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RESEARCH ARTICLE

# Diversification of the stem vascular system in a clade of recent radiation and multiple habit transitions: The Bunchosia clade (Malpighiaceae)

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## Abstract

**Premise:** Within the Malpighiaceae, the Bunchosia clade is distinctive for its significant habit variation and abundance of different vascular variants. However, the processes underlying the diversification of the vascular system over time and the ontogenetic events involved remain unclear. Focusing on the Bunchosia clade, this study explores how new vascular configurations evolve in Malpighiaceae and the factors driving this diversification.

**Methods:** We analyzed stem ontogeny in 19 species representing all six genera of the Bunchosia clade, sampling from the apex to the base of the plants. We used the phytools package in R to map the entire stem ontogenies onto the most recent phylogeny estimate of Malpighiaceae, identifying the developmental modifications and processes involved in stem diversification within the clade.

**Results:** The ancestral condition of the clade was inferred to be a lianescent habit with regular stem anatomy. Over evolutionary time, two independent transitions to a self-supporting habit were inferred to have occurred. We identified five ontogenetic pathways, which led to distinct vascular system arrangements. Additionally, we propose two new records of cambial variants for the family.

**Conclusions:** From a regular secondary growth condition, different vascular variants evolved in a short period of evolutionary time in this clade. The self-supporting habit appeared twice: (1) in *Bunchosia*, maintaining a plesiomorphic regular secondary growth, and (2) another in *Echinopterys*, where the self-supporting habit retained the vascular variant inherited from the ancestor of the subclade. Our study provides insights into how stem vasculature diversified in lianescent clades and how it is related to habit transitions.

KEYWORDS

ancestral character state reconstruction, development, Lianas, Malpighiaceae, self-supporting habit, vascular cambium, vascular variants

The diversity of the vascular system in woody stems and roots is directly linked to the diversification of vascular cambial activity throughout plant evolutionary history (Tomescu and Groover, 2019). The presence of a single (bifacial) vascular cambium producing secondary xylem (wood) inward and secondary phloem (inner bark) outward is the ancestral condition in lignophytes and still the most common form. However, this cambial activity has changed numerous times across various plant lineages (Spicer and Groover, 2010; Angyalossy et al., 2012; Ragni and Greb, 2018). These modifications have traditionally been referred to as cambial variants (Carlquist, 2001), and more recently as vascular variants, a broader term that encompasses not only modifications of the vascular cambium, but also changes involving the procambium and the formation of ectopic cambia (Cunha Neto, 2023). The occurrence of vascular variants is known to

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be influenced by the diversity of plant habits (Quintanar-Castillo and Pace, 2022) and by the presence of plants in different habitats. Both factors have likely favored the adoption of different strategies for success, as seen in mangroves. Mangroves, adapted to waterlogged and saline environments, have developed successive cambia in their stems, a key adaptation that enhances water transport and supports their survival in fluctuating tidal conditions (Angyalossy et al., 2012, 2015). An efficient hydraulic system (Baas and Carlquist, 1985) and mechanical properties that promote a balance between stiffness and flexibility (Rowe and Speck, 2005) are other examples of such strategies, particularly evident in lianas (Carlquist, 1985; Ewers and Fisher, 1991).

Obtaining a better understanding of vascular system diversity requires a more thorough examination of its ontogeny. The study of the development of anatomical features allows us to unravel the nature of morphological changes (Carlquist, 2001; Cunha Neto, 2023) because differences among individuals within a group are linked to their development (Onyenedum and Pace, 2021; Cunha Neto, 2023). Comparing the ontogeny within a phylogenetic framework allows us to understand the morphological diversity and infer which developmental mechanisms generated such variation (Olson, 2007; Pace et al., 2009; Onyenedum and Pace, 2021). Studies that characterize the relevant evolutionary processes and explain morphological diversity considering ontogeny, using a phylogenetic approach, are becoming increasingly more common (e.g., Layahe et al., 2005; Hearn, 2009; Pace et al., 2009; 2011; Chery et al., 2020; Luna-Márquez et al., 2021; Cunha Neto et al., 2022, Quintanar-Castillo and Pace, 2022) and allow us to better to understand the plant evolutionary history.

Understanding the relationship between habit evolution and vascular system development requires studying a group with high morphological variation. Recent studies have confirmed previous suggestions (Anderson, 1990) that the self-supporting habit is the ancestral condition in Malpighiaceae (Quintanar-Castillo and Pace, 2022; Bueno et al., 2025, Sanches et al., 2025; Figure 1). In contrast, while the lianescent habit was originally thought to have evolved numerous times (Anderson, 1990), evidence now suggests it appeared only twice in the evolutionary history of Malpighiaceae (Quintanar-Castillo and Pace, 2022; Figure 1). Subsequent transitions back to a self-supporting habit occurred in several clades, many nested within lianescent lineages. Some of these transitions were envisioned previously (as in *Banisteriopsis* and *Heteropterys*; Anderson, 1977), and some were unexpected (e.g., *Bunchosia*, *Echinopterys*, and *Malpighia*; Quintanar-Castillo and Pace, 2022; Figure 1).

Within the Bunchosia clade, the transition back to a self-supporting habit occurred in two genera, one of them being the genus *Bunchosia*, where all members are trees or shrubs. This clade is one of the 18 major lineages recognized in the most recent phylogenies for the family Malpighiaceae (Davis and Anderson, 2010; Davis et al., 2014; Almeida and van den Berg, 2021). It comprises six genera: *Bunchosia* Kunth, *Echinopterys* A. Juss, *Heladena* A. Juss, *Henleophytum* H. Karst, *Thryallis* Mart., and *Tristellateia* Thouars (Anderson et al., 2006). These genera are mainly distributed in continental America and the Caribbean, except for *Tristellateia*, which occurs in Africa and Asia (Davis and Anderson, 2010). Notably, *Tristellateia* represents one of the nine lineages within the Malpighiaceae that have independently dispersed to Africa and Asia (Davis and Anderson, 2010) (Figure 2A).

*Bunchosia* gives its name to the clade and is also the largest genus in it (Figure 2B), encompassing 75 species. It is one of the most well-known genera of trees and shrubs in Malpighiaceae and is characterized by fleshy, sometimes tomentose fruits (Anderson et al., 2006; Figure 2C). This genus is distributed from Mexico to northern Argentina and the Caribbean (Anderson, 2013). *Echinopterys* is a Mexican endemic genus, with two species of erect to leaning or climbing shrubs (Anderson, 2013), found in xeric shrublands to deciduous forests (León-Velasco, 2014). It is distinguished by eglandular flowers and schizocarpic fruits (Anderson et al., 2006; Figure 2D, E). Among the lianescent genera, *Heladena* is a monospecific genus distributed in Argentina, Brazil, and Paraguay, and occurs in deciduous and subdeciduous forests (Anderson et al., 2006; Barros et al., 2022). It is characterized by flowers with ciliated petals and dry, dehiscent fruits (Anderson et al., 2006; Figure 2F–H). *Henleophytum* is another monospecific genus endemic to Cuba (Anderson, 2013), where it occurs in coastal xeric shrublands and secondary vegetation (González Gutierrez and Meyer, 2019). Its setiferous fruits resemble those of *Echinopterys* (Anderson, 2013; Figure 2J). *Thryallis* is a genus with five species that is distributed in Brazil and extends to Paraguay and Bolivia (Davis and Anderson, 2010). It is typically found in open habitats such as shrublands and caatinga (Anderson et al., 2006). Species of *Thryallis* have also been described as climbing shrubs (Anderson et al., 2006) and are characterized by stellate trichomes and persistent sepals on the fruit (Barros et al., 2022; Figure 2L, M). Finally, *Tristellateia* is the only genus in the clade inhabiting Africa and Asia, with 21 recognized species, 19 of which are endemic to Madagascar (Arènes, 1947), with one widely distributed in Asia. *Tristellateia* is readily distinguished by its laterally winged samaras (Anderson et al., 2006; Figure 2N, O).

The different habits within the Bunchosia clade allow us to address a series of questions regarding the links between the evolution of different habits and how these habits have influenced the diversification of the vascular system in Malpighiaceae, with potential implications for other plant lineages. Lianescent members of Malpighiaceae frequently exhibit conspicuous vascular variants (Carlquist, 1991; Angyalossy et al., 2015; Cabanillas et al., 2017; Pace et al., 2018; Quintanar-Castillo and Pace, 2022; Quintanar-Castillo et al., 2024). However, to date, no vascular variants have been identified in the self-supporting genera within the family.

Several self-supporting habits appear to be nested within lianoid clades (as in the *Bunchosia* clade), suggesting that they evolved from climbing ancestors and may retain some characteristics due to common ancestry. Furthermore, the presence of vascular variants has been recognized as a potential driver of diversity (Chery et al., 2020; Cunha Neto

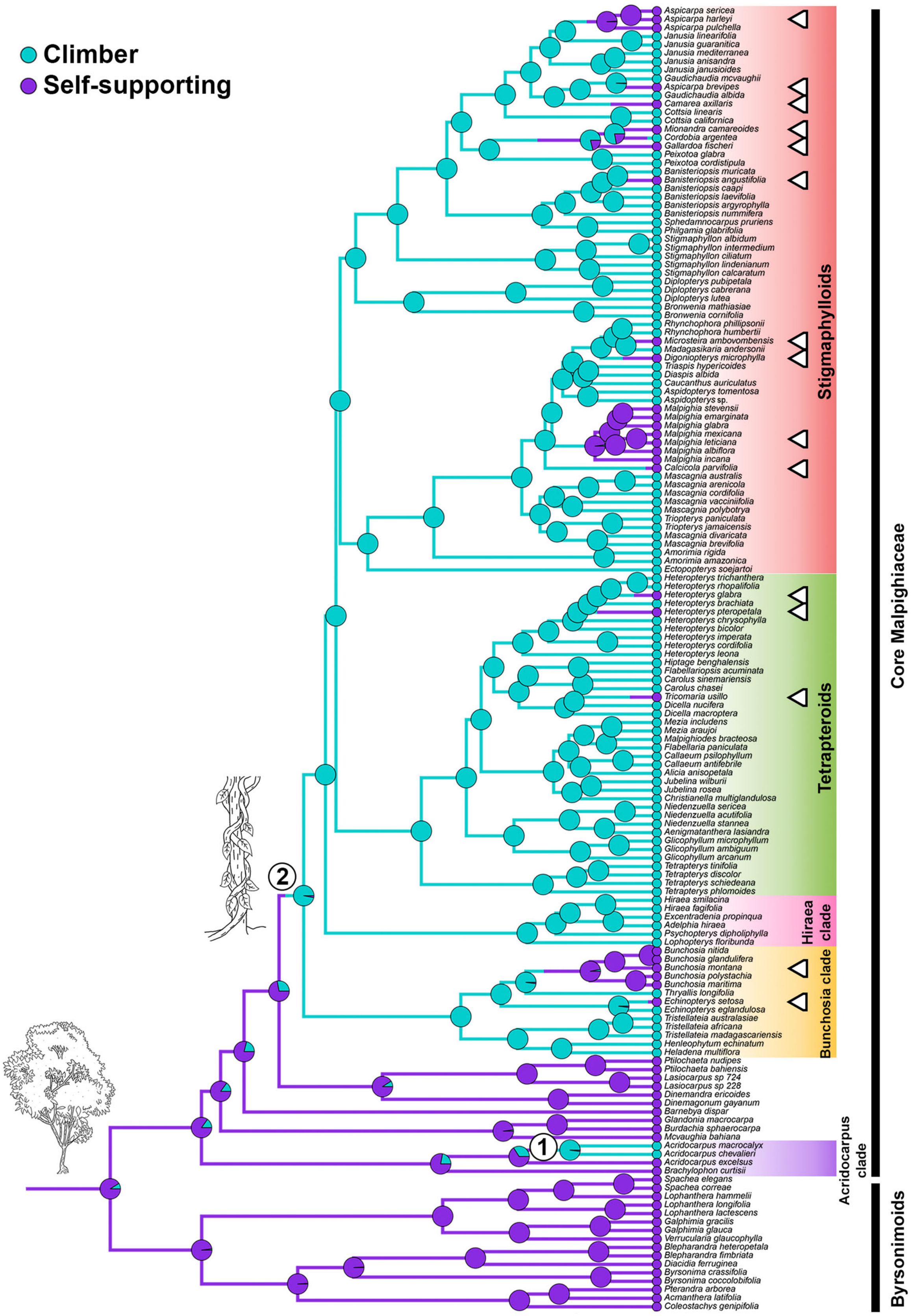

**FIGURE 1** Evolution of the lianescent habit in Malpighiaceae. Purple: self-supporting plants (herbs, trees, shrubs, and subshrubs); turquoise: climbing plants. A self-supporting habit is the ancestral condition with the highest support. Climbing plants evolved twice in Malpighiaceae, once in the Acridocarpus clade (1) and another in the group composed of the Bunchosia clade + Hiraea clade + Tetrapteroids and Stigmaphylloids (2). Multiple reversions to a self-supporting habit are also observed (white arrowheads) (tree from Quintanar-Castillo and Pace, 2022).

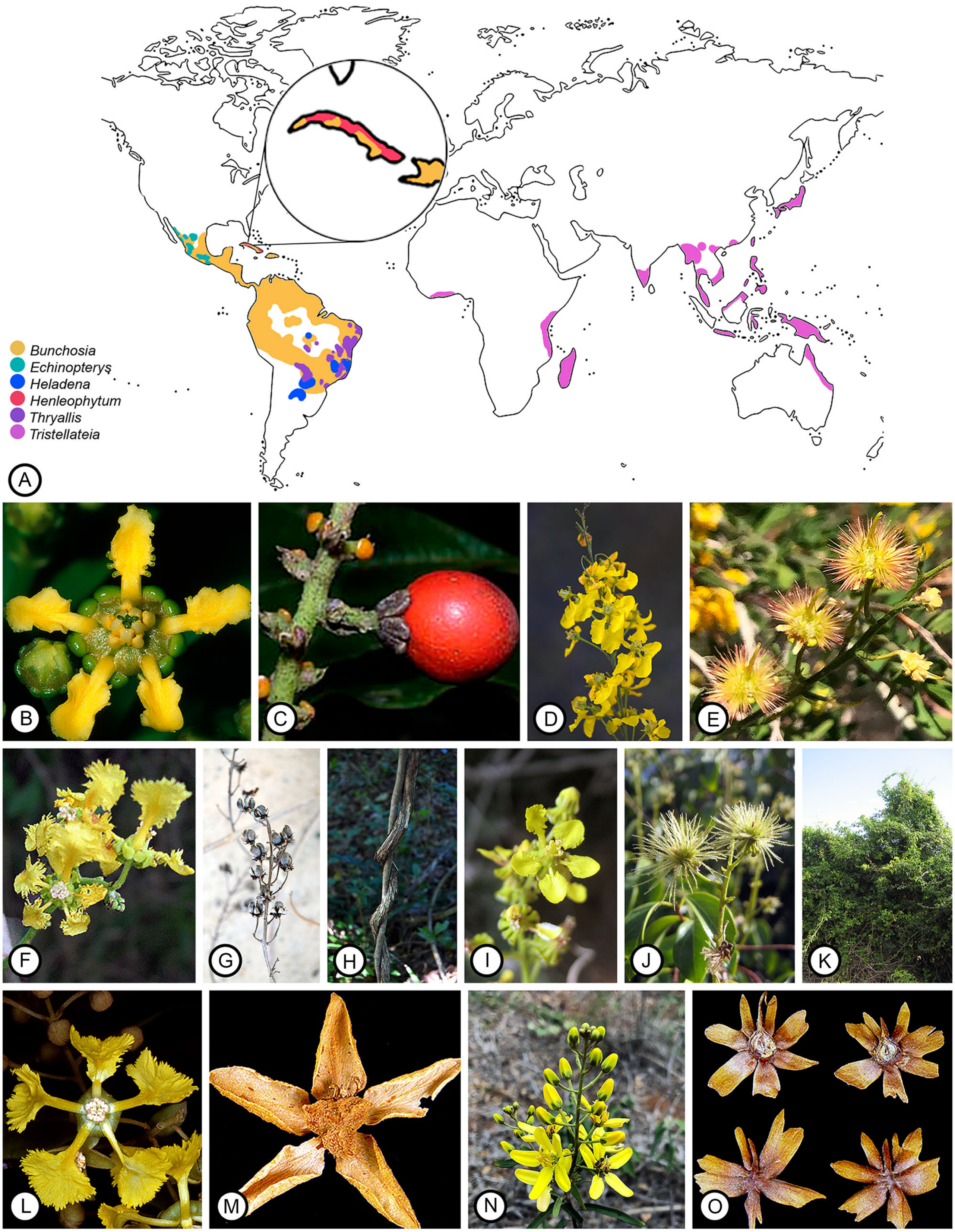

**FIGURE 2** (See caption on next page).

et al., 2022; Quintanar-Castillo and Pace, 2022; Cunha Neto, 2023). However, developmental studies in Malpighiaceae remain limited to a small subset of genera (e.g., Schenck, 1893; Chodat and Vischer, 1917; Obaton, 1960; Caballé, 1993; Cabanillas et al., 2017; Pace et al., 2018; Quintanar-Castillo and Pace, 2022; Quintanar-Castillo et al., 2024). This gap highlights the importance of exploring additional genera to determine whether vascular variants are strictly associated with lianescent habits or also occur in self-supporting taxa, particularly those that have evolved from lianescent ancestors.

Using the Bunchosia clade as a model system, we aimed to compare the modifications of the vascular system during ontogenetic and evolutionary transitions from a phylogenetic perspective. Our objectives were to (1) examine the evolution of habits within the clade and their impact on the vascular system, (2) explore the events in ontogenetic development that may have led to the diversification of the vascular system, and (3) reconstruct the ancestral states and diversity of the vascular system within the clade.

# MATERIALS AND METHODS

## Taxon sampling and anatomical procedures

Building on previous studies that included nearly all genera of the family (Quintanar-Castillo and Pace, 2022), we narrowed down our survey to 24 species of Malpighiaceae. Among these, 19 species belong to the six genera of the *Bunchosia* clade: *Bunchosia*, *Echinopterys*, *Heladena*, *Henleophytum*, *Thryallis*, and *Tristellateia* (Table 1). For these species, we conducted a detailed study of their ontogeny. We deposited vouchers of all collections in several herbaria, a complete list with the data of the collected specimens is in Appendix S1.

We collected stem samples from their thickest portion and sectioned them into pieces approximately 3–4 cm long. We identified different developmental stages progressing toward the apex of the branch using a 10× hand lens. For samples from trees, we collected stem portions at their base using a hammer and a chisel to obtain stem wedges that were approximately 5 cm deep and almost 3 cm high and included a portion of wood, cambium, and bark. To extract a specific stem fragment, we placed the chisel in the desired area and hit the back of the chisel with the hammer. Once the area to be extracted was delimited, we used the chisel to pry and carefully remove the wood sample, avoiding breaking the sample. During collection, samples were fixed in FAA 70 (1:1:18 formalin–acetic acid–70% v/v ethanol) for several days, then transferred to 70% ethanol for long-term preservation (Johansen, 1940). We selected one species per genus for ontogenetic studies and examined the stems with 10× hand lens or with a microscope to establish the ontogenetic stages. These stages range from the onset of secondary growth to the thickest part of the stem (the thickest stem being c. 8 cm in diameter but ranging from 2 to 4 cm).

Because the family is characterized by having very stiff wood, we first softened the samples in a solution of boiling water and glycerin (10:1) for 8 h per day for a week or more (Pace, 2019). We then gradually infiltrated the samples with polyethylene glycol 1500 (Rupp, 1964). We sectioned the samples using a sliding microtome (Leica Hn40) equipped with permanent steel knives sharpened with sandpapers of different grits (Barbosa et al., 2018). To prevent tearing and obtain intact stem sections, we applied a polystyrene resin coat (Styrofoam dissolved in butyl acetate) to the samples (Barbosa et al., 2010). The sections were double stained using safrablau (1:9 safranin–astra blue; Bukatsch, 1972, modified by Kraus and Arduin, 1997), and then mounted in Canada balsam for preservation and analysis.

## Character delimitation

We constructed a database comprising four characters—habit (binary and multistate), ontogeny, and vascular variant status (absent/present) (see Appendix S2: Table S1); character states were scored based on our direct observations. Habit was assessed as a multistate character with four states and as a binary character (climbing/self-supporting) for correlation analysis (Appendix S2: Table S1). However, we present only the multistate reconstruction because the binary reconstruction yielded results that were identical to those of coauthors Quintanar-Castillo and Pace (2022) shown in Figure 1. Additionally, the multistate reconstruction provides a more detailed representation of habit transitions, capturing the complexity of developmental changes more effectively. We divided each ontogenetic trajectory into stages, with each stage representing a detected change in the stem ontogeny. We then scored each trajectory as a single character state. We built a step matrix to represent the number of steps needed for each ontogeny to change into another (Table 2). Each number represents the number of steps needed for a particular ontogeny to change into another; in this way, stages are not lost when considering a complete ontogeny (Mabee and Humpries, 1993).

**FIGURE 2** Distribution and morphology of members of the Bunchosia clade. (A) Distribution of the genera of the Bunchosia clade. Genera of the clade are distributed in the Caribbean, Central, South, and part of North America, only *Tristellateia* is distributed in Africa, Asia, and Oceania. *Bunchosia* Kunth (B, C). (B) *Bunchosia argentea*. Flower. (C) *Bunchosia macrophylla*. Fleshy fruit. *Echinopterys eglandulosa* (A. Juss.) Small (D, E). (D) Inflorescence and flowers. (E) Fruits. *Heladena multiflora* (Hook & Arn). Nied. (F–H). (F) Flower. (G) Fruits. (H) Habit. *Henleophytum echinatum* (Griseb.) Small (I–K). (I) Flower. (J) Fruits. (K) Plant habit. *Thryallis* Mart. (L, M). (L) *Thryallis longifolia*. Flower. (M) *Thryallis brachystachys*. Fruit. *Tristellateia* Thouars (N, O). (N) *Tristellateia greveana*. Flowers. (O) *Tristellateia australasiae*. Dried fruits. Photograph credits: G. Gerlach (B), R. Aguilar (C), R. Nejapa (D, G, H), M. R. Pace (E, N), A. Francener (F), P. González Gutiérrez (I–K) and W. R. Anderson (L, M, O).

**TABLE 1** Species collected for anatomical and ontogeny descriptions. Asterisks indicate species used in the phylogenetic comparative methods.

| No. | Species | Habit | Vascular system arrangement |
|---|---|---|---|
| | **Bunchosia clade** | | |
| 1 | *Bunchosia argentea* | Tree | Regular stem |
| 2 | *B. armeniaca* | Tree | Regular stem |
| 3 | *B. elliptica* | Tree | Regular stem |
| 4 | **B. glandulifera* | Tree | Regular stem |
| 5 | *B. linearifolia* | Tree | Regular stem |
| 6 | *B. macilenta* | Tree | Regular stem |
| 7 | **B. maritima* | Tree | Regular stem |
| 8 | **B. montana* | Tree | Regular stem |
| 9 | **B. nitida* | Tree | Regular stem |
| 10 | **B. polystachia* | Tree | Regular stem |
| 11 | **Echinopterys eglandulosa* | Climbing shrub | Conspicuous wide rays |
| 12 | **E. setosa* | Shrub | Conspicuous wide rays |
| 13 | **Heladena multiflora* | Liana | Wavy cambium |
| 14 | **Henleophytum echinatum* | Liana | Interxylary phloem |
| 15 | *Thryallis brachystachys* | Climbing shrub | Wavy cambium |
| 16 | **T. longifolia* | Climbing shrub | Wavy cambium |
| 17 | *T. laburnum* | Climbing shrub | Wavy cambium |
| 18 | **Tristellateia australasiae* | Liana | Phloem wedges and interxylary phloem |
| 19 | *T. greveana* | Liana | Phloem wedges and interxylary phloem |
| | **Other Malpighiaceae** | | |
| 20 | **Dicella nucifera* | Liana | Interxylary phloem |
| 21 | **Hiptage benghalensis* | Liana | Regular stem |
| 22 | **Malpighia glabra* | Tree | Regular stem |
| 23 | **Niedenzuella stannea* | Liana | Phloem wedges and interxylary phloem |
| 24 | **Tetrapterys schiedeana* | Liana | Regular stem |

## Character reconstruction

To reconstruct the evolution of the habit and vascular system ontogeny in the Bunchosia clade, we used a previously calibrated phylogeny of Malpighiaceae (the MCC tree of Quintanar-Castillo and Pace, 2022). This phylogeny is composed of 154 taxa representing the main clades in Malpighiaceae with 13 outgroup species from Malpighiales and Saxifragales. It was constructed using a data set consisting of three plastids genes (*matK*, *ndhF*, *rbcL*) and one nuclear marker (*PHYC*) and calibrated using fossil data and previously estimated evolutionary rates (Quintanar-Castillo and Pace, 2022). For morphological character reconstructions, we extracted 16 tree tips, 11 representing members of the Bunchosia clade and five from the sister clade of the group. The MCC tree was pruned to cover only the representatives of the Bunchosia clade and a limited number of outgroups using the drop.tip function from the R package phytools v. 2.3-1 (Revell, 2024).

**TABLE 2** Stem matrix that shows the number of steps that would be needed for one ontogeny to change into another.

| Ontogeny | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **1** | | | | | |
| **2** | 1 | | | | |
| **3** | 2 | 3 | | | |
| **4** | 4 | 5 | 4 | | |
| **5** | 2 | 3 | 4 | 6 | |

We estimated the character transition rates and identified the best-fit model (either equal rates or all rates different) for each character using a likelihood ratio test and the Akaike information criterion with the fitMk function in phytools (Revell, 2024). Based on the best-fit model (Appendix S2: Table S2), we reconstructed the evolutionary history of each character using a stochastic mapping approach (Bollback, 2006) by applying the make.simmap function, running 500 iterations and summarizing the results with countSimmap (Revell, 2024). The character histories were visualized on the pruned tree adding the posterior probabilities at the nodes using the plotSimmap function in phytools (Revell, 2024).

To test whether the evolution of habit was correlated with the presence of vascular variants, we performed a Pagel correlation test for discrete character evolution (Pagel, 1994) using the fit.pagel function in phytools (Revell, 2024). We tested an independent model and a dependent model where both traits evolve interdependently (Appendix S2: Table S3). All analyses were performed in R version 4.3.1 (R Core Team, 2021). The underlying phylogenetic tree, database, and codes are available at https://doi.org/10.5281/zenodo.14956639.

## Terminology adopted

Axial region: Refers to the stem region, particularly the wood that is responsible for structural support and vertical water transport. The cells in this region are usually elongated and run parallel to the long axis of the stem (Wiedenhoeft et al., 2005).

Climber: Plants that do not stand upright by themselves in their adult stages and climb different structures that are used as support to grow in height, maintaining the connection with the ground temporarily or permanently. Climbers can include lianas and climbing shrubs (Cabanillas and Hurrell, 2012; Sperotto et al., 2020).

Climbing shrub: Plants with long, semi-rigid stems that initially are self-supporting or grow upright with autonomous mechanical support. When they come into contact with other plants or support structures, they either lean or climb. Typically, they have long hanging branches that seek support to ascend (Acevedo-Rodriguez, 2015; Sperotto et al., 2020).

Radial region: Refers to the stem region, particularly the wood, composed of rays and responsible for the radial transport of water and photosynthetic products. Cells in this region are usually elongated and run perpendicular to the long axis of the stem, extending from the pit to the bark (Wiedenhoeft et al., 2005).

Self-supporting: Plants with autonomous mechanical support such as trees and shrubs with rigid and upright stems (Rowe and Speck, 2005; Cabanillas and Hurrell, 2012).

# RESULTS

## Evolution of habit in the Bunchosia clade

As previous analyses suggested and is shown in Figure 1, there were two independent evolutionary origins of the climbing habit in Malpighiaceae, one in the Old World genus *Acridocarpus* and another encompassing most of the diversity of the family. Within this large lianescent clade (labeled as number 2 in Figure 1), there were at least 15 independent reversals to the self-supporting habit, one of which is the Bunchosia clade. In the Bunchosia clade specifically, the self-supporting habit was re-gained twice, once in *Bunchosia* and once in *Echinopterys*, the latter a genus including both self-supporting and climbing species.

However, the binary division of climber and self-supporting is insufficient to describe all the diversity of growth forms present in Malpighiaceae. Hence, we broke down these two categories into four: climbing shrub, liana, shrub, and tree. This more detailed approach allows us to infer that the common ancestor of the clade was most likely a liana. This habit transitioned to self-supporting at least once, but most likely twice (Figure 3) in the subclade formed by *Bunchosia*, *Echinopterys*, and *Thryallis*. In the *Bunchosia* genus, the most likely ancestral habit is a tree. *Echinopterys* (a genus of two species: self-supporting shrub *E. setosa* and climbing shrub *E. eglandulosa*) has climbing shrub as its most likely ancestral habit, with the subsequent loss of this habit in *E. setosa*. *Thryallis* is the only genus in the subclade that kept the plesiomorphic condition of the subclade as a climbing shrub (Figure 3E).

## Diversity and evolution of the vascular system in the clade

Considering the overall adult stems of the members of the Bunchosia clade and their ontogeny, it is possible to delimit five ontogenetic trajectories (Figure 4) that lead to five different arrangements of the stem vascular system in the group. Although the anatomical structure of the adult stems is different, their trajectories share common developmental stages (Figure 4). All genera begin their secondary growth with a regular stem and a single vascular cambium with regular activity throughout its circumference (Figure 4, first column). Only the self-supporting genus *Bunchosia* maintains this arrangement throughout its development, while the rest of the genera (self-supporting or climbing) eventually undergo modifications (extra steps) from this basic conformation (Figure 4).

### Vascular system diversity in the Bunchosia clade

**Ontogeny 1. Regular stem.** *Bunchosia* is the only genus with a regular configuration in the vascular cambium in both trees and shrubs (Figure 4, Ontogeny 1). A regular stem configuration implies a concentric vascular cambium that forms more secondary xylem to the inside than secondary phloem to the outside.

**Ontogeny 2. Conspicuous wide rays.** This vascular arrangement is found only in *Echinopterys* (Figure 4, Ontogeny 2), a genus with both erect and climbing shrubs. At the beginning of development, the stem has regular secondary growth (Figure 5A), and vessels are organized in radial multiples and begin to form short tangential bands (Figure 5C). In later stages, the wood rays become increasingly more conspicuous within the wood, as seen in the transverse section—and unlike most woods, the rays widen continuously. In addition, these rays are produced by areas of the cambium with higher cell production rates, forming projections toward the secondary phloem (Figure 5D, F). The increase in xylem radial parenchyma production is a result of the variant activity in the radial region of the cambium. Axial cambial regions located between the rays continue to produce secondary xylem to the inside and secondary phloem to the outside at regular rates (Figure 5C). As the stem grows thicker, the vascular cambium remains continuous with differential activity between the axial and radial region, the rays begin to become more conspicuous and have abundant prismatic crystals (Figure 5E, F). These conspicuous rays may consist of both multiseriate and aggregate rays (Figure 5F–H). The axial section of wood has abundant vessels with a semidendritic pattern or in short tangential bands and with vessel dimorphism. (Figure 5D). In the phloem, the axial region has abundant druses, and the sclerenchyma consists of clusters or short bands of sclereids (Figure 5D). The projection of wood rays generates small arcs of axial phloem (Figure 5C).

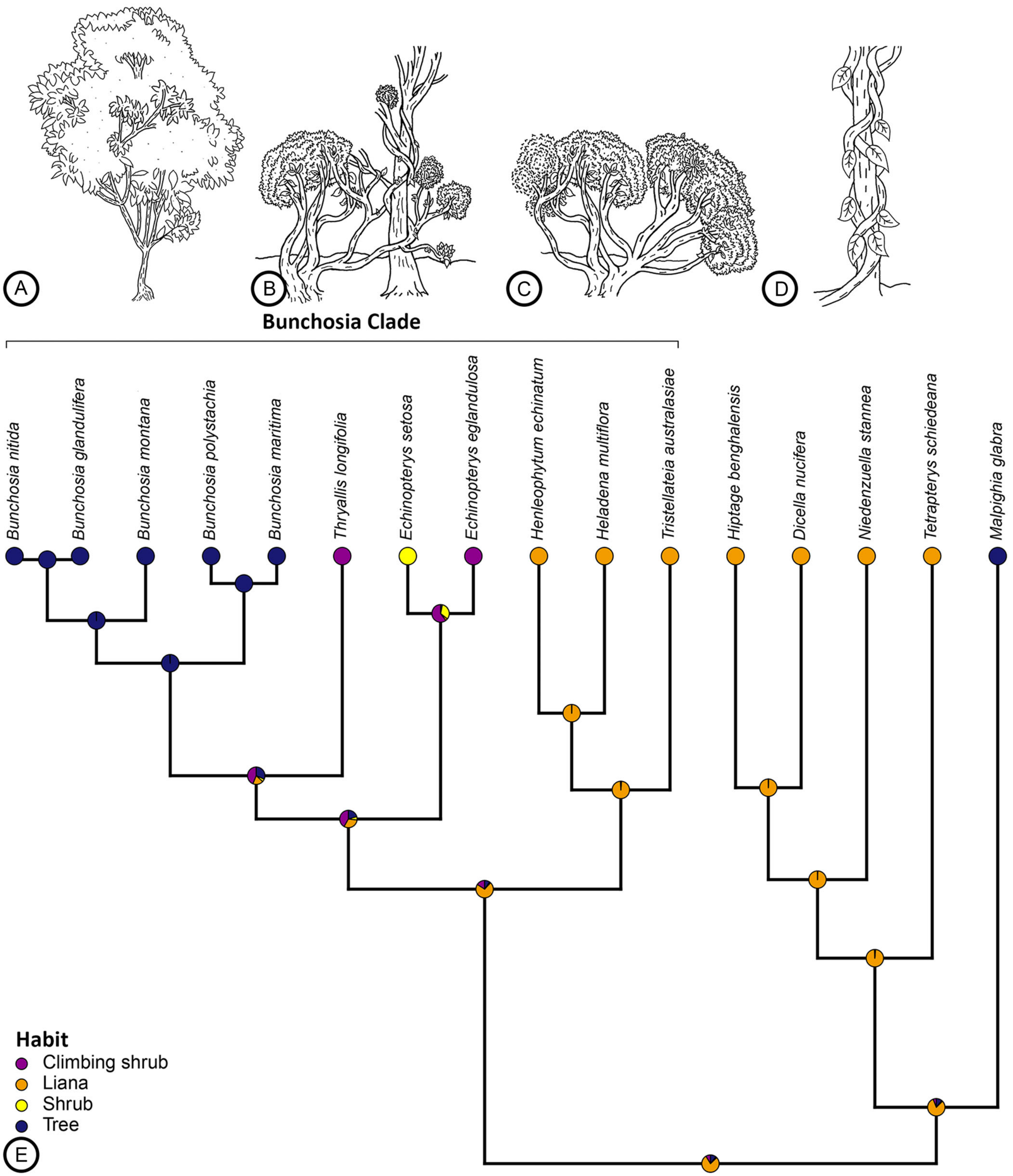

**FIGURE 3** Habit evolution in Bunchosia clade. Line drawings of (A) tree, (B) climbing shrub, (C) shrub, (D) liana. (E) Reconstruction of the ancestral habit in the clade. The model infers that the most likely state for the most recent common ancestor of the Bunchosia clade was a liana.

**Ontogeny 3. Wavy cambium.** This vascular variant is found in *Heladena* and *Thryallis* (Figure 4, Ontogeny 3). Plants with wavy cambium begin secondary growth with a single, continuous, regular vascular cambium (Figure 6A). Vessels are initially arranged in radial multiples, while gradually progressing to adopt an arrangement of short tangential bands (Figure 6C). Later, the shallow phloem arcs begin to have no apparent pattern, but the vascular

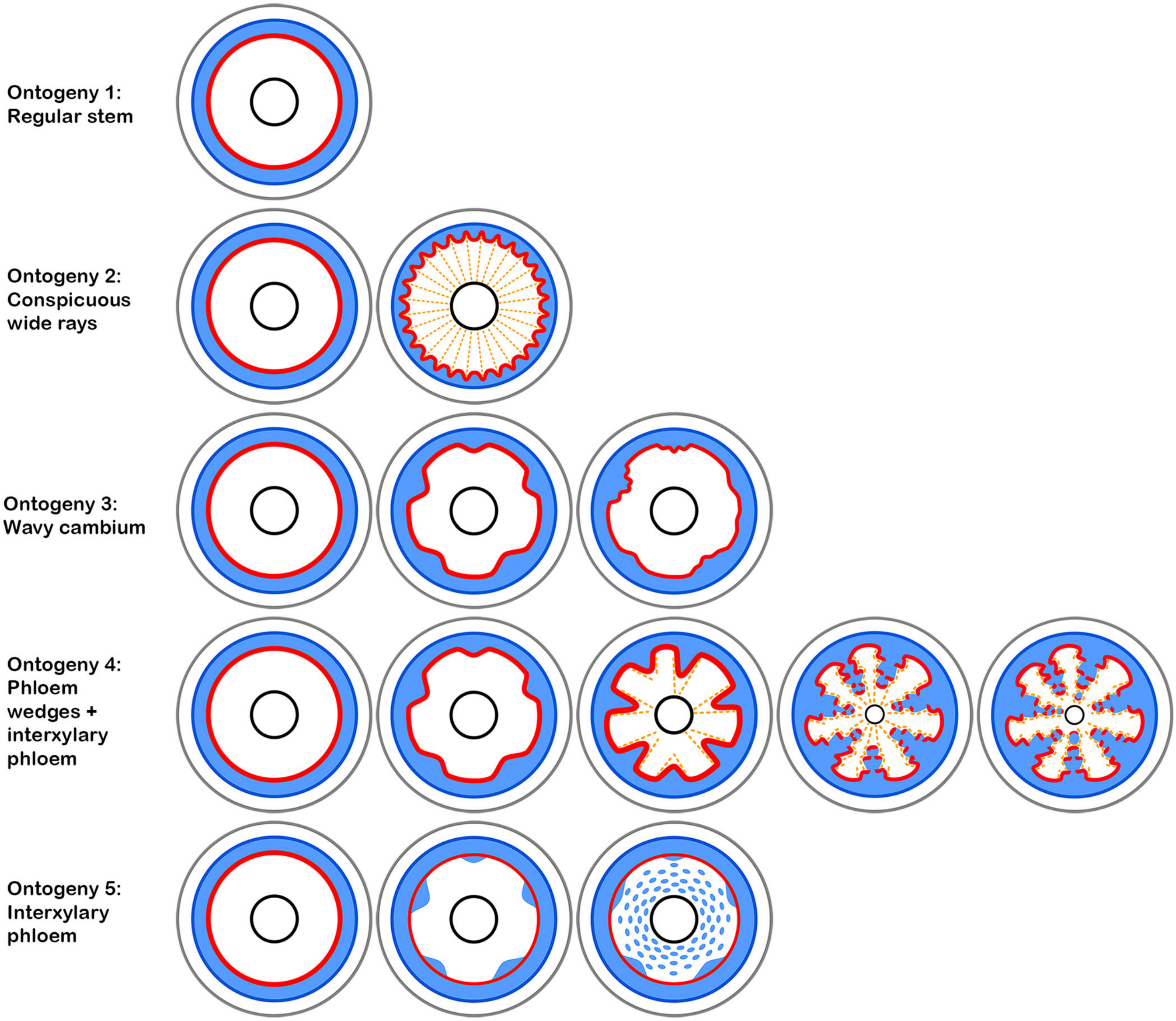

**FIGURE 4** Ontogenetic pathways. Schematic representation of the five different ontogenetic trajectories that were found in the Bunchosia clade. Each trajectory leads to a different final stem anatomy. Blue: secondary phloem; gray: limit of bark; black: limit of the pith; orange: conspicuous wide rays (which act as limiting rays in ontogeny 4); red: vascular cambium.

cambium remains continuous with a sinuous or wavy course (Figure 6B, D). At this stage, the vessels are distributed in a semidendritic pattern or short tangential bands (Figure 6E). In *Heladena*, the axial parenchyma is nonlignified (Figure 6E) and is arranged in short tangential bands with prismatic crystals included. The shallow phloem arcs are formed by differential activity in these cambial regions (variant cambium). In these variant regions, more secondary phloem is produced than secondary xylem, whereas in the regular regions, the production of both tissues is regular. In the adult stage, the vascular cambium has a continuous wavy pattern that is more conspicuous, and the shallow phloem arcs become more prominent and irregular. In *Thryallis*, phloem arcs are flanked by limiting rays that usually contain prismatic crystals (Figure 6F). Regions with phloem arcs have differential production of secondary xylem (more evident in *Heladena*) with a greater density of fibers than vessels and narrower vessels (Figure 6E), whereas in the regions where arcs do not form, the production of wood and phloem is regular. In *Heladena*, the nonlignified axial parenchyma is distributed in short bands, forming a crenate pattern.

**Ontogeny 4. Phloem wedges and interxylary phloem derived from phloem wedges inclusion.** Within the Bunchosia clade, this vascular variant is only found in the lianescent *Tristellateia* (Figure 4, Ontogeny 4). Secondary growth begins with the differentiation of a single, continuous, regular cambium that produces secondary xylem in greater proportion than that of secondary phloem (Figure 7A). The vessels at this stage are arranged in short

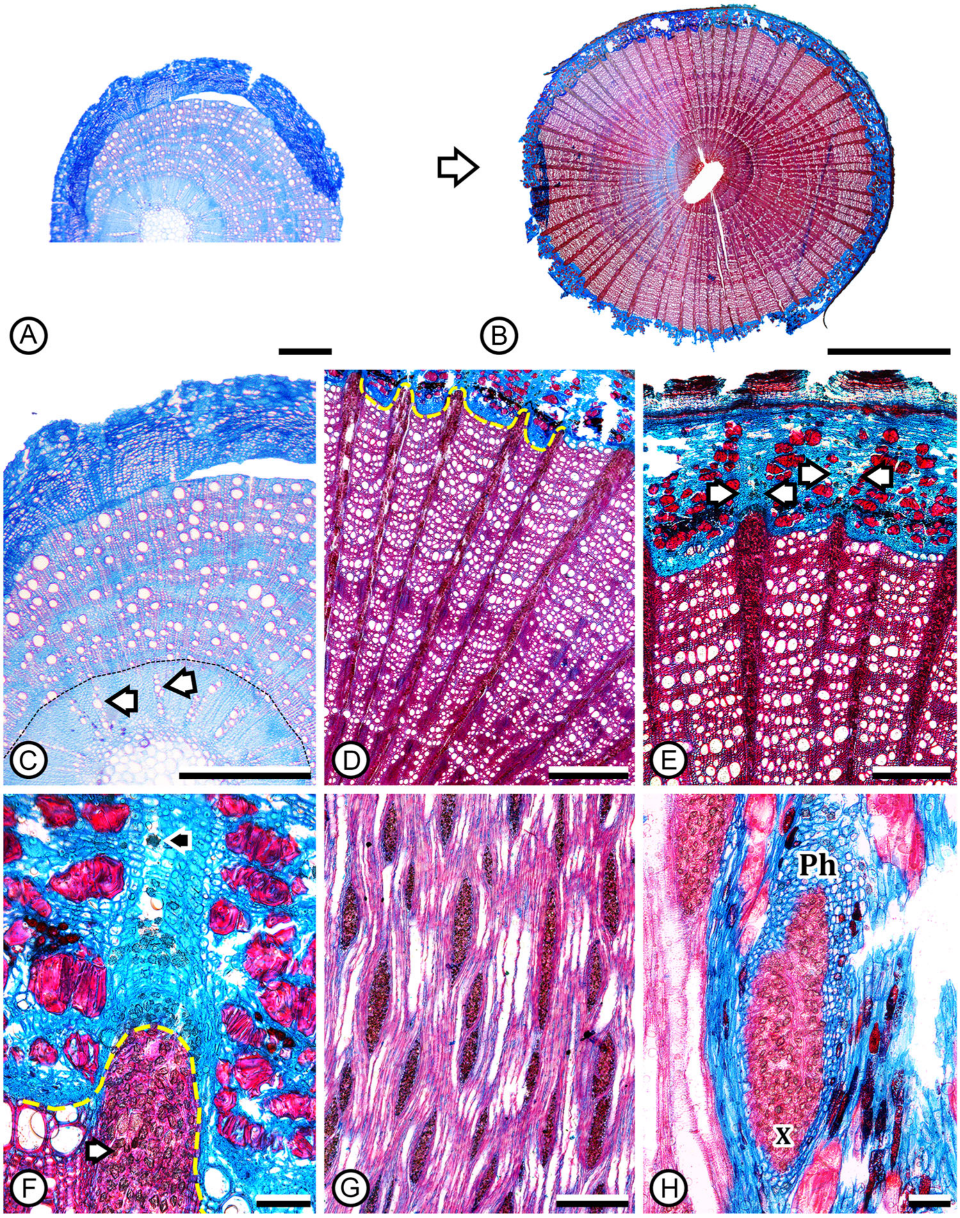

**FIGURE 5** Ontogeny 2. Conspicuous wide rays. (A–F) Transverse sections. (A, B) Stem development. (A) *Echinopterys setosa*. Juvenile stem with regular growth. (B) *Echinopterys eglandulosa*. Adult stem with conspicuous wide rays in wood due to higher production in the radial vascular region. (C) *E. setosa*. Vessel in radial multiples in early stages of development (arrows). (D) *E. eglandulosa*. Detail of the wide, conspicuous rays, and the phloem arcs in the axial region (yellow dotted line). (E) *E. eglandulosa*. Sclereids in the nonconducting phloem are distributed only in the axial region and absent in the radial region (arrows). (F) *E. eglandulosa*. Detail of a conspicuous ray. Vascular cambium remains continuous (yellow dotted line), and more radial cells are produced toward the xylem than the phloem. Prismatic crystals are observed in radial parenchyma cells of the xylem (white arrow) and druses to the radial parenchyma cells of phloem (black arrow). (G, H) Tangential sections. (G) *E. eglandulosa*. Conspicuous rays are multiseriate and sometimes form aggregates. (H) *E. eglandulosa*. Limit between xylem (X) and phloem (Ph) in a multiseriate ray. Scale bars: A, C–E, G = 500 μm, B = 5 mm, F, H = 100 μm.

radial multiples. In the next stage, the phloem begins to form phloem arcs (Figure 7B), and vessel dimorphism appears. Later, more conspicuous wedges, flanked by limiting rays containing prismatic crystals appear (Figure 7C). These phloem wedges are the result of a variant type of activity in the cambium of these regions. The vascular cambium in the wedge region (variant cambia) produces more secondary phloem than secondary xylem, whereas in the interwedge regions (regular cambia), xylem production is greater than secondary phloem. As phloem wedges continue to develop, the vascular cambium loses continuity between the wedge and interwedge regions (Figure 7E). This disruption is caused by a decrease or cessation of anticlinal divisions in the variant cambium. As the wedges enlarge, they widen toward the regular cambium (interwedge region) because of a change in the activity of the cambium in regions adjacent to the wedges. This transition from regular to variant activity also produces a stepped pattern in the wedge

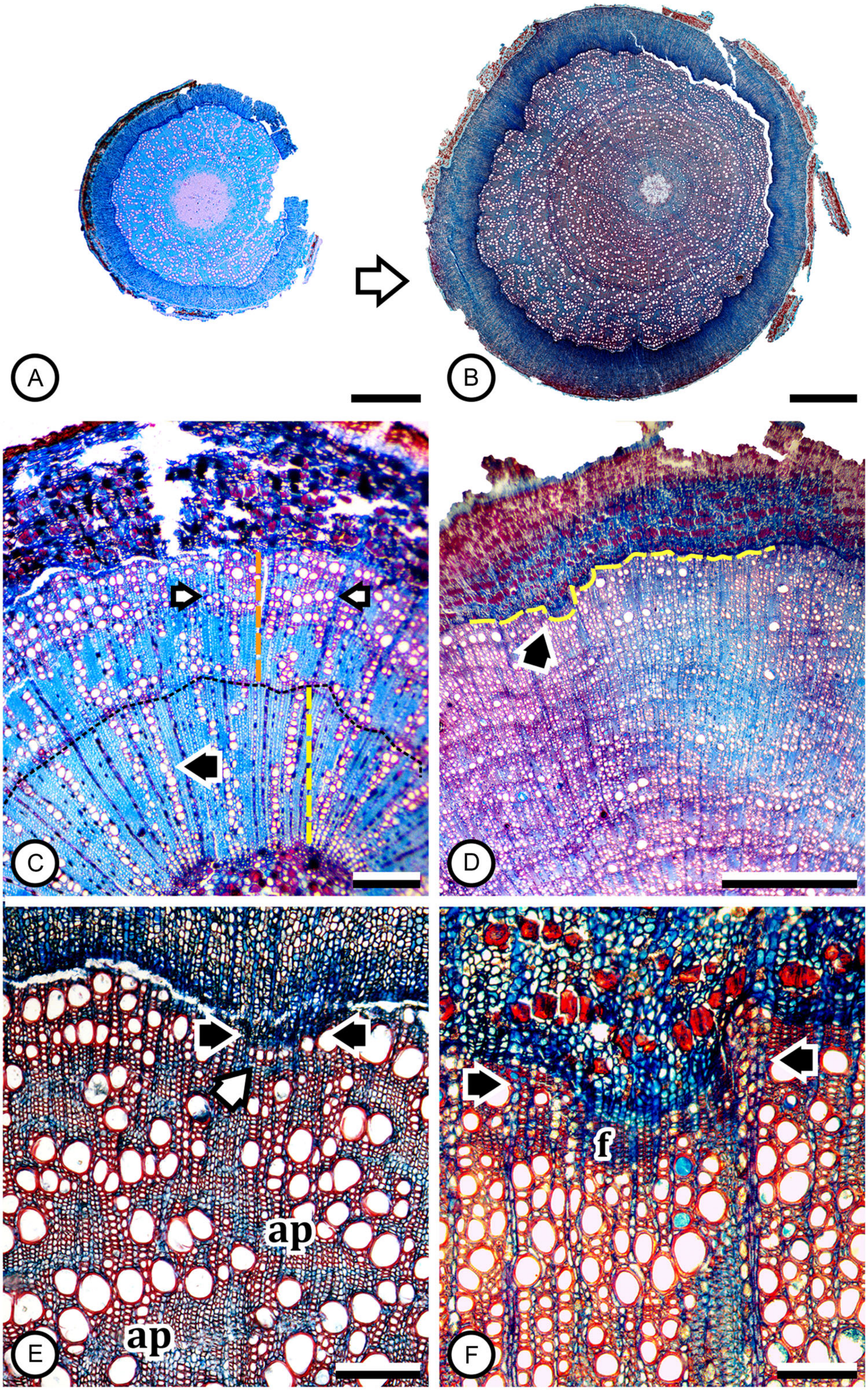

**FIGURE 6** Ontogeny 3. Wavy cambium. (A–F) Transversal sections. (A, B) Ontogenetic trajectory in *Heladena multiflora*. (A) Early secondary growth with a single, regular cambium only slightly wavy. (B) Adult stem with a conspicuously wavy cambial pattern, forming irregular phloem arcs. (C, D) Ontogenetic trajectory in *Thryallis longifolia*. (C) First stage of wavy cambium formation. Stem with regular secondary growth. Vessels form long radial multiples (arrow). Yellow dotted lines indicate the self-supporting phase of the stem, where the vessels are arranged in multiple long radials. Orange dotted lines indicate the change in the arrangement of vessels when the plant becomes a climber. Vessels are in tangential bands (back arrowheads). (D) Adult stem with irregular phloem arcs (arrow). (E) *H. multiflora*. Variant activity in the region of phloem arcs (arrows). The variant cambium produces fibers in higher proportion and narrower vessels. Nonlignified parenchyma (pa). (F) *T. longifolia*. Variant region that produces a greater amount of fibers (f) than in the adjacent regions Shallow phloem arcs flanked by limiting rays. Scale bars: A, B, D = 2 mm. C–F = 200 μm.

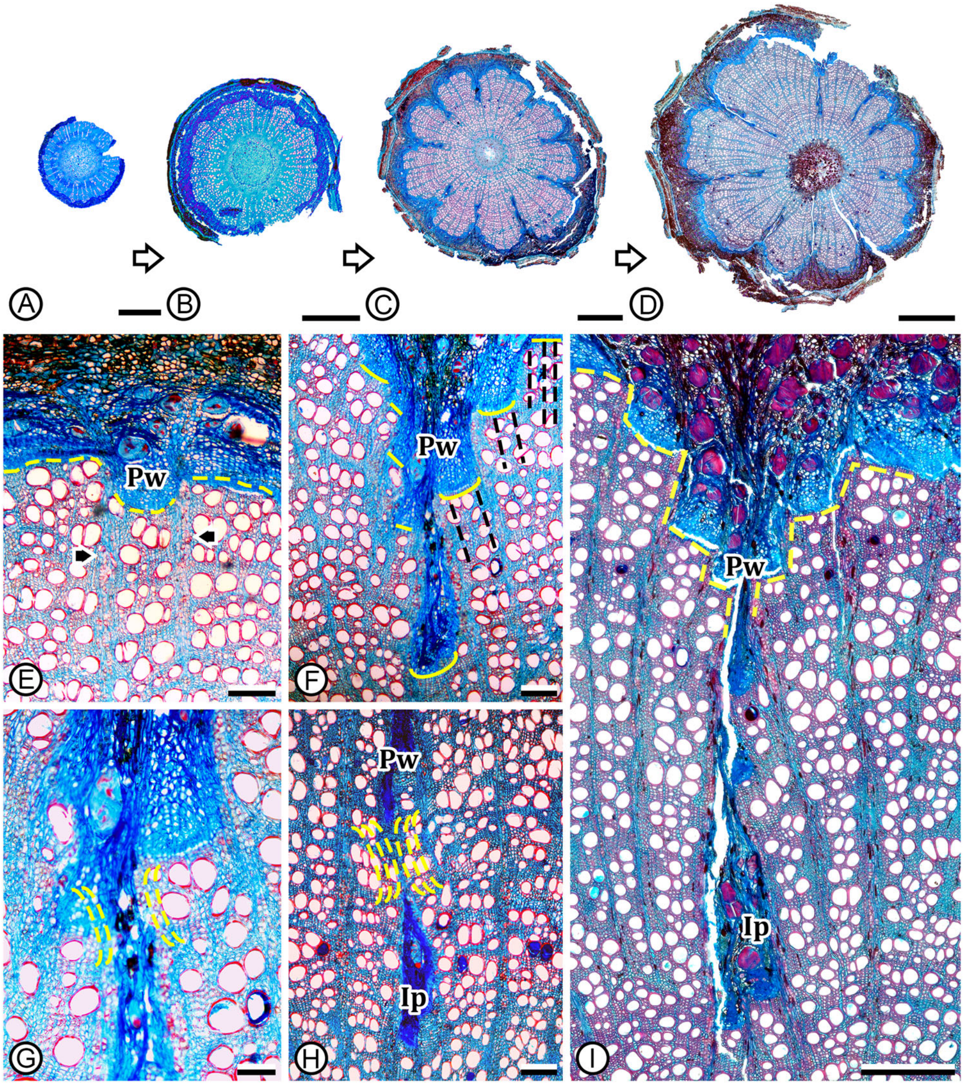

**FIGURE 7** Ontogeny 4. Phloem wedges and interxylary phloem. (A–I) *Tristellateia greveana*. (A–D) Ontogenetic pathways. (A) Onset of secondary growth with a regular, single cambium. (B) Formation of small phloem arcs in the variant regions of the cambium. (C) Formation of phloem wedges and disruption of cambium. (D) Phloem wedges with discontinuous cambium forming a stepped pattern and interxylary phloem. (E–I) Transverse sections. (E) Early phloem wedge (Pw), flanked by limiting rays (arrows). At this stage, the disruption of the cambium is already observed (yellow dotted line). (F) Well-developed phloem wedge with discontinuous cambium (yellow lines) evidenced by the direction in which the cambium products are arranged (black dotted lines). (G) Adjacent regions with regular activity produce more xylem, which exerts pressure toward the wedges (yellow dotted lines) and promotes the inclusion of the innermost portion of the wedges. (H) Interxylem phloem (Ip) resulting from the pressure exerted by the adjacent wood (yellow dotted lines). (I) Phloem wedge on adult stem, with a stepped pattern (yellow dotted line) and discontinuous cambium. Interxylary phloem close to the phloem wedge. Scale bars: A–D = 2 mm. E, F, H = 200 μm. G = 100 μm. I = 500 μm.

borders (Figure 7F, I). The variant cambium (wedge region) disconnects from the regular cambium as the wedges widen, including portions of the variant cambium at the base of the wedge steps. The included cambium remains active, producing secondary xylem and phloem (Figure 7F). In contrast, the regular cambium continues producing secondary xylem in greater quantities. This high production in adjacent regions eventually exerts pressure toward the wedges, including the innermost wedge portions, forming interxylary phloem strands (Figure 7G, H). Finally, in the adult stage, the vascular system is arranged in phloem wedges that widen as they approach the interwedge regions (regular regions), forming a stepped pattern (Figure 7I) and with interxylary phloem (Figure 7D).

**Ontogeny 5. Interxylary phloem.** This vascular variant is only present in *Henleophytum* (Figure 4, Ontogeny 5). At the beginning of secondary growth, a single, regular vascular cambium is established (Figure 8A). Later, small arcs of secondary phloem are formed because of variation in certain regions of the continuous, concentric vascular cambium (Figure 8B). These regions of variant activity begin to produce secondary phloem both centrifugally and centripetally only in these regions (Figure 8D). The sieve tube elements of the variant phloem are visibly wider than

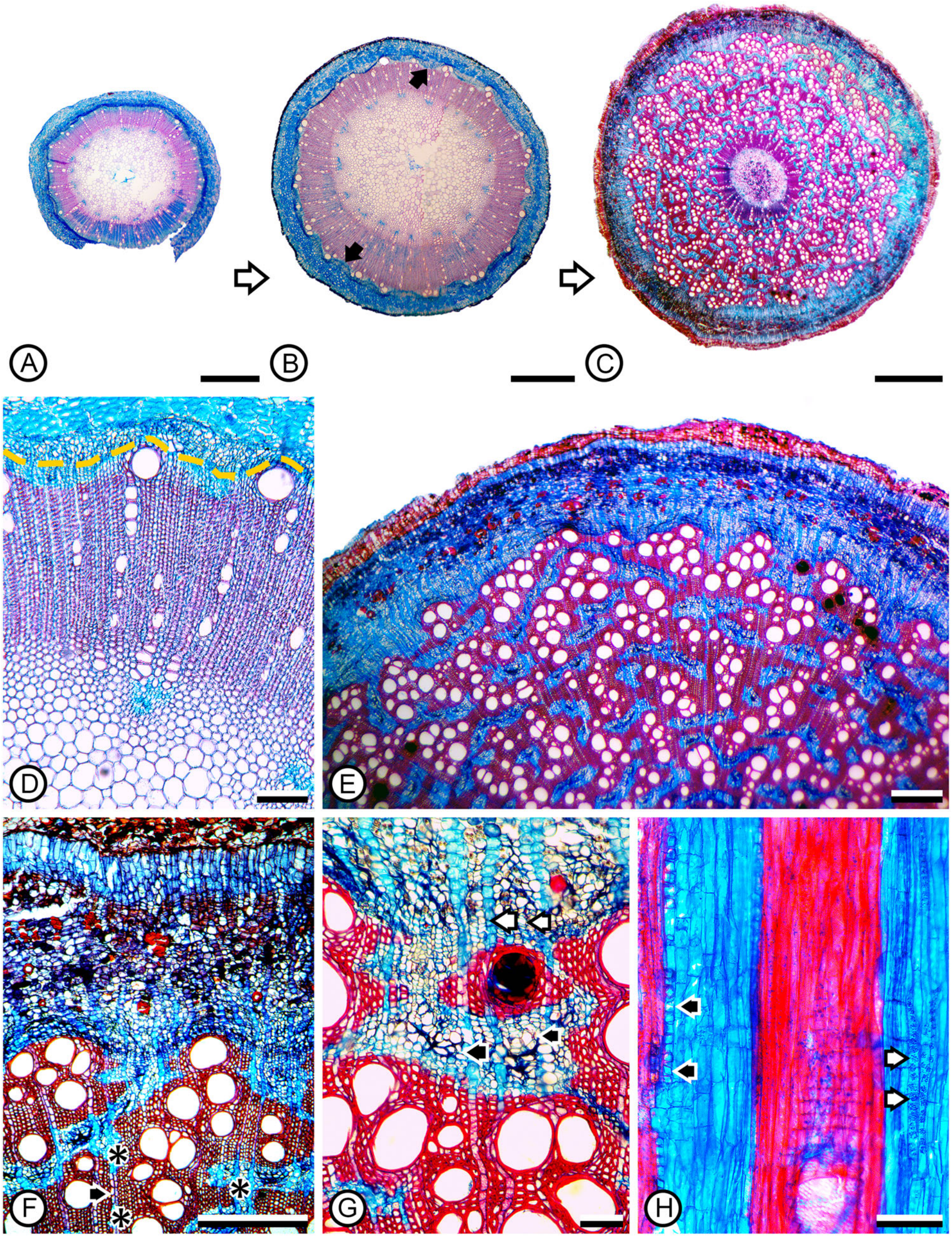

**FIGURE 8** Ontogeny 5. Interxylary phloem. (A–C) Ontogenetic trajectory of the interxylary phloem. (A) At the onset of secondary growth, the stem is regular, with a single continuous cambium of regular activity. (B) Formation of shallow phloem arcs in certain regions of the cambium (black arrows). (C) Adult stems showing a slightly dendritic pattern of interxylary phloem strands with several arcs of phloem in the cambial region. (D–G) Transverse sections. (D) Continuous vascular cambium (yellow dotted line) producing secondary phloem in- and outward in certain regions, forming phloem arcs (asterisks). (E) Production of interxylary, variant phloem (Ip) inward, while producing regular phloem outward, due to a switch in the activity of a single vascular cambium (Vc). (F) Vessels have no contact with the interxylary phloem strands and are surrounded solely by fibers (black arrow), while phloem strands are surrounded by nonlignified axial parenchyma. Interxylary phloem (Ip). (G) Adult stem with interxylary phloem with a dendritic pattern. (H) Radial section. Prismatic crystals in the nonlignified parenchyma (black arrows) surrounding the interxylary phloem. Druses (white arrows) are present in the regular phloem (Rp). Scale bars: C = 2 mm. A, B = 1 mm. G = 500 µm. D–F, H = 100 µm. Color key: black = pith, blue = secondary phloem, grey = cortex, red = vascular cambium (modified from Quintanar-Castillo et al., 2024, reproduced with permission).

those of the regular phloem (Appendix S2: Table S4). Variant phloem is surrounded by nonlignified axial parenchyma with prismatic crystals, while regular phloem parenchyma has druses instead (Figure 8H). In subsequent stages, the variant regions that produced phloem in both directions resume their regular activity forming phloem on the outside and secondary xylem on the inside, initially producing fibers and then vessels. As xylem is produced again in these variant regions, the variant phloem strands previously produced become included (Figure 8F). In addition to nonlignified parenchyma, interxylary phloem strands are surrounded only by fibers, without having direct contact with vessels (Figure 8E, F). Later, other regions of the cambium change their activity to form secondary phloem in both directions. Again, these regions shift into regular activity, so that interxylary phloem appears across the entire wood. Finally, adult stems have strands of interxylary phloem surrounded by nonlignified parenchyma and fibers (Figure 8C, F). These interxylary phloem strands form short sinuous or semidendritic bands (Figure 8G).

### Evolution of the vascular system in the Bunchosia clade

The reconstruction of ontogenetic evolution in the Bunchosia clade indicates that ontogeny 1 (Figure 4), which leads to the formation of a regular stem, is the ontogeny with the highest probability of being the ancestral condition in the group (Figure 9). From this ontogeny, four distinct ontogenetic trajectories are likely derived, each one involving additional developmental steps.

The regular stem development is maintained only at the ancestral node of the *Bunchosia*, *Thryallis*, and *Echinopterys* subclade. This condition persists through the rest of the subclade, with changes in *Echinopterys* leading to the development of conspicuous wide rays (ontogeny 2), whereas in *Thryallis* it evolves into a wavy cambium conformation (ontogeny 3).

A shift to a more complex vascular cambium conformation occurs at the ancestral node of the *Heladena*, *Henleophytum*, and *Tristellateia* subclade. The formation of a wavy cambium (Ontogeny 3) is the most likely condition at this node, which is subsequently maintained only in *Heladena*. In *Henleophytum*, the cambium transitions to the formation of interxylary phloem in *Heladena*, while in *Tristellateia* it evolves into the formation of phloem wedges along with interxylary phloem.

Changes in vascular cambium that occurred in the evolution of the Bunchosia clade (summarized in Figure 9) include that the regular stem condition is conserved exclusively in *Bunchosia*, whereas independent changes led to the development of a wavy cambium in *Thryallis* and *Heladena*. In both genera, certain portions of the cambium reduce secondary xylem production while increasing secondary phloem, resulting in shallow, irregular phloem arcs. In *Tristellateia*, these phloem arcs grow deeper, forming phloem wedges with discontinuous cambium and interxylary phloem strands. Otherwise, in *Henleophytum*, these small arcs develop from a cambium that undergoes alterations in specific areas of their girth, producing phloem internally and externally, giving rise to interxylary phloem. In *Echinopterys*, the radial regions in the vascular cambium produce more wood ray parenchyma than phloem, leading to the formation of wide rays that compress the axial regions of the phloem, giving the appearance of small arcs, which continually widen.

The correlation analysis between habit and vascular variants shows there is no significant relationship between these two traits ($P = 0.1399$, see Appendix S2: Table S3). This result suggests that the habit does not consistently influence the development of different vascular arrangements (Figure 10).

## DISCUSSION

### Self-supporting growth forms are a derived condition in the Bunchosia clade

Based on the reconstruction of ancestral habits, which considers only the character states of self-supporting and climbers, the most recent common ancestor of the Bunchosia clade is reconstructed as being a climbing plant (Figure 1). From this climbing ancestor, two lineages independently transitioned toward the self-supporting habit, one encompassing the entire genus *Bunchosia* and the other including one of the two species of *Echinopterys*—*E. setosa*, from the Chihuahuan desert.

However, categorizing plants as either climbers or self-supporting species does not capture the full diversity of habits within the Bunchosia clade or the Malpighiaceae as a whole. Therefore, we further subcategorized both self-supporting and climbing plants. We sorted self-supporting plants into trees and shrubs, whereas for climbers we distinguished between lianas and climbing/leaning shrubs. We characterized lianas as woody vines with active climbing mechanisms (e.g., twining), and we characterized climbing shrubs, which can be woody or not, as vines without active mechanisms, but which lean on the surrounding vegetation, as suggested by broad studies on climber life forms (Cabanillas and Hurrell, 2012; Sperotto et al., 2020).

Our analysis of habit evolution, considering the different types of self-supporting and climbing plants in the Bunchosia clade, supports lianas as the most likely ancestral condition for the clade. There is a subsequent transition toward climbing shrubs in the most recent common ancestor of the subclade formed by *Bunchosia*, *Echinopterys*, and *Thryallis*. Although the climbing condition is maintained in two genera of this subclade, the mechanism changes to climbing shrubs in *Thryallis* and *Echinopterys*. Subsequently, two independent shifts occur toward trees in *Bunchosia* and shrubs in *Echinopterys setosa*, so self-supporting forms are a derived condition within the clade (Quintanar-Castillo and Pace, 2022).

Self-supporting plants nested within lianescent clades have been associated with transitions to more extreme environmental conditions, such as drier, strongly seasonal habitats (Schmitz et al., 2007; Terrazas et al., 2011; Calió et al., 2022). Additionally, tropical plants that can occupy different habitats have a wide tolerance range that may be related to a variation in their habit (Lohmann et al., 2013). In the Bunchosia clade, the variation in growth forms (vines, trees, and erect or climbing shrubs) may have facilitated transitions to different habitats. In the case of *Echinopterys setosa*, which is exclusively found in xeric

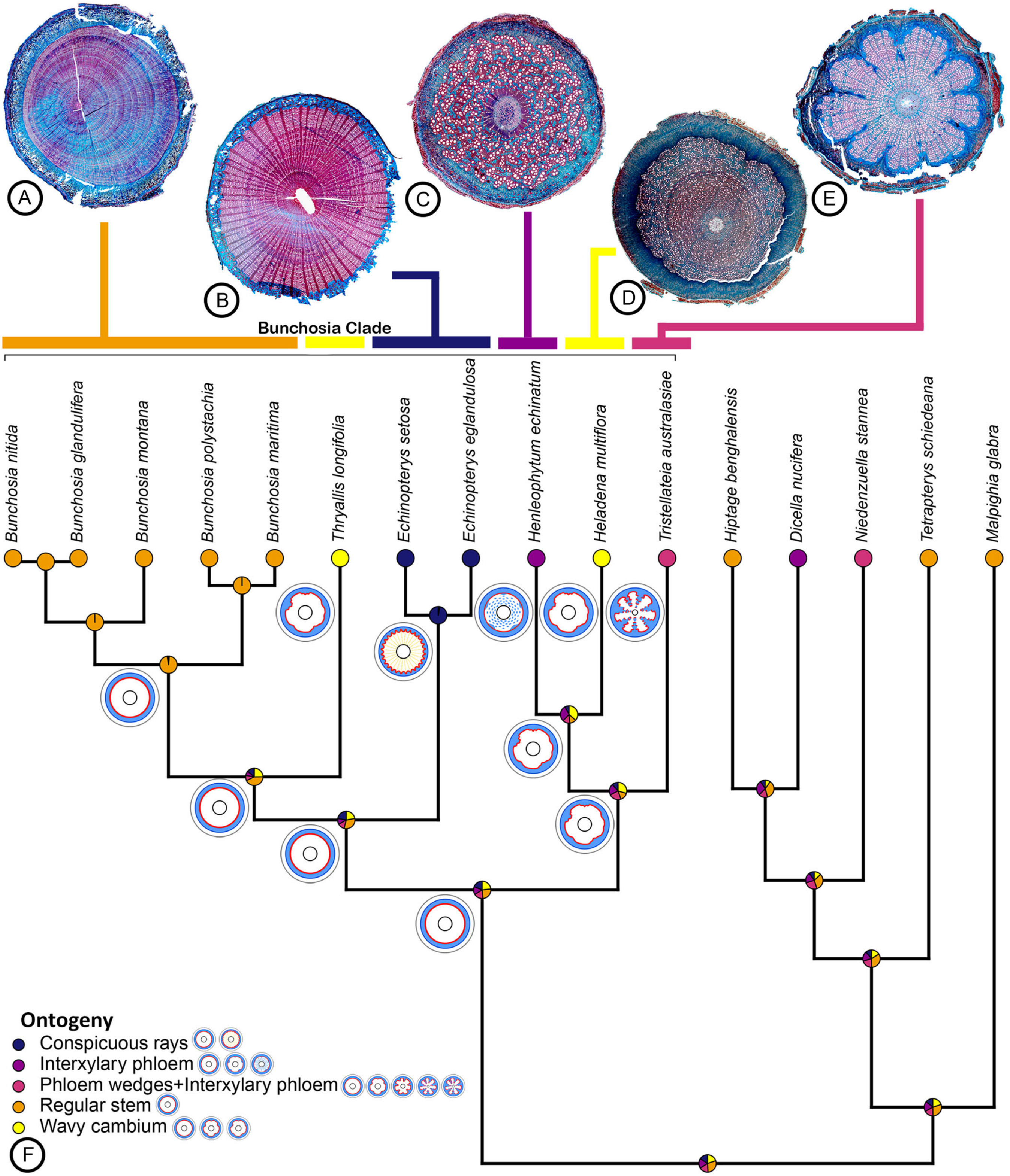

**FIGURE 9** Distribution of vascular variants in Bunchosia clade. (A) *Bunchosia macilenta*. Regular secondary growth (Ontogeny 1). (B) *Echinopterys eglandulosa*. Conspicuous wide rays (Ontogeny 2), synapomorphic vascular variant for the Mexican genus *Echinopterys*. (C) *Henleophytum echinatum*. Interxylary phloem (Ontogeny 5). (D) *Heladena multiflora*. Wavy cambium (Ontogeny 3). (E) *Tristellateia australasiae*. Phloem wedges plus interxylary phloem development (Ontogeny 4), synapomorphic characteristic of the genus *Tristellateia*. (F) Ancestral state reconstruction for ontogenetic trajectories. A regular stem is the likely condition of the common ancestor in the clade, a trajectory that is only conserved in *Bunchosia*.

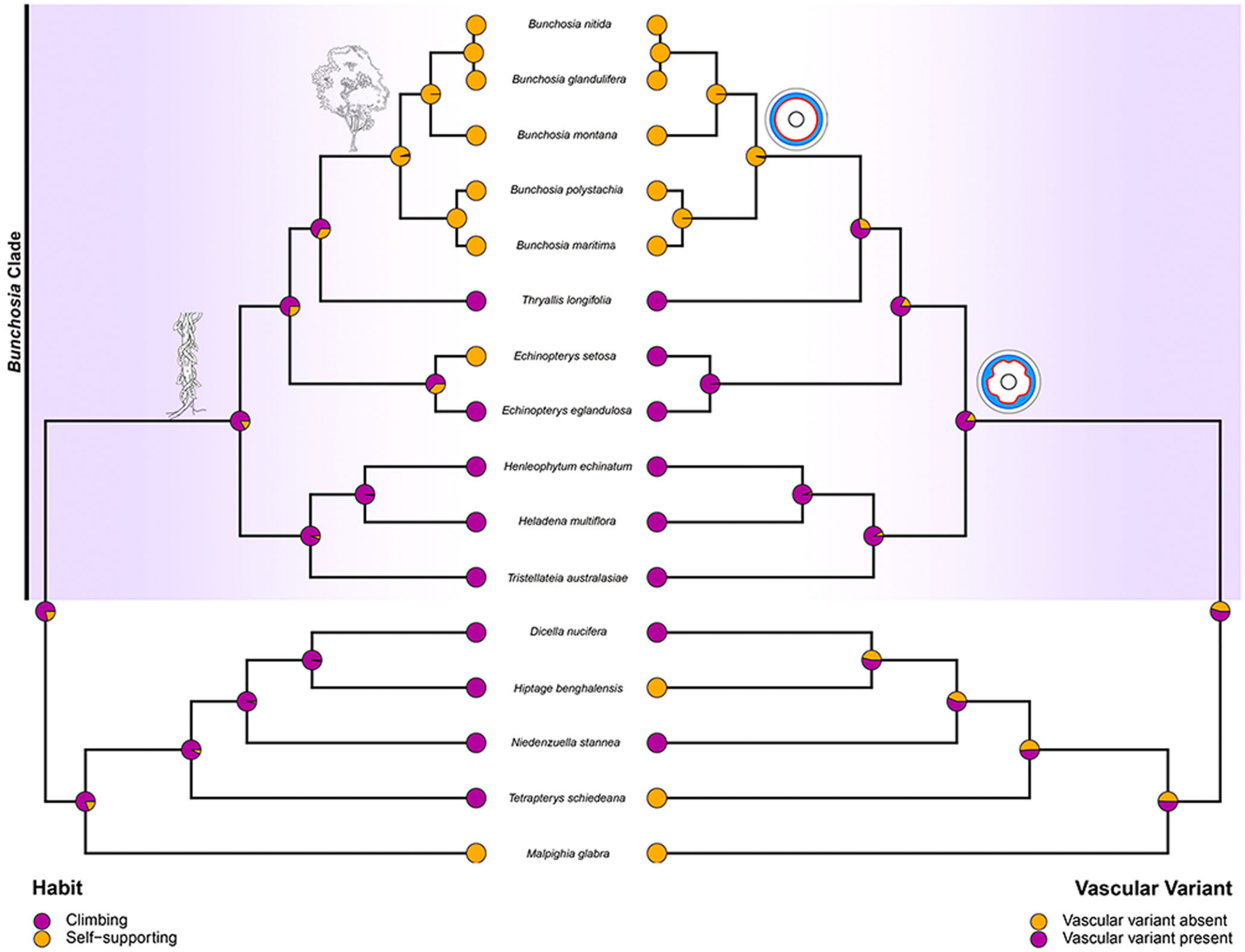

**FIGURE 10** Correlation between habit and vascular variant presence. Trees show the occurrence of habit (climbing/self-supporting) and vascular variant presence in Bunchosia clade.

shrublands, the shift to self-supporting forms may be linked to its occupation of seasonally drier habitats (see Calió et al., 2022, for a similar phenomenon in Bignoniaceae). A biogeographical reconstruction of ancestral habitats in Malpighiaceae would be timely to determine whether a transition to drier habitats could explain the evolution of self-supporting forms in *Bunchosia* and other lineages within the family. The geographical distribution of self-supporting genera nested within lianescent lineages (Figure 1), such as *Calcicola*, *Malpighia*, and *Tricomaria*, and shrubby or tree species within *Banisteriopsis* and *Peixotoa* seems to support this hypothesis for the Malpighiaceae.

## The climbing habit in Bunchosia clade appears not to be related to vascular variants

Correlation analyses on the relationship between habit and the presence of vascular variants indicate that these traits evolve independently in the Bunchosia clade. Specifically, the development of vascular variants does not depend on the type of habit in the Bunchosia clade, as demonstrated in *Echinopterys*, which has vascular variants in both self-supporting and climbing forms. This finding contradicts previous broader analyses with Malpighiaceae that found a positive relationship (Quintanar-Castillo and Pace, 2022).

Vascular variants are common in lianas and other types of climbing plants (Schenck, 1893; Obaton, 1960), but they are not exclusive to these forms; they can also be found in self-supporting plants, although less frequently (Carlquist, 1991). Examples of vascular variants in self-supporting plants include successive cambia in Acanthaceae (*Avicennia*) (Studholme and Philipson, 1966; Schmitz et al., 2007), Convolvulaceae (*Ipomoea* series *Arborescentes*) (McDonald, 1992; Ceja-Romero and Pérez-Olvera, 2010), Leguminosae (*Dalbergia* and *Machaerium*) (Nair and Mohan Ram, 1990; Dias-Leme, 2000), and Amaranthaceae (*Atriplex*) (Fahn and Zimmerman, 1982). However, in many of these lineages (except for *Avicennia*), a lianescent ancestor is likely, meaning that the vascular variant could have been retained due to common ancestry. *Avicennia* is not the only group where vascular variants appear independently from lianescent ancestor lineages. Similar cases have been observed in plants with interxylary phloem such as *Mirabilis*, *Neea*, and *Salpianthus* of the Nyctaginaceae (Hernández-Ledezma et al., 2011; Cunha Neto et al., 2021), in *Aquilaria* of the Thymelaeaceae (Luo et al., 2018, 2019, 2021), and in *Erisma* of the Vochysiaceae (Carlquist, 2001). Additionally, the presence of intraxylary phloem, which is a synapomorphy of all the Myrtales, further highlights this phenomenon (Verhoeven and Van der Schijff, 1974; Carlquist, 1975; Van Vliet and Baas, 1984; Stevens, 2001; Zózimo et al., 2011).

However, our correlation results should be interpreted with caution. *Echinopterys setosa* represents the first record of a vascular variant in a self-supporting Malpighiaceae, and it is nested within a lianescent lineage. Considering our ancestral character-state reconstructions, the ancestor of *E. setosa* likely already had conspicuous rays, suggesting that their presence in the species is a plesiomorphy, a retention of a feature due to common ancestry. Other authors have also proposed that the retention of vascular variants in self-supporting forms may be linked to survival strategies in hostile environments or environments that are more demanding in terms of transport of water and photosynthates (Robert et al., 2011; Terrazas et al., 2011). Indeed, vascular variants in self-supporting forms have been associated with improved efficiency in photosynthate conduction, carbohydrate and water storage, embolism repair, and wound responses (Fisher and Ewers, 1989; Carlquist, 2001; Robert et al., 2011; Zózimo et al., 2011).

In Malpighiaceae, many lianas have vascular variants (Schenck, 1893; Angyalossy et al., 2015; Quintanar-Castillo and Pace, 2022), which have been documented mainly in tetrapteroids and stigmaphylloids (Jussieu, 1843; Schenck, 1893; Chodat and Vischer, 1917; Obaton, 1960; Cabanillas et al., 2017; Pace et al., 2018). Recently, phloem wedges and interxylary phloem have also been documented in the Bunchosia clade (Quintanar-Castillo and Pace, 2022; Quintanar-Castillo et al., 2024). In the next section, we present yet new vascular variants within this group and family.

## Stems in the Bunchosia clade have several vascular variants

We identified five different arrangements of the vascular system in adult stems of the *Bunchosia* clade, which implies five different ontogenetic trajectories, some sharing common developmental stages (Figure 4). Of these five trajectories, one maintains a regular configuration, i.e., genus *Bunchosia*, while four resulted in vascular variants.

The development of vascular variants in Malpighiaceae has been the subject of study for several centuries (e.g., Gaudichaud, 1841; Jussieu, 1843; Van Tieghem, 1891; Schenck, 1893; Chodat and Vischer, 1917; Caballé, 1993; Cabanillas et al., 2017; Pace et al., 2018). So far, five types of vascular variants have been recognized in the Malpighiaceae: (1) interxylary phloem, (2) asymmetrical or noncylindrical stems, (3) phloem wedges, (4) fissured stems, and (5) interxylary cambia (Chodat and Vischer, 1917; Obaton, 1960; Cabanillas et al., 2017; Pace et al., 2018). Some of these variants later promoted the development of more complex forms, such as phloem wedges, which represent a step in the formation of fissured stems, a process observed in some genera like *Banisteriopsis* (for more information see Quintanar-Castillo and Pace, 2022).

In the Bunchosia clade, two of these vascular variants have recently been described, the interxylary phloem found in the Cuban endemic *Henleophytum* (Quintanar-Castillo et al., 2024) and phloem wedges with interxylary phloem found in the Old World *Tristellateia* (Quintanar-Castillo and Pace, 2022). Here we add two new conformations of the vascular system that had not previously been reported in other members of the family: conspicuous wide rays in *Echinopterys* and wavy cambia in both *Heladena* and *Thryallis*.

## Phloem wedges and interxylary phloem

Phloem wedges are one of the most common types of vascular variants in angiosperms and have been found in other lianescent lineages such as Bignoniaceae, Icacinaceae, or Leguminosae (Pace et al., 2009; Angyalossy et al., 2012, 2015). In Malpighiaceae, this configuration is recognized as one of the most widely distributed vascular variants (Jussieu, 1843; Schenck, 1893; Chodat and Vischer, 1917; Carlquist, 1991; Quintanar-Castillo and Pace, 2022).

In Malpighiaceae, phloem wedges can form through two different ontogenetic trajectories, which imply either continuity or discontinuity of the cambium (Quintanar-Castillo and Pace, 2022). Most Malpighiaceae including *Mascagnia*, *Peixotoa*, some *Heteropterys*, and some *Stigmaphyllon* have phloem wedges with a continuous cambium (Quintanar-Castillo and Pace, 2022). In contrast, the formation of phloem wedges in *Tristellateia* causes discontinuity of the vascular cambium. This discontinuity arises from the absence of anticlinal divisions, which creates an imbalance in growth rates between the variant and regular regions in the cambium. As a result, the innermost parts of the wedge may be included through mechanical action from the adjacent wood. Phloem wedges with discontinuous cambium may represent a potential synapomorphy of the genus *Tristellateia*. To address this hypothesis, further comparative studies are needed on the ontogenetic trajectories of additional species within the genus, beyond those observed in this work (*T. australasiae* and *T. greveana*). Considering that *Tristellateia* is in Madagascar, where this genus is most abundant (Arènes, 1947), there are likely many new species of the genus to be described (C. C. Davis, Harvard University, personal communication). Phloem wedges with discontinuous cambium have been documented in other angiosperm lineages, such as Bignoniaceae and Leguminosae (Pace et al., 2009; Angyalossy et al., 2015) where similar disruptions in anticlinal divisions in the variant cambium underlie this phenomenon.

## Interxylary phloem

Interxylary phloem is another common vascular variant found in angiosperms. It has been described in families such as Acanthaceae, Combretaceae, Convolvulaceae, Leguminosae, Nyctaginaceae, and Thymelaeaceae (Schenck, 1893; Chalk and Chattaway, 1937; Den Outer and van Veenendaal, 1981, 1995; van Veenendaal and den Outer, 1993; Carlquist, 2013; Angyalossy et al., 2015; Luo et al., 2018, 2019, 2021; Cunha Neto et al., 2021; Rajput et al., 2022; Wilson et al., 2025). The developmental pathways for interxylary phloem are different among groups, and so far, three ontogenetic origins with some subtypes have been recognized (Den Outer and van Veenendaal, 1995; Pace et al., 2009; Carlquist, 2013; Cunha Neto et al., 2021; Quintanar-Castillo and Pace, 2022; Rajput et al., 2022).

In *Henleophytum*, the development of interxylary phloem follows the *Thunbergia*-type pattern, also called the *Combretum* subtype (Den Outer and van Veenendaal, 1995; Rajput et al., 2022). In this pattern, certain regions of the cambium change their activity to produce phloem in both directions, subsequently resuming their regular activity. This process promotes the inward inclusion of the earlier produced phloem. Previous reports of this variant in Malpighiaceae were described solely for *Dicella* (Chodat, 1892; Schenck, 1893; Chodat and Vischer, 1917; Niedenzu, 1928), a genus distantly related to *Henleophytum* (Davis and Anderson, 2010), and *Tristellateia*. In *Tristellateia*, the ontogeny (described above) results from the mechanical inclusion of portions of phloem wedges rather than from a periodical bidirectional phloem formation originating from the cambium.

## Wavy cambium

A wavy pattern in the vascular cambium is a characteristic that has not previously been documented in Malpighiaceae. Although a wavy cambium in lianas had been previously reported for the Old World and widely cultivated *Podranea ricasoliana* (Bignoniaceae), the authors did not consider it as a cambial variant (Pace and Angyalossy, 2013). In the Bunchosia clade, a wavy cambium was observed in *Heladena* and *Thryallis*, although both present characteristics of the lianescent habit, there are also some differences in tissue production between the phloem arcs and the regular regions. These differences can be considered variations in the vascular cambium, but it is still uncertain whether this variation is sufficient to be considered a vascular variant.

Acevedo-Rodriguez (2015) listed *Thryallis laburnum* as having phloem wedges; however, in a macroscopic view, these wedges do not appear to be particularly conspicuous, and our observations of anatomical preparations show a wavy cambium pattern. Compared to the other species described here (*T. brachystachys* and *T. longifolia*), the previously described wedges in *T. laburnum* are better interpreted as a wavy cambium.

A wavy cambium pattern has also been reported in other families such as Convolvulaceae. Obaton (1960) described it in stems of *Bonamia thunbergiana* (=*B. cymosa*), where the vascular cambium had shallow undulating areas that increased and became more marked as the plant grew. However, for Obaton (1960), these shallow phloem depressions that produced a wavy cambium pattern could not be delimited into any known vascular variant, such as phloem wedges. In lianescent groups, where some members exhibit well-defined vascular variants, some lianas do not exhibit variations. Nevertheless, some members may display an irregular girth, with differential tissue production, as observed in Bignoniaceae and Convolvulaceae, as mentioned above. A wavy cambium may therefore facilitate the evolution of more complex vasculatures because this ancestral condition was reconstructed at the node here, leading to the subsequent evolution of phloem wedges and interxylary phloem.

## Conspicuous wide rays

In *Echinopterys*, the vascular cambium produces unusually wide rays that consistently widen from the center to the periphery. A similar situation of rays constantly widening is only common in plants with a vascular variant named “axial elements in segments” (Angyalossy et al., 2012, 2015), which is typical of some families where the interfascicular cambium gives rise solely to rays (e.g., Piperales; Trueba et al., 2015). However, in *Echinopterys*, the wide rays are not confined to the interfascicular areas. As in most plants, new rays are formed as the stem homogeneously grows in girth, instead of by widening the original rays. However, in other lineages with wide rays, such as Annonaceae, several genera also have this phenomenon of rays widening conspicuously toward the periphery, instead of producing new rays (e.g., as reported for *Uvariopsis* and *Pseuduvaria* by Koek-Noorman and Westra, 2012). To the best of our knowledge, there is still no explanation for this variation in wood anatomy.

In *Echinopterys*, the areas that give rise to the wide rays also have greater cambial activity toward the xylem, forming larger amounts of wood rays and generating small arcs of phloem that are limited by these conspicuous wood rays. In a broad sense, the extensions of the xylem toward the phloem and the formation of phloem arcs by the mechanical action of the xylem rays form a wavy pattern, which could be considered also a type of wavy cambium. If any modification of the typical vascular development from modification in the timing, organization, spatial distribution, and relative abundance of xylem and phloem is considered a vascular variant (Carlquist, 2001; Angyalossy et al., 2015; Cunha Neto, 2023), these radial xylem protrusions toward the secondary phloem can be considered as a vascular variant as well. If this is indeed considered a cambial variant, the conspicuous rays in *Echinopterys* would be the first record in Malpighiaceae of a vascular variant in a group with climbing and self-supporting plants. Climbing species in the Bunchosia clade all seem to have some degree of variation in their secondary growth, although their cambia begin with regular activity. *Echinopterys* has self-supporting representatives that also begin with regular activity in the vascular cambium and develop conspicuous, wide rays that have a greater production of wood concerning the secondary phloem (Figure 4, ontogeny 2).

There is not enough information on the evolution of vascular variants in self-supporting species nested in lianescent groups. However, the presence of vascular variants in both lianescent and self-supporting species of the same group has also been described in families such as Convolvulaceae and Leguminosae (Obaton, 1960; Dias-Leme, 2000; Carlquist, 2001; Terrazas et al., 2011) and appears to be a difficult feature to lose during the transition to new habits.

The retention of vascular variants can be considered a case of exaptation, where the variants provide flexibility and resistance in lianas, while in self-supporting species that evolved from lianas, they may provide other advantages such as storage capacity. Anatomical traits common in lianas and present in self-supporting species have been documented in Cucurbitaceae (*Dendrosicyos*), where abundant radial and axial parenchyma—typically preventing stem damage when twisted in many lianas—was likely co-opted for water storage in self-supporting succulent stems (Olson, 2003). This shift from lianas with abundant parenchyma to self-supporting plants with succulent stems for water storage has also been documented in Passifloraceae (*Adenia*) (Hearn, 2006; Hearn et al., 2013). Very prominent rays are related to storing water and carbohydrates and to promoting stem segmentation that allows for twisting and prevents stem tearing, in addition to increasing radial transport surface (Lev-Yadun and Aloni, 1995; Rajput and Rao, 2003; Ceja-Romero and Pérez-Olvera, 2010). In *Echinopterys*, these conspicuous, wide rays with abundant prismatic crystals can be another barrier preventing tearing due to torsion. In this way, the extensions of radial parenchyma from the xylem toward the phloem region can form a kind of gear that provides resistance to torsion and tearing prevention, which is supported by the number of prismatic crystals in the radial parenchyma. The increase in radial parenchyma area in the form of multiseriate, aggregate rays can also be considered to be an adjustment to the difference in growth rates between the axial and radial tissues, similar to how the phloem rays dilate to adjust to an increase in stem circumference (Evert, 2006).

### Evolution of the vascular system in the Bunchosia clade

Regular secondary growth is reconstructed as the ancestral condition in the Bunchosia clade, with five independent evolutions toward more complex forms in the structure of the vascular system. All development pathways initiate their secondary growth with a regular stem and regular cambial activity. However, in both lianescent genera and *Echinopterys*, alterations in the conformation of the vascular system led to different arrangements. In contrast, *Bunchosia* maintains a regular conformation, suggesting retention of this characteristic from the common ancestor of the clade.

The terminal addition within each developmental trajectory can be considered a case of heterochrony by peramorphosis, where new stages are added to ancestral trajectories to form novel configurations (Onyenedum and Pace, 2021). Furthermore, alterations in the production and spatial arrangement of certain tissues can be explained through other developmental mechanisms. The development of phloem wedges and wide, conspicuous rays implies greater production of phloem and ray parenchyma in certain regions of the vascular cambium, which may be an example of heterometry (Onyenedum and Pace, 2021). The formation of interxylary phloem, occupying spaces originally intended for the secondary xylem, can be considered a case of either heterotopy or homeosis (Sattler, 1988; Onyenedum and Pace, 2021). Finally, the retention of vascular variants in derived self-supporting forms may reflect phylogenetic inertia (Onyenedum and Pace, 2021) or could be an example of exaptation (Gould and Vrba, 1982) if these retained characters serve a function.

Our results underscore the significant role of ontogeny in explaining the morphological variation of the vascular system across different habits. By examining how these variations likely evolved throughout the evolutionary history of the group, we have gained valuable new insights into the modifications that the vascular system experienced in the Malpighiaceae. These insights are particularly relevant for understanding transitions between habits, especially in clades where self-supporting forms are nested within lianescent lineages. The latter nested relationship indicates a complex evolutionary history that requires further investigation to comprehend the ecological and functional consequences of these transitions.

Integrating anatomical studies with ontogenetic perspectives allows us to contextualize our results within a broader evolutionary framework. This combination provides a deeper understanding of why such variation exists and identifies the developmental processes driving this diversity.

Although our findings focus on a specific clade within the Malpighiaceae, they have broader implications for understanding how different vascular system configurations develop in relation to the type of habit. Future research in other plant groups will be essential to identify additional developmental processes involved in forming these cambial variants and to determine whether convergent patterns exist in their evolution.

## CONCLUSIONS

The diversity in the vascular system of the Bunchosia clade is intricately linked to its development. This study shows that different ontogenetic pathways led to different arrangements of the vascular system, which are present in both lianas and in one of the self-supporting liana-descendent species, *Echinopterys setosa*. The common ancestor of the Bunchosia clade is reconstructed to be a climbing plant (liana) that had regular secondary growth. Within the clade, four different vascular variants evolved independently. Although the ontogenetic trajectories of these vascular variants resulted in different arrangements, their initial developmental states are conserved. The addition of developmental stages in the ontogeny of vascular variants can be interpreted through different mechanisms of developmental change. The interxylary phloem may represent a case of heterotopy or homeosis, while the phloem wedges and conspicuous rays exemplify heterometry. Differences in the final stages of development in each ontogenetic trajectory likely are a case of heterochrony by peramorphosis. Furthermore, variations in the cambium of self-supporting species may indicate either retention from common ancestry or an exaptation case. Our findings regarding the evolution of the vascular system also point to the need for a more comprehensive delimitation of vascular variants and the modifications required for them to be recognized. These results emphasize the importance of integrating both anatomical and developmental perspectives to better understand the evolution of habit and the vascular system.

## AUTHOR CONTRIBUTIONS

A.Q.C.: conceptualization, data curation, formal analysis, writing original draft, review, and editing. A.M.A.: resources, review and editing. M.R.P.: conceptualization, data curation, funding acquisition, resources, supervision, writing, review, and editing.

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## DATA AVAILABILITY STATEMENT

Phylogenetic tree, data matrix and codes necessary to reproduce the analyses in this paper are openly available in Zenodo at https://doi.org/10.5281/zenodo.14956639.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Appendix S1.** Species collected for the Bunchosia clade.

**Appendix S2.** List of characters, states, and models used in the reconstruction of ancestral states of habit and ontogeny of the Bunchosia clade.

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