American Journal of Botany

RESEARCH ARTICLE

Striking developmental convergence in angiosperm endoparasites

Luiza Teixeira-Costa[1,2,3], Charles C. Davis[2], and Gregorio Ceccantini[1]

Manuscript received 6 March 2020; revision accepted 7 January 2021.

[1] Institute of Biosciences, University of Sao Paulo, Sao Paulo 05508-090, Brazil

[2] Harvard University Herbaria, Cambridge, MA 02138, USA

[3] Author for correspondence (e-mail: luiza.teixeirac@gmail.com)

Citation: Teixeira-Costa, L., C. C. Davis, and G. Ceccantini. 2021. Striking developmental convergence in angiosperm endoparasites. *American Journal of Botany* 108(5): 1–13.

doi:10.1002/ajb2.1658

PREMISE: A subset of parasitic plants bear extremely reduced features and grow nearly entirely within their hosts. Until recently, most of these endoparasites were thought to represent a single clade united by their reduced morphology. Current phylogenetic understanding contradicts this assumption and indicates these plants represent distantly related clades, thus offering an opportunity to examine convergence among plants with this life history.

METHODS: We sampled species from Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae spanning a range of developmental stages. To provide a broader comparative framework, Santalaceae mistletoes with a similar lifestyle were also analyzed. Microtomography and microscopy were used to analyze growth patterns and the ontogeny of host–parasite vascular connections.

RESULTS: Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae species demonstrated a common development characterized by late cell differentiation. These species were also observed to form direct connections to host vessels and to cause severe alterations of host xylem development. Apodanthaceae and Rafflesiaceae species were additionally observed to form sieve elements, which connect with the host phloem. Endophytic Santalaceae species demonstrated a dramatically different developmental pattern, featuring early cell differentiation and tissue organization, and little effect on host anatomy and cambial activity.

CONCLUSIONS: Our results illuminate two distinct developmental trajectories in endoparasites. One involves the retention of embryonic characteristics and late connection with host vessels, as demonstrated in species of Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae. The second involves tissue specialization and early connection with host xylem, as exemplified by Santalaceae species. These differences are hypothesized to be related to the absence/presence of photosynthesis in these plants.

KEY WORDS Apodanthaceae; *Arceuthobium*; Cytinaceae; endoparasite; holoparasite; mistletoe; Mitrastemonaceae; parasitic plants; plant development; Rafflesiaceae.

Plant parasitism has evolved 12 times within flowering plants (Nickrent, 2020). This phylogenetic diversity is accompanied by a wide variety of habits, geographical distribution, host preferences, morphologies, and developmental patterns (Kuijt, 1969; Heide-Jørgensen, 2008). A broad spectrum of photosynthetic capabilities is also represented among these plants, ranging from species fully capable of photosynthesis, to those that are achlorophyllous during all or most of their life cycle, thus rendering them entirely reliant on host resources (Bromham et al., 2013). A subset of plants in the latter category exhibits a particularly extreme degree of reduction: their vegetative body is reduced to mycelium-like filaments of parenchyma cells embedded within their host tissues. In these cases, the parasite only becomes visible during reproduction when the flower/inflorescence temporarily emerges from its host (Mauseth, 1990; Meijer and Veldkamp, 1993; Nikolov et al., 2014b). Due to this extremely reduced growth form, restricted to life inside its host, these plants are commonly referred to as endoparasites.

This highly modified and cryptic growth habit has been best described in detail from a small number of mistletoe species (Těšitel, 2016). Mistletoes are widely distributed parasitic plants known as keystone species in both natural and human-altered ecosystems (Watson, 2009; Kuijt, 2015; Griebel et al., 2017). They belong to three

families within the order Santalales and germinate directly upon the stems and branches of their hosts (Aukema, 2003; Vidal-Russell and Nickrent, 2008; APG, 2016). In species such as *Arceuthobium pusillum* (Santalaceae), *Tristerix aphyllus* (Loranthaceae), and *Viscum minimum* (Santalaceae), abortion of aerial organs is observed soon after germination and further development is restricted to the embryo root pole, which matures into a profuse endophytic tissue system (Thoday and Johnson, 1930; Mauseth et al., 1985; Kuijt, 1986). Owing to their negative economic impact on timber production (in the case of *Arceuthobium* spp.; Hawksworth, 1983) and the relative ease in cultivating these species ex situ, the germination and ontogeny of these mistletoes have been the subject of numerous detailed analyses (Thoday and Johnson, 1930; Cohen, 1954; Mauseth et al., 1985; Lye, 2006; Mauseth and Rezaei, 2013). Importantly, this literature has formed the primary basis of our understanding of the endophytic lifestyle in plants (Heide-Jørgensen, 2008; Twyford, 2017). In contrast, the bulk of endoparasite diversity remains poorly investigated.

These frequently overlooked endoparasite species represent four plant families: Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae. Together, these clades include 50 species, all of which are endoparasites (Nickrent, 2020). Among these families, Apodanthaceae includes two genera, *Apodanthes* and *Pilostyles* (10 species), which colonize the stems of Fabaceae and Salicaceae (Bellot and Renner, 2014). Cytinaceae also includes two genera, *Bdallophytum* and *Cytinus* (12 species), which parasitize mostly Burseraceae and Cistaceae, respectively (Visser, 1981; Burgoyne, 2006; Alvarado-Cárdenas, 2009; de Vega et al., 2009). Mitrastemonaceae includes only the genus *Mitrastemon* (2 species), which is parasitic in the roots of Fagaceae trees (Makino, 1909; Yamamoto, 1936). Finally, Rafflesiaceae includes three genera, *Rafflesia*, *Rhizanthes*, and *Sapria* (36 species), which are well known for their massive flowers that bloom on *Tetrastigma* host vines (Vitaceae) (Nikolov et al., 2014b). In addition to the cryptic nature of these species, they often occur in fragmented populations with a disjunct geographical distribution, which adds to the difficulties in sampling these endoparasites across comprehensive developmental stages. In fact, these features, together with aspects of floral morphology and ovule and seed structure have previously been used to circumscribe these species into a single family, Rafflesiaceae (Bouman and Meijer, 1994; Takhtajan, 1997). This grouping was later segregated into four families, which were until relatively recently maintained in a single order, Rafflesiales (Nickrent, 2002).

Our traditional understanding of the phylogenetic affinities and circumscription of Rafflesiaceae/Rafflesiales has changed dramatically in the past decade. With the advent of large-scale molecular sequencing, it has been revealed that Rafflesiales as traditionally circumscribed are polyphyletic, with its former members dispersed broadly across numerous flowering plant orders (Nickrent et al., 2004; Filipowicz and Renner, 2010). Large-flowered Rafflesiaceae have been shown to group with Euphorbiaceae, within Malpighiales (Davis et al., 2007). Cytinaceae have been placed within Malvales, as sister to Muntingiaceae (Nickrent, 2007). Mitrastemonaceae, once confidently placed within Ericales (Barkman et al., 2004, 2007), has been tentatively placed as sister to Lecythidaceae (Rose et al., 2018). And Apodanthaceae are placed within Curcubitales (Filipowicz and Renner, 2010). This greatly revised phylogenetic understanding of these four main subclades of endoparasites clarifies that this lifestyle has evolved multiple times and suggests that features of this life mode arose convergently rather than by common ancestry as previously hypothesized (Barkman et al., 2004; Nickrent et al., 2004).

In light of this new phylogenetic understanding, a revised assessment of endoparasitism in angiosperms is warranted and is the goal of our study. Here, we focus our attention on a detailed comparative analysis of development in each of the four families previously grouped as Rafflesiales. Our analysis will help illuminate the cryptic nature of how parasitic plants interact with their hosts. Moreover, we compare the development of species in these four understudied families to well-known endophytic mistletoes species belonging to Santalales: *Arceuthobium douglasii* and *Viscum minimum*. This comparative context will enable important advancements in our understanding of the parasitic life form among plants.

MATERIALS AND METHODS

Plant material and sampling

We analyzed six species representing the four clades that are exclusively composed of endoparasites: Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae. During the sampling, plant material was selected to represent the developmental progression of each species. Because germination and initial host penetration are largely unknown for these plants, our investigation began by sampling sections of infested host organs where no signs of the parasite were externally visible. Based on previous reports estimating the extension of the parasitic tissue in each species, host tissue sampled a few centimeters away from the external signs of the parasite was a landmark to identify the earliest possible stages of endophyte development (Jochems, 1928; Watanabe, 1936; García-Franco and Rico-Gray, 1996; García-Franco et al., 1998; Barkman et al., 2017).

Due to the cryptic nature of these plants, parasitic flower bud size was used as a proxy for the progression of subsequent developmental stages. Thus, in addition to host stems/roots with no external signs of the parasite, sampled material ranged from sections of host roots/stems bearing parasite flower buds of varying sizes, to sections in which only the senescent flower (or flower abscission scar) were present in the host parasitized organ. In all cases, fresh field-collected material was preserved in paraformaldehyde-glutaraldehyde (Karnovsky's solution). Rafflesiaceae specimens were originally preserved in formalin–acetic acid (FAA).

To provide a broader comparison, two Santalaceae species were added to our analyses, representing two of the three genera that include endophytic mistletoes. In both analyzed mistletoe species, the germinated seed remains visible upon the host branch for ca. 2 years (Hawksworth and Wiens, 1996), allowing us to easily trace the initial stages of developmental in these endophytic mistletoes. Inflorescence size and maturation were used as proxies for the developmental progression of the vegetative body. Figure 1 illustrates the morphology and phylogenetic relationships among all analyzed species. Table 1 provides a complete list of the analyzed species, their respective hosts and infested host organs, as well as sampling locations.

Microtomography

To provide an understanding of the parasite's endophytic extension within the host body, we analyzed samples using microtomography at

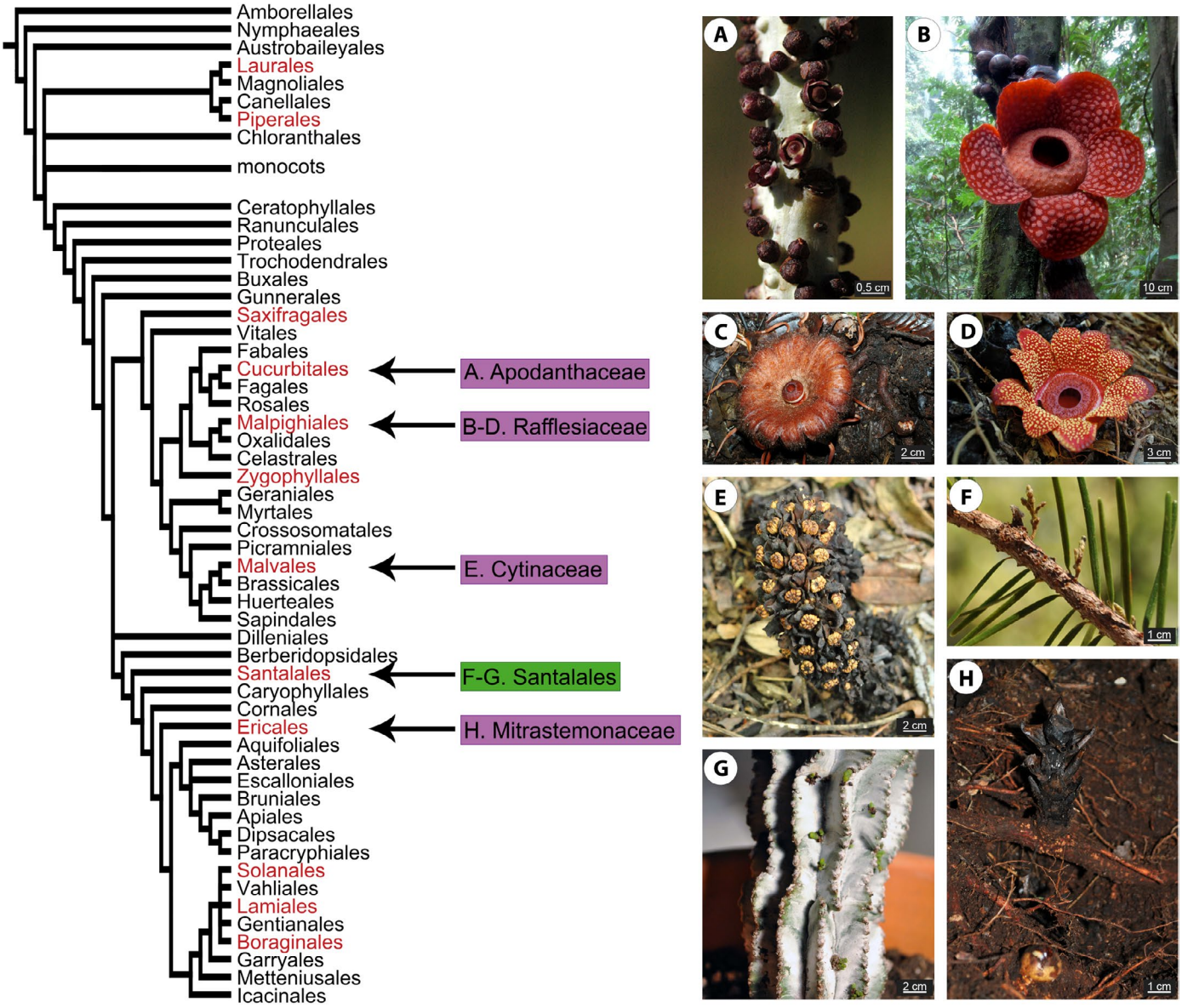


FIGURE 1. Phylogenetic position and general morphology of analyzed species. (A) *Pilostyles blanchetii* (Apodanthaceae). (B) *Rafflesia cantleyi* (Rafflesiaceae). (C) *Rhizanthes lowii* (Rafflesiaceae). (D) *Sapria himalayana* (Rafflesiaceae). (E) *Bdallophytum americanum* (Cytinaceae). (F) *Arceuthobium douglasii* (Santalaceae). (G) *Viscum minimum* (Santalaceae). (H) *Mitrastemon matudae* (Mitrastemonaceae). Tree topology modified from APG IV (APG, 2016). Names in red indicate orders that include parasitic plants. Names highlighted in purple and green indicate clades that include endoparasites and endophytic mistletoes, respectively.

the Center for Nanoscale Systems (Harvard University, Cambridge, MA, USA) and at the Microtomography Laboratory (University of São Paulo, São Paulo, Brazil) using a Nikon X-Tek HMXST225 imaging system (Manassas, VA, USA) and a Bruker Skyscan1176 high performance in vivo scanner (Kontich, Belgium), respectively. Following the method proposed by Teixeira-Costa and Ceccantini (2016), plant samples were either injected or, in the case of potted plants, watered with contrasting solutions to enhance the observation of parasitic tissues within the host.

For all analyzed species, 0.1% w/v Lugol's solution was used to increase the contrast between host and parasite parenchyma and improve endophyte detection in all species. This solution was chosen based on reports of histochemical analyses demonstrating differences between parasite and host tissues in terms of starch storage (Engler and Krause, 1908; do Amaral, 2007; Mauseth and Rezaei, 2013; Nikolov et al., 2014b). Additionally, fixed samples were also injected with aqueous solutions of heavy metal salts, such as 0.2% w/v lead citrate ($C_6H_8O_7Pb$) and 0.2% w/v lead nitrate ($PbNO_3$), to improve the detection of host–parasite xylem connections. Three-dimensional reconstruction was conducted using CT-Pro 3D (version XT 3.1.11, Nikon) and NRecon software (version 1.0.0, Bruker microCT). Analyses and acquisition of images and videos were performed with VG Studio Max (version 3.0, Volume Graphics) and CT-Vox software (version 3.3.1, Bruker microCT).

TABLE 1. Analyzed endoparasitic species, their respective hosts, sampling location and herbaria where voucher material is deposited.

Parasitic plant family (Order)	Parasitic species	Host species	Parasitized host organ	Sampling location	Herbaria and vouchers
Apodanthaceae (Curcubitales)	*Pilostyles blanchetii* (Gardner) R.Br.	*Mimosa maguirei* Barneby and *M. foliolosa* Benth.	Stems	Serra do Cipó, Minas Gerais, Brazil	Universidade de Sao Paulo (SPF), GC 4163
Cytinaceae (Malvales)	*Bdallophytum americanum* (R.Br.) Eichler ex Solms	*Bursera linanoe* (La Llave) Rzed., Calderón & Medina	Roots	Reserva de la Biósfera Sierra de Huautla, Morelos, Mexico	Universidad Autónoma de Aquascalientes (HUAA), LTC 318
Mitrastemonaceae (Ericales)	*Mitrastemon matudae* Yamam.	*Quercus* sp.	Roots	Reserva de la Biósfera la Sepultura, Chiapas, Mexico	Universidad Autónoma de Aquascalientes (HUAA), LTC 324
Rafflesiaceae (Malpighiales)	*Rafflesia cantleyi* Solms	*Tetrastigma* cf. *rafflesiae* Planch.	Roots and stems	Sabah, Malaysian Borneo, Malaysia	Harvard University Herbaria (A), coll. C. Davis 2007
	Rhizanthes lowii (Becc.) Harms	*Tetrastigma* cf. *coriaceum* (DC.) Gagnep.	Roots		Harvard University Herbaria (A), coll. C. Davis 2007
	Sapria himalayana Griff.	*Tetrastigma* cf. *cruciatum* W.G.Craib & Gagnep.	Roots		Harvard University Herbaria (A), coll. C. Davis 2007
Santalaceae (Santalales)	*Arceuthobium douglasii* Engelm.	*Pseudotsuga menziesii* (Mirb.) Franco	Stems	Crater Lake, Oregon, USA	Universidade de Sao Paulo (SPF), GC 4278
	Viscum minimum Harv.	*Euphorbia polygona* Haw. *Euphorbia obesa* Hook. f.	Stems	Cultivated in EEB Greenhouses at University of Connecticut	-

Histology

Due to the nondestructive nature of tomography scanning, we were able to use the same samples for both microtomography and histological preparations. Segments of parasitized host organs of all host–parasite associations were embedded in paraffin for serial microtome sectioning. Specimens were dehydrated in both ethanol (10% to 70%) and butanol–ethanol solutions (50:50 to 100:0) and infiltrated with paraffin using a vacuum incubator (Ruzin, 1999). Serial sections were obtained using a Leica RM2145 rotary microtome (Nussloch, Germany) with a conventional C knife. Histological sections were stained in either toluidine blue or in safranin and astra blue (Kraus and Arduin, 1997). Aniline blue was also applied to detect the presence of callose in phloem sieve cells (Angyalossy et al., 2016).

Confocal microscopy

Thicker histological sections (~50–100 μm) were cut with a Leica SM 2000R sliding microtome (Nussloch, Germany) coupled with a Thermo Fisher R404a cooling system (Asheville, NC, USA). Sections were then stained with aqueous solutions of either 0.1% w/v safranin or 0.01% w/v acridine orange to enhance the observation of tracheary elements (Ruzin, 1999). Analysis and image acquisition were carried out at the Center for Acquisition of Images and Microscopy of the Institute of Biosciences, University of São Paulo using a Zeiss LSM 880 microscope (Jena, Germany). Zeiss Zen Blue software (version 3.2.0) was used for image analysis.

RESULTS

Early endophyte development

The earliest developmental stage analyzed in Apodanthaceae, Cytinaceae, Mitrastemonaceae and Rafflesiaceae species was sampled from areas of the parasitized host organ where no sign of the parasite was visible externally (Fig. 2A). Parasitic endophyte tissue at this stage is composed of extremely reduced parenchyma cells, which are grouped as small masses or thin filaments (Fig. 2B, C). Due to the reduced size of these cell clusters, detection of parasitic tissue within the host using microtomography scanning was not possible at this stage. Conversely, early development of the endoparasitic mistletoes within the host body was readily detected via microtomography scanning just 3 months after seed germination, as shown for *Viscum minimum* (Santalaceae, Fig. 2D).

At a later developmental stage, the tissue of endophytic mistletoes grows to occupy larger extensions of the host bark (Fig. 2E), forming an intricate mesh of parasitic and host tissues (Fig. 2F). In both mistletoe species, initial cell differentiation and organization was already observed at this stage (Fig. 2F). The tissue of endoparasites also grows to colonize larger areas of the host bark. In the endophytic parasites, earliest possible detection of endophyte proliferation via microtomography occurred during this phase, as observed in *Bdallophytum americanum* (Cytinaceae, Fig. 2G). However, these extensive tissues still remained undifferentiated until the onset of flower bud development (Fig. 2E).

Xylem differentiation and host connection

Differentiated vessel elements were observed in all analyzed species. Likewise, direct parasite–host vascular connections were

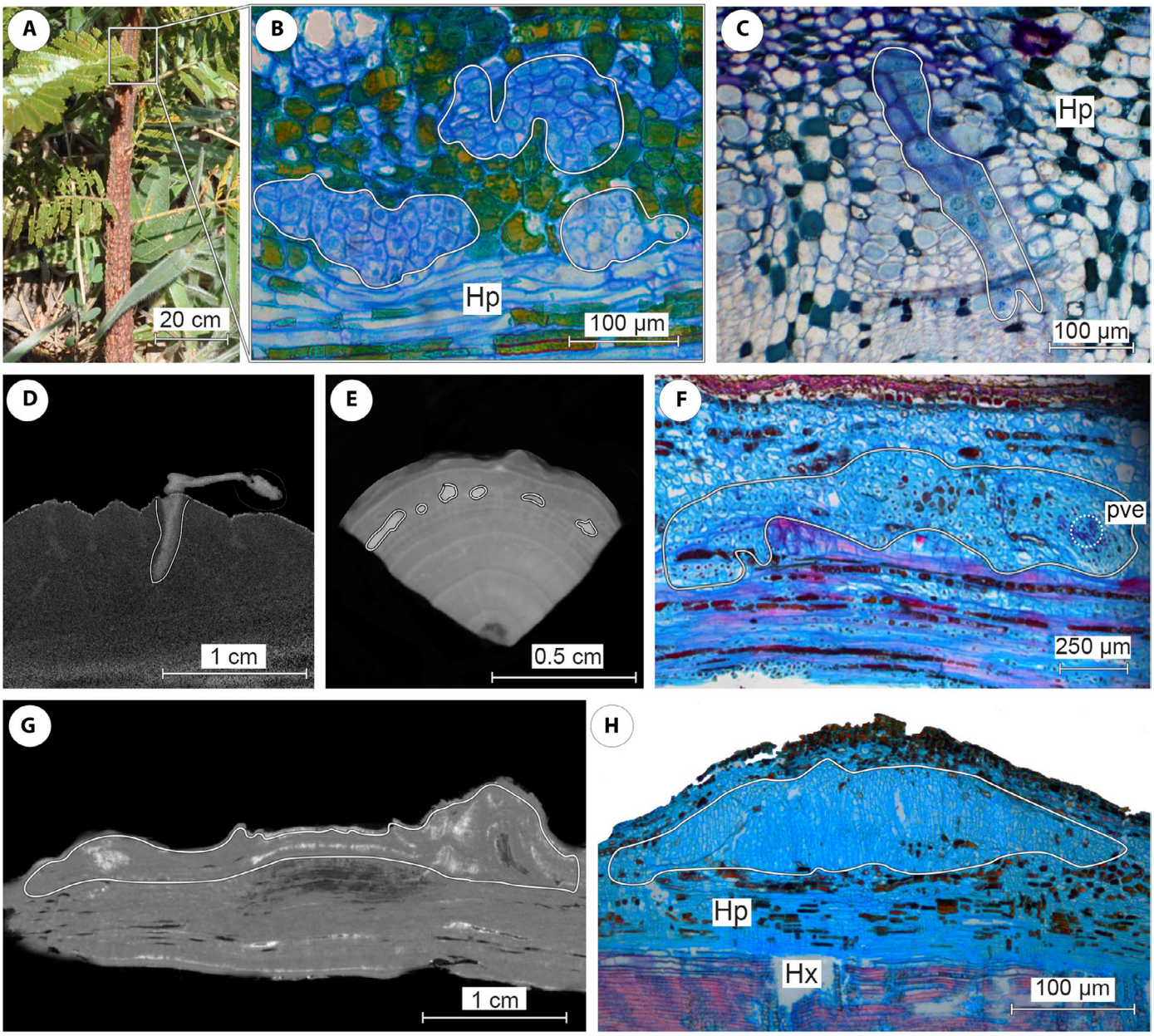


FIGURE 2. Early developmental stages of endoparasites within the host body. (A) Host branch infested by *Pilostyles blanchetii*. (B, C) Cross sections of host phloem stained in toluidine blue showing clusters of parenchyma cells formed by *Pilostyles blanchetii* and filament of parenchyma cells formed by *Rafflesia cantleyi*, respectively. (D) Microtomography scan showing initial penetration of host tissues by *Viscum minimum*. (E, F) Microtomography scan and longitudinal section stained in safranin and astra blue of *Arceuthobium douglasii* occupying the host bark; in (F), note the presence of differentiated parasitic vessel elements. (G, H) Microtomography scan and longitudinal section stained in safranin and astra blue of host root infested by *Bdallophytum americanum* atearly stage of flower bud formation within the host phloem. Hp: host phloem, Hx: host xylem; pve: parasite vessel element.

detected in most cases, with the exception of *Viscum minimum* (Santalaceae). Vessel–vessel connections were formed via growth of parasitic tissue among host ray cells, producing the structure known as the sinker, through which xylary connection with the host is achieved (Fig. 3A-C). It is noteworthy that, in the endophytic mistletoe *A. douglasii*, sinker development and host wood penetration occurred when the parasitic system of cortical strands was already established within the host bark (Fig. 3B). Cortical strands showed a tiered, root-like cell organization, with a central area occupied by vessel elements and a peripheral area occupied either by sieve tubes or by parenchyma cells often containing phenolic compounds (Fig. 3B).

On the other hand, vessel element differentiation in Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae species was observed to be related with flower bud onset (Fig. 3C, D). Sinker proliferation was followed by differentiation of

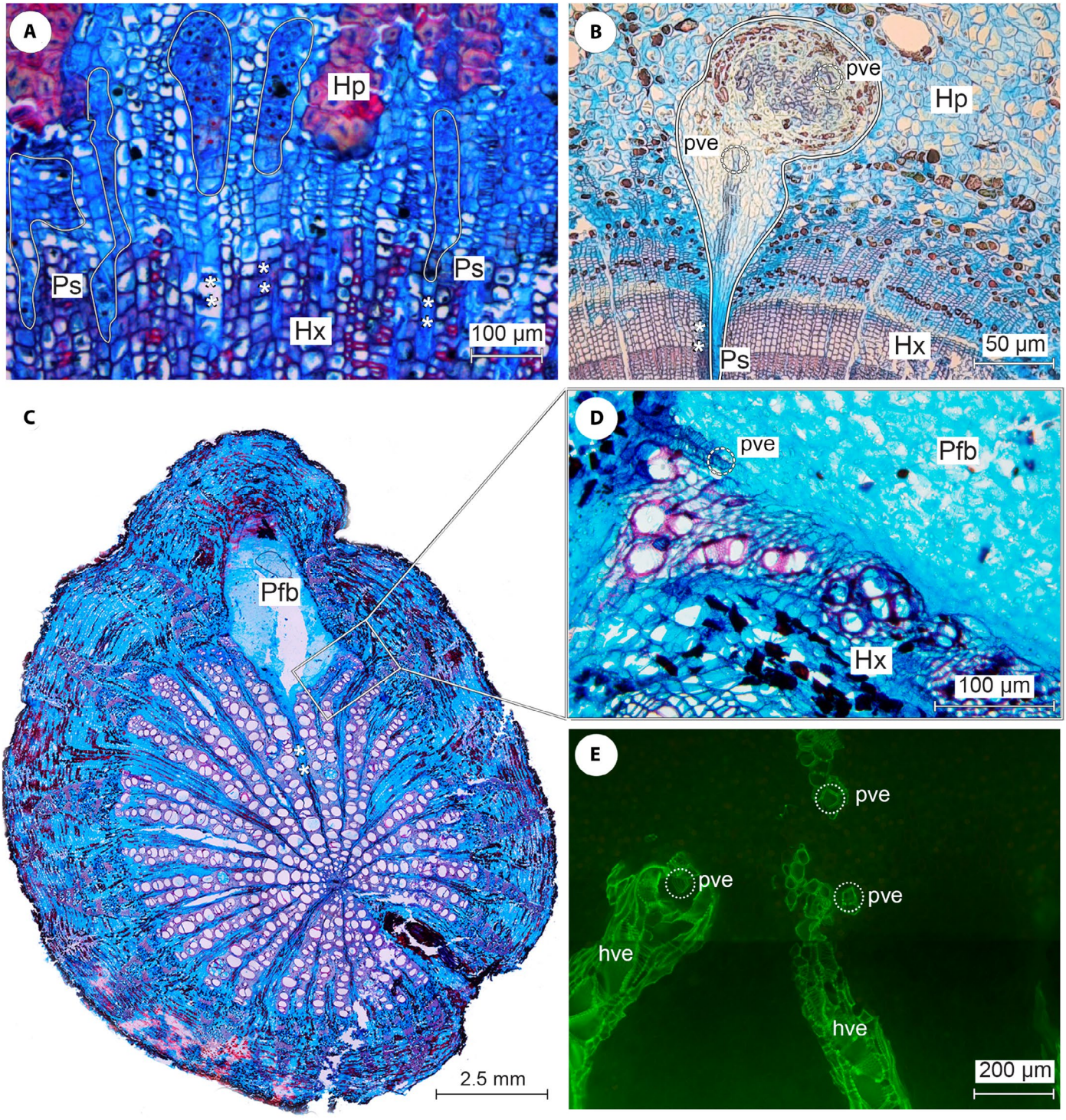


FIGURE 3. Differentiation of parasitic vascular tissue and connection to the host xylem. (A–D) Cross section stained in safranin and astra blue. (A) Parasitic sinkers of *Mitrastemon matudae* extending from the host phloem into the host xylem by growing among ray cells. (B) Parasitic sinkers of *Arceuthobium douglasii* extending from the host phloem into the host xylem by growing among ray cells; note the presence of differentiated parasitic vessel elements. (C) Initial parasite flower bud formation by *Sapria himalayana*. (D) Early differentiation of parasite vessel elements associated with the development of the flower bud in *S. himalayana*. (E) Confocal micrograph stained in acridine orange showing direct connection between parasite vessel elements of *Rafflesia cantleyi* and host vessel elements. Hp: host phloem, hve: host vessel element, Hx: host xylem, Pfb: parasite flower bud, Ps: parasite sinker, pve: parasite vessel element, asterisks: host ray cells.

parenchyma cells into tracheary elements, as found in *Sapria himalayana* (Rafflesiaceae) (Fig. 3D). In all of these species, direct host–parasite luminal connection was established during the early stages of flower bud formation, prior to the rupture through the host bark and anthesis. Frequently, a single host vessel, composed of large vessel elements of the host vine *Tetrastigma* sp. (Vitaceae), was abutted by multiple vessel elements of the parasite, observed to be short and narrow, as in *Rafflesia cantleyi* (Rafflesiaceae; Fig. 3C).

Parallel to the differentiation of parasitic conductive cells in Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae species, host cambial activity also seemed to be enhanced in all respective host species. In certain areas of the host cambium, as new vessels were produced, differentiation occurred toward parasitic tissue, instead of following the axial direction of the wood. Once again, this alteration was prominently detected in Rafflesiaceae species due to the large vessels formed by the host vine (Fig. 4A). Changes in host cambial activity were also observed as an increase in vessel density accompanied by a reduction in vessel lumen size. These alterations were conspicuously noted in the host of *Mitrastemon matudae* (Mitrastemonaceae), *Quercus* sp. (Fagaceae), due its ring-porous wood characterized by the formation of large vessels near the end of each growth ring (Fig. 4B). Changes in host cambial activity regarding xylem production were not observed in the hosts of the analyzed endophytic mistletoes.

A particular form of growth was observed in *Bdallophytum americanum* (Cytinaceae). In this species, endophytic tissue proliferated tangentially underneath the host cambial zone, nearly encircling the host wood (Fig. 3C). Cambial activity was not interrupted by the parasitic growth, as new xylem cells were continuously produced by the host, leading to the formation of a concentric organization of endophytic tissue alternating with layers of host xylem (Fig. 3C). Narrow vessels were observed in the host wood associated with the concentric rings of parasitic tissue (Fig. 3C).

Phloem differentiation and host connection

Direct contact between parasite and host phloem was observed for species representing only two of the analyzed clades. In Rafflesiaceae species (*Rafflesia cantleyi*, *Rhizanthes lowii*, and *Sapria himalayana*), phloem differentiation was restricted to the base of each flower bud (Fig. 4D). Host phloem was observed to form part of the tissue at the base of the parasitic flower bud (Fig. 4D, box). Alterations in the host sieve tube differentiation were also noted, with new sieve elements differentiated toward the parasitic tissue (Fig. 4E). Following flower anthesis and consequent protrusion through the host bark, a collar-like structure containing host phloem tissue was formed around the base of the parasitic flower (Fig. 4E, box). In *Pilostyles blanchetii* (Apodanthaceae), on the other hand, sieve tubes appeared scattered across the host phloem. Parasitic phloem cells also abutted host sieve tubes, indicating the establishment of direct connections (Fig. 4F).

No direct phloem connection was observed between the other four species analyzed, i.e., *A. douglasii* (Santalaceae), *B. americanum* (Cytinaceae), *M. matudae* (Mitrastemonaceae), and *V. minimum* (Santalaceae), and their respective hosts. Nevertheless, parenchyma cells in the parasite were observed close to host sieve tubes, especially in *M. matudae* and *B. americanum* (Fig. 4G). Such proximity could indicate the formation of indirect host–parasite phloem connections mediated by parasitic parenchyma cells.

DISCUSSION

All species analyzed here share a set of similarities often associated with their endoparasitic lifestyle. First, all of these plants exhibit high host-specificity and infest a small subset of species in the areas in which they occur (Meijer and Veldkamp, 1993; Heide-Jørgensen, 2008; Alvarado-Cárdenas, 2009; Bendiksby et al., 2010; Bellot and Renner, 2014). The geographic distribution of most endoparasitic species is also restricted and often disjunct (Meijer and Veldkamp, 1993; Nickrent, 2007; Bendiksby et al., 2010; Bellot and Renner, 2014; Maul et al., 2018), except for *Arceuthobium* species, which are widespread across the northern hemisphere (Hawksworth and Wiens, 1996). Second, most of these plants exhibit a common ontogenetic origin of their flowering shoots. Among these species, flower/inflorescence development is considered to be not only endogenous because parasitic tissues are deeply embedded within their hosts, but in many cases their floral organs also originate from a secondary (endogenous) morphological surface (Kuijt, 1969). This has been described in species of Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae (Solms-Laubach, 1867, 1874; Watanabe, 1936b; Nikolov et al., 2013, 2014a). Inflorescences of another endophytic mistletoe, *Tristerix aphyllus* (Loranthaceae), have also been reported to develop in a similar manner (Mauseth et al., 1984).

Despite these commonalities, the endoparasites investigated here were observed to follow two distinct developmental trajectories. Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae species showed vegetative development characterized by late cell differentiation (Fig. 5A–D). In contrast, the development of the endophytic mistletoes *Arceuthobium douglasii* and *Viscum minimum* is characterized by early cell differentiation and tissue organization (Fig. 5E–H). Differences between these two developmental trajectories are discussed in the following sections in terms of their growth stages before and after the establishment of parasite–host vascular connections.

Early development of endoparasitic plants

Two different patterns of development were detected: *Rafflesia*-type, observed for Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae species; and *Arceuthobium*-type, observed for *A. douglasii* and *Viscum minimum*. Differences among the two patterns were clear early in the life cycle of the analyzed plants. Among species following the *Rafflesia*-type pattern, seed germination and initial host penetration remain largely unknown (Těšitel, 2016). Anecdotal reports indicate that the parasite's seed germinates close to the host root (Heinricher, 1917) (Fig. 5A). Nevertheless, germination assays conducted for Apodanthaceae and Rafflesiaceae species in which parasite seeds have been placed in close contact or even within host tissues have been unsuccessful (Brasil, 2010; Molina et al., 2017). These negative results suggest that these endoparasites might depend on specific host-derived signals for germination, as reported for several other parasitic plants, especially those that lack photosynthetic activity (Baskin and Baskin, 2014). In contrast, mistletoe seed germination is generally host-independent and triggered solely by abiotic factors (Lamont, 1983; Baskin and Baskin, 2014). Autonomous germination has also been reported for the endophytic species following the *Arceuthobium*-type pattern (Kuijt, 1986, 2015; Hawksworth and Wiens, 1996) (Fig. 5E).

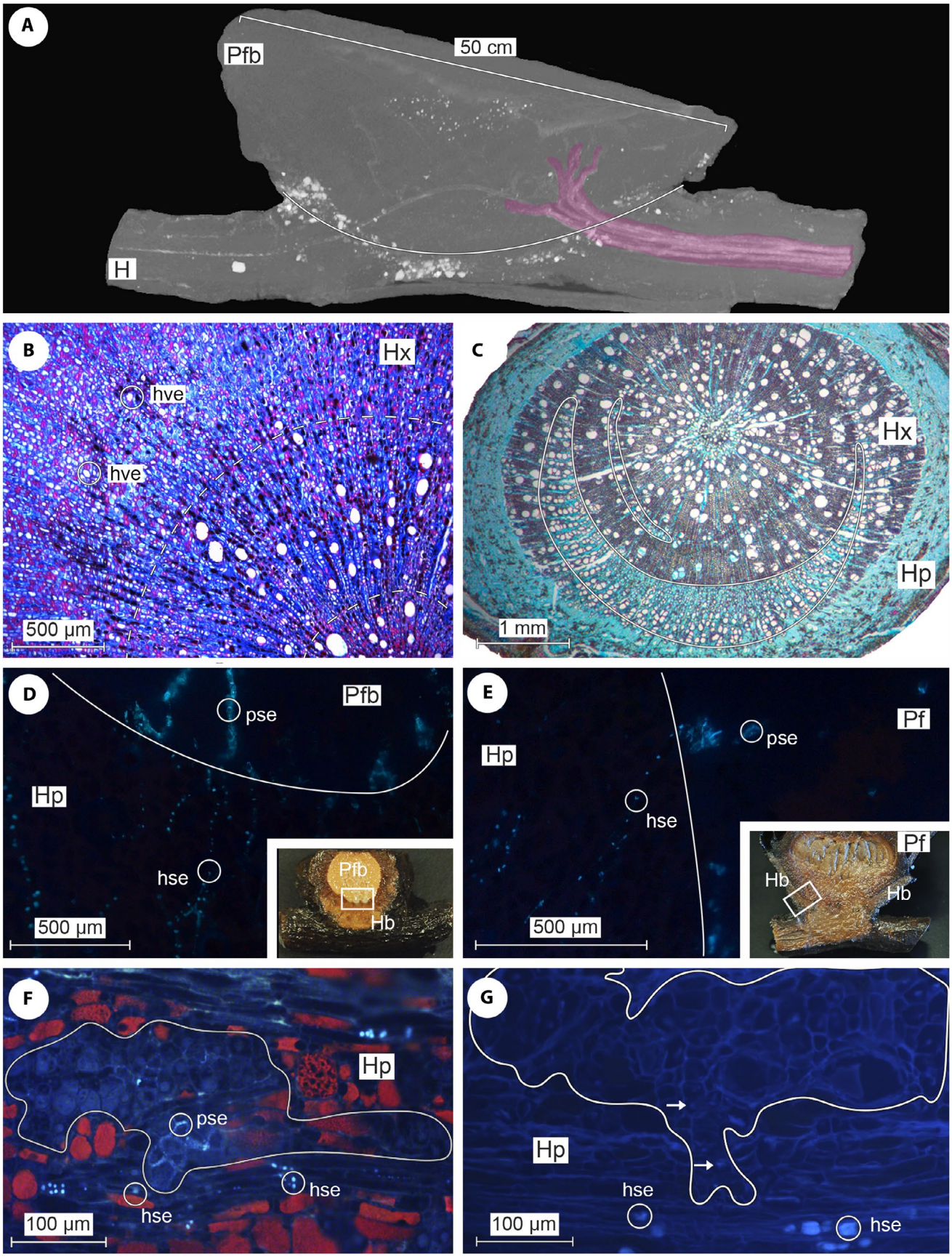

A
Pfb
50 cm
H
B
Hx
hve
hve
500 µm
C
Hx
Hp
1 mm
D
Pfb
pse
Hp
hse
500 µm
Pfb
Hb
E
Hp
pse
Pf
hse
500 µm
Pf
Hb
Hb
F
Hp
pse
hse
hse
100 µm
G
Hp
hse
hse
100 µm

FIGURE 4. Alterations to the host vascular system. (A) Microtomography scan showing vessels (pink) of the host differentiated toward the parasite flower bud formed by *Rafflesia cantleyi*. (B, C) Cross section stained in safranin and astra blue. (B) Host xylem in a root infested by *Mitrastemon matudae*; note narrow host vessels on the outer-most growth ring (delimited by dashed lines). (C) Colonization of host xylem by *Bdallophytum americanum* at later developmental stages. (D–G) Confocal micrographs stained in aniline blue showing longitudinal sections of host phloem. (D) Area of the host bark in contact with the parasite flower bud formed by *Rhizanthes lowii*; note contact between host and parasite sieve elements. (E) Area of the host bark forming a collar-like structure around the parasite flower formed by *Rhizanthes lowii*; note contact between host sieve elements and parasite sieve elements. (F) Sieve elements and clusters of parasitic cells containing parasite sieve elements formed by *Pilostyles blanchetii*. (G) Sieve elements and clusters of parasitic cells formed by *Bdallophytum americanum*; note large nuclei of parasitic cells (arrows). H: host, Hb: host bark, Hp: host phloem, hse: host sieve element, hver: host vessel element, Hx: host xylem, Pf: parasite flower, Pfb: parasite flower bud, pse: parasite sieve element.

Regardless of how host germination and initial host penetration occur in species exhibiting the *Rafflesia*-type pattern, early stages of development consist of reduced parenchymatic filaments or cell masses irregularly dispersed within the host bark (Fig. 5B). The appearance of these filaments or masses has been compared to the proembryonic development stage of other angiosperms, suggesting an instance of pedomorphosis, i.e., protracted juvenilism (Nikolov et al., 2014b). Indeed, ultrastructural analyses suggest endophytic cells to be characteristically undifferentiated (Dell et al., 1982; Kuijt et al., 1985). At the stage of earliest possible detection, we observed that these cell clusters are part of a continuous endophytic body that extends within the host bark. These results corroborate analyses of population genetics reported for adjoined and adjacent inflorescences of *Bdallophytum americanum* (García-Franco et al., 1998) and for Rafflesiaceae individuals spreading over 10 m within the host body (Barkman et al., 2017).

In the *Arceuthobium*-type pattern, initial development is marked by early cell differentiation and tissue organization forming cortical strands (Fig. 5F). Once established, these cortical strands can spread several centimeters within the host body, giving rise to numerous inflorescences (Mauseth et al., 1985; Lye, 2006; Mauseth and Rezaei, 2013). A similar pattern involving the formation of cortical strands soon after initial seed germination is also observed for closely related, non-endophytic mistletoe species (Calvin and Wilson, 1996; Wilson and Calvin, 2006; Mauseth and Rezaei, 2013; Kuijt, 2015). Indeed, analysis of haustorial development and evolution suggests that the endoparasitic lifestyle among mistletoes may have evolved in a similar way on three different occasions, always from ancestral species with a highly specialized system of cortical strands (Teixeira-Costa et al., 2020).

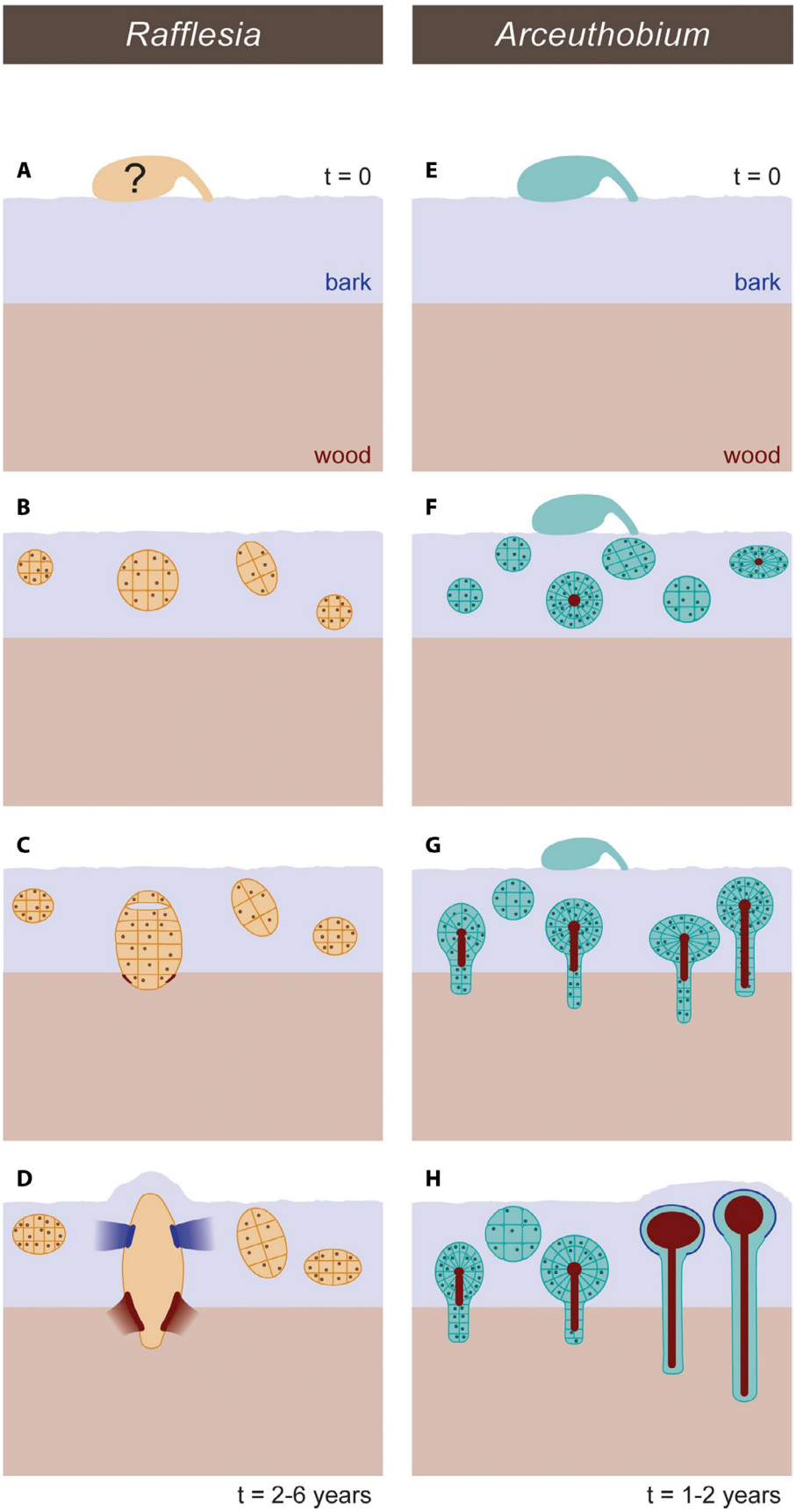
Considering that Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae are exclusively composed of endoparasitic species and that their sister clades are nonparasitic lineages, hypotheses of how this extremely modified lifestyle evolved remains limited. Still, it is noteworthy that the developmental pattern described here for *Bdallophytum americanum* closely corresponds to what has been described for its sister genus, *Cytinus* (De Vega et al., 2007). The same can be said about *Mitrastemon matudae* and its sister species, *M. yamamotoi* (Jochems, 1928; Watanabe, 1933, 1934). Studies addressing the development of *Apodanthes caseariae* appear to be lacking in the literature. Finally, a common developmental pattern was also observed for all analyzed members of Rafflesiaceae, including *Sapria himalayana* and its sister genera *Rhizanthes* and *Rafflesia*. Thus, we hypothesize that while endoparasitism would have evolved among mistletoes via the expansion and specialization of endophytic tissue, this lifestyle would likely have evolved among Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae species via reduction of the endophyte and retention of embryonic cell characteristics (pedomorphosis) until late in these plants' development.

Xylem differentiation and formation of parasite–host connections

The establishment of a vascular connection between parasite and host through the differentiation of a xylem bridge is a crucial stage in the development of all parasitic plants (Heide-Jørgensen, 2008). Nevertheless, direct contact between the tracheary elements of host plants and endoparasites with the *Rafflesia*-type pattern is only established when these plants enter the reproductive stage. Following a yet unknown stimulus, flower bud development initiates with localized cell proliferation and the formation of a secondary morphological surface (Fig. 5C), as described by Nikolov et al. (2014a). Simultaneously, endophytic tissue also reaches the host xylem via wood rays, as observed by previous authors (Brown, 1912; Watanabe, 1934; Dell et al., 1982; Forstmeier et al., 1983). Once in contact with host vessels, endoparasitic parenchyma cells then differentiate into tracheary elements, forming a direct xylem bridge between parasite and host (Fig. 5C, D).

Each developing flower/inflorescence was observed to be individually connected to the host xylem (Fig. 5D). Likewise, host xylem penetration was not detected in portions of the endophyte not directly associated with bud formation. The development of endoparasitic tissue has often been described to disrupt the host bark and the organization of the host secondary phloem and xylem (Unger, 1840; Brown, 1912; Watanabe, 1934; Dell et al., 1982; Forstmeier et al., 1983). We also observed conspicuous host vessel re-orientation, i.e., newly formed host vessel elements being differentiated toward parasitic tissues, as would be observed in branching stems/roots. This form of alteration of the host cambial activity has been discussed for other host–parasite interactions as an indication of parasitic plant manipulation of host tissue differentiation (Aloni, 2015; Spallek et al., 2017).

In endophytic mistletoes, direct xylem connections were rarely detected between endophytic mistletoes and their hosts; instead, abundant specialized parenchyma cells were observed at the host–parasite interface. These parenchyma cells have been reported to mediate most of the vascular connection between the two plants and allow resource uptake from its host (Calvin and Wilson, 1996; Mauseth and Rezaei, 2013). These specialized cells reach the host xylem early in the development of endophytic mistletoes, providing access to host xylem sap, while the green hypocotyl axis of the parasite seedling remains attached to the host stem (Fig. 5E–G). This perennial structure, as well as the adventitious shoots that may emerge from the endophytic tissue subsequently, show some degree of photosynthetic activity (Heide-Jørgensen, 2008). This autotrophic carbon production, albeit limited, plays an important role in providing resources for initial host penetration and sexual reproduction (Kuijt, 1986; Těšitel, 2016). Thus, we hypothesize that the developmental pattern of early cell differentiation and tissue organization in the *Arceuthobium*-type pattern could



be related to acquiring water for photosynthetic activity. In contrast, endoparasitic species with the *Rafflesia*-type pattern, which are completely devoid of chloroplasts, would require a greater volume of water only during flower bud development and anthesis.

Phloem differentiation and formation of host–parasite connections

Despite following a common developmental trajectory, endoparasitic species of the *Rafflesia*-type differed in terms of phloem cell differentiation. The presence of callose, a carbohydrate typically detected in sieve tubes was only detected in Apodanthaceae and Rafflesiaceae species, corroborating earlier findings by Peirce (1893), Brown (1912), and Kuijt et al. (1985). In these plants, direct contact between parasite and host sieve tubes was also detected. In addition, in Rafflesiaceae species, we show that a chimeric tissue, composed of both parasite and host phloem cells, was formed at the base of flower buds and open flowers of the parasite. In this basal region, often termed cupule (Kuijt, 1969; Nikolov et al., 2014b), host sieve tubes were noticed to differentiate toward the parasitic cells (Fig. 5H), which could provide increased access to the host phloem sap. Such improved access could be of great importance in generating energy for the endothermic flowers of Rafflesiaceae species (Patiño et al., 2000, 2002).

Sieve tubes were also detected in both endophytic mistletoes (Fig. 5H), although no direct parasite–host phloem connections were observed. These results agree with observations reported for other endoparasitic mistletoes (Thoday and Johnson, 1930; Mauseth et al., 1985; Lye, 2006; Mauseth and Rezaei, 2013). On the other hand, the fact that sieve tubes were not detected in Cytinaceae and Mitrastemonaceae species should be interpreted with caution because it does not necessarily indicate an absence of parasite–host phloem connections. Unlike the case of endophytic mistletoes, for which extensive ultrastructural studies have been conducted (Tainter, 1971; Alosi and Calvin, 1985; Sadik et al., 1986; Lye, 2006), detailed analyses of the endophyte of Cytinaceae and Mitrastemonaceae are lacking. These analyses should be prioritized and could help elucidate whether parasitic parenchyma cells observed in close proximity to host sieve tubes are capable of

FIGURE 5. Schematic representation comparing the vegetative development over time (t) of endoparasites, represented by *Rafflesia*, and that of endophytic mistletoes, represented by *Arceuthobium*. (A) Endoparasite seed in contact with host root. (B) Early development of the endophyte detected as cell clusters. (C) Cell growth within the clusters and initial flower bud development represented in the largest cluster. (D) Cell proliferation within the clusters and flower bud initial development causing alterations to the host phloem and xylem structure. (E) Germination of endophytic mistletoe seed. (F) Early development of the endophyte detected as abundant cell clusters. (G) Cell growth and proliferation within the clusters and initial development of cortical strands with xylem tissue. (H) Development of phloem tissue and further growth of cortical strands, leading to localized swelling of host tissues. Bark and phloem tissues are in shades of blue; wood and xylem tissues are in shades of red.

forming indirect phloem connections between parasite and host via plasmodesmatal connections.

ACKNOWLEDGMENTS

We thank several people for help provided during sampling efforts in various localities. L.T.C. acknowledges Dr. Rosa Cerros-Tlatilpa and Luis Gil Galván from the Universidad Autónoma de Morlelos in the collection of *Bdallophytum americanum*. Alexander Vázquez, Daniel Gómez, Fernando Vázquez, Juan Martínez, Juvenal Hernández, and Martín Castillo greatly helped collecting *Mitrastemon matudae* at the Reserva Biológica La Sepultura. C.C.D. and L.T.C. acknowledge staff at the EEB Greenhouse at the University of Connecticut (UConn) and Clinton Morse and Matthew Opel for providing specimens of *Viscum minimum*. G.C. and L.T.C. acknowledge David Shaw from Oregon State University and Robert Mathiasen from Northern Arizona University for help and species identification during the sampling of *Arceuthobium douglasii*. We also acknowledge the reviewers and editor for their comments, which helped us improve this paper. L.T.C. was supported by grants from the European Society for Evolutionary Biology and the Harvard University Herbaria. G.C. was supported by the São Paulo Research Foundation (FAPESP-2013/23322-3). This research was also supported by National Science Foundation AToL grant to C.C.D. (DEB-0622764) and DEB-1120243 grant to C.C.D. and Elena Kramer.

AUTHOR CONTRIBUTIONS

L.T.C. and G.C. designed the research. L.T.C., C.C.D., and G.C. secured funding and conducted fieldwork for the original sampling of all analyzed species. L.T.C. conducted microtomography and microscopy analysis. L.T.C., C.C.D., and G.C. analyzed and discussed results. L.T.C. wrote the initial draft of the manuscript; C.C.D. greatly contributed to improving the way questions and hypotheses were framed/discussed and provided extensive early revisions to the manuscript. C.C.D. and G.C. reviewed and edited the manuscript. All authors approved the final version of the manuscript.

DATA AVAILABILITY

Microtomography videos showing the three-dimensional organization of the parasitic tissues of some of the species analyzed here are available at FigShare: *Arceuthobium douglasii* (https://doi.org/10.6084/m9.figshare.12792365.v5), *Mitrastemon matudae* (https://doi.org/10.6084/m9.figshare.12792362), *and Rafflesia cantleyii* (https://doi.org/10.6084/m9.figshare.12792362).

LITERATURE CITED

Aloni, R. 2015. Ecophysiological implications of vascular differentiation and plant evolution. *Trees* 29: 1–16.

Alosi, M. C., and C. L. Calvin. 1985. The ultrastructure of dwarf mistletoe (*Arceuthobium* spp.) sinker cells in the region of the host secondary vasculature. *Canadian Journal of Botany* 63: 889–898.

Alvarado-Cárdenas, L. O. 2009. Sistemática del género *Bdallophytum* (Cytinaceae). *Acta Botanica Mexicana* 87: 1–21.

APG [Angiosperm Phylogeny Group]. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181: 1–20.

Angyalossy, V., M. R. Pace, R. F. Evert, C. R. Marcati, A. A. Oskolski, T. Terrazas, E. Kotina, et al. 2016. IAWA list of microscopic bark features. *IAWA Journal* 37: 517–615.

Aukema, J. E. 2003. Vectors, viscin, and Viscaceae: mistletoes as parasites, mutualists, and resources. *Frontiers in Ecology and the Environment* 1: 212–219.

Barkman, T. J., M. R. Klooster, K. D. Gaddis, B. Franzone, S. Calhoun, S. Manickam, S. Vessabuts, et al. 2017. Reading between the vines: hosts as islands for extreme holoparasitic plants. *American Journal of Botany* 104: 1382–1389.

Barkman, T. J., S.-H. Lim, K. M. Salleh, and J. Nais. 2004. Mitochondrial DNA sequences reveal the photosynthetic relatives of *Rafflesia*, the world's largest flower. *Proceedings of the National Academy of Sciences, USA* 101: 787–792.

Barkman, T. J., J. R. McNeal, S.-H. Lim, G. Coat, H. B. Croom, N. D. Young, and C. W. DePamphilis. 2007. Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evolutionary Biology* 7: 248.

Baskin, C. C., and J. M. Baskin. 2014. Germination ecology of plants with specialized life cycles and/or habitats. seeds: ecology, biogeography, and, evolution of dormancy and germination, 1601. Elsevier Science & Technology, San Diego, CA, USA.

Bellot, S., and S. Renner. 2014. The systematics of the worldwide endoparasite family Apodanthaceae (Cucurbitales), with a key, a map, and color photos of most species. *PhytoKeys* 36: 41–57.

Bendiksby, M., T. Schumacher, G. Gussarova, J. Nais, K. Mat-salleh, N. Sofiyanti, D. Madulid, et al. 2010. Elucidating the evolutionary history of the Southeast Asian, holoparasitic, giant-flowered Rafflesiaceae: Pliocene vicariance, morphological convergence and character displacement. *Molecular Phylogenetics and Evolution* 57: 620–633.

Bouman, F., and W. Meijer. 1994. Comparative structure of ovules and seeds in Rafflesiaceae. *Plant Systematics and Evolution* 193: 187–212.

Brasil, B. 2010. Ciclo de vida, fenologia e anatomia floral de *Pilostyles* (Apodanthaceae - Rafflesiaceae s.l.): subsídios para um posicionamento filogenético da família Apodanthaceae. Master's thesis, University of São Paulo, São Paulo, Brazil.

Bromham, L., P. F. Cowman, and R. Lanfear. 2013. Parasitic plants have increased rates of molecular evolution across all three genomes. *BMC Evolutionary Biology* 13: 1–11.

Brown, W. H. 1912. The relation of *Rafflesia manillana* to its host. *Philippine Journal of Science, C, Botany* VII: 209–226.

Burgoyne, P. M. 2006. A new species of *Cytinus* (Cytinaceae) from South Africa and Swaziland, with a key to the Southern African species. *Novon* 16: 315–319.

Calvin, C. L., and C. A. Wilson. 1996. Endophytic system. *In* F. G. Hawksworth and D. Wiens. [eds.], Dwarf mistletoes: biology, pathology, and systematics. 113–122.Agriculture Handbook 709, U.S. Department of Agriculture, Forest Service, Washington, D.C., USA.

Cohen, L. I. 1954. The anatomy of the endophytic system of the dwarf mistletoe, *Arceuthobium campylopodum*. *American Journal of Botany* 41: 840–847.

Davis, C. C., M. Latvis, D. L. Nickrent, K. J. Wurdack, and D. A. Baum. 2007. Floral gigantism in Rafflesiaceae. *Science* 315: 1812.

de Vega, C., M. Arista, P. L. Ortiz, C. M. Herrera, and S. Talavera. 2009. The ant-pollination system of *Cytinus hypocistis* (Cytinaceae), a Mediterranean root holoparasite. *Annals of Botany* 103: 1065–1075.

de Vega, C., P. L. Ortiz, M. Arista, and S. Talavera. 2007. The endophytic system of Mediterranean *Cytinus* (Cytinaceae) developing on five host Cistaceae species. *Annals of Botany* 100: 1209–1217.

Dell, B., J. Kuo, and A. H. Burbidge. 1982. Anatomy of *Pilostyles hamiltonii* C. A. Gardner (Rafflesiaceae) in stems of *Daviesia*. *Australian Journal of Botany* 30: 1–9.

do Amaral, M. M. 2007. A estrutura da angiosperma endoparasita *Pilostyles ulei* (Apodanthaceae): interface e impacto no lenho de *Mimosa* spp. Master's thesis, University of São Paulo, São Paulo, Brazil.

Engler, A., and K. Krause. 1908. Uber die Lebensweise von *Viscum minimum* Harvey. *Berichte der Deutschen Botanischen Gesellschaft* 26: 524–530.

Filipowicz, N., and S. S. Renner. 2010. The worldwide holoparasitic Apodanthaceae confidently placed in the Cucurbitales by nuclear and mitochondrial gene trees. *BMC Evolutionary Biology* 10: 1–8.

Forstmeier, von L., F. Weberling, and H. C. Weber. 1983. Zum Parasitismus von *Cytinus hypocistis* L. (Rafflesiaceae). *Beiträge zur Bioiogie der Pflanzen* 58: 299–311.

García-Franco, J. G., and V. Rico-Gray. 1996. Distribution and host specificity in the holoparasite *Bdallophyton bambusarum* (Rafflesiaceae) in a tropical decidous forest in Veracruz, Mexico. *Biotropica* 28: 759–762.

García-Franco, J. G., V. Souza, L. E. Eguiarte, and V. Rico-Gray. 1998. Genetic variation, genetic structure and effective population size in the tropical holoparasitic endophyte *Bdallophyton bambusarum* (Rafflesiaceae). *Plant Systematics and Evolution* 210: 271–288.

Griebel, A., D. Watson, and E. Pendall. 2017. Mistletoe, friend and foe: synthesizing ecosystem implications of mistletoe infection. *Environmental Research Letters* 12: 115012.

Hawksworth, F. G. 1983. Mistletoes as forest parasites. *In* M. Calder and P. Bernhardt [eds.], The biology of mistletoes. Academic Press, Sidney, Australia.

Hawksworth, F. G., and D. Wiens. 1996. Dwarf mistletoes: biology, pathology, and systematics. Agriculture Handbook 709, U.S. Department of Agriculture, Forest Service, Washington, D.C., USA.

Heide-Jørgensen, H. S. 2008. Parasitic flowering plants. Brill, Leiden, Netherlands.

Heinricher, E. 1917. Die erste Aufzucht einer Rafflesiaceae, *Cytinus hypocistis* L., aus Samen. *Berichte der Deutschen Botanischen Gesellschaft* 35: 505–512.

Jochems, S. C. J. 1928. Die Verbreitung der Rafflesiaceen-Gattung Mitrastemon. *Recueil des Travaux Botaniques Néerlandais* 25A: 203–207.

Kraus, J. E., and M. Arduin. 1997. Manual básico de métodos em morfologia vegetal. Editora Universidade Rural, Seropedica, Rio de Janeiro, Brazil.

Kuijt, J. 1986. Observations on establishment and early shoot emergence of *Viscum minimum* (Viscaceae). *Acta Botanica Neerlandica* 35: 449–456.

Kuijt, J. 2015. Santalales. *In* K. Kubitzki [ed.], The families and genera of vascular plants, vol. XII, Flowering plants. Eudicots. Santalales, Balanophorales, 209. Springer International. Basel, Switzerland.

Kuijt, J. 1969. The biology of parasitic flowering plants. University of California Press, Berkeley, CA, USA.

Kuijt, J., D. Bray, and A. R. Olson. 1985. Anatomy and ultrastructure of the endophytic system of *Pilostyles thurberi* (Rafflesiaceae). *Canadian Journal of Botany* 63: 1231–1240.

Lamont, B.. 1983. Germination of mistletoes. *In* D. M. Calder and P. Bernhardt [eds.], The biology of mistletoes, 348. Academic Press, Sidney, Australia.

Lye, D. 2006. Charting the isophasic endophyte of dwarf mistletoe *Arceuthobium douglasii* (Viscaceae) in host apical buds. *Annals of Botany* 97: 953–963.

Makino, T. 1909. Observations on the flora of Japan. *Botanical Magazine Tokyo* 23: 325–327.

Maul, K., M. Krug, D. L. Nickrent, K. F. Müller, D. Quandt, and S. Wicke. 2018. Morphology, geographic distribution, and host preferences are poor predictors of phylogenetic relatedness in the mistletoe genus *Viscum* L. *Molecular Phylogenetics and Evolution* 131: 106–115.

Mauseth, J. D. 1990. Morphogenesis in a highly reduced plant: the endophyte of *Tristerix aphyllus* (Loranthaceae). *Botanical Gazette* 151: 384–353.

Mauseth, J. D., G. Montenegro, and A. M. Walckowiak. 1985. Host infection and flower formation by the parasite *Tristerix aphyllus* (Loranthaceae). *Canadian Journal of Botany* 63: 567–581.

Mauseth, J. D., G. Montenegro, and A. M. Walckowiak. 1984. Studies of the holoparasite *Tristerix aphyllus* (Loranthaceae) infecting *Trichocereus chilensis* (Cactaceae). *Canadian Journal of Botany* 62: 847–857.

Mauseth, J. D., and K. Rezaei. 2013. Morphogenesis in the parasitic plant *Viscum minimum* (Viscaceae) is highly altered, having apical meristems but lacking roots, stems, and leaves. *International Journal of Plant Sciences* 174: 791–801.

Meijer, W., and J. F. Veldkamp. 1993. A revision of *Mitrastema* (Rafflesiaceae). *Blumea* 38: 221–229.

Molina, J., W. McLaughlin, K. Wallick, R. Pedales, M. V. Marius, D. N. Tandang, A. II Damatac, et al. 2017. Ex situ propagation of Philippine *Rafflesia* in the United States: challenges and prospects. *Sibbaldia: The Journal of Botanic Garden Horticulture* 15: 77–96.

Nickrent, D. L. 2002. Orígenes filogenéticos de las plantas parásitas. *In* J. A. López-Sáez, P. Catalán, and L. Sáez [eds.], Plantas parásitas de la Península Ibérica e Islas Baleares, 29–56.Mundi-Prensa Libros, S. A., Madrid, Spain.

Nickrent, D. L. 2007. Cytinaceae are sister to Muntingiaceae (Malvales). *Taxon* 56: 1129–1135.

Nickrent, D. L. 2020. Parasitic angiosperms: How often and how many? *Taxon* 69: 5–27.

Nickrent, D. L., A. Blarer, Y. L. Qiu, R. Vidal-Russell, and F. E. Anderson. 2004. Phylogenetic inference in Rafflesiales: the influence of rate heterogeneity and horizontal gene transfer. *BMC Evolutionary Biology* 4: 1–17.

Nikolov, L. A., P. K. Endress, M. Sugumaran, S. Sasirat, S. Vessabutr, E. M. Kramer, and C. C. Davis. 2013. Developmental origins of the world's largest flowers, Rafflesiaceae. *Proceedings of the National Academy of Sciences, USA* 110: 18578–18583.

Nikolov, L. A., Y. M. Staedler, S. Manickam, J. Schönenberger, P. K. Endress, E. M. Kramer, and C. C. Davis. 2014a. Floral structure and development in Rafflesiaceae with emphasis on their exceptional gynoecia. *American Journal of Botany* 101: 225–243.

Nikolov, L. A., P. B. Tomlinson, S. Manickam, P. K. Endress, E. M. Kramer, and C. C. Davis. 2014b. Holoparasitic Rafflesiaceae possess the most reduced endophytes and yet give rise to the world's largest flowers. *Annals of Botany* 114: 233–242.

Patiño, S., T. Aalto, A. A. Edwards, and J. Grace. 2002. Is *Rafflesia* an endothermic flower? *New Phytologist* 154: 429–437.

Patiño, S., J. Grace, and H. Bänziger. 2000. Endothermy by flowers of *Rhizanthes lowii* (Rafflesiaceae). *Oecologia* 124: 149–155.

Peirce, G. J. 1893. On the structure of the haustoria of some phanerogamic parasites. *Annals of Botany* 7: 291–327.

Rose, J. P., T. J. Kleist, S. D. Löfstrand, B. T. Drew, J. Schönenberger, and K. J. Sytsma. 2018. Phylogeny, historical biogeography, and diversification of angiosperm order Ericales suggest ancient Neotropical and East Asian connections. *Molecular Phylogenetics and Evolution* 122: 59–79.

Ruzin, S. E. 1999. Plant microtechnique and microscopy. Oxford University Press, Oxford, UK.

Sadik, A., L. Rey, and S. Renaudin. 1986. Le système endophytique d'*Arceuthobium oxycedri*. II. Aspects ultrastructuraux des zones de contact entre les tissus de l'hôte et du parasite. *Canadian Journal of Botany* 64: 2778–2784.

Solms-Laubach, H. 1867. Ueber den Bau und Entwicklung der Ernahrungsorgane parasitischer Phanerogamen. *Jahrbücher für Wissenschaftliche Botanik* 6: 509–638.

Solms-Laubach, H. G. zu. 1874. Ueber den Thallus von Pilostyles Haussknechtii. *Botanische Zeitung* 32: 49–59.

Spallek, T., C. W. Melnyk, T. Wakatake, J. Zhang, Y. Sakamoto, T. Kiba, S. Yoshida, et al. 2017. Interspecies hormonal control of host root morphology by parasitic plants. *Proceedings of the National Academy of Sciences, USA* 114: 5283–5288.

Tainter, F. H. 1971. The ultrastructure of *Arceuthobium pusillum*. *Canadian Journal of Botany* 49: 1615–1622.

Takhtajan, A. L. 1997. Diversity and classification of flowering plants. Columbia University Press, NY, NY, USA.

Teixeira-Costa, L., and G. C. T. Ceccantini. 2016. Aligning microtomography analysis with traditional anatomy for a 3D understanding of the host–parasite interface – *Phoradendron* spp. case study. *Frontiers Plant Science* 7: 1340.

Teixeira-Costa, L., G. Ocampo, and G. Ceccantini. 2020. Morphogenesis and evolution of mistletoes' haustoria. *In* D. Demarco [ed.], Plant ontogeny, 107–157.Nova Science Publishers, NY, NY, USA.

Těšitel, J. 2016. Functional biology of parasitic plants: a review. *Plant Ecology and Evolution* 149: 5–20.

Thoday, D., and E. T. Johnson. 1930. On *Arceuthobium pusillum*, Peck. I. The endophytic system. *Annals of Botany* 44: 393–413.

Twyford, A. D. 2017. New insights into the population biology of endoparasitic Rafflesiaceae. *American Journal of Botany* 104: 1433–1436.

Unger, F. 1840. Beitrage zur Kenntniss der Parasitischen Pflanzen. Erster oder Anatomisch- Physiologischer Theil. *Annalen des Wiener Museums der Naturgeschichte* 2: 13–60.

Vidal-Russell, R., and D. L. Nickrent. 2008. The first mistletoes: origins of aerial parasitism in Santalales. *Molecular Phylogenetics and Evolution* 47: 523–537.

Visser, J. H. 1981. South African parasitic flowering plants. Juta Publishing, Cape Town, South Africa.

Watanabe, K. 1933. Ungeschlechtliche Fortpflanzung von *Mitrastemon* Yamamotoi. *Proceedings of the Imperial Academy of Japan* 9: 412–415.

Watanabe, K. 1934. Biologie von *Mitrastemon yamamotoi* Makino (Rafflesiaceae). II. Vegetative Fortpflanzung. *Botany Magazine Tokyo* 48: 467–472.

Watanabe, K. 1936a. Morphologisch-biologische Studien uber die Gattung *Mitrastemon* (II). *Journal of Japanese Botany* 12: 698–711.

Watanabe, K. 1936b. Morphologisch-biologische Studien uber die Gattung *Mitrastemon* (III). *Journal of Japanese Botany* 12: 759–773.

Watson, D. M. 2009. Parasitic plants as facilitators: More Dryad than Dracula? *Journal of Ecology* 97: 1151–1159.

Wilson, C. A., and C. Calvin. 2006. Character divergences and convergences in canopy-dwelling Loranthaceae. *Botanical Journal of the Linnean Society* 150: 101–113.

Yamamoto, Y. 1936. Species nova Mitrastemonacearum (Rafflesiacearum) ex Mexico. *Botanical Magazine Tokyo* 50: 539–541.