

First reports and generic descriptions of the achlorophyllous holoparasites Apodanthaceae (Cucurbitales) of Colombia

Primeros registros y descripciones genéricas de la familia de holoparasitos aclorófilos Apodanthaceae (Cucurbitales) de Colombia

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Abstract

The Apodanthaceae (Cucurbitales) are one of the 12 parasitic clades of flowering plants and the only holoparasitic family with a vegetative phase entirely endophytic, growing primarily on Fabaceae and Salicaceae stems. The two genera of the family, *Apodanthes* and *Pilostyles*, are broadly distributed in Colombia, although they remain extremely under-collected and poorly known. Based on field and herbarium work, and a review of the literature, we provide detailed generic-level descriptions of the family. We also update the records of the family in Colombia and discuss the homology and taxonomic implications of floral characters such as perianth scales, pollen sacs, and the central column. Finally, we provide photographic records of Colombian Apodanthaceae, and some guidelines for properly collecting these plants.

Key words: Apodanthaceae, *Apodanthes*, Cucurbitales, Colombian flora, parasitic flowering plants, *Pilostyles*

Resumen

Apodanthaceae (Cucurbitales) es uno de los doce clados de angiospermas parásitas, y la única familia de especies holoparasíticas con la fase vegetativa completamente endofítica en tallos principalmente de especies de Fabaceae y Salicaceae. Los dos géneros de la familia, *Apodanthes* y *Pilostyles* están ampliamente distribuidos en Colombia, aunque han sido muy poco recolectados y permanecen poco conocidos. Con base en trabajo de campo y de herbario, y una extensa revisión bibliográfica, se describen en detalle los caracteres genéricos de la familia. A la vez, se actualizan los registros de la familia en Colombia y se discute la homología e implicaciones taxonómicas de caracteres florales tales como piezas del perianto, sacos polínicos y columna central. Finalmente, presentamos fotografías de las Apodanthaceae colombianas, así como algunas recomendaciones para recolectar adecuadamente estas plantas.

Palabras clave: Apodanthaceae, *Apodanthes*, Cucurbitales, flora colombiana, plantas con flor parásitas, *Pilostyles*

INTRODUCTION

The family Apodanthaceae Takhtajan consists of 11 species in the genera *Apodanthes* Poit. (1 species from the New World) and *Pilostyles* Guill. (10 spp. from North-, Central- and South America, Iran, Irak, Syria, Asia Minor, subtropical eastern Africa and southwestern Australia) (Bellot and Renner, 2014). All members of the family are achlorophyllous holoparasites with an intricate but histologically simple endophyte and an exophyte

consisting of small gregarious flowers that emerge from the parasitized stems of a limited group of hosts (table 1). The wide distribution of the family on four continents strongly suggest a long evolutionary history (Filipowics and Renner, 2010) and make intercontinental dispersal a less plausible biogeographic scenario.

The affinities of Apodanthaceae have long been debated. They have been placed as part of Rafflesiales/Rafflesiaceae primarily based on the parasitic life style, the dioecy

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Table 1. Summary of members of Apodanthaceae, their hosts and their geographical range. Herbarium voucher on the right-hand column are written in *italics* [¹ = species recognized by Bellot and Renner (2014); for a complete synonymy see Bellot and Renner (2014); ² = *Dalea* taxonomy follows Barneby (1977); ³ = Herbarium specimens in *italics*; HUA = Herbario Universidad de Antioquia, Medellín (Antioquia), Colombia]

Species ¹ , autor	Host	Country	Reference/voucher ³
<i>Apodanthes caseariae</i> Poit.	<i>Trema micrantha</i> Blume (Cannabaceae); <i>Casearia macrophylla</i> Vahl, <i>C. nitida</i> (L.) Jacq., <i>C. silvestris</i> Sw. (Salicaceae); Burseraceae; Meliaceae	Bolivia, Brazil, Colombia, Costa Rica, Guyana, Peru, Suriname, Venezuela	Bellot and Renner 2013, 2014, Brown 1845, Gómez 1983, Harms 1935, Idárraga 2011, Yatskievych and Meijer 2004, <i>Callejas et al. 8062</i> (HUA)
<i>Pilostyles aethiopica</i> Welw.	<i>Berlinia eminii</i> Taub., <i>B. paniculata</i> Benth., <i>Brachystegia</i> spp.; <i>Julbernardia globiflora</i> (Benth.) Troupin (Fabaceae-Faboideae)	Angola, Malawi, Tanzania, Zambia, Zimbabwe	Bellot and Renner 2013, 2014, Blarer et al. 2004, Harms 1935, Meijer 1993
<i>P. berteroi</i> Guill.	<i>Adesmia arborea</i> Bertero, <i>A. emarginata</i> Clos (Fabaceae-Faboideae)	Argentina, Bolivia, Chile, Peru	Bellot and Renner 2013, 2014, Brown 1845, Filipowics and Renner 2010, Guillemín 1834, Harms 1935, Jaffuel 1933, Rodríguez et al. 2008
<i>P. blanchetii</i> (Gardner) R. Br.	<i>Calliandra</i> spp. (Fabaceae-Mimosoideae); <i>Inga</i> sp.; <i>Mimosa maguirei</i> Barneby, <i>M. foliolosa</i> Benth., <i>M. maguirei</i> , <i>M. setosa</i> Benth., <i>M. setosissima</i> Taub., <i>Mimosa</i> sp. (Fabaceae-Mimosoideae); <i>Bauhinia</i> sections <i>Caulotretus</i> and <i>Pauletia</i> ; <i>B. lunarioides</i> A. Gray; <i>Cassia</i> sp. (Fabaceae-“Caesalpinioideae”); <i>Dioclea</i> spp.; <i>Galactia jussiaeana</i> HBK; <i>Schnella</i> spp. (Fabaceae-Faboideae)	Argentina, Brazil, Cayman Islands, Costa Rica, Guyana, Jamaica, Panama, Uruguay, Venezuela	Amaral 2007, Amaral and Ceccantini 2011, Bellot and Renner 2013, 2014, Blassingame 1968, Brown 1845, Endriss 1902, Gomes and Fernandes 1994, Harms 1935, Robinson 1891, Rose 1909, Vattimo-Gil 1978
<i>P. boyacensis</i> F. González & Pabón-Mora	<i>Dalea cuatrecasii</i> Barneby ²	Colombia (Eastern Cordillera)	González and Pabón-Mora 2014
<i>P. coccoidea</i> K. R. Thiele	<i>Jacksonia</i> (Fabaceae-Faboideae)	Western Australia	Bellot and Renner 2013, 2014, Filipowics and Renner 2010, Thiele et al. 2008
<i>P. collina</i> Dell	<i>Gastrolobium</i> spp., <i>Oxylobium</i> spp. (Fabaceae-Faboideae)	Western Australia	Bellot and Renner 2013, 2014, Thiele et al. 2008
<i>P. hamiltonii</i> C. A. Gardner	<i>Daviesia</i> sp. (Fabaceae-Faboideae)	Western Australia	Bellot and Renner 2013, 2014, Filipowics and Renner 2010, Thiele et al. 2008

Tabla 1: Continued

Species ¹ , autor	Host	Country	Reference/voucher ³
<i>P. haussknechtii</i> Boiss.	<i>Astragalus pycnocladus</i> Boiss. & Hausskn. ex Boiss.; <i>A. spp.</i> , <i>Halimodendron spp.</i> ; <i>Onobrychis spp.</i> (Fabaceae-Faboideae)	Iran, Syria	Bellot and Renner, 2013, 2014, Harms, 1935, Heide-Jørgensen, 2008, Solms-Laubach 1874
<i>P. mexicana</i> Rose	<i>Calliandra grandiflora</i> Benth. (Fabaceae-“Caesalpinioideae”)	Guatemala, Honduras, Mexico	Bellot and Renner 2013, 2014, Rose 1909
<i>P. thurberi</i> Gray	<i>Dalea bicolor</i> Humboldt & Bonpland ex Willdenow var. <i>canescens</i> (M. Mart. & Galeotti) Barneby, <i>D. formosa</i> Torr., <i>D. frutescens</i> A. Gray; <i>D. hospes</i> (Rose) Bullock; <i>D. lutea</i> (Cavanilles) Willdenow var. <i>lutea</i> ; <i>Psoralea emoryi</i> (A. Gray) Rydberg, and <i>P. schottii</i> (Torr.) Barneby ²	Mexico, Southern United States of America	Barneby 1977, Blassingame 1968, Bellot and Renner 2013, 2014, Cerros-Tlatilpa and Espejo-Serna 1998, Felger 1988, Filipowicz and Renner 2010, Harms 1935, Kuijt et al. 1985, Laudermilk 1945, Robinson 1891, Rose 1909, Yatskievych 1993

and the central column of the flower (e.g., Brown, 1845; Endlicher, 1841; Eichler, 1878; Eliasson, 1994; Engler, 1912; Gómez, 1983; Harms, 1935; Hutchinson, 1959; Karsten, 1856; Kuijt, 1969; Meijer, 1993; Melchior, 1964; Mitchell, 2004; Takhtajan, 1997; Yatskievych and Meijer, 2004) or related to Malvales (Blarer et al. 2004; Heide-Jørgensen, 2008) based on floral development and morphology. However, recent molecular-based phylogenetic analyses have shown that Rafflesiaceae s.l. is polyphyletic, and placed Apodanthaceae as sister to the remaining seven families of the order Cucurbitales (Filipowicz and Renner, 2010; Nickrent, 2008; Nickrent et al. 2004; Schaeffer and Renner, 2011). According to Filipowicz and Renner (2010), inferior ovaries, parietal placentation and dioecy support the inclusion of the Apodanthaceae within Cucurbitales. Thus, Apodanthaceae and the redefined Rafflesiaceae s. str. (now part of Malpighiales) represent two of the 12 independent origins of parasitism among flowering plants (Barkman et al. 2007; Nickrent, 2008).

At a specific level, the equivocal morphological characters in Apodanthaceae have led some authors (e.g., Harms, 1935; Solms-Laubach, 1878) to use extrinsic traits to distinguish species, particularly the hosts on which they grow, or the geographical area they occupy. This is evident in names such as *Apodanthes caseariae* Poit., *Pilostyles aethiopica* Welw., *P. mexicana* (Brandege) Rose, or *P. ingae* or *P. calliandrae*, the latter two lacking any differential trait in Solms-Laubach's (1878) treatment besides the hosts they parasitize.

Although members of Apodanthaceae from the United States, Mexico, Venezuela, Brazil and Chile have been collected and are thus relatively well documented, they remain poorly known and undercollected in Colombia. This is mainly because of their small flowers, the endophytic life form, the absence of conspicuous hypertrophy of the infected tissues in the host, and the short flowering time. Thus, the goals of this work are: (1) to report new findings of Apodanthaceae in Colombia, (2) to update and revisit historical data regarding the presence of the family in Colombia, (3) to contribute to the ongoing discussion of the homology of the perianth, the central column, the androecium, and the fruit and (4) to provide guidelines to help Colombian botanists collect and make careful field observations of this extremely undercollected family. At present, limited collections prevent detailed developmental, morphological, ecological and taxonomic studies of these interesting holoparasitic flowering plants.

MATERIALS AND METHODS

We examined all the Colombian specimens (ca. 2500) of the genera expected or reported as hosts of Apodanthaceae, e.g., *Adesmia*, *Bauhinia*, *Calliandra*, *Cassia*, *Dalea*, *Dioclea*, *Galactia*, *Inga*, *Mimosa*, and *Schnella* (Fabaceae), and *Banara*, *Casearia* and *Xylosma* (Salicaceae) housed at Herbario Nacional Colombiano (COL; Universidad Nacional de Colombia, Bogotá, Colombia), Herbario Universidad de Antioquia (HUA; Universidad de Antioquia, Medellín, Colombia), New York Botanical

Garden (NY; New York, U. S. A.), and Herbario Toli (TOLI; Universidad del Tolima, Ibagué, Colombia). The historical collections of Apodanthaceae cited below and deposited at Gray Herbarium (GH; Harvard University, Massachusetts, U. S. A.) and Royal Botanic Gardens, Kew (K; London, U. K.) were also examined for the present study. Based on this herbarium search, fieldwork was carried out in selected localities where Apodanthaceae and their hosts are expected to occur in Boyacá (Colombia).

The descriptions that follow are based on our field and herbarium observations, as well as relevant literature, especially Bellot and Renner (2013, 2014), Blarer et al. (2004), Blassingame (1968), Dell et al. (1982), Endriss (1902), Felger (1988), Guillemin (1834), Harms (1935), Heide-Jørgensen (2008), Kuijt (1969), Kuijt et al. (1985), Nickrent (2006), Robinson (1891), Rutherford (1970), Solms-Laubach (1874), Takhtajan (1997), Thiele et al. (2008), and Vattimo-Gil (1955, 1956, 1978). The taxonomy of *Dalea* follows Barneby (1977).

RESULTS AND DISCUSSION

Morphological traits. Apodanthaceae are perennial, achlorophyllous holoparasites (figures 1-4) and their vegetative tissues are reduced to a mycelium-like system that is completely endophytic, that is, it develops entirely within the host. Both monoecy and dioecy have been reported to occur in the family. In *Pilostyles aethiopica* Welw. (formerly *Berlinianche aethiopica*) from Zimbabwe and *P. haussknechtii* Boiss. from Iran, Bellot and Renner (2013) reported a higher number of host individuals carrying staminate flowers. They also found that the presence of flowers of both sexes on the same host is occasional or nule. This issue is further complicated by the fact that it is not easy to determine an individual in Apodanthaceae (see below). Nevertheless, we have detected the presence of staminate and carpellate flowers in the same floral row in *P. boyacensis*, which indicates monoecy at least in this species.

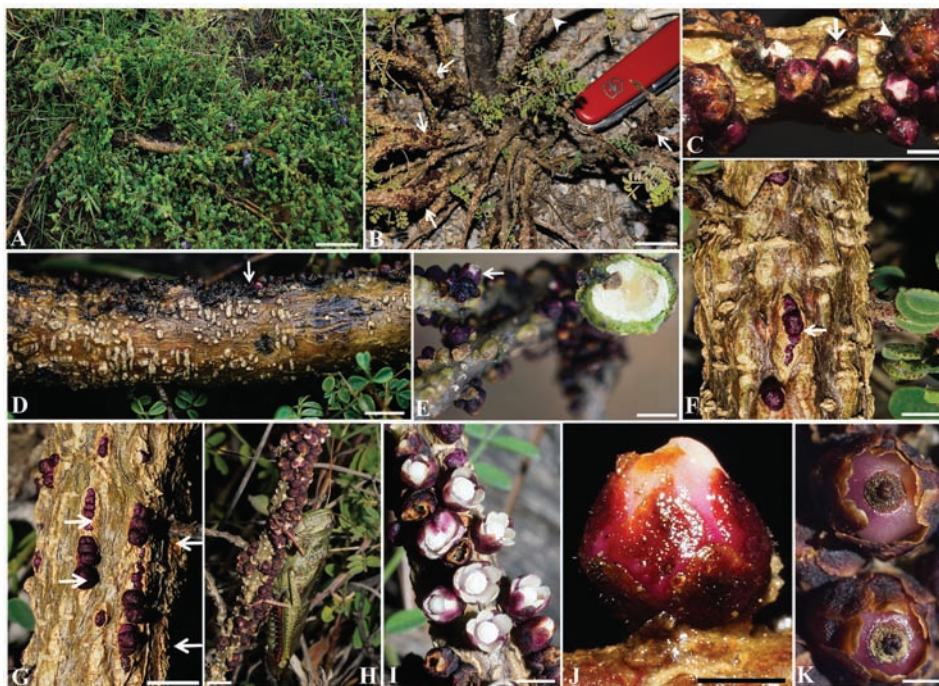


Figure 1. *Pilostyles boyacensis* (Apodanthaceae) parasitizing *Dalea cuatrecasasii*. A, B. Top view of an infected host; note the gregarious flowers (arrows) and fruits (arrowheads) of *P. boyacensis*; C. Carpellate flowers (arrow) and fruits (arrowhead) of *P. boyacensis*; D. Rows of floral buds and flowers of *P. boyacensis* (arrow); E. Transverse section of a host branch through an infection site; an anthetic flower (arrow) is apparent in the background; F, G. *Dalea* branches with rows of flowers of *P. boyacensis* breaking through the host tissue (arrows); H. Branch with a grasshopper, the largest floral visitor observed so far; I. Detail of (mostly) staminate flowers; note the pileus-like central column formed by white papillae covering the pollen sacs; J, K. Carpellate flowers (J, side view) and bacciform fruits (K, frontal view) of *P. boyacensis*. (A-E, from González 4508; F, G, from González 4509; H-K, from González 4519) (scale bars: 5 cm in A; 2 cm in B; 2 mm in C, I, K; 1 cm in D, H; 5 mm in E-G; 0.5 mm in J).



Figure 2. *Pilostyles* sp. specimen I. J. Holton 84 (GH). A. Herbarium specimen; B. Label details and the two main fragments of the specimen; C. Additional leaf fragment (probably *Calliandra* sp.) with the label “Leaf of the Acacia on which the Pilostyles of N Granada was found by Holton” (sic); D. Additional label that includes the heading “Flora Neogranadina Caucana”, the identification “*Pilostyles thurberi* Gray”, and the locality “La Paila” in which the specimen was collected on February 1853; E. Detail of a leaf and portion of a twig with at least 17 flowers and floral buds of *Pilostyles* sp.; F. Detail of a flower (scale bars in C and E: 1 cm)

The flowers of Apodanthaceae are gregarious, sessile (figures 1-4), unisexual, globose, ovoid or ellipsoid in side view, small (< 6 mm in length and 8 mm in diameter, some of the smallest among the holoparasitic plants; Kuijt, 1969), bright yellow, white, pink, red or purple colored, with a tannoid or “indoloid” odor (pers. obs.; Blassingame, 1968; Sipes et al. 2014). Prior to the emergence from the host tissue, floral buds are apparent as they form wart-like outgrowths (called “floral cushions” by Solms-Laubach, 1874; figure 1E). Flower emergence occurs from endogenous primordia by rupturing the outer layer of the host stem (figures 1E-G, 3) such that several

to many flowers can break through (Kuijt, 1969: figure 1, 3O), thus forming floral rows that presumably belong to a single individual (figure 1E, F). The perianth is formed by 12 to 15 opposite to alternate organs, in 3 or 4 poorly defined whorls; aestivation of these organs in the floral bud is irregular, although cochleate appear to be the most frequent type in both the staminate and the carpellate flowers (figures 1F, 3C, D). The outer perianth whorl is formed by two or three scale-like free organs more or less integrated to the rest of the flower (figures 1, 3 B, E, 4D), whereas the middle and inner whorls (calyx and corolla *sensu* Guillemain; figure 3) are tri- to hexamerous. At least in *Pilostyles*, the outer and middle perianth organs are purple and the inner ones are yellow, pink, red, brown, purple or, less often, white (figure 1 D, G, H). The inner perianth organs in *P. aethiopica* have a hair carpet at the base of its adaxial side. A tubular column basally surrounded by a nectary disk is formed at the center of both staminate and carpellate flowers; stomata are present in the outer epidermis of the nectary disk.

Staminate flowers. The androecium is a synandrium composed of two to four rings of globose, sessile pollen sacs located laterally to the central column (figure 3). These dehisce through a transversal slit between adjacent rings. The number of pollen sacs per ring varies from 15 to 30, and usually decreases centripetally. The pollen sacs lack endothecia. Pollen grains are tricolpate in *Apodanthes* and *Pilostyles*, except in *P. aethiopica*, which has non-aperturate pollen. Above the rings of pollen sacs, a rooflike ring of vesicular papillae is formed. These papillae were interpreted as rudiments of the stigma by Solms-Laubach (1874). Gynocelial rudiments in the form of a style with a stylar canal and a mound-like, massive stigmatic surface are present in the staminate flowers. Vestiges of locules or placentae have not been found, although ovule-like rudimentary tissue is occasionally formed in the stylar canal.

Carpellate flowers. Rudiments of androecium have not been found. The gynoecium is four-carpellate with a unilocular ovary that is quadrangular or circular in transverse section. There are four (sometimes more due to proliferating placentae) discrete parietal placentae or a broad and diffuse placenta with the ovules covering the whole cavity may be present. The position of the ovary appears to be inferior or half-inferior; however, this assessment implies that the outer scales, clearly located below the ovary, are not part of the flower (figures 3, 4). The central column is differentiated into a short, massive and vascularized style with a stylar canal bearing transmitting tissue, and a stigma formed by a series of large vesicular

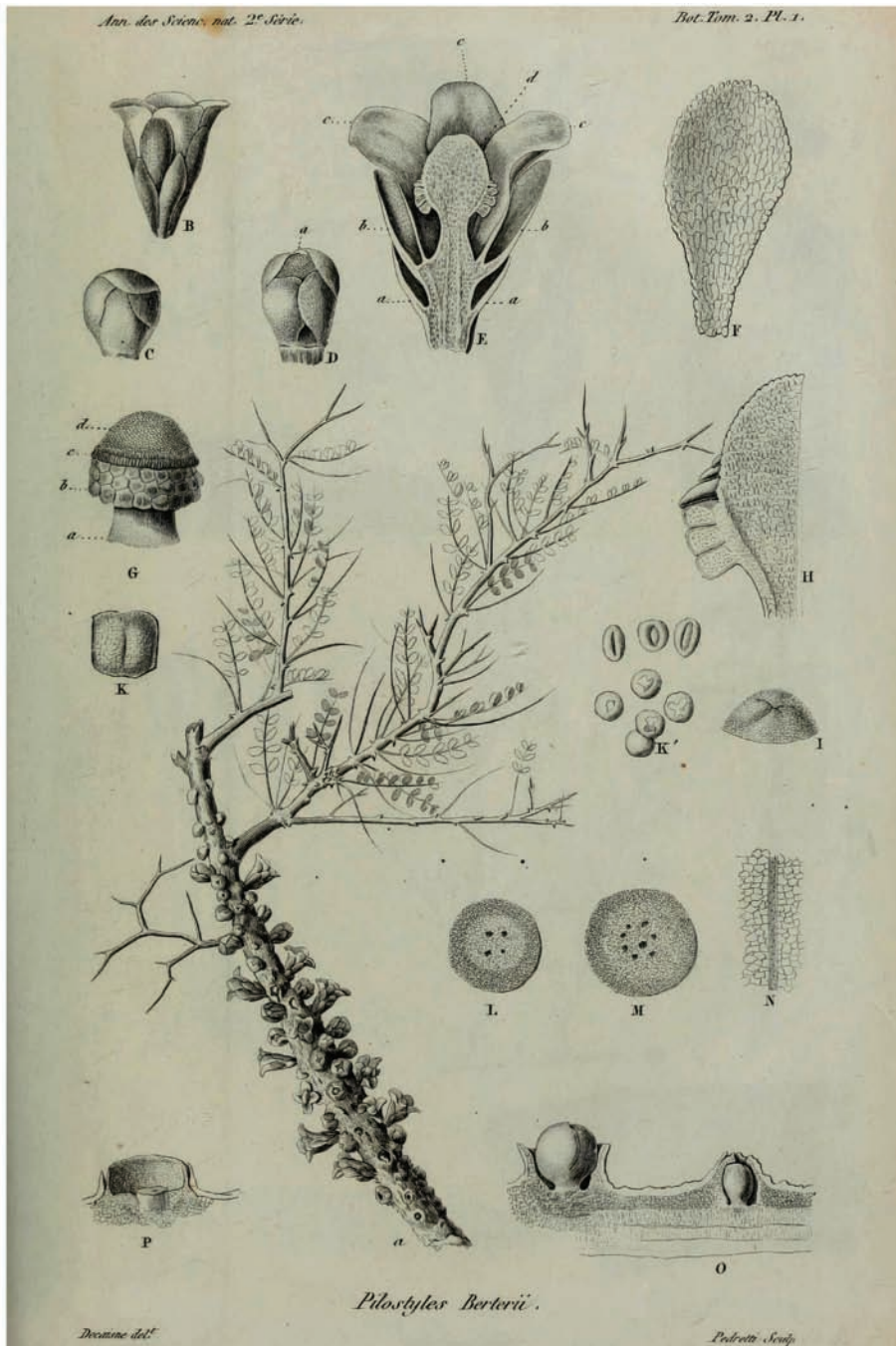


Figure 3. "*Pilostyles berterii*". A. Branch of *Adesmia microphylla* parasitized by *P. berteroi*; B. Flower, side view; C, D. Floral buds; note in D, the tip of the central column "chapeau" (a); E. Longitudinal section of a flower, showing the "bracts" (a), the calyx (b), the petals (c), and the central column ("colonne génitale", d); F. Petal; G-I. Dissections of the "colonne génitale", showing the basalmost portion of the central column ("pied", a), the lowermost pollen sac ring ("anneau antheral", b), the stigmatic marginal papillae ("papilles marginales du chapeau"), and the "chapeau" (d), and (in I) the apical lobes of the "chapeau"; K. Pollen sac; K'. Pollen grains; L-M. Transverse sections of the "colonne" at the levels of its apex (L) and its base (M); N. Floral vascular bundle; O. Longitudinal section of a branch of *Adesmia* and two emerging floral buds of *Pilostyles*; P. Floral scar of *Pilostyles* (from Guillemin, 1834)

papillae located just below the apex of the column. The ovules are bitegmic, tenuinucellate, anatropous to halfway anatropous; the embryo sac is *Polygonum* type. Fruits have been described either as berries or as fleshy capsules; they are small (<1 cm in diameter), ovoid-turbinate, conical to

globose, orange, yellow or red, and are either exposed (figure 4D) or enclosed by the persistent perianth organs (figure 1G, I), they contain up to 100 or more minute (< 0.5 mm long) ovoid to pyriform seeds, which are sticky and hyaline. Both the embryo and the endosperm are reduced.

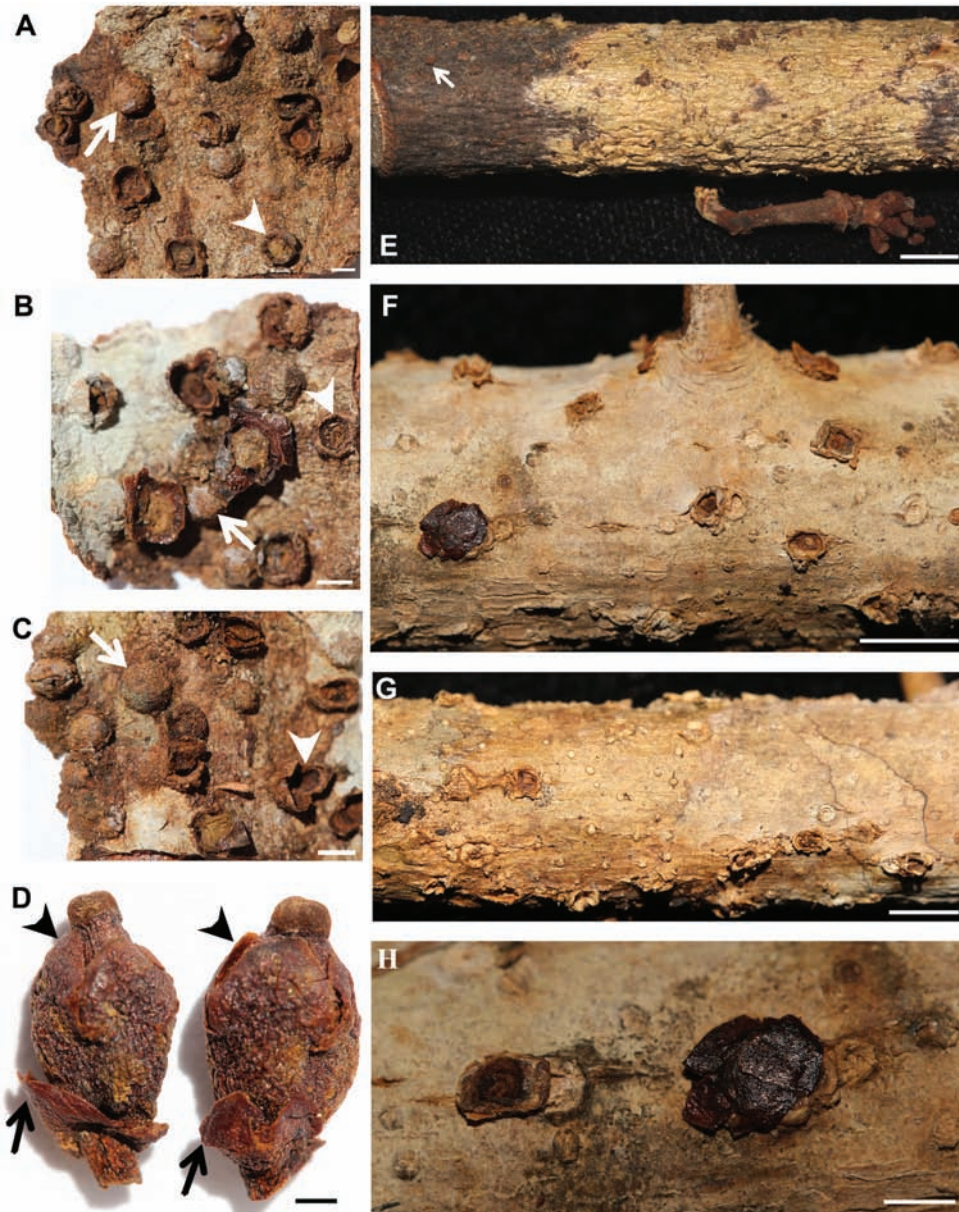


Figure 4. *Apodanthes caseariae* parasitizing *Casearia* spp. [Salicaceae]; figures A-D, *A. caseariae* in the specimen *Idrobo 5225* (COL) of *C. sylvestris* Sw.]. A-C. Bark fragments of *C. sylvestris* with floral buds (arrows) and floral scars (arrowheads) of *A. caseariae*; D. Two carpellate flowers of *A. caseariae*, lateral view, note the massive exserted stigma at the top, and the outer (arrows) and inner (arrowhead) perianth organs; E. Portion of a stem of *C. megacarpa* (*Cuatrecasas 15408*, COL) with minute floral buds of *A. caseariae* (arrow); F-H. Portions of a branch of the specimen *de Bruijn 1093* (COL) with a floral bud, an anthetic flower, and several scars of *A. caseariae* (scale bars: 1 mm in A-D, H; 5 mm in E-G)

Floral and fruit traits of uncertain homology. The origin and homology of several floral organs in Apodanthaceae have long been in dispute, and developmental studies are urgently needed for a better understanding of the perianth organs, the central column, the androecium, and the fruit type.

Perianth organs. The homology of the outer, middle and inner perianth parts has been controversial, although a detailed comparative study by Blarer et al. (2004) suggests that the outer whorl does not correspond to the perianth. This is consistent with the lack of integration of the outer scale-like bracts with the rest of the flower, especially in *Apodanthes* (figure 4D), as a short internode separates the scale-bracts and the middle perianth organs, rendering the ovary as inferior or halfway inferior.

Central column. The central column in the flowers of Apodanthaceae (figure 3 E, G) has long been interpreted as a gynostemium despite the fact that flowers are functionally unisexual. In fact, Guillemin (1834) placed his new genus *Pilostyles* as a member of the class *Gynandria Polyandria*. However, the vestigial gynoeceum in the staminate flowers and the absence of vestigial androecium in the carpellate flowers complicates the interpretation of the central column as the result of the fusion between carpellary and androecial tissue.

A close relationship between Apodanthaceae (as members of Rafflesiaceae) and Aristolochiaceae (cf. Huber, 1960; Solms-Laubach, 1891) was based on the superficial resemblance of the ring-shaped stigmatic surface in these families. A detailed comparison shows, however, that the central column of the former is mainly carpellary tissue, either vestigial or functional (cf. Blarer et al. 2004; Igersheim and Endress, 1998), whereas the gynostemium of the latter is formed by the fusion of 5, 6 or more stamens and stigmatic lobes, both functional and extremely specialized for pollination (González and Stevenson, 2000).

Androecium. The structure of the androecium in Apodanthaceae is unique because the individual, monosporangiate pollen sacs are sessile and do not conform the typical dithecal, tetrasporangiate anthers (pers. obs.; Blarer et al. 2004; Endress and Stumpf, 1990; Guillemin, 1834; Kuijt, 1969; figure 3). In addition, the presence of numerous, contiguous pollen sacs per ring, the transverse dehiscence between two contiguous pollen sacs, and the lack of endothecium do not support the interpretation of each pollen sac as a monosporangiate anther.

Fruits. Fruits in Apodanthaceae have been described as either capsules or berries (cf. Harms, 1935; Karsten, 1856; Solms-Laubach, 1878). However, the histological changes after fertilization and during fruit formation are still unknown. Thus, a developmental/ anatomical study to examine the histology of the pericarp and the mechanism(s) of fruit dehiscence, if any, is underway to unequivocally answer this question. It will also be useful to better understand the plesiomorphic state in fruit evolution in Cucurbitales, as within each of the remaining families of the order (Anisophyllaceae, Begoniaceae, Coriariaceae, Cucurbitaceae, Datisceae, and Tetramelaceae) both fleshy (drupes, pepos or berries) and dry (capsules or samaras) fruits occur.

Systematic and taxonomic considerations. The molecular-based placement of Apodanthaceae in Cucurbitales appears to be well supported. Filipowicz and Renner (2010) stated that the inferior ovary, the parietal placentation, and the dioecy are the morphological synapomorphies for the order. However, these traits need both developmental studies and extensive fieldwork (currently in progress by the authors), as they are still unclear in the family. For instance, the occurrence of dioecy or monoecy in the species of Apodanthaceae is not easy to discern, because of the persistence of many fruits from previous years in a single host individual, the formation of numerous seeds per fruit, and the uncertainty to assert what constitutes an individual in Apodanthaceae (figure 1A, B). According to Bellot and Renner (2013:1091), the endoparasitic lifestyle in the family “makes it difficult to decide whether cases of monoecy in fact represent multiple infections with two or more parasite individuals, each representing one sex”.

The systematics and taxonomy within Apodanthaceae is challenging, mainly due to the extreme reduction of the individuals as obligate parasites, the endoparasitic lifestyle, and the lack of clear-cut characters to distinguish genera and species. A first taxonomic approach to distinguish *Apodanthes* and *Pilostyles* used the features of the floral scales as the primary criteria to key out the two genera (Harms, 1935; Solms-Laubach, 1878). These authors distinguished *Apodanthes* by the “perigonial scales” always epygynous, clawed, caducous, and inner scales slightly connate, versus *Pilostyles*, which has hypogynous outer scales, persistent, with a broad (not clawed) base, and free inner ones. In addition, Harms’s (1935) classification included characters such as the number of perianth series and organs, the number of rings of pollen sacs, and the extent of the placenta. However, these characters are variable at specific and generic levels and therefore are

taxonomically unreliable. For instance, the number of rings of pollen sacs is equivocal, as there are two rings in *Apodanthes* (see Blarer et al. 2004), two to four in *Pilostyles*, and one to two rings in *Berliniache* (treated as *Pilostyles* section *Berliniache* by Harms, 1935).

The recognition of *Berliniache* as a distinct genus from *Pilostyles* (Vattimo-Gil, 1955) has been followed by other authors (e.g., Blarer et al. 2004; Heide-Jørgensen, 2008), who have provided several morphological characters to key out the three genera. We have summarized them as follows:

1. Whorls of scales three or six (rarely seven)-merous; perianth formula 3-6-6 or 3-3-5; inner whorl organs with a hair carpet at the base of the adaxial surface. Androecium forming a tubular synandrium with two whorls of pollen sacs, the inner ones forming a cylindrical column at the center of the flower, and the outer ones forming a hollow tube; sterile gynoecium in staminate flowers not fused to the synandrium; nectary disk conspicuous. Pollen inaperturate. ***Pilostyles (Berliniache) aethiopica***
- 1' Whorls of scales di- or tetramerous; perianth formulas 2-4-4 or 3-4-4; inner whorl organs lacking a basal hair carpet on the adaxial surface. Stamens in one whorl; sterile gynoecium in staminate flowers almost completely fused to the androecium; disk nectary inconspicuous. Pollen tricolpate. **2**
2. Flowers subtended by alternate bracts; perianth formula 2-4-4; organs of the outer whorl of scales caducous; organs of the inner whorl of the perianth fused in a small extension at their bases. Locule of ovary cruciform in transverse section, placentae not diffuse. Parasites primarily on Salicaceae. ***Apodanthes***
- 2'. Flowers subtended by imbricate bracts; perianth formula 3-4-4; organs of the outer whorl of scales persistent; organs of the inner whorl of the perianth fused by a large portion of their bases. Locule of the ovary nearly circular in transverse section, placentae diffuse. Parasites on legumes. ***Pilostyles* spp.**

Alternatively, Blarer et al. (2004) identified the following sets of shared features, which are inconsistent with the key presented above: **a)** *Apodanthes* and *Pilostyles* (as *Berliniache*) *aethiopica* share the doubling in the number of scales from the outer to the middle whorl, the isomerous

middle and inner whorl, the stigmatic papillae covering the whole apical platform in carpellate flowers, and the style not narrower than the stigma; **b)** *P. aethiopica* and the remaining species of *Pilostyles* share legume parasitism, the perianth organs of the inner whorl caducous, with broad insertion, the presence of hairs in a ring in staminate flowers, the simple stigmatic unicellular papillae in carpellate flowers, and the placentae less protruding and bearing fewer ovules than in *Apodanthes*.

The recent combined (morphological plus nuclear plus mitochondrial) analysis carried out by Bellot and Renner (2014) for all members of the family renders the genus *Berliniache* as nested within *Pilostyles*. Thus, the morphological traits of *P. aethiopica* described in the *Apodanthes* + *P. aethiopica* taxonomic scenario mentioned above appear to be homoplastic.

Apodanthaceae in Colombia. Two historical records of Apodanthaceae in Colombia are known. The first was reported by Karsten (1856), who briefly described *Pilostyles ingae* (under the generic name of *Sarna*), from the department of Cauca (“*Ramis Ingae* specierum insidens, in fluminis Cauca valle prope oppidum Popayan observata”; Karsten 1856: 415). The second record comes from the herbarium specimen *I. F. Holton 84* (GH!, K!) collected on February 24, in La Paila, Valle del Cauca (Colombia) (figure 2). This specimen, initially identified as *Apodanthes* sp., bears two latter identifications as *Pilostyles thurberi* Gray in the GH specimen, and *P. ingae* (H.Karst.) Hook.f. (now *P. blanchetii*) in the K specimen. Isaac Farwell Holton (1812-1874) collected by mid-XIX century in Colombia as part of the project “Flora Neogranadina Caucana”. The Kew specimen (available at <http://plants.jstor.org/specimen/viewer/k000601220>) is not fragmented and bears at least 52 flowers and floral buds that are densely arranged on the proximal portion of the host’s branch. It also has two drawings of a flower and a floral bud. The Harvard specimen consists of approximately 20 flowers in different developmental stages, the largest of about 4 mm in diameter. The flowers are scattered in a short branch of ca. 5.5 cm long and 6 mm in diameter (figure 2). In both specimens, the leaves of the host are pinnately-compound, and the leaflets are oblong and minute (ca. 3 x 1 mm), which suggests that it belongs to the genus *Calliandra* (Mimosaceae). Both of these historical collections cannot be further identified as the material is rather fragile and fragmented, although according to their hosts, they could correspond to *P. blanchetii*.

Currently, the collections of Apodanthaceae from Colombia sufficiently documented are here identified at a species level as follows:

Apodanthes caseariae Poit.: PANAMA/COLOMBIA

border: Southernmost peak of Cerro Pirre massif, Alto de Nique, 1300-1520 m, cloud forest, parasitizing a trunk of *Casearia* sp., flowers yellow, fruits brown, 19 Apr 1980 (fl, fr), A. Gentry et al. 28659 (COL)". **COLOMBIA:** **Department of Antioquia:** Remedios, eastern slopes of the Central Cordillera, 500-1500 m of elevation, parasitizing *Trema micrantha*, 4 Jul 1989 (fl, fr), R. Callejas et al. 8062 (HUA); San Roque, Granja Experimental Corpoica, vereda Marbella, 800-1200 m, parasitizing *Casearia*, 14 Fb 1998 (fl, fr), R. Fonnegra et al. 6726 (HUA). **Department of Santander:** 150 km of Barrancabermeja, 74°4'W, 8°15'N, Mico Ahumado camp, 22 Aug 1966 (fl), 900 m, found by the authors in the specimen *J. de Bruijn* (COL; figure 4 F-H) filed under *Casearia aculeata* Jacq. **Department of Casanare:** carretera a Yopal, bosque de galería, río Charte, 19 Oct 1962, found by the authors in the specimen *J. M. Idrobo* 5225 (COL) filed under *Casearia sylvestris* Sw. (figure 4 A-D). **Department of Valle del Cauca:** Western Cordillera, "vertiente occidental, hoya del río Sanquinini, lado izquierdo, La Laguna, 1250-1400 m, 10-20 Dic 1943", found by the authors in the specimen *J. Cuatrecasas* (COL) filed under *Casearia megacarpa* Cuatr. (figure 4 E); finca Kyburz, eastern slope above the Bitaco River, Bitaco valley 1 km above and east of Bitaco, Pacific slope of the Western cordillera, 4500 ft, 16 Nov 1963, found by the authors in the specimen *P. C. Hutchison & J. M. Idrobo* 3010 (COL), filed under *Casearia megacarpa* Cuatr.

Pilostyles boyacensis F. González & Pabón-Mora:

COLOMBIA: **Department of Boyacá:** Chíquiza, vereda Juan Díaz, 2415 m, 30 Nov 2013 (fl, fr), *F. González* 4508 (COL, HUA, NY); Sáchica, vereda El Espino, 2202 m, 01 Dec 2013 (fl fr), *F. González* 4509, 4510 (COL, HUA, NY); Sáchica, vereda El Espino, 2200 m, 15 Dec 2013 (fl fr), *F. González* 4517, 4518 (COL, HUA, NY); Sáchica, vereda El Espino, 2205 m, 01 Mar 2014 (fl fr), *F. González* 4519 (COL, HUA, NY); Villa de Leyva, vereda La Cañuela, 2135 m, 450 m después de la entrada al parque Gondava, 23 Mar 2014 (fl, fr), *F. González et al.* 4521 (COL, HUA, NY); Soatá, Cañón del Chicamocha, 2000-2100 m, Dec 1952 (fl, fr), found by the authors on the *Dalea cuatrecasasii* specimen *J. Hernández* 665 (COL).

Ecological and chorological aspects. The two genera *Apodanthes* and *Pilostyles* do not share hosts, as the first parasitizes primarily Salicaceae (formerly Flacourtiaceae) and the second parasitizes Fabaceae (table 1). Worldwide, the geographic areas of distribution of the hosts are much wider than those of the parasites (table 1). This might also be the case in Colombia, as the distribution of Apodanthaceae

known to occur in the country (summarized in figure 5) are considerably narrower than those of the hosts (not mapped), either members of Fabaceae, Burseraceae, Meliaceae or Salicaceae, or the genus *Trema* (Cannabaceae).

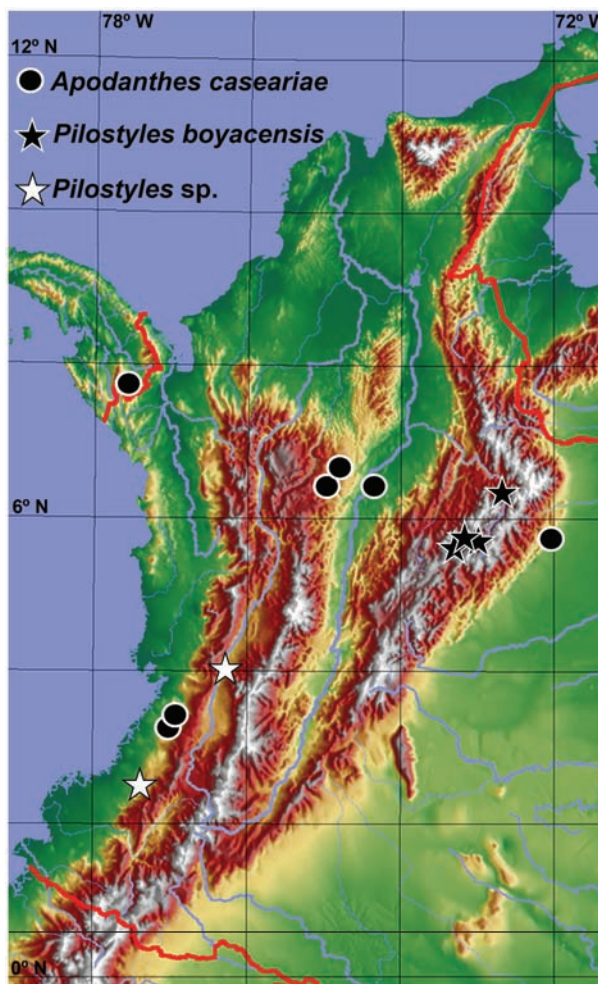


Figure 5. Distribution map of Apodanthaceae in Colombia

A map of the distribution of Apodanthaceae in Colombia (figure 5) indicates that they can grow in wet or dry forests, at elevations between 500 and 2415 m, on both sides of the Andes, as well as in mid-elevation forests of the Cauca and the Magdalena valleys, and in inter-Andean dry valleys of the Eastern Cordillera. In Colombia, they appear to set flowers and fruits mainly during or after the rains, in April, and from October to December.

A swelling in the host stem appears to be the first indication of infection. Flower emergence moves progressively up the

host stem such that the next season's flowers emerge from the distalmost parts of the host twigs (Dell et al. 1982). According to Solms-Laubach (1874), the branching of the primary root of *Pilostyles hausknechtii* is not superficial but it occurs in the cambial region of its host. However, this observation requires further corroboration. The endophyte consists of uniseriate filaments and radial sinkers (Kuijt et al. 1985), formed by cells with large nuclei, two nucleoli and a dense cytoplasm (Riopel and Timko, 1995). At least in *Pilostyles*, the endophyte develops isophasically with respect to the host branch (Kuijt, 1969), that is, it keeps pace with the primary growth of the host shoot apex; however, the vegetative cells of the parasite do not reach the host apical meristem (but see Heide-Jørgensen, 2008, who described that the endophyte develops multicellular strands that can penetrate host tissue to the very shoot tip at least in *Pilostyles*). The endophytic cells of *Pilostyles* develop extensions from the host cortex to the vascular tissue. According to Dell et al. (1982), the vegetative endophyte of *Pilostyles* takes primarily phloem-derived nutrients, whereas the reproductive portion takes nutrients both from the host phloem and xylem. *Pilostyles ingae* affects its host *Mimosa maguirei* by causing an increase in branching but a decrease in the branch length (Gomes and Fernandes, 1994). Host anatomical responses also occur in *Mimosa* spp. parasitized by *P. ulei* and include changes in the size of the plant, vessel diameter, length and number and fiber length, perhaps to compensate for the required hydraulic properties of the host (Amaral, 2007; Amaral and Ceccantini, 2011).

The life cycle, reproduction and dispersal mechanisms of the New World species have not been investigated in detail. The flowers of Apodanthaceae last only a few days. *Pilostyles thurberi* is perennial, but plants appear to flower for only two years, dying afterwards (Heide-Jørgensen, 2008). African and Iranian members of Apodanthaceae are fly-pollinated, although flowers are also visited by ants, bees, butterflies and wasps (Bellot and Renner, 2013; Sipes et al. 2014). At least in the ripened fruits remain attached to the host until the following flowering period (figure 1G, I); thus, the same individual host can have floral buds, anthetic flowers, fruits and seeds simultaneously (figure 1).

In the New World *Apodanthes caseariae*, *Trigona* bees and *Thraupis* birds have been reported as flower visitors and fruit eaters, respectively (Gómez, 1983). According to Heide-Jørgensen (2008), harvest ants (*Messor pergandei*) are likely the pollinators and fruit dispersers of *P. thurberi*, as they need to walk over the parasite on their way up to collecting the rewards of the host *Dalea emoryi*. We have

also seen frequent visits of a number of floral visitors (including Camponotus ants, Vespidae, shield bugs, and small beetles) in the dry areas where the *Pilostyles-Dalea* association occurs in Colombia. According to Sipes et al. (2014), only the bee *Augochloropsis metallica* and potter wasps are effective pollinators or *Pilostyles thurberi*, a process that could be mediated by the secretion of raspberry ketones and eugenols during anthesis of this species.

Guidelines to collect Apodanthaceae. The following field observations are necessary to improve the information contained in the herbarium specimens of Apodanthaceae, and in turn, to better understand the natural history, morphology, and taxonomy of these plants:

1. Occurrence of isophasic development of the parasite in the case of *Pilostyles*. This can be detected if the floral buds are found primarily towards the distal portions of the branch host, and the anthetic flowers and fruits towards the proximal portions of the host.
2. Detection of dioecy versus monoecy on each floral row. The staminate flowers can be detected by the presence of a white, collar row of digitiform, vesicular hairs above the pollen sacs, which is visible from the outside of the flower. The carpellate flowers are more globose at the base, and lack the collar-like roof of hairs on the central column.
3. Discharge of floral scent, particularly strong at noon.
4. Presence of flower and/or fruit visitors. The peaks of visitors detected by the authors, at least in *Pilostyles*, occur at noon.
5. Given that dry specimens are extremely hard to be dissected and lose much of the diagnostic characters, it is highly recommended to fix either in FAA or 70% ethanol each infected host branch in individual tubes or jars with its distal end visually marked, and to record each sample with a good quality and scale documented photographic series. These preserved samples will be critical for further dissection and standard transverse and longitudinal anatomical sections in the laboratory.
6. Silica gel preserved tissues are also critical, as molecular markers have shown to be promising for species recognition (e.g., Thiele et al. 2008; Bellot and Renner, 2014). Here also the flower rows (preferably at pre-anthesis) become the "sampling units" at least in *Pilostyles*, assuming that all flowers of each row are connected underneath by the same endophyte, and that

they all conform a single individual. Thus, each floral row tangentially cut-off from the host tissue needs to be collected as a separate sample in silica gel.

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