The systematics of the worldwide endoparasite family Apodanthaceae (Cucurbitales), with a key, a map, and color photos of most species

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Abstract
Using morphological, nuclear, and mitochondrial data, we here revise the taxonomy of Apodanthaceae and allocate the 36 names published in the family to ten biological species in two genera, Apodanthes and Pilostyles. All species are endo-parasites that live permanently inside trees or shrubs of the families Salicaceae or Fabaceae and that only emerge to flower. Because of this life history, Apodanthaceae are among the least known families of flowering plants. Nevertheless, the World’s herbaria as of 2013 hold at least 785 collections that, in combination with DNA phylogenies, permit well-founded species circumscriptions and geographic range maps. We also provide a key to all species, discuss the newly accepted or synonymized names, and make available color photos of six of the ten species.

Keywords
Apodanthaceae, genus circumscriptions, mitochondrial DNA sequences, nuclear DNA sequences, parasitic plants, species circumscriptions

Introduction
Apodanthaceae Tiegh. ex Takht. (Cucurbitales) is a family of endoparasites that live entirely in their host’s stems and only become visible once the strictly unisexual flowers have burst through the bark. This life style, added to the small size of the flowers and...
patchy occurrence of the apparently mostly dioecious populations, has made it difficult to collect good and complete herbarium material (including both sexes and flowering and fruiting specimens). While populations once identified may be recollected at the same time year after year, usually only local botanists will have the opportunity to carry out such recollections. Apodanthaceae are disjunctly distributed in North and South America, mainland Africa, Iran, and Australia. They occur in arid as well as humid tropical environments. Two genera have been validly described, the worldwide Pilostyles, and Apodanthes from Central and South America.

The taxonomy of the genera and species of Apodanthaceae has not been studied since the work of Ida de Vattimo-Gil (Vattimo-Gil 1950, 1955, 1971, 1973). Modern molecular-phylogenetic work based on representatives of most of the so-far named species (Bellot and Renner in review), together with study of collections deposited in the World’s herbaria since the end of the 19th century, has revealed the need to synonymize many superfluous names, a task that we carry out here. We also up-date the circumscription of the family and its two genera, and clarify that they have specialized on different hosts, namely Salicaceae (mainly Casearia) and Fabaceae.

To achieve a better understanding of species boundaries and relationships, and to clarify the species’ geographic and host ranges, we compared loans from numerous herbaria, dissected flowers, and isolated DNA from multiple collections. Molecular markers useful for these obligatory holoparasites are the nuclear 18S ribosomal RNA region and mitochondrial matR (Barkman et al. 2004; Bellot and Renner in review), and we show here that these markers can be used to place incomplete collections (for example, those of only one sex or only with fruits) in the correct species. Lastly, we provide an annotated key to all species that we recognize, and brief descriptions of their diagnostic traits along with color images and comments on their geographic and host ranges.

Methods

Plant material, DNA extraction and sequencing, phylogenetic analyses

We enlarged the DNA data matrix of Bellot and Renner (in review) by extracting DNA from additional specimens representing either unusual individuals or potential new species. No DNA sequences could be obtained from Pilostyles stawiarskii, known only from two collections in R, and Pilostyles holtzii, the only collection of which was destroyed in World War II. Suppl. material 1 shows species names and their authors, herbarium vouchers, and GenBank accession numbers. In total, 10 sequences (3 of 18S and 7 of matR) were newly generated for this study.

Total genomic DNA was extracted from herbarium specimens using the commercial plant DNA extraction Invisorb® Spin Plant Mini Kit (Stratec molecular, Berlin, Germany). The mitochondrial matR and the nuclear 18S genes were amplified using the primers listed in Bellot and Renner (in review). PCR products were purified with the ExoSAP or FastAP clean-up kits (Fermentas Life Sciences, St. Leon-Rot, Germa-
ny), and sequencing relied on the Big Dye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3130-4 automated capillary sequencer. Chromatograms were checked and sequences were edited using Geneious R7 (Biomatters, available from http://www.geneious.com), and contigs were then blasted against GenBank to rule out contamination. Alignments of the clean sequences were performed using the program MAFFT v. 7 (Katoh 2013) resulting in matrices of 1626 and 1727 aligned nucleotides for *matR* and 18S, respectively. We failed to amplify the gene *matR* from the African *Pilosyles aethiopica* and from the Iranian *P. haussknechtii*. Phylogenetic reconstructions relied on maximum likelihood (ML) as implemented in RAxML-7.2.8-ALPHA (Stamatakis 2006), using the GTR + G model of nucleotide substitution with 100 bootstrap replicates under the same model. Trees were rooted on *Corynocarpus laevigatus* (Corynocarpaceae; Cucurbitales), based on Filipowicz and Renner (2010).

**Morphological data and assessment of the host ranges of Apodanthaceae**

We geo-referenced locality data from 785 herbarium collections on loan from the herbaria B, G, C, GH, K, M, MO, MSB, W, NA, PMA, and SI and added data from the Global Biodiversity Information Facility (GBIF Backbone Taxonomy, 2013-07-01, http://www.gbif.org/species/7279680). We also recorded host names, up-dating their taxonomy as relevant. All label information was compiled in a database using the Botanical Research and Herbarium Management System (BRAHMS, http://herbaria.plants.ox.ac.uk/), and maps were produced using DIVA-GIS 7.5 (http://www.diva-gis.org). Collections were sorted by geography, flowering specimens were sexed to evaluate sexual dimorphism, and a representative number of flowers were then dissected under a stereoscope. For each dissected flower, the first author recorded the number, arrangement and size of the tepals, shape and ornament of the pistil/central column, number of pollen sacs, presence of hairs and presence of a nectary at the base of the flower. Pictures of representative organs were taken using a Dino-Lite USB microscope model AM413ZT (Dino-Lite Europe) and the DinoCapture Imaging software version 2.0 of the same company.

**Results and discussion**

**Genus and species boundaries in Apodanthaceae**

The dissections showed that species have characteristic flower sizes, number of tepals, tepal cilia, and number of anthers rings. For the American species, we use these differences in the key (below). Suppl. material 2 shows measurements and counts from the 123 dissected flowers. Six collections could not reliably be assigned to these groups because their flowers were slightly unusual: *R. Callejas et al. 8062*, a male plant from
Colombia identified as *Apodanthes caseariae* by A. Idarraga in 2002; *Y. Mexia 4540*, a female plant from Brazil that is the type of the name *A. minarum*; H. S. Irwin *et al.* 20350, a female plant from Brazil identified as *Pilostyles ulei* by Ida de Vattimo in 1975; H. S. Irwin 31560, a male plant identified as *P. blanchetii* by the first author but parasitizing an uncommon host (*Dioclea*, Fabaceae); J. Rzedowski 11303, a female plant from Mexico identified by the collector as *P. thurberi*; and F. Chiang 9034, a female plant from Mexico identified as *P. thurberi* by J. Henrickson in 1972.

The 18S and *matR* molecular trees show the *Pilostyles* collections that we wanted to identify (in red on Fig. 1) grouped with *P. thurberi* or *P. blanchetii*. The collections *R. Callejas et al.* 8062 and *Y. Mexia 4540* grouped with two undoubted representatives of *A. caseariae*. *R. Callejas et al.* 8062 is a male plant and comes from the border with Panama, a country where *A. caseariae* has been repeatedly collected. The host of *R. Callejas et al.* 8062 was originally identified as *Trema* (Cannabaceae), but a partial *matR* sequence of this host BLASTed to *Casearia nitida*, making it likely that the host was in fact *Casearia*. If that is the case, this would suggest that the collection represents an *Apodanthes*. The few male flowers of *Apodanthes caseariae* that have so far been dissected (Suppl. material 2) do not allow assessing the full morphological variability of the male flowers of this species. Therefore we had to rely on DNA for identification. In terms of its *matR* (Fig. 1A) *R. Callejas et al.* 8062 was embedded among other sequences of *A. caseariae*, while in terms of its 18S (Fig. 1B), it was sister to them. We identified the specimen as *A. caseariae*. Other *matR* and 18S sequences in the *A. caseariae* clade are from the type of the name *A. minarum* (*Mexia 4540*) from Brazil. Its host was a *Casearia* and its (female) flowers match those of *A. caseariae* (Suppl. material 2). We therefore synonymize *A. minarum* under *A. caseariae* (an action carried out below).

In combination, the present morphological and molecular results show that Apodanthaceae comprise at least ten biological species that can be allocated to two mutually monophyletic genera. In the Americas, these are *Pilostyles thurberi* in the southern United States of America and Mexico, *P. mexicana* in Mexico, Guatemala and Honduras, the widely distributed *P. blanchetii* from Panama to Jamaica to Brazil and Uruguay, and *P. berteroi* in Chile and Argentina. The Americas also harbor *A. caseariae* from Guatemala to Brazil (Fig. 2). Australia has three species, *P. coccoidea*, *P. collina*, and *P. hamiltonii*; Iran has *P. haussknechtii*, and Africa has *P. aethiopica*. The second African species, *P. holtzii* has not been recollected since 1907, when its type collection was made. Another species, the southern Brazilian *P. stawiarskii*, is only known from two specimens (one of them the type) collected at the same locality in Jan./Feb. 1948 and Dec. 1949; morphologically it resembles *P. blanchetii* (Vattimo, 1950). The host ranges of our accepted genera and species do not overlap. *Apodanthes* parasitizes only Salicaceae, whereas *Pilostyles* parasitizes only Fabaceae. As seen on Figure 3, there is a correspondence, although not perfect, between the phylogenies of host genera and parasitic species, and host specialization may have played a role in speciation of Apodanthaceae. At the species level, Table 1 shows that species of Apodanthaceae can grow on one or up to thirteen host species. As seen in Figures 2 and 3, our species concepts are corroborated by geographic and host ranges, except in the
Figure 1. Phylogenetic relationships in Apodanthaceae obtained under maximum likelihood from the mitochondrial gene matR (A) and the nuclear ribosomal RNA gene 18S (B). Shown in red are the specimens we wanted to identify to species. Numbers indicate bootstrap support >70%.
Figure 2. Geographic distribution of Apodanthaceae based on label information from 785 herbarium collections.

Figure 3. Phylogenetic relationships among the hosts of Apodanthaceae (legume relationships from Wojciechowski et al. 2006) and among the species of Apodanthaceae (from Bellot and Renner, in review). Identical colors link parasite species and their host(s) and are also used in Figure 2. Dashed lines represent associations with rarely reported hosts; hosts in bold are the most common ones.
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Table 1. Hosts of Apodanthaceae based on label information from 785 herbarium collections. Upper case numbers refer to the references below the table.

| Parasite       | Host genera                          | Host species                                                                 |
|----------------|--------------------------------------|------------------------------------------------------------------------------|
| *P. aethiopica*| Berlinia¹, Brachystegia, Julbernardia, Pseudoberlinia¹, Westia¹ | Brachystegia boehmii Taub., Brachystegia glaucescens x spiciformis, Brachystegia spiciformis Benth., Brachystegia taxifolia Harms., Julbernardia globiflora (Benth.) Troupin |
| *P. berteroii* | Adesmia                              | Adesmia arbores Bert. ex Savi, Adesmia aff. spinosissima Meyen, Adesmia obovata Clos, Adesmia bedwellii Skotts., Adesmia miraflorensis Remy, Adesmia uspallatensis Gill ex H. & A., Adesmia gracilis Meyen ex Vogel, Adesmia microphylla H. & A., Adesmia monosperma Clos, Adesmia pinifolia Gillies, Adesmia trrigia Gillies |
| *P. blanchetii*| Bauhinia, Cassia, Dioeclea, Galactia², Mimosa, Schnellla | Bauhinia candida Benth., Bauhinia diversicata L., Mimosa clausenii Benth., Mimosa cyclophylla Taub., Mimosa aff. setosa Benth., Mimosa maguirei Barneby, Mimosa scabrella Benth., Mimosa setosissima Taub., Mimosa uraguensis H. & A., Mimosa cf. xanthocentra Martius, Schnella cumanensis Britton & Rose |
| *A. caseariae* | Casearia, Xylosma                     | Casearia aculeata Jacq., Casearia arborea Urb., Casearia decandra Jacq., Casearia grandiflora Cambessèdes, Casearia guianensis Urb., Casearia hirsute Swartz, Casearia nitida Jacq. |
| *P. eucoida*   | Jacksonia                            | Gastrolobium erythrophylum Chandler & Crisp |
| *P. collina*   | Gastrolobium, Oxycloibium             | Gastrolobium erythrophylum Chandler & Crisp |
| *P. hamiltonii*| Daviesia                             | Daviesia angulata Benth., Daviesia decurrens Meissner, Daviesia pectinata Meissner, Daviesia preissii Lindley |
| *P. haussknechtii* | Astragalus, Halimodendron, Onobrychis | Astragalus brachycalyx Fisch., Astragalus brachystachys DC., Astragalus cephalanthus DC., Astragalus chalaranthus Boiss. & Hausskn., Astragalus compacta Reich, Astragalus florosus Boiss., Astragalus gossypinus Fisch., Astragalus microcephalus Willd., Astragalus rhodoesinum Boiss. & Hausskn., Astragalus spinosus Muschler, Astragalus susianus Boiss., Astragalus versus Olivier, Halimodendron halodendron (Pall.) Druce |
| *P. mexicana*  | Calliandra                           | Calliandra houstoniana (Miller) Standley |
| *P. thurberi*  | Dalea, Psorothamnus, Piasula³         | Dalea bicolor Humb. & Bompl. in Willd., Dalea formosa Torrey, Dalea frutescens Gray, Psorothamnus emory (Gray) Rydberg |

¹Verdecourt, B., 1998. Flora of tropical East Africa - Rafflesiaceae. Flora of tropical East Africa 175, 1–2. CRC Press.
²Ule, E., 1915. Rafflesiaceae. Notizblatt des Königl. botanischen Gartens und Museums zu Berlin-Dahlem 6, 292–293.
³Rose, J. N., 1909. Studies of Mexican and Central American Plants n°6. Contributions from the United States National Herbarium 7, 26–265.

The case of *Apodanthes caseariae* and *Pilostyles blanchetii*, both widespread in Brazil. These two species have different sized flowers (see below), and parasitize phylogenetically distantly related hosts (Fig. 3).

**Description of the family**

Stem-endoparasites, non-photosynthetic. No leaves, stem or roots, instead an endophytic system of cells inside the stem parenchyma of the host, flowers bursting
through the host bark. Flowers unisexual, plants dioecious or monoecious, a point still insufficiently known; flowers of both sexes on the same host or not. Pollination by flies and bees, possibly also wasps (Bellot and Renner 2013; Sipes et al. 2014), based on the fruit color and size, dispersal is probably by birds. Flowers white or yellow (*Apodanthes*), or white, pink, orange, red, purple or brown (*Pilostyles*), aggregated on the host stems, minute (1.5 to 15 mm long when dried), usually

**Figure 4.** Tepals of Apodanthaceae. A Tepal of the outer whorl of *Apodanthes caseariae* B Tepal of the middle whorl of *A. caseariae* C Tepal of the inner whorl of *A. caseariae* D Tepal margin of *A. caseariae*. The scale bar corresponds to 0.2 mm.

**Figure 5.** Sexual organs of Apodanthaceae from rehydrated herbarium material. A Androecium of *Pilostyles haussknechtii*, note the two rings of pollen sacs topped by a ring of hairs B Style and stigma of *P. haussknechtii* C Ovary locule and ovules of *P. haussknechtii* D Androecium of *Apodanthes caseariae* after bursting of the pollen sacs, note the hairs covering the column apex E Gynoecium of *A. caseariae*. The scale bar corresponds to 0.2 mm.
with radial symmetry. Perianth composed of 2 or 3, rarely 4, whorls of tepals (Fig. 4A–C), the latter sometimes with hairs along their margins (Fig. 4D), or a hair cushion at their basis (Blarer et al. 2004). In male flowers, the staminal filaments completely fused and forming a tube around a central column that is usually fused to the column (Fig. 5A, D), the up to 72 pollen sacs arranged in 1–4 rings around the column apex (Fig. 5A), the column apex dome-shaped and circled or covered by single-celled hairs (Fig. 5A, D), a basal nectar cushion in both sexes (Fig. 5E). Female flowers without staminodes and with a single thick style topped by the dome-shaped stigma (Fig. 5B, C, E). Ovary semi-inferior, placenta parietal with 50–300 ovules (Fig. 5C, E). Seeds ca. 0.5 mm long (Bouman and Meijer 1994). Fruit a fleshy berry.

Key to the genera and species of Apodanthaceae

1a From the Neotropics, parasitizing Salicaceae, tepals always in 3 whorls with (from the outside) 2, 4, and 4 tepals, the inner whorl easily deciduous, female flowers >5 mm long .............................. 1. Apodanthes caseariae

1b From the Neotropics, Africa, Iran, or Australia, parasitizing Fabaceae, number of outer tepals usually >2, female flowers <5 mm long ...................... 2

2a Occurring in Australia ....................................................... 3

2b Not in Australia ................................................................. 5

3a Tepals in 3 whorls ............................................................. 2. Pilostyles collina

3b Tepals in 2 whorls ............................................................. 4

4a Flower diameter >3 mm ................................................ 3. P. hamiltonii

4b Flower diameter <3 mm ................................................ 4. P. cocoidea

5a Occurring in Africa .......................................................... 5. P. aethiopica

5b Not in Africa ................................................................. 6

6a Occurring in Iran ............................................................. 6. P. haussknechtii

6b Occurring in the America .................................................. 7

7a Tepals in 3 whorls, each with 2 to 7 tepals, anthers in 4 whorls (spiral), number of anther lobes >70, on Adesmia ...................................................... 7. P. berteroi

7b Tepals in 3 whorls, each with 3 or 4 (rarely more) tepals, anthers in 2 or 3 whorls, number of anther lobes <70, not on Adesmia .............................. 8

8a Middle tepals ovoid, on Calliandra, Dalea, Parosela or Psorothamnus, anthers in 3 whorls (at least if on Dalea, Parosela or Psorothamnus) .................. 8. P. blanchetii

8b Middle tepals mostly diamond-shaped, apparently never on Calliandra, Dalea, Parosela or Psorothamnus, anthers in 2 whorls (females of the three species cannot be securely distinguished) ............................. 8. P. blanchetii

9a On Calliandra, tepals in 3 whorls, each with 4 tepals .......................... 9. P. mexicana

9b On Dalea, Parosela, or Psorothamnus, tepals in 3 whorls, each with 3 or 4 tepals. ......................................................... 10. P. thurberi
Allocation of all species names so far described in Apodanthaceae

1. **Apodanthes caseariae** Poiteau, *Ann. Sci. Nat. (Paris)* 3: 422, t. 26. 1824.  
http://species-id.net/wiki/Apodanthes_caseariae

**Apodanthes flacourtiae** Karsten, Linnaea 28: 413. 1857. Type: Venezuela, Aragua, Choroni, parasitic on “Flacourtiaceae” [most like a species that today would be placed in Salicaceae], *H. Karsten s.n.* (W, destroyed in WWII), **syn. nov.**

**Apodanthes tribracteata** Rusby, *Descr. S. Amer. Pl.* 15. 1920. Type: Bolivia, near Ing-lis-Inglis, 8 Aug. 1902, *R. S. Williams 1580* (NY), **syn. nov.**

**Nom. inval. Apodanthes matogrossensis** Vattimo, *Rodriguésia* 26(38): 45. 1971, without Latin descr. Type: Brazil, Mato Grosso, parasitic on *Casearia*, *J. G. Kuhlmann 53076* (R, not seen).

**Apodanthes panamensis** Vattimo-Gil, *Rodriguésia* 26(38): 45. 1971, without Latin descr., Latin diagnosis in *Rev. Brasil. Biol.*, 33(1): 140. 1973. Type: Panama, Canal Zone, Aug. 1984, *R. E. Woodson Jr. and R. W. Schery 965* (NY, MO). Already synonymized by A. Gentry (1973).

**Apodanthes surinamensis** Pulle, *Recueil Trav. Bot. Néerl.* 6: 259. 1909. Type: Suriname, along the Marowijne River, July-Dec. 1903, parasitic on Flacourtiaceae [most like a species that today would be placed in Salicaceae], *G. M. Versteeg s.n.* (U0007645), **syn. nov.**

**Apodanthes roraimae** Ida de Vattimo, *Rodriguésia* 29(44): 48-49. 1978. Type: Brazil, Roraima, 24 Jul. 1974, parasitic on Flacourtiaceae [most likely a species that today would be placed in Salicaceae], *G. T. Prance et al. 21353* (NY), **syn. nov.**  
Comment: George Yatskievych, a curator at the Missouri Botanical Garden, also studied the NY isotype in 2004 and annotated it as *A. caseariae*.

**Apodanthes minarum** Vattimo-Gil, *Rodriguésia* 26 (38): 45. 1971, without Latin descr.; Latin diagnosis in *Rev. Brasil. Biol.*, 33(1): 140. 1973. Type: Brazil, Minas Gerais, Viçosa, 31 Mar. 1930, *Y. Mexia 4540* (L, MO), **syn. nov.**

**Type.** French Guiana, Karouany, c. 1802, parasitic on *Casearia spec.*, *P. A. Poiteau s.n.* (P: P00686413).

**Note.** Tepals white to yellow, female flowers >5 mm long, tepals in 3 whorls, the outer with 2 tepals, the inner one easily deciduous (Figs 4A–D; 6C, D). Growing in trunk and branches of *Casearia* and occasionally *Xylosma* (Salicaceae, Fig. 3) in Guatemala, Honduras, Costa-Rica, Panama, Colombia, Venezuela, Suriname, French Guiana, Brazil, Peru and Bolivia (Fig. 2).

2. **Pilostyles collina** Dell, *Nuytsia* 4: 293–294. 1983.  
http://species-id.net/wiki/Pilostyles_collina

**Type.** Australia, Western Australia, Peak Charles, 10 Jan. 1982, parasitic on *Oxylobium*, *B. D. Dell 8216* (G, MO).
Note: Tepals orange to red, in 3 whorls. Growing in young stems of *Gastrolobium* and *Oxylobium* in Western Australia (Figs 2, 3, see Thiele et al. 2008 for pictures of flowers).

3. *Pilostyles hamiltonii* Gardner, J. Roy. Soc. Western Australia 32: 77. 1948.  
http://species-id.net/wiki/Pilostyles_hamiltonii

Type. Australia, Western Australia, Darling District, Helena Rover, Mundaring Weir, Mar. 1946, parasitic on *Daviesia pectinata* Lindl., C. D. Hamilton s.n. (PERTH, not seen).  
Note: Tepals dark burgundy, in 2 whorls, flowers >3 mm in diameter. Growing in young stems of *Daviesia* in Western Australia (Figs 2, 3, see Thiele et al. 2008 for pictures of flowers).

4. *Pilostyles coccoidea* K.R.Thiele, Nuytsia 18: 273–284. 2008.  
http://species-id.net/wiki/Pilostyles_coccoidea

Type. Australia, Western Australia, Waddi Road, 30°33’26”S, 115°28’10”E, 7 Mar. 2008, parasitic on *Jacksonia*, K.R. Thiele 3495 (PERTH 07692447).  
Note. Tepals pale orange to brown, in 2 whorls, flowers <3 mm in diameter. Growing in stems of *Jacksonia* in Western Australia (Figs 2 and 3, see Thiele et al. 2008 for pictures of flowers).

5. *Pilostyles aethiopica* Welwitsch, Trans. Linn. Soc. London 27: 66–70. 1871 = *Berlinianche aethiopica* (Welw.) Vattimo-Gil, nom. inval.  
http://species-id.net/wiki/Pilostyles_aethiopica

*Pilostyles boltzii* Engler, Bot. Jahrb. Syst. 46: 293. 1912 = *Berlinianche boltzii* (Engl.) Vattimo-Gil, not validly published. Type: Tanzania, Kilimatinde, July 1907, parasitic on *Berlinia eminii* Taub., *W. Holtz* 1422 (B, destroyed during World War II),  
syn. nov. (based on the protologue).

Syntypes. Angola, Huila, 12 May 1860, parasitic on *Berlinia paniculata* Benth. = *Pseudoberlinia paniculata* (Benth.) P.A.Duvign., *F. M. J. Welwitsch* 529, 529b (C, G).  
Note. Tepals pink to brown, in 3 to 4 whorls each with 3-6 tepals. Male flowers with 1 or 2 ring(s) of ca. 15 pollen sacs, stamen filaments free from the central column (Fig. 6F), hair cushion at the basis of the inner tepals (Blarer et al. 2004). Growing in branches of *Julbernardia* and *Brachystegia*, maybe also on *Berlinia, Westia* and *Pseudoberlinia*, in Zimbabwe, Zambia, Tanzania, Angola and Malawi (Figs 2, 3).
6. *Pilostyles haussknechtii* Boissier, Arch. Sci. Phys. Nat. 25: 255–261. 1866. http://species-id.net/wiki/Pilostyles_haussknechtii

**Type.** Middle East, parasitic on *Astragalus, J. E. Haussknecht s.n* (G-BOISS, not seen).

**Note:** Tepals pink to brown in 2 whorls, each with 6 to 10 tepals (Fig. 6G). Found at the basis of young branches of *Astragalus* and occasionally *Onobrychis* and *Halimodendron* in Iran (Figs 2, 3).

7. *Pilostyles berteroi* Guillemin, Ann. Sci. Nat., Bot., sér. 2, 2: 21. 1834 = *Apodanthes berteroi* (Guill.) Gardner, Hooker’s Icon. Pl. 7: t. 655. 1844. syn. nov. http://species-id.net/wiki/Pilostyles_berteroi

**Syntypes.** Chile, Quillota, parasitic on *Adesmia*, [in Chile 1828-1831] C. L. G. Bertero s.n. (P, not seen); Chile [from the collection number this was in “various localities in the Andes”, during the period from 27 Oct.-26 Nov. 1841], *T. Bridges 1273* (BM, not seen, K, not seen).

**Note:** Tepals purple to brown with clearer margins (Fig. 6A), 9–18 in number, stamens in 4 whorls (spirals), with > 70 pollen sacs. Growing in older stems of *Adesmia* shrubs in Chile, Argentina, Peru, and Bolivia (Figs 2, 3). Our morphological (Suppl. material 2) and molecular data (Fig. 1) show that the species is nested among other species of *Pilostyles*, indicating that Gardner’s transfer was erroneous.

8. *Pilostyles blanchetii* (Gardner) R.Br., Trans. Linn. Soc. London 19(3): 247. [6 Nov 1844] = *Apodanthes blanchetii* Gardner, Icon. Pl. 7: t. 655 b. 1844 [Jul 1844] = *Frostia blanchetii* (Gardner) H. Karst., Nov. Acta Acad. Caes. Leop.-Carol. Nat. Cur. 26: 922. 1858. http://species-id.net/wiki/Pilostyles_blanchetii

*Pilostyles calliandrae* (Gardner) R.Br., Trans. Linn. Soc. London 19(3): 247. [6 Nov 1844] = *Apodanthes calliandrae* Gardner, Icon. Pl. 7: t. 644. 1844 [Jan 1844] = *Frostia calliandrae* (Gardner) H. Karst., Nov. Acta Acad. Caes. Leop.-Carol. Nat. Cur. 26: 921. 1858. Type: Brazil, Amazonas, near Maynas [Manaus], Feb. 1840, *G. Gardner 3639* (K000601222), **syn. nov.**

*Pilostyles caulotreti* (Karsten) Hook.f., Prodr. (DC.) 17: 116. 1873 = *Sarna caulotreti* Karsten, Linnaea 28: 415. Jun 1857 [1856]. Type: Venezuela, “H. Karsten s.n.” (W, destroyed in WWII). Comment: Gentry (1973) considered this name as synonym of *P. blanchetii*, and we agree with this assessment.

*Pilostyles ingae* (Karsten) Hooker f., Prodr. (DC.) 17: 116. 1873 = *Sarna ingae* H. Karst., Linnaea 28: 415. Jun 1857 [1856]. Type: Colombia, Cauca, Popayán, parasitic on *Inga, H. Karsten s.n.* (W, destroyed in WWII), **syn. nov.** (based on the protologue).
Pilostyles galactiae Ule, Notizbl. Königl. Bot. Gart. Berlin 6: 292. 1915. Type: Brazil, Amazonia, Surumu River, tributary of the Rio Branco, Oct. 1909 and Mar. 1910, parasitic on Galactia jussiaeana Kunth., E. Ule 7895 (B, holotype destroyed in WWII; isotype NY), syn. nov.

Pilostyles goyazensis Ule, Ber. Deutsch. Bot. Ges. 33: 475. 1915. Syntypes (all parasitic on Mimosa): Brazil, Goias, region near city of Corumba, Sobradinho, Aug. 1892, E. Ule 3097; Serra dos Pyreneos, Mun. Corumba, Dec. 1892, E. Ule 3098; same location, Dec. 1892, E. Ule 3099; in the Corumba region, Aug. 1892, not flowering, E. Ule s.n.; Serra dos Pyreneos, Aug. 1892, not reproductive, E. Ule s.n. (all in B, material destroyed in WWII), syn. nov. (based on the protologue).

Pilostyles globosa (S.Watson ex Robinson) Hemsl., J. Linn. Soc., Bot. 31: 311. 1896 = Apodanthes globosa S.Watson ex Robinson., Bot. Gaz. 16: 83, tab. 9, 1891. Type: Mexico, Northern part, Sierra Madre, parasitic on Bauhinia lunarioides A. Gray, C. G. Pringle 1950 (G), syn. nov.

Pilostyles stawiarskii Vattimo-Gil, Revista Brasil. Biol. 10: 196. 1950. Type: Brazil, Paraná, Mun. de Palmas, parasitic on Mimosa scabrella Benth. [incl. its synonym Mimosa bracaatinga Hoehne], Jan. 1948 and Feb. 1948, V. Stawiarski R50.591 and 50.592 (R, photos). There is also a topotypical collection from Dec. 1949, syn. nov. (based on the protologue).

Pilostyles ulei Solms-Laub., in Goebel, Organogr. Pfl. 2,1: 434. Figure 292 (1900), descr. in Endriss, Flora, Ergänz.-Bd. 91: 209. 1902. Type: Brazil, Goias, parasitic on Fabaceae, E. Ule s.n. (B, destroyed in WWII; R has E. Ule 34, E. Ule 36, E. Ule 38, E. Ule 148, E. Ule 367, E. Ule 482, and E. Ule 483 labeled as this species, not seen). Comment: already Solms-Laubach (1901) and Endriss (1902) considered P. ulei as a synonym of P. ingae.

Type. Brazil: Bahia, 1839, J. S. Blanchet 2861 (NY).

Note. Tepals purple to brown sometimes with clearer margins (Fig. 6E), in 3 whorls with usually 4 (rarely 3-6) tepals, the middle tepal diamond-shaped. Stamens in 2 whorls. Found in branches of Mimosa and Bauhinia, but also Cassia, Dioclea, Galactia and Schnella, in Jamaica, Cayman Islands, Costa-Rica, Panama, Colombia, Venezuela, Guyana, Brazil, Argentina and Uruguay (Figs 2, 3).

9. Pilostyles mexicana (Brandegee) Rose, Contr. U.S. Natl. Herb. 12(7): 264. 1909 = Apodanthes mexicana Brandegee, Zoe 5(11): 245. 1908.
http://species-id.net/wiki/Pilostyles_mexicana

Type. Mexico, near Zacuapan, Tenampa, parasite on Calliandra grandiflora Benth., Oct. 1906, C.A. Purpus 2207 (NY).

Note. Tepals red to brown, in 3 whorls, each with 4 tepals. Growing in branches of Calliandra in Guatemala, Honduras and Mexico (possibly further south; Figs 2, 3).
10. *Pilostyles thurberi* Gray, Pl. Nov. Thurb. 326–327. 1854.  
http://species-id.net/wiki/Pilostyles_thurberi

*Pilostyles covillei* Rose, Contr. U.S. Natl. Herb. 12: 263. 1909. Type: USA, Texas, Dickens county, Matador ranch, 14 June 1894, parasitic on *Parosela formosa* (Torr.) Vail, *F. V. Coville* 1860 (US, not seen).

*Pilostyles glomerata* Rose, Contr. U.S. Natl. Herb. 12: 263. 1909. Type: Mexico, Puebla, near Tehuacán, 31 Aug. 1905, parasitic on *Parosela*, *J. N. Rose and J. H. Painter* 9942 (NY, G). The protologue gives the collection number as 8942.

*Pilostyles palmeri* Rose, Contr. U.S. Natl. Herb. 12: 263. 1909. Type: Mexico, San Luis Potosí, near Alvarez, May 1887, parasitic on *Parosela*, *E. Palmer* 584 (US-570088).

*Pilostyles pringlei* (Watson) Hemsl., J. Linn. Soc., Bot. 31: 311. 1896 = *Apodanthes pringlei* Watson ex B.L.Rob., Bot. Gaz. 16: 83, tab. 9. 1891, no Latin descr.; *Pilostyles pringlei* (Watson) Rose, Contr. U.S. Natl. Herb. 12: 264. 1909, superfluous transfer. Type: Mexico, Sierra Madre, near Monterey, 27 June 1888, parasitic on *Dalea frutescens* A. Gray, *C. G. Pringle* 1949 (NY, M, G).

*Pilostyles sessilis* Rose, Contr. U.S. Natl. Herb. 12: 263. 1909. Type: male flowers: Mexico, Hidalgo, Ixmiquilpan, 1905, parasitic on *Parosela*, *J. N. Rose* 9041 (NY); female flowers: Mexico, Querétaro, hacienda Ciervo, 20 Aug. 1905, parasitic on *Parosela tuberculata* (Lag.) Rose, *J. N. Rose and J. H. Painter* 9636 (NY, US).

**Type.** USA, probably Arizona, near Gila river, June 1850, parasitic on *Psorothamnus emoryi* (A. Gray) Rydb., *G. Thurb* 682 (NY).

**Note.** Tepals white, red to brown, in 3 whorls, each with 3 or 4 tepals, rarely more (Fig. 6B). Growing in branches of *Dalea*, *Parosela* and *Psorothamnus* in the southern United States of America and Mexico (Figs 2, 3). New York (NY) has a specimen from Mexico of this species annotated as “*Pilostyles mortoni*”, a nomen nudum, by Ida de Vattimo in 1952.

**Note on an invalid genus name**

Harms (1935) tried to place the two African names, *Pilostyles aethiopica* Welw. and *P. holtzii* Engl., in a separate section, *Pilostyles* section *Berlinianche*, named for their legume host species in the genus *Berlinia*, but failed to include a Latin diagnosis for the new section. Later, Vattimo-Gil (1955, 1971) decided to rank this section as a separate genus because of the hair cushions on the inner perianth whorl and strictly tri- and hexamorous flowers compared to the tetramorous flowers of the American species of *Pilostyles*. This assessment, however, could only have been based on specimens of *P. aethiopica*, since the only collection of *P. holtzii* burnt in World War II. Unfortunately, Vattimo-Gil also neglected to provide a Latin diagnosis, and the genus name is therefore not valid. Based on our results (Fig. 1), *P. aethiopica* does not deserve generic status because it is embedded among the other species of *Pilostyles*. 
Note on a possible new species of Pilostyles

Flavio González and Natalia Pabón-Mora, at the university of Antioquia in Colombia, are studying the ecology and morphology of Apodanthaceae in Colombia (González and Pabón-Mora accepted a) and are describing a new species of Pilostyles (González and Pabón-Mora accepted b). This species is the first Pilostyles parasitizing the legume genus Dalea in South America and occurs in dry valleys of the Colombian Eastern Cordillera at altitudes above 2000 m. Morphologically, the new species is most similar to P. berteroi, which grows in the Chilean and Peruvian Andes at up to 3000 m of altitude (Fig. 2) and parasitizes Adesmia (closely related to Dalea, see Fig. 3).

Conclusion

By combining morphological and molecular information, we show that Apodanthaceae comprise 10 species and that morphological distinctions fit well with geographical disjunctions and specializations on different hosts (Salicaceae vs. Fabaceae). DNA sequences of mitochondrial matR and nuclear 18S rDNA, along with morphology and geography permit identifying any collection of Apodanthaceae. A wider sampling of the morphological variation, especially of male Apodanthes caseariae and female Pilostyles blanchetii, P. mexicana and P. thurberi, however, is needed to determine whether some unusual specimens might deserve to be ranked as subspecies.

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**Supplementary material 1**

**List of accessions used in this study with author names for each species, herbarium vouchers, and GenBank accession numbers**

Authors: Sidonie Bellot, Susanne S. Renner

Data type: species list

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**Supplementary material 2**

**Results of the dissections of 123 flowers from 82 specimens of Apodanthaceae**

Authors: Sidonie Bellot, Susanne S. Renner

Data type: measurements

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