

## COLLETTERS IN AMERICAN SPERMACOCEAE GENERA (RUBIACEAE): MORPHOANATOMICAL AND EVOLUTIONARY ASPECTS

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**Premise of research.** The primary goal of this study is to describe the collectors on vegetative and reproductive organs in the Spermacoceae tribe and to characterize their morphoanatomy, their distribution, and some functional and evolutionary aspects.

**Methodology.** The anatomy, development, and vascularization of collectors from 28 species were studied using standard light and scanning electron microscopy based on fresh and fixed material. Our results are interpreted within the framework of recent molecular phylogenies.

**Pivotal results.** Standard-type collectors were found on stipules, calyx, and bracteoles, and they were also found on underground buds in five species of *Galianthe*. Six species have green collectors, and two have vascularized collectors. We discuss previously unknown functional aspects based on the *Staelia glandulosa* collectors and propose a new term: long-lived collectors.

**Conclusions.** This article describes the anatomy of collectors in the Spermacoceae tribe of the family Rubiaceae in detail. The presence of underground collectors in *Galianthe grandifolia* is confirmed and extended to other species of the genus. Vascularization is recorded for the first time in collectors of *Mitracarpus* and *Staelia*. This study provides data about the type, distribution, and anatomy of collectors in Spermacoceae, contributing important information for ongoing phylogenetic studies in the tribe. The proposal of a new functional type of collector based on anatomical, morphological, and ecological aspects, named long-lived collector, reflects the need to perform further comprehensive studies based on multidisciplinary approaches.

**Keywords:** green collectors, long-lived collectors, Spermacoceae, vascularized collectors.

### Introduction

The Rubiaceae is one of the five most species-rich flowering plant families, with ca. 13,000 species (Goevarts et al. 2011), divided into three subfamilies (Rubioidae, Ixoroideae, and Cinchonoideae), 40 tribes, and ca. 620 genera. In Rubioidae, the tribe Spermacoceae is one of the most species-rich lineages. It has 61 genera and ca. 1235 species, which are distributed in intertropical and subtropical areas of the Americas, Africa, Oceania, and Australia (Groeninckx et al. 2009). Recent phylogenetic studies on the tribe Spermacoceae demonstrate the existence of several monophyletic clades, of which the *Spermacoce* clade is deeply nested among the remaining genera of the tribe (Groeninckx et al. 2009). The clade is an often-herbaceous group, with plants generally characterized

by the following combination of characters: fimbriate stipules, uniovulate ovary locules, and pluriaperturate pollen grains. In the Americas, the *Spermacoce* clade comprises 23 genera with more than 400 species (Salas et al. 2015; R. M. Salas, unpublished data).

Collectors are structures that secrete sticky substances. Their main function is to protect the developing meristem against desiccation (Solereeder 1908; Robbrecht 1988; Thomas and Dave 1989; Thomas 1991; Appezzato-da-Gloria and Estelita 2000; Klein et al. 2004; Mayer et al. 2013). They also protect the meristems of shoot apices and lateral buds against insect and pathogen attacks (Miguel et al. 2006). However, the occurrence of collectors is not limited to vegetative organs; they also occur on reproductive organs (Gonzalez and Tarragó 2009; Mayer et al. 2011; Gonzalez et al. 2012). In Rubiaceae, it has further been proposed that collector secretion participates in nutritional aspects of the bacterial leaf nodule symbiosis (Lersten 1975).

Morphologically, collectors are multicellular trichomes consisting of a parenchymatous middle axis surrounded by a layer

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of secretory palisade-like epidermal cells. Because colleters develop from both protoderm and the underlying meristem, they are considered as emergences (Dave et al. 1988; Robbrecht 1988; Thomas 1991). This structural organization is seen in the most common type of colleter, known as the “standard type” (Lersten 1974*a*, 1974*b*). However, other types of colleters are also recognized in Rubiaceae depending on their morphology (Lersten 1974*a*, 1974*b*; Robbrecht 1988; Thomas 1991; Coelho et al. 2013).

Colleters are present in more than 60 angiosperm families, and the number is increasing with research progress (Thomas and Dave 1989; Muravnik et al. 2014). They have received special attention in Rubiaceae in recent years, because their morphology, abundance, and distribution are of taxonomic relevance (Robbrecht 1988; Thomas 1991; De Block 2003; Taylor 2003).

The aim of this study was to describe the colleters on vegetative and reproductive organs in the tribe Spermacoeae and characterize their morphoanatomy and distribution. The results are interpreted and discussed based on phylogenetic trees, so that some evolutionary questions can be answered, such as whether genera with the same pattern of colleter distribution are phylogenetically related and how many times vascularized colleters appear independently. A new insight in their functionality is discussed based on *Staelia glandulosa*, and a new functional type is proposed: “long-lived colleters.”

## Material and Methods

### Studied Taxa

We studied 28 species belonging to 13 genera of the tribe Spermacoeae. Voucher specimens were deposited in the herbarium of the Instituto de Botánica del Nordeste, Corrientes, Argentina (appendix). Living material was collected in the field, and vegetative and reproductive parts were preserved in formalin–acetic acid–alcohol (5 mL formalin, 5 mL acetic acid, and 90 mL 50% ethanol). Samples from two species were analyzed from herbarium specimens; they were previously expanded in 2.5% aqueous NaOH. Most of the species analyzed in this study belong to the *Spermacoe* clade, whereas other genera, such as *Manettia* Mutis ex L. and *Oldenlandia* L., belong to the former tribes Manettieae and Hedyotideae, respectively. The last two tribes were recently incorporated into Spermacoeae, mainly on the basis of molecular studies. Our sampling represents native species from Argentina, Brazil, and Mexico; only *Oldenlandia corymbosa* is an Asian weed that is widespread in the tropics.

### Light Microscopy

Fixed and rehydrated herbarium material was analyzed and imaged under a Leica MZ6 (Leica Microsystems) dissecting microscope to determine the number and morphology of the colleters and their positions on the organs. For light microscopy observations, fixed material was dehydrated and embedded in paraffin (Johansen 1940; modified according to Gonzalez and Cristóbal 1997), then cut into 10–12- $\mu$ m sections

using a Microm HM350 rotary microtome (Microm International, Walldorf, Germany). Both cross and longitudinal sections were stained with safranin and Astra blue (Luque et al. 1996) and mounted in synthetic Canada balsam. Observations and digital images were acquired using a Leica DM LB2 (Leica Microsystems) light microscope equipped with a Leica digital camera. A camera lucida was used for the drawings. The presence of crystals and xylem was confirmed by observations with polarized filters. Fresh material was hand-sectioned, and the autofluorescence of plastid chlorophyll was detected using a Leica DM 1000 (Leica Microsystems) fluorescence microscope with a UV-2B filter. The node and stipules in some species were also cleared with sodium hypochlorite (NaOCl) to examine their vasculature (Johansen 1940).

### Scanning Electron Microscopy

For scanning electron microscopy, fixed material was dehydrated in an increasing acetone series and then critical-point dried using liquid CO<sub>2</sub> (Denton Vacuum, DCP-1, Pleasanton, NJ) and sputter-coated with gold-palladium (Denton Vacuum, Desk II, Pleasanton, NJ). The gold-coated samples were photographed with a Jeol LV 5800 (JEOL, Tokyo, Japan) at 10 kV in the Service of Electron Microscopy facility at the Universidad Nacional del Nordeste.

The general terminology of Rubiaceae colleters follows Robbrecht (1988). Different terminologies have been used for the projections found on the stipules and interlobular sinuses of the calyx in the taxonomic descriptions of the species analyzed, such as fimbriae (Bacigalupo and Cabral 2007), laciniae (Delprete 2010; Salas and Cabral 2010), lobes (Borhidi 2006; Bacigalupo and Cabral 2007), setae (Kirkbride 1979; Delprete 2010), teeth (Bacigalupo 1996; Delprete 2010), and intercalary appendices (Bacigalupo 1996). To unify the terminology, in this study, the term “fimbria” describes those structures that are longer than they are wide, whereas the term “teeth” describes structures of a similar length and width, both in vegetative and reproductive organs.

## Results

In the analyzed species of the tribe Spermacoeae, the colleters occur on the calyx, bracteoles, and stipules, and *Galianthe* also has colleters on underground buds. The distribution, characteristics, and number of colleters in the analyzed species are given in table 1.

### Colleters on Calyx

All species have a two- to four-lobed calyx (six-lobed calyx in *Richardia*) with short teeth and/or fimbriae on the interlobular sinuses (fig. 1). The fimbriae can be glabrous (fig. 1C–1F, 1I) or pubescent (fig. 1G, 1H). The colleters occur at several locations: (i) on the tips of teeth and fimbriae of the interlobular sinuses (in all species except *Oldenlandia corymbosa*; fig. 1A–1J); (ii) on the inner surface of the calyx (in *Manettia cordifolia* and *Manettia paraguariensis*; fig. 1I); and (iii) on

**Table 1**  
**Distribution and Characteristics of Colleters in the Analyzed Species of Spermaceae**

Species, organ	Distribution of colleters				Morphology	Axis with raphides	Axis with chlorenchyma
	No. teeth tipped with colleter	No. fimbriae tipped with colleter	No. colleters on border	No. colleters on inner surface			
<i>Borreria brachystemonoides:</i>							
Calyx	1-3	...	...	...	Conical, rare: rounded	-	...
Bracteoles	...	>10	...	...	Conical, rare: rounded	-	...
Stipules	...	7-9	...	...	Conical, rare: rounded	-	...
<i>Borreria orientalis:</i>							
Calyx	1-3	...	...	...	Conical	-	+
Bracteoles	...	7-9	...	...	Conical	-	+
Stipules	...	7-9	...	...	Conical	-	+
<i>Borreria palustris:</i>							
Calyx	1-3	...	...	...	Conical	+	...
Bracteoles	...	6-7	...	...	Conical	+	...
Stipules	...	7-9	...	...	Conical	+	...
<i>Diodia saponariifolia:</i>							
Calyx	2	...	...	...	Conical, rare: rounded, pad-like	+	+
Bracteoles	...	4-6	...	...	Conical, rare: rounded, pad-like	+	+
Stipules	3-4	1-3	2	...	Conical, rare: rounded, pad-like	+	+
<i>Ernodea littoralis:</i>							
Calyx	2-3	...	...	...	Conical, rare: rounded	+	...
Bracteoles	3-5	5	...	...	Conical, rare: rounded	+	...
Stipules	2-4	1-3	2-3	...	Conical, rare: rounded	+	...
<i>Hexasepalum apiculatum:</i>							
Calyx	2-4	...	...	...	Conical, rare: cylindrical	+	...
Bracteoles	...	6-8	...	...	Conical, rare: cylindrical	+	...
Stipules	...	>10	...	...	Conical, rare: cylindrical	+	...
<i>Galianthe centranthoides:</i>							
Calyx	1-2	...	...	...	Conical	+	...
Bracteoles	...	2-3	...	...	Conical	+	...
Stipules	...	7-9	...	...	Conical	+	...
Underground buds	...	ND	...	...	Conical	+	...
<i>Galianthe fastigiata:</i>							
Calyx	1-2	...	...	...	Conical	+	...
Bracteoles	...	2	...	...	Conical	+	...
Stipules	...	7-9	...	...	Conical	+	...
Underground buds	...	ND	...	...	Conical	+	...
<i>Galianthe hispidula:</i>							
Calyx	1-2	...	...	...	Conical	+	+
Bracteoles	...	2-3	...	...	Conical	+	+
Stipules	...	7-9	...	...	Conical	+	+
Underground buds	...	ND	...	...	Conical	+	+
<i>Galianthe valerianoides:</i>							
Calyx	1-2	...	...	...	Conical	+	...
Bracteoles	...	2-3	...	...	Conical	+	...
Stipules	...	7-9	...	...	Conical	+	...
Underground buds	...	ND	...	...	Conical	+	...
<i>Manettia cordifolia:</i>							
Calyx	3-6	0-1	3-4	...	Conical, rare: rounded	+	...
Bracteoles	-	-	-	...	Conical, rare: rounded	+	...
Stipules	>10	0-3	>10	4-8	Conical, rare: rounded	+	...
<i>Manettia paraguariensis:</i>							
Calyx	5-7	0-1	2-6	...	Conical	+	+
Bracteoles	-	-	-	-	Conical	+	+
Stipules	1-2	0-2	>10	...	Conical	+	+

Table 1 (Continued)

Species, organ	Distribution of colleters				Morphology	Axis with raphides	Axis with chlorenchyma
	No. teeth tipped with colleter	No. fimbriae tipped with colleter	No. colleters on border	No. colleters on inner surface			
<i>Mitracarpus megapotamicus:</i>							
Calyx	...	1-2	...	...	Conical, rare: cylindrical	+	...
Bracteoles	...	3-5	...	...	Conical, rare: cylindrical	+	...
Stipule (IF)	...	12	...	...	Conical, rare: cylindrical	+	...
Stipule (EF)	...	8-9	...	...	Conical, rare: cylindrical	+	...
<i>Mitracarpus pusillus:</i>							
Calyx	...	1-2	...	...	Conical	+	...
Bracteoles	...	>10	...	...	Conical	+	...
Stipule (IF)	...	6-7	...	...	Conical	+	...
Stipule (EF)	...	7	...	...	Conical	+	...
<i>Mitracarpus polygonifolius:</i>							
Calyx	...	1-2	...	...	Conical	+	...
Bracteoles	...	3-5	...	...	Conical	+	...
Stipule (IF)	...	15	...	...	Conical	+	...
Stipule (EF)	...	7-8	...	...	Conical	+	...
<i>Oldenlandia corymbosa:</i>							
Calyx	-	-	-	-	Conical, rare: rounded	-	...
Bracteoles	-	-	-	-	Conical, rare: rounded	-	...
Stipules	...	5-8	...	...	Conical, rare: rounded	-	...
<i>Oldenlandia salzmanni:</i>							
Calyx	1-3	...	...	...	Conical, rare: cylindrical	+	+
Bracteoles	2-3	...	1-2	...	Conical, rare: cylindrical	+	+
Stipules	1-2	...	1-2	...	Conical, rare: cylindrical	+	+
<i>Planaltina capitata:</i>							
Calyx	...	3	...	...	Conical, elongated	-	...
Bracteoles	...	>10	...	...	Conical, elongated	-	...
Stipules	...	7-9	...	...	Conical, elongated	-	...
<i>Psyllocarpus asparagoides:</i>							
Calyx	...	2-4	...	...	Conical, cylindrical, rounded	-	...
Bracteoles	...	>6	...	...	Conical, cylindrical, rounded	-	...
Stipules	...	>8	...	...	Conical, cylindrical, rounded	-	...
<i>Psyllocarpus laricoides:</i>							
Calyx	...	2-4	...	...	Conical, rare: cylindrical	-	...
Bracteoles	1-2	4-7	...	...	Conical, rare: cylindrical	-	...
Stipules	...	>8	...	...	Conical, rare: cylindrical	-	...
<i>Psyllocarpus phyllocephalus:</i>							
Calyx	...	4-7	...	...	Conical	-	...
Bracteoles	...	6-7	...	...	Conical	-	...
Stipules	...	>8	...	...	Conical	-	...
<i>Richardia brasiliensis:</i>							
Calyx	1-2	1-2	...	...	Conical, rare: cylindrical	-	+
Bracteoles	...	5-6	...	...	Conical, rare: cylindrical	-	+
Stipules	...	4-6	...	...	Conical, rare: cylindrical	-	+
<i>Richardia scabra:</i>							
Calyx	0-1	1-2	...	...	Conical, rare: cylindrical	-	...
Bracteoles	...	>5	...	...	Conical, rare: cylindrical	-	...
Stipules	...	4-6	...	...	Conical, rare: cylindrical	-	...
<i>Spermacoce prostrata:</i>							
Calyx	1-3	...	...	...	Conical, rare: cylindrical	+	...
Bracteoles	...	>4	...	...	Conical, rare: cylindrical	+	...
Stipules	...	7-9	...	...	Conical, rare: cylindrical	+	...
<i>Staelia domingosii:</i>							
Calyx	...	3	...	...	Conical	-	...
Bracteoles	...	>4	...	...	Conical	-	...
Stipules	...	3	...	...	Conical	-	...

Table 1 (Continued)

Species, organ	Distribution of colleters				Morphology	Axis with raphides	Axis with chlorenchyma
	No. teeth tipped with colleter	No. fimbriae tipped with colleter	No. colleters on border	No. colleters on inner surface			
<i>Staelia glandulosa</i> :							
Calyx	...	1–3	...	...	Cylindrical, rare: conical	–	...
Bracteoles	...	>5	...	...	Cylindrical, rare: conical	–	...
Stipules	...	3	...	...	Cylindrical, rare: conical	–	...
<i>Staelia virgata</i> :							
Calyx	...	3	...	...	Conical	–	...
Bracteoles	...	2–3	...	...	Conical	–	...
Stipules	...	3	...	...	Conical	–	...

Note. A minus sign indicates absence, and a plus sign indicates presence. EF = external fimbriae; IF = internal fimbriae; ND = no data.

the margin of the calyx lobes (e.g., *Richardia brasiliensis* and *Richardia scabra*; fig. 1J).

#### Colleters on Bracteoles

A bracteole is a foliar organ markedly reduced to a stipular sheath and its fimbria, in which the blade is absent (fig. 1A, 1K–1L). The fimbriae may be pubescent (fig. 1L) or glabrous (fig. 1K). Colleters are located on the apices of the fimbriae. *Oldenlandia corymbosa* and the two analyzed species of *Manettia* lack bracteoles.

#### Colleters on Stipules

In general, the Rubiaceae has opposite leaves with interpetiolar stipules. In the tribe Spermacoceae, the leaf bases are fused, forming a generally fimbriate stipular sheath (fig. 2A, 2B). The fimbriae are glabrous (fig. 2E–2G, 2J–2L) or pubescent (fig. 2H, 2I). *Staelia domingosi* has ternate leaves, and *Staelia glandulosa* has three to four leaves per node. The four-leaf condition has not previously been documented for the tribe. The margin of the stipular sheath is mostly multifimbriate in the *Spermacoce* clade (fig. 2A). In *Manettia*, the margin of the stipule is entire (fig. 2C), with some teeth and/or fimbriae. The colleters occur on the margins of the stipules of all the species studied, without any teeth or fimbriae below (fig. 2C), and also on the teeth and/or the fimbriae (fig. 2E–2L). The fimbriae of *Mitracarpus* are arranged in two rows, an inner one with small fimbriae and an outer one with large fimbriae (fig. 2J). The colleters on *M. cordifolia* also occur irregularly on the inner distal surface of the stipules (fig. 2M).

#### Colleters on Underground Buds

The *Galianthe* species have a rhizome (*Galianthe hispidula* and *Galianthe valerianoides*) or xylopodium (*Galianthe centranthoides*, *Galianthe fastigiata*, and *Galianthe grandifolia*). In both cases, the species have buds with reduced leaf blades (fig. 2N). The interpetiolar stipules have a small sheath with fimbriae tipped with small colleters (fig. 2O).

#### Colleter Morphology and Anatomy

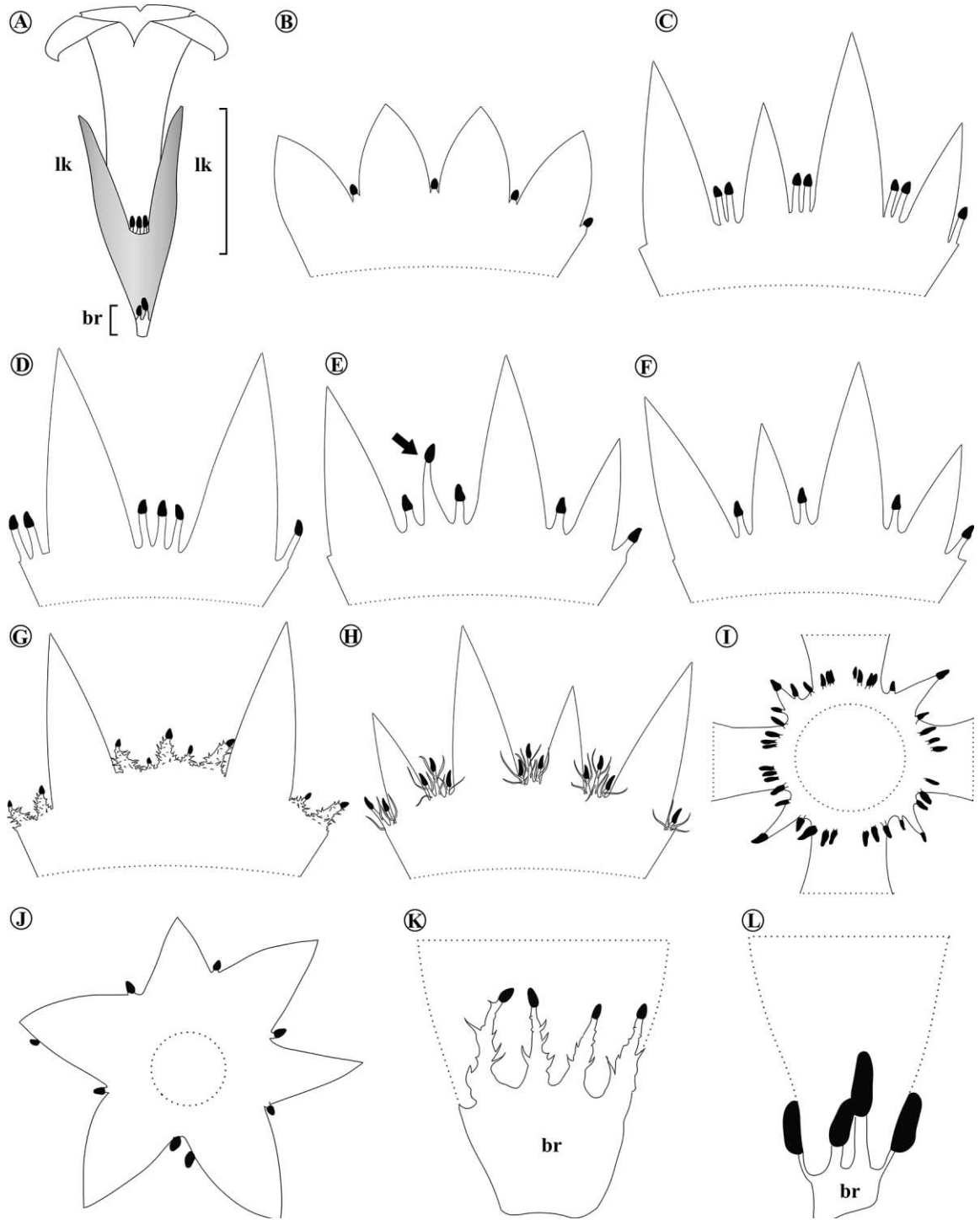
In all cases, the colleters are of the standard type. The colleter shape is rather uniform. The most common shape is conical, and it is rarely cylindrical or rounded (table 1; fig. 3). The colleters present on stipules are larger than those on the calyx or bracteoles (table 2). The largest stipular colleters are present in *S. glandulosa* (520.37  $\mu\text{m}$ ) and *M. paraguariensis* (372.94  $\mu\text{m}$ ), and the smallest ones are found on *Psyllocarpus* stipules (44.51  $\mu\text{m}$ ). Among the calycine colleters, the largest ones occur on *M. paraguariensis* and *S. glandulosa*, whereas the smallest are found on *Psyllocarpus laricoides*, *Psyllocarpus asparagoides*, and *Borreria palustris*.

The colleters consist of a central axis of parenchymatous tissue covered with a palisade epidermis (fig. 4A–4D, 4F). A thin cuticle, 2–4- $\mu\text{m}$  thick, covers the entire structure. The cells of the axis are rectangular in longitudinal section, and they are longitudinally arranged, without any intercellular spaces. In some species, these cells have acicular needles of calcium oxalate packed in raphides (fig. 4A, 4B, 4E, 4J, 4K). The epidermal cells are typically glandular with a dense cytoplasm, densely packed around the axis. The colleters are circular or oval in cross section (fig. 4H). The analysis of fresh material of six species showed that the central axis of colleters from stipules and calyx is composed of chlorenchyma (fig. 4I–4L).

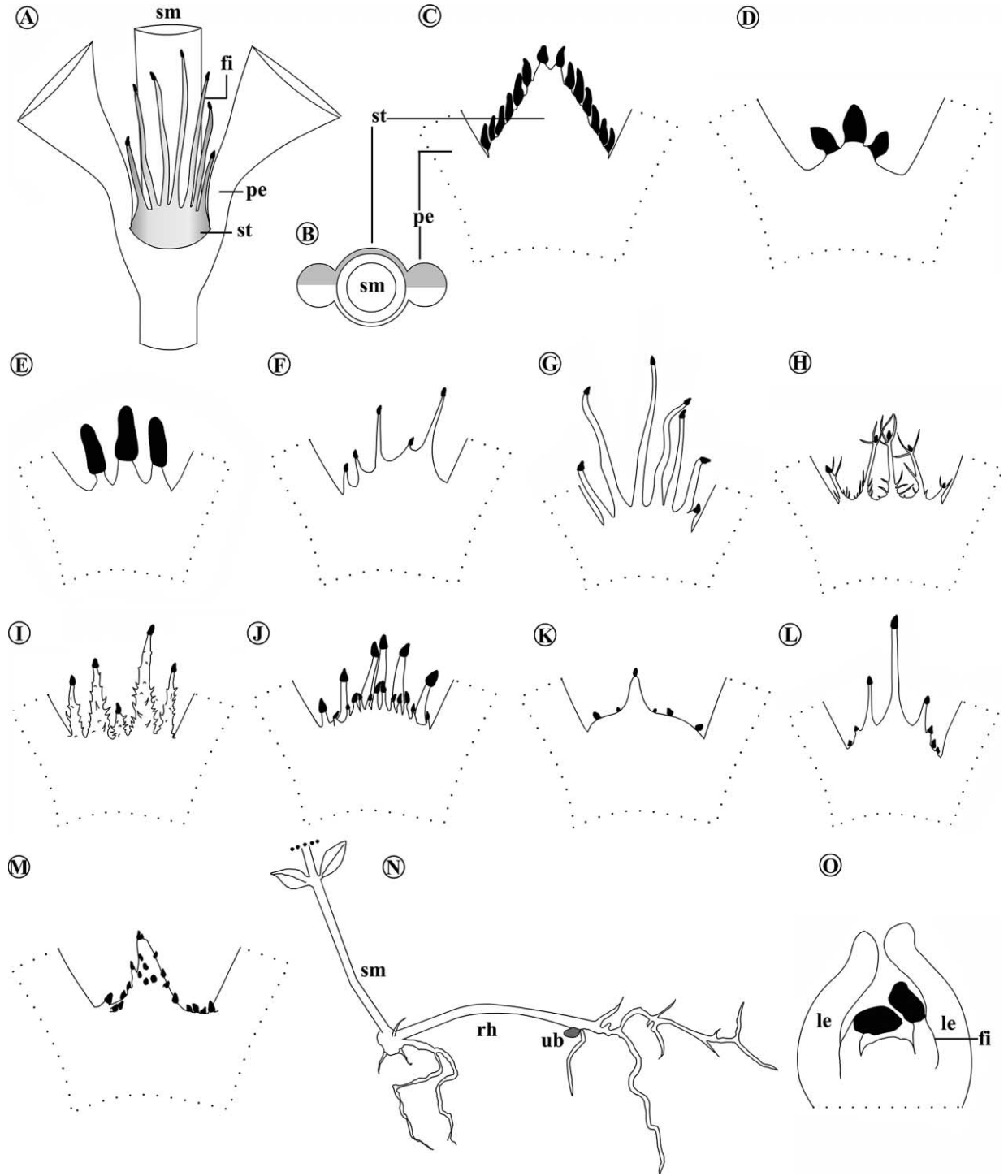
#### Vascularization

Only *Mitracarpus polygonifolius* (figs. 4D, 4E, 6E, 6F) and *S. glandulosa* (fig. 4F–4H) of the 28 species analyzed have vascularized colleters. Such colleters have a single vascular bundle composed of xylem and phloem elements (figs. 4D–4H, 6E, 6F). They are present on the stipules and on the tip of the reduced lobes of the calyx of *S. glandulosa* and on the longest fimbriae of the stipules in *M. polygonifolius*.

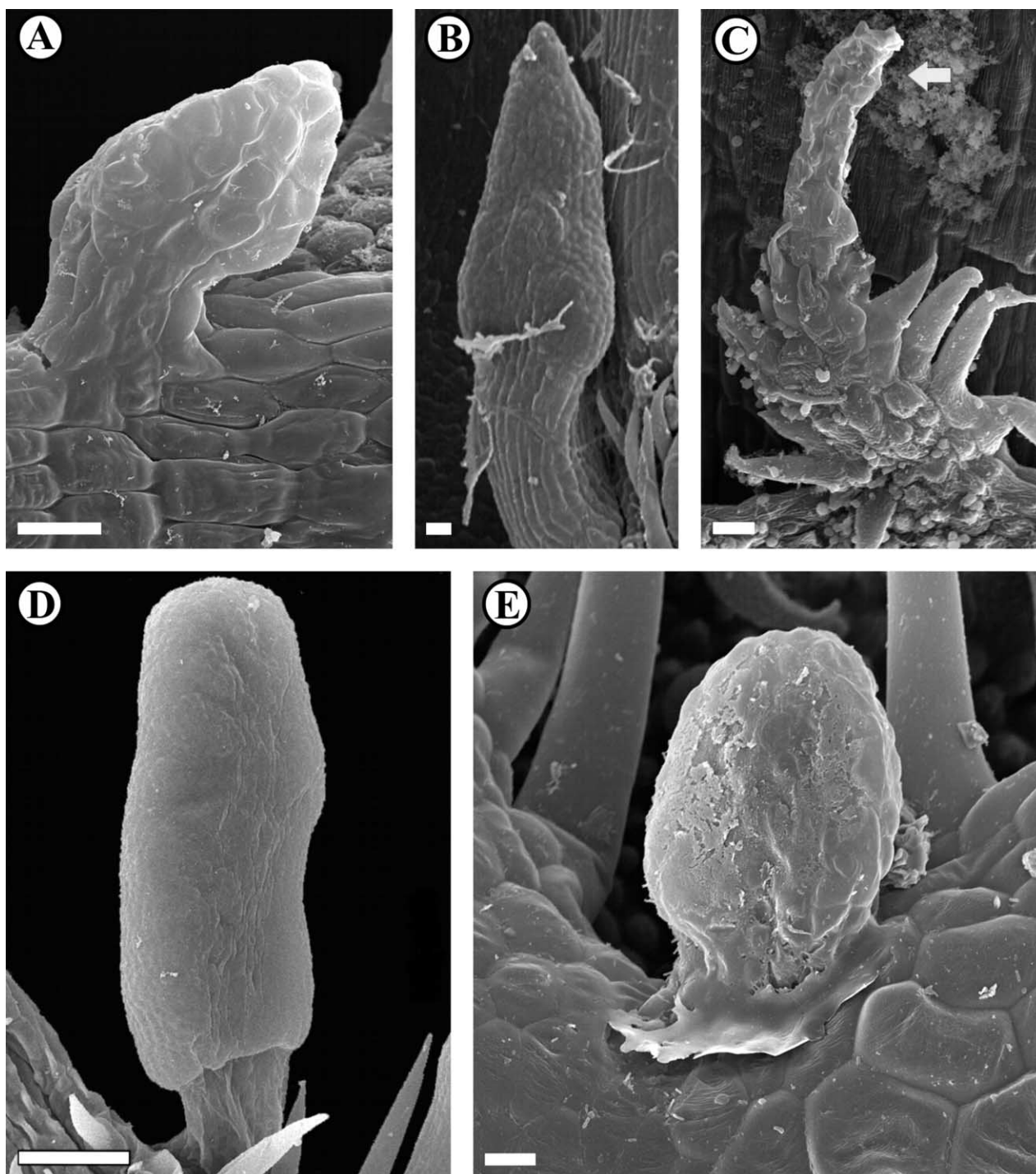
As described above, *M. polygonifolius* has opposite leaves (fig. 5A), and *S. glandulosa* has three or four leaves per node (fig. 5B–5J). Nodal vasculature is bi-, tri-, or tetralacunar, depending on the leaf arrangement. In both of these species,



**Fig. 1** Distribution of colleters in calyx and bracteoles. A, Outline of a Spermaceace flower with colleters (in black) in the interlobular sinus of calyx (lk; in gray) and on bracteoles (br). B–H, Diagrams of unfolded calyx showing the number of sepals, fimbriae, teeth, and position of colleters (in black). B, *Borreria*, *Ernodea littoralis*, *Galianthe*, *Hexasepalum apiculatum*, *Oldenlandia salzmannii*, *Spermaceoce prostrata*. C, *Mitracarpus*. D–F, *Staelia glandulosa*; note the reduction of the fourth calyx lobe to a fimbria tipped by a colleter in E (arrow). G, *Psyllocarpus*; note the pubescent fimbriae. H, *Planaltina capitata*; note the pubescent fimbriae. I–J, Adaxial view of calyx tube and lobes. I, *Manettia*. J, *Richardia*. K–L, Detail of a flower with colleters on bracteoles (br). K, *Psyllocarpus*; note the presence of papillae and hairs on the fimbriae. L, Typical situation in the studied species (fimbriae glabrous).



**Fig. 2** Distribution of colleters on stipules and underground buds. *A*, Outline of a nodal region in surface view showing the position of stem (sm), petiole (pe), interpetiolar stipules (st), and fimbria (fi) with colleters in black. *B*, Outline of a node in cross section. *C–L*, Portions of interpetiolar stipules (marked in gray in *B*). *C*, *Mannettia paraguayensis*. *D*, *Oldenlandia salzmannii*. *E*, *Staelia*. *F*, *Oldenlandia corymbosa*. *G*, *Borreria*, *Galianthe*, *Planaltina capitata*, *Spermacoce prostrata*. *H*, *Richardia*; note the pubescent fimbriae. *I*, *Psyllocarpus*; note the presence of papillae and hairs on the fimbriae. *J*, *Mitracarpus*, *Hexasepalum apiculatum*; note the two rows of fimbriae. *K*, *Ernodea littoralis*. *L*, *Diodia saponarifolia*. *M*, *Manettia cordifolia*; note the colleters on the inner surface. *N*, Outline of a stem (sm) and rhizome (rh) with underground bud (ub). *O*, Longitudinal section of subterranean bud in *Galianthe*, with modified leaves protecting the apex (le), reduced fimbriae (fi), and colleters (black).



**Fig. 3** Morphology of colleter (SEM). A–C, Conic colleter. A, *Ernodea littoralis*. B, *Galianthe hispidula*. C, Fimbriae of *Psyllocarpus* with conic colleter (arrow). D, Cylindrical colleter of *Staelia glandulosa*. E, Rounded colleter of *Richardia brasiliensis*. Scale bars: 20  $\mu\text{m}$ .

each petiole has one median trace departing from a single gap in the central vascular cylinder (fig. 5E–5G). Two lateral traces depart from this central bundle and split immediately; the median strands complete the vascular supply of the petiole, and the external bundles extend along the stipules (fig. 5H). Several traces depart from these vascular bundles and penetrate the fimbriae, reaching the colleter (fig. 5A, 5B, 5I, 5J). The vascular bundles

from two adjacent leaves are connected in the interpetiolar stipule (fig. 5A–5D).

#### *Ontogeny and Secretion*

Observations of the colleter development showed a close similarity between all the studied species, and therefore only



Table 2

## Measures of Colleters in the Analyzed Species of Spermaceae

Species, distribution	Length ( $\mu\text{m}$ )			Width ( $\mu\text{m}$ )			Length:width ratio
	Median	Maximum	Minimum	Median	Maximum	Minimum	
<i>Borreria brachystemonoides:</i>							
Stipule	143.17	192.61	71.12	97.13	136.20	53.90	1.47:1
Calyx	103.76	145.48	60.25	50.51	87.56	34.76	2.05:1
Bracteole	133.57	159.18	79.12	76.68	135.13	53.83	1.74:1
<i>Borreria orientalis:</i>							
Stipule	393.68	520.08	267.91	204.48	238.22	146.23	1.92:1
Calyx	177.46	213.85	133.77	55.89	74.32	37.78	3.17:1
Bracteole	209.90	302.15	126.16	82.86	140.37	49.09	2.53:1
<i>Borreria palustris:</i>							
Stipule	314.99	547.43	209.8	96.95	107.01	78.95	3.25:1
Calyx	59.55	66.68	52.42	40.97	46.88	35.06	1.45:1
Bracteole	89.18	180.30	50.01	45.07	88.59	30.81	1.98:1
<i>Diodia saponariifolia:</i>							
Stipule	339.89	461.66	149.00	284.03	488.18	87.19	1.19:1
Calyx	126.54	169.10	90.89	106.65	183.76	49.10	1.18:1
Bracteole	204.42	257.60	136.14	125.46	191.93	84.88	1.63:1
<i>Ernodea littoralis:</i>							
Stipule	100.66	171.18	56.20	77.38	147.79	41.33	1.30:1
Calyx	124.42	172.22	71.49	82.35	113.13	60.11	1.51:1
Bracteole	112.34	137.57	83.59	84.30	120.32	42.19	1.33:1
<i>Galianthe centranthoides:</i>							
Stipule	110.17	112.54	107.80	77.44	83.46	71.42	1.42:1
Calyx	104.35	112.84	95.87	74.08	77.52	70.64	1.41:1
Bracteole	ND	ND	ND	ND	ND	ND	ND
Underground buds	143.38	192.88	99.85	ND	ND	ND	ND
<i>Galianthe fastigiata:</i>							
Stipule	126.28	166.11	87.69	59.01	67.63	50.40	2.14:1
Calyx	76.80	95.59	46.06	43.21	59.52	31.64	1.77:1
Bracteole	110.71	116.20	106.82	ND	ND	ND	ND
Underground buds	109.43	164.15	54.71	ND	ND	ND	ND
<i>Galianthe hispidula:</i>							
Stipule	238.49	353.70	140.41	109.51	203.67	64.08	2.17:1
Calyx	115.74	133.43	90.32	67.52	101.82	45.96	1.71:1
Bracteole	144.29	174.71	75.95	70.08	96.26	59.36	2.05:1
Underground buds	204.56	298.26	78.77	118.46	159.70	70.22	1.73:1
<i>Galianthe valerianoides:</i>							
Stipule	112.02	139.19	84.86	74.58	83.11	66.05	1.50:1
Calyx	161.52	176.93	152.24	82.29	90.72	76.41	1.96:1
Bracteole	ND	ND	ND	ND	ND	ND	ND
Underground buds	175.54	249.07	117.75	ND	ND	ND	ND
<i>Hexasepalum apiculatum:</i>							
Stipule	142.01	229.63	96.34	58.00	72.76	41.11	2.45:1
Calyx	98.32	130.06	58.93	49.21	77.19	33.37	1.99:1
Bracteole	120.65	145.84	89.27	62.46	80.48	41.20	1.93:1
<i>Manettia cordifolia:</i>							
Stipule	125.47	165.97	90.84	102.97	119.04	72.37	1.22:1
Calyx	121.05	199.00	75.27	62.62	98.59	37.21	1.93:1
Bracteole	ND	ND	ND	ND	ND	ND	ND
<i>Manettia paraguariensis:</i>							
Stipule	319.79	372.94	189.93	117.67	217.00	74.13	2.71:1
Calyx	310.14	375.26	205.75	88.26	125.63	60.30	3.51:1
Bracteole	ND	ND	ND	ND	ND	ND	ND
<i>Mitracarpus megapotamicus:</i>							
Stipule (IF)	95.83	133.18	64.26	61.97	71.09	50.46	1.55:1
Stipule (EF)	151.54	168.65	122.91	98.16	104.77	88.91	1.54:1
Calyx	79.55	91.58	53.95	54.46	65.01	49.21	1.46:1
Bracteole	77.75	87.95	56.97	53.08	76.55	42.52	1.46:1

Table 2 (Continued)

Species, distribution	Length ( $\mu\text{m}$ )			Width ( $\mu\text{m}$ )			Length:width ratio
	Median	Maximum	Minimum	Median	Maximum	Minimum	
<i>Mitracarpus pusillus:</i>							
Stipule (IF)	76.22	117.32	54.46	58.75	110.65	27.15	1.29:1
Stipule (EF)	117.94	139.12	95.55	87.41	119.82	41.89	1.35:1
Calyx	79.53	123.12	51.59	47.10	54.58	39.85	1.68:1
Bracteole	114.74	166.14	85.77	69.31	82.85	41.94	1.65:1
<i>Mitracarpus polygonifolius:</i>							
Stipule (IF)	174.53	301.02	119.43	85.70	118.32	55.04	2.04:1
Stipule (EF) <sup>a</sup>	360.07	467.37	242.20	154.20	206.32	80.75	2.33:1
Calyx	87.26	101.92	54.67	42.96	49.29	36.68	2.03:1
Bracteole	109.88	123.98	95.04	49.19	61.19	36.76	2.23:1
<i>Oldenlandia corymbosa:</i>							
Stipule	118.92	143.29	79.72	86.54	133.62	58.94	1.37:1
<i>Oldenlandia salzmannii:</i>							
Stipule	143.80	175.52	102.49	118.33	156.33	92.32	1.21:1
Calyx	102.82	169.21	56.28	78.42	116.55	40.17	1.31:1
Bracteole	98.47	119.35	71.20	71.71	112.27	48.15	1.37:1
<i>Planaltina capitata:</i>							
Stipule	232.88	320.82	181.92	78.47	128.57	51.60	2.97:1
Calyx	165.26	192.36	141.45	50.95	54.76	43.46	3.24:1
Bracteole	157.95	189.22	135.22	56.32	66.28	41.36	2.80:1
<i>Planaltina asparagoides:</i>							
Stipule	59.55	81.87	44.51	41.84	50.83	32.20	1.42:1
Calyx	44.28	50.07	39.15	39.79	42.43	35.91	1.11:1
Bracteole	59.48	68.29	47.25	47.98	60.49	37.81	1.24:1
<i>Planaltina laricoides:</i>							
Stipule	62.60	75.49	50.18	44.88	58.06	33.71	1.39:1
Calyx	52.75	67.84	46.27	39.72	45.30	31.52	1.33:1
Bracteole	56.39	75.80	45.54	39.99	45.50	33.23	1.41:1
<i>Planaltina phyllocephalus:</i>							
Stipule	55.97	61.97	47.85	40.36	50.56	32.14	1.38:1
Calyx	ND	ND	ND	ND	ND	ND	ND
Bracteole	61.85	65.92	54.06	41.40	47.00	36.32	1.49:1
<i>Richardia brasiliensis:</i>							
Stipule	156.10	187.54	124.02	101.83	134.52	76.03	1.53:1
Calyx	98.00	127.96	68.35	64.87	87.00	51.94	1.51:1
Bracteole	116.84	135.40	103.15	72.90	115.52	43.05	1.60:1
<i>Richardia scabra:</i>							
Stipule	127.25	156.90	96.33	98.47	128.90	71.52	1.29:1
Calyx	94.81	128.55	61.49	65.25	82.08	33.29	1.45:1
Bracteole	90.19	118.92	39.85	56.78	72.76	33.46	1.59:1
<i>Spermacoce prostrata:</i>							
Stipule	89.05	103.13	67.79	41.28	51.38	25.20	2.15:1
Calyx	46.15	48.79	43.52	28.74	30.85	26.63	1.61:1
Bracteole	74.76	95.51	65.21	41.24	46.40	37.27	1.81:1
<i>Staelia domingosii:</i>							
Stipule	178.37	228.75	145.85	95.33	114.74	75.18	1.87:1
Calyx	111.94	148.06	95.34	64.77	74.54	38.36	1.73:1
Bracteole	149.22	200.62	115.54	73.70	88.22	52.43	2.02:1
<i>Staelia glandulosa:</i>							
Stipule <sup>a</sup>	441.51	520.37	367.23	160.61	186.24	80.64	2.75:1
Calyx <sup>a</sup>	205.38	292.61	142.16	81.82	101.15	70.26	2.51:1
Bracteole	202.47	260.25	104.02	130.25	177.99	69.95	1.55:1
<i>Staelia virgata:</i>							
Stipule	146.28	215.12	94.82	90.86	120.38	56.13	1.61:1
Calyx	93.34	149.00	65.73	64.56	104.36	48.53	1.44:1
Bracteole	109.29	131.00	80.28	65.93	79.86	44.56	1.66:1

Notes. EF = external fimbriae; IF = internal fimbriae; ND = no data.

<sup>a</sup> Vascularized colleter.

the development of *S. glandulosa* will be described in detail. The colletar arises as protrusions composed of protoderm, ground meristem, and procambial cells. These meristematic cells are characterized by a dense cytoplasm and conspicuous nuclei. Both the protoderm and the ground meristem form a small dome (fig. 6A). The protoderm cells are radially elongated and undergo only anticlinal cell division; the ground meristem cells are small and isodiametric and undergo cell division in several planes (fig. 6B). Some cells of the central core of the colleters are thin and longitudinally arranged, finally forming procambial cells (fig. 6C). The colletar becomes enlarged with the successive divisions in different planes; the palisade epidermis develops from the protoderm, first becoming radially elongated and then finally palisade shaped and secretory (fig. 6D, 6E). The underlying tissue elongates and forms the central axis. The vascular system originates from the procambial cells near the colletar. The base of some colleters is constricted, and so they have a short stalk formed of shortened epidermal cells (fig. 6F).

In *S. glandulosa*, accumulated secretion was observed under the cuticle in some colleters (fig. 7A), whereas in others the secretion flowed through the broken cuticle (fig. 7B). In other species, secretion was detected on the colleters, but no rupture or presence of pores in the cuticle was observed (fig. 7C–7G).

Mature colleters on fresh material are senescent and ochre-brown in color (fig. 7H). In cross section, both epidermal and parenchyma cells appear deformed and collapsed (fig. 7I, 7J). Once the development of the organ is complete, the colleters collapse and generally fall off. Occasionally, they may persist as blackish, dehydrated structures; this is common in colleters found on calyces that persist on fruits.

#### Long-Lived Colleters

Field observations indicated that secretion was still present in old stipular colleters on *S. glandulosa*. The secretion was copious. The secretion left the stem surface shiny in the dried material. *Staelia glandulosa* is a scarcely branched shrub. The branches have numerous nodes with regularly distributed whorled leaves; each leaf has an axillary brachyblast. More than 60 foliar nodes were observed, of which 10–30 apical nodes bear multiflowered axillary inflorescences. Active colleters were observed on these nodes, both flowering and vegetative. As these colleters function over an extended time span, we named them long-lived colleters. In contrast, the remaining species that we analyzed in the tribe Spermaceae have collapsed stipular colleters on the younger apical nodes that are visible to the naked eye. Normally, the collapse of the stipular colletar starts when the leaf primordia slightly surpass the stipular sheath. Therefore, completely collapsed colleters are visible on the second or third node, immediately beneath the apical node.

### Discussion

#### *Distribution of Colleters on Calyx, Stipules, and Bracteoles*

Colleters are present on the teeth and fimbriae of the calyx, bracteoles, and stipules in all the species studied of the tribe

Spermaceae, as was indicated in an earlier study (Thomas 1991). They are usually on the interlobular sinus of the calyx and on the margin of stipules. Colleters rarely occur on the margin of the calyx lobes or the calyx or inside the stipules. However, in *Manettia cordifolia*, the colleters cover the inner surface of the stipules, which was previously reported in the taxonomic treatment of this species (Delprete 2010). *Manettia paraguayensis* has no colleters on the inner surface of the stipules but shares the presence of colleters on the inner surface of the calyx lobes with *M. cordifolia*. Results of this study suggest that the latter feature is exclusive to the genus *Manettia*. Marginal colleters on the calyx lobes occur only in the genus *Richardia*.

The presence of colleters on the internal surface of the calyx and stipules is common in other tribes of the Rubiaceae, even in Rubioideae (e.g., on the stipules of *Psychotria* L., tribe Psychotrieae; Miguel et al. 2009; Vitarelli and Santos 2009; Tresmondi et al. 2015). The latter pattern is also found in *Cephalanthus occidentalis* L. of the tribe Naucleae (Cinchonoideae; Rutishauser 1983) and in species of Gardenieae (Ixoroideae), such as *Randia* L. (Judkevich et al. 2015; Tresmondi et al. 2015). In other Gardenieae, the co-occurrence of colleters inside the stipules and calyx is very common, such as in *Gardenia* J. Ellis (Dave et al. 1988; Low and Wong 2007), *Cordia* A. Rich. (Barreiro and Machado 2007; Tresmondi et al. 2015), *Rosenbergiodendron* Fagerl. (Gustafsson 1998), and *Tocoyena* Aubl. (Machado et al. 2007; Tresmondi et al. 2015). According to Robbrecht (1988), the distribution of colleters on the internal surface of stipules is perhaps the primitive condition.

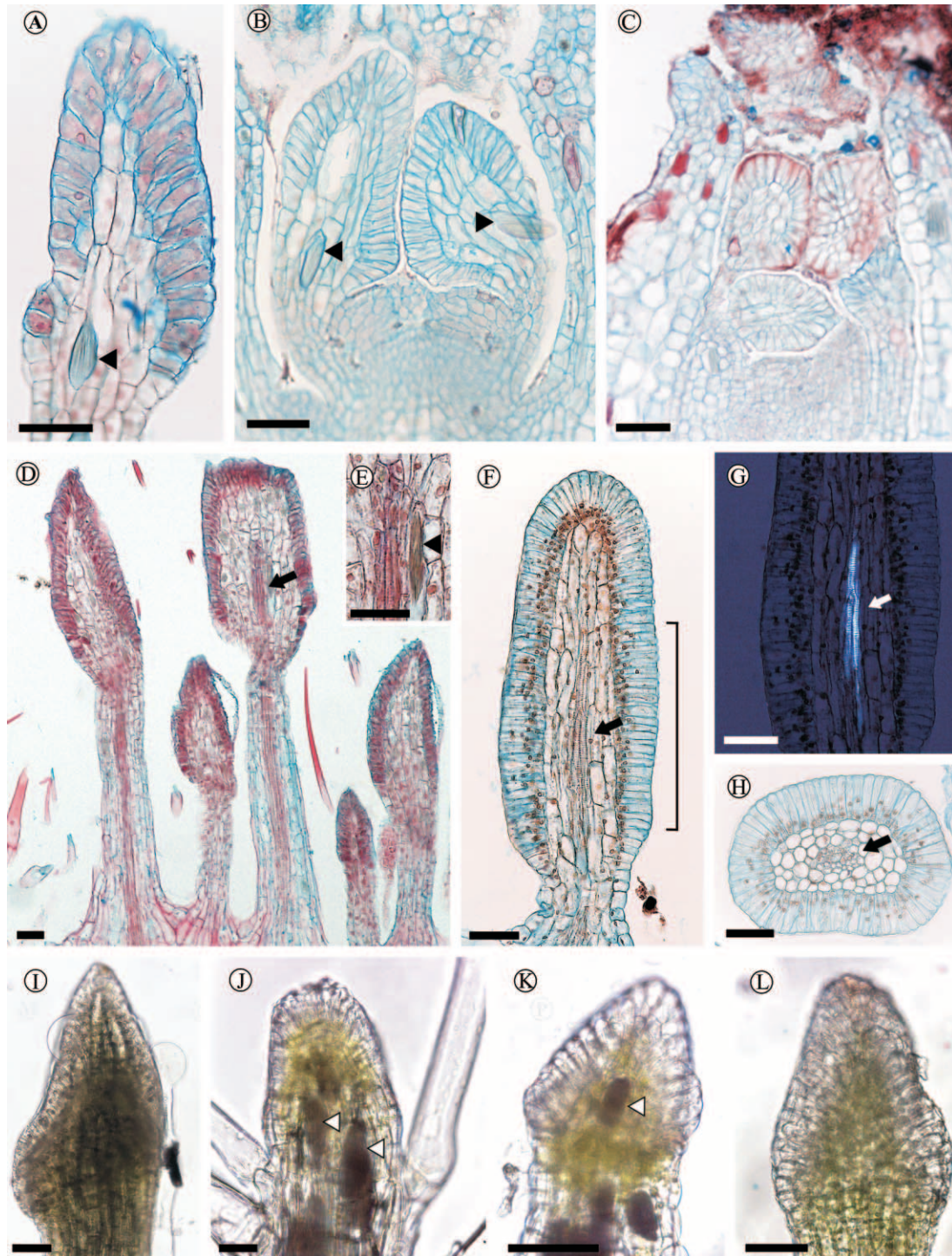
#### *Colleters on Underground Buds*

Colleters were found on underground organs (rhizome and xylopodium) of four additional *Galianthe* species. This location of colleters has been previously documented only for the xylopodium of *Galianthe grandifolia* (Vilhalva 2008). In this study, we also extended this location to *Galianthe centranthoides* and *Galianthe fastigiata*. The buds of *Galianthe valerianoides* and *Galianthe hispidula* rhizomes were analyzed, and the presence of colleters is reported here for the first time in a rhizomatous herb.

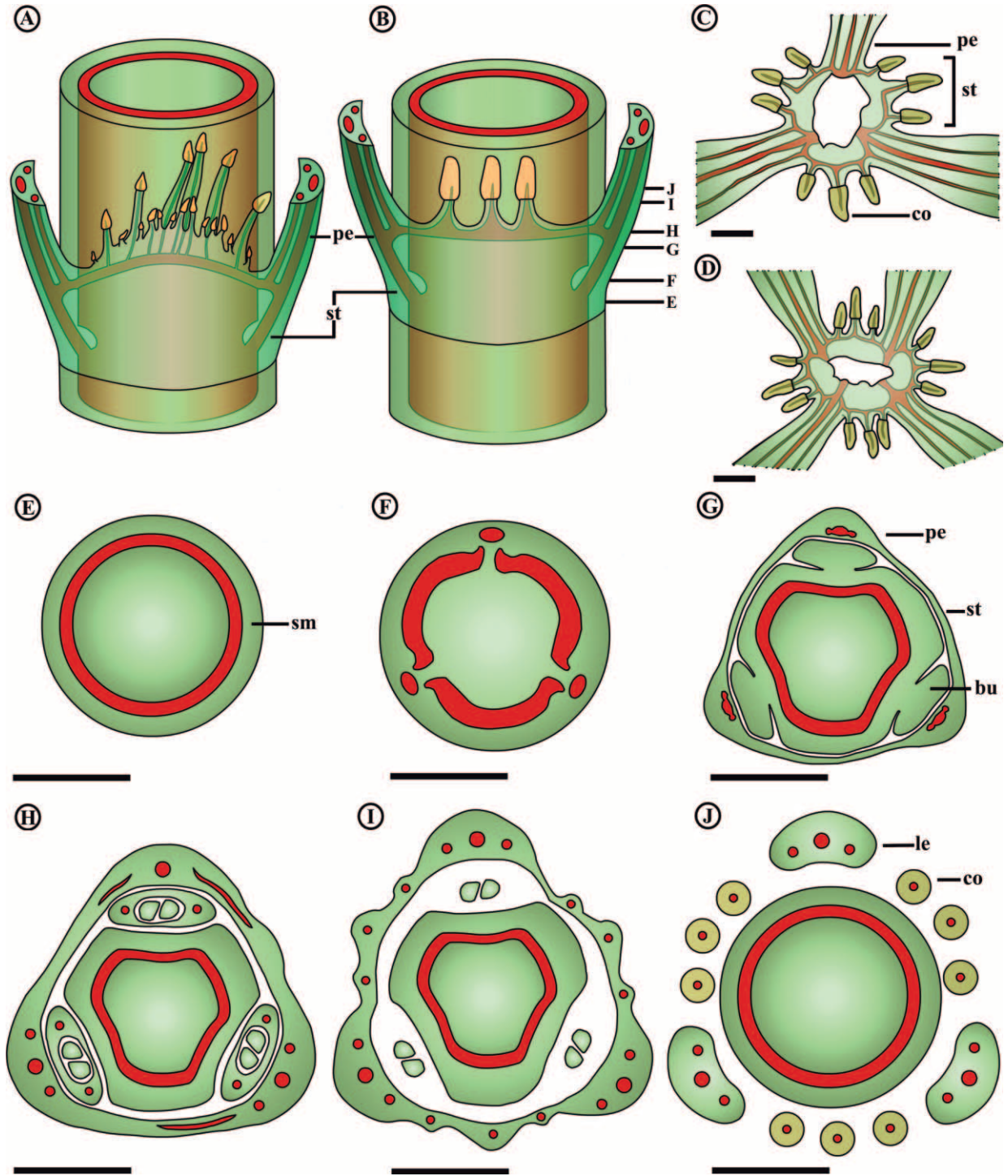
#### *Morphology and Anatomy of Colleters*

According to the classification of colleters in Rubiaceae proposed by Lersten (1974a, 1974b), the anatomical type of colletar in Spermaceae belongs to the standard type. These results are consistent with earlier studies of the group (*Borreria hispidula* K. Schum., *Diodia sarmentosa* Swartz., *Ernodea littoralis* Swartz., and *Oldenlandia friesorium* Bremek.; Lersten 1975). Standard colleters may be conical, cylindrical, round, or pad-like (Robbrecht 1988), with the first being the most common shape. According to Hallé (1967), in species of the tribe Gardenieae, identical colleters are usually found on the calyx lobes and the stipules, and we observed the same conditions in Spermaceae.

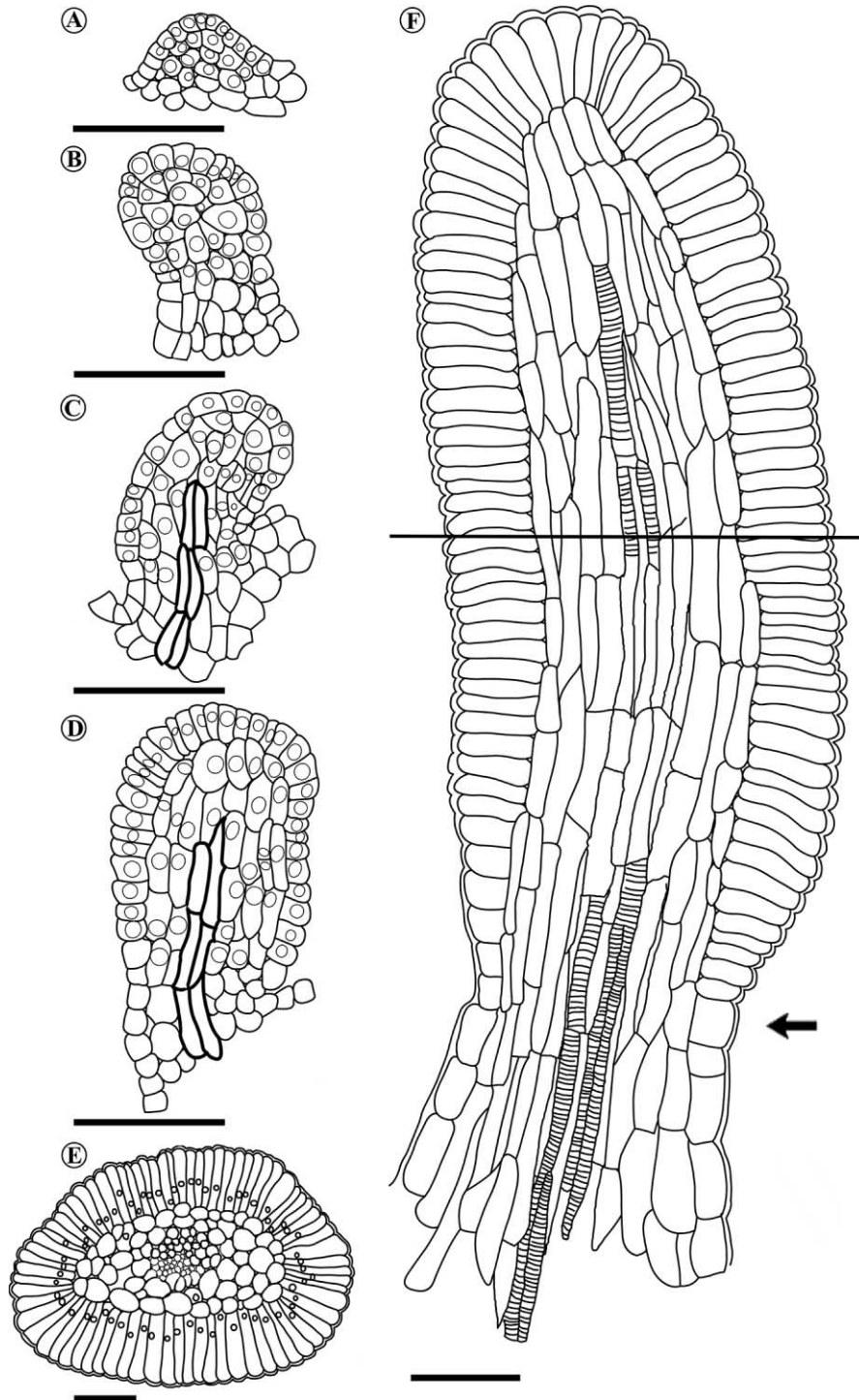
We observed packages of raphides in the axes of the colleters in certain species. The presence of crystals in cells of



**Fig. 4** Anatomy of colleters (LM). A–G, Longitudinal section of colleters. A, *Manettia paraguariensis*. B–C, *Galianthe hispidula*, colleters of the stipules (B) and underground bud (C). D–H, Vascularized colleters. D–E, *Mitracarpus polygonifolius*, note in (E) the raphide next to the vascular bundle. F–H, *Staelia glandulosa*; note in G the xylem seen with polarized light, corresponding to section marked in F. I–L, LM of fresh, unstained colleters showing chlorenchyma. I, *Borreria palustris*. J, *Diodia saponariifolia*. K, *Galianthe hispidula*. L, *Richardia brasiliensis*. Arrows indicate the vascular bundle, and arrowheads indicate raphides. Scale bars: 50  $\mu\text{m}$ .



**Fig. 5** Diagrams of nodal anatomy showing vascular supply to colleters of *Mitracarpus polygonifolius* (A) and *Staelia glandulosa* (B–J). A–B, Outline of nodal region showing stem, petioles, and interpetiolar stipules. C–D, Top view of node with vascular supply. C, Node with three leaves. D, Node with four leaves. E–J, Cross sections of a node (marked in B) showing vascularization of stem (sm), petiole (pe), stipules (st), leaves (le), and colleters (co). The position of the buds (bu) in the node is indicated only in the transverse drawings (G–I). Scale bars: 0.5 mm.

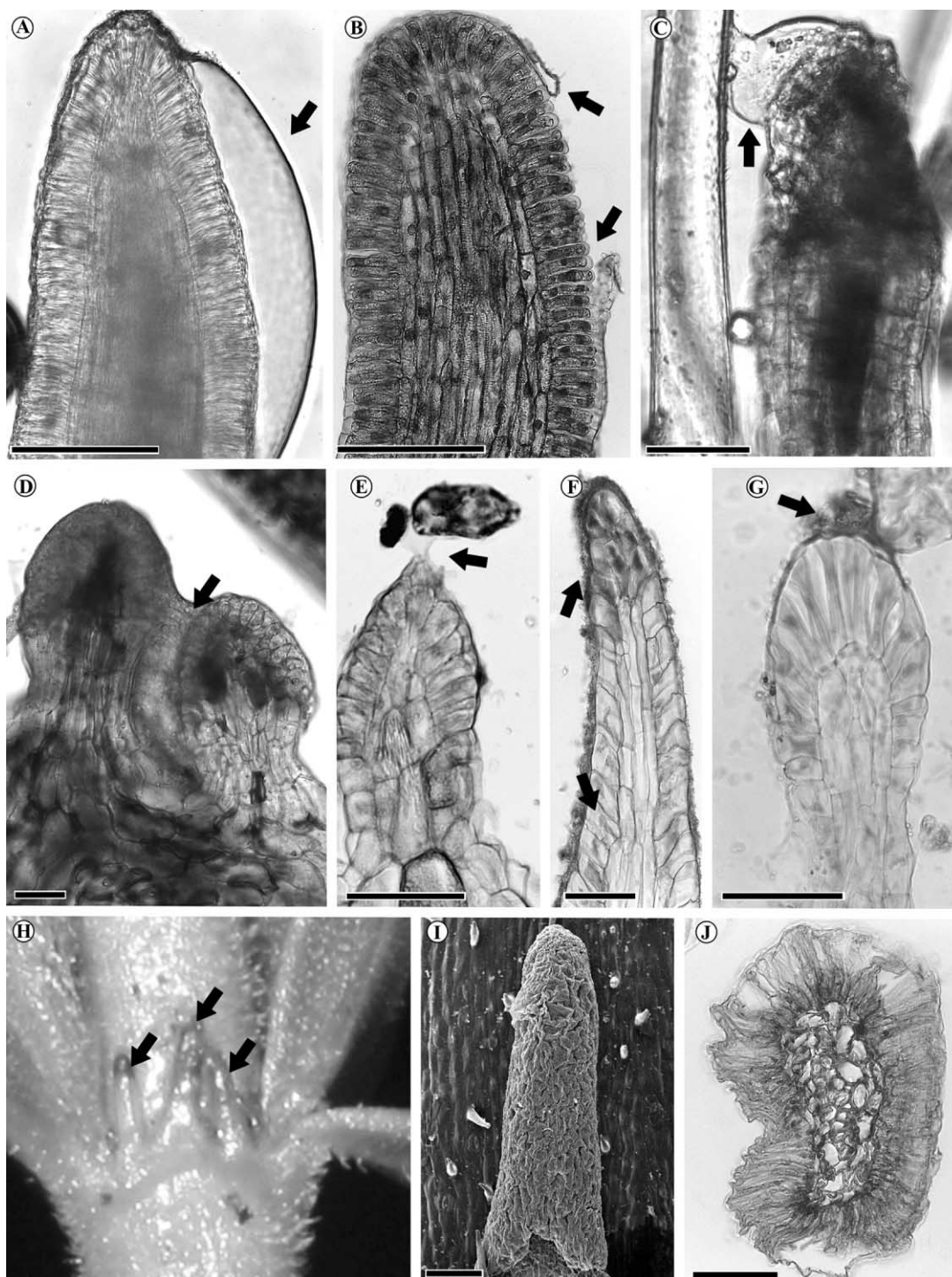


**Fig. 6** Ontogeny of stipular colleters of *Staelia glandulosa*. A–D, Longitudinal sections of different development stages; note the procambial cells in C and D (darker-colored cells). E–F, Mature colleter. E, Cross section at level indicated in F. F, Longitudinal section; note the constricted base of the colleter (arrow). Scale bars: 50  $\mu\text{m}$ .

the central core of colleters has been documented for several species of Rubiaceae (Lersten 1974a, 1974b; Dave et al. 1988; Miguel et al. 2009; Muravnik et al. 2014; Vitarelli and Santos 2009). Species with crystaliferous idioblasts in colleters also

had idioblasts in other organs (Gonzalez and Tarragó 2009; Machado Martins 2012).

We found green colleters on stipules and calices in six of the analyzed species. The presence of chlorenchyma has



**Fig. 7** Secretion and senescence of colleters, observed under LM (A–G, J) and SEM (I). A, Colleters in secretory phase showing cuticle displacement and accumulated secretion in the subcuticular space (arrow). B, Colleter with broken cuticle (arrows). C–D, Colleters in secretory phase. E–H, Old colleters with secretion residues (arrows). I–J, Senescent colleters. A–B, H–J, *Staelia glandulosa*. C, *Richardia brasiliensis*. D, *Oldenlandia salzmännii*. E, *Galianthe hispidula*. F, *Manettia paraguariensis*. G, *Staelia virgata*. Scale bars: 50  $\mu$ m.

been mentioned in stipular colleters of a few species of Rubiaceae (Tresmondi et al. 2015), in the stalk of colleters of *Allamanda* L. of Apocynaceae (Ramayya and Bahadur 1968), and in the developing colleters of *Macrocarpaea obtusifolia*,

Gentianaceae (Dalvi et al. 2014). It has been mentioned that the presence of chlorenchyma in the latter species might be related to the production of the precursors of the secreted compounds. It is unknown whether the colleters of

the Spermaceae could play another role as well as the photosynthetic function. Further observations of fresh material in other genera of Spermaceae are needed to confirm the presence of this type of tissue throughout the tribe. The presence of chlorenchyma is recorded for the first time in *Borreria*, *Diodia*, *Galianthe*, *Manettia*, and *Oldenlandia*. Tresmondi et al. (2015) mentioned this tissue in stipules of *Richardia grandiflora*, and we also observed it in stipules and calyx of *Richardia brasiliensis*.

#### Vascularization

In Rubiaceae, vascularized collectors were described on stipules of several species of *Bathysa* C. Presl (Coelho et al. 2013) and on *Simira glaziovii* (K. Schum.) Steyerl. (Klein et al. 2004). Anderson (1972) indicated the presence of vascularization in the collectors of the longest fimbriae located in the interlobular position on the calyx in the genus *Crusea* Cham. & Schltdl., which represented the only record for the tribe Spermaceae. However, collectors with vascularization were also found on two species analyzed in this study: *Staelia glandulosa* (always in stipules, rarely in calyx) and *Mitracarpus polygonifolius* (only in stipules).

Two different hypotheses have been made regarding vascularization in collectors. Thomas (1991) proposed that it is always connected to the organ to which it is attached. On the other hand, Carlquist (1969) conducted a review of the evolutionary interpretations of floral structure and proposed that the vascular tissue bears a certain relationship with the size of an organ. For the collectors in Spermaceae, the size hypothesis should be excluded. There are other analyzed species with stipular collectors that have similar sizes to those found in *S. glandulosa* (520.37  $\mu\text{m}$ ) and *M. polygonifolius* (467.37  $\mu\text{m}$ ), such as *Borreria orientalis* (520.08  $\mu\text{m}$ ), *Borreria palustris* (547.43  $\mu\text{m}$ ), and *Diodia saponariifolia* (461.66  $\mu\text{m}$ ). However, these latter species are nonvascularized. In *S. glandulosa*, the Thomas proposition is the most acceptable, given the proximity of the collector to the vascular traces of the stipule (and to the reduced lobes of calyx when present). In *M. polygonifolius*, the collectors of the internal and external fimbriae have the same proximity to the vascular traces of the stipule, but the first are nonvascularized; therefore, more studies on this species regarding the relationship between the collectors and vascularization are required.

#### Development of Collectors

The collectors of *S. glandulosa* are developed from both protoderm and ground elements and are, therefore, emergences, as is the case in other Rubiaceae species (Hallé 1967; Dave et al. 1988; Thomas 1991; Coelho et al. 2013; Muravnik et al. 2014). Morphologically, a change in color from greenish (due to the presence of chlorophyll) to ochre-brown was observed during the development of collectors in this study. Microscopically, alteration of cell shape is observed, indicating the senescent phase of the collectors. These changes are similar to those observed by Thomas (1991) in other species. According to Esau (1965), collectors wither away after ceasing their secretory function; however, in Spermaceae, the col-

lectors may be persistent structures, especially the long-lived collectors of *S. glandulosa*.

#### Long-Lived Collectors and Copious Secretion

The secretion produced by the long-lived collectors in *S. glandulosa* cover all herbaceous parts of the plant. The secretion apparently provides the plant with a defense mechanism, because ants and mites get stuck in the sticky secretion covering the stems. The exudates in *S. glandulosa* also cover older foliar nodes as well as meristems, because the collectors continue to secrete for a longer time. The cause of the longer activity of the collectors should be studied. Other cases of collectors producing an abundant secretion have been mentioned in the Rubiaceae. In *Robbrechtia* De Block (tribe Pavetteae, subfamily Ixoroideae), secretion from collectors (of the standard conical type) covers young shoots and parts of the inflorescences (De Block 2003). Another case is *Colleteria* D. W. Taylor (a rare genus without an infrafamiliar or tribal assignment), in which the copious secretion covers both apical and axillary buds (Taylor 2003). In the cases that were mentioned having profuse secretion (*Robbrechtia* and *Colleteria*), this is produced by several collectors occurring on the inner surface of stipules, whereas in *S. glandulosa*, there are only a few collectors located on the short fimbriae on the stipular margin. Additional field observations are necessary in *S. glandulosa* to explore the functional aspects of their collectors.

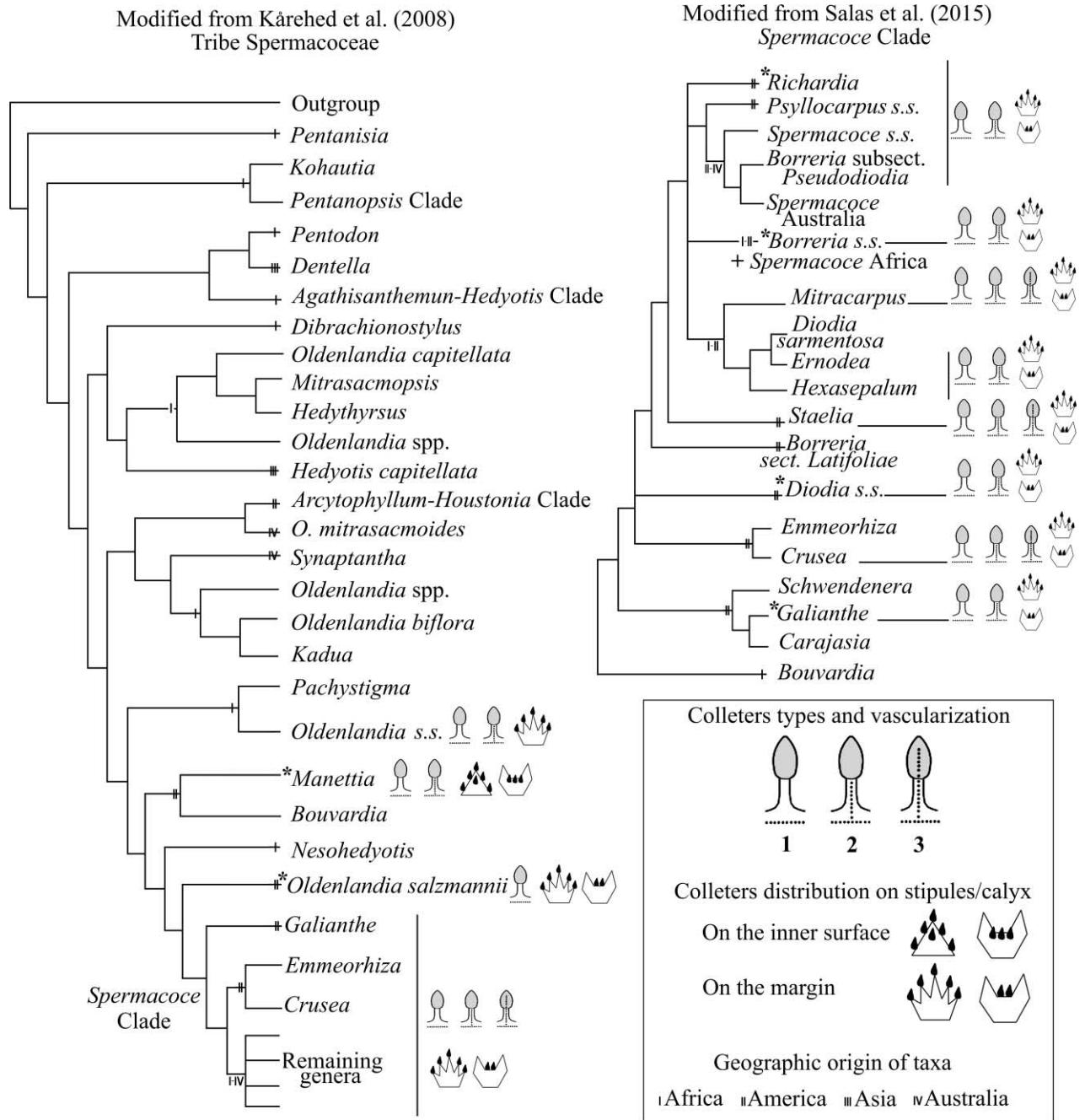
#### Evolutionary Interpretations

Three basic types, mainly based on the presence or absence of the vascular supply of the collectors and underlying tissues, have been defined (fig. 8): (i) type 1, collector and underlying tissues (teeth or fimbria) without any vascular supply (teeth never seen with vascular supply); (ii) type 2, collector nonvascularized but fimbriae are vascularized; and (iii) type 3, collectors and fimbriae are both vascularized.

The tribe Spermaceae is one of the few tribes in the Rubiaceae that is supported only by molecular synapomorphies (Groeninckx et al. 2009). The presence/absence of the three collectors types have been compared with one of the most comprehensive phylogenies of the tribe performed by Kårehed et al. (2008). The phylogenetic tree was adapted from Kårehed et al. (2008; fig. 8). Most of the genera studied belong to the monophyletic *Spermaceae* clade, which appears internally unresolved. In this tree, the presence of collectors of the three types on stipules and calyx has been mapped. Likewise, a phylogenetic tree of the *Spermaceae* clade recently published by Salas et al. (2015) has been used.

Type 1 collectors occur in all clades in which the species analyzed are included (*Oldenlandia* s.s., *Manettia*, *Oldenlandia* from South America, and the *Spermaceae* clade). They occur on the tip of teeth and small fimbriae. Like type 1, type 2 collectors are found in all studied genera. Type 3 collectors are much rarer. The three types of collectors may coexist in a single species or even in a single organ (e.g., stipules of *M. polygonifolius*). To date, type 3, the vascularized collector, has been recorded only in the *Spermaceae* clade, which appears to be an independent acquisition for the *Staelia*, *Mitracarpus*, and





**Fig. 8** Two phylogenetic trees, one representing the tribe Spermacoceae, modified from Kårehed et al. (2008; left), and the other representing the Spermacoceae clade, modified from Salas et al. (2015; right). Below the right tree is a schematic reproduction of the three colleters types based on patterns of vascularization. Dotted lines indicate vascular bundles. Asterisks indicate green colleters.

*Crusea* genera. However, more studies are needed to elucidate this aspect.

### Conclusion

Our study describes the anatomy of the colleters in the tribe Spermacoceae of the Rubiaceae in detail. The presence of underground colleters in *Galianthe* is confirmed and extended to

other species of the genus. The existence of vascularization in colleters of *Mitracarpus polygonifolius* and *Staelia glandulosa* is recorded for the first time. A new term for colleters is proposed based on anatomical, morphological, and ecological aspects: long-lived colleter. We provide data about the type, distribution, and anatomy of colleters in the Spermacoceae, contributing an important source of information for ongoing phylogenetic studies in the tribe.

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### Appendix

List of the analyzed species of Spermatoceae. Information about their location, habitat, and distribution is provided as follows: species; voucher information; habitat and distribution. Note: two asterisks indicate fresh material (in addition to fixed material), and the pound sign denotes herbarium specimens.

*Borreria brachystemonoides* Cham. & Schltdl.; Argentina. Corrientes: Riachuelo, 27°33'42.96"S, 58°44'35.31"W, 54 m, 30 Mar 2012, *Judkevich and Salas 3* (\*\*). Misiones: San Ignacio, Teyú Cuaré Park, 27°16'36"S, 55°33'25"W, 176 m, 02 Mar 2013, *Sobrado 163*; decumbent herbs, savannas, sometimes rocky fields; NE Argentina, South Brazil, Paraguay, and Uruguay.

*Borreria orientalis* E.L. Cabral, R.M. Salas & L.M. Miguel; Argentina. Misiones: Iguazú, Iguazú National Park, 02 Mar 2013, *Sobrado 138* (\*\*); decumbent herbs. Seasonally dry forest, mostly primary forest; W Paraguay, Argentina (Misiones), and Brazil (Paraná).

*Borreria palustris* (Cham. & Schltdl.) Bacigalupo & E.L. Cabral; Argentina. Misiones: San Pedro, Moconá Falls Park, 27°09'11.73"S, 53°53'21.21"W, 138 m, 07 Mar 2013, *Judkevich 46*; decumbent herbs. Atlantic humid forest, in riverine vegetation; NE Argentina, and SE and S of Brazil.

*Diodia saponariifolia* (Cham. & Schltdl.) K. Schum.; Argentina. Misiones: San Pedro, Moconá Falls Park, 27°09'28.3"S, 53°53'43.7"W, 536 m, 10 Dic 2011, *Miguel 20* (\*\*); prostrate herbs. Swampy areas, river banks; Disjunct, Mexico, SE and S Brazil, and NE Argentina.

*Ernodea littoralis* Sw.; Mexico. Guerrero: Acapulco, sea dunes, 20 Jan 2013, *Cabral 905*; much-branched decumbent shrubs. Mostly sandy soils in dunes close to the sea; Mexico and Caribbean islands.

*Galianthe centranthoides* (Cham. & Schltdl.) E.L. Cabral; Argentina. Corrientes: Riachuelo, 27°33'42.96"S, 58°44'35.31"W, 54 m, 30 Mar 2012, *Judkevich and Salas 2* (#); geoxylic shrub. Savannas with sandy soils; NE Argentina, Paraguay, SE and S Brazil, and Uruguay.

*Galianthe fastigiata* Griseb.; Argentina. Misiones: Candelaria, Loreto, 27°19'54.24"S, 55°32'55.14"W, 178 m, 08 Dec 2011, *Sobrado 105*; San Javier, 2.5 km from Itacuararé, 27°52'2.28"S, 55°15'36.42"W, 134 m, 13 Dec 2011, *Sobrado 130*; erect to geoxylic subshrubs. Savannas, sometimes rocky fields; NE Argentina, South Brazil, Paraguay, and Uruguay.

*Galianthe grandifolia* E.L. Cabral; Brazil. Distrito Federal: Planaltina, Apr 2009, *Queiróz et al. 14015*; geoxylic shrub. Savannas with lateritic and sandy soils; Central, SE, NE, and N Brazil.

*Galianthe hispidula* (A. Rich. ex DC.) E.L. Cabral & Bacigalupo; Argentina. Misiones: Manuel Belgrano, Urugua-í Park, 25°51'27.8"S, 54°10'06.1"W, 240 m, 03 Apr 2013, *Miguel 62* (\*\*); rhizomatous herbs, Atlantic humid forest, in riverine vegetation; NE Argentina, Paraguay, and SE and S of Brazil.

*Galianthe valerianoides* (Cham. & Schltdl.) E.L. Cabral; Argentina. Misiones: Manuel Belgrano, 26°16'29.04"S, 53°41'59.52"W, 815 m, 10 Dec 2011, *Miguel 16* (#); rhizomatous subshrubs. Lowland savanas; NE Argentina, Paraguay, and S Brazil.

*Hexasepalum apiculatum* (Willd.) Delprete & J.H. Kirkbr.; Brazil. Bahia: Santo Sé, 20°20'30.8"S, 41°18'47.7"W, ca. 1200 m, 28 May 2010, *Salas 457*; shrub or herbs, decumbent or erect. Savannas, rocky fields, edge of deciduous forest; America, from Mexico to Paraguay, including Caribbean region. Introduced in tropical Africa.

*Manettia cordifolia* Mart.; Argentina. Misiones: Iguazú, Iguazú National Park, 02 Mar 2013, *Sobrado 147*; climbing subshrubs. Edge of dry or humid forest, occasionally bush savannas; South America.

*Manettia paraguariensis* Chodat.; Argentina. Misiones: Iguazú, Iguazú National Park, 02 Mar 2013, *Sobrado 141*; San Pedro, Cruce Caballero Park, 26°31'08.1"S, 53°59'20.2"W, 626 m, 05 Mar 2013, *Judkevich 31* (\*\*); climbing subshrubs. Edge of humid forest; NE Argentina, Paraguay, and S Brazil.

*Mitracarpus megapotamicus* (Spreng.) Kuntze.; Argentina. Formosa, Guaycolec, 11 Set 2014, *Judkevich and Salas 60*; decumbent subshrub. In savannas or disturbed areas, mainly in sandy soils; N Argentina, S Bolivia, Paraguay, S Brazil, and NE Uruguay.

*Mitracarpus pusillus* Steyerl.; Brazil. Minas Gerais: Joaquim Felício, 17°41'57"S, 44°16'29"W, 1140 m, 17 Apr 2012, *Viana 5888*; erect herbs. In deep and sandy soils of Espinhaço Mountains Range; Minas Gerais, Brazil.

*Mitracarpus polygonifolius* (A. St.-Hil.) R.M. Salas & E.B. Souza; Brazil. Minas Gerais: Santana do Riacho, 19°15'53.1"S, 43°31'22.5"W, 1325 m, 15 Apr 2012, *Viana 5906*; erect shrub. Open, bush or rocky savannas; widespread in NE Brazil and French Guiana.

*Oldenlandia corymbosa* L.; Brazil. Minas Gerais: Januaria, 15°29'30"S, 44°21'35"W, 548 m, 18 Apr 2012, *Viana 5896*; herbs. Large ecological amplitude, disturbed areas; widespread in tropics of America, Africa and Asia.

*Oldenlandia salzmannii* (DC.) Benth. & Hook. f. ex B.D. Jacks; Argentina. Corrientes: San Cosme, RN 12, 1075 km, 15 Jun 2015, *Salas 498* (\*\*); prostrate herbs. Lowland savannas; South America, introduced in USA.

*Planaltina capitata* (K. Schum.) R. M. Salas & E. L. Cabral; Brazil. Goiás: Colinas do Sul, 14°12'07.2"S, 47°51'22.9"W, 913 m, 25 Apr 2009, *Cardoso 2573*; erect shrub. Bush savannas; Central Brazil.

- Psyllocarpus asparagoides* Mart. ex Mart. & Zucc.; Brazil. Minas Gerais: Diamantina, 22 Dec 2012, 18°1'15.8"S, 43°42'26"W, 1255 m, *Sobrado 153*; erect shrub. Rocky fields; Bahia and Minas Gerais, Brazil.
- Psyllocarpus laricoides* Mart. ex Mart. & Zucc.; Brazil. Minas Gerais: Santana de Riacho, 19°15'53.1"S, 43°31'22.5"W, 1325 m, 15 Apr 2012, *Sobrado 136*; erect shrub. Rocky fields; Bahia and Minas Gerais, Brazil.
- Psyllocarpus phyllocephalus* K. Schum.; Brazil. Minas Gerais: Joaquim Felício, 17°41'38"S, 44°12'44"W, 1128 m, 17 Apr 2012, *Viana 5885*; erect shrub. Rocky fields; Goiás and Minas Gerais, Brazil.
- Richardia brasiliensis* Gomes; Argentina. Corrientes: Riachuelo, 27°33'42.96"S, 58°44'35.31"W, 54 m, 30 Mar 2012, *Judkevich and Salas 4* (\*); herbs. Large ecological amplitude, disturbed areas; widespread in tropics of the Americas.
- Richardia scabra* L.; Brazil. Minas Gerais: Olhos d'Água, MG-451 59–60 km, 17°27'54"S, 43°34'04"W, 794 m, 19 Apr 2012, *Viana 5912*; herbs. Large ecological amplitude, disturbed areas; widespread in tropics of the Americas.
- Spermacoce prostrata* Aubl.; Argentina. Misiones: San Javier, 2.5 km de Itacuararé, 27°52'2.28"S, 55°15'36.42"W, 134 m, 13 Dic 2011, *Sobrado 129*; herbs. Large ecological amplitude, including humid and dry forest, riverine vegetation, rocky fields; widespread, tropical areas of America, Asia, and Oceania.
- Staelia domingosii* R.M. Salas & E.L. Cabral; Brazil. Bahía, Andaraí, 12°49'21"S, 41°19'21"W, 330 m, 16 May 2009, *Queiroz 14599*; subshrub. Deep sandy dunes. Caatinga; Bahia, Brazil.
- Staelia glandulosa* R.M. Salas & E.L. Cabral; Brazil. Bahía: Umburanas, Serra do Curral Frio, 10°24'13.3"S, 41°18'41.7"W, 905 m, 28 May 2010, *Queiroz 14813*; *Salas 451*; *Cardoso 2989*; shrub. Deep sandy dunes. Caatinga; Bahia, Brazil.
- Staelia virgata* (Link. ex Roem. & Schutl.) K. Schum.; Argentina. Salta: Coronel Juan Solá, 23°28'40"S, 62°53'52.06"W, 223 m, 9 Jan 2010, *Salas 469*; subshrub. Large ecological amplitude deep. Mostly sandy dunes; N Argentina, Bolivia, Brazil, and Paraguay.

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