*, 2004; Choat *et al.*, 2012) applies at the island-wide scale; (3) mean temperature of the driest quarter (MTDQ) to quantify species tolerance to drought and heat stress that peaks during the driest period characterized by a high evaporative demand; (4) elevation as a driver of the fine-scale distribution of climate on high-elevation islands; and (5) the frequency of occupation of rainforest habitats as a proxy of micro-climatic conditions, such as local water availability, light exposure, and disturbance regimes tolerated by the selected species. MAP data were extracted from a 1 km-resolution grid produced by Météo-France through the AURELHY model by interpolating rainfall records from 1991 to 2000

(Météo-France, 2007). MAT and MTDQ data were extracted from the WorldClim database (Hijmans *et al.*, 2005). Elevation was derived from a 10 m-resolution digital elevation model (DEM) provided by the *Direction des Infrastructures, de la Topographie et des Transports Terrestres* (DITTT) of the Government of New Caledonia. Each location was associated with the appropriate pixel on which it was centered. Finally, percentage of occurrence in rainforest was estimated using a vegetation map in the form of a shapefile published in the Atlas of New Caledonia (Jaffré *et al.*, 2012).

5.2.5 Data analysis

To recognize groups of species with similar embolism vulnerabilities, we used one-way ANOVAs with post-hoc Tukey's HSD using 95% confidence intervals to compare P_{50} values, along with other embolism vulnerability indexes across species. Independent *t*-tests were used to compare embolism vulnerability parameters between vessel-bearing and vesselless species. Linear regressions were used to determine the relationship of P_{50} with environmental data. Regression lines are shown only when relationships were significant. Pearson's correlation analyses were used to evaluate the relationship between xylem embolism vulnerability, leaf and stem functional traits, and environmental correlates of species distribution. Correlations were considered as significant at $P < 0.05$. Finally, to place the New Caledonian rainforest species in a global perspective, we combined our data with the worldwide dataset of Choat et al. (2012). To confirm a link between MAP and P_{50} at a global scale, we estimated this relationship by assessing a linear relationship on $\log_{10}$-transformed data. To facilitate the $\log_{10}$ transformation of P_{50} values, we used the method of Maherali et al. (2004) converting P_{50} values from negative to positive prior to data transformation. All analyses were performed using R v.3.1.2 (R Development Core Team, 2014).

5.3 Results

5.3.1 Xylem embolism vulnerability of New Caledonian basal angiosperms

P_{50} varied two-fold across species (Fig. 5.4), with significant inter-specific variation (F = 28.34; P < 0.001) (Fig. 5.4). Similar significant variation in P_{12} (F=30.63; P < 0.001), P_{88} (F=23.54; P < 0.001), and vulnerability curve slopes (F=14.82; P < 0.001) was observed across species. Mean P_{50} of rainforest angiosperms was -2.60 MPa, with most species falling into a narrow range of P_{50} values between -2.0 and -2.7 MPa (Fig. 5.4). The highest P_{50} was -2.0 MPa for *Cryptocarya aristata.* Both *Hedycarya* species had a similar vulnerability level with P_{50} more negative than -3.0 MPa. *Zygogynum crassifolium* had the lowest P_{50}, at -4.03 MPa, standing out from the rest of the species (Fig. 5.4). Slopes of the vulnerability curves, which reflect the rate at which embolisms occur, varied seven-fold across species (Table 5.1), with the lowest slope of 31 % MPa^{-1} recorded for *Hedycarya cupulata*, and the steepest slope of 214 % MPa^{-1} measured in *Zygogynum stipitatum*. The three embolism vulnerability indexes measured (P_{12}, P_{50}, P_{88}), which indicate xylem tensions at which different percentages of hydraulic conductivity are lost, all correlated well with one another. P_{50} was significantly correlated with P_{12} (r = 0.76; P = 0.002) and P_{88} (r = 0.72; P = 0.004), suggesting that embolism vulnerability acts similarly at different drought intensities. P_{50} was not correlated with the cavitation curve slope (r = -0.05; P = 0.85), suggesting that the speed of embolism occurrence is not related to embolism resistance across the sampled species.

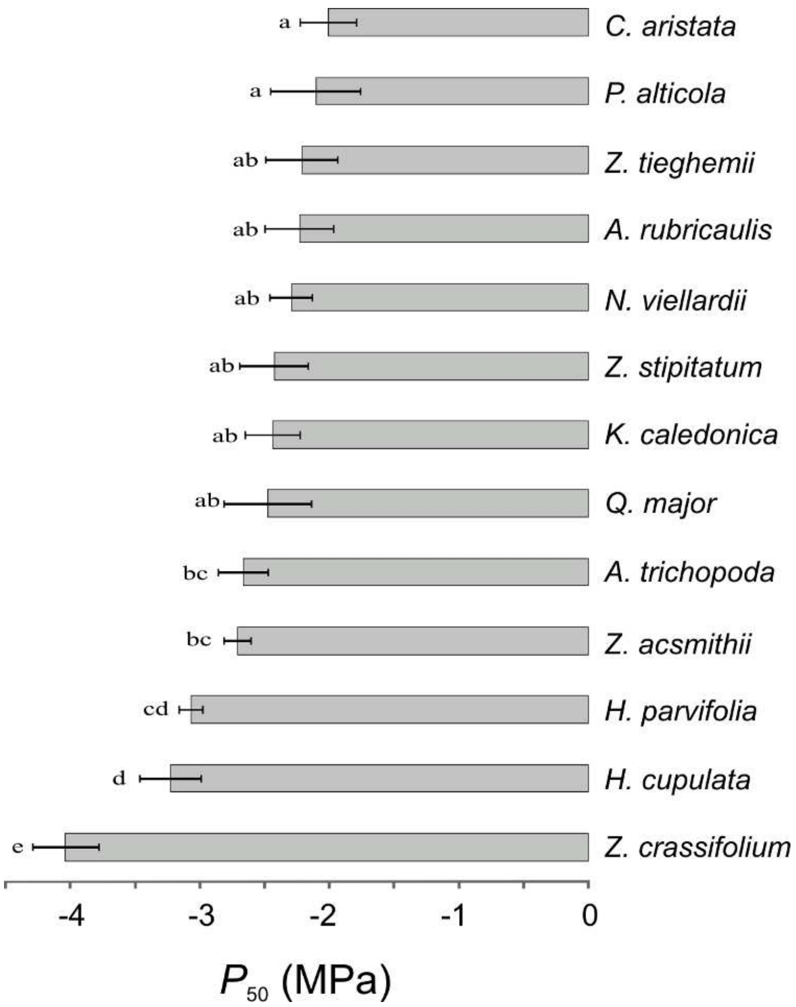


FIGURE 5.4 P_{50} , xylem pressure inducing 50% loss in conductance, of 13 basal angiosperm species. Different letters indicate significant differences between species at P < 0.05. Standard errors are represented by bars.

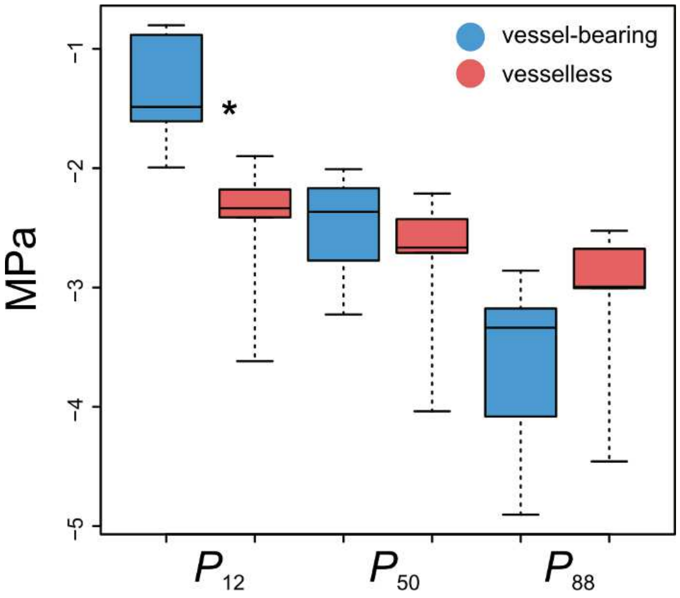


FIGURE 5.5 Box plot of embolism vulnerability indexes for vessel-bearing species, and vesselless species. Boxes show the median, 25th and 75th percentiles, and bars indicate maximum/minimum values. Significant differences of embolism vulnerability indexes at $P \leq 0.05$ between conduit morphologies are indicated with an asterisk.

Mean P_{50} of vessel-bearing species (-2.48 MPa) was not significantly different from the mean P_{50} of vesselless species (-2.81 MPa) (t = -0.92; P = 0.391) (Fig. 5.5). P_{88} was also similar between both groups of xylem conduit elements morphologies (t = 1.16; P = 0.28). Differences were only observed for P_{12} (t = 3.43; P = 0.013), with vessel-bearing species having higher P_{12} values (-1.34 MPa) than vesselless species (-2.49 MPa) (Fig. 5.5). A significant difference was detected when comparing the slopes of the vulnerability curves between both xylem conduit morphologies (t = 7.42; $P \leq 0.001$). On average, vessel-bearing species had much lower slopes (53% MPa^{-1}) than vesselless species (172% MPa^{-1}).

5.3.2 Xylem embolism vulnerability and functional traits in relation to environmental variables and species distribution

P_{50} was positively correlated with the proportion of species occurrences located in rainforest areas (Fig. 5.6a), indicating that species which are less present in rainforest areas have greater xylem embolism resistance. For instance, *Paracryphia alticola*, which had the most rainforest-restricted distribution, with 91% of its occurrence records in rainforest areas, had a high P_{50} of -2.10 MPa, ranking among the least resistant species (Fig. 5.4). On the other hand, *Zygogynum crassifolium*, for which only 24% of occurrences were in rainforest areas, had the most negative P_{50} (-4.03 MPa) (Fig. 5.4). P_{50} was also related to species mean elevational distribution (Fig. 5.6b), indicating that species from lower elevations are less vulnerable to xylem embolism. P_{50} was negatively associated with MAT (Fig. 5.6c). P_{12} was also related to species distribution variables and MAT (Table 5.2). Both P_{12} and P_{50} were correlated with the MTDQ (Table 5.2). For other stem and leaf traits, significant relationships were detected only between LMA and rainforest occupancy (Table 5.2). Embolism vulnerability indexes were the only biological variable that correlated with more than one environmental variable (Table 5.2). Vulnerability to embolism was not associated with MAP within our group of species (Fig. 5.6d; Table 5.2). The probability of an association between P_{50} and MAP was marginally significant (P = 0.0509) when considering a 5% significance level. The relationship between P_{50} and MAP became significant when including our data in the global P_{50}-MAP dataset of Choat et al. (2012), suggesting that New Caledonian rainforest species fit this global-scale relationship (Fig. 5.7).

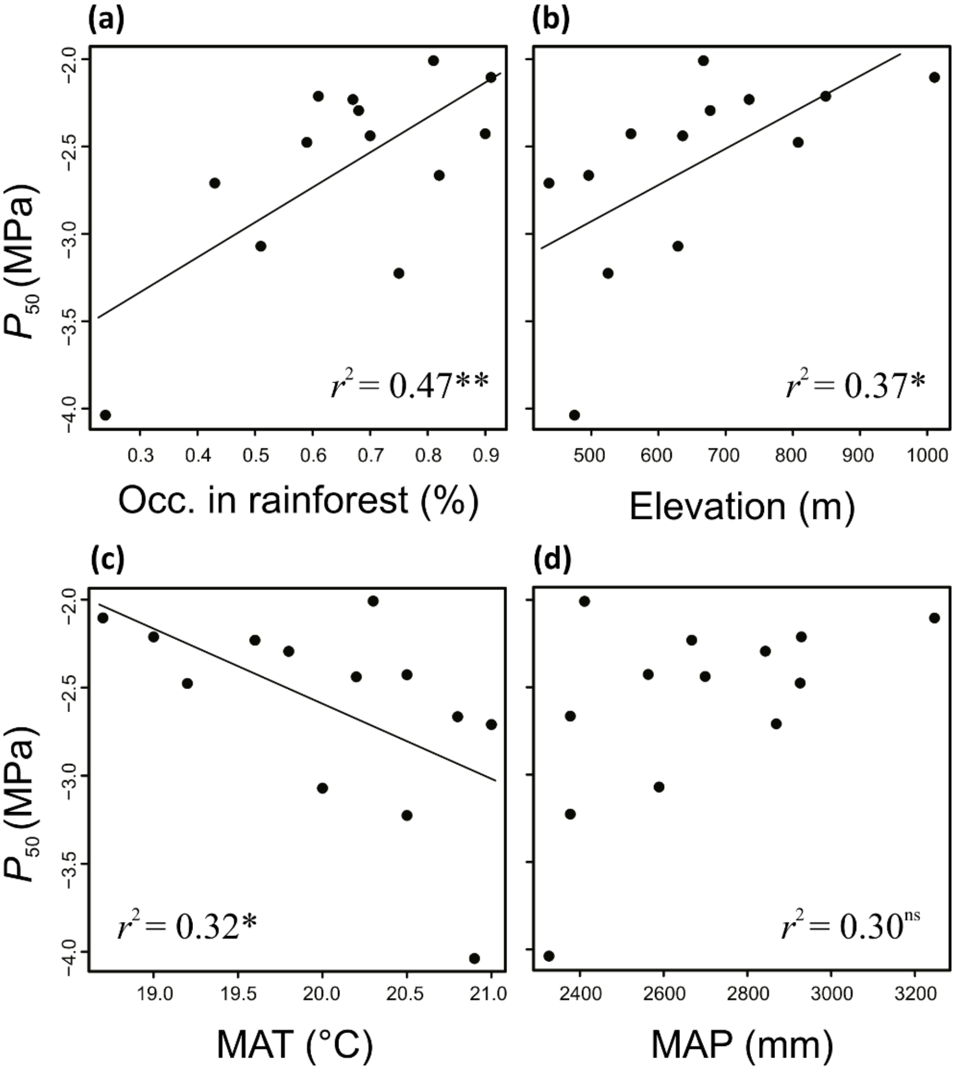


FIGURE 5.6 Relationships between proportion of occurrences in rainforest (A), elevation (B), mean annual temperature (C), mean annual precipitation (D), and vulnerability to embolism (P_{50}) of insular rainforest species. Points represent mean values per species. ns = non significant at $P = 0.0509$; * $P \leq 0.05$; ** $P \leq 0.01$.

TABLE 5.2 *Correlations of environmental variables with embolism vulnerability parameters and functional traits*

		MAP (mm)	MAT (°C)	MTDQ (°C)	Elevation (m)	Rainforest occupancy (%)
P_{12} (MPa)	*r*	0.48	**-0.65**	**-0.71**	**0.69**	**0.64**
	P	0.094	**0.016**	**0.006**	**0.008**	**0.017**
P_{50} (MPa)	*r*	0.55	**-0.56**	**-0.65**	**0.61**	**0.68**
	P	0.051	**0.044**	**0.015**	**0.025**	**0.009**
P_{88} (MPa)	*r*	0.33	-0.18	-0.25	0.21	0.37
	P	0.263	0.559	0.401	0.484	0.213
Slope (% MPa^{-1})	*r*	-0.15	0.40	0.36	-0.41	-0.07
	P	0.630	0.180	0.232	0.164	0.816
WD (g cm^{-3})	*r*	0.36	-0.24	0.07	0.24	-0.48
	P	0.22	0.422	0.816	0.431	0.092
VD (mm mm^{-2})	*r*	-0.38	0.24	0.28	-0.16	-0.27
	P	0.200	0.420	0.348	0.588	0.376
LMA (g m^{-2})	*r*	-0.06	0.003	0.30	-0.02	**-0.71**
	P	0.839	0.990	0.321	0.934	**0.006**

Pearson's correlation coefficients (r) and P-values (*P*) of bivariate cross-correlations. Bold values indicate significant correlations at $P \leq 0.05$. Abbreviations: MAP, mean annual precipitation; MAT, mean annual temperature; MTDQ, mean temperature of the driest quarter; WD, wood density; VD, leaf vein density; LMA, leaf mass per area.

5.3.3 Relationships between vulnerability to embolisms and leaf and xylem functional traits

P_{50} was not associated with any of the wood and leaf functional traits. WD scaled negatively with P_{50}, but the relationship was not significant (r = -0.24; P = 0.435). Similarly, LMA was not correlated with P_{50} (r = -0.37; P = 0.214). VD was not related to P_{50} (r = -0.06; P =0.840). P_{12} and P_{88} were also not associated with any of the traits measured (not shown).

5.4 Discussion

5.4.1 Association between xylem embolism vulnerability and habitat occupation of New Caledonian basal angiosperms

A main result of our investigation is that vulnerability to xylem embolism correlates with the percentage of occupancy of the rainforest by angiosperm species. Indeed, P_{50} explained 47% of the occurrence of angiosperm species in rainforest areas (Fig. 5.6a), suggesting that embolism vulnerability influences habitat occupation even within a moderate latitudinal gradient. For instance, species such as *Amborella trichopoda*, which occurs with a frequency of 82% in rainforest, may be restricted to moist habitats because of the risk of suffering hydraulic failure in drier locations. Moreover, *Zygogynum crassifolium*, the species with the most highly negative P_{50} in our study and thus the most drought-resistant (Fig. 5.4), has the lowest occurrence in rainforest areas (24%). It thus appears that higher resistance to xylem embolism allows this species to occur on both rainforest and drier habitats such as scrublands. It has been shown that mean trait values of plant species correspond to their position along environmental gradients (Violle & Jiang, 2009). The occurrence of species with high embolism resistance in drier habitats likely reflects the importance of resistance to xylem embolism as an adaptive response to water-stress (Maherali *et al.*, 2004). For instance, *Callitris tuberculata*, the species with the highest embolism resistance ever measured (P_{50} = -18.8 MPa), inhabits extremely dry areas of western Australia in zones with MAP as low as 180 mm at its most extreme margin (Larter *et al.*, 2015). At the other end of the embolism vulnerability spectrum are species from moist habitats such as the tropical rainforest, which experience xylem embolisms under much less negative xylem pressures (Choat *et al*. 2012).

Our work provides the first evidence of a relation between species elevational distribution and embolism vulnerability, with highland species being more vulnerable to xylem embolism (Fig. 5.6b). For instance, *Paracryphia alticola*, the second most vulnerable species measured here (Fig. 5.4), had the highest elevational range (mean = 1011 m). This relation between species elevational range and embolism vulnerability, along with the negative relation between embolism resistance and MAT (Fig. 5.6c), has very important conservation implications for the flora of the New Caledonian rainforest. Temperature increases have already been recorded in New Caledonia over the last three decades at a rate of 0.25°C per decade. Using the same rate, local climate models suggest that MAT could increase by *c.* 2.5 °C over the next century (Cavarero *et al.*, 2012). Upward shifts of organisms' elevational distribution are expected as climate changes (Walther *et al.*, 2002; Parmesan & Yohe, 2003) and have already been documented in temperate forest plants (Lenoir *et al.*, 2008; Urli *et al.*, 2014). Extensive upslope shifts toward cooler areas have also been documented by several studies of tropical plant species, indicating that this displacement is already ongoing within the tropics (Colwell *et al.*, 2008; Feeley *et al.*, 2011; Feeley *et al.*, 2013; Morueta-Holme *et al.*, 2015). Given that temperature-induced upward shifts in species distribution are ultimately restricted by dispersal and resource availability (Walther *et al.*, 2002), montane-rainforest angiosperms can be assumed to have a limited ability to respond to increasing temperatures because of a reduced range to disperse into suitable microrefugia. Populations of New Caledonian rainforest angiosperms restricted to high elevational ranges, which have the lowest drought resistances, could therefore be at significant conservation risk if temperature keeps increasing at the same pace.

Previous analyses have shown that average annual rainfall explains species embolism vulnerability across biomes (Brodribb & Hill, 1999; Maherali *et al.*, 2004; Choat *et al.*, 2012). Among the environmental variables analyzed in our study within a single biome, MAP was the only one marginally related to embolism vulnerability (P = 0.0509; Fig. 5.6d). This discrepancy in the relationship linking P_{50} and MAP likely stems from the difference in scale between global approaches and our island-wide study. At such a fine scale, the MAP raster we used probably had a resolution (1 km) too coarse to render the actual amount of water available for plants, which depends on microclimatic effects that were better captured by fine-scale layers such as the DEM and the rainforest map. In addition, averaged variables such as MAP appear to be of lower predictive power than extreme climate variables like MTDQ, which are recognized as good predictors of species distribution as they are related to plant mortality (Zimmermann *et al.*, 2009). Finally, we can question the ability of the MAP raster interpolated from 121 points (i.e., one meteorological station per 150 km^2), most of which are located at low elevation, to account for the complex distribution of MAP resulting from a double gradient of elevation and windwardness.

In contrast to MAP, the distribution of MAT is much easier to estimate because it arises from a single elevational gradient through the environmental lapse rate (Maitrepierre, 2012). In spite of the lack of relation between MAP and embolism vulnerability at the island-wide scale, a strong relationship was detected between both variables when including our data in the global dataset of Choat *et al*. (2012) (Fig. 5.7). Our study increases the representation of angiosperm species from humid habitats, which were less represented in that study as compared to plant species from habitats with lower rainfall regimes (Fig. 5.7). We show that species endemic to the rainforest of New Caledonia fit the pattern described by this global sample, occupying one of the ends of the embolism vulnerability range (Fig. 5.7). This finding confirms that within continental and island ecosystems, high embolism vulnerability is observed in species growing in high rainfall conditions.

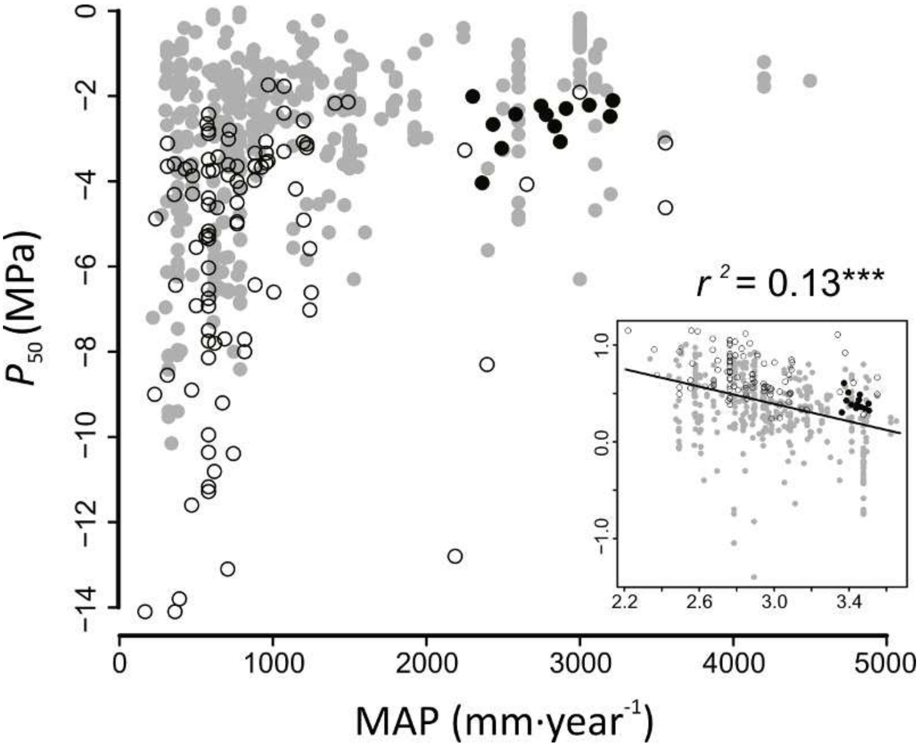


FIGURE 5.7 Vulnerability to embolism as a function of mean annual precipitation (MAP) at a global scale. The 13 New Caledonian basal angiosperm species analyzed in this study fit the global pattern of P_{50}-MAP. Different symbols represent gymnosperm species (open circles), angiosperm species (gray circles), and New Caledonian rainforest angiosperm species (black circles). Inset: Negative relationship between MAP and embolism resistance using $\log_{10}$-transformed data. P_{50} values were converted from negative to positive to facilitate $\log_{10}$ transformation. The coefficient of determination corresponds to the relationship after $\log_{10}$-transformation. Additional data obtained from Choat et al. (2012). *** $P \leq 0.001$.

5.4.2 Poor differentiation of embolism resistance between vessel-bearing and vesselless basal angiosperms: insights into vessel evolution

Angiosperm vessel elements are thought to have evolved from tracheids. The concentration of pits on tracheid endwalls, along with lysis of primary membranes, gave rise to scalariform perforation plates, which in turn evolved to simple perforation plates after loss of the perforation bars (Carlquist & Schneider, 2002). Some functional adaptive advantages of xylem conduit variation have been suggested: for instance, it has been shown that the loss of bars in perforation plates enhances water conductivity because tracheids and scalariform perforation plates confer significant flow resistance compared to simple perforation plates (Pittermann *et al.*, 2005; Sperry *et al.*, 2007; Christman & Sperry, 2010). Moreover, it has been shown that angiosperm venation networks are influenced by xylem conduit type, with vesselless angiosperms having lower vein densities compared to vessel-bearing angiosperms (Feild & Brodribb, 2013). Hence, xylem conduit morphology influences water conductivity, stomatal conductance and photosynthetic capacities. These observations support the hypothesis that vessel evolution was driven by selection for increasing efficiency in water conductivity.

With regard to water transport safety, it has been suggested that species bearing vessel elements with scalariform perforation plates are more vulnerable than tracheid-bearing angiosperms (Sperry *et al.*, 2007). However, we did not observe significant differences in embolism vulnerability between vesselless and vessel-bearing angiosperms (Fig. 5.5). Only slight differences were observed at the onset of embolism formation (P_{12}), with vessel-bearing species being less resistant (Fig. 5.5). This suggests a lack of differentiation in embolism vulnerability across species with different xylem conduit anatomies, with slight differences at low xylem tensions. The low values of embolism resistance in species with "primitive" vessels, and the lack of differentiation of embolism resistance between tracheids and vessels, supports the hypothesis that angiosperm vessels could not have evolved in xeric habitats because of limitations caused by embolism risk (Sperry *et al.*, 2007; Carlquist, 2012). Despite suffering xylem embolisms at less negative pressures, vessel-bearing species had much lower embolism vulnerability curve slopes compared to vesselless species, suggesting that after the start of cavitation, embolism propagation proceeds slower in vessel-bearing species. Current research highlights the great importance of xylem ultrastructure, with characters such as pit membrane structure playing a key role in embolism resistance (Lens *et al.*, 2011; Lens *et al.*, 2013; Jansen & Schenk, 2015; Schenk *et al.*, 2015). The lack of differentiation in embolism resistance between vesselless and vessel-bearing angiosperms suggests that evolutionary changes in xylem conduit types are not associated with ultrastructural anatomical changes. Further research on ultrastructural characteristics of interconduit pits would be needed in order to discern which xylem properties allow variation in embolism vulnerability in tropical rainforest angiosperms with primitive xylem conduits.

5.4.3 Weak association between xylem embolism vulnerability and common functional traits in a tropical rainforest

It has been shown that, to maintain a safe water conductivity under substantial negative pressures, the investment in the cell walls of conduits and fibers is increased, resulting in increases of wood density (Hacke *et al.*, 2001). In this sense, previous studies have proposed a negative relation between P_{50} and wood density in angiosperm species (Jacobsen *et al.*, 2005; Pratt *et al.*, 2007; Hao *et al.*, 2008; Markesteijn *et al.*, 2011). Surprisingly, despite being negatively related, we did not find a significant association between wood density and embolism vulnerability in our sample of New Caledonian rainforest species. Significant negative relationships between WD and P_{50} showed in previous studies were obtained from measurements of nine (Pratt *et al.*, 2007) and six (Jacobsen *et al.*, 2005) chaparral species. Using the same number of species that we studied here, Markesteijn *et al*. (2011) showed a strong negative relation between WD and P_{50} in species of tropical dry forest, another drought-prone biome. Finally, Hao *et al*. (2008) showed a negative relation between WD and P_{50} when considering ten species from savanna and forest, which are very contrasting environments. Hacke *et al*. (2001) showed that the relationship between P_{50} and wood density across a wide range of species is curvilinear. In their analysis, the slope of the curve is lower in species with wood densities between 0.4 and 0.7 g cm^{-3}, corresponding to the WD values of the species measured in this study (Table 5.1). The curve then becomes much steeper with increasing embolism resistance, corresponding to wood density values above *c*. 0.7 g cm^{-3}. This suggests a lack of selective pressure for greater structural investment to increase conductive safety in the wood of tropical rainforest species. Wood structure can therefore be modulated for diverse competitive functions in environments where water stress does not exert an important pressure.

Higher values of leaf mass per unit area (LMA) in drier environments reduce leaf water loss by the increase of leaf tissue density or thickness (Wright *et al.*, 2002). Therefore, the higher LMA values observed in drought-exposed species can be considered to represent an adaptive response to water stress operating at the leaf level (Niinemets *et al.*, 1999). This is supported by several studies showing that LMA increases with water stress (Cunningham *et al.*, 1999; Fonseca *et al.*, 2000; Wright *et al.*, 2002; Jordan *et al.*, 2013). A relation between xylem embolism vulnerability and LMA could be expected, since both traits are related to plant drought resistance. However, our study shows that LMA and P_{50} are decoupled in New Caledonian rainforest species. This result agrees with similar findings showing the lack of a relationship between P_{50} and LMA (or SLA) in Neotropical dry forests (Markesteijn *et al.*, 2011; Méndez-Alonzo *et al.*, 2012). Moreover, it has been shown that leaf life span, which is strongly related to LMA (Wright *et al.*, 2004), is not related to P_{50} in plants of an Asian tropical dry forest (Fu *et al.*, 2012). Furthermore, Maréchaux *et al*. (2015) found no significant relationship between LMA and leaf water potential at turgor loss point, a leaf-level indicator of drought tolerance. The lack of relationship between LMA and embolism vulnerability observed in rainforest species and within drier habitats (Markesteijn *et al.*, 2011; Méndez-Alonzo *et al.*, 2012) suggests that the two traits are not evolutionarily coordinated.

The absence of a relationship between a key hydraulic trait such as xylem embolism resistance and typically measured functional traits such as WD and LMA forewarn further research on the risk of using these traits as indicators of drought tolerance.

It has been shown that P_{50} is positively related to photosynthetic capacity among conifer species (Pittermann *et al.*, 2012). Moreover, according to the so-called hydraulic safety-efficiency tradeoff hypothesis, safety in hydraulic conductivity should be selected against conductive efficiency (Zimmermann, 1983). The second foliar considered in our study, leaf vein density (VD), has been shown to be strongly positively related to functions such as leaf hydraulic conductivity and photosynthetic capacity (Brodribb *et al.*, 2007; Brodribb *et al.*, 2010; Feild & Brodribb, 2013; Sack & Scoffoni, 2013). In this sense, a positive relation between VD and P_{50} can be expected, with conductive-efficient species also being more vulnerable. However, this correlation was the weakest of those involving the traits measured in our study. In support of the safety-efficiency hypothesis, several studies have shown a tradeoff between stem hydraulic conductivity and embolism vulnerability (Pockman & Sperry, 2000; Martínez-Vilalta *et al.*, 2002; Sperry *et al.*, 2008; Zhu & Cao, 2009; Markesteijn *et al.*, 2011).

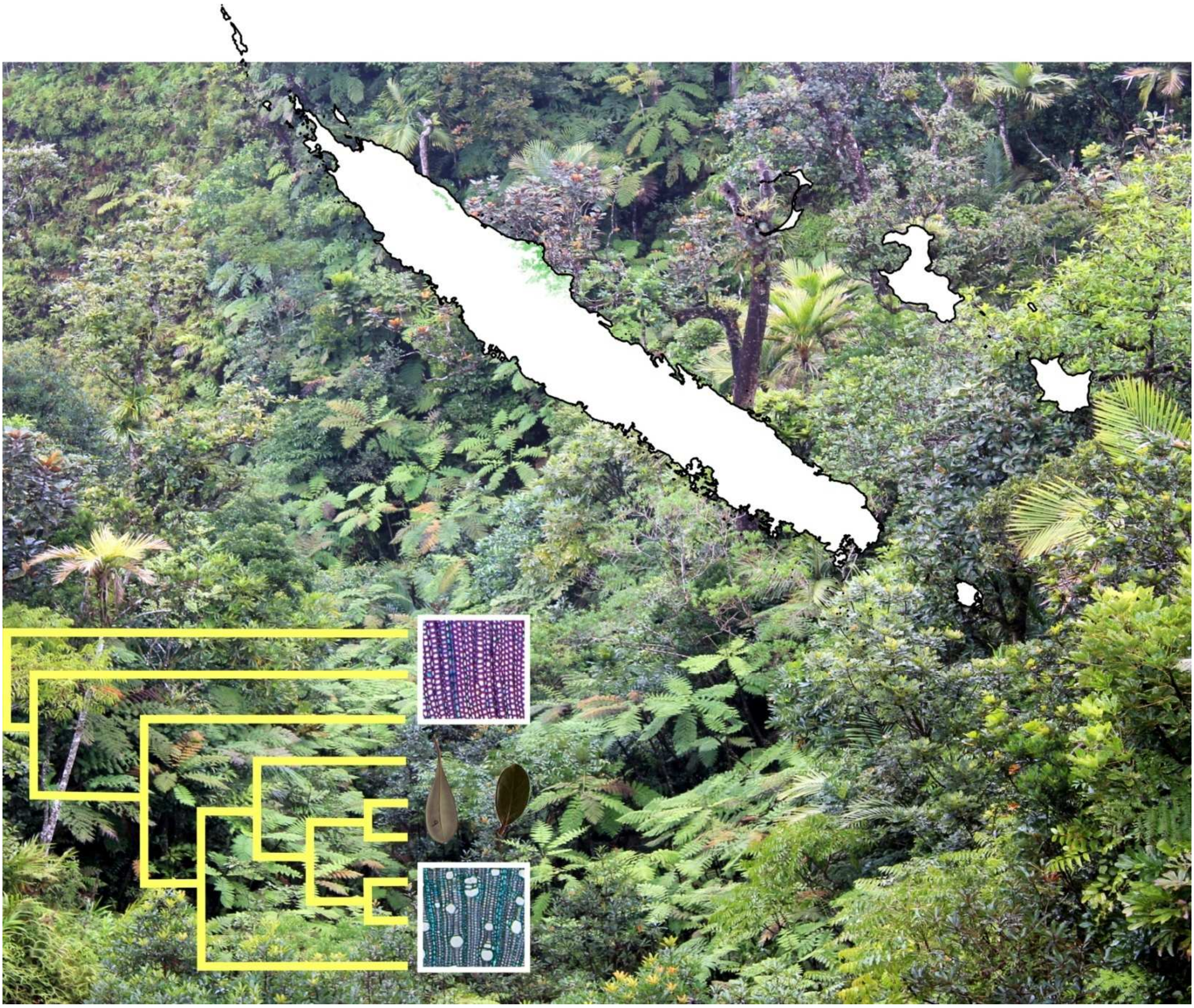
In a large scale analysis, Maherali *et al*. (2004) did not detect a correlation between xylem-specific hydraulic conductivity and P_{50} when exclusively considering angiosperm species. More recently, a global meta-analysis by Gleason *et al*. (2015) has shown that there is a weak tradeoff between hydraulic safety and efficiency. In this regard, previous studies have shown that leaf P_{50} and leaf hydraulic efficiency, a trait which is strongly related to VD, are not correlated in woody species across different biomes (Blackman *et al.*, 2010; Nardini & Luglio, 2014). Our results provide evidence of the lack of such a tradeoff within New Caledonian rainforest species, which show low hydraulic efficiency, as reflected by their low values of VD, but also low embolism resistance. Gleason *et al*. (2015) have recently shown that many species share this profile, having both low hydraulic efficiency and safety. Future research would be needed to identify the environments favouring the presence of species with this apparently non-optimal hydraulic profile.

Current plant ecological research focuses on the measurement of phenotypic attributes that influence plant fitness within local environments (Westoby & Wright, 2006). By using this functional approach, several structural traits have been proposed as indicators of drought tolerance. However, it has been stressed that the use of easy-to-measure traits, such as LMA, as indicators of drought tolerance is controversial and can potentially yield misleading results

(Delzon, 2015; Maréchaux *et al.*, 2015). Among the traits measured in our study, xylem embolism resistance was the most closely related to environmental variables (Table 5.2), being associated with the ecological differentiation of tropical angiosperms. Plant hydraulic physiology is strongly linked with photosynthetic assimilation and derived carbon uptake (Brodribb, 2009). Drought-induced rainforest dieback may therefore alter the primary production and functional composition of one of the richest ecosystems of the world, consequently diminishing extensive amounts of biomass and carbon storage (Malhi *et al.*, 2009; Phillips *et al.*, 2009; Phillips *et al.*, 2010). It has recently been proposed that hydraulic failure is the main underlying mechanism of rainforest tree mortality (Rowland *et al.*, 2015). Xylem embolism resistance may thus play a major role in the maintenance of primary productivity and plant function (Brodribb, 2009). Our findings emphasizes the importance of incorporating key ecophysiological traits such as xylem embolism resistance into process-based models in order to estimate the response mechanisms of vegetation to global climate change.

CHAPTER 6

General Discussion and Conclusions



- Synthesis
- Perspectives
- Conclusions

This chapter summarizes our main results. Moreover, we provide novel insights into: I) the ancestral form, function and ecology of angiosperms; II) the evolution of angiosperm xylem conduits and its ecological implications; III) the natural history of New Caledonia and the disharmony of its flora; and IV) the conservation of the New Caledonian flora. Finally, we provide main concluding remarks and research perspectives.

6.1 Insights into the form, function, and ecology of early flowering plants

The ancestral form, function, and ecology of angiosperms, and the influence of these characteristics on the rise of the ecological dominance of angiosperms, are some of the aspects of flowering plant evolution that remain uncertain (Feild *et al.*, 2009). Several hypotheses have been proposed using extant species as modern analogs of the early angiosperms. The 'paleoherb' hypothesis (Taylor and Hickey, 1992; Taylor and Hickey, 1996) is supported by a cladistic analysis that placed the families Chloranthaceae and Piperaceae as the first divergent lineages of the angiosperm tree. Using this topology, a reconstruction of the ancestral secondary growth of angiosperms was performed, suggesting that the protoangiosperm was a "diminutive, rhizomatous to scrambling perennial herb". The long-standing paleoherb hypothesis has been supported by the observation of paedomorphic wood features in Piperales (Carlquist, 1993; 1995), suggesting an herbaceous ancestral habit. The assumption of Piperales as a secondarily woody lineage has persisted over almost two decades (Spicer and Groover, 2010). However, the ancestral habit of Piperales remained very ambiguous and previous studies have alternatively coded Piperales as 'herbaceous', 'woody', or 'secondarily woody' (Kim *et al.*, 2004; Feild and Arens, 2005; Spicer and Groover, 2010).

By performing the most extensive overall synthesis to date of the anatomical organization and cambial activity of Piperales, the largest basal angiosperm order, we propose that the common ancestor of Piperales had an active bifacial cambium and a truly woody habit (Trueba *et al.*, 2015). Therefore, our results challenge the long-held vision of secondary woodiness in Piperales and contrast with the paleoherb hypothesis. As stressed above, wood paedomorphisms (*sensu* Carlquist 1962; 2009) have been proposed as anatomical evidence of secondary woodiness in Piperales. However, our study suggests that instead of being an evidence of secondary woodiness, wood characters pointing to protracted juvenilism, such as raylessness and multiseriate rays, could be the result of anatomical adaptations to specific growth habits such as the woody herb habit of *Saruma* or the cane-like shrub habit of *Piper* (see Chapter 3 for a detailed explanation). Additionally, the exhaustive review of Lens *et al.*

(2013) has shown examples of primarily woody taxa that exhibit paedomorphic wood features. By showing the lack of a relation between wood paedomorphisms and secondary woodiness, we challenge the use of paedomorphic features as reliable evidence of herbaceous ancestry. The hypothesis of an herbaceous ancestor of the angiosperms, which is based on Piperaceae as a modern analog (Taylor and Hickey, 1992; Taylor and Hickey, 1996), seems very unlikely given that our optimization proposes that the putative ancestor of Piperales had a woody habit.

Recent changes in the topology of the angiosperm phylogenetic tree have greatly influenced hypotheses about the form and function of the earliest flowering plants. Sun *et al*. (2002) proposed that early angiosperms were aquatic herbs growing in stable habitats similar to modern Nymphaeales and *Ceratophyllum*. This hypothesis is supported by fossil evidence of herbaceous aquatic angiosperms (Sun *et al.*, 1998; Sun *et al.*, 2002; Gomez *et al.*, 2015). However, it has been proposed that aquatic angiosperms were derived from plants that previously occupied terrestrial environments (Philbrick and Les, 1996). Moreover, recent studies have proposed that Nymphaeales, *Ceratophyllum* and monocots are herbaceous lineages derived from woody ancestors (Kim *et al.*, 2004; Spicer and Groover, 2010). Based on the most recent angiosperm phylogeny, Feild and colleagues have analyzed the ecophysiology of extant Chloranthales and species of the ANA grade, proposing the "damp, dark, and disturbed" and "ancestral xerophobia" hypotheses (Feild *et al.*, 2003; Feild *et al.*, 2004; Feild and Arens, 2005; 2007; Feild *et al.*, 2009). Our study on basal angiosperm representatives from New Caledonia provides new insights into these last hypotheses.

Amborella trichopoda Baill. has a multi-stemmed habit with scandent stems, similar to the cane-like habit of other basal angiosperm representatives such as *Aristolochia*, *Eupomatia*, *Illicium*, *Piper*, *Sarcandra* and *Trimenia* (Carlquist, 1996; 2001; Feild and Arens, 2005; Carlquist, 2009; Isnard *et al.*, 2012). By providing a detailed description of the architecture of *Amborella*, we show that its habit is made up of a series of sympodial branched complexes. It has been proposed that sympodiality is coupled with a moderate cambial activity (Blanc, 1986). Moreover, sympodiality has previously been proposed as a widespread feature among early-diverging angiosperms and as a putative ancestral feature from which woodier lineages have been derived (Carlquist 2009). In this sense, previous studies have described a similar sympodial growth in other basal angiosperm representatives, and it has been reconstructed as an ancestral feature in Piperales (Isnard *et al.*, 2012). This suggests that sympodiality is related to the development of cane-like habits in basal angiosperms. Furthermore, we show the absence of a typical non-self supporting mechanical construction behind the scandent stems of *Amborella*. A similar mechanical organization has been observed in the lianescent vesselless angiosperm *Tasmannia cordata* (Feild *et al.*, 2012). Despite its lianescence, this species possesses a stiff wood whose mechanical properties do not change during stem development. This suggests that the tracheid-based vasculature probably prevents the ontogenetic changes in mechanical properties observed in vessel-bearing mesangiosperm lianas, which exhibit increasing elasticity towards the base of the stem (Rowe and Speck, 2005; Rowe *et al.*, 2006). Our architectural observations on *Amborella*, along with the cambial activity reconstruction of Piperales, coincides with the ancestral growth habit proposed by Feild *et al.* (2004), suggesting that early angiosperms were shrubs or small trees with a sympodial growth construction.

Feild's hypotheses proposes that early angiosperms were fundamentally drought intolerant plants, preferring moist and shady habitats (Feild *et al.*, 2004; Feild *et al.*, 2009). Our assessment of the preferred habitat of New Caledonian basal angiosperms supports this hypothesis by showing that the highest habitat overlap of basal angiosperms in the archipelago occurs in mild, moist, and aseasonal environments (Chapter 4). In accordance with this habitat preference, we further show that representative basal angiosperms, including *Amborella* and species of Chloranthaceae, are drought intolerant. This is reflected by a relatively high vulnerability to xylem embolism (Chapter 5; mean P_{50} = -2.6 MPa). By providing new measurements of both the habitat preference and the drought vulnerability of basal angiosperms, we confirm the potential xerophobia of early angiosperms.

The reconstruction of the ecology of early angiosperms also suggests that these plants were exposed to understory disturbances such as frequent soil washouts and mechanic disturbances induced by falling debris (Feild and Arens, 2005; 2007). The sympodial growth and multi-stemmed habit of *Amborella*, with resprouting axes originating from dormant buds, may confer the capability to tolerate this type of mild-perturbation regime. Indeed, these morphological attributes have been suggested as key features that confer the ability to form new shoots after destruction of living tissues (Pausas *et al.*, 2016). Moreover, we have also observed adventitious roots in the aerial stems of *Amborella*, a developmental feature that could allow *Amborella*'s axes to re-root after a disturbance event, adopting a persistence strategy (Bond and Midgley, 2001). Our architectural observations suggest that *Amborella*'s axes are not strongly differentiated morphologically, suggesting a lack of strong architectural organization and reflecting a morphological 'flexibility', which may have conferred early flowering plants with significant competitive advantages during their early evolutionary stages in disturbed understory habitats.

Our research has further described pervasive patterns of trait covariation that are well known across vascular plants. For instance, our results have shown that across a gradient of canopy openness, leaf traits of *Amborella* such as leaf mass per area (LMA) and leaf dry matter content (LDMC) varied 6-fold and 5-fold, respectively (Chapter 2). It has been recently stressed that intraspecific trait variation accounts for a considerable percentage of the total trait variation within plant communities (Violle *et al.*, 2012; Siefert *et al.*, 2015). Our research on leaf trait variability in *Amborella* confirms the adaptive importance of this phenotypic response to patchy habitat openings. Moreover, this suggests that this phenotypic response is observed across the major lineages of vascular plants. Within this phenotypic variation, a coordination of leaf and stem traits seems to occur in *Amborella*, something that has been previously documented across vascular plant species at a global scale (Díaz *et al.*, 2004; Díaz *et al.*, 2015). For instance, the significant coordination between LMA and stem specific density (SSD) observed at the intraspecific level in *Amborella* ($r = 0.48$, $P = 0.01$) has also been observed in a recent interspecific analysis considering 2870 woody species (Díaz *et al.*, 2015). This may reflect that carbon allocation and organ longevity are coordinated in both stems and leaves within and across species. The pervasiveness of this trait coordination suggests the existence of an evolutionary bottleneck that may result from inescapable physical and physiological constraints. These constraints seem to preclude the combination of certain traits across and within plant species.

6.2 Insights into the evolution and ecology of the xylem hydraulic apparatus in angiosperms

Vessel evolution is not linear and reversals may have occurred

It has been suggested that angiosperms are ancestrally vesselless (see Box 1 in section 1.5.1). Vessel elements are thought to have evolved from tracheids by loss of the inter-tracheid pit membranes in both end walls, resulting in large scalariform perforations connecting multiple vessel elements within a single vessel (Carlquist and Schneider, 2002; Christman and Sperry, 2010). Further, vessel elements with simple perforation plates seem to have evolved *via* a progressive loss of bars from scalariform perforation plates (Frost, 1930). Bailey and Tupper (1918) published the first work that documented this structural gradient in angiosperm xylem conduits (see figure 1.6A). This anatomical transition has long been considered as a major trend of xylem evolution, which has been frequently interpreted as a phyletic ladder of evolutionary progress from tracheids to vessel elements with simple perforation plates (Olson, 2012). However, some studies have shown that xylem conduit evolution is not linear and reversals to an ancestral vasculature may occur. For instance, Feild *et al*. (2002) suggested that, as a consequence of the environmental pressure of freezing conditions, tracheids re-evolved from a vessel-bearing ancestor within Winteraceae.

Our inference of the ancestral state of vessel element perforation plates in Piperales suggests that the scalariform perforation plates observed in Saururaceae (except in *Anemopsis*) are the result of an evolutionary reversal (Chapter 3). This reversal to scalariform perforation plates in Saururaceae could be the result of limited secondary growth and retention of primary xylem features. It has been shown that scalariform perforation plates significantly increase water flow resistivity in comparison to simple perforation plates (Christman and Sperry, 2010), but such an increase of flow resistivity is probably not physiologically limiting in the wet and semi-aquatic environments of Saururaceae. To our knowledge, only few studies have provided similar evidence of evolutionary reversals of the morphology of perforation plates (Lens *et al.*, 2007; Oskolski and Jansen, 2009). Our analysis provides new evidence of such evolutionary reversals, which differ from the classical Baileyan trend of vascular evolution. This invites further studies to use similar optimizations in clades where various types of perforation plates are observed. Finally, further research would be needed to detect the environmental pressures and selective advantages behind the reversal to such 'unfashionable' and inefficient perforation plates.

Xylem conduit type and its ecological implications

It has been suggested that species with "primitive" vessel elements bearing scalariform perforation plates are often confined to humid environments because of their hydraulic limitations (Carlquist, 1975; Carlquist, 2001). This thesis provides a first estimation of a possible xylem conduit-driven habitat distribution (Chapter 4). We show that basal angiosperm species bearing vessel elements with simple perforation plates tend to occupy drier and more seasonal environments. Indeed, some members of the families Lauraceae, Annonaceae, and Piperaceae, which have hydraulically efficient vessel elements with simple perforation plates, seem to escape the humid habitats in which most species of the other basal angiosperm families are restrained. The restricted distribution of species with 'primitive' vessels supports the hypothesis that vessel evolution may have been limited to wet habitats (Carlquist, 2001; Sperry *et al.*, 2007). The subsequent evolution of vessel elements with simple perforation plates may have allowed flowering plants to escape from the humid forests and diversify into more seasonal environments.

A main result of our study is the relationship between vulnerability to xylem embolism and habitat differentiation. By showing that embolism vulnerability is strongly related to habitat occupation, we confirm that xylem hydraulic safety has a strong influence on the geographic distribution of plant species. It has been suggested that 'primitive' vessels, such as those observed in basal angiosperms, are more vulnerable to drought than angiosperm tracheids (Sperry *et al.*, 2007). However, our study suggests that xylem embolism resistance acts equally regardless of xylem conduit type. The only difference between vesselless and vessel-bearing species was observed in the cavitation curve slopes. Vessel-bearing species had much lower embolism vulnerability curve slopes, suggesting that after the onset of cavitation, embolism propagation acts more slowly in vessel-bearing species. However, while cavitation seems to operate differently in the two types of conduit types, P_{50} and P_{88} values were similar, suggesting that xylem embolism vulnerability does not differ between vessel-bearing and vesselless species.

Given that we did not observe compelling differences in embolism vulnerability across xylem conduit types, fine-scale anatomical features may underlie the variation in embolism vulnerability that we observed across species. Recent research has highlighted the importance of anatomic ultrastructure in xylem embolism resistance (Schenk *et al.*, 2015). Among the ultrastructural features involved, the thickness and porosity of pit membranes seems to be one of the most important (Lens *et al.*, 2011; Lens *et al.*, 2013; Schenk *et al.*, 2015). Therefore, our results suggest that variation in pit membrane features occurs independently of the type of xylem conduit. Further studies including measurement of micro-anatomical and ultrastructural features may shed light on the xylem structures that confer embolism resistance across different angiosperm xylem conduit types.

6.3 Climate stability and drought sensitivity: new insights into the disharmonic composition of the New Caledonian flora

Because of their old age and their narrow geographic distribution, several New Caledonian species of basal angiosperms could be considered as potential palaeoendemics. It has been proposed that palaeoendemism is associated with niche conservatism (Jordan *et al.*, 2015). The niche (*sensu* Hutchinson) describes the set of abiotic and biotic conditions where a species is able to persist (Holt, 2009). According to Wiens and Graham (2005), niche conservatism is the tendency of species to conserve ancestral ecological characteristics. Crisp *et al*. (2009) have proposed that evolution rarely induces biome transitions even on large time scales. Indeed, it has been underscored that ecological niche evolution is a very slow process (Losos, 2008). For instance, by comparing the biome of disjunct sister taxa, Crisp *et al*. (2009) have shown that biome stasis during plant speciation overweighed biome shifts by a ratio of more than 25:1. Because it may be "easier to move than to evolve" (Donoghue, 2008) and because changes in biomes operate slowly, on scales of tens of millions of years, palaeoendemic species may therefore contain "signals of the nature of past environments" (Jordan *et al.*, 2015). Regarding these assumptions, we can consider that the habitat currently occupied by extant basal angiosperms reflects characteristics similar to those occupied by members of these lineages since their arrival in the archipelago.

Our research has shown that the highest habitat overlap (*i.e.* convergence of suitable abiotic conditions) of 62 basal angiosperms in New Caledonia occurs in warm locations with mean annual precipitation ranging from 1,500 mm to 2,300 mm and with low diurnal variations in temperature (below 7.5 °C). Therefore, we can deduce that basal angiosperm species of New Caledonia have a remarkable preference for humid and stable environments. These environmental characteristics correspond to rainforest habitats that exhibit high moisture levels and low diurnal and seasonal variations in temperature. Considering the niche conservatism theory, our results suggest that rainforest-type environments have been the preferred habitat of basal angiosperms since their early diversification. This assumption coincides with the widespread distribution of other extant basal angiosperms in tropical rainforests of Asia-Australasia (Morley, 2001; Buerki *et al.*, 2014).

Given that the New Caledonian landmass was submerged during the Palaeocene, emerging only during the late Eocene (Pelletier, 2006), the archipelago could only have been a secondary centre of dispersal for basal angiosperm lineages. Therefore, rainforest habitats may have persisted in nearby territories during the submersion of the New Caledonian landmass. This is a plausible scenario because it has been shown that moist climates in Northern Australia were contemporary with the late Eocene reemergence of New Caledonia (Bowman *et al.*, 2010). Moreover, a relative global climatic stability was observed during the Eocene (Zachos *et al.*, 2001). This past environmental stability allowed the widespread presence of an aseasonal-wet biome in Australia (Crisp *et al.*, 2004), which may have been the habitat of basal angiosperm lineages before their arrival to the New Caledonian archipelago.

After a period of climatic stability, Australia and nearby regions suffered a progressive desiccation over the past 25 Myr culminating in extreme aridity events over glacial cycles (Crisp *et al.*, 2004; Byrne *et al.*, 2008). The aridification of the Australian landmass during several Neogene and Quaternary events may have resulted in the depauperation of basal angiosperm species there. Because of its geographic and topographic characteristics, the New Caledonian archipelago has an oceanic climate with trade winds that can supply significant amounts of orographic cloud water. Orographic cloud water is an integral component of the hydrology of many coastal and mountain environments and it represents almost a half of the water input in these habitats (Scholl *et al.*, 2007). The tropical oceanic climate of New Caledonia may have been a major mitigating variable in the survival of basal angiosperm lineages in New Caledonia. Indeed, our study suggests that New Caledonian rainforest habitats have acted as a Pleistocene refugium for basal angiosperms during the major climatic fluctuations of the last glacial maximum (LGM). Previous studies have proposed similar LGM rainforest refugia for *Amborella* (Poncet et al., 2013) and microendemic palm species (Pintaud *et al.*, 2001). Our study thus confirms the importance of the maintenance of rainforest habitats in New Caledonia during past climatic fluctuations for the survival of rainforest lineages.

Jordan *et al*. (2015) suggested that the geographic restriction of palaeoendemic species mainly results from ecological selection rather than dispersal limitation, since over such long evolutionary time the probability of dispersal cannot be null. Pillon *et al*. (2010) have also proposed that dispersal is not a prevailing factor in explaining the over-representation of some angiosperm families in New Caledonia, given that other families with high effective dispersal capacities tend to be under-represented. Ultramafic substrates might instead play the role of an ecological filter in New Caledonia and over-representation of angiosperm families could result from exaptation to ultramafic soils (Pillon *et* al. 2010). However, our results show that the hypothesis proposed by Pillon *et al*. (2010) does not apply for the over-represented families of basal angiosperms and other rainforest lineages that preferentially occur on non-ultramafic substrates. Many studies have shown the critical role that refugia have played in the survival and diversification of biota during and after the Pleistocene glaciations (see Keppel *el al*., 2012). Crisp *et al*. (2009) suggested that a large proportion of the Australian plant species that colonized New Caledonia dispersed into their same original biome. The presence of rainforest refugia in New Caledonia may thus have prevented the local extinction of basal angiosperms during the periods of global climatic instability that severely affected the humid biomes of Australia (Gallagher *et al.*, 2003; Dodson and Macphail, 2004; Byrne, 2008; Byrne *et al.*, 2008; Byrne *et al.*, 2011; Kooyman *et al.*, 2013). The disharmony between the flora of New Caledonia and Australia may therefore be partially explained by the presence of rainforest refugia that could have prevented the extinction of angiosperm lineages in the New Caledonian archipelago. The permanence of rainforest habitats may have also allowed the local diversification of rainforest lineages.

We have shown that geographic restriction to rainforest habitats is strongly related to xylem embolism vulnerability (Chapter 5), which is an indicator of drought intolerance. Because most of the basal angiosperms in New Caledonia occur in rainforest habitats, our results suggest that most of the local species in these groups are vulnerable to drought. The drought vulnerability of New Caledonian basal angiosperms can be regarded as a potential physiological signature of an uninterrupted occupancy of humid habitats during long periods of evolutionary time. Similar relationships between xylem embolism vulnerability and the environmental distributions of conifer species have been outlined (Brodribb and Hill, 1999; Piñol and Sala, 2000). New Caledonia has the largest assemblage of conifers in the Pacific region (Jaffré *et al.*, 1994; De Laubenfels, 1996). Among them, the genus *Araucaria* (Araucariaceae) is particularly well represented given that 13 (out of the 19 worldwide) species occur in the archipelago (Gaudeul *et al.*, 2014). A recent study has shown that Australian *Araucaria* species have high drought vulnerability compared to other gymnosperm species (Zimmer *et al.*, 2015). Moreover, they showed an isohydric behaviour (*i.e.* water loss control through stomatal closure), which generally corresponds to high embolism vulnerability and occupation of mesic habitats (Piñol and Sala, 2000). The three Australian *Araucaria* species measured by Zimmer *et al.* (2015) have stem P_{50} values between -2.64 MPa and -3.01 MPa, which correspond to the range of P_{50} values (-2.0 to -4.0 MPa) of New Caledonian rainforest angiosperms measured in this study. New Caledonian *Araucuaria* mostly occur in humid forests on ultramafic substrates (Jaffré *et al.*, 2010; Gaudeul *et al.*, 2014) and they are among the most drought sensitive conifers of the archipelago (Delzon, pers. com.). The over-representation of relict palaeoendemic conifers, such as *Araucaria,* could therefore be a combination of both mechanisms, an ultramafic filtering, and a past climatic stability.

6.4 Implications for the conservation of the New Caledonian flora: basal angiosperms as potential barometers of climate change

New Caledonia is one of the world's hotspots for biodiversity conservation because of its rich, endemic and threatened biodiversity (Myers *et al.*, 2000). Several studies have outlined particular threats to the New Caledonian biodiversity. Mining activities (Jaffre *et al.*, 1998; Pascal *et al.*, 2008; Jaffré *et al.*, 2010), along with wildfires (Ibanez *et al.*, 2013; Gomez *et al.*, 2014; Curt *et al.*, 2015) and introduced invasive species (Beauvais *et al.*, 2006; Soubeyran *et al.*, 2015), have a major impact on local plant biodiversity. Surprisingly, although climate change represents the most pervasive of the various threats on the global biodiversity (Malcolm *et al.*, 2006), to date no study has assessed its potential impact on the flora and vegetation of New Caledonia. Moreover, oceanic islands are thought to be particularly vulnerable to climate change due to inherent ecological features such as low habitat availability and small population sizes (Harter *et al.*, 2015). This particular vulnerability requires that climate change be considered as a significant potential threat to the flora of the New Caledonian archipelago.

The climate of our planet is changing, global temperature is increasing (IPCC, 2014), and although forecasted changes in precipitation remain uncertain, climatic models predict more frequent and severe droughts in the near future (Meehl and Tebaldi, 2004; Burke *et al.*, 2006; Chadwick *et al.*, 2015). It has been suggested that increases in the frequency, length, and severity of droughts would have a major impact on plant species mortality (Allen *et al.*, 2010; Allen *et al.*, 2015). Recent syntheses have documented over 90 worldwide localities manifesting drought-induced forest mortality (Allen *et al.*, 2015; Hartmann *et al.*, 2015). Despite these reports, to our knowledge, this alarming phenomenon has not yet been documented in New Caledonia. Because basal angiosperm species are abundant in rainforest communities of New Caledonia and because they seem to be very sensitive to drought, we propose that basal angiosperm populations are potential indicators of the effects of drought on the flora of the archipelago. A monitoring program of selected populations of basal angiosperms could provide an early warning of the impact of drought on the New Caledonian vegetation.

By analyzing climatic trends of the Western Pacific region, Whan *et al.* (2014) have shown a significant increase of MAT over the past 50 years. Moreover, it has been shown that MAT, along with minimal and maximal temperatures, have increased in New Caledonia by 0.25°C per decade over the last 39 years (Cavarero *et al.*, 2012). According to climate models analyzing probable climatic changes in the archipelago, minimum and maximum temperatures are expected to increase by +1.5 to +2.7°C by the end of the 21st century (Cavarero *et al.*, 2012). Global warming is expected to induce upward shifts of the elevational distributions of organisms (Walther *et al.*, 2002; Parmesan and Yohe, 2003). Such upward migrations have already been observed in plant species in different biomes (Colwell *et al.*, 2008; Lenoir *et al.*, 2008; Feeley *et al.*, 2011; Feeley *et al.*, 2013; Urli *et al.*, 2014; Morueta-Holme *et al.*, 2015). Previous studies have stressed that New Caledonian plant species from high-altitude habitats could be endangered due to a climate-driven contraction of their suitable habitats (Munzinger *et al.*, 2008; Pillon and Nooteboom, 2009; Hopkins *et al.*, 2009). This thesis has shown a significant positive relation between drought-induced xylem embolism vulnerability and the elevational distribution of species. In this context, species occurring at high elevations, such as *Paracryphia alticola* and *Zygogynum tieghemii,* identified among the most drought-vulnerable species in our study, are thus at significant conservation risk because of drought vulnerability and because their upward migration is limited to very restricted available areas.

Our research represents the first effort to estimate the link between drought vulnerability and the distribution patterns of plant species at the island-wide scale. However, single traits such as P_{50} should not be used in isolation to predict drought survival (Zimmer *et al.*, 2015). Moreover, plant species can have a wide array of drought survival strategies (Delzon, 2015). In this sense, it would be important to include new predictors of physiological drought tolerance such as leaf turgor loss point (Bartlett *et al.*, 2012), stomatal control (Skelton *et al.*, 2015), and leaf-level embolism resistance (Brodribb *et al.*, 2016). Combining such leaf traits with our data on stem embolism vulnerability could round out the drought vulnerability profile of the New Caledonian basal angiosperms. Ongoing efforts on the description of drought tolerance of New Caledonian gymnosperms (S. Delzon pers. comm.), along with future studies dealing with the drought tolerance of other angiosperm clades and across different vegetation types, could provide a thorough overview of the drought vulnerability of the flora of the archipelago. Finally, increasing efforts to gather data on physiological traits, such as xylem embolism vulnerability, could help to develop species distribution models that take into accounts trait values to predict future distributional patterns (Scheiter *et al.*, 2013; van Bodegom *et al.*, 2014).

Despite New Caledonia's status as a biodiversity hotspot, only 3.4% of the archipelago's surface is protected (Morat *et al.*, 2012). Basal angiosperm species are highly distinctive elements of the New Caledonian biota and some of them are representative of presumably larger groups that are assumed to have partly disappeared. From this perspective, they can be strongly informative about past diversity and therefore have high patrimonial value (Grandcolas *et al.*, 2014). Pillon and Munzinger (2005) have outlined that although *Amborella* has been the subject of many scientific studies, none of them has considered the conservation of this species or its habitat. Unfortunately, 11 years later, the great interest of the international scientific community in local species such as *Amborella* has not been reflected in stronger conservation policy. Our research has stressed that basal angiosperm species restricted to humid habitats could be threatened by global warming. We therefore underscore that *Amborella*, along with other distinctive basal angiosperms, should be regarded as flagship species to highlight the need to preserve local biodiversity.

We have suggested that basal angiosperm richness in New Caledonia stems from past climatic stability, inherent to the oceanic climate of the archipelago, which allowed the persistence of refugia during major climatic fluctuations. Montane rainforests may have acted as a past refugia for basal angiosperms as well as other forest species (Pintaud *et al.*, 2001). Because species contracted to and persisted in rainforest refugia when regional climates were unfavorable in the past, these refugia are likely to facilitate survival during projected anthropogenic climate change (Taberlet and Cheddadi, 2002; Keppel *et al.*, 2012). Moreover, it has been suggested that refugia have shaped the current intra- and interspecific diversity in the New Caledonian flora (Poncet *et al.*, 2013). Therefore, the conservation of refugial areas would preserve a significant part of its richness and the evolutionary processes involved in generating the island's diversity. Some of the refugial areas proposed in this study include the mountain range delimited by Roche de la Ouaïeme and Gwâ Rùvianô, including the Massif des Lèvres and Görö Tâné. It should be noted that none of these areas is protected by the local legislation.

6.5 Conclusions and outlook

Understanding the set of evolutionary events associated with the origin and early radiation of flowering plants is one of the main goals of research in plant evolutionary biology. The research carried out during this Ph.D. thesis project provides novel insights into the structural and ecological features of early angiosperms. The observation of a sympodial habit in *Amborella*, combined with previous documentation of sympodiality in basal angiosperms, suggests that sympodial growth was acquired early during the evolution of flowering plants, conferring numerous competitive advantages to the group. Moreover, our analysis of the anatomical evolution of Piperales suggests that they are ancestrally woody, refuting the long-held vision of an herbaceous ancestry for the order. These results support the notion that early angiosperms were woody plants, probably with sympodial growth. Future analysis of lineage-specific evolution of woodiness and sympodiality, covering as many angiosperm lineages as possible, would be needed to understand whether sympodiality and woodiness characterized the growth form of the common ancestor of all flowering plants.

This thesis has shown that New Caledonian basal angiosperms are mostly restricted to humid environments with relatively stable temperatures. This environmental restriction is particularly remarkable in species bearing primitive wood features. Our results agree with the hypotheses that early angiosperms occupied wet environments and that vessel evolution occurred in habitats with high moisture availability and low evaporative demand. This is supported by the observation that representative basal angiosperms have low resistance to drought-induced xylem embolism. Future analyses comparing drought-induced hydraulic failure across angiosperms would be necessary to understand this theory of vessel evolution. In addition to the observed habitat restriction among basal angiosperms, the proposition of past climatic refugia suggests that a past climatic stability may have preserved basal angiosperm species from extinction in the archipelago. These results expand our understanding of the mechanisms that underlie the local over-representation of some angiosperm groups and support a novel scenario to explain the high distinctiveness of the New Caledonian flora. Future studies modeling the past distribution of species may allow us to understand to what extent these refugia preserved other plant lineages from extinction.

Drought- and heat-related impacts on vegetation are being observed around the world, and these events are expected to continue to intensify. During the work done for this thesis, in 2015, our planet had one of the hottest years in recorded history according to NOAA and NASA. This disturbing scenario requires that current ecological studies analyze the potential effects of climate change on vegetation in order to design appropriate conservation strategies. By demonstrating the strong effect of drought vulnerability on the distribution of plant species in the archipelago, this thesis opens up a critical research topic for the conservation of the New Caledonian biodiversity. The accumulation of drought vulnerability data from other plant species, along with the use of new indicators of drought sensitivity, are fundamental for understanding the fate that awaits the unique New Caledonian flora in the current context of anthropogenic climate change.

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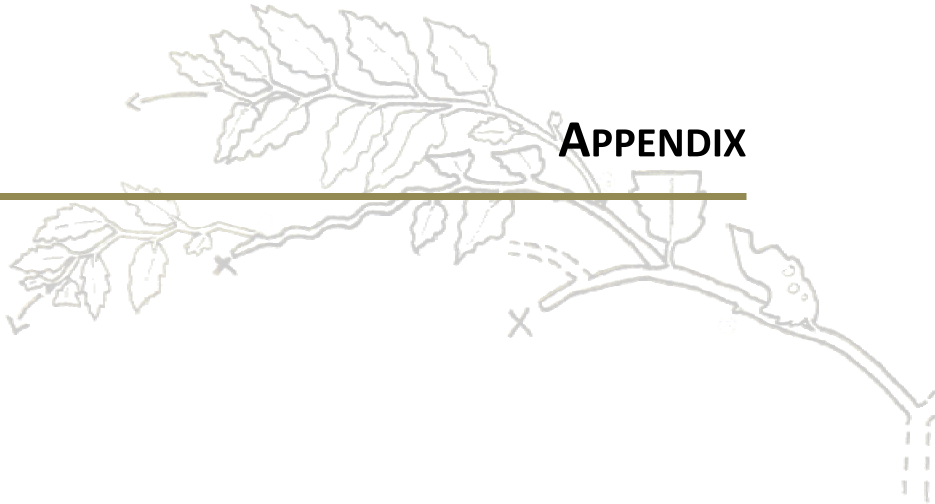
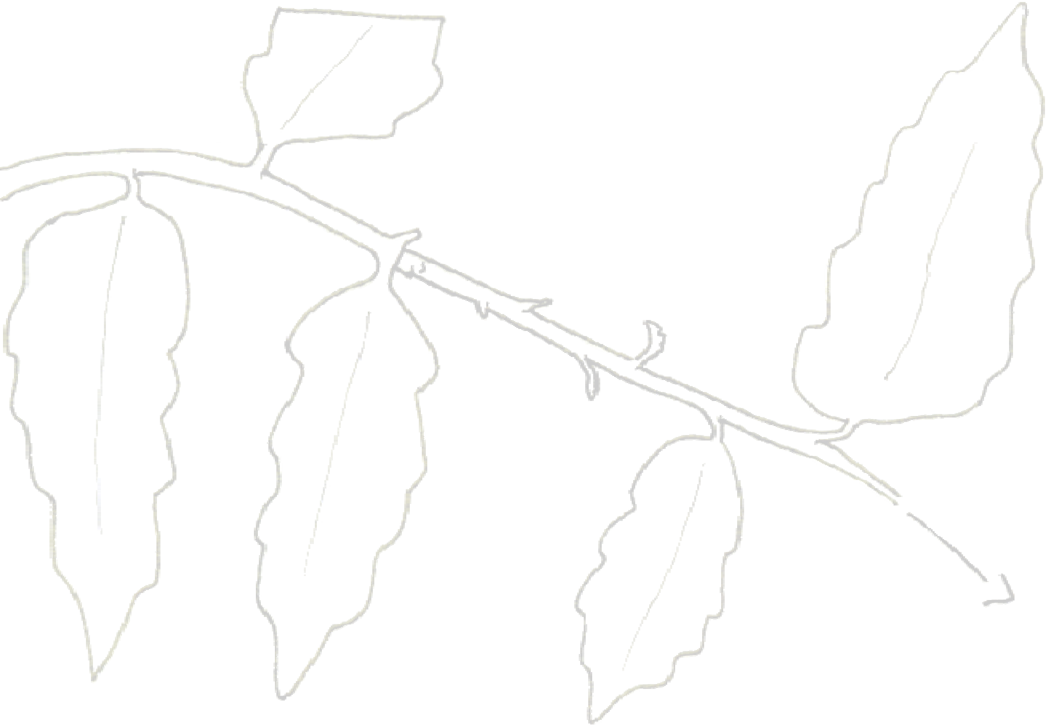
APPENDIX





APPENDIX A1

Voucher Information and Localities for Wood Samples

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

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

APPENDIX A2

Review of anatomical features of Piperales species reported in previous works

FAMILY	SPECIES	GENERAL ANATOMY Number of cycles of vascular bundles	Medullary bundles	1 exclusive medullary bundle	Presence of canals	Active phellogene, bark production	Stem endodermis with a Casparian band	Sclerenchyma ring geometry	Width of the sclerenchyma ring	Aerenchyma	Calcium oxalate	Cambial activity
Aristolochiaceae	*Aristolochia asclepiadifolia* Brandegee	1				+		C				5
Aristolochiaceae	*Aristolochia baetica* L.	1				+		C				4
Aristolochiaceae	*Aristolochia californica* Torr.	1	-	-	-	+	-			-	D	5
Aristolochiaceae	*Aristolochia chiapensis* J.F. Ortega & R.V. Ortega	1				+		C				/
Aristolochiaceae	*Aristolochia clematitis* L.	1						C				2
Aristolochiaceae	*Aristolochia fimbriata* Cham. & Schltdl.	1						C				4
Aristolochiaceae	*Aristolochia gigantea* Mart.	1				+		C				5
Aristolochiaceae	*Aristolochia grandiflora* Sw.	1						C				5
Aristolochiaceae	*Aristolochia griffithii* Hook. F.	1	-	-	-	+	-	C		-	D	5
Aristolochiaceae	*Aristolochia iquitensis* O.C. Schmidt	1	-	-	-	+	-			-		5
Aristolochiaceae	*Aristolochia kaempferi* Willd.	1	-	-	-	+	-	C		-	D	5
Aristolochiaceae	*Aristolochia kalebii* Beutelsp.	1				+		C				4
Aristolochiaceae	*Aristolochia leuconeura* Linden	1				+		C				5
Aristolochiaceae	*Aristolochia lindneri* A. Berger	1						C				4
Aristolochiaceae	*Aristolochia malacophylla* Standl.	1				+		C				5
Aristolochiaceae	*Aristolochia ovalifolia* Duch.	1				+		C				5
Aristolochiaceae	*Aristolochia promissa* Mast.	1				+		C				5
Aristolochiaceae	*Aristolochia rotunda* L.	1						C				2
Aristolochiaceae	*Aristolochia serpentaria* L.	1						C				2
Aristolochiaceae	*Aristolochia sipho* L'Hérit.	1	-	-	-	+	-	C		-	D	5
Aristolochiaceae	*Aristolochia tomentosa* Sims	1				+		C				5
Aristolochiaceae	*Aristolochia triactina* Hook. F.	1	-	-	-	+	-	C		-	D	5
Aristolochiaceae	*Aristolochia tricaudata* Lem.	1				+		C				5
Aristolochiaceae	*Aristolochia veracruzana* J.F. Ortega	1				+		C				5
Aristolochiaceae	*Aristolochia westlandii* Hemsl.	1				+		C				5
Aristolochiaceae	*Thottea grandiflora* Rottb.	1										/
Asaraceae	*Asarum canadense* L.	1	-	-				-	-			3
Asaraceae	*Asarum cardiophyllum* Franch.	1	-	-		-		-	-			3
Asaraceae	*Asarum hartwegii* S. Wats.	1	-	-	-	-	-			-		3
Hydnoraceae	*Hydnora longicollis* Welw.	1	-	-	-	+		-	-	-		2
Hydnoraceae	*Hydnora triceps* Drège & Meyer	1	-	-	-	+	-	-	-	-		2
Hydnoraceae	*Hydnora visseri* Bolin, E. Maass & Muss.	1	-	-	-	+		-	-	-		2

APPENDIX A2
(*Continued*)

FAMILY	SPECIES	GENERAL ANATOMY										
		Number of cycles of vascular bundles	Medullary bundles	1 exclusive medullary bundle	Presence of canals	Active phellogene, bark production	Stem endodermis with a Casparian band	Sclerenchyma ring geometry	Width of the sclerenchyma ring	Aerenchyma	Calcium oxalate	Cambial activity
Hydnoraceae	*Prosopanche americana* (R. Br.) Baill.	1	-		P							2
Lactoridaceae	*Lactoris fernandeziana* Phil.	1	-	-	-	+	-			-		5
Piperaceae	*Macropiper excelsum* Miq. [a]											4
Piperaceae	*Manekia urbani* Trel.	>2	+	-	P, C							5
Piperaceae	*Ottonia martiana* Miq. [b]	2	+	-			+	U				4
Piperaceae	*Peperomia argyreia* E. Morr.	>2	+									1
Piperaceae	*Peperomia cookiana* C. DC.	>2	+					-			D	1
Piperaceae	*Peperomia dahlstedtii* C. DC.	>2	+	-		-	+		-	-	D	1
Piperaceae	*Peperomia ellipticibacca* C. DC.	>2	+					-		+	R	1
Piperaceae	*Peperomia expallescens* C. DC.	>2	+					-			R	1
Piperaceae	*Peperomia hirtipetiola* C. DC.	>2	+					-			-	1
Piperaceae	*Peperomia latifolia* Miq.	>2	+					-			D	1
Piperaceae	*Peperomia leptostachya* Hook. & Arn.	>2	+					-		+	-	1
Piperaceae	*Peperomia lilifolia* C. DC.	>2	+					-			D,R	1
Piperaceae	*Peperomia membranacea* Hook. & Arn.	>2	+					-			D,R	1
Piperaceae	*Peperomia metallica* Lindl. Rodig.	>2	+								D	1
Piperaceae	*Peperomia oahuensis* C. DC.	>2	+		+			-			R	1
Piperaceae	*Peperomia obtusifolia* A. Diertr.	>2	+								D	1
Piperaceae	*Peperomia pellucida* H.B.K.	>2	+									1
Piperaceae	*Peperomia reflexa* Kunth	>2	+					-		+	-	1
Piperaceae	*Peperomia rockii* C. DC.	>2	+					-			-	1
Piperaceae	*Peperomia sandwicensis* Miq.	>2	+					-			D	1
Piperaceae	*Piper betle* L.	2		-	P, C						D	4
Piperaceae	*Piper brachystachyum* Wall.										+	/
Piperaceae	*Piper colubrinum* Link.	2	+	-	P		+	U	4			5
Piperaceae	*Piper cubeba* L. f.										+	4
Piperaceae	*Piper diospyrifolium* Kunth	2	+	-			+	U			R	5
Piperaceae	*Piper kadsura*(Choisy) Ohwi	2	+	-	-		-			-		5
Piperaceae	*Piper longum* L.	2		-	-						-	4
Piperaceae	*Piper nepalense* Miq.										+	/
Piperaceae	*Piper nigrum* L.	2	-	-	P, C		+					4
Piperaceae	*Piper palauense* Horok.											/
Piperaceae	*Piper pedicellosum* Wall.										+	/
Piperaceae	*Piper subrubrispicum* C. DC.	>2		-	C						D	5
Saururaceae	*Anemopsis californica* Hook.	1	-	-	-	-	-					3
Saururaceae	*Saururus cernuus* L.	1	-	-	-	-	+					2

APPENDIX A2
(*Continued*)

SPECIES	WOOD ANATOMY												
	Growth rings boundaries distinct (1)	Vessels in diagonal and / or radial pattern (7)	Vessels exclusively solitary (90% or more) (9)	Vessels in radial multiples (10)	Vessel clusters common (11)	Simple perforation plates (13)	Scalariform perforation plates (14)	Intervessel pits scalariform (20)	Intervessel pits opposite (21)	Intervessel pits alternate (22)	Axial parenchyma absent or extremely rare (75)	Axial parenchyma diffusse (76)	Axial parenchyma diffuse-in-aggregates (77)
Aristolochia asclepiadifolia Brandegee			+										
Aristolochia baetica L.													
Aristolochia californica Torr.						+	-			+		+	+
Aristolochia chiapensis J.F. Ortega & R.V. Ortega													
Aristolochia clematitis L.													
Aristolochia fimbriata Cham. & Schltdl.													
Aristolochia gigantea Mart.													
Aristolochia grandiflora Sw.													
Aristolochia griffithii Hook. F.						+	-			+		+	+
Aristolochia iquitensis O.C. Schmidt						+	-			+		+	
Aristolochia kaempferi Willd.	+					+	-	-	+	+	-	-	+
Aristolochia kalebii Beutelsp.													
Aristolochia leuconeura Linden													
Aristolochia lindneri A. Berger													
Aristolochia malacophylla Standl.													
Aristolochia ovalifolia Duch.													
Aristolochia promissa Mast.													
Aristolochia rotunda L.													
Aristolochia serpentaria L.													
Aristolochia sipho L'Hérit.						+	-			+		+	+
Aristolochia tomentosa Sims	+												
Aristolochia triactina Hook. F.						+	-			+		+	
Aristolochia tricaudata Lem.													
Aristolochia veracruzana J.F. Ortega													
Aristolochia westlandii Hemsl.													
Thottea grandiflora Rottb.													
Asarum canadense L.													
Asarum cardiophyllum Franch.													
Asarum hartwegii S. Wats.			-		+	+	-	+		+			
Hydnora longicollis Welw.													
Hydnora triceps Drège & Meyer						+	-	+	-	-			
Hydnora visseri Bolin, E. Maass & Muss.													
Prosopanche americana (R. Br.) Baill.													

APPENDIX A2
(*Continued*)

SPECIES	WOOD ANATOMY												
	Growth rings boundaries distinct (1)	Vessels in diagonal pattern (7)	Vessels exclusively solitary (90% or more) (9)	Vessels in radial multiples (10)	Vessel clusters common (11)	Simple perforation plates (13)	Scalariform perforation plates (14)	Intervessel pits scalariform (20)	Intervessel pits opposite (21)	Intervessel pits alternate (22)	Axial parenchyma absent or extremely rare (75)	Axial parenchyma diffusse (76)	Axial parenchyma diffuse-in-aggregates (77)
Lactoris fernandeziana Phil.	-	+				+	-	-	-	+	-	-	-
Macropiper excelsum Miq.[a]						+	-						
Manekia urbani Trel.													
Ottonia martiana Miq. [b]													
Peperomia argyreia E. Morr.													
Peperomia cookiana C.DC.													
Peperomia dahlstedtii C. DC.	-					+	-	+	-	+	-		
Peperomia ellipticibacca C. DC.													
Peperomia expallescens C. DC.													
Peperomia hirtipetiola C. DC.													
Peperomia latifolia Miq.													
Peperomia leptostachya Hook. & Arn.													
Peperomia lilifolia C. DC.													
Peperomia membranacea Hook. & Arn.													
Peperomia metallica Lindl. Rodig.													
Peperomia oahuensis C. DC.													
Peperomia obtusifolia A. Diertr.													
Peperomia pellucida H.B.K.													
Peperomia reflexa Kunth													
Peperomia rockii C. DC.													
Peperomia sandwicensis Miq.													
Piper betle L.						+	-			+			
Piper brachystachyum Wall.						+							
Piper colubrinum Link.						+	-			+			
Piper cubeba L. f.						+							
Piper diospyrifolium Kunth													
Piper kadsura(Choisy) Ohwi								+	-	+			
Piper longum L.													
Piper nepalense Miq.						+							
Piper nigrum L.													
Piper palauense Horok.													
Piper pedicellosum Wall.						+							
Piper subrubrispicum C. DC.						+	-						
Anemopsis californica Hook.	+		-	-	+	+	-	+	-	-	-	-	-
Saururus cernuus L.													

APPENDIX A2
(*Continued*)

SPECIES	WOOD ANATOMY (*Continued*)									REFERENCES
	Axial parenchyma scanty paratracheal (78)	Axial parenchyma vasicentric (79)	Axial parenchyma in narrow bands (86)	Ray width – commonly 4- to 10- seriate (98)	Ray width commonly > 10 seriate (99)	Wood rayless (117)	All ray cells upright and / or square (105)	Ray lignification	Multi-seriate rays	
Aristolochia asclepiadifolia Brandegee								+	+	Wagner et al. 2012; Wagner et al. 2014
Aristolochia baetica L.									+	Wagner et al. 2014
Aristolochia californica Torr.			+			-	+	+	+	Carlquist 1993
Aristolochia chiapensis J.F. Ortega & R.V. Ortega									+	Wagner et al. 2014
Aristolochia clematitis L.									+	Wagner et al. 2014
Aristolochia fimbriata Cham. & Schltdl.								-	+	Wagner et al. 2014
Aristolochia gigantea Mart.									+	Wagner et al. 2014
Aristolochia grandiflora Sw.									+	Wagner et al. 2014
Aristolochia griffithii Hook. F.			+			-	+		+	Carlquist 1993
Aristolochia iquitensis O.C. Schmidt	+	+				-	+		+	Carlquist 1993
Aristolochia kaempferi Willd.	-	-	-	-	+	-	+		+	InsideWood 2004-onwards
Aristolochia kalebii Beutelsp.								+	+	Wagner et al. 2012
Aristolochia leuconeura Linden									+	Wagner et al. 2014
Aristolochia lindneri A. Berger								-	+	Wagner et al. 2014
Aristolochia malacophylla Standl.								+	+	Wagner et al. 2012; Wagner et al. 2014
Aristolochia ovalifolia Duch.									+	Wagner et al. 2014
Aristolochia promissa Mast.									+	Wagner et al. 2014
Aristolochia rotunda L.									+	Wagner et al. 2014
Aristolochia serpentaria L.								+	+	Wagner et al. 2014
Aristolochia sipho L'Hérit.			+			-	+		+	Carlquist 1993
Aristolochia tomentosa Sims									+	Wagner et al. 2012; Wagner et al. 2014
Aristolochia triactina Hook. F.		+				-	+		+	Carlquist 1993; Wagner et al. 2014
Aristolochia tricaudata Lem.								+	+	Wagner et al. 2012
Aristolochia veracruzana J.F. Ortega								+	+	Wagner et al. 2014
Aristolochia westlandii Hemsl.								-	+	Wagner et al. 2012; Wagner et al. 2014
Thottea grandiflora Rottb.	+					-	+	+	+	Carlquist 1993
Asarum canadense L.										Wagner et al. 2014
Asarum cardiophyllum Franch.										Wagner et al. 2014
Asarum hartwegii S. Wats.						-	+		+	Carlquist 1993
Hydnora longicollis Welw.		+								Wagner et al. 2014
Hydnora triceps Drège & Meyer										Tennakoon et al. 2007

APPENDIX A2
(*Continued*)

SPECIES	WOOD ANATOMY (*Continued*)									REFERENCES
	Axial parenchyma scanty paratracheal (78)	Axial parenchyma vasicentric (79)	Axial parenchyma in narrow bands (86)	Ray width – commonly 4- to 10- seriate (98)	Ray width commonly > 10 seriate (99)	Wood rayless (117)	All ray cells upright and / or square (105)	Ray lignification	Multi-seriate rays	
Hydnora visseri Bolin, E. Maass & Muss.		+								Wagner et al. 2014
Prosopanche americana (R. Br.) Baill.										Schimper 1880; Wagner et al. 2014
Lactoris fernandeziana Phil.	+	-		-	+	-	+	+	+	Carlquist 1990*b*; Metcalfe and Chalk 1957; Wagner et al. 2014
Macropiper excelsum Miq.[a]							+		+	Carlquist 2013
Manekia urbani Trel.									+	Silva-Sierra et al. 2014
Ottonia martiana Miq. [b]										Souza et al. 2004
Peperomia argyreia E. Morr.										Datta and Dasgupta 1977
Peperomia cookiana C.DC.										Yuncker and Gray 1934
Peperomia dahlstedtii C. DC.	-	+	-	-	+		+			Souza et al. 2004
Peperomia ellipticibacca C. DC.										Yuncker and Gray 1934
Peperomia expallescens C. DC.										Yuncker and Gray 1934
Peperomia hirtipetiola C. DC.										Yuncker and Gray 1934
Peperomia latifolia Miq.										Yuncker and Gray 1934
Peperomia leptostachya Hook. & Arn.										Yuncker and Gray 1934
Peperomia lilifolia C. DC.										Yuncker and Gray 1934
Peperomia membranacea Hook. & Arn.										Yuncker and Gray 1934
Peperomia metallica Lindl. Rodig.										Datta and Dasgupta 1977
Peperomia oahuensis C. DC.										Yuncker and Gray 1934
Peperomia obtusifolia A. Diertr.										Datta and Dasgupta 1977
Peperomia pellucida H.B.K.										Datta and Dasgupta 1977
Peperomia reflexa Kunth										Yuncker and Gray 1934
Peperomia rockii C. DC.										Yuncker and Gray 1934
Peperomia sandwicensis Miq.										Yuncker and Gray 1934
Piper betle L.					+				+	Murty 1959
Piper brachystachyum Wall.									+	Datta and Dasgupta 1977
Piper colubrinum Link.										Ravindran and Remashree 1998
Piper cubeba L. f.									+	Datta and Dasgupta 1977
Piper diospyrifolium Kunth										Souza et al. 2004
Piper kadsura(Choisy) Ohwi		+			+		+			InsideWood 2004-onwards
Piper longum L.										Murty 1959
Piper nepalense Miq.									+	Datta and Dasgupta 1977

APPENDIX A2
(*Continued*)

SPECIES	WOOD ANATOMY (*Continued*)									REFERENCES
	Axial parenchyma scanty paratracheal (78)	Axial parenchyma vasicentric (79)	Axial parenchyma in narrow bands (86)	Ray width – commonly 4- to 10- seriate (98)	Ray width commonly > 10 seriate (99)	Wood rayless (117)	All ray cells upright and / or square (105)	Ray lignification	Multi-seriate rays	
Piper nigrum L.										Ravindran and Remashree 1998
Piper palauense Horok.									+	Carlquist 2013
Piper pedicellosum Wall.									+	Datta and Dasgupta 1977
Piper subrubrispicum C. DC.							+		+	Murty 1959
Anemopsis californica Hook.	-	+	-	-	+	-	+	-	+	Carlquist et al. 1995; Schneider and Carlquist 2001
Saururus cernuus L.										Carlquist et al. 1995

Notes: This table synthesizes the anatomical information found in literature for Piperales species, using the same anatomical features we use in the current study (table 1 in the main text). Presence (+) or absence (-) of a given character is marked only when it has been explicitly declared in the consulted reference; blank cells denote the absence of information for a given character. **Presence of canals**: *P*, Peripheral canals, several canals occurring at the perimeter of the stem as seen in transverse section. *C*, Central canal, a single canal occurring in the center of the stem. **Sclerenchyma ring geometry**: *C*, Circular continuous band of sclerenchyma fibers seen in transverse section. *D*, Discontinuous bands of sclerenchyma, fibers occurring as islands or caps contiguous to vascular bundles. *U*, Undulating and continuous band of sclerenchyma. **Calcium oxalate**: *D*, Druses, *P*, Prismatic crystals. *R*, Raphides. **Cambial activity**: 1, Absent. 2, Restricted to fascicular areas. 3, Woody herb. 4, Slightly woody. 5, Truly woody. /, Not specified. **Ray lignification**: *E*, Complete lignification over the entire width of the stem. *I*, Incomplete or partial ray lignification. Numbers between brackets correspond to the IAWA wood feature numbers. Species names correspond to those mentioned in the cited reference. [a]*Macropiper excelsum* Miq. is considered a synonym of *Piper excelsum* G. Forst. a species belonging to the *Macropiper* group which is part of the "South Pacific" clade according to Jaramillo *et al.* (2008). [b]*Ottonia martiana* Miq. is considered a synonym of *Piper miquelianum* C. DC.

APPENDIX A3

Proportion of species included in the study, infrageneric clades representation and growth habits for each Piperales genera

Genera	Species no.	References	Species no. in the current study			Infrageneric clades	% of species covered	% of clades covered	Growth habit
			Studied	Reviewed	Total				
Saruma	1	Wanke et al. 2006 (1)	1	0	1	NA	100	100	Rhizomatous herb
Asarum	*ca.* 85	Wanke et al. 2006 (1); Kelly 1998	0	3	3	*Asarum s.s.**, *Asiasarum* + *Hexastylis* + *Heterotropa*	3.5	50	Rhizomatous herb
Lactoris	1	Wanke et al. 2007*b*	0	1	1	NA	100	100	Shrub
Hydnora + *Prosopanche*	7 + 3	Naumann et al. 2014	0	4	4	NA	40	100	Subterranean holoparasitic
Thottea	35	Oelschlägel et al. 2011	9	1	10	"India"*, "SE-Asia"*, "India + SE-Asia"*	28.6	100	Shrub, subshrub
Aristolochia	*ca.* 450	Wagner et al. 2014	2	25	27	*Aristolochia**, *Pararistolochia**, *Siphisia**	6	100	Climber, shrub, subshrub, herb
Saururus	2	The Plant List 2013; Wanke et al. 2007*b*	1	1	2	NA	100	100	Rhizomatous herb
Gymnotheca	3	The Plant List 2013; Wanke et al. 2007*b*	1	0	1	NA	33.3	100	Rhizomatous herb
Anemopsis	1	The Plant List 2013; Wanke et al. 2007b	0	1	1	NA	100	100	Rhizomatous herb
Houttuynia	1	The Plant List 2013; Wanke et al. 2007*b*	1	0	1	NA	100	100	Rhizomatous herb
Verhuellia	*ca.* 3	Wanke et al. 2007*a*	1	0	1	NA	100	100	Herb
Zippelia	1	Wanke et al. 2007*b*	1	0	1	NA	100	100	Herb
Manekia	*ca.* 5	Wanke et al. 2007*b*	1	1	2	NA	40	100	Climber
Peperomia	*ca.* 1600	Samain et al. 2009	2	17	19	*Micropiper**, *Oxyrhynchum*, *Leptorhynchum**, *Peperomia**, "unnamed 1"*, "unnamed 2"*, *Panicularia*, "unnamed 3"*, *Pleurocarpidium*, *Tildenia*.	1.2	60	Herb
Piper	*ca.* 2000	Quijano-Abril et al. 2006; Jaramillo et al. 2008	8	14	22	"Neotropical"*, "Tropical Asian"*, "South Pacific"*	1.1	100	Treelet, climber, shrub, subshrub

Note: NA = Not Applicable, denotes monotypic and poorly diversified genera for which any infrageneric divisions have been proposed. Infrageneric clades represented by our sampling and literature survey are marked with an asterisk (*).

APPENDIX A4

List of relict angiosperm genera and corresponding dominant xylem conduit structure

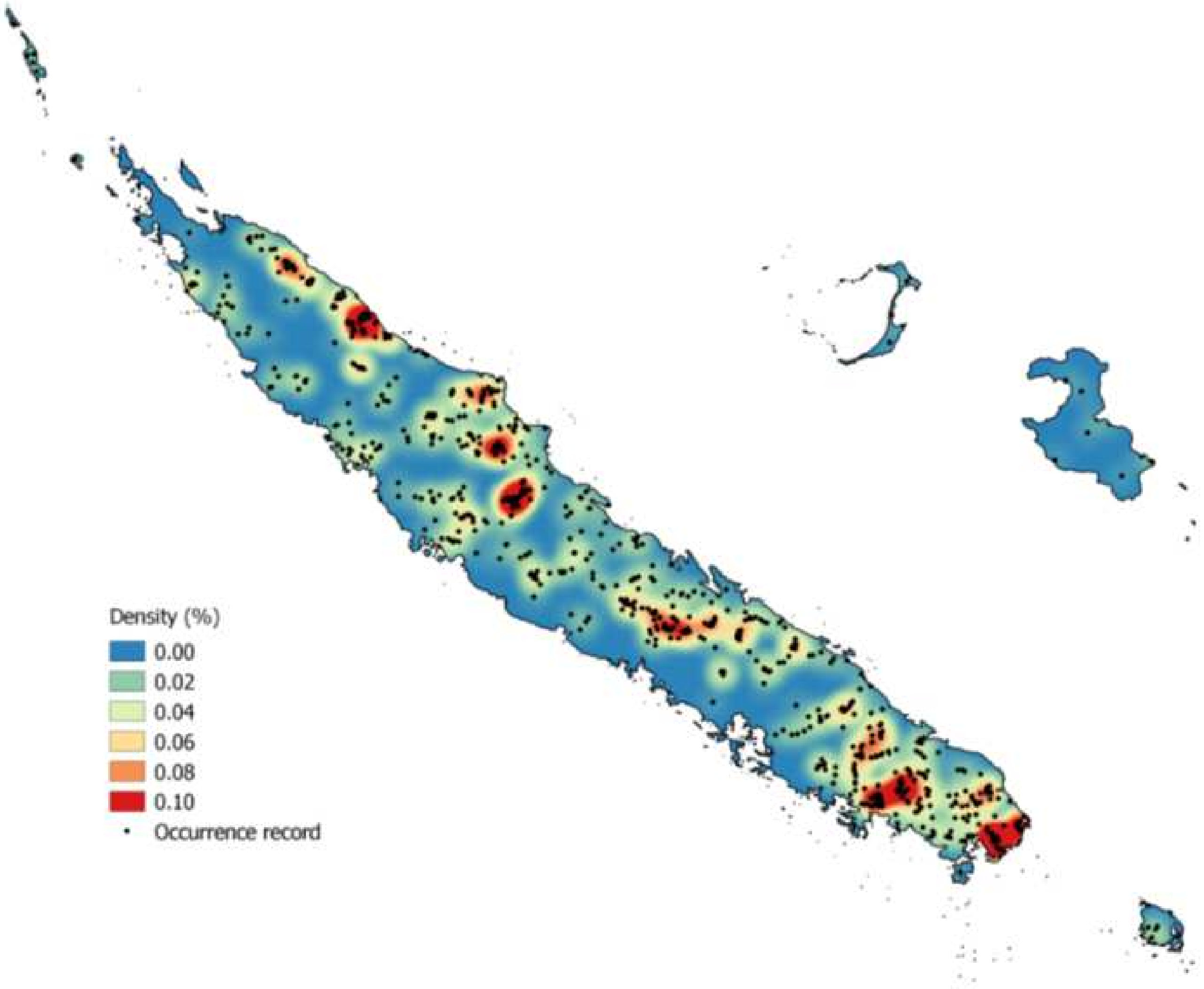
T = tracheids; Sc = vessel elements with scalariform plates; Si = vessel elements with simple perforation plates. References correspond to previous works reporting xylem element structure of related species within the same genus (previously reported species are specified). See below for full references.

Family	**Genus**	**Xylem**	**Reference(s)**	**Reported species**
Amborellaceae	*Amborella*	T	Bailey (1957) Feild *et al.* (2000)	*A. trichopoda*
Annonaceae	*Goniothalamus*	Si	Ingle & Dadswell (1953)	*G. grandifolius*
	Hubera	Si	ST pers. obs.	*Hubera nitidissima*
	Meiogyne	Si	InsideWood (2004-onwards) Metcalfe & Chalk (1950)	*M. mindorensis* *M. virgata*
	Xylopia	Si	Ingle & Dadswell (1953) InsideWood (2004-onwards) Metcalfe & Chalk (1950)	*X. acutiflora* *X. aethiopica* *X. aromatica* *X. aurantiiodora* *X. bemarivensis* *X. buxifolia* *X. cupularis* *X. danguyella* *X. ferruginea* *X. humblotiana* *X. hypolampra* *X. lamii* *X. longipetala* *X. perrieri* *X. pynaertii* *X. phloiodora* *X. acutiflora*
Atherospermataceae	*Nemuaron*	Sc	ST pers. obs.	*N. vieillardii*
Chloranthaceae	*Ascarina*	Sc	Carlquist (1990*a*)	*Ascarina rubricaulis* *Ascarina solmsiana*
Hernandiaceae	*Hernandia*	Si	InsideWood (2004-onwards)	*Hernandia spp.*
Lauraceae	*Beilschmiedia*	Si	ST pers. obs.	*B. oreophila*
	Cryptocarya	Si	InsideWood (2004-onwards) Richter (1990)	*C. alba* *C. alseodaphnifolia* *C. caryoptera* *C. chinensis* *C. crassinervia* *C. enervis* *C. ferrea* *C. griffithiana* *C. mannii* *C. kurzii* *C. wrayi*

	Endiandra	Si	InsideWood (2004-onwards)	*E. palmerstonii* *E. spp.*
	Litsea	Si	InsideWood (2004-onwards) Patel (1987)	*L. acuminata* *L. calicaris* *L. coreana* *L.faberi* *L.glutinosa* *L.japonica* *L. lancifolia* *L. polyantha* *L. sessilis*
Monimiaceae	*Hedycarya*	Sc	Garratt (1934) Patel (1973) Poole & Gottwald (2001)	*Hedycarya angustifolia* *Hedycarya arborea*
	Kibaropsis	Sc	ST pers. obs.	*K. caledonica*
Piperaceae	*Peperomia*	Si	Trueba *et al.* (2015)	*Peperomia blanda* *Peperomia incana*
	Piper	Si	Trueba *et al.* (2015)	*Piper comptonii* *Piper flaviflorum* *Piper gorgonillense* *Piper hispidonervum* *Piper insectifugum* *Piper sarmentosum*
Trimeniaceae	*Trimenia*	Sc	Carlquist (1984)	*T. neocaledonica*
Winteraceae	*Zygogynum*	T	Carlquist (1982, 1983) Feild *et al.* (2002) Hacke *et al.* (2007)	*Z. baillonii* *Z. balansae* *Z. crassifolium* *Z. pancheri* *Z. pommiferum* *Z. queenslandiana* *Z. stipitatum* *Z. spp.*

APPENDIX A5

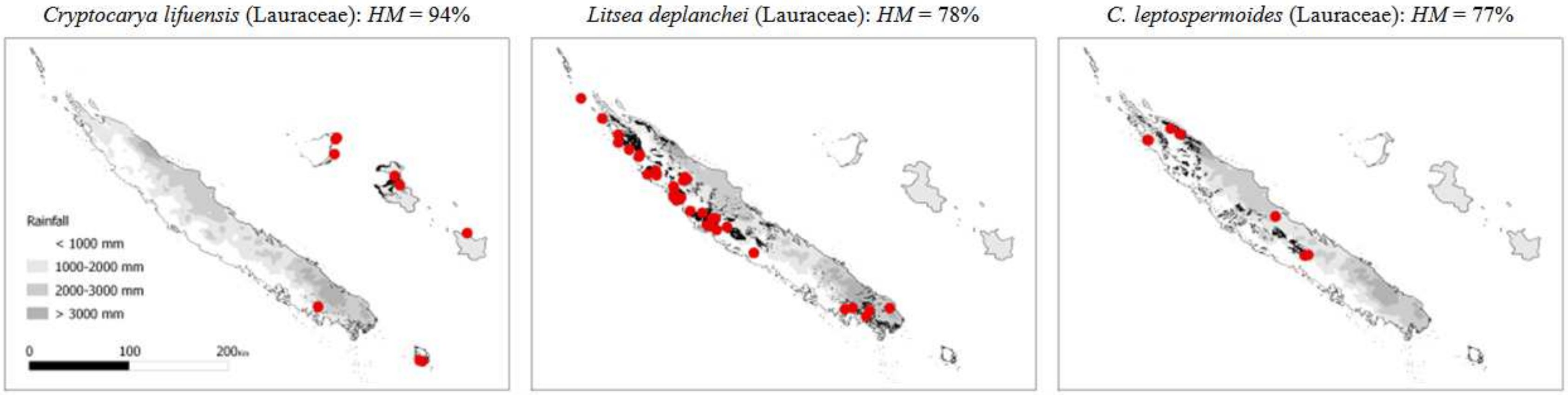
Density of relict angiosperm occurrence data from the NOU Herbarium and the NC-PIPNN plot network over New Caledonia.



APPENDIX A6

Distribution of relict angiosperm species occupying the three most (first line) and the three least marginal habitats (second line).

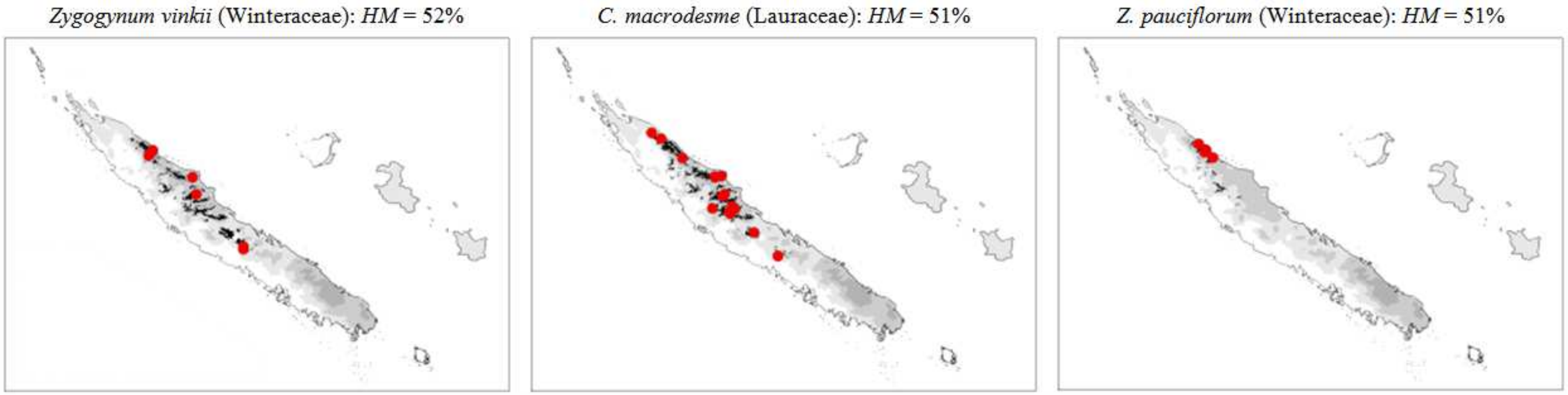
Red circles denote observed occurrences and black areas modelled species distribution. Grey levels correspond to 1000 mm isohyetal lines as shown in the legend.



Cryptocarya lifuensis (Lauraceae): *HM* = 94%

Litsea deplanchei (Lauraceae): *HM* = 78%

C. leptospermoides (Lauraceae): *HM* = 77%



Zygogynum vinkii (Winteraceae): *HM* = 52%

C. macrodesme (Lauraceae): *HM* = 51%

Z. pauciflorum (Winteraceae): *HM* = 51%

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Ecologie, formes et fonctions des angiospermes basales de Nouvelle-Calédonie

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Résumé en Français

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Chapitre 1. Introduction générale

Il y a *c.* 150 MA, lors du Jurassique supérieur, les premières plantes à fleurs (angiospermes) sont apparues. Leur apparition a été suivie d'une invasion majeure dans la niche écologique des gymnospermes durant le Crétacé, conduisant à un bouleversement de la composition de la végétation terrestre sur une période relativement courte. Depuis cette époque, les angiospermes se sont extraordinairement diversifiées et dominent aujourd’hui la végétation de la plupart des écosystèmes terrestres. En plus de leur forte richesse en espèces, les angiospermes présentent également une grande polyvalence morphologique, écologique et fonctionnelle. En outre, elles représentent la base structurelle et énergétique de la grande majorité des écosystèmes terrestres actuels. La richesse spécifique des angiospermes surpasse largement celle de tous les autres groupes de plantes terrestres combinés. Ainsi on estime que 89,4% des espèces d'embryophytes existantes sont des angiospermes. Les plantes à fleurs comportent actuellement c. 350 000 espèces. Les progrès de la systématique moléculaire ont fourni des bases solides pour la reconnaissance des principaux clades d’angiospermes et l'établissement de leurs relations de parenté.

Les angiospermes basales, les premières divergences des plantes à fleurs

Au sein des plantes à fleurs, les eudicotylédones constituent le groupe le plus riche car contenant 73% des espèces d’angiospermes existantes. Le deuxième groupe d’angiospermes le plus riche, les monocotylédones, contient 23,3% des plantes à fleurs. Les "angiospermes basales" sont un ensemble de lignées largement moins diversifiées qui ont divergé temporellement avant le clade massif formé par les monocotylédones-eudicotylédones. Leurs temps de divergence sont les plus anciens pour les angiospermes. En effet, les ordres des Amborellales, Nymphaeales et Austrobaileyales ont été identifiés par plusieurs études phylogénétiques comme des groupes frères successifs de toutes les autres plantes à fleurs. Ces ordres forment un groupe paraphylétique généralement dénommé le grade ANA. Au sein de ce grade, l’ordre des Amborellales est la plus ancienne lignée ayant divergé il y a environ 139,4 MA. Les âges des autres lignées d’angiospermes basales datent elles aussi du Crétacé inférieur. Divergeant immédiatement après le grade ANA, les Chloranthales et les Magnoliidées correspondent à un autre clade de divergence précoce. Malgré leur paraphylie, les espèces incluses dans le grade ANA, les Chloranthales + les Magnolidées sont souvent considérées comme formant un ensemble non naturel, celui des "angiospermes basales" compte tenu de leurs divergences très anciennes. Ce travail de thèse suit cette nomenclature et nous considérons comme angiospermes basales toutes les plantes à fleurs ayant divergé avant le nœud reliant les monocotylédones et les eudicotylédones.

Que peut-on apprendre sur l'évolution des plantes à fleurs en étudiant les angiospermes basales ?

Il est erroné de penser que les angiospermes basales sont des "fossiles vivants" qui sont restés figés dans le temps depuis leur apparition. Les angiospermes basales ne sont pas primitives *per se* car elles sont contemporaines de toutes les autres plantes à fleurs actuelles. Ces lignées ont eu le temps d'éprouver de nombreux changements depuis leurs divergences précoces. Cependant, l'étude des angiospermes basales peut nous fournir des informations cruciales pour la compréhension de l'évolution des angiospermes, car elles correspondent au groupe frère des monocotylédones + eudicotylédones. Dans un contexte comparatif nous pouvons donc émettre des hypothèses sur les caractéristiques des premières angiospermes. Il a ainsi été suggéré que les angiospermes basales portent des caractères plésiomorphiques (*i.e.* des caractères primitifs). Parmi ces caractères observés chez ces lignées, il a été proposé que les éléments de conduction dans le bois portent des caractéristiques primitives. Nous pourrions ainsi lister la présence des bois formés exclusivement par des trachéides, ou encore, des éléments de vaisseaux montrant des perforations scalariformes, lesquels ont été considérés comme une forme transitoire entre les trachéides et les éléments de vaisseaux à perforations simples.

En raison de leur position phylogénétique, mais aussi de la présence de traits primitifs, plusieurs études ont proposé que les angiospermes basales peuvent être des bons indicateurs pour comprendre l'écophysiologie, les formes de vie et l'habitat des premières angiospermes. Il a été proposé que les premières angiospermes auraient été des herbacées habitant des sites ensoleillés, des herbes aquatiques, ou encore des buissons habitant des milieux secs. L'avancement des techniques moléculaires et la compréhension des relations phylétiques des angiospermes ont beaucoup influencé ces théories. En s'appuyant sur l’observation des angiospermes basales des groupes ANA et Chloranthales, une des hypothèses les plus récentes propose que les premières angiospermes auraient été des plantes ligneuses buissonnantes ou arbustives qui ont diversifié dans des milieux humides et ombragés fréquemment soumis à des perturbations liées à la vie en sous-bois.

Nouvelle-Calédonie, terre d'angiospermes basales

La Nouvelle-Calédonie est un archipel situé dans le Pacifique sud-ouest. Elle présente une très grande richesse floristique avec 3371 espèces de plantes vasculaires et un taux d'endémisme très élevé de 74.7% pour la flore vasculaire. L'une des originalités les plus remarquables de la flore néo-calédonienne repose sur l'abondance d'espèces appartenant aux angiospermes basales. Ces lignées sont présentes dans d'autres régions telles que l'Asie du sud-est et l'Australie, cependant la Nouvelle-Calédonie montre concentration exceptionnelle en espèces issues de lignées basales. Les angiospermes basales de Nouvelle-Calédonie sont ainsi représentées par 109 espèces, la plupart endémiques à l'archipel (90 %). L'espèce la plus emblématique, *Amborella* trichopoda, seule représentante des Amborellaceae et espèce sœur de toutes les plantes à fleurs, est également endémique de l'archipel.

De façon remarquable, la plupart des familles d'angiospermes basales appartiennent aux familles surreprésentées de la flore de Nouvelle-Calédonie, par comparaison avec les flores des régions voisines telles que l'Australie. Des études précédentes ont proposé qu'un filtre écologique imposé par les substrats géologiques ultramafiques, très répandus en Nouvelle-Calédonie, pourrait être à l'origine d'une telle disharmonie floristique. En effet les substrats ultramafiques qui couvrent un tiers de la surface de l'archipel sont très chargés en métaux lourds, pauvres en nutriments, et ont une faible capacité de rétention d'eau. Ces substrats induisent ainsi une contrainte très importante au développement des plantes, imposant une préadaptation des espèces colonisatrices. Si la surreprésentation des angiospermes basales a été signalée il y a quelques années, depuis aucun travail n'a encore proposé de mécanismes qui pourraient expliquer cette disharmonie, ainsi que le maintien dans l'archipel de ces lignées si particulières.

Objectifs

Ce travail de thèse a pour objectif de compléter nos connaissances sur la forme, les fonctions et l'écologie des angiospermes basales en s'appuyant principalement sur l'étude des espèces néo-calédoniennes. Plusieurs objectifs ont été fixés : évaluer l'influence de l'ouverture de la canopée dans la variation de la forme de vie et de la coordination des traits chez *Amborella trichopoda*, la seule représentante de la plus ancienne lignée des angiospermes (Chapitre 2) ; proposer des patrons évolutifs pour différents traits de bois liés aux formes de vie chez les Piperales, ordre le plus diversifié des angiospermes basales (Chapitre 3) ; tester des hypothèses écologiques et climatiques qui pourraient expliquer la surreprésentation des angiospermes basales en Nouvelle-Calédonie (Chapitre 4) ; et investiguer les corrélations entre la distribution environnementale des espèces et leur résistance à la sécheresse (Chapitre

5). A travers ces travaux, nous espérons donner de nouvelles perspectives pour la compréhension des caractéristiques des premières angiospermes, et apporter un nouvel éclairage sur les mécanismes expliquant le maintien des lignées d'angiospermes basales en Nouvelle-Calédonie.

Chapitre 2. Coordination des traits, biomécanique, et plasticité de la forme de croissance d'*Amborella trichopoda* sous différentes ouvertures de canopée

Comprendre la distribution des traits de vie à travers la phylogénie des angiospermes peut aider à connaître la hiérarchie imbriquée des fonctionnalités qui caractérise des nœuds évolutifs clés. La découverte de la position d'*Amborella* en tant qu'espèce sœur de toutes les angiospermes soulève la question de savoir si elle partageait avec elles certains traits fonctionnels clés, ainsi que des réponses plastiques aux variations environnementales qui caractérisent les angiospermes d'un point de vue global. Avec cet objectif, nous avons (1) étudié l'architecture végétative d'*Amborella* et analysé la coordination de 12 traits structurels et fonctionnels des tiges et des feuilles (2) testé l'hypothèse d'une présence de réponses plastiques de ce traits et de leur coordination, induits par l'ouverture de la canopée. Nous avons ainsi montré que la croissance d'*Amborella* est réalisée selon une série de complexes ramifiés à croissance sympodiale, lesquels varient en taille et en mode de ramification selon l'ouverture de la canopée. L'absence de hiérarchie architecturale marquée contribue à la plasticité morphologique de l'espèce. Des corrélations entre la plupart des traits foliaires et des tiges ont été observées. Nous constatons également une importante modulation de la structure foliaire induite par les variations de lumière disponible. Toutefois les branches se développant dans différents milieux lumineux présentent une allométrie similaire. Des changements dans les taux d'élongation des tiges, ainsi que dans la taille des feuilles, engendrent des morphologies distinctes chez *Amborella* lorsqu'elle croît sous des conditions lumineuses différentes. Cependant, les propriétés mécaniques des tiges ne montrent aucune différence significative entre ces environnements lumineux contrastés. La croissance sympodiale observée chez *Amborella* pourrait ainsi correspondre à une synapomorphie des angiospermes. Notre étude apporte des preuves d'une coordination intra-spécifique entre les spectres économiques des feuilles et des tiges. La variation des traits le long de ces spectres permet la réalisation de stratégies adaptatives fonctionnelles sous différents milieux lumineux. L'observation de cette variation chez *Amborella* suggère que ces réponses plastiques étaient présentes chez l'ancêtre hypothétique commun à toutes les angiospermes.

Chapitre 3. Evolution de la croissance secondaire chez les Piperales, un ordre diversifié d'angiospermes basales

Les Piperales représentent l'ordre le plus diversifié d'angiospermes basales avec *c.* 4090 espèces. Cette diversité d'espèces s'accompagne d'une grande diversité des formes de vie qui serait liée aux variations de l'activité cambiale fréquente au sein de l'ordre. En dépit des nombreuses études s'intéressant aux Piperales, aucun travail de synthèse n'a traité l'évolution de l'activité cambiale. Ce chapitre se propose de compiler des informations anatomiques de toutes les lignées de Piperales, et d'effectuer une reconstruction phylogénétique des états des caractères. L'origine du caractère ligneux chez les Piperales est discutée ainsi que la fonctionnalité écologique de certains caractères anatomiques. Les observations anatomiques ont été réalisées sur 28 espèces incluant des représentants néo-calédoniens appartenant au genre *Piper*, et des espèces représentatives de toutes les lignées de Piperales d'autres régions du monde. En combinant les données originales du présent travail et celles d'études précédemment publiées, nous établissons une reconstruction de l'activité cambiale et des types de perforation des éléments de vaisseaux afin d'estimer l'origine du caractère ligneux et l'évolution vasculaire des Piperales. Différents patrons de l'activité cambiale sont ainsi décrits; variant d'une croissance secondaire localisée dans les régions intra- et inter-fasciculaires chez *Aristolochia* et *Thottea* (Aristolochiaceae), *Saruma* (Asaraceae), *Manekia* et *Piper* (Piperaceae), à une activité cambiale restreinte aux régions fasciculaires chez les Saururaceae. En outre, une absence totale de croissance secondaire est observée chez *Verhuellia* (Piperaceae) ainsi que dans quelques espèces de *Peperomia* (Piperaceae). Les éléments de vaisseaux de Piperaceae, Aristolochiaceae et Asaraceae ont des perforations simples tandis que ceux des Saururaceae sont principalement scalariformes. Un endoderme possédant une bande de Caspary (caractère atypique dans les tiges aériennes) est observé pour tous les genres de Piperaceae, ainsi que pour *Saururus* et *Houttuynia* (Saururaceae). Nos travaux suggèrent que l'ancêtre hypothétique commun des Piperales possédait très probablement un cambium actif et une forme de vie ligneuse, avec une vascularisation caractérisée par des éléments de vaisseaux à perforations simples. Cette hypothèse est appuyée par l'homogénéité anatomique des espèces ligneuses des Piperales qui partagent toutes des caractères du bois tels que des rayons très larges et longs, suggérant une origine commune du bois pour l'ordre. La grande diversité des formes de vie observée chez les Piperales est certainement liée à une histoire évolutive témoignant de changements fréquents de l'activité cambiale par le passé, associés à des changements de forme de vie.

Dans le contexte local de la Nouvelle-Calédonie, les formes grimpantes et herbacées des Piperaceae, inhabituelles au regard du reste des angiospermes basales néo-calédoniennes, pourraient être à l’origine de la grande marginalité de niche de cette famille témoignant ainsi d'un lien entre la diversité des formes de vie et la diversité écologique.

Chapitre 4. Caractérisation de l'habitat des angiospermes basales de Nouvelle-Calédonie, distribution présente et passée des espèces

La flore de Nouvelle-Calédonie présente une forte disharmonie et les angiospermes basales font partie des lignées de plantes à fleurs surreprésentées. Cependant, les mécanismes derrière leur prévalence restent inconnus. Cette section de thèse explore deux hypothèses majeures pour expliquer cette surreprésentation: (1) la diversité d'angiospermes basales est la conséquence d'une adaptation aux sols ultramafiques qui fonctionnent comme un filtre contre des nouveaux colonisateurs; et (2) cette diversité découle d'un climat humide qui aurait persisté en Nouvelle-Calédonie pendant le Quaternaire tandis que l'Australie et d'autres îles de la région ont souffert des variations climatiques plus importantes causant des possibles événements d'extinction. Etant donné que les angiospermes basales présentent une grande diversité anatomique des conduits du xylème, nous testons si la présence des différents types de conduits du xylème peut expliquer une différentiation écologique au sein de notre groupe d'étude. Nous avons utilisé des modèles de distribution pour déterminer des corrélats environnementaux pour 60 espèces d'angiospermes basales. Des variables telles que le type de végétation, le substrat et le climat ont été utilisées pour caractériser l'habitat type. Nous avons ensuite testé si le type d'élément de conduction dans le bois, lequel devrait affecter directement leur capacité hydraulique, est corrélé avec la préférence d'habitat des espèces. Finalement, nous avons analysé une possible prédominance des espèces sur différents substrats et projeté la taille de leur habitat et de leur distribution lors du dernier maximum glaciaire. Les résultats montrent une préférence des angiospermes basales pour des habitats de forêt humide localisés sur des substrats non ultramafiques. Seules les espèces possédant des éléments de vaisseaux à perforations simples présentent un habitat plus étendu. Nous avons également montré que ces habitats de forêt humide ont subi une réduction très importante de leur surface et un déplacement géographique pendant la dernière période glaciaire vers des zones refuges localisées sur la côte est, laquelle est plus pluvieuse et plus chaude. Le maintien des angiospermes basales dans des habitats caractérisés par de faibles demandes d'évaporation semble être lié aux limitations hydrauliques de ces espèces. La surreprésentation des angiospermes basales en Nouvelle-Calédonie peut donc être expliquée par la persistance des forêts humides dans l'archipel malgré les fluctuations climatiques du Quaternaire qui ont affecté la végétation de la région. Cette étude propose ainsi un nouveau modèle pour expliquer la représentation disharmonique de certaines familles d'angiospermes en Nouvelle-Calédonie.

Chapitre 5. Influence de la vulnérabilité à la sécheresse dans la répartition géographique des angiospermes basales en Nouvelle-Calédonie

Les angiospermes basales de Nouvelle-Calédonie ont une préférence marquée pour des écosystèmes forestiers humides (Chapitre 4). Or nous avons également montré que ces habitats forestiers ont pu constituer des refuges pour ces lignées basales au cours du dernier maximum glaciaire. La prévalence actuelle des angiospermes basales dans les forêts humides de Nouvelle-Calédonie pourrait s’expliquer par une forte vulnérabilité à la sécheresse. Dans le contexte contemporain de changements climatiques, une augmentation de la mortalité des arbres provoquée par la sécheresse est observée dans de nombreuses forêts humides de la planète. La sécheresse est en outre susceptible d'affecter la distribution géographique de la végétation tropicale mondiale. Cependant, le lien entre la vulnérabilité à la sécheresse et la distribution environnementale des espèces tropicales a été très peu étudié. Dans ce chapitre nous avons mesuré la vulnérabilité aux embolies (P_{50}) de 13 espèces d'angiospermes basales endémiques de Nouvelle-Calédonie présentant des morphologies distinctes des conduits du xylème. Nous avons examiné la relation entre la variable P_{50} et une gamme de variables environnementales d'une part, et d'autres traits fonctionnels comme la teneur de matière sèche des feuilles, la densité des nervations des feuilles et la densité du bois. Les angiospermes basales sélectionnées ont des valeurs de P_{50} variant entre -4.03 et -2.00 MPa, la plupart des espèces se situant dans une gamme étroite de résistance inférieure à - 2.7 MPa. La vulnérabilité aux embolies est significativement corrélée à l'altitude, à la température annuelle moyenne, et au pourcentage d'occurrences géographiques des espèces dans des régions de forêt humide. Le type de conduit du xylème n’explique pas la variation de résistance à la sècheresse (P_{50}) entre les espèces. Les traits fonctionnels mesurés, couramment utilisés comme proxy de la résistance à la sécheresse, ne sont pas liés à la P_{50}. La vulnérabilité aux embolies dans le xylème se distingue comme un trait physiologique étroitement lié à la distribution des espèces de forêt humide. Notre étude suggère que la différentiation écologique associée à la résistance à la sécheresse est découplée de la densité de bois chez les espèces de forêt humide. Ces résultats suggèrent que la sécurité hydraulique et la densité de bois ont pu suivre des chemins évolutifs distincts chez les espèces d'habitats humides. Finalement, nous soulignons un risque important pour la conservation des angiospermes basales des forêts humides d'altitude en Nouvelle-Calédonie.

Chapitre 6. Discussion générale, Conclusions et Perspectives

Un aperçu de la forme de vie, la fonction, et l'écologie des premières angiospermes

La forme de vie et les préférences écologiques des premières angiospermes sont parmi les aspects les plus incertains de l'histoire évolutive des angiospermes. L'une des hypothèses les plus fameuses est celle des 'paléoherbes'. Cette hypothèse s'appuie sur l'observation des espèces des Piperaceae et des Chloranthaceae, et propose que les premières angiospermes étaient représentées par herbes rhizomateuses. L'hypothèse des paléoherbes s'est appuyée sur l'observation de caractères juvéniles dans le bois des plantes adultes chez les Piperales, qui indiquerait un ancêtre putatif herbacée selon l'hypothèse du « secondary woodiness ». Cependant, nos travaux sur l'évolution anatomique des Piperales indiquent que l'ancêtre commun de l'ordre possédait plutôt un cambium actif et présentait une forme de vie ligneuse. Nos résultats s'opposent ainsi à l'hypothèse des paléoherbes et suggèrent que les caractères juvéniles dans le bois ne sont pas un indicateur fiable d'un ancêtre putatif herbacé.

Une hypothèse plus récente, s'appuyant sur des données fossiles et écophysiologiques, en accord avec nos résultats, suggère que les premières angiospermes étaient des plantes ligneuses qui habitaient des habitats humides, ombragés, et fréquemment soumis à des perturbations (hypothèse « dark and disturbed »). La croissance sympodiale ainsi que la capacité à produire des rejets basitones chez *Amborella* sont des caractéristiques qui sont très rares, voir absentes, chez les gymnospermes. Ces caractéristiques de développement ont été proposées comme des réponses adaptatives à la vie dans des milieux fréquemment perturbés car permettant d'adopter une stratégie de persistance après une perturbation. L'observation d'une croissance sympodiale chez *Amborella trichopoda*, laquelle a été précédemment décrite chez d'autres angiospermes basales, suggère que ce mode de développement était déjà présent chez l'ancêtre commun de toutes les angiospermes. Ces caractéristiques ont pu fournir aux premières angiospermes des capacités de compétition qui ont participé à leur dominance des écosystèmes terrestres.

En plus de ces caractéristiques particulières aux angiospermes basales, nos travaux ont pu montrer des patrons de covariation des traits qui sont connus dans des nombreuses plantes vasculaires. Par exemple, nos résultats ont montré que des traits fonctionnels foliaires d'*Amborella* tels que la teneur en masse sèche, ainsi que le rapport de masse sèche par unité de surface fraiche, varient respectivement 6 à 5 fois en fonction des conditions lumineuses dans lesquels la plante se développe. Etant donné que ce type de réponses foliaires a été largement documenté chez d'autres groupes d'angiospermes, l'observation de cette variation chez l'espèce sœur de toutes les angiospermes confirme l'importance adaptative de cette réponse phénotypique. Nous avons également montré qu'il existe une relation forte entre la densité des tiges et la teneur de masse sèche par surface des feuilles. Cette coordination des traits entre les organes de la plante a été largement documentée dans d'autres groupes de plantes ligneuses (angiospermes et non-angiospermes). Le caractère universel de cette coordination de traits suggère donc l'existence des contraintes physiques et physiologiques qui pourraient empêcher la production de formes 'inaccessibles' chez les végétaux.

Evolution et écologie de l'appareil vasculaire des angiospermes. Contributions de cette thèse

La grande majorité des études s'accordent sur l'absence de vaisseaux dans le bois de l'ancêtre commun des angiospermes. Les trachéides étaient alors les seules structures responsables de la conduction de l'eau dans les tiges. A partir de ces trachéides, les plantes à fleurs auraient ainsi développé des vaisseaux exhibant une grande diversité structurelle. Cette tendance évolutive a longtemps été perçue comme une trajectoire linéaire depuis les trachéides vers les éléments de vaisseaux à perforation simple. Les éléments de vaisseaux à perforation scalariforme représenteraient une forme transitoire. Cependant, nos travaux sur l'évolution de l'anatomie des éléments de conduction dans le bois des Piperales proposent une possible réversion des perforations simples vers des perforations scalariformes. Ces résultats remettent en cause le caractère irréversible de cette tendance évolutive et fournissent un exemple de ce type de réversion dans l'appareil vasculaire des angiospermes. D'autres cas épars de réversion ont été mis en évidence par le passé. De futures investigations sur l'évolution des clades d'angiospermes présentant les deux types de vascularisation seraient pertinentes afin de mieux comprendre les processus qui ont conduit à ces reversions.

Au delà des conclusions sur l'évolution structurelle du bois, nos recherches fournissent aussi des nouvelles informations sur l'influence de la vascularisation du bois dans l'écologie des plantes. Il a été suggéré que les premières angiospermes munies d'éléments de vaisseaux étaient restreintes à des milieux humides en raison d'une limitation hydraulique imposée par la structure de leurs vaisseaux 'primitifs', présentant des perforations scalariformes. Nos travaux illustrent la première relation directe entre l'occupation des habitats et le type d’élément de conduction dans le bois. Ces résultats suggèrent que les espèces qui présentent une vascularisation formée par des éléments de vaisseaux à perforations scalariformes, ainsi que par des trachéides, sont restreintes à des habitats humides et peu variables en température. Les espèces qui présentent des vaisseaux à perforation simple, lesquels confèrent une meilleure performance hydraulique, seraient capables d’occuper des habitats plus diversifiés tels que le maquis ou les forêts sèches de Nouvelle-Calédonie où les demandes évapotranspiratives sont plus importantes.

Un autre résultat majeur de cette thèse repose sur la mise en évidence d’une forte influence de la physiologie du bois dans la distribution des espèces. En effet, nous avons montré un lien très fort entre la capacité à résister aux embolies provoquées par la sécheresse et l’occupation des habitats. De plus, nous n’avons pas trouvé de différence significative entre la résistance à la sécheresse des plantes avec ou sans vaisseaux. L’absence de différences malgré les différences anatomiques dans le bois suggère que d’autres caractéristiques anatomiques des conduits telles que les membranes des ponctuations doivent jouer un rôle majeur dans la résistance aux embolies. Finalement, l’absence de différentiation entre les espèces sans vaisseaux et les espèces pourvues des vaisseaux primitifs suggère que les premiers vaisseaux ont été développés dans des zones avec une humidité stable et peu soumises à la sécheresse.

La stabilité climatique du passé et les zones refuge, une nouvelle explication pour la disharmonie de la flore néo-calédonienne

En se basant sur des études globales et régionales, de nombreux travaux ont montré que les plantes qui colonisent de nouvelles aires géographiques ont tendance à occuper des biomes similaires à ceux de leurs aires d'origine. Cette hypothèse connue sous le terme de « conservatisme de niche » repose sur l’idée qu’il est plus facile pour une espèce de se déplacer plutôt que d’évoluer. A partir de ce principe, nous pouvons émettre l’hypothèse que l’environnement occupé actuellement par les angiospermes basales de Nouvelle-Calédonie possède des caractéristiques similaires à celui qu’elles ont occupée avant leur arrivée sur l’archipel. Nos travaux ont montré que le plus grand chevauchement d'habitat de 62 espèces d’angiospermes basales se trouve dans des zones avec une précipitation relativement forte (1,500 à 2,300 mm) et de faibles variations journalières de température. Ces caractéristiques correspondent aux zones de forêt humides, suggérant ainsi que les angiospermes basales ont occupé des habitats de forêt humide depuis leur arrivée en Nouvelle-Calédonie. Etant donné que la Nouvelle-Calédonie a été complètement immergée pendant le Pléistocène et que sa réémergence a eu lieu pendant l'Eocène supérieur, les angiospermes basales de Nouvelle-Calédonie ont du occuper des forêts humides dans des régions voisines telles que l'Australie, ou des îles aujourd'hui disparues, pour coloniser par la suite l'archipel Calédonien.

L'Australie a souffert d'une dessiccation progressive au cours des dernières 25 MA, culminant dans des événements d'aridité extrême pendant les cycles glaciaires. Cette dessiccation a pu provoquer l'extinction d'espèces appartenant aux angiospermes basales en Australie. Grace à ses caractéristiques géographiques et topographiques, la Nouvelle-Calédonie présente un climat océanique avec des alizés qui apportent une grande quantité d'humidité. Le climat océanique de Nouvelle-Calédonie a probablement constitué un atout majeur à la survie des lignées angiospermes basales sur le territoire pendant ces périodes des changements climatique. En effet, notre étude suggère que des habitats de forêt humide ont pu subsister en Nouvelle-Calédonie, agissant comme un refuge pendant les grandes fluctuations climatiques du dernier maximum glaciaire. Notre étude confirme donc l'importance de la présence des refuges des forêts humides pour le maintien des espèces végétales en Nouvelle-Calédonie. Cette stabilité climatique peut-être un mécanisme majeur pour expliquer la surreprésentation de certains groupes des plantes à fleurs en Nouvelle-Calédonie.

Implications de cette thèse pour la conservation de la flore de Nouvelle-Calédonie

La Nouvelle-Calédonie est considérée comme l'un des points chauds pour la conservation de la biodiversité mondiale en raison de sa riche biodiversité, mais aussi en raison des nombreuses menaces qui pèsent sur celle-ci, telles que les activités minières, les feux de forêt, et les espèces envahissantes. Etonnamment, bien que le changement climatique représente une menace potentiellement forte sur la biodiversité globale, aucune étude n'a évalué son impact sur la végétation de la Nouvelle-Calédonie. En outre, les îles océaniques sont considérées comme fortement vulnérables aux changements climatiques en raison de certaines caractéristiques inhérentes telles que la faible disponibilité de surface et les petites tailles des populations. Cette vulnérabilité particulière exhorte à considérer le changement climatique comme une menace potentielle sur la flore de l'archipel calédonien.

Le climat de notre planète est en train de changer, la température globale augmente et on atteint des chiffres record. De même, on attend des changements dans le régime des précipitations à travers le monde. Il a été suggère que l'augmentation de la fréquence et de la gravité des sécheresses aurait un impact majeur sur la mortalité des espèces végétales. Malgré ces constats, à notre connaissance, ce phénomène n'a pas encore été observé en Nouvelle-

Calédonie. Etant donné que les angiospermes basales sont très abondantes dans les communautés forestières de Nouvelle-Calédonie, mais aussi parce qu'elles semblent être très vulnérables à la sécheresse, les angiospermes basales pourraient constituer des indicateurs potentiels de l'effet de la sécheresse sur la flore de l'archipel. Un suivi des populations d’angiospermes basales pourrait fournir une alerte précoce de l'impact de la sécheresse sur la végétation en Nouvelle-Calédonie.

En analysant les tendances climatiques de l’archipel à travers les dernières 39 années, il a été suggéré que la température est en train de monter à un rythme de 0,25 °C par décennie. Selon les modèles climatiques, les températures devraient augmenter de *c.* 2 °C à la fin du 21ème siècle. Le réchauffement climatique pourrait entraîner des déplacements altitudinaux dans la distribution d'organismes. Ces migrations ont déjà été observées chez des espèces végétales au sein de différents biomes. Cette thèse a montré une relation positive entre la vulnérabilité du xylème face aux embolies induites par la sécheresse et la distribution altitudinale des espèces. Dans ce contexte, notre étude a montré que les espèces qui se trouvent à des altitudes élevées comme *Paracryphia alticola* et *Zygogynum tieghemii*, sont parmi les espèces les plus vulnérables à la sécheresse. La vulnérabilité de ces espèces serait d’autant plus exacerbée que leur migration est limitée par une réduction de la surface disponible avec l’altitude.

Ces travaux de thèse représentent la première tentative d'évaluer le lien entre la vulnérabilité à la sécheresse et les patrons de distribution des espèces végétales à l’échelle de l’archipel. Cependant, des traits tels que la résistance du bois aux embolies ne devraient pas être utilisés seuls pour évaluer la vulnérabilité à la sécheresse car les végétaux disposent des nombreuses stratégies pour faire face à la sécheresse. En ce sens, il serait pertinent d’inclure d’autres indicateurs de résistance à la sécheresse tels que le point de perte de turgescence des feuilles ou le control de l'activité stomatique. L’intégration de traits physiologiques des feuilles pourrait compléter nos connaissances sur la vulnérabilité à la sécheresse des angiospermes basales. Finalement, des travaux considérant la tolérance à la sécheresse d’autres lignées de plantes présentes en Nouvelle-Calédonie ainsi que d'autres types de végétation, pourraient fournir un aperçu plus intégral de la vulnérabilité à la sécheresse de la flore de l'archipel. Enfin, ces traits écophysiologiques pourraient être intégrés aux modèles de distribution d’espèces qui prennent en compte les valeurs des traits pour projeter la distribution des espèces.

Malgré son statut de *hotspot* pour la conservation de la biodiversité seulement 3,4% de la surface de Nouvelle-Calédonie est protégée. Les angiospermes basales sont des taxons très distinctifs de la biodiversité Calédonienne. De ce point de vue, il serait pertinent de mettre en avant des taxons tels qu'*Amborella*, qui ont une forte valeur patrimoniale, pour attirer l'attention mondiale sur la conservation de la flore locale. Nous avons suggéré que la richesse d'angiospermes basales en Nouvelle-Calédonie pourrait être le résultat de la stabilité du climat passé, qui aurait permis la présence de refuges forestiers pendant des grandes fluctuations climatiques globales. Ces refuges pourraient faciliter la survie des espèces pendant les changements climatiques d'origine anthropique qui sont en train d'affecter la planète. Par conséquent, la préservation de ces zones refuges pourrait permettre de conserver une part importante de la flore locale. Certaines zones refuges évoquées dans notre étude comprennent la zone de montagnes située entre Roche de la Ouaïeme et Gwâ Rùvianô, cette zone comprend le Massif des Lèvres et Görö Tâné. Il convient de noter qu'aucune de ces zones est protégée par la législation de la Nouvelle-Calédonie. La compréhension de l'impact du changement climatique sur les espèces de l'archipel, ainsi que la préservation des potentielles zones refuge, sont essentielles pour la protection de la richesse floristique de la Nouvelle-Calédonie.

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Conference abstracts

Trueba, S., Pouteau, R., Delzon, S., Lens, F., Olson, M.E., Feild, T.S., Isnard, S. (2015). Basal angiosperm species as listening posts for climate change in New Caledonia. *International Congress for Conservation Biology – European Congress for Conservation Biology (ICCB-ECCB).* Montpellier, France.

Trueba, S., Pouteau, R., Delzon, S., Lens, F., Feild, T.S., Isnard, S., Olson, M.E. (2015). Basal angiosperm species as barometers for climate change in New Caledonia, an ecophysiological and biogeographical approach. *Association of Tropical Biology and Conservation ATBC annual meeting*. Honolulu, Hawaii.

Pouteau R., **Trueba S.**, Isnard S. (2014). The ecological niche of extant basal angiosperms native to New Caledonia: Biogeographical and evolutionary implications. *Island Biology 2014,* Honolulu, Hawaii.

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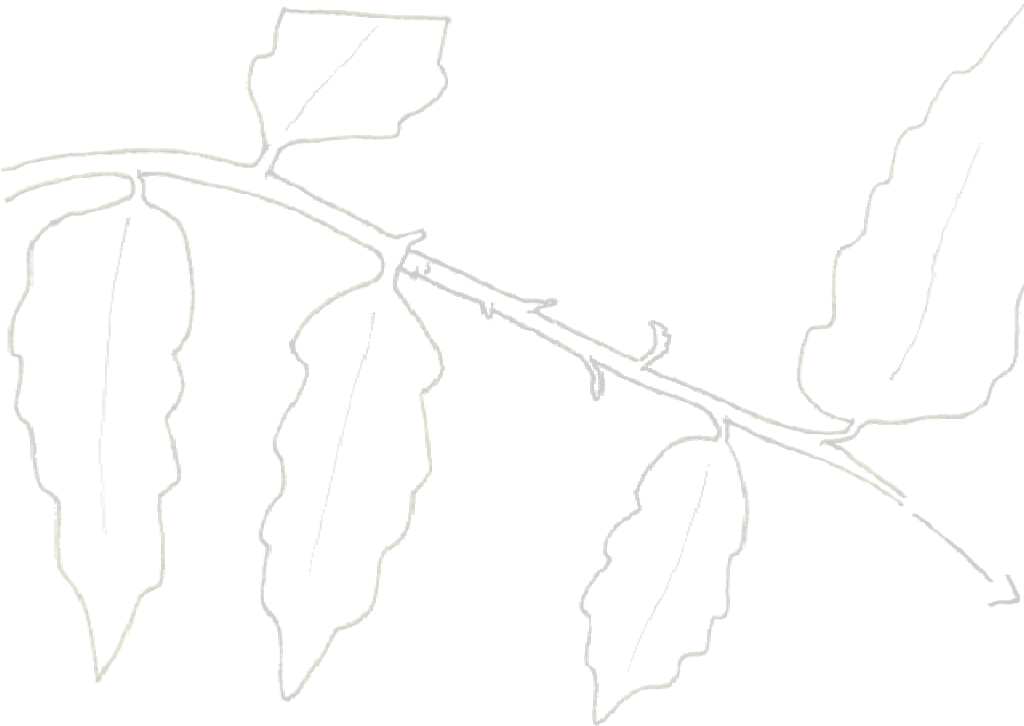

Résumé

L’une des remarquables originalités de la flore néo-calédonienne repose sur la présence de nombreuses lignées correspondant aux premières divergences des plantes à fleurs, compte tenu de leurs positions phylogénétiques. Au sein de ces lignées, certaines espèces sont susceptibles de porter des traits morpho-anatomiques ancestraux. Par conséquent, dans un contexte comparatif, l'étude de ces espèces peut fournir des informations cruciales pour comprendre les premières étapes évolutives des angiospermes. Un premier volet de cette thèse vise à étudier des caractéristiques structurelles et fonctionnelles des groupes représentatifs d’angiospermes basales. L'étude d'*Amborella trichopoda*, espèce sœur de toutes les angiospermes, nous a montré une covariation des traits fonctionnels (tige-feuille) et une plasticité morphologique en réponse à des variations de l'environnement lumineux. Cela suggère que ces réponses plastiques étaient déjà présentes chez l'ancêtre commun de toutes les plantes à fleurs. En parallèle, l’étude de l'évolution anatomique des Piperales, ordre le plus riche parmi les angiospermes basales, suggère que leur ancêtre commun aurait possédé un cambium actif. Ces résultats supportent que les premières angiospermes avaient une forme de vie ligneuse et une probable structure sympodiale.

Les angiospermes basales comptent parmi les lignées d'angiospermes surreprésentées en Nouvelle-Calédonie. Cependant, les mécanismes à l’origine de cette dysharmonie demeurent inexplorés. Un deuxième volet de cette thèse analyse la répartition environnementale des angiospermes basales de l'archipel afin de connaitre leurs préférences en termes d’habitats, ainsi que leurs exigences environnementales. Cette distribution environnementale a également été analysée au regard de leur résistance à la sécheresse. Nous montrons que la plupart des espèces présentent une préférence marquée pour des habitats de forêt humide avec des faibles variations en température. La vulnérabilité hydraulique face à la sécheresse apparait comme un trait majeur qui confine la distribution de ces espèces dans des habitats humides. Ces conditions auraient persisté dans des zones refuges dans l'archipel lors de la dernière période glaciaire, permettant ainsi le maintien de certaines lignées d’angiospermes basales. Une stabilité climatique passée pourrait donc être à l'origine de la surreprésentation de certains groupes d'espèces forestières qui ont disparu dans les régions voisines. La distribution des angiospermes basales néo-calédoniennes, ainsi que leur sensibilité à la sécheresse, supportent l'hypothèse suggérant que les premières angiospermes habitaient des milieux humides et stables.

Mots-clés: anatomie du bois, angiospermes basales, biogéographie, écologie fonctionnelle, écophysiologie, évolution, Nouvelle-Calédonie, vulnérabilité à la sécheresse.

Abstract

One of the remarkable characteristics of the New Caledonian flora is the presence of numerous angiosperm lineages recognized as the earliest divergences of the flowering plants, due to their phylogenetic positions. Within these lineages, some species are likely to bear ancestral morpho-anatomical features. Therefore, under a comparative perspective, the study of these species can provide compelling information for understanding the early evolutionary stages of angiosperms. The first part of this thesis aims to study the structural and functional characteristics of representative groups of basal angiosperms. The study of *Amborella trichopoda*, sister species to the remaining flowering plants, shows a covariation of functional traits (stem-leaf) and a morphological plasticity in response to changes in the light environment. This suggests that these plastic responses were already present in the common ancestor of angiosperms. In parallel, the study of the anatomical evolution of Piperales, the most diversified basal angiosperm order, suggests that their common ancestor had an active cambium. These results support the hypothesis that early angiosperms had a woody habit and that sympodial growth may have been acquired early during angiosperms evolution.

Basal angiosperms are among the over-represented angiosperm lineages of New Caledonia. However, the mechanisms underlying this disharmony remain unexplored. A second component of this thesis analyzes the environmental distribution of New Caledonian basal angiosperms to know their habitat preferences and their environmental requirements. Further, we assess the influence of their drought tolerance on their environmental distribution. We show that most species have a preference for rain forest habitats with small variations in temperature. Drought-induced hydraulic vulnerability stands as a major trait that restricts the distribution of these species to humid habitats. These stable conditions seem to have persisted in refugial areas in the archipelago during the last glacial maximum, allowing the persistence of basal angiosperm species. Therefore, a past climatic stability could explain the over-representation of some groups of forest species that may have disappeared in neighboring regions. The distribution of basal angiosperms in New Caledonia, as well as their drought sensitivity, support the hypothesis suggesting that early angiosperms lived in humid and stable environments.

Keywords: basal angiosperms, biogeography, drought vulnerability, ecophysiology, evolution, functional ecology, New Caledonia, wood anatomy.