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The rise and evolution of the cambial variant in Bignonieae (Bignoniaceae)

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SUMMARY Cambial variants represent a form of secondary growth that creates great stem anatomical diversity in lianas. Despite the importance of cambial variants, nothing is known about the developmental mechanisms that may have led to the current diversity seen in these stems. Here, a thorough anatomical analysis of all genera along the phylogeny of Bignonieae (Bignoniaceae) was carried out in order to detect when in their ontogeny and phylogeny there were shifts leading to different stem anatomical patterns. We found that all species depart from a common developmental basis, with a continuous, regularly growing cambium. Initial development is then followed by the modification of four equidistant portions of the cambium that reduce the production of xylem and increase the production of phloem, the former with much larger sieve tubes and an extended lifespan. In most species, the formerly continuous cambium becomes disjunct, with cambial portions within phloem wedges and cambial portions between them. Other anatomical modifications such as the formation of multiples of four phloem wedges, multiple-dissected phloem wedges, and included phloem wedges take place thereafter. The fact that each novel trait raised on the ontogenetic trajectory appeared in subsequently more recent ancestors on the phylogeny suggests a recapitulatory history. This recapitulation is, however, caused by the terminal addition of evolutionary novelties rather than a truly heterochronic process. Truly heterochronic processes were only found in shrubby species, which resemble juveniles of their ancestors, as a result of a decelerated phloem formation by the variant cambia. In addition, the modular evolution of phloem and xylem in Bignonieae seems to indicate that stem anatomical modifications in this group occurred at the level of cambial initials.

INTRODUCTION

The major goal of evolutionary developmental biology is to understand the evolution of morphological diversity. One way to achieve this understanding is through ontogenetic comparisons of closely related species in a phylogenetic context so that the exact ontogenetic shifts that may have led to different morphologies can be detected (Gould 1977; Guerrant 1988). Because differences between morphological traits of distinct organisms are linked to their development, a comparison of the development of different organisms in a phylogenetic framework allows us to infer which developmental mechanisms may have generated diversity among clades (Gould 1977; Li and Johnston 2000). Specifically, this approach allows us to test whether developmental mechanisms such as heterochrony, heterotopy, novelty, or homeosis (Table 1) were involved in the generation of different morphologies in various organisms.

Comparative studies of ontogeny and phylogeny in plants have traditionally focused on flowers and leaves, organs of determinate growth (Li and Johnston 2000) while, more recently, studies have also been conducted with wood anatomical traits (Olson 2007). The fact that the secondary xylem is a tissue that is produced by the vascular cambium in concentric layers and the derivative xylem cells are kept forever in the center of the stem, allows the study of all ontogenetic steps of a plant stem from the center to the periphery (Olson and Rosell 2006; Olson 2007). Such an approach mitigates the problem of setting a comparable stage in organisms of indeterminate growth (Guerrant 1988). In fact, the entire developmental history of the stem is printed in its secondary xylem, regardless of the time at which the wood is collected, allowing for a reconstruction of all developmental stages (Olson 2007). Additionally, stems with similar diameters have been shown to represent good surrogates of reference points for comparisons, even in plants that have grown under different conditions, allowing studies of ontogeny and phylogeny in plants to use the diameter as the criterion for the selection of reference point for analyses (Rosell and Olson 2007). However, little is known about the exact developmental mechanisms that may have generated the diversity seen in current stem anatomical forms.

Table 1. Types of developmental mechanisms that may generate new morphologies

Developmental mechanism	Definition
Heterochrony	Changes in relative time of developmental events (Gould 1977)
Heterotopy	Change in the site of expression of a trait (West-Eberharal 2003)
Novelty	The rise of a feature not homologous to anything else in the organism (Müller and Wagner 1991)
Homeosis	The replacement of one structure for another (Sattler 1988)

The stem anatomy of species of Bignonieae is quite variable due to the presence of a cambial variant (unusual cambial activity) in their stems. The tribe Bignonieae represents the largest group of lianas in the neotropics (Gentry 1991) and includes 383 species, approximately half the species of the family (Fischer et al. 2004; Lohmann 2006). The presence of cambial variants and stems with various morphologies in Bignonieae, combined with the existence of a robust phylogeny for this tribe (Lohmann 2006), makes Bignonieae an amenable group in which to address questions associated with developmental mechanisms (e.g., heterochrony, novelty, heterotopy, or homeosis) that may have led to the current anatomical stem diversity in this group.

Cambial variants represent a form of secondary growth that is especially common in lianas of various families (Carlquist 1988). During this growth form, the cambium changes its activity in one or many aspects, leading to the production of soft-walled tissues mixed with thick-walled tissues, which presumably provide higher flexibility to climbing (Rowe et al. 2004; Isnard and Silk 2009), while allowing twisting without damaging the xylem (Carlquist 1988). Alternatively, these structures might also play a role in photosynthate storage (Carlquist 1988) and wound repair (Dobbins and Fisher 1986). Different plant families have independently evolved cambial variants along the course of angiosperm evolution, with different variants being so characteristic of each plant group that these plants families can be identified based only on their stems (Caballé 1993). For instance, the Menispermaceae exhibit successive cambia (Schenck 1983; Jacques and De Franceschi 2007), the Sapindaceae show multiple steles (Schenck 1983; Carlquist 1988), some Fabaceae show asymmetric growth (Shenck 1983; Fisher and Ewers 1992), while the Bignoniaceae exhibit a xylem that is furrowed by phloem wedges (Schenck 1983; Dobbins 1971; Carlquist 1988).

Despite the functional importance of cambial variants for lianas in general, it is still unclear how evolution has acted in order to produce the unusual stem anatomical features that we see today in many liana families. The high diversity of anatomical forms present in Bignonieae makes it a suitable model group to address this question. During stem development, the cambium of species of Bignonieae changes its activity in four portions, in each of these portions the vascular cambium reduces the production of xylem and increases the production of phloem in a way that four phloem wedges are formed, interrupting the secondary xylem (Dobbins 1971). More interestingly, the phloem produced by the variant portions exhibit much larger sieve tube elements than the adjacent normal regions (Dobbins 1971).

Four different adult stem configurations result from the differential cambial activity in the Bignonieae (Dos Santos 1995): (i) species that present four phloem wedges during their whole lifespan, (ii) species that develop additional phloem wedges between the four wedges initially formed, generating multiples of four phloem wedges, (iii) species whose multiple of four wedges get dissected while the stem grows in diameter, forming multiple-dissected phloem wedges, and finally, (iv) species that exhibit several included phloem wedges at the same radii in mature stems. These features have been mapped onto a molecular phylogeny of Bignonieae and have emerged as key synapomorphies of well-supported clades (Lohmann 2003). The development of phloem wedges has been associated with the vascular pattern and the position of the decussate leaves (Dobbins 1969, 1970, 1981). However, what exactly causes the formation of more than four phloem wedges in certain stems is still unclear (Dobbins 1970).

Although the functional importance of cambial variants is widely recognized, no ontogenetic comparative research has been conducted in a phylogenetic framework to understand which developmental modifications may have happened to generate such forms. The aim of the present study was to survey the ontogeny of the cambial variant in stems of Bignonieae in order to elucidate which developmental mechanisms acted during the rise and diversification of the cambial variant in this group.

MATERIALS AND METHODS

Taxon sampling

Collections were mostly made in natural habitats; some samples were also gathered from private living collections (see Appendix S1). During fieldwork, stems with a 2 cm diameter were collected and fixed in FAA 50 (10% formalin, 5% acetic acid, 50% alcohol). Stems of this diameter are known to present fully developed cambial variants and to represent adults of reasonable size (Ewers and Fisher 1991). Such standardization allowed the analysis of the entire cross section of each species and the diameter to be used as a reference point on which to base comparisons among species (Rosell and Olson 2007). In addition, stems of larger diameters were also collected to ensure that further differences in cambial variants would not be missed and that 2-cm stems did indeed rep-

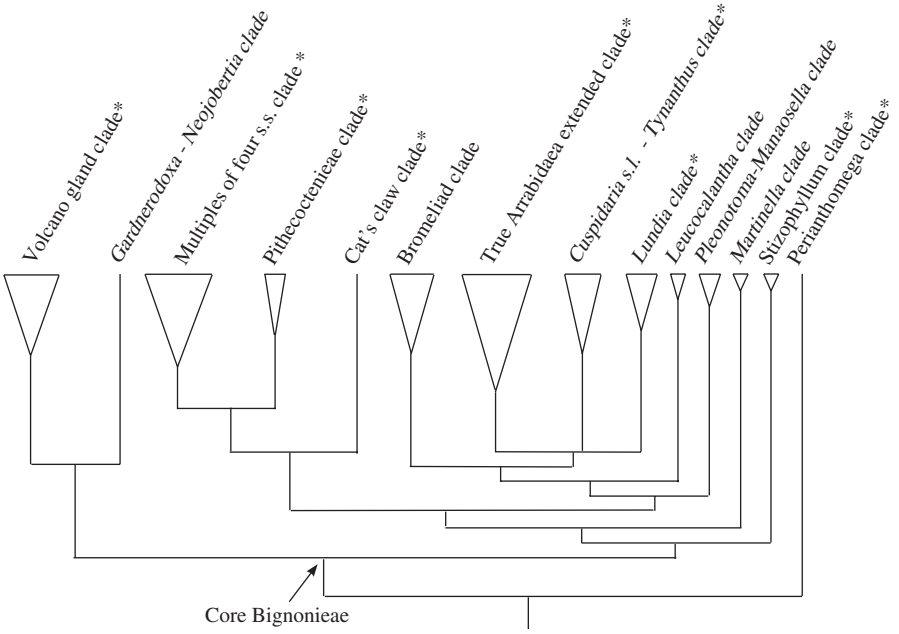

Fig. 1. Phylogenetic relationship of major Bignonieae clades as proposed by Lohmann (2006). In the present study, adult species of all clades were analyzed, but ontogenetic studies were carried out exclusively on the clades indicated by asterisks.

resent the last stage in development. Two sampling schemes were used for the present study: one for the ontogenetic analyses and another for adult stem analyses.

For the ontogenetic analyses of the stem, 10 species belonging to 10 different clades of Bignonieae were selected in accordance with their phylogenetic distribution (Fig. 1). One species belonging to the tribe Tecomeae was used as an outgroup. Species names follow Lohmann (in press) and were grouped according to the clades they belong to in the phylogeny of Bignonieae (Fig. 1). The selected species were the following: *Podranea ricasoliana* (the outgroup), *Perianthomega vellozoi* (the earliest diverging lineage in Bignonieae), *Adenocalymma divaricatum* (Volcano-gland clade), *Stizophyllum riparium* (*Stizophyllum* clade), *Tanaecium pyramidatum* (Bromeliad clade), *Tynanthus cognatus* (*Cuspidaria* s.l.-*Tynanthus* clade), *Fridericia platyphylla* (True *Arrabidaea* extended clade), *Lundia corymbifera* (*Lundia* clade), *Pyrostegia venusta* (Multiples of four s.s. clade), *Amphilophium crucigerum* (Pithecoctenieae clade), and *Dolichandra unguis-cati* (Cat's claw clade). For these species, sections were taken from each internode beginning at the apex until reaching the fully developed stem.

For the adult stem analyses, 50 species of Bignonieae and two species of Tecomeae were sampled (Appendix S1). Analyses of adult stems were carried out to ensure that the cambial variant types, whose ontogenies were studied in detail, did represent all the diversity of pathways found in the group. Information on species authorities, collection localities, number of individuals sampled, and name of species analyzed per group are presented in Appendix S1.

Anatomical procedures

After collection and fixation, specimens were transferred to a conserving solution of 70% ethanol. Obtaining good anatomical sections of liana stems is challenging as the presence of soft-walled cells embedded in the xylem causes the tissues to tear apart during anatomical procedures. To mitigate this problem, entire stems were softened in 10% ethylenediamine for up to 4 days (Carlquist 1982), gradually embedded in increasingly more concentrate solutions of polyethylene glycol 1500 overnight starting at 10% until a 100% polyethylene glycol solution was obtained (Rupp 1964 with modifications). Samples were subsequently sectioned with the help of antitearing resins made of an expanded polystyrene (foam) solution applied upon the stem before sectioning in a sliding microtome (Barbosa et al. unpublished data). Sections were double stained in Astra Blue- Safranine 9:1, a modification of Bukatsch (1972), and mounted in Canada Balsam to make permanent slides. Smaller parts and details of cell anatomy were analyzed after embedding in Historesin® (Leica Mycrosystem, Wetzlar, Germany), sectioning in a rotary microtome, and staining in 0.05% toluidine blue in glacial acetic buffer at pH 4.7 (O'Brien et al. 1964).

Anatomical terminology adopted

Two anatomical terms, "phloem arcs" and "phloem wedges" are adopted here. These terms refer to two different types of homologous forms of variant secondary growth that differ in their cambium continuity. In the case of phloem arcs, the cambium is continuous, while in the case of phloem wedges the cambium is disjunct, with portions included in the interior of the wedges and portions in between wedges (Fig. 2). The portions in between arcs and wedges are here called interarcs and interwedges.

Character state delimitation, ancestral character state reconstruction, and evolutionary inferences

The development of cambial variants is a linear process. This means that one structure cannot be formed without the formation of the preceding structure, facilitating the division of the ontogeny into recognizable stages. We considered as a stage any new anatomical structure (e.g., new phloem wedge) raised during the de-

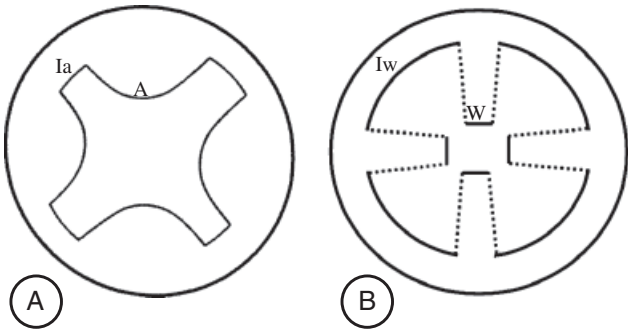

Fig. 2. Schemes of stems present in the Bignonieae. (A) Stem with a continuous cambium (full line), but with four variant portions that form arcs. Regions between the arcs are called interarcs. (B) Stem with four phloem wedges. The cambium (full line) is disjunct, with portions in the interior of the phloem wedges and portions in between them (interwedges). The dashed lines correspond to the rays. A, arcs; Ia, interarcs; W, wedges; Iw, interwedges.

velopment of the stem. Nonetheless, the development of a cambial variant involves several different tissues that change concurrently in the stem. Instead of dismembering and treating each involved tissue as a character, we focused on the whole process of phloem wedge development in a way that the whole ontogeny could be described and treated as a single character. Equating the entire ontogenetic pathway as a single character state avoids losing known sequences of events involved in an ontogeny. In addition, it allows mapping the ontogenies as character states onto a phylogeny. This approach was suggested by Mabee and Humphries (1993) and has been successfully used in comparisons of ontogeny and phylogeny in other plant groups (e.g., Jaramillo et al. 2004). This methodology involves the creation of a step matrix that includes distance information between different character states (Table 2). The distance between character states reflects the likelihood of evolutionary transformations between two characters, with a higher score indicating a more complicated transformation between states.

Ancestral ontogenies were reconstructed with a parsimony algorithm using MacClade 4.0 (Maddison and Maddison 2000). A combined *ndh*F and *Pep*C phylogeny for 104 Bignonieae species produced by Lohmann (2006) was used as basis. We combined the results of the 50 species studied here with the 73 species previously studied by Dos Santos (1995) to obtain a broader sampling for the stems of Bignonieae. Because a number of species sampled here were not included in the phylogeny of Lohmann (2006), the remaining 272 species of Bignonieae not included in that phylogeny were placed at the deepest nodes of clades that represented genera in the latest classification of the group (Lohmann in press). This led to a final tree that included all 383 species of Bignonieae that was used as basis to map character states. Character mapping allowed us to detect the ancestral ontogenies at each node, to compare ontogenies across the whole phylogeny, to determine which traits were common to particular clades (synapomorphies), and which developmental mechanisms might have been involved in the generation of diversity in different lineages. Finally, all anatomical modifications found in the rise and evolution of cambial variants in Bignonieae were summarized in a simplified version of the phylogeny.

Table 2. Step matrix illustrating the likelihood of ontogenetic transformation between character states, from the most basal to the most terminal nodes in the phylogeny

	ONT 0	ONT 1	ONT 2	ONT 3	ONT 4	ONT 5	ONT 6
ONT 0							
ONT 1	1						
ONT 2	2	1					
ONT 3	2	1	2				
ONT 4	3	2	1	3			
ONT 5	4	3	2	4	1		
ONT 6	5	4	3	5	4	5	

RESULTS

Ontogeny of cambial variants

Six different ontogenetic pathways were found in the stems of Bignonieae (Fig. 3) leading to six different types of anatomical architectures (Fig. 4; Table 3). Regardless of the apparent differences in the anatomy of the adult stems, their ontogenies share common stages of great resemblance (Figs. 3 and 5). Therefore, a single ontogenetic description is given to avoid a reiterative description of identical events, while highlighting the differences or deviations that each clade experienced.

All species initiate their secondary growth with a continuous vascular cambium that shows regular activity throughout its girth (Figs. 5, stage 1 and 6A). The outgroups, *P. ricasoliana* (Figs. 4A and 5A) and *Tecoma capensis* maintain a regular growth throughout their developmental trajectory.

The first sign of variance

After a period of regular growth, all Bignonieae modify the activity of four equidistant portions of the vascular cambium and start to display four curved arcs (Fig. 5, stage 2). The four recently formed arcs represent segments of the cambium that have reduced the production of xylem and increased the production of phloem (Fig. 2A). During this ontogenetic stage, the vascular cambium still forms a continuous cylinder (Figs.

Table 3. Types of cambial variants in adult stems of Bignonieae

Type 1	Four phloem arcs derived from an increased activity of variant cambia; continuous cambium
Type 2	Four deeply embedded phloem wedges; disjunct cambium
Type 3	Four phloem arcs derived from reduced activity of variant cambia; continuous cambium
Type 4	Multiples of four phloem wedges; disjunct cambium
Type 5	Multiple-dissected phloem wedges; disjunct cambium
Type 6	Included phloem wedges at the same radii; disjunct cambium

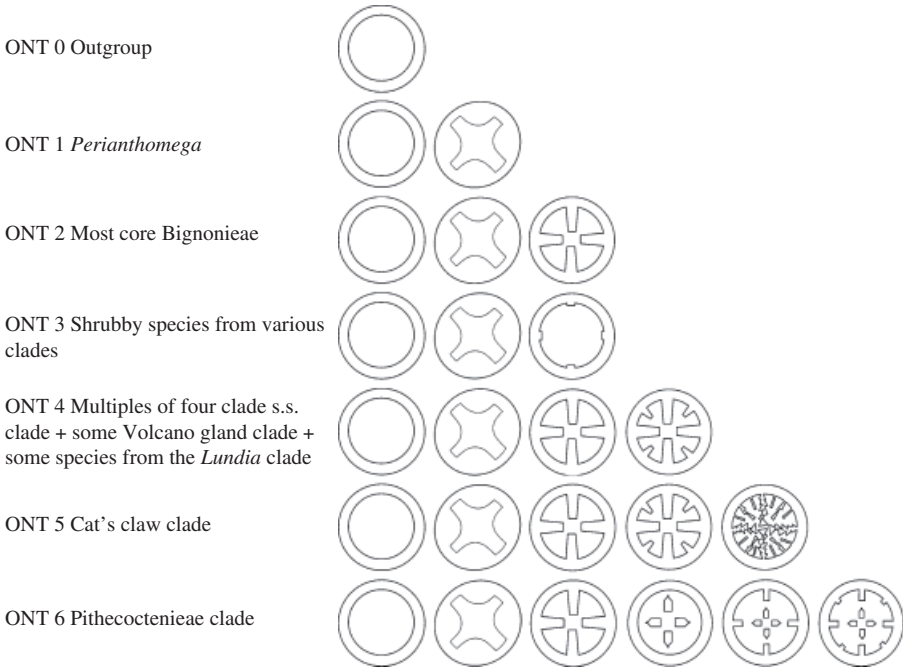

Fig. 3. Schematic ontogenetic pathways present in the stems of Bignonieae and outgroup (not to scale).

5, stage 2 and 6B), but the phloem formed possesses much larger sieve tubes than the adjacent regions that maintain regular secondary growth (Figs. 6B and 7, D–E). Moreover, while the phloem at the interarcs possesses a reduced conductivity span (Fig. 7D), the sieve tubes at the arcs are conductive over a much larger extent from the cambium to the outside (Fig. 7E). This conclusion is based on anatomical observations: in these plants phloem conducting failure can be seen by definitive callose deposition at the sieve plates and/or swelling of the neighboring parenchyma cells (Fig. 8). Sometimes this is also accompanied by sieve tubes breakdown (Fig. 8) and ray dilatation. A single genus of Bignonieae, *Perianthomega* (the earliest diverging lineage in the group), persists perpetually in this stage, with four variant portions but always with a continuous cambium, even in mature stems (Figs. 3, ontogeny 1 and 5B). All other Bignonieae (i.e., the core Bignonieae clade) include a series of subsequent additional developmental stages (Fig. 5, C–D).

The formation of phloem wedges (Fig. 3, ontogeny 2)

At this stage, the four-phloem arcs transform into four phloem wedges (Fig. 2B). In these stems the cambium becomes disjunct, with portions included on the interior of the phloem wedges and portions in between these wedges (Figs. 5, stage 3 and 6C). A thorough analysis of the variant cambia of all species was carried out in order to understand why wedges would form here and not in *Perianthomega*. Our analyses indicate that the presence of wedges in the core Bignonieae clade is associated with the type of cambial divisions that these variant regions execute. The wedges are formed in the stems of the core Bignonieae because the four regions of variant cambia stop dividing radial anticlinally (Fig. 7, B and C), a type of division that allows the cambium to grow in girth. As a result, these cambia remain restricted in width (Fig. 7A). Because the variance occurs in only four regions and not in the whole extension of the cambium, regular growing portions alternate with variant portions, each one with different rates of xylem and phloem differentiation, creating, therefore, two reverse movements. Regular growing cambia is displaced outwards at each side of the wedges while xylem is produced. In addition, the variant cambia remains almost stationary, producing large quantities of phloem outwards and very small amounts of xylem inwards (Fig. 6, B and C). These reverse displacements promote cambium dissection and phloem wedge formation (Fig. 6, B and C). Limiting rays enlarge around the periphery of the wedges leaving marks on the xylem as the plant grows. This process allows us to trace precisely when the cambium inclusion occurred during the organism's ontogeny (Fig. 7A). Furthermore, the limiting rays represent evidence that the variant cambia virtually does not increase in girth after cambial inclusion (Fig. 7A). Alternatively, cambial regions adjacent to the phloem wedges may change from a regular to a variant activity, developing a stair-like pattern along the phloem wedges (Figs. 4G and 6C).

Decelerated cambial activity (Fig. 3, ontogeny 4)

Two clades here analyzed, the True *Arrabidaea* extended clade and the Volcano gland clade possess shrubby species (*F. platyphylla*, *Adenocalymma campicola*, and *Adenocalymma peregrinum*) that grow in the Brazilian cerrado. The ontogenies of these species follow all the steps mentioned above,

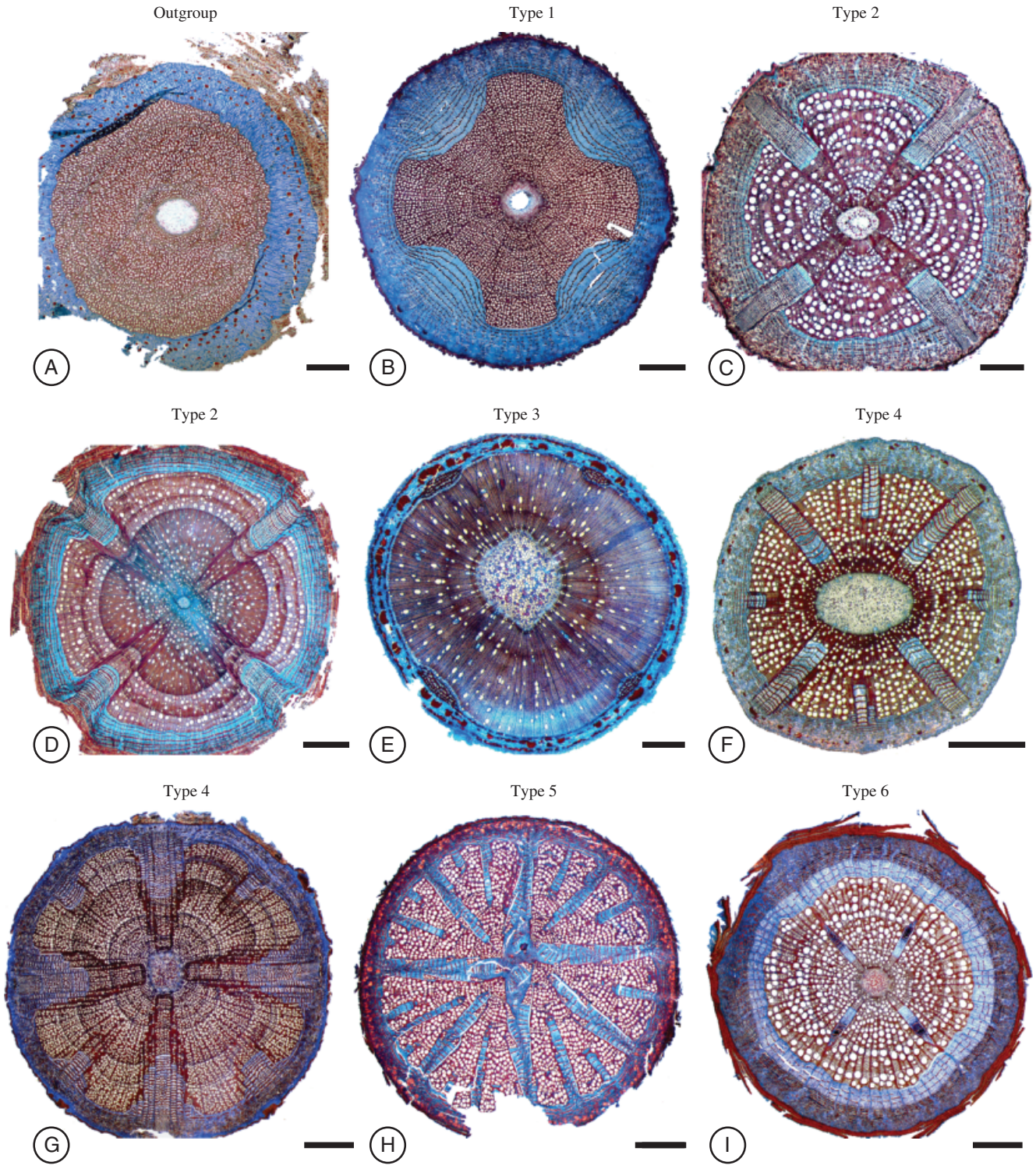

Fig. 4. Stem cross-sections illustrating the diversity of anatomical architectures resulting from six different ontogenetic pathways. (A) *Podranea ricasoliana* (the outgroup), regular secondary growth. (B) *Perianthomega vellozoi* (the first diverging lineage within Bignonieae), type 1 = four phloem arcs, variant cambium with anticlinal divisions. (C) *Tanaecium pyramidatum* (bromeliad clade), type 2 = four phloem wedges. (D) *Fridericia chica* (true *Arrabidaea* extended clade), type 2 = four phloem wedges. (E) *Fridericia platyphylla* (true *Arrabidaea* extended clade), type 3 = four phloem arcs, cambium without anticlinal divisions. (F) *Bignonia binata* (Multiples of four clade), type 4 = multiples of four phloem wedges. (G) *Adenocalymma divaricatum* (volcano gland clade), type 4 = multiples of four phloem wedges. (H) *Dolichandra unguis-cati* (Cat's claw clade), type 5 = multiple-dissected phloem wedges. (I) *Amphilophium crucigerum* (Pithecoctenieae clade), type 6 = included phloem wedges. Scale bars: 2 mm.

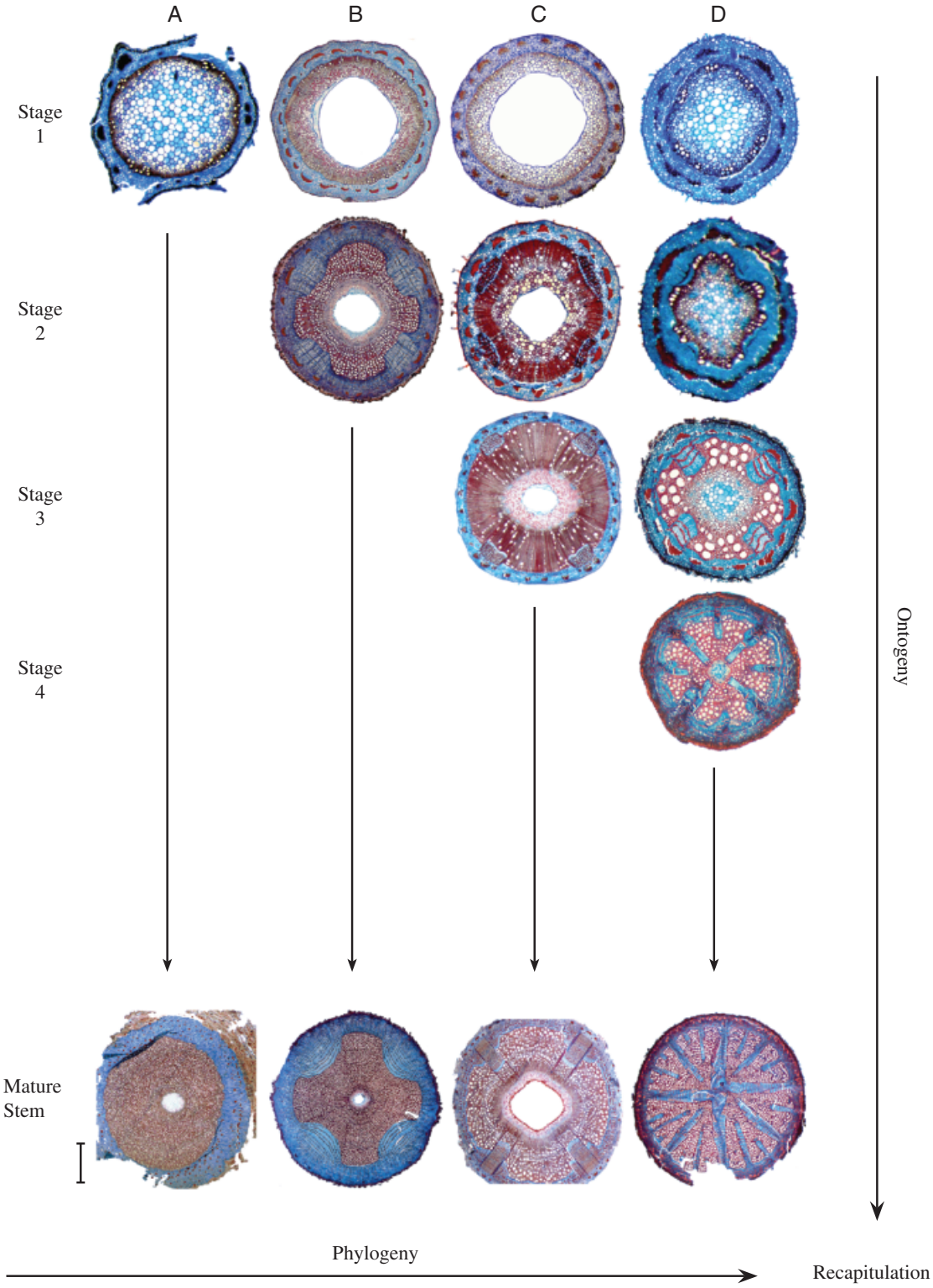

Fig. 5. Four examples of ontogenetic pathways found in Bignonieae and the outgroup. Arrows connect the last ontogenetic event to the mature stems. Species are organized from the most basal to the most terminal nodes. (A) *Podranea ricasoliana* (outgroup). (B) *Perianthomega vellozoi* (first diverging lineage). (C) *Styzophyllum riparium* (*Stizophyllum clade*, similar to most Core Bignonieae). (D) *Dolichandra unguis-cati* (Cat's claw clade). Scale bar: 500 µm (Stages 1, 2d, 3d); 1 mm (Stages 2b–d, 3c, 4d); 2 mm (Stages 2c); 4 mm (Stage 4); 5 mm (Adult).

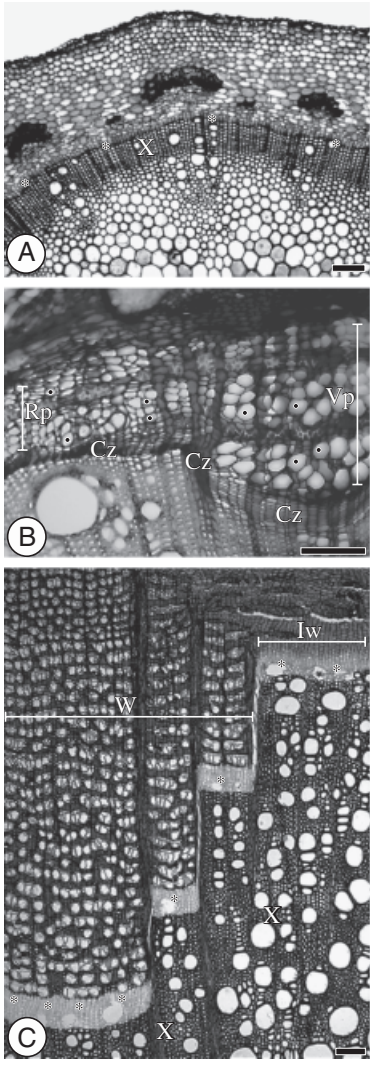

Fig. 6. Stem cross-sections. (A) *Perianthomega vellozoi*; stage 1, cambium with regular activity in its entire girth (asterisks). (B) *Amphilophium crucigerum*; stage 2, variant cambium increases the production of phloem and reduces the production of xylem, sieve tubes (dots) are much larger at the variant portion, and the cambium is continuous. (C) *Cuspidaria convoluta*; stage 3, cambium disjunct (asterisks), with portions included in the interior of the phloem wedge and portions on the interwedges. Scale bars = 100 μm. Cz, cambial zone; Vp, variant phloem; Rp, regular phloem; Iw, interwedge; W, wedge; X, xylem.

including the cessation of anticlinal divisions. However, they never form wedges, remaining as shallow arcs during their entire lifespan (Fig. 4E). Their variant cambia have a limited phloem formation activity and wedges are therefore not formed. In fact, there is a minor reduction in the production of xylem with a discrete increase in the production of phloem. As the rates are so inexpressive, the cambium dissection never occurs, nor are limiting rays formed.

The formation of multiples of four phloem wedges (Fig. 3, ontogeny 4)

Within the core Bignonieae clade there are plants that go even further in their ontogenies, but always following the formation of four phloem wedges. One example is the formation of additional phloem wedges between the 4 initial phloem wedges, making 8 wedges, then 16, and so forth in a geometric progression (Fig. 4, F and G). This feature was found in all species of the Multiples of four s.s. clade, and in some species of both the *Lundia* clade (*L. corymbifera* and *Lundia glazioviana*), and the Volcano gland clade (*A. divaricatum* and *Adenocalymma tanaeciicarpum*).

Multiple-dissected phloem wedges (Fig. 3, ontogeny 5)

This type of wedge formation is exclusive to the species in the Cat's claw clade. In their ontogeny, their stems develop in a very similar way to the multiples of four, but unlike any other species of Bignonieae their limiting rays and axial parenchyma have large portions that do not lignify. These portions eventually start to divide in all planes compressing the phloem wedges, dissecting and including them (Fig. 9). This situation creates a mosaic of wedges and islands of included phloem with large parenchymatous limits (Figs. 4H, 5D, and 9).

The formation of included phloem wedges (Fig. 3, ontogeny 6)

This last type of wedge formation is exclusive to the Pithecoctenieae clade. In these species, after four phloem wedges are produced, the regular cambia present at the outside limits of the wedges start to grow toward the wedge by means of anticlinal divisions of radial initials and redifferentiation of parenchyma cells, gradually recovering its continuity and including the phloem wedges (Figs. 4I and 10A). New phloem wedges differentiate at the same radius of the included wedges, with some species developing additional wedges between the four included phloem wedges; in some species these wedges also get included as growth continues. Another distinct feature found in Pithecoctenieae stems regards their phloem conductivity. Unlikely other genera, the genera in this clade also lose phloem conductivity in the wedges just a few layers away from the cambium (Fig. 10B), with most of the wedges lacking any conductivity whatsoever (Fig. 10A).

Character state distance and ancestral character state reconstruction

Each of the six ontogenetic pathways found among members of the Bignonieae were categorized as character states, from ONT 0 to ONT 6 (Fig. 3), and a step matrix was created to indicate the distance between these ontogenies (Table 3). Results from the step matrix indicate a continuous increase in

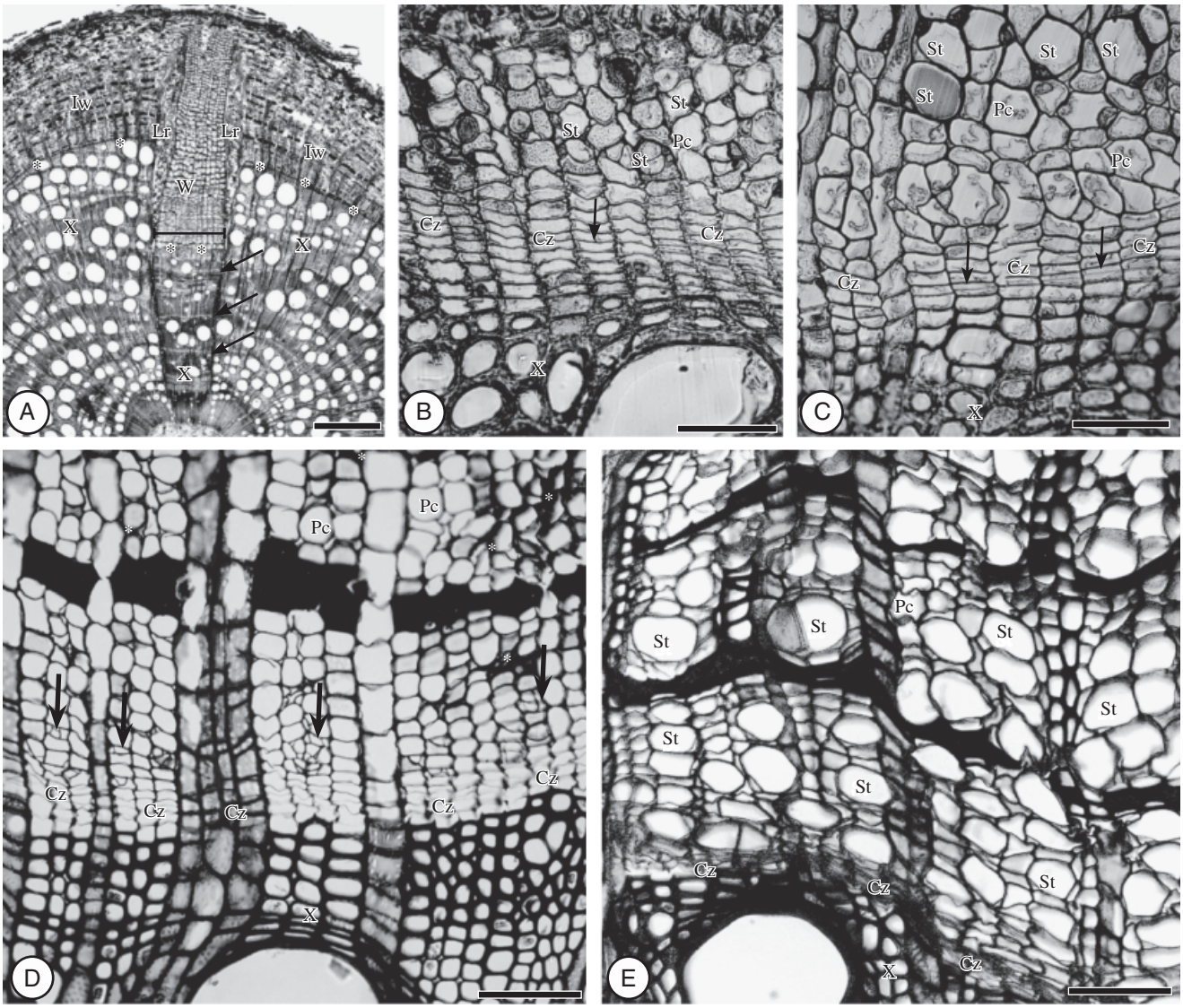

Fig. 7. (A, D–E) *Tanaecium pyramidatum*. (A) Cambium in the interior of the phloem wedge has a virtually determinate length (bar), as evidenced by the limiting rays (arrows). (B and C) *Bignonia magnifica*. (B) Interwedge cambium with two types of division, radial anticlinal (arrow) and periclinal. (C) Variant cambium with only periclinal divisions (arrows). (D) Sieve tube elements at the interwedges (arrow), which are minute and rapidly lose conductivity, evidenced by total sieve tube element breakdown (asterisk) and parenchyma swelling. (E) Sieve tube elements in the phloem wedges are wider and with a greater conductivity. Scale bars: 1 mm (A), 50 μm (B–C), 100 μm (D–E). Cz, cambial zone; Iw, interwedge; Lr, limiting rays; W, phloem wedge; St, sieve tube; Pc, parenchyma cell.

complexity from the outgroup ontogeny through the most terminal nodes of Bignonieae (Table 3). Each number represents the number of steps needed for one particular ontogeny to evolve into another. Whereas some ontogenies are separated by a lower number of steps, other ontogenies require much more complex changes. For example, ontogeny 2 is only distant from ontogeny 4 by one step, while ontogeny 1 is five steps away from ontogeny 6 (Table 3).

Mapping ontogenetic pathways onto the phylogeny indicate that the ancestral ontogeny of the core Bignonieae is ontogeny 2, an ontogeny that leads to four phloem wedges in mature stems. The ancestral character of all Bignonieae, on the other hand, is uncertain because *Perianthomega* presents four phloem arcs and the core Bignonieae, its sister group, presents four phloem wedges, resulting in an ambiguous ancestral character state ontogeny to represent the ancestral condition in the group.

Within the core Bignonieae clade, there were two independent evolutions to a decelerated cambial activity (Fig. 3, ontogeny 3), one at the Volcano gland clade and another at the True *Arrabidaea* expanded clade. In addition, three independent evolutions of the formation of multiples of four

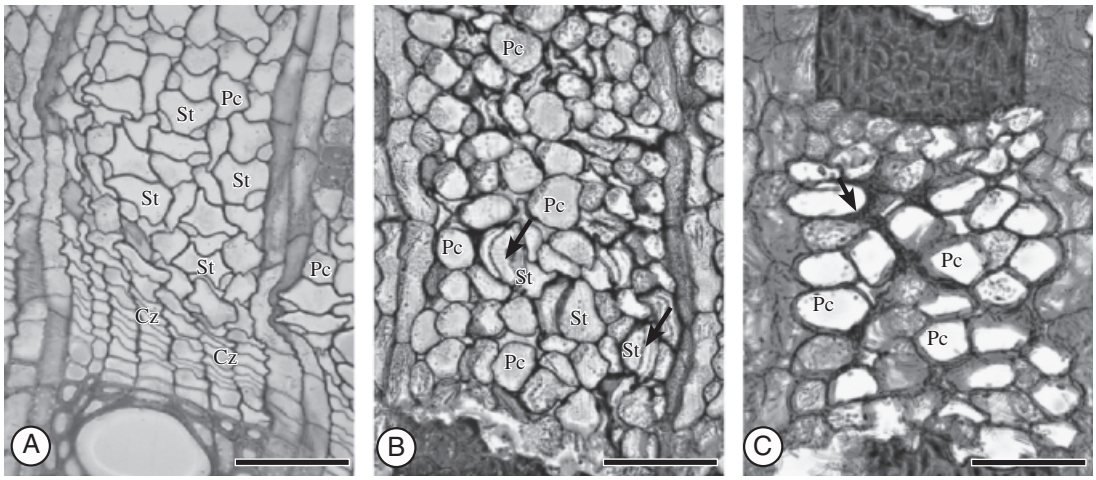

Fig. 8. *Perianthomega vellozoi.* (A–C) Process leading to nonconductivity of sieve tube elements. (A) Conducting sieve tube elements (arrows) in regions close to the cambial zone. (B) Definitive callose deposition at the sieve plates (arrows) and swelling of the parenchyma cells around the sieve tube elements. (C) Total collapse of the sieve tube elements (arrow) with their space being occupied by swollen parenchyma cells. Scale bars = 50 μm.

phloem wedges (Fig. 3, ontogeny 4) were also observed: one at a clade formed by the "Multiples of four s.s. clade+Cat's claw clade+the Pithecoctenieae clade," one at the Volcano gland clade, and another at the *Lundia* clade (Fig. 11). At the node that reunites the "Multiples of four s.s. clade+Cat's claw clade+the Pithecoctenieae clade," an ancestor with four phloem wedges is suggested, leading to three independent evolutions of three different types (types 4–6).

All anatomical events that have taken place in the rise and evolution of the cambial variant are summarized in Fig. 11. This figure illustrates that the first developmental event corresponds to a reduction in the production of xylem and an increase in the production of a phloem with wider sieve tube elements and an extended lifespan in four arcs. Secondarily, at the next node, the end of anticlinal divisions and subsequent inclusion of the cambium inside four wedges occurred. Thirdly, the evolution of multiple of four phloem wedges independently occurred in the "Multiples of four s.s. clade+Pithecoctenieae clade+Cat's claw clade," the Volcano gland clade and the *Lundia* clade. Fourthly, the presence of nonlignified parenchyma led to the formation of multiple-dissected phloem wedges in the Cat's claw clade. Finally, the inclusion of the phloem wedges occurred in the Pithecoctenieae clade.

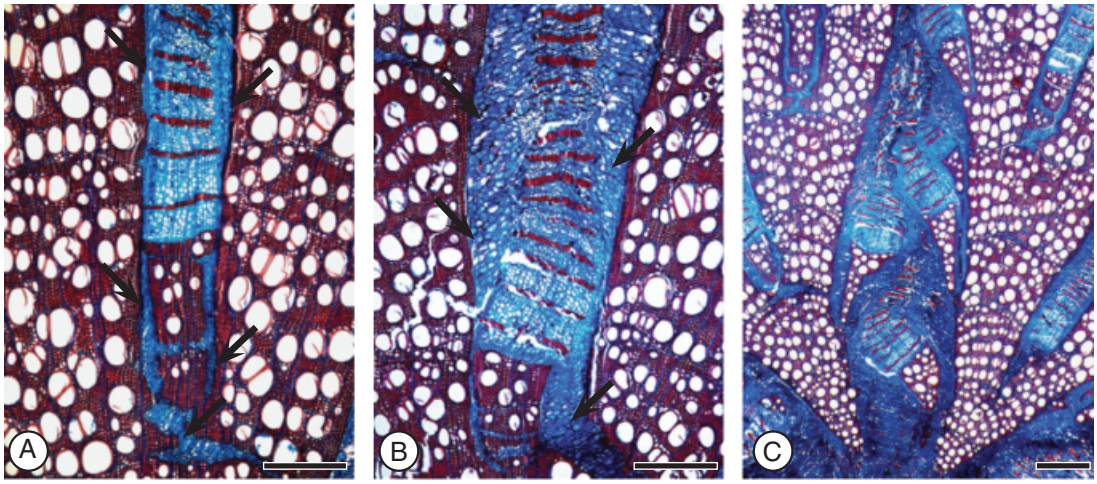

Fig. 9. *Dolichandra unguis-cati.* (A) Nonlignified parenchyma at the limiting rays and portions of axial parenchyma (arrows). (B) Dividing parenchyma compressing the phloem wedge (arrows). (C) Random divisions of the parenchyma cells including portions of the phloem wedges and dissecting the xylem. Scale bars: (A and B) 300 μm and C, 1 mm.

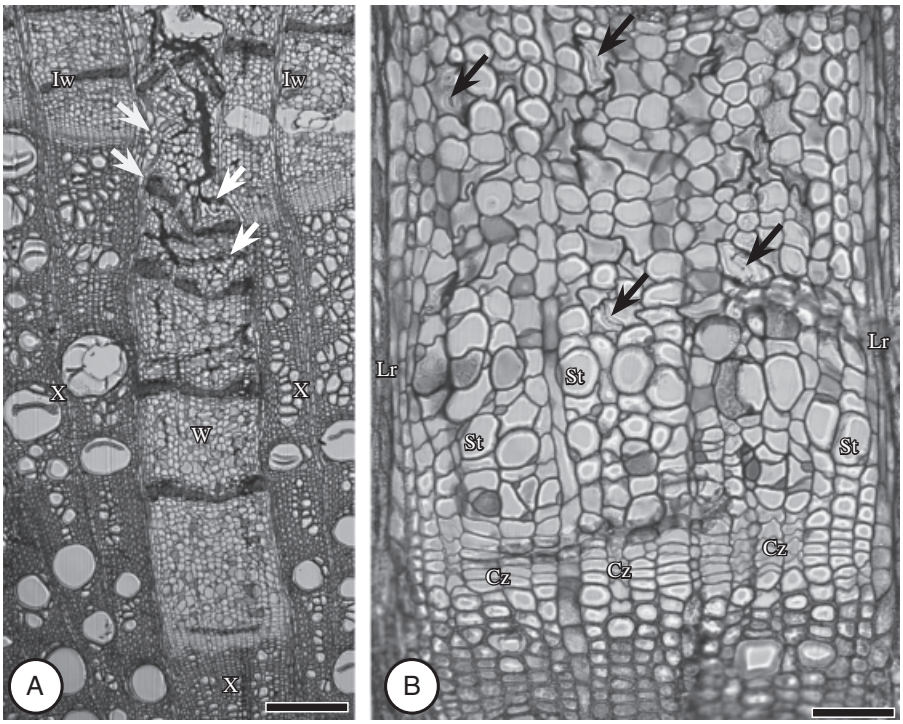

Fig. 10. *Amphilophium crucigerum.* (A) Coalescence of the phloem wedge at its nonconducting portion (arrows). (B) Detail of sieve tubes showing early loss of conductivity at phloem wedges, evidenced by definitive callose deposition (arrows) near the cambial zone. X, xylem; W, phloem wedge; Iw, interwedge; Lr, limiting rays; St, sieve tube; Cz, cambial zone. Scale bars: (A) 200 μm, (B) 50 μm.

DISCUSSION

This study elucidates the nature of the ontogenetic transformations underlying the anatomical changes that have occurred during the rise and evolution of the cambial variant in Bignonieae.

Ontogenetic transformations leading to augmented complexity

This study illustrates that even though a great diversity of forms is seen in the adult stems of Bignonieae (Dos Santos 1995), the various morphologies depart from a common developmental basis. Overall, we observe an increase in the complexity of the anatomical forms from the most basal nodes to the most terminal nodes of the phylogeny, with anatomies ranging from almost "normal" configurations like that found in *Perianthomega*, to mosaic-like stems like those found in the Cat's claw clade. This increased number of ontogenetic steps is evidenced both by the step matrix and the mapping of character states onto the phylogeny (Fig. 11).

If we consider that the various types of cambial variants that evolved in the stems of Bignonieae are features that are not homologous to anything else present outside this tribe, it becomes evident that these new features represent evolutionary novelties (*sensu* Müller and Wagner 1991) that evolved within this group. The first evolutionary novelty was the formation of four regions that reduce the production of xylem and increase the production of phloem, combined with sieve tubes of wider diameters and greater longevity. The difference in the sieve tube diameters, longevity, and frequency is so different between the variant portions and regular portions that it is very likely that most of the conduction of assimilates may be restricted to the phloem arcs/wedges, similar to what was suggested for the included phloem strands in the stems of *Strychnos millepunctata* (Veenendaal and Den Outer 1993). This evolutionary novelty, shared by all species of Bignonieae (synapomorphy) provides a much larger and more efficient area for photosynthate conduction. It is well known that although lianas possess narrow stems, their canopies may be as large as those of a tree (Ewers and Fisher 1991), contributing up to 20% of the total biomass of a forest (Pérez-Salicrup et al. 2001). The xylem of lianas has been shown to be more efficient for water conduction (Ewers 1985; Ewers et al. 1990), with some of the widest vessels recorded (up to 500 μm) (Carlquist 1985). Similarly, it is expected that the phloem would exhibit features allowing for a better conduction of photosynthates. In fact, the variant cambia produce wider sieve tube elements not only in Bignonieae, but also in the Hippocrateaceae (Obaton 1960), Loganiaceae (Veenendaal and Den Outer 1993), and Icacinaceae (see figures in Lens et al. 2008).

The interruption of anticlinal divisions by the variant cambia, transforming the phloem arcs into phloem wedges is

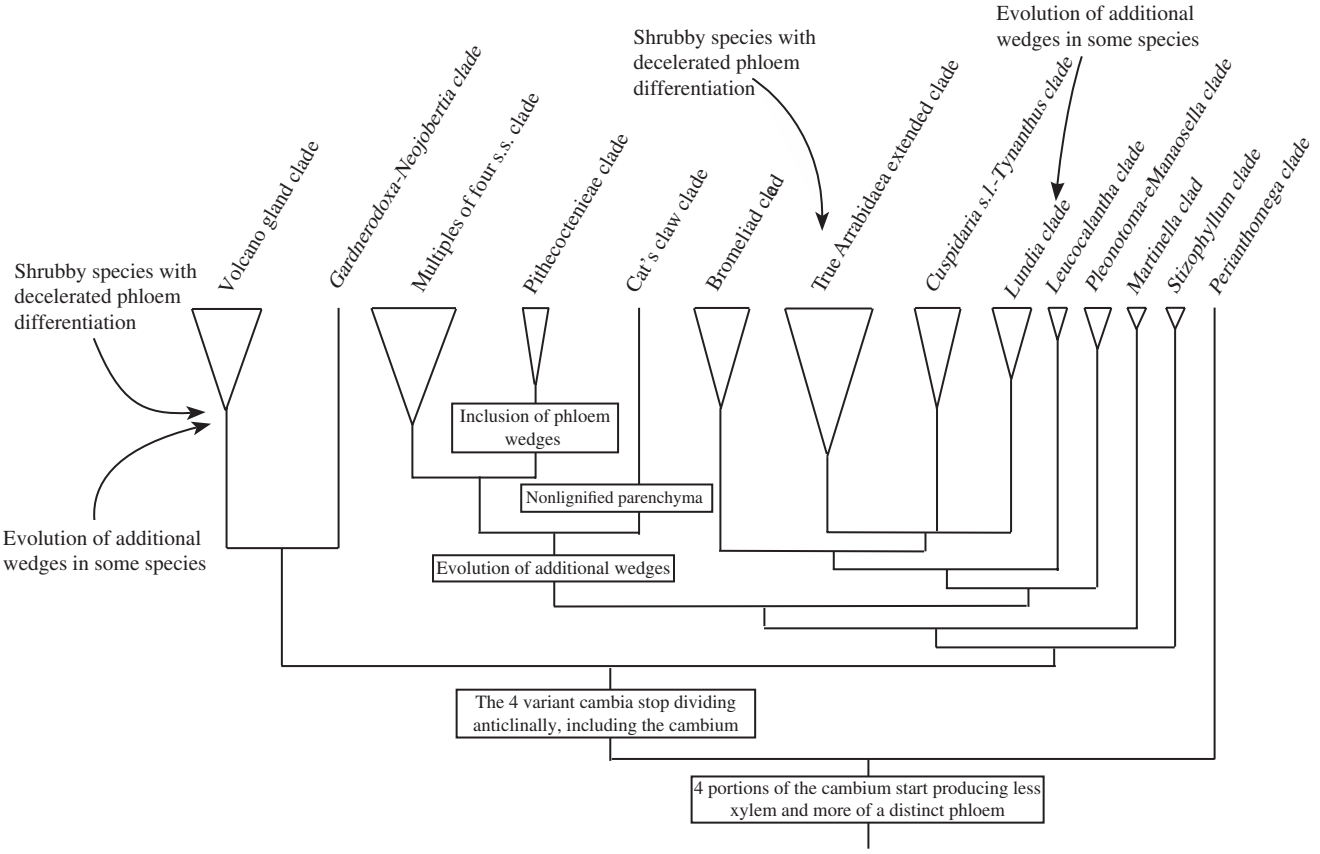

Fig. 11. Anatomical modifications that occurred during the evolution of the cambial variant in Bignonieae. Rectangles illustrate synapomorphies of clades and arrows indicate modifications that appear in only a few species inside a clade, with other species maintaining plesiomorphic traits.

the second evolutionary novelty raised. Here the variation becomes fully evident and may gain new function, not only that of efficient conduction of photosynthates, but also that of conferring higher flexibility to climbing by intercalating soft and rigid tissues at the stem. The formation of phloem wedges is common to all species of the core Bignonieae clade. The formation of phloem wedges was, moreover, accompanied by the cambial disjunction, derived from the two reverse displacements of the cambium caused by the coexistence of regular growing sectors alternating with variant sectors. This disjunction causes the cambium to form isolated sectors, instead of its most common, cylindrical and continuous form. The reason why the cambium does not restore its continuity is anatomically intriguing because experimental studies showed that once the cambium loses one part or even if half the stem is removed, its tendency is to restore its continuity, always paralleling the surface (Lang 1965). In fact, a disjunct cambium is formed in the Loganiaceae by the asymmetric activity of regular and variant regions growing together, but very soon these variant portions get included by the regular cambium that advances from both sides of the strands, until the cambial continuity is restored, creating islands of included phloem within the stem (Boureau 1957; Veenendaal and Den Outer 1993). Nevertheless, in 19 of the 20 genera that possess phloem wedges in the Bignonieae the cambium never restores its continuity. However, a single clade in Bignonieae (Pithecoctenieae clade) has members whose phloem wedges become coalesced by the regular cambium that advances and includes the phloem wedges, similar to the Loganiaceae.

The reason why this occurs is still uncertain, but perhaps the rapid loss of conductivity of the sieve tubes of members of the Pithecoctenieae clade may be involved because all other Bignonieae tend to have a larger area of conducting phloem from the cambium to the outside. The cambium restoration occurs at a region of collapsed nonconducting phloem, converting phloem parenchyma cells into cambial initials, eventually restoring its continuity. Inclusion of phloem wedges to the level of nonconducting phloem parenchyma cells is in agreement with the formation of other types of secondary meristems within the secondary phloem. For instance, when the phellogen or the ray dilatation meristem is formed within

the secondary phloem, these are always formed on its nonconducting portion (Evert 2006). In addition, successive cambia are also formed at the nonconductive phloem portion in *Dalbergia paniculata* (Nair and Ram 1990), and cambium inclusion is also formed in *Strychnos* at the level of parenchyma cells within the variant secondary phloem (Veenendaal and Den Outer 1993).

Given the evidences presented above it is possible that phloem wedges may have been of great importance in the diversification of Bignonieae. Moreover, *Perianthomega* and the core Bignonieae represent two lineages that descend from a common ancestor, but they present an astonishing difference: while *Perianthomega* is a monospecific genus, the core Bignonieae includes over 300 species in 20 different genera. Besides the formation of phloem wedges, tendrils are also different between the two lineages. Specifically, in *Perianthomega* they are modified petioles, while they are modified terminal leaflets in the core Bignonieae (Lohmann 2006). Perhaps these various features together may represent key innovations that may have led to much greater diversification in the core Bignonieae clade (Gentry 1980). Descending from ancestors with four phloem wedges, other anatomical architectures have evolved within the core Bignonieae: the shrubby type, the multiples of four type, the multiple-dissected type, and the included phloem wedges at the same radii type. Each of these novelties may have evolved due to different constraints. This possibility is discussed in further detail below.

The shrubby type has much reduced variant phloem production. In addition, their variant cambia produce almost as much xylem as the regular cambium. Phloem wedges in the lianas occupy a large portion of the stem that would otherwise be involved in water conduction. Therefore, flexibility would have been favored at the expense of water conducting tissue, an issue that in turn would have been solved in the lianas by their very large vessel elements (Ewers et al. 1990). In fact, the xylem of free-standing plants exhibit much narrower vessels and at smaller frequencies than the lianas (Ewers 1985), implying that more water conducting and sustaining tissues are critical to these plants. The shrubby type is present in all the analyzed shrubs of the tribe, from both the Volcano gland clade and the True *Arrabidaea* extended clade, two distantly related lineages, showing a convergence for the reduction of phloem wedges in the shrubs.

The multiples of four phloem wedges and the multiple-dissected phloem wedges, on the other hand, are likely anatomical novelties that have evolved as additional specializations for climbing. In these plants much more soft tissue is embedded within rigid tissues, conferring even more flexibility to climbing. The formation of additional phloem wedges in multiples of four is a synapomorphy of the clade containing "Multiples of four s.s. clade+Cat's claw clade+Pithecoctenieae clade," but appeared independently in the *Lundia* and in the Volcano gland clade. Multiple evolutions of this feature are not surprising, as just one step is needed for stems with four phloem wedges to develop into multiples of four phloem wedges. The formation of new wedges between the previously formed wedges, following a geometric progression and in an equidistant fashion, indicates that the primary vascular pattern and leaves are likely not the only factors associated with wedge formation, as postulated by Dobbins (1970), but that a positional control on variants distribution at the stem might also be important.

The multiple-dissected stems derive from random divisions executed by nonlignified parenchyma present in these stems, forming a parenchymatized secondary xylem. The parenchymatized xylem represent an evolutionary novelty exclusive to the Cat's claw clade and may have increased the flexibility of these stems, given that these are recorded as the most twining among all Bignoniaceae (Gentry 1980). The formation of nonlignified axial parenchyma within the secondary xylem is uncommon among most plant groups but has been frequently recorded in two groups of plants: the succulents and the lianas (Carlquist 1988). In the succulents, the nonlignified parenchyma has been related to storage (Gibson 1973), while in the lianas it has been suggested that it increases stem flexibility, facilitates wound repair (Dobbins and Fisher 1986), and promotes water storage (Carlquist 1988). Within Bignoniaceae, however, this feature is exclusively found in the eight species that form the Cat's claw clade and not elsewhere in the family. The nonlignified parenchyma occupies a space where fibers, vessels, and lignified axial parenchyma would otherwise be present. Such replacement of features has been hypothesized for Caricaceae as a case of homeosis within the secondary xylem (Olson 2007).

Heterochrony in the evolution of the phloem wedges

Two major types of heterochrony exist: paedomorphosis and recapitulation (also called peramorphosis) (De Beer 1940; Alberch et al. 1979). Recapitulation directly connects us to the ideas of Ernst Haeckel (as discussed in Gould 1977), who imagined the ontogeny of organisms as recapitulating the adult forms of their ancestors. Recapitulation in this sense was shown to be flawed (Gould 1977), although the resemblance of an ontogenetic stage of a species to the adult of one of its ancestors could simply indicate that the ancestor did not deviate much from a common ontogenetic stage (De Beer 1940). In Bignonieae an evident recapitulation (more specifically a hypermorphosis, *sensu* De Beer 1940) is illustrated when the ontogeny of a Cat's claw species, for instance, is compared with the ontogeny of a species from a lower node (see Fig. 5). This clear recapitulative result derives, however, from a nonheterochronic process. In fact, evolutionary novelties were terminally added in different ontogenies lead-

ing to this recapitulative result. Raff and Wray (1989) have clearly shown how nonheterochronic processes can yield heterochronic results, arguing that even the classic example of paedomorphic development (*Ambystoma mexicanum*, Amphibia; De Beer 1940) is derived from a nonheterochronic process (hormonal production failure, instead of a timing control process).

Nevertheless, Bignonieae also provides us with an example of a heterochronic result derived from a truly heterochronic process: the decelerated development of phloem wedges in the shrubby species. These species were shown to derive from ancestors with fully developed phloem wedges and to possess the same characteristics of cambium activity, except for low rates of secondary phloem formation by the variant cambia. Such slowed down development generates adult species that resemble the juveniles of more basal species in the phylogeny and are, therefore, paedomorphic.

Modular evolution of xylem and phloem

A module is a complex of characters that are integrated to form an evolutionary unit, in a way that the evolution of each unit of the complex of characters leads to the evolution of the other integrated characters (Wagner 1996). Most of the research in plant modular evolution is concentrated in flower morphology (Armbruster et al. 2004) and little is known about the existence of anatomical integration in the evolution of vegetative traits in general.

Secondary phloem and xylem are tissues derived from the same meristems (i.e., the vascular cambium), and therefore could be expected to evolve as part of the same module (Olson and Rosell 2006). Recent work on the Moringaceae secondary growth has shown, however, that different evolutionary pathways can be taken by these tissues. Specifically, paedomorphosis can be present in the xylem, but regular development can be present in the phloem demonstrating that phloem and xylem can behave as belonging to different modules (Olson and Rosell 2006). Our results indicate, however, a different evolutionary scenario, with the reduction in xylem differentiation always being accompanied by increased phloem differentiation. Hence, one could assume that these tissues belong to the same module in Bignonieae, while belonging to two modules in the Moringaceae. Another possibility would be the presence of three modules related to vascular differentiation, one specific to the cambial initials (known for being composed of a single layer of cells), a second being specific to the phloem derivatives, and a third specific to the xylem derivatives. In this case, while the modification would have occurred at the level of the derivative cells in the Moringaceae, the modification would have occurred at the level of cambial initials in the case of Bignonieae.

CONCLUSION

Our results indicate how few anatomical modifications can connect a plant with regular secondary growth to a plant with highly modified, variant secondary growth. Developmental changes caused by evolutionary novelties lead to different anatomical architectures in Bignonieae, likely improving photosynthate conduction and flexibility, and greatly influencing the evolution of morphology in Bignonieae. Other developmental mechanisms such as heterochrony also seem to have played important roles during the diversification of this group. These results illustrate the importance of studies that integrate ontogeny and phylogeny in plant groups and its potential to test hypotheses associated with the developmental mechanisms that led to current anatomical diversity patterns.

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Appendix S1. Species authorities and collection details.

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