

**EVOLUTION OF DISPARITY BETWEEN THE REGULAR AND  
VARIANT PHLOEM IN BIGNONIEAE (BIGNONIACEAE)<sup>1</sup>**

MARCELO R. PACE<sup>2</sup>, LÚCIA G. LOHMANN, AND VERONICA ANGYALOSSY

Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, Cidade Universitária, CEP 05508-090, São Paulo, SP, Brazil

- *Premise of the study:* The phloem is a plant tissue with a critical role in plant nutrition and signaling. However, little is still known about the evolution of this tissue. In lianas of the Bignoniaceae, two distinct types of phloem coexist: a regular and a variant phloem. The cells associated with these two phloem types are known to be anatomically different; however, it is still unclear what steps were involved in the evolution of such differences.
- *Methods:* Here we studied the anatomical development of the regular and variant phloem in representatives of all 21 genera of Bignoniaceae and used a phylogenetic framework to investigate the timing of changes associated with the evolution of each phloem type.
- *Key results:* We found that the variant phloem always appears in a determinate location, between the leaf orthostichies. Furthermore, the variant phloem was mostly occupied by very wide sieve tubes and generally included a higher concentration of fibers, indicating an increase in conduction and mechanical support. On the other hand, the regular phloem included much more parenchyma, more and wider rays, and tiny sieve tubes that resembled terminal sieve tubes from plants with seasonal formation of vascular tissues; these findings suggest reduced conduction and higher storage capacity in the regular phloem.
- *Conclusions:* Overall, differences between the regular and variant phloem increased over time, leading to further specialization in conduction in the variant phloem and an increase in storage specialization in the regular phloem.

**Key words:** Bignoniaceae; cambial variant; development; evolution; liana; pedomorphosis; phloem; sieve tubes.

The phloem is a plant tissue with a critical role in plant nutrition and signaling, through the conduction of photosynthates and a multitude of other molecules (e.g., RNAs). Despite its importance, the evolution of the phloem has never been investigated within a phylogenetic context. In Bignoniaceae (Bignoniaceae), two distinct types of secondary phloem are present in the stems: a regular phloem (present in the interwedges) and a variant phloem (present in the wedges; see Fig. 1). This feature results from the presence of a cambial variant in the stems and is distinctive of representatives of Bignoniaceae. A cambial variant is an unusual type of secondary growth that varies from the presence of multiple cambia to the differential production of secondary tissues or even the inward formation of phloem instead of outward (Schenck, 1893; Carlquist, 2001). Because many cambial variants are taxon-specific, various plant families can be recognized exclusively by the anatomical configura-

tion of their stems (Schenck, 1893; Caballé, 1993). Moreover, cambial variants are thought to have played important roles in the diversification of individual plant families (e.g., Bignoniaceae; Pace et al., 2009).

The presence of a cambial variant is a synapomorphy of the tribe Bignoniaceae (Lohmann, 2006), the most abundant and diverse group of lianas in the neotropics (Gentry, 1991; Lohmann, 2006). In addition, the different forms that these variants can adopt represent synapomorphies of clades within this tribe (Lohmann, 2006, 2011; Pace et al., 2009). The presence of cambial variants is much more common in lianas than in any other life form and has been suggested to increase the flexibility of stems for climbing by mixing soft tissues (e.g., phloem and parenchyma) with the stiff secondary xylem (Fisher and Ewers, 1992; Rowe et al., 2004; Isnard and Silk, 2009). Conversely, cambial variants may also act in injury repair (Dobbins and Fisher, 1986) and water storage (Carlquist, 2001). Specifically in the Bignoniaceae, cambial variants were shown also to directly influence the development of secondary xylem (Lima et al., 2010). The evolution of cambial variants occurred multiple times during the history of plants, from fossil pteridosperms such as *Medullosa steinii* (Dunn et al., 2003) to the extant core eudicots (Bignoniaceae, Fabaceae, Malpighiaceae, Sapindaceae, among others; Schenck, 1893; Carlquist, 2001; Spicer and Groover, 2010), suggesting that these alternative forms of secondary growth may confer great advantage to the lianoid habit.

Specifically in Bignoniaceae, four to multiples of four portions of an initially regular growing cambium modify their activity, producing less xylem and more phloem and creating a pattern of phloem wedges that furrow the xylem (Dobbins, 1971; Pace et al., 2009). The uniqueness of this cambial variant, however, comes from the fact that the four cambial regions with altered activity coexist with regions that maintain a regular secondary

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<sup>2</sup> Author for correspondence (e-mail: marcelorpace@yahoo.com.br; marcelo.pace@helsinki.fi); present address: Department of Biological and Environmental Sciences, Institute of Biotechnology, University of Helsinki, Viikinkaari 1 00014 Helsinki, Finland

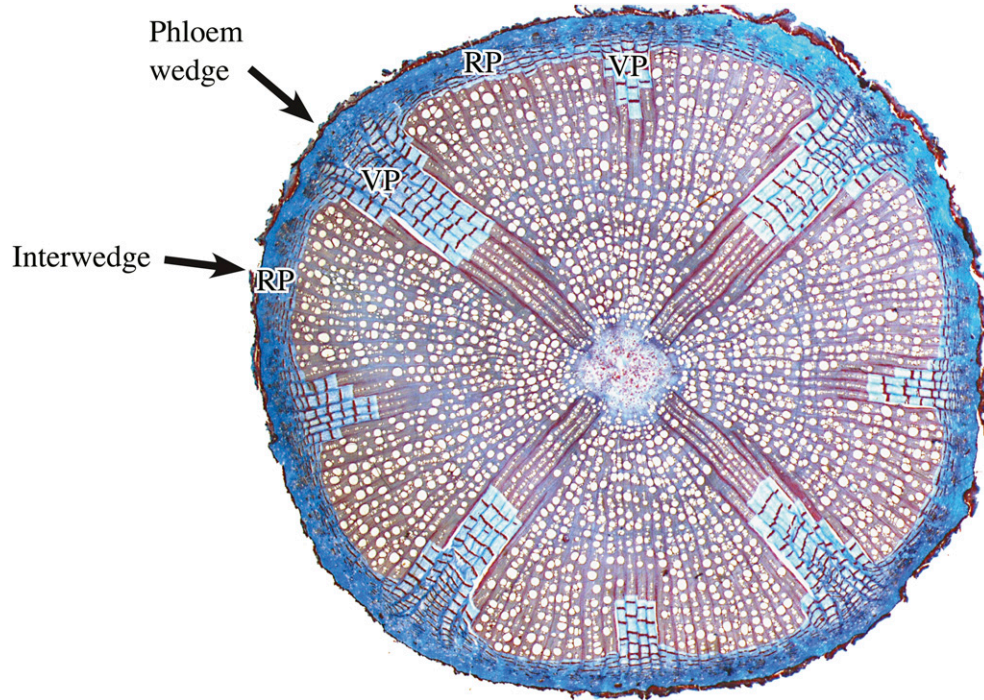


Fig. 1. Transverse section of *Mansoa difficilis* illustrating the position of the variant phloem (VP) within phloem wedges and the position of the regular phloem (RP) in the interwedges.

growth within the stem (Solereder, 1908; Dobbins, 1971), a very unusual feature. Thus, the variant cambium produces more phloem than xylem inside the phloem wedges, while the regular cambium has regular activity producing more xylem than phloem in the regions between the wedges (interwedges). To our knowledge, such a situation is present exclusively in the Bignoniaceae (Dobbins, 1971), Celastraceae s.l. (Hippocrateaceae in Obaton, 1960), and Icacinaceae (Bailey and Howard, 1941; Lens et al., 2008).

Another remarkable feature of this cambial variant is the fact that the phloem produced by the variant portions is not identical to the phloem produced by the regular portions (Dobbins, 1971; Pace et al., 2009). In Bignoniaceae, four main differences were recorded: (1) a difference in the rate of differentiation of xylem and phloem between the regular and variant portions (Schenck, 1893; Solereder, 1908), (2) more divisions in the regular phloem of the fusiform phloem derivatives that give rise to the sieve tube

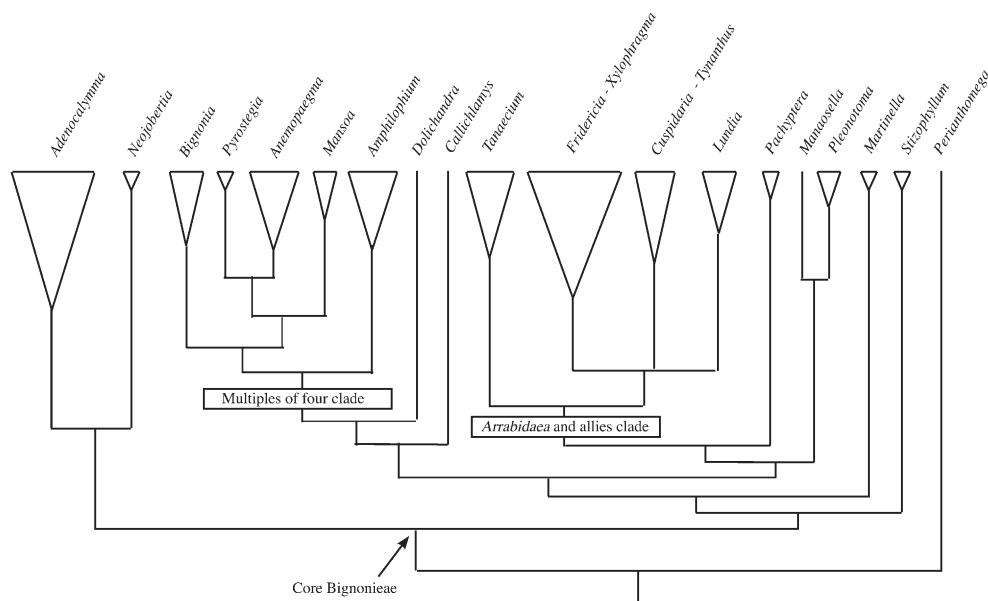


Fig. 2. Summary of phylogenetic relationships between the 21 genera of Bignoniaceae as proposed by Lohmann (2006, 2011).

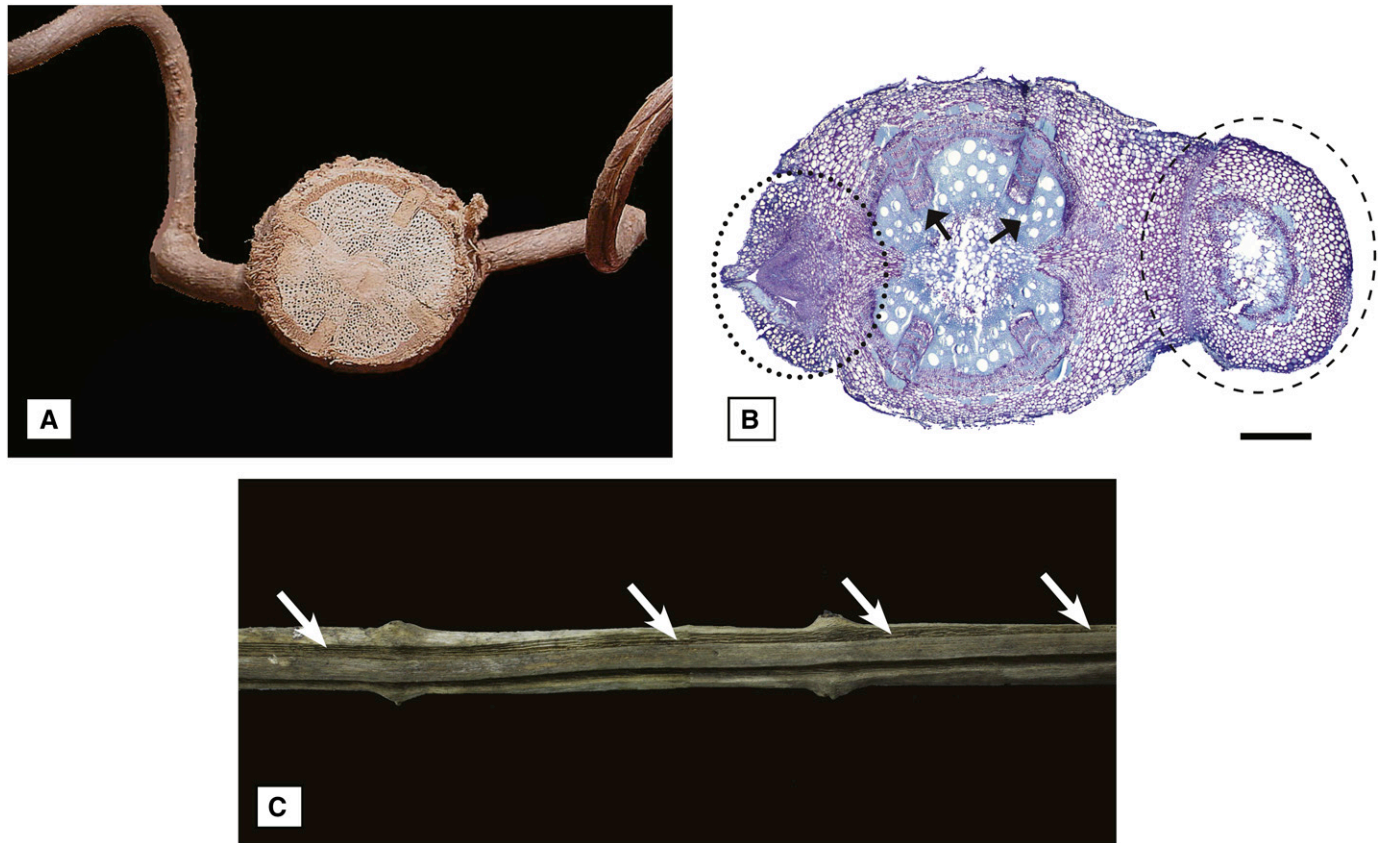


Fig. 3. Position of phloem wedges (variant phloem). (A) Macroscopic view of a stem in transverse section with four phloem wedges in alternation with two leaf petioles departing from the stem (unidentified species of Bignoniaceae). (B) Nodal anatomy of a young stem at the beginning of secondary growth, with four phloem wedges in alternation with the leaves (*Dolichandra unguis-cati*). Dotted lines indicate an axillary bud with its leaf primordia, and dashed lines indicate a petiole departing from the stem. (C) Spiral pattern of phloem wedges indicated by the furrows in a dead branch (unidentified species of Bignoniaceae). Scale bar: 500  $\mu$ m.

elements and associated companion cells (Dobbins, 1971), (3) wider sieve tubes in the variant phloem (Dobbins, 1971; Pace et al., 2009), and (4) a lack of radial anticlinal divisions in the variant cambium (Pace et al., 2009). Even though these differences have long been documented, it is still unclear how exactly these differences evolved over time.

Here we thoroughly compared the regular phloem and the variant phloem in Bignoniaceae and investigated the patterns of change in each phloem type within a phylogenetic framework. More specifically, we detected how and when particular differences evolved in a comparative fashion and tested whether differences between phloem types increased or decreased over time.

## MATERIALS AND METHODS

**Taxon sampling and anatomical procedures**—Fifty-nine species of Bignoniaceae, representing all 21 genera currently recognized in the tribe (Lohmann, 2006, 2011; Fig. 2) were collected in the field or obtained from living collections; only a few specimens were obtained from wood collections (see Appendix 1). Because stem diameter has been shown to represent a good proxy for the multiple qualities of the stem (Rosell and Olson, 2007), we used stems with ca. 2-cm diameter for all analyses; this diameter is the most common diameter of lianas growing in their natural environments (Schnitzer et al., 2006) and represents adults of a reasonable size (Gerwing et al., 2006). For most species, stems of thicker diameters were also collected to ensure that no develop-

mental information was being missed from the analyses conducted with 2-cm diameter stems.

Since the phloem represents a very fragile tissue that rapidly collapses when dried, analyses of the phloem require liquid-preserved materials. Therefore, as soon as stems were collected in the field, they were immediately sectioned into portions no more than 5 cm in length and fixed in Karnovsky's (1965) solution or 70% formalin-acetic acid alcohol (FAA; Berlyn and Miksche, 1976) for up to 10 d under vacuum. After that, materials were transferred to a solution of 70% ethanol. To obtain good anatomical slides, all samples were first softened in ethylenediamine for up to 4 d (Carlquist, 1982), then gradually embedded in polyethylene glycol 1500 (Rupp, 1964), and finally sectioned with the help of an anti-tearing resin made from an expanded polystyrene dissolved in butyl acetate and placed on upon the samples to be sectioned with the aid of an adhesive tape (Barbosa et al., 2010). This technique prevents tissues from tearing apart, allowing us to obtain entire cross sections of stems. Sections were double-stained in astra-blue and safranin (Bukatsch, 1972), then mounted in a synthetic resin to make permanent slides. Whenever we wished to obtain anatomical sections less than 10  $\mu$ m thick, samples were cut into small cubes of 3 mm (including phloem and contiguous cambium and xylem), and subsequently embedded in Histo-resin (Leica Microsystem, Wetzlar, Germany). In this case, materials were sectioned in a rotary microtome and stained in 0.05% toluidine blue O in glacial acetic buffer at pH 4.7 (O'Brien et al., 1964).

**Characterization of the variant and regular phloem**—Anatomical sections were used as a basis to characterize the variant and regular phloem types present in the stems of representatives of Bignoniaceae. First, we described the position of the regular and variant phloem in the stem. Second, we characterized each of the four cell types encountered in the secondary phloem (i.e., sieve

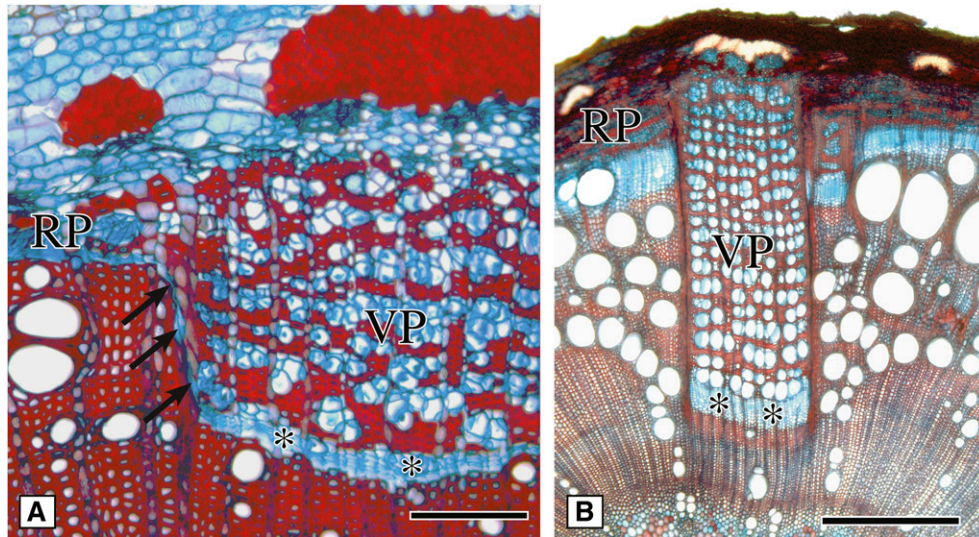


Fig. 4. Cambial dissection and inclusion. (A) Continuous cambium about to dissect, indicated by arrows (*Adenocalymma divaricatum*). (B) Variant cambium (asterisks) included within a phloem wedge (*Tynanthus cognatus*). Scale bar: A = 100  $\mu$ m, B = 500  $\mu$ m, VP = variant phloem, RP = regular phloem, Asterisks: A = variant cambium, B = variant cambium + xylem in differentiation.

tubes with their associated companion cells, parenchyma, fibers, and rays) for each phloem type. Cell types were characterized both through qualitative and quantitative traits, with measurements taken in transverse section using the free software ImageJ version 1.39d (<http://rsb.info.nih.gov/ij/>). For all measurements, at least 30 cells were randomly sampled per phloem type, and subsequently had their average calculated per species. A paired *t* test with  $\alpha = 0.05$  was performed to test whether the averages for regular and variant phloem were significantly different. The numerical data collected for the regular and variant phloem are presented in Tables 1 and 2; species received from wood collections were not included in the quantitative analyses, since their phloem was dehydrated and therefore not perfectly preserved. A question mark was used to indicate a missing datum whenever we were unable to calculate a particular value. Graphics and statistical tests were performed in Excel (Microsoft, Redmond, Washington, USA) and using the free software R (<http://www.R-project.org>). Details on the specific traits and measurements made for each cell type are presented next.

**Sieve tube elements**—Sieve tubes were characterized in terms of their shape, arrangement, frequency (total cells per transverse area), total area occupied (percentage of area occupied by sieve tubes), individual area (the average area occupied by a single sieve tube), and individual diameter. Frequencies were

obtained by counting the number of cell types in a grid of 50  $\text{mm}^2$  within the variant phloem (with at least four repetitions per specimen) and in grids of 10  $\text{mm}^2$  for the regular phloem given the smaller thickness of the regular phloem tissue. To make the data from the regular and variant phloem comparable, we multiplied the results from the regular phloem by five. The same approach was used to estimate the percentage of cells per area.

Because sieve tubes are not circular and have irregular shapes, we documented the area of each cell instead of the diameter. However, we further calculated the square root of the areas to obtain an approximate diameter for the individual cells and, hence, make our data comparable to the diameter data available in the literature.

**Parenchyma**—Parenchyma was characterized in terms of the frequency of the axial parenchyma and the total area occupied. Frequencies were obtained following the same procedure described for sieve tubes.

**Sclerenchyma**—Sclerenchyma was characterized in terms of its type (fibers or sclereids) and arrangement (equal or different arrangement between regular and variant portions). Fibers and sclereids were distinguished following Evert (2006).



Fig. 5. Different developmental stages of a stem showing the amount of xylem (stroke with bars) and phloem (simple stroke) produced at each stage (*Tynanthus cognatus*). X = xylem, P = phloem.

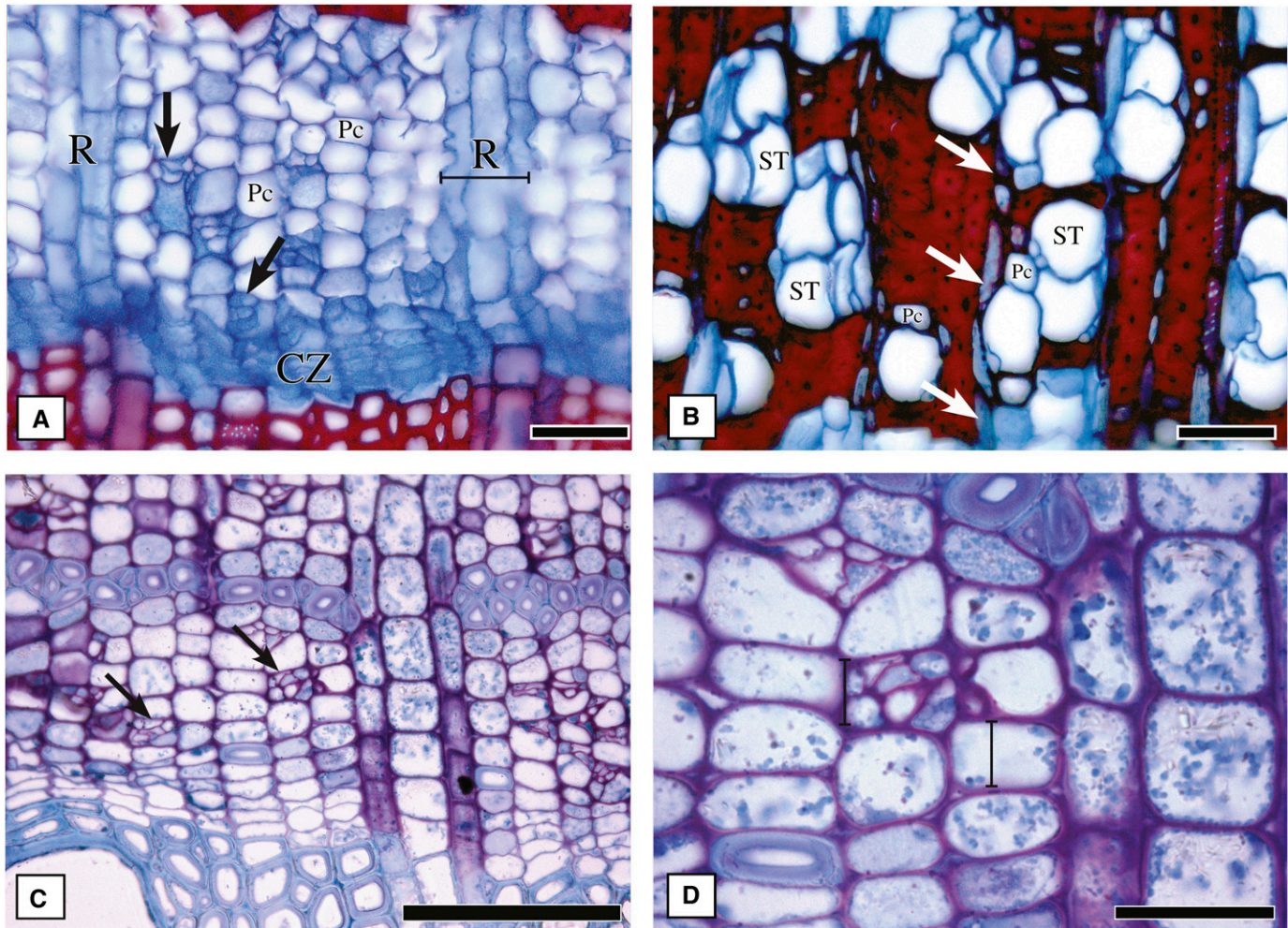


Fig. 6. (A, B) Regular and variant phloem types in *Styzophyllum riparium*. (A) Regular phloem, with narrow and rectangular sieve tubes indicated by arrows, arranged in clusters and groups of 2–3 cells; note the relative abundance of parenchyma in the phloem and the presence of multiseriate rays. (B) Variant phloem, with wide round-shaped sieve tubes, arranged in multiples of 2–3 cells; note the scanty parenchyma in the phloem and the predominantly uniseriate rays indicated by arrows. (C, D) Regular phloem in *Tynanthus cognatus*. (C) Sieve tubes arranged in assemblages (arrows). (D) Detail of an assemblage. Note that each assemblage has exactly the same shape as a neighboring parenchyma cell, indicating that assemblages are derived from the division of a single fusiform phloem derivative. CZ = cambial zone, Pc = parenchyma cell, R = ray, ST = sieve tube. Scale bars = A, B, 50  $\mu$ m; C, 100  $\mu$ m; D, 30  $\mu$ m.

**Rays**—Ray cells were characterized by their width and height in tangential sections.

**Crystals**—Whenever present, crystals were characterized in terms of their shape (e.g., acicular, prismatic), according to the types described by the IAWA Committee (1989).

**Phylogenetic independent contrasts and ancestral character state reconstructions**—All characters considered in this study are variable between the regular and variant phloem. Character state reconstructions of discrete characters were carried using parsimony algorithms in the program MacClade 4.06 (Maddison and Maddison, 2003) and likelihood assumptions in the program Mesquite 2.06 (Maddison and Maddison, 2009). Character state reconstructions of continuous characters also used both parsimony and likelihood assumptions and were performed in Mesquite 2.06 (Maddison and Maddison, 2009). In addition, an analysis of phylogenetic independent contrast was performed using the Phenotypic Diversity Analysis Program (PDAP) implemented in Mesquite 2.06 (Midford et al., 2003) to estimate whether the patterns observed in the continuous characters were independent from their phylogenetic history.

A robust molecular phylogeny of Bignoniaceae including 104 species was used as the basis for all analyses (Lohmann, 2006). However, since this phylogeny did not contain all the 383 species currently recognized in Bignoniaceae (Lohmann and Ulloa Ulloa, 2010), we collapsed the branches in the deepest nodes of genera and introduced the remaining 279 species that were not included in this phylogeny following the most recent classification of the group (Lohmann, 2011). This approach led to a tree that included all species of Bignoniaceae currently recognized. We then used this tree to prepare a second tree from which we pruned all species that had not been sampled for the anatomical analysis. Both trees were used to reconstruct ancestral character states and to perform PDAP calculations. Because no differences were encountered in the analyses using the two trees, we only present the results based on the tree that exclusively included the species with complete anatomical sampling.

## RESULTS

**Characterization of the regular and variant phloem**—*Position of the regular and variant phloem in the stem*—The four phloem wedges present in the stems of Bignoniaceae are always

TABLE 1. Data for the sieve tubes and parenchyma cells in the variant and regular phloems.

| Species                            | Habit | Sieve tube elements                      |         |                            |         |  |            |                  |         | Parenchyma                               |          |                            |         |
|------------------------------------|-------|--|---------|----------------------------|---------|--|------------|------------------|---------|--|----------|----------------------------|---------|
|                                    |       | Frequency<br>(cells/50 mm <sup>2</sup> ) |         | Total occupied<br>area (%) |         | Individual areas<br>(μm <sup>2</sup> ) |            | Diameter<br>(μm) |         | Frequency<br>(cells/50 mm <sup>2</sup> ) |          | Total occupied<br>area (%) |         |
|                                    |       | Regular                                  | Variant | Regular                    | Variant | Regular                                | Variant    | Regular          | Variant | Regular                                  | Variant  | Regular                    | Variant |
| <i>Adenocalymma bracteatum</i>     | Liana | ?  | ?       | ?                          | ?       | 244 ± 88                               | 1356 ± 276 | 15 ± 4           | 38 ± 14 | ?  | ?        | ?                          | ?       |
| <i>Adenocalymma comosum</i>        | Liana | 48 ± 18                                  | 34 ± 5  | 4.1                        | 45.6    | 336 ± 120                              | 2108 ± 640 | 18 ± 8           | 46 ± 11 | 198 ± 52                                 | 64 ± 15  | 56.3                       | 29.7    |
| <i>Adenocalymma divaricatum</i>    | Liana | 46 ± 19                                  | 24 ± 5  | 1.5                        | 26.9    | 124 ± 32                               | 2236 ± 444 | 11 ± 4           | 47 ± 12 | 274 ± 15                                 | 57 ± 7   | 40.8                       | 10.6    |
| <i>Adenocalymma flaviflorum</i>    | Liana | 31 ± 9                                   | 36 ± 4  | 2.8                        | 37.5    | 252 ± 80                               | 1728 ± 169 | 16 ± 4           | 41 ± 10 | 210 ± 35                                 | 73 ± 8   | 42.5                       | 32.2    |
| <i>Adenocalymma neoflavidum</i>    | Liana | 57 ± 17                                  | 38 ± 3  | 5.9                        | 31.5    | 248 ± 62                               | 1604 ± 207 | 16 ± 3           | 40 ± 4  | 245 ± 60                                 | 80 ± 10  | 52.7                       | 20.9    |
| <i>Adenocalymma nodosum</i>        | Shrub | 95 ± 45                                  | 57 ± 10 | 6.6                        | 23.4    | 192 ± 47                               | 516 ± 104  | 14 ± 4           | 23 ± 17 | 215 ± 37                                 | 98 ± 19  | 44.8                       | 42.1    |
| <i>Adenocalymma peregrinum</i>     | Shrub | 35 ± 18                                  | 61 ± 4  | 3.1                        | 24.9    | 168 ± 43                               | 652 ± 120  | 13 ± 5           | 25 ± 8  | 190 ± 17                                 | 120 ± 12 | 47.6                       | 32.3    |
| <i>Adenocalymma salmoneum</i>      | Liana | 46 ± 14                                  | 39 ± 7  | 3.0                        | 33.0    | 124 ± 40                               | 1752 ± 240 | 11 ± 4           | 42 ± 4  | 168 ± 14                                 | 64 ± 2   | 41.9                       | 21.2    |
| <i>Amphilophium bracteatum</i>     | Liana | 76 ± 21                                  | 28 ± 3  | 24.1                       | 56.4    | 498 ± 140                              | 2389 ± 370 | 22 ± 4           | 49 ± 5  | 167 ± 15                                 | 66 ± 9   | 74.5                       | 42.1    |
| <i>Amphilophium crucigerum</i>     | Liana | 86 ± 12                                  | 32 ± 7  | 16.5                       | 20.6    | 340 ± 101                              | 1156 ± 250 | 18 ± 4           | 34 ± 14 | 150 ± 16                                 | 120 ± 9  | 78.7                       | 66.3    |
| <i>Amphilophium dolichoides</i>    | Liana | ?  | ?       | ?                          | ?       | 640 ± 98                               | 3400 ± 349 | 25 ± 6           | 58 ± 7  | ?  | ?        | ?                          | ?       |
| <i>Amphilophium elongatum</i>      | Liana | 63 ± 22                                  | 61 ± 15 | 14.9                       | 71.9    | 488 ± 109                              | 2848 ± 361 | 22 ± 7           | 53 ± 18 | 136 ± 26                                 | 68 ± 10  | 73.9                       | 17.2    |
| <i>Amphilophium magnoliifolium</i> | Liana | ?  | ?       | ?                          | ?       | 912 ± 107                              | 1668 ± 238 | 30 ± 4           | 41 ± 12 | ?  | ?        | ?                          | ?       |
| <i>Amphilophium paniculatum</i>    | Liana | 58 ± 18                                  | 29 ± 8  | 46.5                       | 70.4    | 2156 ± 230                             | 5500 ± 472 | 46 ± 5           | 74 ± 16 | 156 ± 25                                 | 78 ± 4   | 48.5                       | 25.2    |
| <i>Anemopaegma chamberlaynii</i>   | Liana | 49 ± 13                                  | 34 ± 3  | 9.4                        | 29.4    | 504 ± 67                               | 1588 ± 222 | 24 ± 2           | 40 ± 7  | 170 ± 19                                 | 102 ± 6  | 47.8                       | 29.9    |
| <i>Bignonia binata</i>             | Liana | 61 ± 25                                  | 60 ± 5  | 6.3                        | 42.1    | 80 ± 44                                | 1256 ± 245 | 9 ± 4            | 35 ± 4  | 195 ± 39                                 | 122 ± 15 | 49.6                       | 26.5    |
| <i>Bignonia campanulata</i>        | Liana | 53 ± 37                                  | 59 ± 1  | 3.1                        | 46.5    | 148 ± 35                               | 1316 ± 218 | 12 ± 4           | 36 ± 6  | 218 ± 116                                | 81 ± 14  | 65.8                       | 22.5    |
| <i>Bignonia corymbosa</i>          | Liana | ?  | ?       | ?                          | ?       | 340 ± 99                               | 1560 ± 209 | 18 ± 3           | 39 ± 9  | ?  | ?        | ?                          | ?       |
| <i>Bignonia magnifica</i>          | Liana | 61 ± 25                                  | 59 ± 17 | 9.7                        | 33.5    | 1200 ± 164                             | 2880 ± 349 | 35 ± 7           | 54 ± 5  | 161 ± 30                                 | 88 ± 13  | 66.3                       | 43.0    |
| <i>Callichlamys latifolia</i>      | Liana | 35 ± 15                                  | 34 ± 3  | 8.0                        | 44.1    | 528 ± 130                              | 2112 ± 289 | 23 ± 5           | 46 ± 5  | 115 ± 29                                 | 63 ± 6   | 57.1                       | 28.0    |
| <i>Cuspidaria convoluta</i>        | Liana | 19 ± 14                                  | 21 ± 4  | 1.2                        | 37.2    | 124 ± 33                               | 3340 ± 462 | 11 ± 3           | 58 ± 6  | 211 ± 25                                 | 76 ± 7   | 64.7                       | 18.0    |
| <i>Cuspidaria pulchra</i>          | Shrub | 25 ± 16                                  | 27 ± 9  | 2.1                        | 8.3     | 180 ± 39                               | 372 ± 97   | 13 ± 5           | 19 ± 5  | 165 ± 31                                 | 91 ± 6   | 91.4                       | 16.7    |
| <i>Dolichandra unguiculata</i>     | Liana | 48 ± 22                                  | 41 ± 11 | 12.4                       | 54.7    | 600 ± 90                               | 2300 ± 243 | 24 ± 5           | 48 ± 7  | 101 ± 10                                 | 77 ± 7   | 54.2                       | 29.3    |
| <i>Dolichandra unguis-cati</i>     | Liana | 73 ± 19                                  | 49 ± 10 | 13.2                       | 36.5    | 276 ± 48                               | 716 ± 168  | 17 ± 6           | 27 ± 5  | 263 ± 18                                 | 109 ± 21 | 56.2                       | 40.1    |
| <i>Fridericia chica</i>            | Liana | 43 ± 5                                   | 31 ± 2  | 6.7                        | 25.0    | 328 ± 149                              | 1376 ± 289 | 18 ± 7           | 37 ± 12 | 268 ± 49                                 | 74 ± 11  | 63.3                       | 25.9    |
| <i>Fridericia conjugata</i>        | Liana | 30 ± 24                                  | 24 ± 10 | 6.6                        | 37.4    | 492 ± 154                              | 3584 ± 485 | 22 ± 3           | 60 ± 8  | 223 ± 23                                 | 78 ± 7   | 56.2                       | 35.3    |
| <i>Fridericia platyphylla</i>      | Shrub | 19 ± 6                                   | 36 ± 5  | 1.6                        | 25.7    | 156 ± 42                               | 1272 ± 297 | 12 ± 4           | 36 ± 7  | 119 ± 19                                 | 56 ± 9   | 51.8                       | 16.8    |
| <i>Fridericia samydoidea</i>       | Liana | 39 ± 7                                   | 20 ± 3  | 3.6                        | 31.3    | 200 ± 46                               | 4172 ± 489 | 14 ± 4           | 64 ± 8  | 150 ± 18                                 | 66 ± 8   | 61.5                       | 23.4    |
| <i>Fridericia speciosa</i>         | Liana | 38 ± 7                                   | 35 ± 7  | 3.9                        | 39.5    | 220 ± 30                               | 2620 ± 320 | 14 ± 4           | 51 ± 7  | 160 ± 37                                 | 70 ± 6   | 59.1                       | 19.0    |
| <i>Lundia cordata</i>              | Liana | 46 ± 16                                  | 17 ± 5  | 1.6                        | 23.8    | 76 ± 21                                | 2412 ± 311 | 9 ± 3            | 49 ± 6  | 214 ± 56                                 | 46 ± 17  | 67.5                       | 11.6    |
| <i>Lundia corymbifera</i>          | Liana | 88 ± 38                                  | 30 ± 5  | 1.6                        | 16.6    | 60 ± 12                                | 1029 ± 401 | 8 ± 3            | 32 ± 7  | 135 ± 78                                 | 67 ± 18  | 47.0                       | 31.4    |
| <i>Lundia damazii</i>              | Liana | 60 ± 13                                  | 28 ± 3  | 4.1                        | 30.0    | 164 ± 60                               | 2008 ± 428 | 13 ± 4           | 45 ± 8  | 280 ± 45                                 | 81 ± 21  | 82.5                       | 18.9    |
| <i>Lundia glazioviana</i>          | Liana | 36 ± 16                                  | 18 ± 2  | 1.0                        | 17.6    | 72 ± 43                                | 1500 ± 309 | 8 ± 2            | 39 ± 10 | 170 ± 18                                 | 66 ± 8   | 63.3                       | 25.1    |
| <i>Manaosella cordifolia</i>       | Liana | 46 ± 15                                  | 33 ± 3  | 10.7                       | 45.0    | 448 ± 81                               | 1904 ± 235 | 21 ± 4           | 44 ± 7  | 156 ± 27                                 | 92 ± 10  | 66.1                       | 30.6    |
| <i>Manaosella difficilis</i>       | Liana | 74 ± 21                                  | 24 ± 5  | 7.6                        | 32.9    | 168 ± 34                               | 2416 ± 305 | 13 ± 3           | 49 ± 9  | 219 ± 16                                 | 101 ± 19 | 56.5                       | 37.4    |
| <i>Mansoa onohualcoidea</i>        | Liana | ?  | ?       | ?                          | ?       | 180 ± 43                               | 1908 ± 310 | 13 ± 2           | 45 ± 5  | ?  | ?        | ?                          | ?       |
| <i>Mansoa standleyi</i>            | Liana | 83 ± 19                                  | 48 ± 6  | 6.6                        | 30.8    | 156 ± 46                               | 1032 ± 169 | 12 ± 2           | 32 ± 5  | 290 ± 46                                 | 142 ± 23 | 74.3                       | 36.1    |
| <i>Martinella obovata</i>          | Liana | ?  | ?       | ?                          | ?       | 260 ± 22                               | 1804 ± 62  | 16 ± 2           | 42 ± 3  | ?  | ?        | 45.6                       | 30.5    |
| <i>Neoberberia mirabilis</i>       | Liana | 44 ± 18                                  | 17 ± 8  | 5.9                        | 28.8    | 316 ± 87                               | 3576 ± 426 | 18 ± 6           | 60 ± 6  | 146 ± 38                                 | 55 ± 6   | 52.6                       | 18.8    |
| <i>Pachyptera kerere</i>           | Liana | ?  | ?       | ?                          | ?       | 112 ± 20                               | 740 ± 85   | 10 ± 2           | 28 ± 4  | ?  | ?        | 38.6                       | 37.4    |
| <i>Perianthomega vellozoi</i>      | Liana | 35 ± 4                                   | 42 ± 3  | 2.2                        | 29.0    | 164 ± 45                               | 1280 ± 450 | 13 ± 3           | 36 ± 10 | 119 ± 28                                 | 68 ± 9   | 66.6                       | 37.4    |
| <i>Pleonotoma stichadenia</i>      | Liana | 40 ± 12                                  | 25 ± 4  | 1.7                        | 18.3    | 96 ± 33                                | 960 ± 289  | 10 ± 2           | 31 ± 12 | 215 ± 21                                 | 67 ± 12  | 79.2                       | 22.2    |
| <i>Pleonotoma tetraquetta</i>      | Liana | 25 ± 14                                  | 15 ± 6  | 4.0                        | 27.3    | 324 ± 45                               | 2484 ± 401 | 18 ± 3           | 50 ± 8  | 125 ± 14                                 | 61 ± 23  | 67.8                       | 32.5    |
| <i>Pyrostegia venusta</i>          | Liana | 74 ± 16                                  | 34 ± 4  | 9.5                        | 40.4    | 324 ± 40                               | 2188 ± 512 | 18 ± 2           | 47 ± 6  | 258 ± 64                                 | 73 ± 9   | 65.8                       | 37.6    |
| <i>Stizophyllum riparium 1</i>     | Liana | 101 ± 25                                 | 60 ± 12 | 8.0                        | 39.6    | 224 ± 34                               | 1408 ± 342 | 14 ± 3           | 37 ± 8  | 243 ± 23                                 | 64 ± 9   | 71.9                       | 11.9    |
| <i>Stizophyllum riparium 2</i>     | Liana | 98 ± 15                                  | 40 ± 10 | 5.7                        | 28.5    | 152 ± 14                               | 1600 ± 102 | 12 ± 2           | 40 ± 6  | 195 ± 32                                 | 54 ± 12  | 65.8                       | 11.9    |
| <i>Stizophyllum riparium 3</i>     | Liana | 111 ± 50                                 | 29 ± 2  | 5.0                        | 35.6    | 96 ± 34                                | 2480 ± 501 | 10 ± 4           | 50 ± 5  | 209 ± 32                                 | 30 ± 6   | 77.3                       | 9.6     |
| <i>Tanaecium bilabiatum</i>        | Liana | 36 ± 13                                  | 32 ± 4  | 1.9                        | 17.8    | 92 ± 16                                | 1756 ± 310 | 9 ± 2            | 42 ± 8  | 184 ± 50                                 | 79 ± 12  | 61.0                       | 21.0    |

TABLE 1. Continued.

| Species                        | Habit | Sieve tube elements                   |          |                         |          |                                     |             |               |          | Parenchyma                            |          |                         |          |
|--------------------------------|-------|---------------------------------------|----------|-------------------------|----------|-------------------------------------|-------------|---------------|----------|---------------------------------------|----------|-------------------------|----------|
|                                |       | Frequency (cells/50 mm <sup>2</sup> ) |          | Total occupied area (%) |          | Individual areas (μm <sup>2</sup> ) |             | Diameter (μm) |          | Frequency (cells/50 mm <sup>2</sup> ) |          | Total occupied area (%) |          |
|                                |       | Regular                               | Variante | Regular                 | Variante | Regular                             | Variante    | Regular       | Variante | Regular                               | Variante | Regular                 | Variante |
| <i>Tanaecium pyramidatum</i> 1 | Liana | 55 ± 9                                | 21 ± 2   | 5.3                     | 31.1     | 196 ± 43                            | 2832 ± 634  | 14 ± 10       | 53 ± 16  | 141 ± 39                              | 60 ± 9   | 68.6                    | 28.4     |
| <i>Tanaecium pyramidatum</i> 2 | Liana | 46 ± 9                                | 25 ± 5   | 5.0                     | 13.1     | 128 ± 50                            | 1336 ± 489  | 11 ± 5        | 36 ± 12  | 136 ± 45                              | 79 ± 18  | 55.2                    | 31.6     |
| <i>Tynanthus cognatus</i>      | Liana | 65 ± 19                               | 16 ± 2   | 2.7                     | 35.6     | 96 ± 13                             | 3936 ± 1000 | 10 ± 2        | 63 ± 17  | 150 ± 23                              | 67 ± 12  | 40.9                    | 22.6     |
| <i>Tynanthus elegans</i>       | Liana | 69 ± 17                               | 24 ± 3   | 2.3                     | 38.5     | 80 ± 18                             | 3712 ± 870  | 8 ± 2         | 61 ± 12  | 171 ± 22                              | 86 ± 11  | 39.2                    | 20.6     |
| <i>Xylophragma myrianthum</i>  | Liana | 53 ± 31                               | 12 ± 3   | 2.2                     | 28.8     | 96 ± 21                             | 4704 ± 580  | 10 ± 2        | 68 ± 16  | 220 ± 38                              | 86 ± 12  | 57.2                    | 16.4     |

produced between the leaf orthostichies (Fig. 3A, B). Because the phyllotaxy is decussate, the four variant portions constantly rotate in the stems, promoting a spiral pattern usually visible from outside the stem (Fig. 3C). The regular portions of the cambium keep producing more xylem inward than phloem outward, while the variant cambium greatly decreases the production of xylem and increases the production of phloem. As a result, two reverse cambial displacements are established in these stems. These different displacements finally lead to cambial dissection (Fig. 4A) and inclusion inside phloem wedges (Fig. 4B). Even though the regular and variant cambia produce xylem and phloem at different rates, this production continues throughout the plant's life, as evidenced by the comparison of different developmental stages of the same branch (Fig. 5).

**Sieve tube elements**—Sieve tube elements from the regular phloem were shown to differ from those in the variant phloem in terms of their shape, width, and arrangement. In the regular portions of the phloem, sieve tubes were generally rectangular and presented a small radial diameter (Fig. 6A). In addition, sieve tubes from the regular portions of the phloem were encountered in clusters, solitary, or in pairs scattered among parenchyma cells (Fig. 6A). Furthermore, a group of tiny sieve tubes was often found in association with their companion cells and smaller parenchyma cells, all of which formed an assemblage (Fig. 6C, D). Assemblages generally presented the same shape and size as their neighboring parenchyma cells (Fig. 6C, D), suggesting that both cell types originated from a single fusiform derivative. On the other hand, most sieve tube elements from the variant portions of the phloem were large and round (Fig. 6B), generally arranged in multiples of two or more (Fig. 6B).

Overall, the regular phloem presented a higher number of sieve tubes per square millimeter (54 cells/50 mm<sup>2</sup>) than the variant phloem (34 cells/50 mm<sup>2</sup>; Table 1). However, only 7% of the total area of the regular phloem was occupied by sieve tubes, contrasting with 34% of the total area occupied by sieve tubes from the variant phloem (Table 1). This is the result of the remarkable difference in width between sieve tubes of the regular and variant phloem types, as confirmed by a *t* test (Table 2); this pattern was found in all species analyzed (Fig. 7). Overall, sieve tubes from the variant phloem had an average area of 1756 μm<sup>2</sup> and an average diameter of 42 μm, while sieve tubes from the regular phloem had an average area of 192 μm<sup>2</sup> and an average diameter of 14 μm.

The difference in sieve tube areas was documented in both lianas and shrubs of Bignoniaceae. However, we encountered the narrowest sieve tubes of the tribe in the variant phloem of shrubs. The area of such narrow sieve tubes ranged from 372 to 1272 μm<sup>2</sup>. In the regular phloem, however, shrubs had sieve tube diameters that were similar to those of the lianas (Table 1).

**Parenchyma**—In the regular phloem, the parenchyma generally covered more than 50% of the total phloem area (Figs. 6A, 6C, 8A). In the variant phloem, on the other hand, the parenchyma generally covered less than 30% of the total phloem area (Figs. 6B, 8B; Table 1)

**Sclerenchyma**—Most genera in the tribe presented phloem fibers in the regular and variant portions of the conducting phloem, except for *Pleonotoma*, which only presented sclereids in the regular portion of the phloem (Fig. 8A). Individual phloem fibers did not differ in shape or diameter between the regular and variant portions of the phloem. However, in the variant phloem, two different arrangements of fiber bands were encountered. The first fiber arrangement was composed of fiber bands of identical distribution in both the regular and variant portions of the phloem, forming a continuum (Fig. 8F). In the second fiber distribution, however, fiber bands were present in the regular phloem, while a less-defined matrix of closely spaced fibers was encountered in the variant portion of the phloem (Fig. 8G).

**Rays**—In most species of Bignoniaceae (the core Bignoniaceae clade), rays of the regular phloem were high (>1 mm) and multiseriate (Fig. 9A), while rays from the variant phloem were short (<1 mm) and uniseriate to biseriate (Fig. 9B). On the other hand, phloem rays of *Perianthomega* were equally distributed, all being high and wide, both in the regular and variant portions of the phloem (Figs. 9C, D).

**Crystals**—Three different types of crystals were encountered in the axial and ray parenchyma of Bignoniaceae: prismatic, acicular, and styloid. Virtually, all species analyzed have the same pattern of crystal content both in the regular and variant portions, except for one genus, *Pleonotoma*, whose differences are very strong. The regular phloem of *Pleonotoma* contains axial and ray parenchyma fully occupied by acicular crystals (Fig. 8C, D), while its variant phloem contains exclusively prismatic crystals (Fig. 8E).

TABLE 2. Results from the paired *t* test between sieve tube areas of the regular and variant phloem.

| Species                            | <i>P</i> value       | df | <i>t</i> | Significant difference between means |
|------------------------------------|----------------------|----|----------|--------------------------------------|
| <i>Adenocalymma bracteatum</i>     | $87 \times 10^{-4}$  | 3  | 06.13    | Yes                                  |
| <i>Adenocalymma comosum</i>        | $24 \times 10^{-15}$ | 29 | 16.60    | Yes                                  |
| <i>Adenocalymma divaricatum</i>    | $14 \times 10^{-22}$ | 29 | 25.84    | Yes                                  |
| <i>Adenocalymma flaviformum</i>    | $14 \times 10^{-14}$ | 29 | 12.91    | Yes                                  |
| <i>Adenocalymma neoflavidum</i>    | $14 \times 10^{-22}$ | 29 | 25.80    | Yes                                  |
| <i>Adenocalymma nodosum</i>        | $48 \times 10^{-9}$  | 29 | 07.30    | Yes                                  |
| <i>Adenocalymma peregrinum</i>     | $18 \times 10^{-13}$ | 29 | 11.66    | Yes                                  |
| <i>Adenocalymma salmoneum</i>      | $94 \times 10^{-16}$ | 29 | 14.40    | Yes                                  |
| <i>Amphilophium bracteatum</i>     | $20 \times 10^{-4}$  | 17 | 04.79    | Yes                                  |
| <i>Amphilophium crucigerum</i>     | $12 \times 10^{-15}$ | 29 | 09.76    | Yes                                  |
| <i>Amphilophium dolichoides</i>    | $42 \times 10^{-11}$ | 29 | 14.22    | Yes                                  |
| <i>Amphilophium elongatum</i>      | $82 \times 10^{-14}$ | 29 | 12.04    | Yes                                  |
| <i>Amphilophium magnoliifolium</i> | $72 \times 10^{-7}$  | 29 | 05.44    | Yes                                  |
| <i>Amphilophium paniculatum</i>    | $25 \times 10^{-14}$ | 29 | 11.49    | Yes                                  |
| <i>Anemopaegma chamberlaynii</i>   | $95 \times 10^{-13}$ | 29 | 10.89    | Yes                                  |
| <i>Bignonia binata</i>             | $12 \times 10^{-11}$ | 28 | 09.86    | Yes                                  |
| <i>Bignonia campanulata</i>        | $25 \times 10^{-14}$ | 28 | 12.92    | Yes                                  |
| <i>Bignonia corymbosa</i>          | ?                    | ?  | ?        | ?                                    |
| <i>Bignonia magnifica</i>          | $37 \times 10^{-11}$ | 28 | 09.38    | Yes                                  |
| <i>Callichlamys latifolia</i>      | $44 \times 10^{-10}$ | 29 | 08.23    | Yes                                  |
| <i>Cuspidaria convoluta</i>        | $25 \times 10^{-17}$ | 29 | 16.55    | Yes                                  |
| <i>Cuspidaria pulchra</i>          | ?                    | ?  | ?        | ?                                    |
| <i>Dolichandra unguiculata</i>     | $23 \times 10^{-17}$ | 29 | 16.60    | Yes                                  |
| <i>Dolichandra unguis-cati</i>     | $13 \times 10^{-5}$  | 29 | 04.41    | Yes                                  |
| <i>Fridericia chica</i>            | $42 \times 10^{-10}$ | 36 | 07.68    | Yes                                  |
| <i>Fridericia conjugata</i>        | $16 \times 10^{-18}$ | 29 | 18.37    | Yes                                  |
| <i>Fridericia platyphylla</i>      | $59 \times 10^{-11}$ | 29 | 09.05    | Yes                                  |
| <i>Fridericia samydoidea</i>       | ?                    | ?  | ?        | ?                                    |
| <i>Fridericia speciosa</i>         | ?                    | ?  | ?        | ?                                    |
| <i>Lundia cordata</i>              | $29 \times 10^{-17}$ | 29 | 16.46    | Yes                                  |
| <i>Lundia corymbifera</i>          | $13 \times 10^{-8}$  | 29 | 06.92    | Yes                                  |
| <i>Lundia damazii</i>              | ?                    | ?  | ?        | ?                                    |
| <i>Lundia glazioviana</i>          | $84 \times 10^{-11}$ | 29 | 08.90    | Yes                                  |
| <i>Manaosella cordifolia</i>       | $84 \times 10^{-11}$ | 28 | 10.24    | Yes                                  |
| <i>Mansoa difficilis</i>           | $25 \times 10^{-12}$ | 29 | 16.56    | Yes                                  |
| <i>Mansoa onohualcoides</i>        | $49 \times 10^{-4}$  | 29 | 07.45    | Yes                                  |
| <i>Mansoa standley</i>             | $10 \times 10^{-14}$ | 29 | 13.98    | Yes                                  |
| <i>Martinella obovata</i>          | $21 \times 10^{-4}$  | 3  | 10.07    | Yes                                  |
| <i>Nejobertia mirabilis</i>        | ?                    | ?  | ?        | ?                                    |
| <i>Pachyptera kerere</i>           | $52 \times 10^{-4}$  | 3  | 07.33    | Yes                                  |
| <i>Perianthomega vellozoi</i>      | $17 \times 10^{-5}$  | 30 | 04.50    | Yes                                  |
| <i>Tanaecium bilabiatum</i>        | $46 \times 10^{-9}$  | 15 | 10.04    | Yes                                  |
| <i>Tanaecium pyramidatum</i>       | $13 \times 10^{-11}$ | 41 | 08.49    | Yes                                  |
| <i>Tynanthus cognatus</i>          | $94 \times 10^{-16}$ | 29 | 09.75    | Yes                                  |
| <i>Tynanthus elegans</i>           | $94 \times 10^{-16}$ | 29 | 14.39    | Yes                                  |
| <i>Xylophragma myrianthum</i>      | $46 \times 10^{-12}$ | 29 | 10.66    | Yes                                  |

Note: ? = missing data.

**Ancestral state reconstructions and phylogenetic independent contrasts—Sieve tube elements**—In ancestral state reconstructions of sieve tube areas for the regular phloem, sieve tube areas of ca.  $195 \mu\text{m}^2$  were inferred to represent the ancestral condition in the tribe (Fig. 10A). Furthermore, a relatively constant area (range  $160\text{--}360 \mu\text{m}^2$ ) was encountered throughout Bignoniaceae, with the widest sieve tubes (ca.  $700 \mu\text{m}^2$ ) in *Amphilophium* and other representatives from the “multiples of four clade” (e.g., *Bignonia magnifica* and *Dolichandra unguiculata*); this sieve tube area was significantly higher than that inferred for the ancestor of the tribe (Fig. 10A). The “*Arrabidaea* and allies clade,” on the other hand, presented the lowest sieve tube areas in the regular phloem ( $129 \mu\text{m}^2$ ). Phylogenetic independent contrasts failed

to indicate that the variation in sieve tube areas would be independent of the phylogenetic history of the species (PDAP with  $P > 0.05$ ;  $R^2 = 0.003$ ).

Ancestral state reconstructions of the area of sieve tubes from the variant phloem led to a rather different scenario. More specifically, we observed a gradual increase in the area of sieve tubes from the most basal lineages toward more derived clades (Fig. 10B). We inferred that the ancestor of Bignoniaceae presented sieve tubes with ca.  $1616 \mu\text{m}^2$  in the variant phloem. The highest sieve tube areas were found in the “*Arrabidaea* and allies clade” ( $2600 \mu\text{m}^2$ ) and other derived lineages (e.g., *Amphilophium*, *Nejobertia*, *Xylophragma*). This observation was further corroborated through phylogenetic independent contrasts (PDAP with  $P < 0.05$ ;  $R^2 = 0.216$ ), which indicated that the concentration of the widest sieve tubes in more derived lineages was independent of phylogenetic history. Furthermore, the difference between sieve tube areas from the regular and variant phloem types was also shown to increase toward more derived lineages (PDAP with  $P < 0.05$ ;  $R^2 = 0.186$ ).

**Parenchyma**—Ancestral state reconstructions of parenchyma indicated an increase in the amount of phloem parenchyma toward more derived lineages in the regular phloem (Fig. 11). This observation was corroborated by phylogenetic independent contrasts (PDAP with  $P < 0.05$ ;  $R^2 = 0.193$ ), which indicated that the concentration of increased amounts of parenchyma in more derived lineages was independent of phylogenetic history. On the other hand, ancestral state reconstructions indicated a random pattern of variation in the phloem parenchyma of the variant phloem, with taxa that presented high amounts of parenchyma being more closely related to taxa with low amounts of parenchyma.

**Fibers**—Ancestral state reconstructions of fibers indicated that the different fiber arrangements evolved at least once in *Adenocalymma*, once within the “*Arrabidaea* and allies clade,” once in *Pleonotoma*, and once in *Styzyphyllum* (Fig. 12). The ancestral condition for Bignoniaceae and for the core Bignoniaceae was equivocal in the parsimony reconstruction (Fig. 12A), but presented a high probability of presenting equal fiber arrangements in both phloem types in the likelihood reconstruction (81% and 65%, respectively; Fig. 12B). The highest liability in fiber arrangement was found in *Adenocalymma*, whose ancestral node had a 53% probability of having an equal fiber arrangement in both the regular and variant phloem types (Fig. 12B).

**Rays**—Ancestral state reconstructions of ray type indicated that unequal ray width and height are exclusive to the core Bignoniaceae clade, while rays with equal width and height are exclusive to *Perianthomega*.

## DISCUSSION

This study describes in detail the anatomical differences between the regular and variant phloem types that coexist in stems of Bignoniaceae. It further describes how these differences evolved over time, suggesting a subfunctionalization of the phloem and indicating that the regular phloem may have specialized in storage, while the variant phloem may have specialized in the conduction of photosynthates.



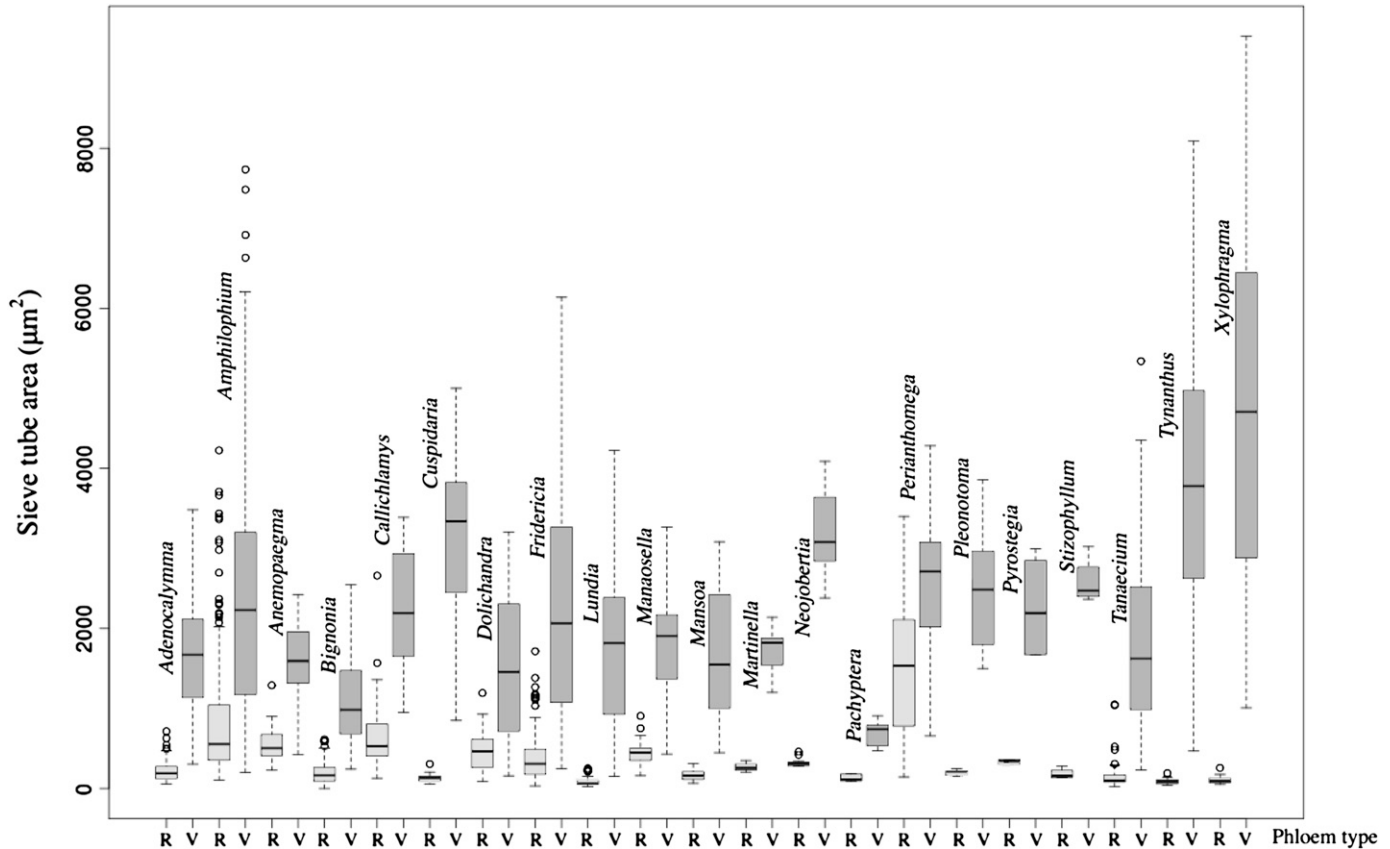


Fig. 7. Comparison between sieve tube areas from the variant (V) and regular (R) phloem types in all genera analyzed.

**Position of the regular and variant portions of the phloem**—It has been hypothesized that the primary vascular pattern and position of decussate leaves are critical for the formation and regulation of the cambial variant in Bignoniaceae (Dobbins, 1970, 1981, 2005). In this study, phloem wedges were always found between leaves, corroborating previous observations (Schenk, 1893; Solereder, 1908) and the hypothesis that phloem wedges and their associated variant phloem are indeed related to leaf position. Phloem wedges were also found to present a spiral pattern following the decussate phyllotaxis of Bignoniaceae.

Leaves represent centers of production of auxins, a hormone of pivotal role in vascular differentiation and pattern formation (Ye, 2002). As a consequence, it is likely that leaves may be directly correlated to the control of the regular and variant phloem (Dobbins, 2005). However, the suggestion that the variant cambia may be unidirectional, i.e., that in some cases, the formation of xylem ceases completely (Boureau, 1957; Philipson and Ward, 1965; Dobbins, 1971; Philipson et al., 1971; Philipson, 1990; Gabrielli, 1993), was never observed in the present study nor in more specific studies of xylem development within the group (Lima et al., 2010). Even though the variant cambia clearly reduces the differentiation of xylem, it appears that xylem production never ceases.

**Anatomical differences between the regular and variant phloem**—All phloem cell types observed presented some degree of divergence between the regular and variant portions of the phloem (see Fig. 13). In particular, sieve tubes presented a

much smaller area in the regular portions of the phloem than in the variant portions, probably due to the number of divisions of the fusiform phloem derivatives of this zone, as described in *Dolichandra unguis-cati* (Dobbins, 1971). Unlike the fusiform phloem derivatives of the variant portions of the phloem, the regular portions are subject to many more divisions, leading to narrower sieve tubes. Moreover, wider sieve tubes in the variant phloem were shown to be widespread in Bignoniaceae, which probably also holds true for other lianas in which the regular and variant portions coincide (see pictures in Bailey and Howard, 1941 and Lens et al., 2008).

Sieve tube shape, arrangement, and frequency also differed between the regular and variant portions of the phloem in all studied taxa. Sieve tubes from the regular phloem were smaller and rectangular, while sieve tubes from the variant portions were larger and round. The smallest sieve tubes from the regular portions of the phloem greatly resembled sieve tubes from the terminal phloem of plants with seasonal formation of secondary tissues with regards to their narrow radial diameter (Schneider, 1945; Derr and Evert, 1967; Deshpande and Rajendrababu, 1985; Rajput and Rao, 1998; Angyalossy et al., 2005) and arrangement (Angyalossy et al., 2005). Sieve tubes were also shown to be more frequent in the regular phloem than in the variant phloem; however, since sieve tubes from the regular phloem were much narrower than sieve tubes from the variant phloem, the difference in frequency likely reflects the smaller area occupied by the individual sieve tubes from the regular phloem, in transverse section. In fact, sieve tubes of the variant

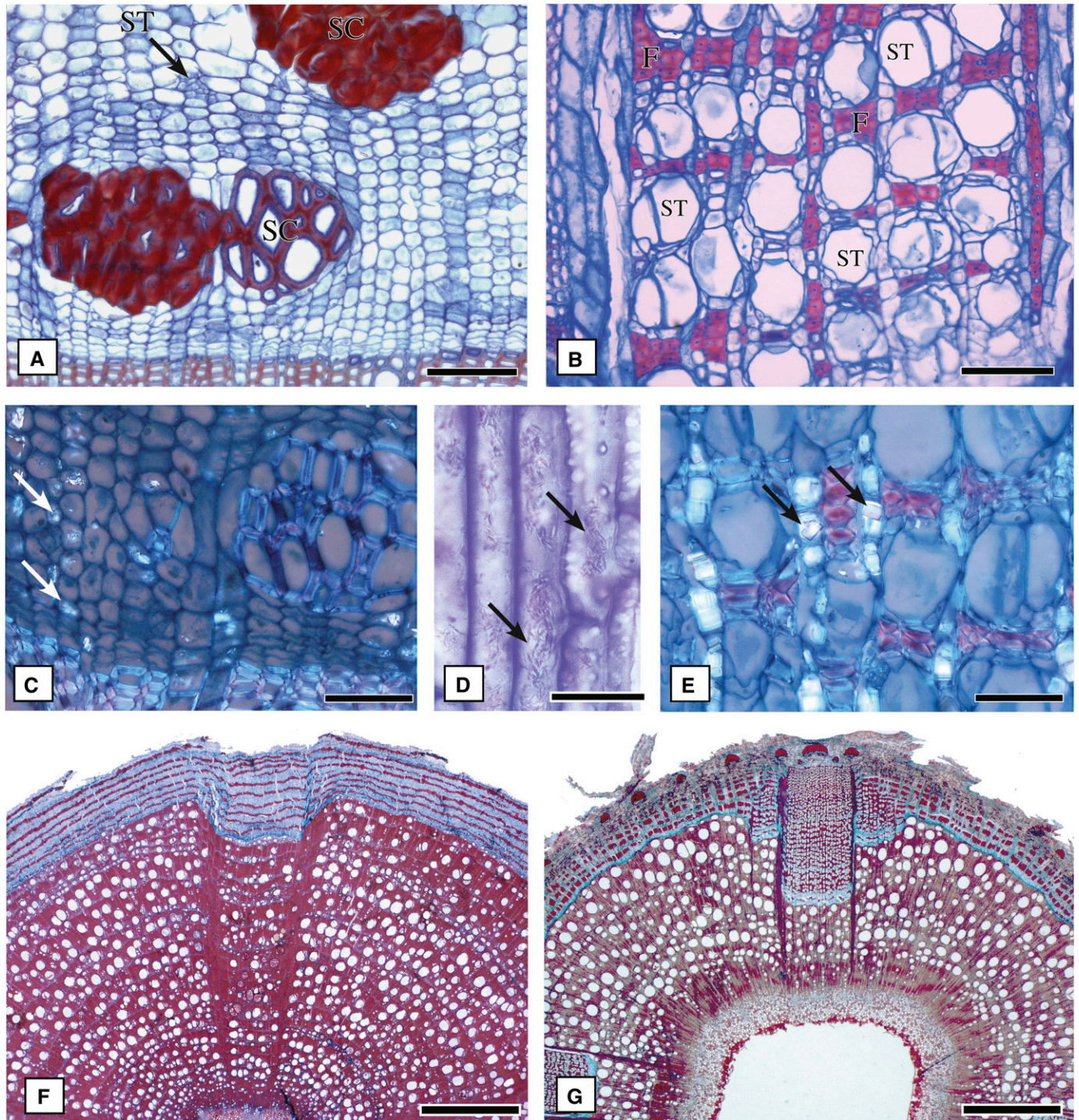


Fig. 8. (A–E) Regular and variant conducting phloem types in *Pleonotoma tetraquetra*. (A) Regular phloem with abundant phloem parenchyma and sclereids, (B) Variant phloem with less phloem parenchyma and fibers. (C, D) Regular phloem with acicular crystals (arrows) seen in detail in tangential section in (D). (E) Variant phloem with prismatic crystals (arrows). (F, G) Pattern of fiber distribution in the regular and variant phloem types. (F) Same pattern of distribution in the regular and variant phloem (*Adenocalymma bracteatum*). (G) Distinct pattern of distribution in the regular and variant phloem (*Stizophyllum riparium*). ST = sieve tube, SC = sclereids, F = fibers. Scale bars: A, B = 100  $\mu$ m; C, E = 50  $\mu$ m; D = 25  $\mu$ m; E, F = 2 mm.

phloem occupy 34% of the total area of the phloem, while only 7% of the total area is occupied by sieve tubes in the regular phloem. In addition, sieve tubes are generally scattered in a matrix of phloem parenchyma in the regular phloem, similarly to the terminal phloem of plants subject to seasonal growth of the

vascular tissues. Overall, these features suggest that the sieve tubes from the regular portion conduct less photosynthates.

Parenchyma was much more abundant in the regular than in the variant portions of the phloem in all species. Furthermore, the amount of parenchyma was shown to increase in the regular

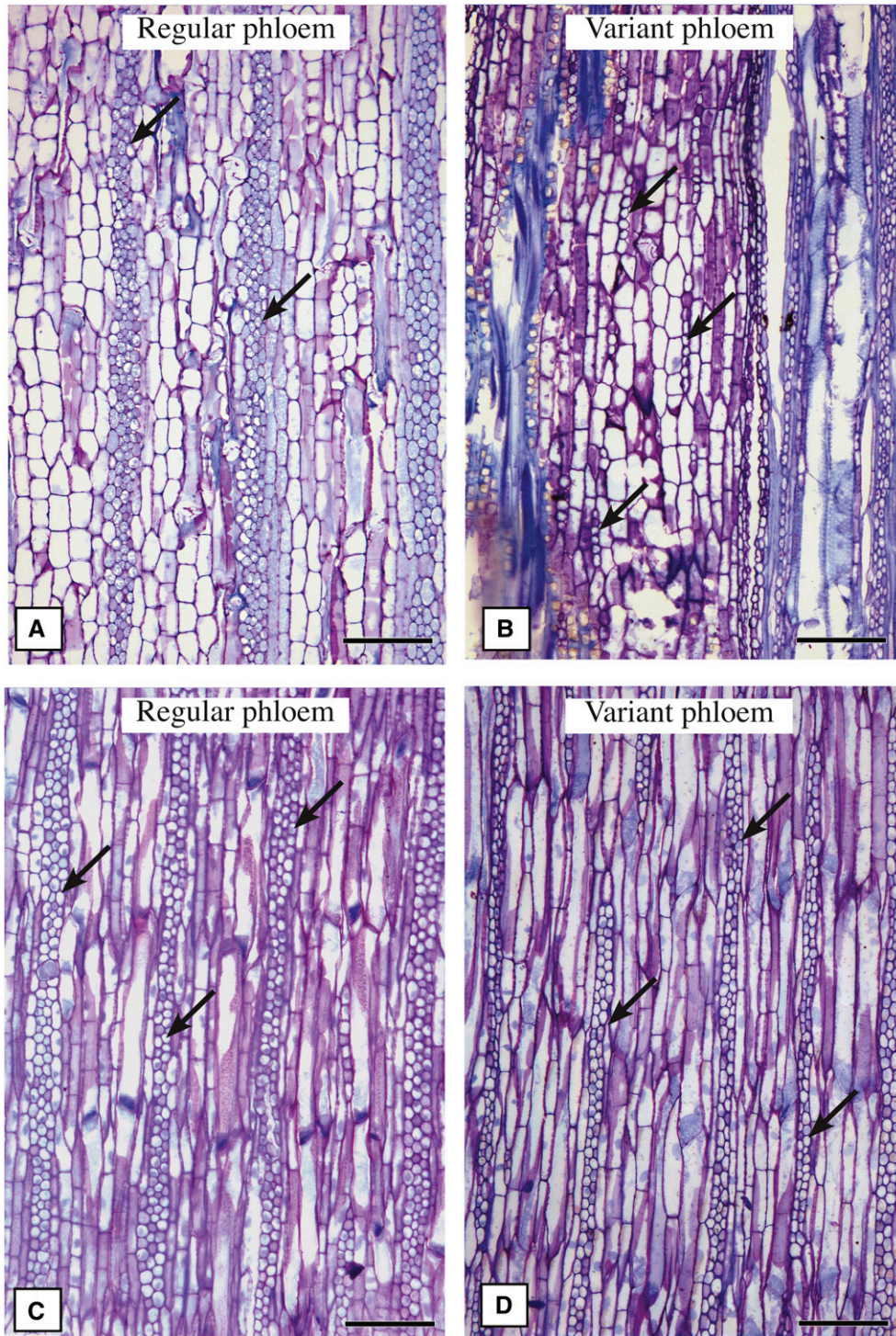


Fig. 9. Ray width and height. (A, B) Differential ray width and height between phloem types; high and multiseriate rays in the regular phloem vs. short and uniseriate rays in the variant phloem, as indicated by arrows (*Amphilophium crucigerum*). (C, D) Identical ray width and height in the regular and variant phloem types indicated by arrows (*Perianthomega vellozoi*). Scale bar: 200  $\mu$ m.

phloem toward more derived lineages. Such an increase in the amount of parenchyma associated with the reduction in size of sieve elements in the regular phloem toward more derived lineages suggests a gradual specialization for storage.

Two different patterns of fiber distribution were documented in Bignoniaceae. While fibers had the same pattern of distribution in the

regular and variant portions of the phloem in some species, others had a differential distribution pattern in the regular and variant portions of the phloem (i.e., *Adenocalymma*, the "Arrabidaea and allies clade," *Pleonotoma*, and *Stizophyllum*). It is possible that the evolution of the differential pattern of fiber distribution in the regular and variant portions of the phloem might be associated with

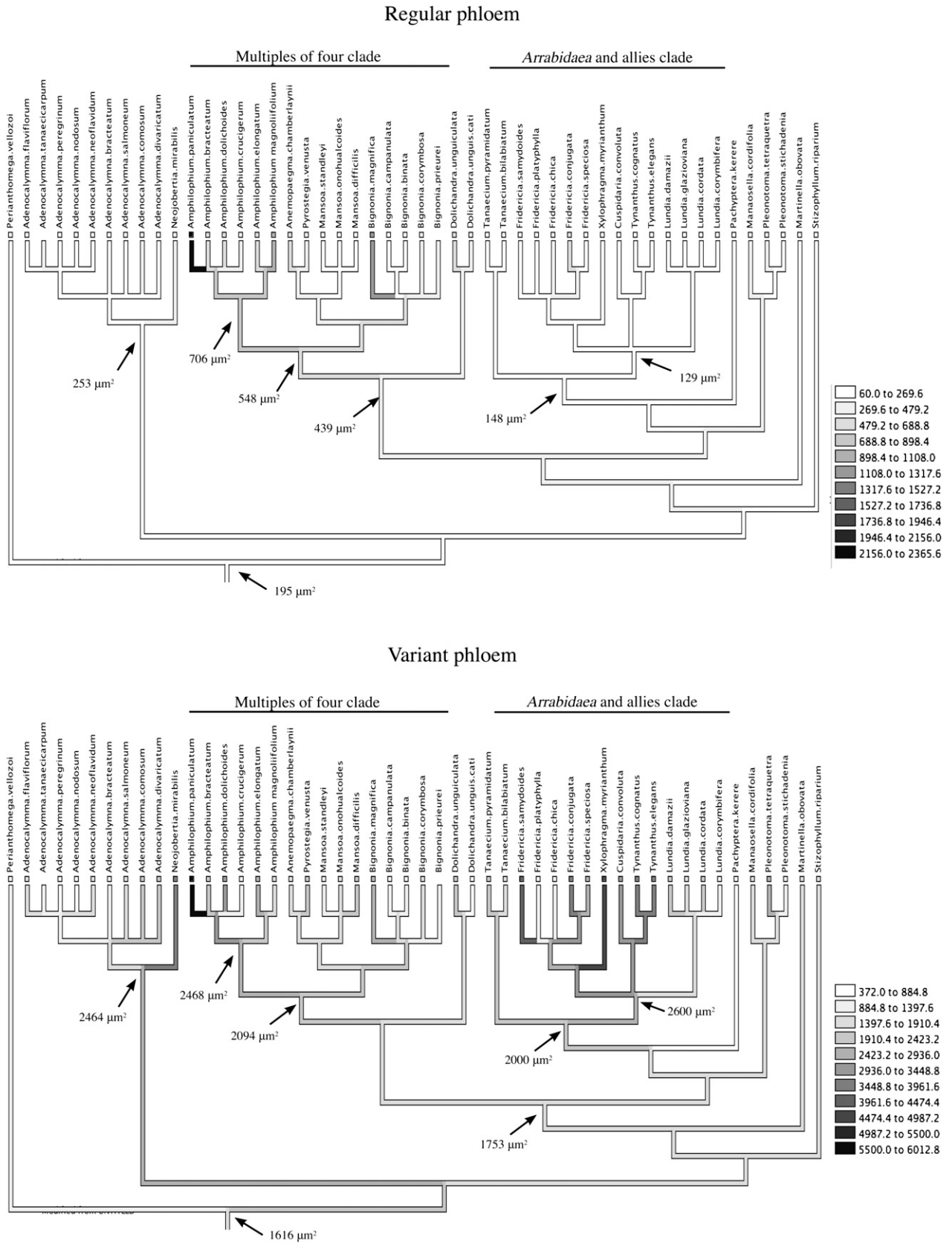


Fig. 10. Parsimony ancestral state reconstruction of sieve tube areas. (A) Regular phloem, (B) variant phloem.

## Regular phloem

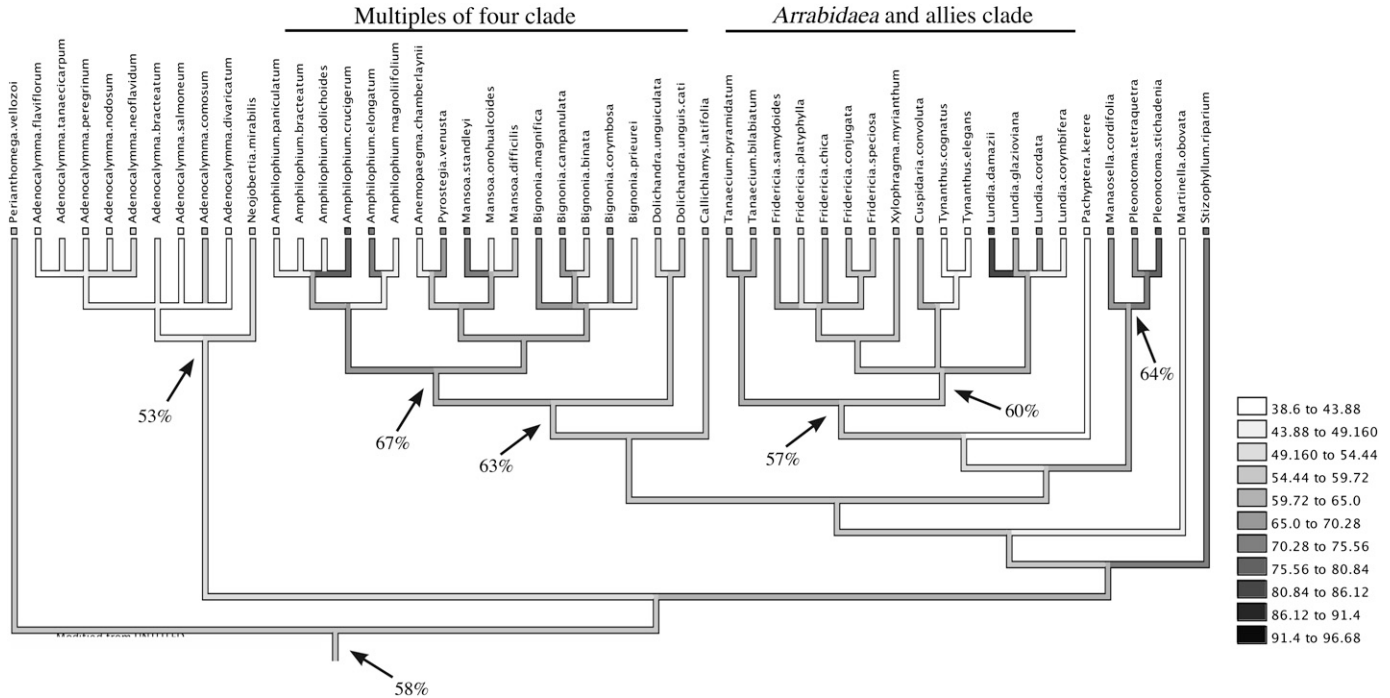


Fig. 11. Parsimony ancestral state reconstruction of the percentage of phloem parenchyma per transactional area in the regular phloem.

higher mechanical support and protection against injury by girdling and herbivores; however, this hypothesis needs testing.

Phloem rays had the same width and height throughout the regular and variant portions of the phloem in *Perianthomega*, but differed in width and height between the regular and variant phloem types of its sister group (i.e., the core Bignoniaceae), hence representing a synapomorphy of the core Bignoniaceae clade. In *Perianthomega* and in the regular phloem of the core Bignoniaceae clade, rays were large and multiseriate as expected for lianas (Carlquist, 1985; Gasson and Dobbins, 1991; Dias-Leme, 2000). Conversely, they were short and uniseriate in the variant portions of all species of the core Bignoniaceae clade, exactly the opposite pattern expected. The presence of uniseriate rays inside the variant portions of the phloem is intriguing, especially since all other rays of stems are multiseriate. Given that studies with the cambium and secondary xylem have shown that rays are usually narrower at the beginning of the secondary growth (Barghoorn, 1940; Chattaway, 1951), it is possible that the maintenance of this characteristic inside the variant phloem represents retention of a juvenile feature in the adult and is a case of true paedomorphosis in the secondary phloem. This paedomorphic feature may derive from the fact that the variant cambium does not divide radial anticlinally from the very beginning of the secondary growth (Pace et al., 2009) when the phloem wedges start their development and the cambium becomes included. Because phloem wedges are formed very early in the stem ontogeny and the variant cambia stop dividing anticlinally at the same time, this juvenile feature is maintained as a developmental drift. Alternatively, the short rays of the variant portions of the phloem might have evolved more recently, similar to the pattern of ray specialization that has been documented for the “dicotyledons” as a whole (Kribs, 1935). This suggestion should, however, be

taken with caution, given that the line of specialization previously suggested for rays (Kribs, 1935) seems to be more complex than a simple conversion from multiseriate to uniseriate rays along evolutionary time (Barghoorn, 1941). In this context, it seems more likely that the reduced ray height is another character retained from the initial development of the cambium that was maintained in the variant portions of Bignoniaceae.

Calcium oxalate crystals of three main types (prismatic, acicular, and styloid) were found in the axial and ray parenchyma of all Bignoniaceae. The occurrence of those crystal types varied randomly across clades, indicating that crystals are evolutionarily very labile. The distribution of crystals was often equal between the regular and variant portions of the phloem, except for *Pleonotoma*, in which the regular phloem possessed acicular crystals and the variant phloem presented prismatic crystals exclusively. *Pleonotoma* also showed a differential pattern between the regular and variant portions of the phloem, with sclereids found exclusively in the regular phloem and fibers in the variant phloem. These results are very important because they indicate that the regular and variant phloem portions of the phloem evolve independently of each other (nonmodularly) within the same plant.

**Sieve tube evolutionary patterns in the regular and variant portions of the phloem**—The study of the variation of the individual sieve tube areas along the phylogeny provided interesting insights. First, the sieve tubes of the regular phloem illustrate an evolutionary pattern that goes in two opposite directions: the “Multiples of four clade”, and especially the genus *Amphilophium*, showed an increase in the area of its sieve tube elements, while genera from the “*Arrabidaea* and allies clade” showed a reduction in the area of their sieve tubes. The increase in the area of sieve tubes of the regular phloem of

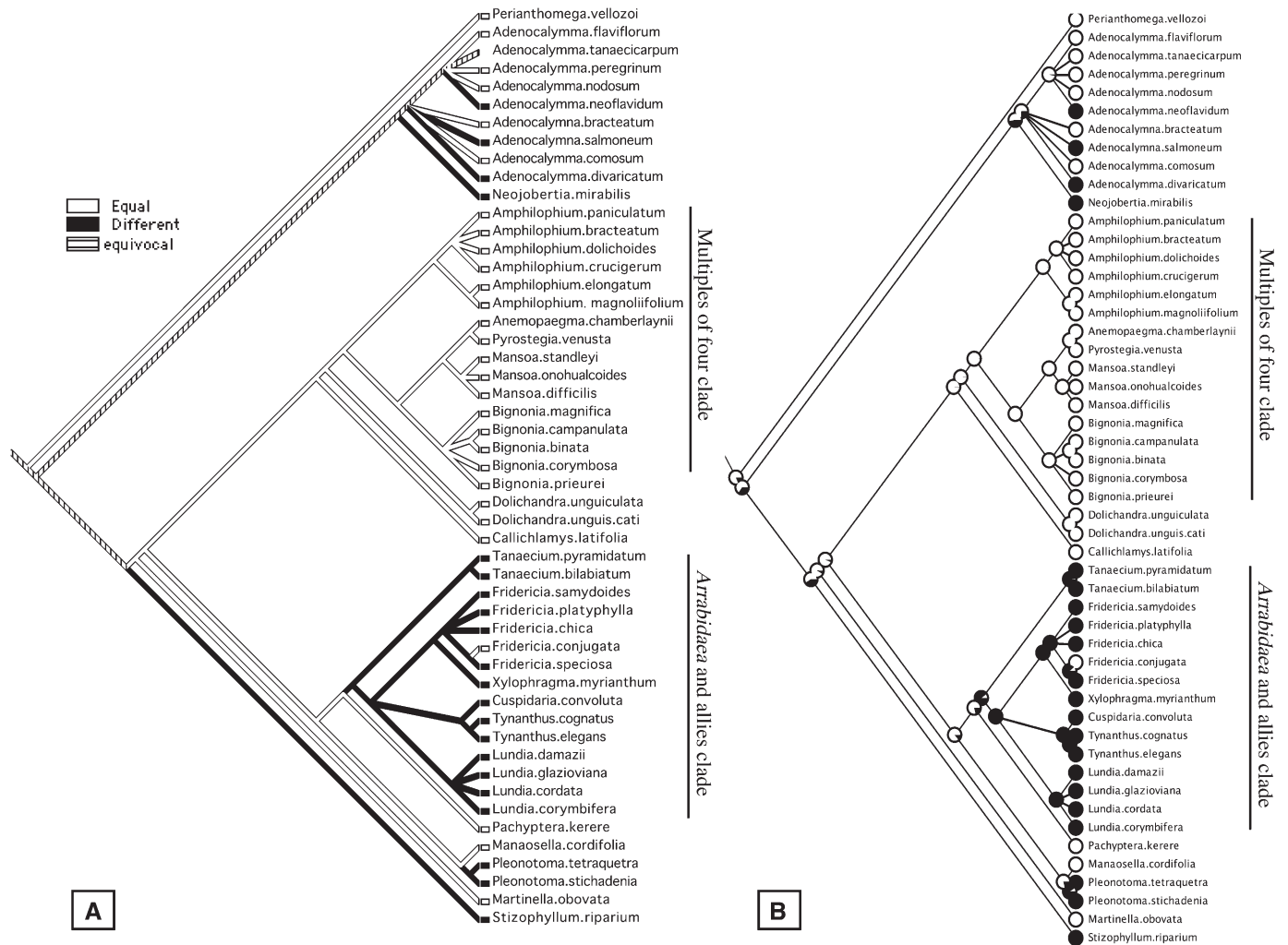


Fig. 12. Ancestral state reconstruction of the fiber distribution in the regular and variant phloem types using parsimony (A) and maximum likelihood assumptions (B).

*Amphilophium* is extremely interesting, given that *Amphilophium* is the only genus of Bignoniaceae whose phloem wedges become included once the stems grow in diameter, precociously losing their functionality (Pace et al., 2009). This finding suggests that wider sieve tubes in the regular phloem of representatives of this genus may have been selected to account for the lack of functionality in the variant portions of the phloem. On the other hand, the reduction of the area of sieve tubes within the “*Arrabidaea* and allies clade” and several other clades may indicate that the variant phloem in this clade is becoming more important for the conduction of photosynthates, a scenario that will be further discussed below.

In the variant phloem, a gradual increase in diameter of the area of sieve tubes was observed toward more derived lineages. This pattern shows that the variant phloem of Bignoniaceae may have specialized in the conduction of photosynthates over time. Therefore, apart from conferring flexibility (Carlquist, 2001; Rowe et al., 2004; Isnard and Silk, 2009), aiding in injury repair (Dobbins and Fisher, 1986), and having an important role in water storage (Carlquist, 2001), these findings further suggest that the variant phloem of Bignoniaceae might have specialized in the conduction of photosynthates over time. A higher

conduction of photosynthates may be one of the reasons why more secondary xylem is produced by the regular cambium portions that flank the phloem wedges (Lima et al., 2010). Such specialization for increased conduction has also been recorded for the secondary xylem of lianas, which often have very wide vessel elements (Carlquist, 1985; Ewers, 1985; Ewers and Fisher, 1989; Ewers et al., 1990; Gartner et al., 1990; Gasson and Dobbins, 1991), which are known to require additional water supply to compensate for the water loss due to the high transpiration derived from their large canopies (Ewers and Fisher, 1991). The evolution of larger sieve tubes may, therefore, represent an additional specialization for the liana habit, a hypothesis that is further corroborated by the narrower sieve tubes present in the variant phloem of Bignoniaceae shrubs. Other lianas such as those in Icacinaceae (Bailey and Howard, 1941; Lens et al., 2008), Fabaceae (Dias-Leme, 2000), Sapindaceae (Tamaio and Angyalossy, 2009), and Loganiaceae (Van Veenendaal and Den Outer, 1993) also have wider sieve tubes in the variant portions of their phloem when compared to the diameter of the regular phloem of the same stem (e.g., Icacinaceae; Bailey and Howard, 1941) or when compared to shrubs and tree species that belong to the same families (e.g., Fabaceae; Dias-Leme, 2000).

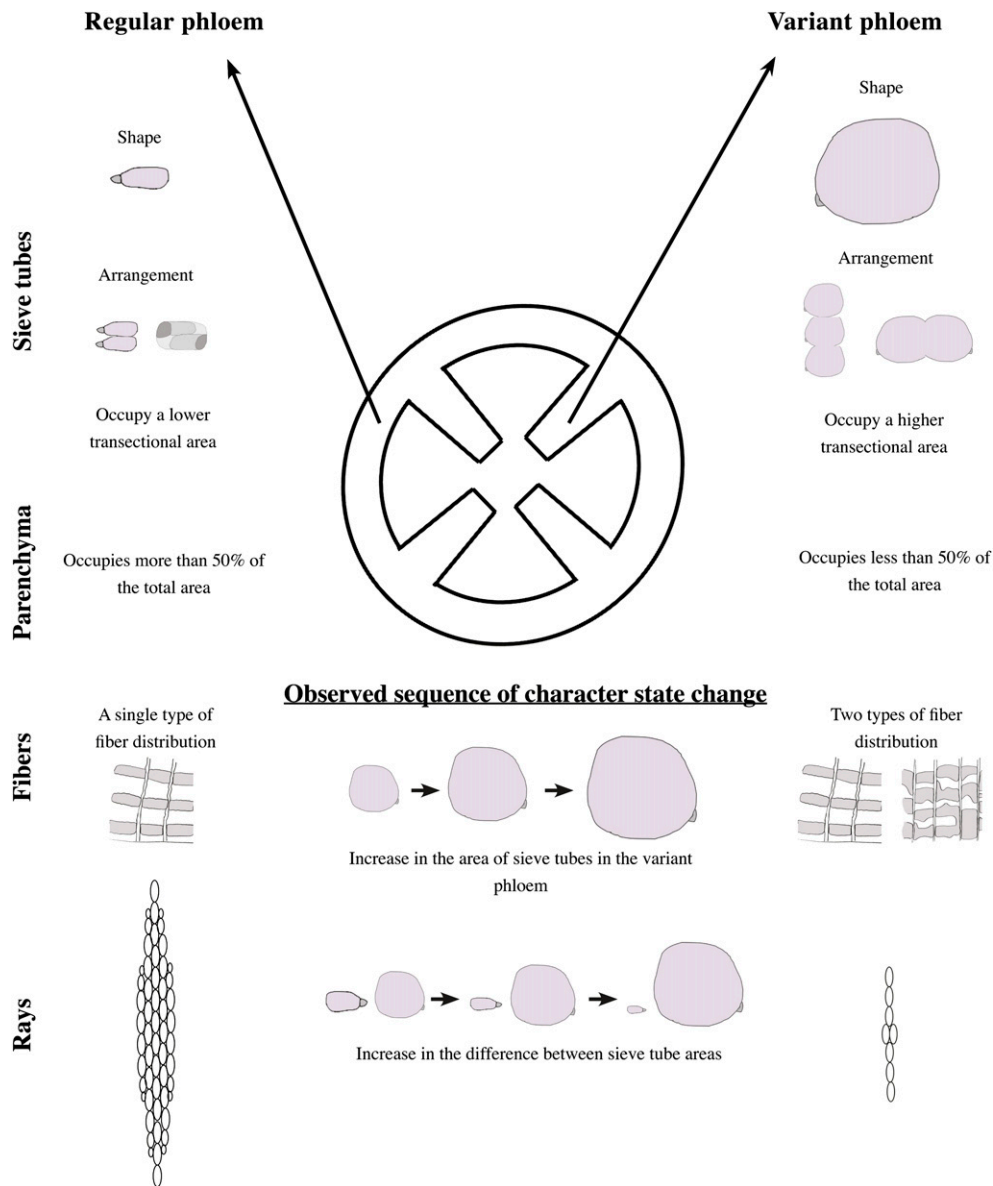


Fig. 13. Comparison between the regular and variant phloem types in Bignoniaceae.

**Conclusions**—Our results indicate that the regular and variant phloem types of Bignoniaceae have accumulated a series of anatomical differences over time. Perhaps the most remarkable differences are the increase in sieve tube area in the variant phloem of all species of the tribe and the increase in the abundance of phloem parenchyma over time recorded for the regular phloem. Wider sieve tubes in the variant phloem likely contributed to an increase in the importance of the variant phloem in conduction of photosynthates, while the increased abundance of parenchyma likely enhanced the storage capacity of the regular phloem. An increased conductance role for the variant phloem provides an additional significance for the evolution and maintenance of the cambial variant in Bignoniaceae. Another remarkable difference between the regular and variant portions of the phloem is the distribution of fibers, which became more closely arranged in the variant portions of the phloem in several lineages, likely enhancing mechanical support and protection in those

lineages. Last, rays were found to be higher and wider in the regular portions of the phloem and shorter and narrower in the variant portions, suggesting different ontogenetic processes in the different phloem types. More specifically, the reduced rays in the variant portions of the phloem suggest paedomorphosis. This study illustrates the value of detailed comparative anatomical studies for a better understanding of the role of individual plant tissues for the diversification of lianas and plants as a whole.

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APPENDIX 1. Taxa, collectors, and localities. Vouchers for all specimens were deposited in the University of São Paulo Herbarium (SPF), unless specified otherwise after the voucher information. Other herbaria listed are: MAD = Madison Forest Products Laboratory (WI, USA); MG = Museu Paraense Emílio Goeldi (Pará, Brazil); MO = the Missouri Botanical Garden (MO, USA).

*Adenocalymma bracteatum* (Cham.) DC., *Castanho 153*, Lohmann 861, Rio Negro, Amazonas, Brazil. *Adenocalymma comosum* (Cham.) DC., *Pace 53*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Adenocalymma divaricatum* Miers, *Udulutsch 2808*, Lençóis, Bahia, Brazil. *Adenocalymma flaviflorum* (Miq.) L.G. Lohmann, *Sousa-Baena 2*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Adenocalymma neoflavidum* L.G. Lohmann, *Zuntini 23*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Adenocalymma nodosum* (Silva Manso) L.G. Lohmann, *Pace 20*, Uberlândia, Minas Gerais, Brazil. *Adenocalymma peregrinum* (Miers) L.G. Lohmann, *Pace 26*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Adenocalymma salmoneum* J.C. Gomes, *Lohmann 658*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Adenocalymma tanaecicarpum* (A.H. Gentry) L.G. Lohmann, *Dos Santos 263*, Porto de Moz, Pará, Brazil, received from the MADw wood collection, vouchers in MAD, MO and MG. *Amphilophium bracteatum* (Cham.) L.G. Lohmann, *Ozório-Filho 8*, São Paulo, São Paulo, Brazil. *Amphilophium crucigerum* (L.) L.G. Lohmann, *Pace 1*, *Pace 2*, *Pace 3*, *Pace 34*, São Paulo, São Paulo, Brazil. *Amphilophium dolichoides* (Cham.) L.G. Lohmann, *Ozório-Filho 9*, São Paulo, São Paulo, Brazil. *Amphilophium elongatum* (Vahl) L.G. Lohmann, *Pace 45*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Amphilophium magnoliifolium* (Kunth) L.G. Lohmann, *Lohmann 851*, Rio Negro, Amazonas, Brazil; *Dos Santos 272*, Porto de Moz, Pará, Brazil, received from the MADw wood collection, vouchers in MAD, MO and MG. *Amphilophium paniculatum* (L.) Kunth, *Pace 46*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Anemopaegma chamberlaynii* (Sims) Bureau & K. Schum., *Zuntini 15*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Anemopaegma chrysoleucum* (Kunth) Sandwith, *Dos Santos 144*, Marabá, Pará, Brazil, analyzed at the MADw wood collection, vouchers in MAD, MO and MG. *Anemopaegma longidens* Mart. ex DC., *Dos Santos 394*, Parauapebas, Pará, Brazil, analyzed at the MADw wood collection, vouchers in MAD, MO and MG. *Bignonia binata* Thunb., *Galvanese 22*, Rio Negro, Amazonas, Brazil. *Bignonia campanulata* Cham., *Pace 39*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Bignonia corymbosa* (Vent.) L.G. Lohmann, *Zuntini 2*, *Zuntini 17*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Bignonia magnifica* W. Bull., *Pace 51*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Bignonia priurei* DC., *Zuntini 13*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Bignonia scuripabula* (K. Schum.) L.G. Lohmann, *Zuntini 8*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Callichlamys latifolia* (Rich.) K. Schum., *Zuntini 175*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil; *Pace 42* Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Cuspidaria convoluta* (Vell.) A.H. Gentry, *Pace 48* Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Cuspidaria pulchra* (Cham.) L.G. Lohmann, *Pace 24*, *Pace 25*, Uberlândia, Minas Gerais, Brazil. *Dolichandra unguiculata* (Vell.) L.G. Lohmann, *Zuntini 176*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Dolichandra unguis-cati* (L.) L.G. Lohmann, *Ceccantini 2687*, Matozinhos, Minas Gerais, Brazil; *Groppa 322*, São Paulo, São Paulo,

Brazil. *Fridericia chica* (Bonpl.) L.G. Lohmann, *Pace 50*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Fridericia conjugata* (Vell.) L.G. Lohmann, *Pace 44*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Fridericia platyphylla* (Cham.) L.G. Lohmann, *Pace 22*, *Pace 23*, Uberlândia, Minas Gerais, Brazil. *Fridericia samyoides* (Cham.) L.G. Lohmann, *Pace 49*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Fridericia speciosa* Mart., *Pace 40*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Lundia cordata* (Vell.) DC., *Zuntini 1*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Lundia damazii* C. DC., *Pace 55*, *Pace 56*, São Paulo, São Paulo, Brazil. *Lundia glazioviana* Kraenzl., *Zuntini 126*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Lundia nitidula* DC., *Nogueira 139*, Oliveiras, Minas Gerais, Brazil. *Manaosella cordifolia* (DC.) A.H. Gentry, *Pace 41*, Brazil, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Mansoa difficilis* (Cham.) Bureau & K. Schum., *Pace 35*, São Paulo, São Paulo, Brazil; *Zuntini 4*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Mansoa onohualcoides* A.H. Gentry, *Zuntini 276*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Mansoa standleyi* (Steyer.) A.H. Gentry, *Pace 43*, Living collection Plantarum Institute, Nova Odessa, São Paulo, Brazil. *Martinella obovata* (Kunth) Bureau & K. Schum., *Zuntini 7*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Neojobertia mirabilis* (Sandwith) L.G. Lohmann, *Dos Santos 48*, Buriticupu Forest Reserve, Maranhão, Brazil, received from the MADw wood collection, vouchers in the MAD, MO, and MG. *Neojobertia sp. nov.*, *Zuntini 18*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Pachyptera kerere* (Aubl.) Sandwith, *Castanho 143*, *Lohmann 834*, Rio Negro, Amazonas, Brazil. *Perianthomega vellozoi* Bureau, *Pace 10*, *Pace 15*, Viçosa, Minas Gerais, Brazil; *Pace 28*, *Pace 29*, Santa Cruz de la Sierra, Santa Cruz, Bolivia. *Pleotoma dendrotricha* Sandwith, *Dos Santos 173*, Parauapebas, Pará, Brazil, analyzed at the MADw wood collection, vouchers in MAD, MO and MG. *Pleotoma melioides* (S. Moore) A.H. Gentry, *Dos Santos 298*, Senador José Pontifício, Pará, Brazil, analyzed at the MADw wood collection, vouchers in MAD, MO, and MG. *Pleotoma orientalis* Sandwith, *Dos Santos 160*, Parauapebas, Pará, Brazil, analyzed from the MADw wood collection, vouchers in MAD, MO and MG. *Pleotoma tetraquetra* (Cham.) Bureau, *Ozório-Filho 11*, São Paulo, São Paulo, Brazil. *Pleotoma stichadenia* K. Schum., *Zuntini 7*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil; *Dos Santos 187*, Parauapebas, Pará, Brazil, analyzed at the MADw wood collection, vouchers in MAD, MO and MG. *Pyrostegia venusta* (Ker Gawl.) Miers, *Pace 17*, Campinas, São Paulo, Brazil; *Pace 36*, São Paulo, São Paulo, Brazil. *Stizophyllum riparium* (Kunth) Sandwith, *Pace 16*, *Pace 33*, São Paulo, São Paulo, Brazil; *Zuntini 9*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Tanaecium bilabiatum* (Sprague) L.G. Lohmann, *Lohmann 850*, Rio Negro Amazonas, Brazil. *Tanaecium pyramidatum* (Rich.) L.G. Lohmann, *Pace 14*, *Pace 35*, São Paulo, São Paulo, Brazil. *Tynanthus cognatus* (Cham.) Miers: *Pace 9a*, *Pace 9b*, São Paulo, São Paulo, Brazil. *Tynanthus elegans* Miers, *Zuntini 147*, Vale do Rio Doce Forest Reserve, Espírito Santo, Brazil. *Xylophragma myrianthum* (Cham. ex Steud.) Sprague.