An Acad Bras Cienc (2022) 94(2): e20201975 DOI 10.1590/0001-3765202220201975
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CELLULAR AND MOLECULAR BIOLOGY

Colleters in *Cephalanthus* (Rubiaceae) and reevaluation of occurrence of corolline colleters in Angiosperms

MARÍA FLORENCIA ROMERO, ROBERTO MANUEL SALAS & ANA MARIA GONZALEZ

Abstract: Colleters are glandular structures related to young shoots protection. In several Angiosperm families, as in Rubiaceae colleters are taxonomically important. This study reports presence, location, morpho-anatomical structure, ontogeny and histochemistry of colleters in vegetative and reproductive organs in 6 species of *Cephalanthus* and 20 species representatives of the tribe Naucleeae. Primarily based on their color and their secretion color, they were classified into light (LC) and dark (DC) colleters. The LC are exclusive to the inner side of the stipule, while the DC are found in several vegetative and reproductive organs. Histochemical studies revealed that higher mucilage and phenol content in DC could explain the dark color secretion. Results were analyzed at genus, tribe and subtribe levels, evidencing the taxonomic relevance in the family. Finally, the presence of corolline colleters in angiosperm flowers was reviewed. It was confirmed that, besides *Cephalanthus*, they are only present in *Adenoa* (Passifloraceae) and *Chamaecrista* (Fabaceae). Therefore, structures described as colleters in *Nerium* (Apocynaceae) and *Cuscuta* (Cuscutaceae) corollas do not correspond to colleters. This proves that corolline colleter is a rare character in Angiosperms. To conclude we provide a record of presence/absence, type and distribution of corolline colleters in an Angiosperm phylogenetic tree.

Key words: anatomy, corolline colleters, dark colleters, histochemical, light colleters, Naucleeae.

INTRODUCTION

Colleters stand out among the diversity of glandular structures in the Angiosperms because they are involved in the protection of developing vegetative and reproductive organs (Robbrecht 1988, Thomas 1991). According to Dickison (2000) colleters are multicellular structures that produce a sticky secretion which protects the developing organs. Colleters are conical, pyramidal or digitiform glandular structures, with a head and a short peduncle, structurally composed of a central axis covered with a palisade-like epidermis (Thomas 1991). This type of colleter is considered as the standard type and it is the most frequent and ancestral according to Lersten (1974a).

The presence, structure and variability of colleters have been recorded in several species (Thomas 1991). In Angiosperm families, such as Rubiaceae, Apocynaceae and Passifloraceae, the colleters have taxonomic significance, even being used as a character in the keys for the identification of species and phylogenetic analysis (Lersten 1974a, Robbrecht 1988, Gonzalez 1998, Gonzalez et al. 2012, Judkevich et al. 2017, Capelli et al. 2017, Endress et al. 2018).

The Rubiaceae family is represented by 650 genera, 13,000 species and 44 tribes (Bremer & Eriksson 2009). Krause (1909) examined representatives of 21 tribes and only in the Rubieae no colleters were detected. To date, the presence of colleters has been described in numerous species of the family (Horner & Lersten 1968, Lersten 1974a, b, Robbrecht 1988, Klein et al. 2004, Barreiro & Machado 2007, Coelho et al. 2013, Muravnik et al. 2014, Judkevich et al. 2017). In this family, another five types of colleters have been described in addition to the standard type: reduced standard, dendroid, brush, winged and filiform, demonstrating its great variability (Lersten 1974a, b).

The genus *Cephalanthus* L. belongs to the monogenetic subtribe Cephalanthinae, tribe Naucleeae, Rubiaceae (Razafimandimbison & Bremer 2002). *Cephalanthus* is a genus of pantropical distribution, with six species (Ridsdale 1976): three in America (*C. glabratus* (Spreng.) K. Schum., *C. occidentalis* L. and *C. salicifolius* Bonpl.), two in Asia (*C. angustifolius* Lour. and *C. tetrandrus* (Roxb.) Ridsdale & Bakh. f.) and one in Africa (*C. natalensis* Oliv.). One of the main synapomorphs of the subtribe is the presence of the so-called "glands" on the stipule and corolla (Ridsdale 1976). *Cephalanthus natalensis* is the only species without corolla "glands" according to Ridsdale (1976) and Razafimandimbison & Bremer (2002). Razafimandimbison & Bremer (2002) and Romero et al. (2017) mentioned that these "glands" correspond to colleters, but there are no morpho-anatomical descriptions or any histochemical evidence of them.

In general, colleters are essentially associated with vegetative structures. If they are present in flowers, they are usually related to the calyx and their presence in the corolla is scarce, reduced to four genera of Angiosperms. In Apocynaceae flowers, colleters are common glandular structures in the calyx (Thomas 1991, Endress & Bruyns 2000, Valente & Costa 2005, Demarco 2008, Endress et al. 2018). In *Nerium oleander* (under *N. indicum* Mill.) Thomas & Dave (1989, 1991) described the presence of colleters in the corolla, but the figures showing them are unclear and confusing. Also, in the *Cuscuta* spp. (Cuscutaceae), Thomas & Dave (1991) mentioned the presence of corolline colleters, based on the work of Solereder (1908) who called the scale structures present in the corolla tube "glandular shaggy hairs". These elaborations have also received other denominations, such as "corona" by Johri & Tiagi (1952), and "infrastaminal scales" by Riviere et al. (2013). Another two genera with corolline colleters, in which a detailed anatomical exploration confirmed their nature, were: *Adenoa* Arbo (Passifloraceae: Turneroideae, Gonzalez et al. 2012) and *Chamaecrista* (L.) Moench Cotta (Fabaceae, Cotta Coutinho et al. 2015).

Due to the scarce morpho-anatomical studies of colleters located on reproductive organs and their importance in the Rubiaceae family, the objectives of this study were to: a) describe the morpho-anatomical structure of colleters in the vegetative and reproductive organs in *Cephalanthus glabratus* and evaluate the secretion by means of histochemical tests; b) determine the presence, location and external morphology of the colleters and their taxonomic importance in the six species of *Cephalanthus* (subtribe Cephalanthinae) and in species representative of the other six subtribes of Naucleeae (Rubiaceae); c) re-evaluate the structure of the so-called "corolline colleters" described in *Cuscuta* and *Nerium* in order to record the presence of colleters in corollas of the Angiosperms.

MATERIALS AND METHODS

The present study was conducted on 26 species of the seven subtribes of Naucleeae (Rubiaceae), including the six species of genus *Cephalanthus*. For each species, five to 17 specimens from herbaria were analyzed, and in *C. glabratus* fresh material was analyzed and fixed in FAA (alcohol 70º, acetic acid and formaldehyde, 90:5:5). In addition, fresh and fixed flowers of *Cuscuta campestris* Yunck. and *Nerium oleander* L., species of the Cuscutaceae and Apocynaceae families, respectively, were analyzed. These species had been cited in the bibliography as having corolline colleters. The material analyzed is in Appendix 1.

Morphological analysis

In order to determine the presence/absence, number and location of colleters, the different young vegetative and reproductive organs from fresh, fixed in FAA and herborized material were analyzed. A minimum of 15 organs in at least five specimens of each species were analyzed to carry out the measurements. Vegetative organs were: leaf blade, stipules, bracts, bracteoles and reproductive organs: calyx and corolla of flower buds and open flowers. The studies were carried out using a Leica MZ6 stereomicroscope equipped with a Canon Power Shot S50 camera for recording the observations. For morphometric analysis of the species studied, measurements were taken of 10 colleters for each organ, using Electronic digital caliper (Schwyz) and ImageJ software (Rasband 1997-2018, Gonzalez 2018).

The classifications proposed for the Rubiaceae by Lersten (1974a, b) and Robbrecht (1988) were followed for identifying the types of colleters.

Light Microscopy (LM)

For anatomy and histological analysis, young branches, flowers and fresh fruits fixed in FAA of *C. glabratus* were used. To obtain histological sections, the material was dehydrated and included in paraffin (Johansen 1940, Gonzalez & Cristóbal 1997). Transverse (TS) and longitudinal (LS) serial cuts of 10-12 µm in thickness were made using a Microm HM350 rotary microtome (Microm International, Walldorf, Germany) and were stained with Safranin-Astra blue combinations (Luque et al. 1996). The observations and photographs were made with a Leica DM LB2 light microscope (Leica Microsystems) equipped with a Leica ICC50HD digital camera. The identification of calcium oxalate crystals was made by observation with light microscopy with polarized filters (PLM).

Scanning electron microscopy (SEM)

The samples of herbarium material were previously rehydrated with 5% neutral detergent for 72 hours, then rinsed in tap water and finally fixed in the FAA. The fixed material was dehydrated in an ascending series of acetone, dried to the critical point with $CO2$ and sputter-coated with Gold-Palladium. The observations were made with the MEB Jeol LV 5800, at 20 Kv of the Electronic Microscopy Service of the UNNE, Corrientes.

Histochemical tests

For the identification of the chemical compounds in the colleters and their secretion, fresh stipules were used, preferably from between the apex and the 3rd node in *Cephalanthus glabratus*. We used Lugol for the detection of starch (insoluble carbohydrates), Sudan III for lipid compounds, Ferric chloride for generic phenolic compounds, Cresil blue for mucilage, Toluidine blue for phenols and pectins (Johansen 1940, D'Ambrogio de Argüeso 1986, Ruzin 1999).

RESULTS

Morpho-anatomy of colleters in *Cephalanthus*

The secretory structures present in vegetative and reproductive organs of *Cephalanthus* correspond to the standard type of colleters. Based on the color of the colleters (both in fresh and herbarium material) and the color of its secretion, two types are described: light (LC) and dark colleters (DC) (Figure 1a). Secretion of LC is colorless, whereas the DC secretion is black to dark red. DC. In addition to the different coloration, these colleters differ in plant position, grouping, measurements and vascularization. The detailed characteristics of both types are presented in Table I. Both LC and DC are obpiriform to conical structures, composed of a central axis of compact parenchymatous cells, elongated in LS and polygonal in TS (Figure 1b-i). Vascular bundles are present in the DC located on stipules (Figure 1c-d). The parenchymatous cells present crystalline sand or druses and tannic compounds (Figure 1 j-k). The axis is surrounded by a layer of palisade-like epidermal cells organized radially (Figure 1-d, g). These are secretory cells with dense cytoplasm and conspicuous nuclei. A thick, smooth cuticle covers the epidermis. The light colleters may have a short peduncle or be sessile (Figure 1b), while dark colleters have a short or long peduncle (Figure 1c, f, g).

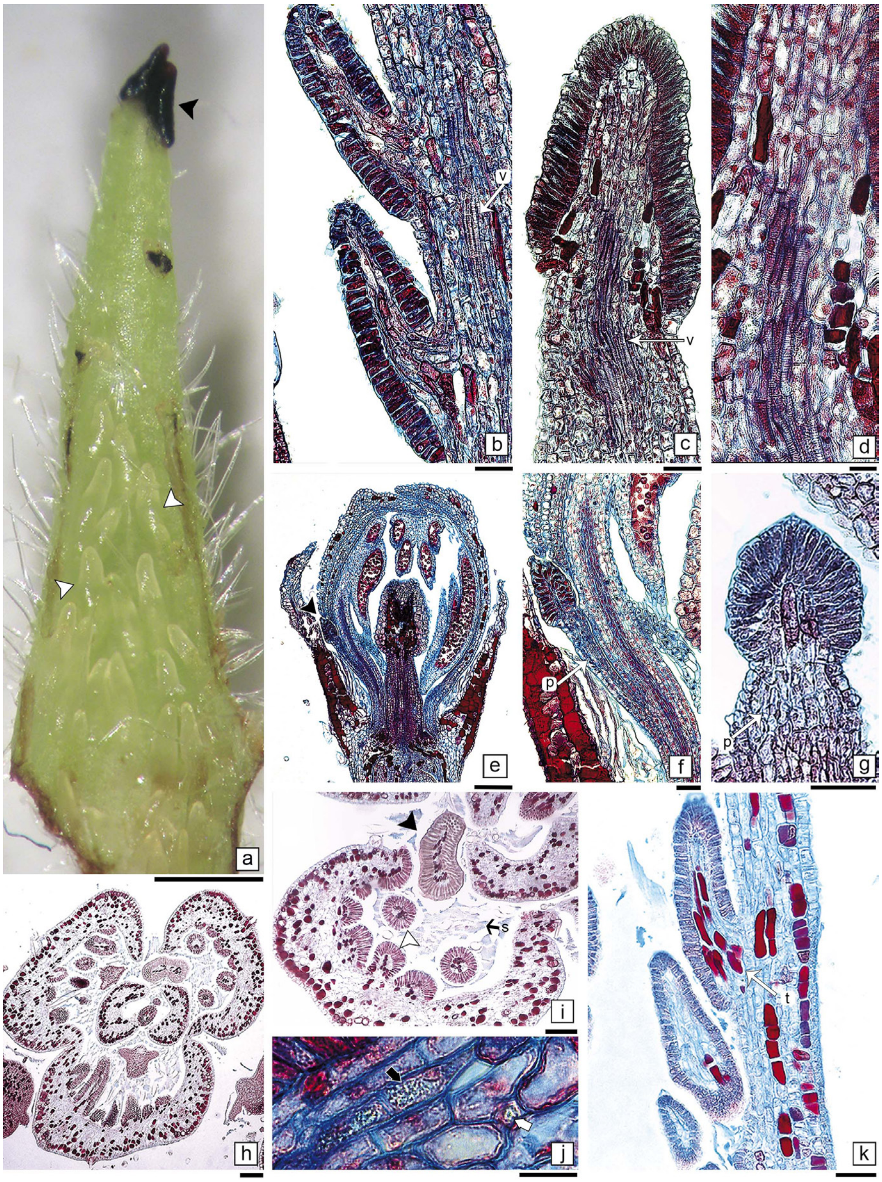

Figure 1. Anatomy of the standard colleters in *C. glabratus*. (a) Colleters on a fresh stipule with stereoscopic microscope, LC (*white arrowhead*) and DC (*black arrowhead*). (b-k) LM. (b) Obpiriform LC in stipule. (c) DC in stipule, vascularized in basal portion. (d) Detail of the vascular bundle of the DC of the stipule. (e) Floral bud with DC on the corolla (*black arrowhead*). (f) Detail of DC of E. (g) DC of calyx. (h) Transection of three stipules with colleters on adaxial side. (i) Detail of stipule with LC (*white arrowhead*) and DC (*black arrowhead*), note the secretion (s) of the colleters. (j) Detail of parenchymatic cells of the colleter axis with crystalline sand (*black arrow*) and druse (*white arrow*). (k) Stipular colleter with taniferous cells. Abbreviations: *p* peduncle, *s* secretion, *t* tannin, *v* vascular bundle. *Scale bars* a = 500 µm; e, h = 200 µm; i = 100 µm; b, c, g, k = 50 µm; d, j = 20 µm.

Table I. Morpho-anatomical characteristics of colleters of *Cephalanthus*.

	Light colleters (LC)	**Dark colleters (DC)**
Location	stipule	leaf, stipule, bract, bracteole, calyx and corolla
Organization	grouped	solitary or grouped
Shape	obpiriform	conical
Length (µm)	84.08-548.60 µm	130.34-291.45 µm
Axis (TS)	3 to 5 cell layers	3 to 7 cell layers
Cuticle (µm)	3.0-6.0 µm	3.6-11.0 µm
Peduncle length (µm)	short: 28.55-32.0 µm or sessile	short: 16.67-26.85 µm stipule, bracts and bracteoles) long: 94.94-236.07 µm (calyx and corolla)
Vascularization	without vascular bundles, the bundles of the stipule pass near them	vascularized, the vascular bundles of the stipule partially enter the colleter, reaching to the base

Ontogeny

The development of colleters was analyzed in stipules of *C. glabratus*, where both types of colleters are present simultaneously. Both types of colleters have the same development. Colleters start from a group of protodermal cells and the underlying meristematic cells, which stand out due to their rectangular shape, dense cytoplasm and conspicuous nuclei (Figure S1a). The protodermal cells undergo several anticlinal divisions. The underlying meristematic cells divide both anticlinally and periclinally to form a protuberance (Figure S1b-c). After successive divisions in both strata, the lump lengthens (Figure S1d). The secretory cells continue to divide and lengthen in their palisade form. The cells of the central axis expand progressively as the head of the colleter lengthens. It is the protodermal cells that give rise to the secretory epidermis, and the underlying tissue to the cells of the colleter axis. In the apical portion, the development of the palisade-like epidermis determines the formation of a more or less elongated head; the basal portion remains as a short peduncle where the epidermis is not palisade-like (Figure S1e-f). Some colleters on the inner side of the stipule do not form peduncles, becoming sessile colleters (Figure S1g).

Location of the colleters in species of *Cephalanthus*

Analysis of fresh material from *Cephalanthus glabratus* and herbarium material of the five other species of the genus, shows that the colleters are presented in various organs (Figures 2, S2, S3). A summary of the locations is in Table II.

Stipules: the colleters are located on the adaxial side, on the margin and apex of the stipules. The two types of colleter have different positions:

Light colleters (LC): are located on the inner face of the stipules, accompanied by simple eglandular trichomes that vary in quantity and disposition between the species (Figures 1a, S2a-i, 2a).

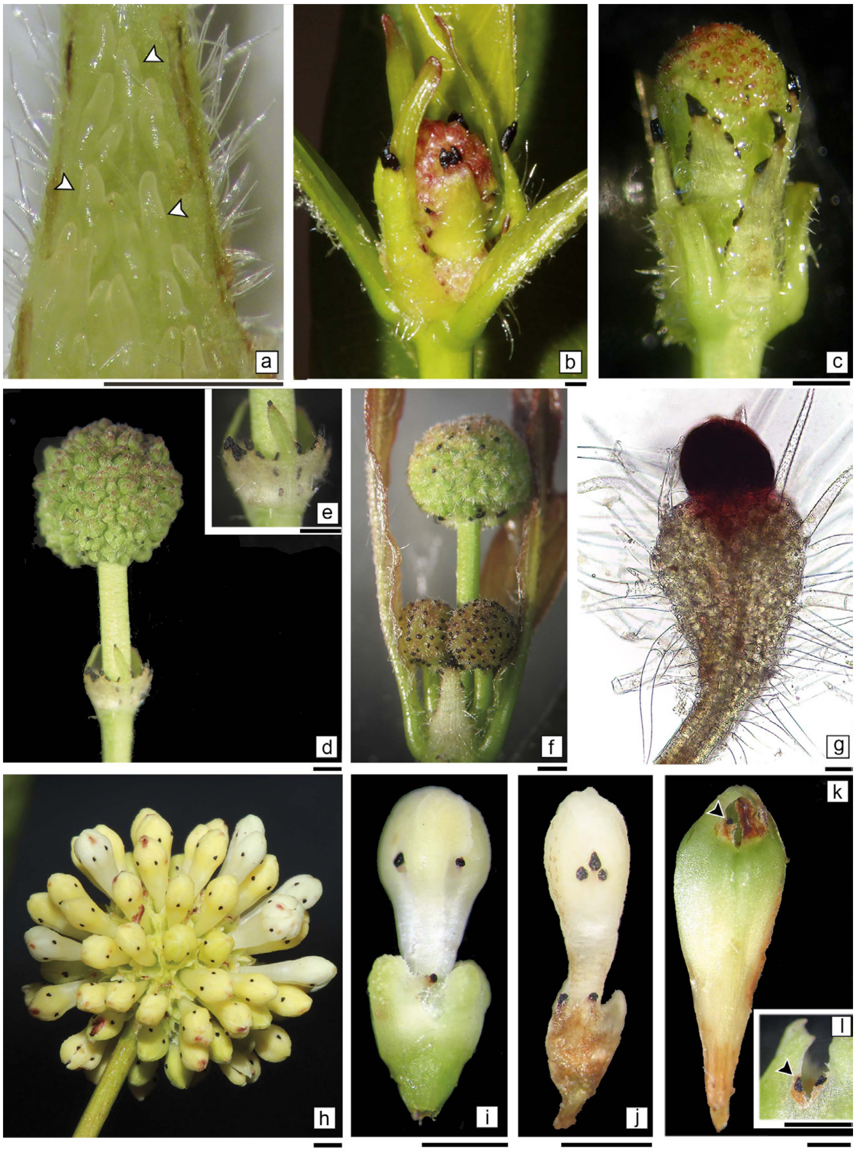

Figure 2. Location of colleters in *Cephalanthus glabratus*. (a) LC (*arrowhead*) covering the inner face of the stipule. (b) DC at apex of the stipule in young undeveloped inflorescence. (c) DC at apex and margin of stipule. (d) DC in bract. (e) Detail of DC at apex and bract margin. (f) DC in bracteoles of the developing inflorescence. (g) Bracteole with DC at apex. (h) DC in corolla. (i) DC solitary in the interlobular sinuses of the corolla and calyx. (j) DC grouped in the interlobular sinus of the corolla and DC at the apex of the calyx lobes. (k) DC in calyx persistent in the fruit (*arrow*). (l) Detail of the DC in k (*arrow*). *Scale bars* a-f, i-l = 1 mm; g = 40 µm; h = 3 mm.

Dark colleters (DC): found at the apex and margins of the stipules. May be solitary or grouped at the apex. They are accompanied, or not, by simple eglandular trichomes (Figures 1a, S2c, d, g, 2b-c, S3a-c).

Colleters on adaxial surface of stipules may be sessile or have a short peduncle, while all those on the margin and apex have a peduncle.

Leaf lamina: a solitary DC is found on the acumen of the young leaves (Figure S3d).

Inflorescence: has DC in different positions:

Bracts: there are tubular structures located on the main axis of the inflorescence, formed by the fusion of three bracts and three stipules, both with their laminar portions reduced. DC are found at the margin and apex. On the inner side of the tubular structure, there are abundant simple eglandular trichomes and no colleters (Figures 2d-e, S3e-f).

Bracteoles: they are spatulate in shape and have one DC in the apical region (Figures 2f-g, S3g). At flower maturity, the colleters on the bracts and bracteoles become detached.

Table II. **Characteristics of the light colleters (LC) and dark colleters (DC) colleters and trichomes in *Cephalanthus*.**

Taxón	Type of colleter	Position	Grouping	Quantity	Size	Simple eglandular trichomes	
						Quantity	**Disposition**
C. angustifolius	LC	adaxial side st	dispersed	10-15	medium	very abundant, colleters overlooked	along the st
	DC	margin/apex st	grouped	2	medium	scarce	margin of st
		margin /apex br	grouped	5-10		absent	
		apex brl	solitary	1			
		interlobular sine ca	solitary	1 per sinus			
		interlobular sinus co	solitary	1 per sinus			
C. glabratus	LC	adaxial side st	trapezoidal-triangular	30-60	large	abundant	along the stipula or up to the middle region
	DC	margin/apex st	grouped	2-7	medium	scarce	margin and ápex st
		margin /apex br	grouped	6-12		absent	
		apex brl	grouped	1			
		interlobular sine ca	solitary	1 per sinus		scarce	sinus of calyx
		apex of ca lobe	solitary	1 per lobe		absent	
		interlobular sinus co	solitary / grouped	1-3 per sinus			
C. natalensis	LC	adaxial side st	rectangular	10-16	short/ medium	moderate	base st
	DC	absent					
C. occidentalis	LC	adaxial side st	triangular	18-30	medium / large	absent	
	DC	margin/apex st	solitary / grouped	1-2	medium	absent	
		margin /apex br	grouped	5-8			
		apex brl	solitary	1			
		interlobular sine ca	solitary	1 per sinus			
		interlobular sinus co	solitary	1 per sinus			
C. salicifolius	LC	adaxial side st	trapezoidal	15-25	medium	scarce to moderate	at the base of the stipule, in a row
	DC	Leaf lamina	solitary	1	medium	absent	
		margin/apex st	solitary / grouped	1-2			
		margin /apex br	grouped	3-6			
		apex brl	solitary	1			
		apex ca lobe	solitary	1 per lobe			
		interlobular sinus co	solitary	1 per sinus			
C. tetrandrus	LC	adaxial side st	disperse	10-12	short	absent	
	DC	margin/apex st	solitary / grouped	1-5	medium	absent	
		margin /apex br	grouped	3-5			
		apex brl	solitary	1			
		interlobular sine ca	solitary	1 per sinus			
		interlobular sinus co	solitary	1 per sinus			

Abbreviations: *br* bract, *brl* bracteole, *ca* calyx, *co* corolla, *st* stipule. Colleter size (µm): *short* 85-130, *medium* 131-350, *large* 351-600.

Flowers: in the floral bud stage, DC are located on the calyx; they are well developed both in the interlobular sinus and the apex of the lobes. In the corolla of the flower bud and the open flower, the DC are generally solitary and located on the interlobular sinus. In *C. glabratus,* colleters were found in groups of 2-3 per corolline sinus, but this character was less frequent. Of all the floral organs where DC were found only those located on the corolla were active at the anthesis stage. A few calyx colleters persist and dry up in the fruits (Figures 2h-l, S3h-k).

Cephalanthus natalensis was the only species in the genus where no DC were found in any of the organs analyzed.

Secretion of the colleter in *Cephalanthus glabratus*

In the young, green stipules the colleters are turgid structures (Figure 3a-b, e). The secretion is profuse in the colleters at the apexes of the branches, and diminishes notoriously from the

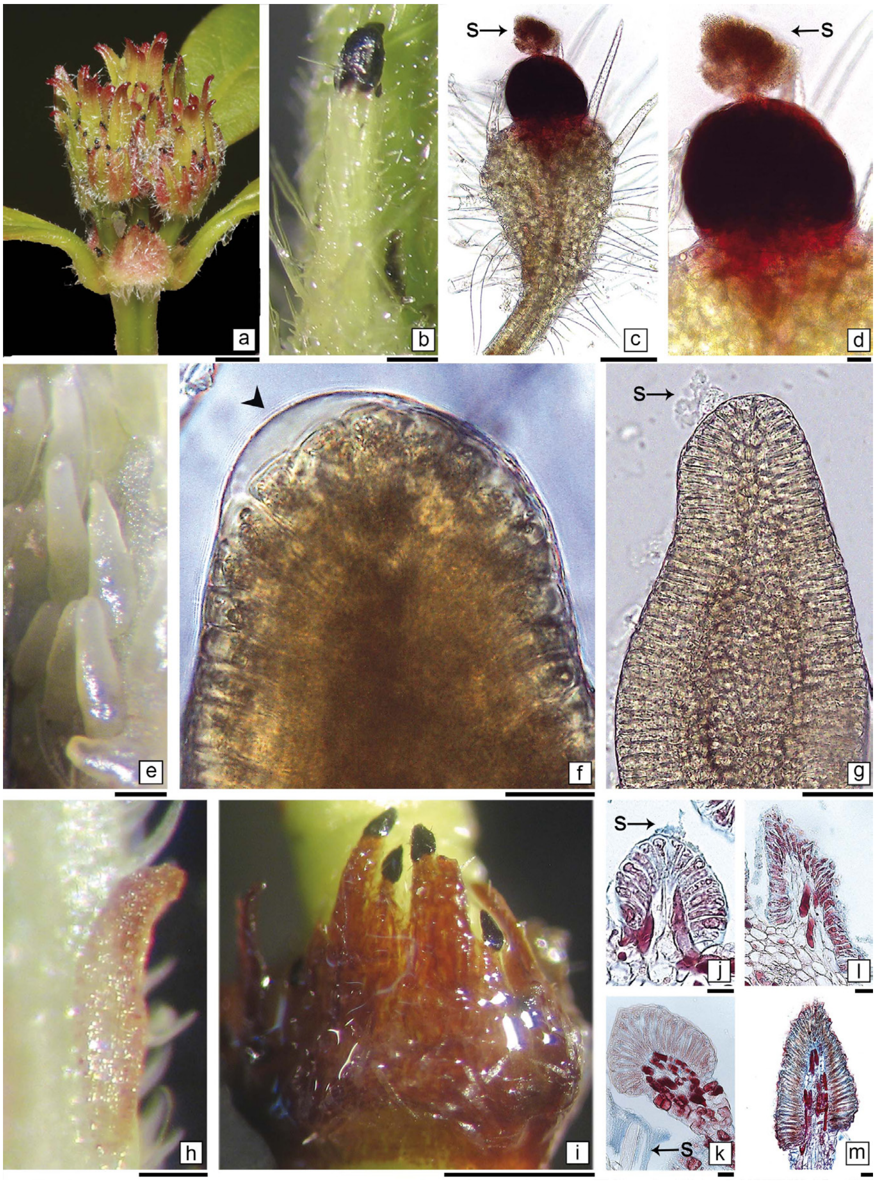

Figure 3. Secretion of the colleters in *C. glabratus*: in fresh material (a, b), LM (c, d, f, g, j-m) and stereoscopic microscope (e, h, i). (a) Vegetative apex with DC at the apex of young stipules. (b) Turgid DC at the apex of the stipule. (c) Bracteole with secreting DC at the apex. (d) Detail of the LC showing blackish-red secretion. (e) Turgid LC. (f) LC with colorless secretion between the cuticle and epidermal cells (*arrowhead*). (g) LC with colorless secretion released. (h) LC withered and brownish. (i) Brown bract with DC in its senescent phase. (j-k) Anatomy of colleters during their secretory phase. (j) LC. (k) DC. (l-m) Anatomy of the colleter in its senescent phase. (l) LC. (m) DC. Abbreviation: *s* secretion. *Scale bars* a, h, i = 1 mm; b = 300 µm; c, e = 100 µm; g = 50 µm; d, f, j-m = 20 µm.

third node. The secretion of DC is black to dark red, while that of the LC is colorless (Figure 3c-g). The release of the secretion occurs by rupture of the cuticle in the apical region of the colleter (Figure 3c-d, f-g).

After the secretory activity, the colleters undergo a change in color and become senescent. In the LC, senescence is evident because the turgid, light colleters wither, changing in color to brown progressively from the apex to the base of the colleter (Figure 3h). In DC, no change in color can be seen in the colleters due to the intense coloration they present, but changes in color are observed in the organ that carries them (e.g. from green to brown in the stipules and bracts) (Figure 3i). Anatomically, both types of senescent colleters are characterized by collapsed epidermal cells (Figure 3j-m).

After senescence, the colleters may fall from the organ that carries them, or occasionally persist in the bracteoles and calyx; they remain for longer on the stipules, bracts and corolla (Figure 3h, i).

Histochemistry of the colleter secretion

Histochemical tests with different reagents were performed on the secretions of the colleters from a stipule of *Cephalanthus glabratus*. The DC reacted more intensely to the different reagents than the LC (Table III) (Figure S4a-j).

The colleter secretion in *C. glabratus* is mainly composed of a phenol-rich mucilage. In the different histochemical tests, it was observed that the axis cells generally present different staining in respect to the epidermal cells. The difference between both types of colleters was observed in the epidermis coloration of dark colleters, which have higher contents of mucilage, phenolic compounds and starch than the light colleters.

Colleters in the Naucleeae tribe

The presence/absence, shape, locations, abundance and size of colleters were determined in species of the Naucleeae tribe, and recorded in Table IV. The colleters were only found on the internal face of the stipules and on the interlobular sinus of the calyx (Figure S5a-j). Except for *Cephalanthus*, no colleters were found on the corollas in any of the species analyzed from the Naucleeae tribe (Figure S6a-b).

The colleters correspond to the standard light type, as described above for *Cephalanthus*. In *Nauclea orientalis*, no colleters were observed

Table III. Histochemical tests performed on colleters of *C. glabratus*.

Histochemical treatment	Substance detected	Color of positive reaction	Light colleters				Dark colleters			
			e	ep	c	s	e	ep	c	s
Cresil blue	mucilage	bright blue	-	-	-	+	+	+	-	+
Ferric chloride	Phenolic compound, mucilage with proteins	black	-	-	-	ns	+	+	-	ns
Toluidine blue	phenols	bright blue	-	+	-	+	+	+	-	ns
	pectins	purple	-	+	-	ns	+	+	-	ns
Lugol	starch	blue or black	-	+	-	ns	-	+	-	ns
Sudan III	lipids	red	-	-	+	ns	-	-	+	ns

Abbreviations: *(+)* positive *(-)* negative, *(c)* cuticle, *(e)* axis, *(ep)* epidermis, *(ns)* organ not seen in specimen, *(s)* secretion.

on the inner side of the stipule, where numerous trichomes were found covering it (Figure S5g).

Colleters located in the interlobular sinuses of the calyx were only found in *Corynanthe pachyceras* (Figure S5h-j). These colleters are solitary, pyramidal and short, morphologically resembling the dark colleters described for *Cephalanthus*, but in *C. pachyceras* they lack coloration.

Occurrence of Corolline Colleters in Angiosperms

In addition to the corolline colleters recorded for *Cephalanthus*, material from *Nerium* and *Cuscuta* was revised. In *Nerium oleander* and *Cuscuta campestris*, no colleters were observed on the corolla (Figure 4a-i). In flowers of *N. oleander,* both single and double corolla, the colleters are located only on the base of the sepals. These colleters are of the standard type, cylindrical and long (320.76- 959.98 μm), pale yellow in color and they are grouped in a row of 5 to 7 per sepal (Figure 4a-d, g). The inner side of the corolla presents a corolline corona composed of filiform and petaloid appendages (Figure 4f).

Cuscuta campestris does not have any colleters associated with the corolla. However, it has non-glandular infrastaminal scales (IFS) on the inner face of stamens. IFS are flat formations that measure 621.19-809.27 μm in length, which bear 20 to 25 secretory fimbriae with a length of 191.03-484.45 μm (Figure 4h-i).

In Figure 5 we record the presence/absence, types and distribution of corolline colleters in a Phylogenetic Angiosperm Tree. According to our bibliographical revision, the corolline colleters are found exclusively in Fabaceae (*Chamaecrista*), Passifloraceae (ex Turneraceae, *Adenoa*) and Rubiaceae (*Cephalanthus*).

DISCUSSION

Colleters in *Cephalanthus* and the Naucleeae tribe

In *Cephalanthus* only a few taxonomic papers mentioned the presence of “glands” on the stipules and corolla (Schumann 1889, Bacigalupo 1974, Ridsdale 1976). From our study, it was proven that the structures described as “glands” for *Cephalanthus* correspond structurally to colleters of the standard type.

According to differences in their general appearance and notably in their color (both in fresh and dry herbarium material) and the color of their secretions, the colleters of *Cephalanthus* were classified into subtypes: light and dark, which vary in morphology, distribution and number with respect to the organ where they are found.

The light colleters lack vascularization, unlike the dark colleters of the stipule that have a vascular bundle in the basal region. Carlquist (1969) points out that the vasculature of a structure is directly proportional to its size and is not necessarily related to any state of advancement. On the other hand, Lersten (1974a, b) and Thomas (1991) indicate that the vascularization in the colleter is connected to the organ on which it is attached. According to the hypotheses mentioned above, the one raised by Carlquist (1969) does not match with our observations since the vascularization was found in the dark colleter which is smaller than the light ones. Therefore, the hypothesis proposed by Lersten (1974a) and Thomas (1991) is the one that agrees with our observations, since the vascular bundle of the dark colleters is a continuity of the vascularization of the stipule. In Apocynaceae, the presence of the vascular bundle in the colleter would be an evolutionary step in the family (Thomas & Dave 1991). Although the vascularized colleters in Rubiaceae

Table IV. Location and characteristics of colleters in species of the Naucleeae tribe.

Taxon	Type	Shape	Location	Abundance	Length	Shape of grouping	Notes
Adina pilulifera	no record		stipule calyx	no record			In *Adina* there are records of colleters in stipule and calyx (Cooke 1958, Lersten 1975). In this study, the analyzed material did not present stipules, they are deciduous. No colleter was observed in calyx.
Breonadia salicina	standard	cylindrical	stipule	25-40	large	linear	No record
Breonia macrocarpa	absent						
Breonia madagascariensis	absent						
Corynanthe mayumbensis	absent						
Corynanthe pachyceras	standard	conical	calyx	1 per sinus	short	solitaire	
Corynanthe paniculata	absent						
Lukedia bernardoi	absent						
Mitragyna inermis	absent						In *Mitragyna* there are records of colleters in stipules (Lersten 1975, Thomas & Dave 1990). In this study their presence is confirmed.
Mitragyna rubrostipuleta	standard	cylindrical	stipule	25-40	medium/ large	triangular	
Mitragyna stipulosa	absent						
Nauclea latifolia	absent						No record
Nauclea orientalis	absent						
Neolamarkia cadamba	absent						
Neonauclea calycina	absent						
Pausinystalia johimbe	absent						
Pertusadina hainanensis	absent						
Sarcocephalus latifolius	standard	obpiriform	stipule	30-60	medium/ large	disperse	In *Sarcocephalus* there are records of colleters in stipule and calyx (Groom 1892, Cooke 1958, Lersten 1975). In this study, the presence of colleters in stipule is confirmed. No colleters were observed in calyx.
Uncaria africana	absent						In *Uncaria* there are records of colleters in stipule (Lersten 1975). This study confirms their presence.
Uncaria rhychophylla	standard	cilindrical	stipule	5-10	medium/ large	disperse	

Abbreviations: Colleter size (µm): ***short*** **85-130,** ***medium*** **131-350,** ***large*** **351-600.**

have been described in several genera (*Crusea* Cham. & Schltdl.: Anderson 1972; *Simira glaziovii* (K. Schum.) Steyerm.: Klein et al. 2004; *Bathysa cuspidata* (St. Hil.) Hook. f.: Coelho et al. 2013; *Mitracarpus polygonifolius* (A. St.-Hil.) R.M Salas & E.B. Souza and *Staelia glandulosa* R.M. Salas & E.L. Cabral: Judkevich et al. 2017) further studies addressing this aspect are necessary in order to interpret the vascularization of the colleter as an evolutionary character in the Rubiaceae. With our study, it was the first time that the vascularization of the colleter has been recorded in the Naucleeae tribe.

The two types of colleters also differ in their distribution on the plant. The LC are restricted to a single position: on the inner side of the stipule. However, the DC are present in various organs, both reproductive and vegetative, including the margin/apex of stipules. In the genus *Cephalanthus* the co-occurrence of colleters on the margin and internal face of the stipule in Rubiaceae is unpublished. The only antecedent of this aspect was that of

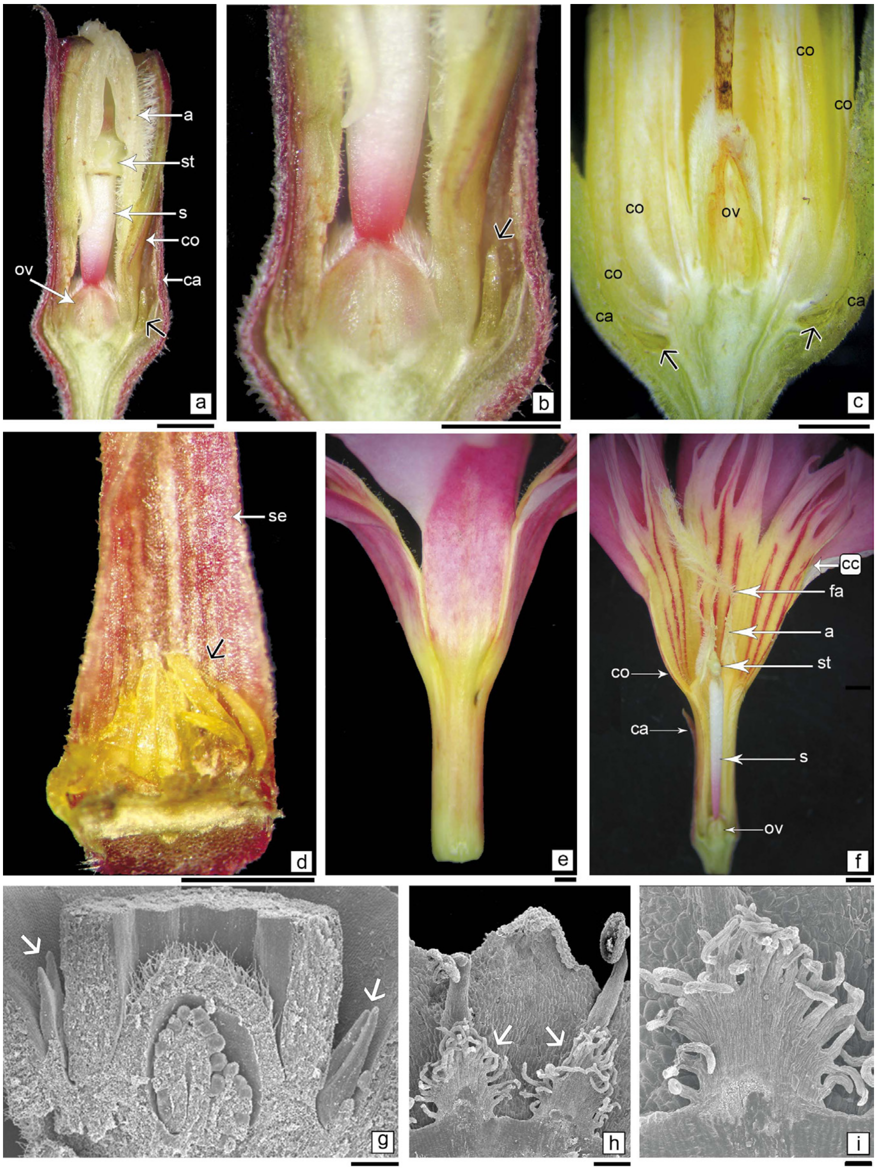

Figure 4. Colleter in the sepal of *Nerium oleander* and infrastaminal scales fimbriae in *Cuscuta campestris* seen with stereoscopic microscope (a-f) and SEM (g-i). *N. oleander* (a- g) and *Cuscuta campestris* (h-i). (a) Flower bud with colleter located in the calyx (arrow). (b) Detail of the callicine colleter in single corolla flower *(arrow)*. (c) Detail of the callicine colleter *(arrows)* in double corolla flower. (d) Distribution of the colleters *(arrows)* located in the inner face of sepals. (e) Outer side of the corolla without colleters. (f) Longitudinal section of flower showing corona position. (g) Detail of the colleter on the inner side of the sepal *(arrows)* (h) Infrastaminal scales fimbriae *(arrow)* welded to the base of the stamen and the corolla. (i) Detail of the infrastaminal scales fimbriae. Abbreviations: *a* anther, *ca* calyx, *cc* corolline corona, *co* corolla, *fa* feathery appendage of the anther, *s* style, *se* sepal, *st* stigma, *ov* ovary. *Scale bars* a-f = 1 mm; g, h = 300 µm; i = 100 µm.

Rutishauserin Thomas (1991) who mentioned it in *C. occidentalis*. According to Robbrecht (1988) the colleters located on the inner side of the stipule would represent the primitive condition in Rubiaceae, from which it could be inferred that the light colleter is more ancestral in relation to the dark ones. *Cephalanthus natalensis* is the only species that lacks dark colleters on all its organs, which in addition to other morphological characteristics would explain its position as sister to the rest of the species in the genus, according to the cladogram proposed by Löfstrand et al. (2014). Therefore, we consider the DC as an exclusive derivative character of *Cephalanthus*.

As for their function, DC acquire importance during the growth of the inflorescence because they are present on bracts, bracteoles, calyx and corolla. As the inflorescence grows, the protagonism of the DC is different, i.e. initially the bracts are the first to be active and protect the meristems of the inflorescence, then follow those of the bracteoles that protect the young glomeruli. Later, it is the calyx, and then the corolla, that protects the buds up to anthesis. Unlike the DC, the LC are only present on the inner side of the stipule, protecting the young developing offspring. The first records of leaf-colleters in Rubiaceae were the DC found in *C. salicifolius* and on the reduced lamina of the bracts in species of *Cephalanthus*, except for *C. natalensis*.

From the ontogenetic point of view, the colleters are structures that can originate from the protodermis or have a mixed origin, with the contribution of protodermis and a group

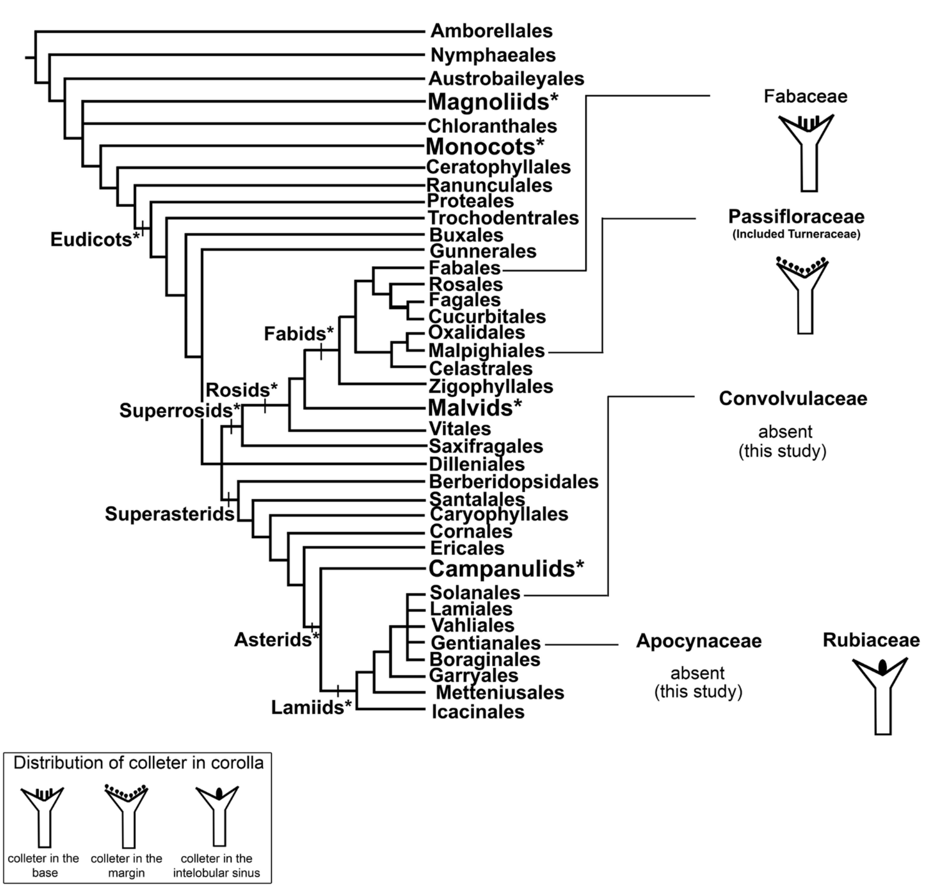

Figure 5. Distribution of presence/absence and types of corolline colleters in a Phylogenetic Angiosperms Tree, modified from APG IV (2016). Only corolline colleters are plotted in taxa previously mentioned in the literature and which were re-investigated in this work: Apocynaceae (*Nerium*), Convolvulaceae (*Cuscuta*), Fabaceae (*Chamaecrista*) Passifloraceae (ex Turneraceae, *Adenoa*) and Rubiaceae (*Cephalanthus*). Some large groups were reduced to a single branch, Magnoliids, Monocots, Malvids and Campanulids in order to facilitate interpretation.

of meristem cells (Coelho et al. 2013). The standard type colleter generally arises from a series of anticlinal divisions of protodermis cells and anticlinal and periclinal divisions of subepidermal cells (Patel & Zaveri 1975, Dave et al. 1988). In *Cephalanthus glabratus* both the light and dark colleters develop from a group of protodermal cells and underlying meristem cells that suffer a series of anticlinal and periclinal and anticlinal divisions, respectively. Therefore, the origin of colleters in *C. glabratus* is mixed, as already described for other Rubiaceae (Dave et al. 1988, Thomas 1991, Coelho et al. 2013, Muravnik et al. 2014, Judkevich et al. 2017). The ontogenetic study carried out in this study represents a contribution to the Naucleeae tribe and is the first for the genus *Cephalanthus*.

Colleters are structures capable of secreting mucilage which contains polysaccharides (Lersten 1974b, Mohan & Inamdar 1986), proteins (Thomas & Dave 1989), specifically hydrolytic enzymes (Mangalan et al. 1990, Miguel et al. 2006); resinous substances (Fahn 1979, Durkee et al. 1984, Leitão & Cortelazzo 2008) and phenolic compounds (Rio et al. 2002, Muravnik & Kostina 2011). In the Rubiaceae several authors studied the nature of the secretion of the colleter detecting the presence of mucilages and proteins, alone or combined (Thomas 1991, Klein et al. 2004, Coelho et al. 2013).

According to our results, the secretion of colleters of *Cephalanthus glabratus* is mainly composed of mucilage rich in phenols. The mucilage was detected in the secretion of the LC and also in the secretion of the epidermal cells of DC. Various authors (Fahn 1990, Thomas 1991, Mayer et al. 2013) consider that a mucilaginous secretion has favorable properties against dehydration, thus protecting the developing vegetative and reproductive organs.

Another compound identified was starch, obtaining an intense positive reaction in dark colleters. The presence of starch grains in the secretory cells of the colleter could be related to an energetic demand for the maintenance of precursors that intervene in the secretory activity of the colleters (Thomas 1991, Paiva 2009, Machado et al. 2012).

The difference observed between the two types of colleters described was that DC have a higher mucilage and phenol content than LC. This could explain the difference in coloration between the colleters present in *Cephalanthus*, since the secretion of the dark colleters without a reagent is blackish-red whereas that of the light colleters is translucent.

Tresmondi et al. (2015) hypothesized that a large proportion of Rubiaceae species living in savannas have developing apexes with colleters covered with lipid exudate which could be a response to environmental problems (elevated temperatures, pronounced periods of drought and high rates of solar radiation).

According to Dell (1977) and Paiva (2009, 2012), in addition to playing a protective role against herbivores and pathogens, colleters could be considered as a xeromorphic character, since the exudate would protect developing organs, preventing water loss through cuticular transpiration. Our results shows that the colleters present in numerous developing organs in *Cephalanthus* species could be related to the environment where they live, being the only genus of the tribe (with the exception of *C. natalensis*) that inhabits areas with mixed conditions: seasonal aquatic environments that occasionally endure long periods of drought. It might be that the prolonged period of drought that the five species of *Cephalanthus* (*C. angustifolius, C. glabratus, C. occidentalis, C. salicifolius* and *C. tetrandrus*) are able to support is a determining factor for the development of colleters in numerous young organs of the plant, protecting them against dehydration.

Cephalanthus natalensis is the only species in the genus where no dark colleters were found in any of the organs analyzed and only a few light colleters were observed on the inner face of the stipule. This species grows on the margins of mountainous forests, rocky outcrops or mountain pastures, places that although they are rainy are not floodable (Bridson & Verdcourt 2003). This in turn could explain why *C. natalensis* is the only species that lacks colleters.

According to Thomas (1991) the number of colleters present in species of a genus may vary in relation to its geographic distribution. In this study we observed that the American species of *Cephalanthus* had the highest number of colleters, the species with the highest number of LC (up to 60 colleters on the stipule) and DC (up to 7 DC at the apex of the stipule and up to 3 DC on the corollary sinus) being *C. glabratus* (South America). Then followed the Asian species, *C. angustifolius* and *C. tetrandrus* (with up to 12 LC in stipules and a solitary DC on various organs) and finally *C. natalensis* (up to 16 LC on stipules and absence of DC).

Lersten (1975) also observed considerable differences in the size of the colleters in relation to distribution in the Rubiaceae: the largest colleters were recorded in South America (up to 2600 μm) in *Psychotria* L. species and they decline towards Asia (up to 410 μm) and Africa (up to 334 μm). According to the distribution of *Cephalanthus* species, our results indicate that the largest colleters were observed in the South American species: *C. glabratus*, followed by the North American species: *C. occidentalis* and *C. salicifolius*, then the Asian species: *C. angustifolius* and *C. tetrandrus*. The African species: *C. natalensis* presented only short/medium light colleters and according to their size they are in-between the American and Asian species.

The data mentioned above show that there is a relationship between the geographical distribution, number and size of the colleters in *Cephalanthus* and therefore the colleter is an important taxonomic character within the genus. In Rubiaceae the variation in the colleters in their morphology, distribution and number has been used as a character of taxonomic importance (Robbrecht 1988, Thomas 1991). However few studies have been carried out on the Naucleeae tribe. The presence of standard colleters has only been described on stipules in *Mitragyna*, *Nauclea* and *Uncaria,* and on stipules and the calyx in *Sarcocephalus* and *Adina*, and in no case was the type of colleter specified (Groom 1892, Cooke 1958, Lersten 1975, Thomas & Dave 1990). Razafimandimbison & Bremer (2002) mentioned the presence of colleters in the corolla in *Cephalanthus*, except for *C. natalensis*. Our results suggest that both dark and light colleters have taxonomic importance at the genus and species level. In order to establish the phylogenetic importance of the morphological data obtained, they were contrasted with the classification of the Naucleeae tribe proposed by Löfstrand et al. (2014). According to this classification, the subtribe Cephalanthinae is in the most basal group and a sister of the remaining subtribes. The dark and light colleters analyzed in this study are shown in Figure S6a-b.

Based on the observations made, it can be concluded that the way in which the LC are aggregated on the inner side of stipules has subtribal importance: Mitragyninae, Adininae and Breoniinae share the linear aggregation, they are disperse in Uncariinae, triangular in Naucleinae, and Cephalanthinae is the subtribe with the greatest variability, presenting the colleters in scattered, rectangular, trapezoidal and triangular aggregates. The form of aggregation also varies between species of *Cephalanthus*, which allows them to be recognized: *C. glabratus* (trapezoidal and triangular), *C. natalensis* (rectangular), *C. occidentalis* (triangular), *C. salicifolius* (trapezoidal) and *C. angustifolius* and *C. tetrandrus* (scattered).

In our study the calyx colleters were only found in *Corynanthe pachyceras*, which according to the morphological aspect are similar to the dark colleters, but no such coloration was observed in them. In *Adina* (Cooke 1958, Lersten 1975) and *Sarcocephalus* (Groom 1892, Cooke 1958, Lersten 1975) they mention the presence of calyx colleters without specifying their location.

On the other hand, the DC acquire taxonomic importance when comparing the subtribes, since this character is characteristic of the subtribe Cephalanthinae and supports it as a brother group of the rest of the subtribes as proposed by Löfstrand et al. (2014). Dark colleters are also important at the species level when comparing absence/presence, *C. natalensis* being the only species that lacks these colleters.

Corolline colleters in Angiosperms

Of the four genera in which "corolline colleters" have been described, they had only been anatomically confirmed in *Adenoa* (Passifloraceae) by Gonzalez et al. (2012) and in *Chamaecrista* (Fabaceae) by Cotta Coutinho et al. 2015). Gonzalez et al. (2012) described the presence of abundant lacrimiform (teardrop shaped) colleters on the margins of the petals in *Adenoa*. In several species of *Chamaecrista* short digitiform colleters are at the base of the petals, in addition to the calicinal colleters of diverse forms (short to long digitiform; club shaped; racket shaped; short to long bottle shaped) at the base and margins of the sepals (Cotta Coutinho et al. 2015).

In *Nerium* (Apocynaceae), Thomas & Dave (1989, 1991) described the existence of colleters in the corolla; the illustrations of these structures on the flowers in both papers are obscure, and in the sections of colleters shown it is not indicated whether they correspond to the corolla, calyx or vegetative parts of this species. Other studies of secretory structures in other Apocynaceae taxa reiterate the existence of corolla colleters, based exclusively on these two papers (Ribeiro et al. 2017). According to our observations, the colleters of *N. oleander* flowers are inserted only at the base of the inner face of the sepals. These calicinal colleters are of the standard type, cylindrical and long (320.76- 959.98 μm long), pale yellow in color and they are grouped in rows of between five and seven colleters per sepal. There are no colleters on the corolla, on either the single or double corolla flowers. The confusion in the papers of Thomas & Dave (1989, 1991) could be because these authors analyzed *Nerium* buds and the structures that appear on the internal face of the petals are fragments of the crown not yet unfolded. Taxonomic studies and recent reviews, such as Endress et al. (2018), no longer cite this detail of the presence of corolline colleters for either *Nerium* or the family.

The structures in flower of *Cuscuta* described by Thomas & Dave (1991) as corolline colleters have been studied by several authors, such as Engelmann (1859) and Riviere et al. (2013), who described them as: infrastaminal scales fimbriae (IFS) in allusion to their location: at the base of the stamens; and they are connected to each other basally by a "bridge" (Yuncker 1932). Although the role of the IFS in *Cuscuta* is still unknown, Tiagi (1966) proposed that they secrete nectar and that they serve to attract/reward pollinators. However, Riviere et al. (2013) consider that the IFS in *Cuscuta* do not secrete nectar, but that they evolved in relation to a modification of their function in the flower: from nectar protection (in the *Monogynella* subgenus) to ovarian/ovular protection against herbivorous insects (in the *Grammica* and

Cuscuta subgenera). None of these authors considers that IFSs have the function of protecting developing organs through the secretion of mucilage and/or resin, a function that has been widely reported for colleters. According to our observations, the structures named: glandular shaggy hairs or IFS in flowers of *Cuscuta* do not correspond structurally with corolline colleters.

In this study, corolline colleters were analyzed in 26 species of the Naucleeae tribe and were only found in the five species of the genus *Cephalanthus*. Therefore, *Cephalanthus* is the only genus of the order Gentianales, of the family Rubiaceae and of the tribe Naucleeae with colleters in the corolla.

To conclude, the presence of colleters in the corolla in species of Angiosperms, in addition to *Cephalanthus* (Rubiaceae), is restricted to *Adenoa* (Passifloraceae) and *Chamaecrista* (Fabaceae). This shows that the corolline colleter is a rare character in Angiosperms.

Acknowledgments

The first author thanks CONICET for the doctoral grant, this study being part of her doctoral thesis. This work was funded by the Universidad Nacional del Nordeste (grants PICTO 199-2011 and PI 01-2012 to AMG, and PI A013-2013 and PI 16P001 to MFR and RMS) and from ANPCyT - Foncyt (PICT 2016-3517 to MFR and RMS). We thank Prof. Charlotte Taylor for providing fixed material of *C. occidentalis*, to Rosemary Scoffield for reading the English manuscript critically.

REFERENCES

ANDERSON WR. 1972. A monograph of the genus Crusea (Rubiaceae). Mem N YBot Gard 22: 1-128.

APG IV. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc 181: 1-20.

BACIGALUPO NM. 1974. Rubiaceaein Flora Ilustrada de Entre Ríos. Burkart AE (Ed) Buenos Aires: Colección Científica del INTA 6(6): 3-50.

BARREIRO DP & MACHADO SR. 2007. Coléteres dendróides em *Alibertia sessilis* (Vell.) K. Schum., uma espécie não-nodulada de Rubiaceae. Rev Bras Bot 30: 387-399.

BREMER B & ERIKSSON T. 2009. Time tree of Rubiaceae: Phylogeny and dating the family, subfamilies, and tribes. Int J Plant Sci 170: 766-793.

BRIDSON DM & VERDCOURT B. 2003. Rubiaceae. In: Pope GV (Ed.), Flora Zambesiaca, Royal Botanic Gardens, Kew, UK 5(part 3): 379-720.

CAPELLI NV, RODRIGUES BA & DEMARCO D. 2017. Stipules in Apocynaceae: an ontogenetic perspective. AoBP 9: 1-11.

CARLQUIST T. 1969. Toward acceptable evolutionary interpretations of floral anatomy. Phytomorphology 19: 332-362.

COELHO VPM, LEITE JPV, FIETTO LG & VENTRELLA MC. 2013. Colleters in *Bathysa cuspidata* (Rubiaceae): development, ultrastructure and chemical composition of the secretion. Flora 208: 579-590.

COOKE T. 1958. The flora of the presidency of Bombay. Vol. 11, Calcutta.

COTTA COUTINHO IA, FRANCINO DMT & STROZI ALVES MEIRA RM. 2015. New Records of Colleters in *Chamaecrista* (Leguminosae, Caesalpinioideae s.l.): Structural Diversity, Secretion, Functional Role, and Taxonomic Importance. Int J Plant Sci 176: 72-85.

DAVE YP, KURIACHEN M & THOMAS V. 1988. Development, structure and senescence of colleters in *Gardenia lucida* Roxb. (Rubiaceae). Acta Soc Bot Pol 57: 3-7.

D'AMBROGIO DE ARGÜESO A. 1986. Manual de Técnicas en Histología Vegetal. Ed. Hemisferio Sur, Buenos Aires.

DELL B. 1977. Distribution and function of resin and glandular hairs in Western Australian Plants. J R Soc West Aust 59: 119-123.

DEMARCO D. 2008. Glândulas de orgãos vegetativos aéreos e florais de Asclepiadeae (R.BR.) Duby (Asclepiadoideae, Apocynaceae) de mata atlântica do estado de Sâo Paulo. PhD Thesis, Universidade de Campinas, Brasil.

DICKISON WC. 2000. *Integrative Plant Anatomy*. Harcourt Academic Press, San Diego.

DURKEE LT, BAIRD CW & COHEN PF. 1984. Light and electron microscopy of the resin glands of *Passiflora foetida* (Passifloraceae). Am J Bot 71: 596-602.

ENDRESS ME & BRUYNS PV. 2000. A revised classification of Apcynaceae s.l. Bot Rev 66: 1-56.

ENDRESS ME, MEVE U, MIDDLETON DJ & LIEDE-SCHUMANN S. 2018. Apocynaceae. In: Flowering Plants. Eudicots (p. 207-411). Springer, Cham.

ENGELMANN G. 1859. Systematic arrangement of the species of the genus *Cuscuta*, with critical remarks on old species and descriptions of new ones. Acad Sci St Louis 1: 453-523.

FAHN A. 1979. Secretory Tissues in Plants. Academic Press, London.

FAHN A. 1990. Plant Anatomy. Oxford: Pergamon Press.

GONZALEZ AM. 1998. Colleters in *Turnera* and *Piriqueta* (Turneraceae). Bot J Linn Soc 128: 215-228.

GONZALEZ AM. 2018. ImageJ: una herramienta indispensable para medir el mundo biológico. Folium 1: 1-17

GONZALEZ AM & CRISTÓBAL CL. 1997. Anatomía y ontogenia de semillas de *Helicteres lhostzkyana* (Sterculiaceae). Bonplandia 9: 287-294.

GONZALEZ AM, SALGADO CR, FERNÁNDEZ A & ARBO MM. 2012. Anatomy, pollen, and chromosomes of *Adenoa* (Turneraceae), a monotypic genus endemic to Cuba. Brittonia 64: 208-225.

GROOM P. 1892. *IX.* On Bud-protection in Dicotyledons. Trans Linn Soc Lond 2nd Series: Botany 3: 255-266.

HORNER HT & LERSTEN NR. 1968. Development, structure and function of secretory trichomes in *Psychotria bacteriophila* (Rubiaceae). Am J Bot 55: 1089-1099.

JOHANSEN DA. 1940. *Plant Microtechnique*. New York: McGraw-Hill Book Co. Inc.

JOHRI BM & TIAGI B. 1952. Morfología floral y formación de semillas en *Cuscuta reflexa* Roxb. Phytomorphology 2: 162-180.

JUDKEVICH MD, SALAS RM & GONZALEZ AM. 2017. Colleters in American Spermacoceae genera of Rubiaceae, morpho-anatomic and evolutionary aspects. Int J Plant Sci 178: 378-397.

KLEIN DE, GOMES VM, DA SILVA-NETO SJ & DA CUNHA M. 2004. The structure of colleters in several species of *Simira* (Rubiaceae). Ann Bot 94: 733-740.

KRAUSE K. 1909. Über harzsecernierende Drüsen an den Nebenblättern von Rubiaceen. Ber Dtsch Bot Ges 27: 446-452.

LEITÃO CA & CORTELAZZO AL. 2008. Structural and histochemical characterisation of the colleters of *Rodriguezia venusta* (Orchidaceae). Aust J Bot 56: 161-165.

LERSTEN NR. 1974a. Colleter morphology in *Pavetta*, *Neorosea* and *Tricalysia* (Rubiaceae) and its relationship to the bacterial leaf nodule symbiosis. Bot J Linn Soc 69: 125-136.

LERSTEN NR. 1974b. Morphology and distribution of colleters and crystals in relation to the taxonomy and bacterial leaf nodule symbiosis of *Psychotria* (Rubiaceae). Am J Bot 61: 973-981.

LERSTEN NR. 1975. Colleter types in Rubiaceae, especially in relation to the bacterial leaf nodule symbiosis. Bot J Linn Soc 71: 311-319.

LÖFSTRAND SD, KRÜGER A, RAZAFIMANDIMBISON SG & BREMER B. 2014. Phylogeny and generic delimitations in the sister tribes Hymenodictyeae and Naucleeae (Rubiaceae). Syst Bot 39: 304-315.

LUQUE R, SOUSA HC & KRAUS DJE. 1996. Métodos de coloração de Roeser (1972) modificado de Kropp (1972) visando a substituição do azul de astra por azul de alcião 8 GS ou 8 GX. Acta Bot Brasil 10: 199-212.

MACHADO SR, BARREIRO DP, ROCHAC JF & RODRIGUES TM. 2012. Dendroid colleters on vegetative and reproductive apices in *Alibertia sessilis* (Rubiaceae) differ inultrastructure and secretion. Flora 207: 868-877.

MANGALAN S, KURIEN KP, JOHN P & NAIR GM. 1990. Development structure and cytochemistry of resin-secreting colleters of *Gardenia gummifera* (Rubiaceae). Ann Bot 66: 123-132.

MAYER JLS, CARMELLO-GUERREIRO SM & MAZZAFERA P. 2013. A functional role for the colleters of coffee flowers. AoBP 5: 1-13.

MIGUEL EC, GOMES VM, DE OLIVEIRA MA & DA CUNHA M. 2006. Colleters in *Bathysa nicholsonii* K. Schum. (Rubiaceae): ultrastructure, secretion protein composition and antifungal activity. Plant Biol 8: 715-722.

MOHAN JSS & INAMDAR JA. 1986. Ultrastructure and secretion of extrafloral nectaries of *Plumeria rubra* L Ann Bot 57: 389-401.

MURAVNIK LE & KOSTINA OV. 2011. Stipule colleters of the *Galium aparine* and *G. album* (Rubiaceae): fluorescent microscopy and histochemistry. Botanicheskiĭ Zhurnal 96: 1070-1076.

MURAVNIK LE, KOSTINA OV & SHAVARDA AL. 2014. Development, structure and secretion compounds of stipule colleters in *Pentas lanceolata* (Rubiaceae). S Afr J Bot 93: 27-36.

PAIVA EAS. 2009. Occurrence, structure and functional aspects of the colleters of *Copaifera langsdorffii* Desf. (Fabaceae, Caesalpinioideae). CR Biol 332: 1078-1084.

PAIVA EAS. 2012. Colleters in *Cariniana estrellensis* (Lecythidaceae): structure, secretion and evidences for young leaf protection. J Torrey Bot Soc 139: 1-8.

PATEL JD & ZAVERI M. 1975. Development of leaf and stipular glands in *Coffea arabica*. Flora 164: 11-18.

RASBAND WS. 1997-2018. ImageJ, US National Institutes of Health, Bethesda, Maryland, USA. https://imagej.nih.gov/ij. (accessed March 2018).

RAZAFIMANDIMBISON SG & BREMER B. 2002. Phylogeny and classification of Naucleeae s.l. (Rubiaceae) inferred from molecular (ITS, rbcL, and trnT-F) and morphological data. Am J Bot 89: 1027-1041.

RIDSDALE CE. 1976. A revision of the tribe Cephalantheae (Rubiaceae). Blumea 23: 177-188.

RIBEIRO JC, FERREIRA MJP & DEMARCO D. 2017. Colleters in Asclepiadoideae (Apocynaceae): protection of meristems against desiccation and new functions assigned. Int J Plant Sci 178: 465-477.

RIO MCS, CASTRO MM & KINOSHITA LS. 2002. Distribuição e caracterização anatômica dos coléteres foliares de *Prestonia coalita* (Vell.) Woodson (Apocynaceae). Rev Bras Bot 25: 339-349.

RIVIERE S, CLAYSON C, DOCKSTADER K, WRIGHT MAR & COSTEA M. 2013. To attract or to repel? Diversity, evolution and role of the ‘‘most peculiar organ’’ in the *Cuscuta* flower (dodder, Convolvulaceae)—the infrastaminal scales. Plant Syst Evol 299: 529-552.

ROBBRECHT E. 1988. Tropical woody Rubiaceae. Opera Bot Belg 1: 1-176.

ROMERO MF, SALAS RM & GONZALEZ AM. 2017. Pollen development and orbicules and pollen grains morphology in species of *Cephalanthus* (Rubiaceae-Naucleeae) from the Americas. Aust J Bot 65: 233-247.

RUZIN SE. 1999. Plant Microtechnique and Microscopy. Oxford University Press, Nueva York.

SCHUMANN KM. 1889. Tribus X. Naucleeae. In:. von Martius CFP, Eichler AG, and Urban I (Eds), Fl Bras 6: 126-131.

SOLEREDER H. 1908. Systematic Anatomy of the Dicotyledons. Oxford: Clarendon Press.

TIAGI B. 1966. Floral morphology of *Cuscuta reflexa* Roxb. and *C. lupuliformis* Krocker with a brief review of the literature on the genus *Cuscuta*. Bot Mag (Tokyo) 79: 89-97.

THOMAS V. 1991. Structural, functional and phylogenetic aspects of the colleter. Ann Bot 68: 287-305.

THOMAS V & DAVE Y. 1989. Structure, origin, development and senescence of colleters in *Nerium indicum* Mill. (*N. odorum* Soland., Apocynaceae). Korean Journal of Botany 32: 163-172.

THOMAS V & DAVE Y. 1990. Structure and necrosis of stipular colleters in *Mitragyna parvifolia* (Rubiaceae). Belg J Bot 123: 67-72.

THOMAS V & DAVE Y. 1991. Comparative and phylogenetic significance of the colleters in the family Apocynaceae. Feddes Repert 102: 177-182.

TRESMONDI F, NOGUEIRA A, GUIMARÃES E & MACHADO SR. 2015. Morphology, secretion composition, and ecological aspects of stipular colleters in Rubiaceae species from tropical forest and savana. Sci Nat-Heidelberg 102: 73.

VALENTE MC & COSTA CG. 2005. Estudo anatômico da flor de *Marsdenia loniceroides* E. Fournier (Asclepiadoideae - Apocynaceae). Rodriguésia 56: 51-66.

YUNCKER TG. 1932. The genus *Cuscuta*. Mem. Torrey Bot Club 18: 113-331.

SUPPLEMENTARY MATERIAL

Figures S1-S6

Appendix 1. List all specimens analyzed, with * were highlighted specimens fixed in FAA.

APOCYNACEAE

Nerium oleander L. **Argentina** — CORRIENTES. Corrientes, 05 Dec 2019, double corolla flowers, *M. F. Romero* 69*. CHACO. Las Breñas, 2015, single corolla flowers, *A.M. Gonzalez* 520* (CTES).

CONVOLVULACEAE

Cuscuta campestris Yunck. **Argentina**— CORRIENTES. Corrientes, Campo experimental Facultad de Ciencias Agrarias UNNE, 17 Nov 2010, *A.M. Gonzalez* & *R. Medina* 318* (CTES).

RUBIACEAE

Subtribu Cephalanthinae

Cephalanthus angustifolius Lour. ANNAM. Bên Du pro, Thua Thiên Récolte sur le bord de Sông Bô, 16°31'40.8''N; 107°34'22.8''W, 13 May 1920, *M. Poilane 1410* (K); Nha Trang, sine data, *M. Krempf s.n.* (P03820121); idem, *M. Krempf s.n.* (P03820124); Khanh Hoa, Dien Khanh district, road transact Suoi Cat-Hon Ba, at km 19 to Hon Ba, forest along rocky riverbank of Da Giang River, 12°06'53"N, 109°00'12"E, 22 Jun 2004, *D.D. Soejarto 13284* (F, P); idem, Suoi Cat Village, lowland tropical rain forest formation, 12°7.97'N, 109°1.28'E, 110 m, 26 Nov 2004, *D.D. Soejarto 13284* (F, P).

Cephalanthus glabratus (Spreng.) K. Schum. **Argentina**— CORRIENTES: San Cosme, Las Lomas, 29 Ago 2015, *M.F. Romero 60** (CTES); Concepción, Ea Yatay Cora, 50 km NE de Chavarría, 23 Oct 1996, *M.M. Arbo 6797* (CTES); Ituzaingó: Ea San Pedro, 56°52' W 27°45' S, 13 Nov 1976, *M.M. Arbo 1250* (CTES); Itá Ibaté, 13 Oct 1949, *G.J. Schwarz 8235* (CTES); Mburucuyá, 06 Nov 1949, *G.J. Schwarz 8564* (CTES); Riachuelo, 09 Sep 2015, *M.F. Romero 61** (CTES); Saladas, Rincón de Ambrosio, 14 Oct 974, *A. Schinini & C. Cristobal 9984* (CTES); San Luis del Palmar, 26 Ago 2017, *M.F. Romero 67** (CTES); ENTRE RÍOS: Paraná, 12 Dec 1944, *A. Schulz 90* (CTES). MISIONES: Iguazú, Parque Nacional Iguazú, circuito superior, salto Rivadavia, 27 Nov 1995, *J. Herrera 113* (CTES). **Brazil**— RIO GRANDE DO SUL: Cruz Alta, 10 Km S de Cruz Alta, 30 Dec 1980, *A. Krapovickas & R. Vanni 37068* (CTES). MATO GROSO DO SUL: Aquidauana, 19°15´S 55°58´W, 21 Nov 1989, *A. Pott 5406* (CTES). **Paraguay**— ALTO PARANÁ: 14 km W de Itaquyry, 12 Oct 1995, *A. Schinini & G. Caballero M. 30213* (CTES); Concepción, Paso Horqueta, Río Aquidabán, 19 Oct 1984, *A. Duré 389* (CTES). Cordillera, Valenzuela, 06 Dec 1989, *R. Vanni 1172* (CTES). **Uruguay**— CERRO LARGO: Ruta 8 Km 374, ayo. del Parao, 32°44'34"S, 54°13'14"W, 113 m, 29 Nov 2001, *G. Seijo 2714* (CTES). COLONIA: Punta Gorda, confluencia del Río Uruguay con el Río de la Plata, E. de Rincón de Darwin, 33°54'57"S, 58°24'49"W, 35 m, 24 Nov 2007, *V. Solis Neffa & G. Seijo 2121* (CTES).

Cephalanthus natalensis Oliv. **Malawi**. Southern Mt. Mulanje - Big Ruo Valley, 1800 m, 3 Nov 1988, *J.D. Chapman & E.G. Chapman 9390* (K, MO). **South Africa**. Nkangala, 12 Jun 1932, *J.C. Smuts 21* (K); 2 miles N of Potholes, 6 Mar 1968, *O.A. Leistner 3261* (K). TRANSVAAL: Barbestón, 2 Oct 1922, *F.A. Rogers 21605* (K). Soutpansberg, 9 Jul 1937, *J.C. Smuts 3283* (K). Wavervol ouder, 1 Oct 1979, *Ruprossev 1227* (K). Sabie, Langverwag, 24 Ago 1963, *W.J. Louw 2836* (AAU). **Tanzania**. Mbeya, Rungwe Livingstone Mountains; foot trail from Bumbigi on steep ridge top N of Isalala River, 9°11'S, 33°52'E, 1900 m, 3 Mar 1991, *R.E. Gereau & C.J. Kayombo 4174* (MO).

Cephalanthus occidentalis L. **Canada**— OTTAWA: Pontiac Station, 30 miles west Hull, north shore of Ottawa river, 28 Ago 1948, *A.J.J. Breitung 7327* (SI) — QUÉBEC: Rigaud, comté de Vaudreuil, 26 Jul 1934, *E. Roy 3337* (SI). USA— ARIZONA: Maricopa Co., 628 m, 6 Jul 2012, *L. Markings & L. Butler 3959* (ASU, SI); idem, Bosque Nacional de Tonto, 33°47.93'N, 11°29.49'W, 25 Jun 1999, *L. Landrum 9539* (CTES) — ILLINOIS: Jackson Co., 37°35'08"N, 89°27'44"W, 110 m, 21 Oct 2014, *M.H. Nee 61696* (MO, SI) — MISSOURI: Saint Louis City, Missouri Botanical Garden's Monsanto Research Center campus, by southwest corner of building, south of chiller equipment, 06 Sep 2017, *C.M. Taylor 12977** (CTES, MO). — NEW JERSEY: Burlington Co., 23 Jul 1981, *L. Ley 42199* (CTES). New York, Bronx, 40°52'03"N, 73°52'34"W, 16 m, 6 Jun 2006, *M.H. Nee 54470* (SI). — TEXAS: Aransas Pass Co., on N. Mc Campbell Road, between Rabbit Road and Jacoby Lane, 3 Jun 1998, *P.A. Fryxell 5137* (CTES); Comanche Co., Near Cache, 25 Jun 1913, *G.W. Stevens 1319* (SI). — WISCONSIN: Richland Co., 210 m, 14 Jul 1978, *M. H. Nee 16308*

(SI); idem, 2 miles NW OF Blue River, floodplain of the Wisconsin River, along the channel (usually with flowing water) flowing into Garner Lake, 43°11'55"N, 90°36'00"W, 204 m, 27 Sep 2014, *M.H. Nee 61662* (SI).

Cephalanthus salicifolius Bonpl. **El Salvador**. — MORAZÁN: Río Sapo, area burnt ca. 20 years prior, 13°55'47"N, 88°06'01"W, 678 m, 25 March 2002, *A.K. Monro 3808* (MO). **Honduras**. El Paraíso, Yuscarán, Aldea El Rodeo, quebrada Las Zarcas, road to Oropolí, 13°53'06"N, 86°46'47"W, 420 m, 3 May 2000, *A. Molina R. et al. 34926* (MO). **México**. — HIDALGO: *Jojutla*, 31 Ago 1902, *C.G. Pringle 9822* (SI). —NAYARIT: San Blas, 3,8 Km al SE de Tecuitata sobre camino a tierra El Cora, carr. Jalcocotan-Miramar, 21°26´16"N, 105°08´13"W, 10 Nov 1994, *G. Flores F. & O. Téllez V. 3435* (CTES). — MORELOS: Cuernavaca, 16 Jan 1901, *C.G. Pringle 8474* (SI). —COLIMA: Ruta 110, Colima a Pihuamo, Km 215 a Río Salado, 19°11'32"N, 103°41'45"W, 300 m, 18 Jan 1982, *D.H. Lorence et al. 3798* (MO).

Cephalanthus tetrandrus (Roxb.) Ridsdale & Bakh. f. **India**. [Uttar Pradesh], Pilibhit, 25 May 1898, *K. Inayat 23668* (K); [Uttar Pradesh], [Lakhimpur] Kheri, idem, 24 Jun 1900, *K. Inayat 22377* (K). Assam, without precise locality, *s. col.*, (K000265513); "M. Sillet", 1832, *N. Wallich s.n.* (K001067160); Katya, Keshampur, South Kheri, 17 May 1920, *S. Ram s.n.* (K); without precise locality, *s. col.* (K000265509). **China**. Yizhang, Hunan, in plain, by river side, 15 Jul 1942, *S.H. Chen 1896* (AAU), locality and collector in Chinese language, 19 May 1983, collection number *323* (AAU).

Subtribu Mitragyninae

Mitragyna inermis (Willd.) Kuntze. **Douna**. near River Bani, 45 km SE of Segov. 270 m. 17 Jan 1977. s.n. Loutfy Boulos. **Senegal**. TANLICOUNDA. Niokolo-Kolia. 05 Sep 1963, *Jaques 8822*. **Senegal**. Dahra – Linguere. 15°20'W, 15°25'N. 8 Jun 1989, *Tybirk 41* (MO).

Mitragyna stipulosa (DC.) Kuntze. **Cameroun**. 10 km S. of Ngaounderé, Station Fouragére Wakwa, alt. 1100 m. Grovem creek bank, 31 Jan 1966, *Leeuwenberg 7625*. — **Liberia**: NIMBA. Harbel, behind botanical reaerch garden Firestone. 18 Feb 1969, *Jansen 1555*. Sierra Leona. Kabala. Loma Mansonia. 16 Feb 1966, *Jacques-Georges 23743*. Manovo-Gounda St Floris National Park. 13 km S of Pende Koumbala confluence on small tributary that branches E off Pende Creek. 8°19' N, 21°14' E. elev. 630 m; prec. 1350mm, 23 Mar 1984, *Fay 6530* (MO).

Mitragyna rubrostipulata (K. Schum.) Havil. **Burundi**. — BURURI. Kumuyange. 3°58' S 29°45' E. alt. 1980 m. 26 Sep 1971, *Lewalle 6107*. Muyange. Alt. 2000 m. 26 Sep1971, *Reekmans 1004*. Deutsch-ost, África, 12 Jul 1915, *Peter 55762*. — TANZANIA Bukoba Rural District. 01°05' S 31°33' E. 1130 m. 28 Nov 1999, *Gereau et al. 6354* (MO).

Subtribu Adininae

Adina pilulifera (Lam.) Franch. ex Drake. **China**. — Hunan. Alt.350 m. 10 Jul 2004, Duan *Lindong 2409*. Zhejiang. 25 Sep 1998. Hu ZJ 62, 16 Jun 1957, *Chen Dezhao 232* (MO).

Lukedia bernardoi (Merr.) Ridsdale. **Filipinas**. —SAMAR. Apr 1914, *Ramos 1707*. Aug 1916 *Wenzei 1661*(MO).

Neonauclea calycina (Bartl. ex DC.) Merr. — PALAWAM. Municipality Puerto Princesa, Irawan Impapaí forest behind BFD field station 9°50'N, 118°38'E. 14 Mar 990. *Soejarto & Madulia 6772*, Dec 1976, *Whales 16777*. —MALASIA. Pahang, 8 Jul 1979, *Sider 28746*. Sn. *16777* (MO).

Pertusadina hainanensis (F.C. How) Ridsdale. **China**. Hunan, alt. 700 m. 4 Aug 2004, *Lin Qinzhong s.n.* (MO).

Subtribu Breoniinae

Breonadia salicina (Vahl) Hepper & J.R.I. Wood. **Madagascar**—ANTSIRANANA: Fivondronana 13°35'44" S 50°00'04" E, alt. 60-100 m., 30 Mar 2001, *Ranaivojaona et al. 363*.

—MALAWI: foot of mt. Mulanje at the Likhubula River. 900 m. 29 Dec 1985, *Chapman 7001*. **Mozambique**. —MAPUTO: Namaacha. Estrada para Matianine, junto à linha da agua, 27 Jan 1981, *Koning et al. 8640*. **Tanzania**. — IRINGA: Lukosi Valley at base of kitonga gorge. Riverine Woodland. 07°40' S 36°10' E, 16 Feb 1988, *Lovett & Congdon 3113* (MO).

Breonia macrocarpa Homolle. **Madagascar**. Taomasina. 19°09'36'' S 48°33'50'' E, alt. 612 m. 24 Nov 2004, *Ranaivojoana & Razanatsima 934*. 19°09'27'' S 48°35'23'' E, alt. 620 m. 10 Oct 2005, *Randrianasolo et al. 1052* (MO).

Breonia madagascariensis A. Rich. ex DC. **Madagascar**. —TAOMASINA: Mantady National Park. 18°49'33'' S 48°26'04'' E, alt. 1110 m. *Birkinshaw et al. 310* (MO).

Subtribu Corynantheinae

Corynanthe mayumbensis (R.D. Good) Raym.-Hamet ex N. Hallé. **Gabón**.— OGOOUÉ-MARITIME: Rabi, near rabi 22. 1°54' S, 9°52' E. alt. 40 m. 14 May 1994, *Van der Burgt 105*. GABÓN OGOOUÉ-LOLO: East of Lastoursville, near Bambidie, C.E.B. chantier. Tall forest. 0°49' S 13°08' E. alt 250 m. 20 Sep 1996, *McPherson 16645*. GABÓN OGOOUÉ-LOLO. Foret des Abeilles, Makandé, just NE of campsite. Primary forest. 0°40.8' S, 11°54.8' E, alt. 250 m. 12 Jan 2001, *Wieringa 4083*. Gabón , Estuaire, au platform de forage, Remboué 1. Foret primaire, bord d'un ruisseau. 0°13' S, 10°02' E, 21 Jan 1991, *Louis 3284* (MO).

Corynanthe pachyceras K.Schum. **Cameroon**. —Station du Cacaoyer de N`Koemvone, 14 km on the road from Ebolowa to Ambam. Low hill with more or less original forest on Souther border of plantation. 2°49' N, 11° 08' E, 13 May 1975, *Wilde 8168*. **Liberia**. Yekepa. 8 Oct 1969. *Jacques-Georges 25096*. Southwest Province, Meme Division. 4°38' N, 9°25' E. elev. 200 m. Scrub and disturbed forest around Kumba. 15-20 Dec 1985. D.W., *Thomas et al. 5218* (MO).

Corynanthe paniculata Welw. **Kongo**. Lukula. Luki. 30-III. *s.n*. Surroundings of Kodjina, 15 km south west of Abengourou, 29 Jul 1969, *Amshoff 581*. Zaire. Bena Longo. Alt. 535 m, 25 May 1958, *Dechamps 92* (MO).

Pausinystalia johimbe (K. Schum.) Pierre ex Beille. **Cameroon**. Kribi-Campo road in HFC logging concession, On skid trail in recently-logged forest, 3 Dec 1997, *Sunderland 1893/1894*. **Gabon**. Ngounié, along a forestry road W of Bembodié. Roadside near primary forest. 1°28.8' S, 10°28.4' E. alt. 170 m. 27 Oct 1994, *Wieringa 2931* (MO).

Subtribu Uncariinae

Uncaria africana G. Don. **Cameroon**. 21 Jan 1969. Bos 3701. GABÓN, Ogooué-Maritime, 22.6 km from Central Station, direction Echira. 2°05' S, 9°48' E, 31 Oct 1990, *Van Nek 158*. **Liberia**—NIMBA: Mt Gangra Forest edge. 7°33.30' N, 8°37.78' W. 785 s.n.m. 11-I-2009, *Jongkind 8393*. **Nigeria**.— IJEBU. Bay the wayside from Omo sawmill to Etemi Forest Reserve, 21 Sep 1968, *Charter & Daramola 61574* (MO).

Uncaria rhynchophylla (Miq.) Miq. ex Havil. **China**. 3 Jun 1956, *Ap 1247*. 10 Aug 1995, *Ye Cun-su 499* (MO).

Subtribu Naucleinae

Nauclea latifolia Sm. **Cameroon**. 5 km S of Banyo, near road to Mayo Darlé. Alt 1080 m. Woodland. 4 Jul 1972. *Leeuwenberg 10127 (MO).*

Nauclea orientalis (L.) L. **Ceylán**. Thunmodera Polonnaruwa District. North CentralProvince, 7 Jun 1974, *Waas 579*. Queenstalnd. Brinsbane Botanic Garden, 23 Dec1959, *Thorne 25362*. Kathiraveli ad occidentem usque ad Manicka-Kulan, in nemore vel in fruticetis, Batticoloa regione. Alt. 30 m. 4 Nov 1975, *Bernardi 15671* (MO).

Neolamarckia cadamba (Roxb.) Bosser. **Tailandia**. —CHIANG MAI: Ban Pa Deng along the Mae Lie-Bai Highway, Bah Bae Subdistric, alt. 900 m., 15 Jun 1992, *Larsen et al. 43433*. 06 Jan

1990, *Maxwell 90-35*. —KRABI. Tham Bok Koroni nat. res. at Ao Luk. 98°45' E 08°35' N. alt 60 m. Nakhon Nayok. Kung. Kew Yai National Park, Hin Dahng Subdistrict, training centro, c. 3 km. south of Maw Sing Dto Reservoir, alt.750 m. 26 May 2001, *Maxwell 01-277* (MO).

Sarcocephalus latifolius (Sm.) Bruce. **Estados Unidos**. —FLORIDA: Martin, 14 Jul 1976, *Kral 58588*. **Gabon**. Around Doussala, in low secondary vegetation. Alt. 200 m. 25 Mar 1988, *Wilde & Jongkind 9570*. **Madagascar**. Savanna between Cosrou and Nandibo, east of Bandama River, alt. 60 m. *Fosberg 40606*.

How to cite

ROMERO MF, SALAS RM & GONZALEZ AM. 2022. Colleters in *Cephalanthus* (Rubiaceae) and reevaluation of occurrence of corolline colleters in Angiosperms. An Acad Bras Cienc 94: e20201975. DOI 10.1590/0001-3765202220201975.

Manuscript received on December 29, 2020;
accepted for publication on December 20, 2021

MARÍA FLORENCIA ROMERO[1]
https://orcid.org/0000-0003-4780-3401

ROBERTO MANUEL SALAS[1,2]
https://orcid.org/0000-0001-7799-9017

ANA MARIA GONZALEZ[1,3]
https://orcid.org/0000-0002-9311-0967

[1]Instituto de Botánica del Nordeste, Consejo
Nacional de Investigaciones Científicas y Técnicas,
Universidad Nacional del Nordeste , Sargento Cabral
2131, C. C. 209, C. P. 3400, Corrientes, Argentina

[2]Facultad de Ciencias Exactas y Naturales y Agrimensura,
Universidad Nacional del Nordeste, Avenida
Libertad 5470, C. P. 3400. Corrientes, Argentina

[3]Facultad de Ciencias Agrarias, Universidad Nacional del
Nordeste, Sargento Cabral 2131, C. P. 3400, Corrientes, Argentina

Correspondence to: **María Florencia Romero**
E-mail: mariafloromero@gmail.com

Author contributions

MFR and RMS collected material from the field and consulted the herbaria cited in the Appendix. All images used on the manuscript were taken and analyzed by MFR and AMG. Anatomical observations were interpreted by MFR and AMG.