Colleters in *Casearia* (Salicaceae): a new interpretation for the theoid teeth

VALÉRIA F. FERNANDES[1], MARCELA THADEO[2], VALDNÉA C. DALVI[3], RONALDO MARQUETE[4,5] and RENATA M. S. A. MEIRA[1]*

[1]*Programa de Pós-Graduação em Botânica, Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Av. P.H. Rolfs, s/n, Campus Universitário, CEP: 36.570-000 Viçosa, Minas Gerais, Brazil*
[2]*Departamento de Biologia, Universidade Estadual de Maringá, Av. Colombo, 5790, Jardim Universitário, CEP 87.020-900 Maringá, Paraná, Brazil*
[3]*Instituto Federal de Educação, Ciência e Tecnologia Goiano – Câmpus Rio Verde, Rod. Sul Goiana Km 01, Zona Rural, Caixa Postal 66, CEP: 75.901-970, Rio Verde, Goiás, Brazil*
[4]*Instituto Brasileiro de Geografia e Estatística, Av. República do Chile 500, 7° andar, CEP: 20.031-170 Rio de Janeiro, Brazil*
[5]*Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rua Pacheco Leão, 915, CEP: 22.460-030 Rio de Janeiro, Brazil*

Salicaceae possess a range of leaf teeth types, many characterized as nectariferous salicoid or violoid teeth. One large genus, *Casearia*, deviates from this generalization in having theoid teeth. Although taxonomic descriptions of *Casearia* have included reports of glands at the apex of teeth, there have been no descriptions of their anatomy or functional role. Here, we aim to describe the anatomy of the theoid teeth of 43 *Casearia* spp. Leaf samples from herbarium specimens were processed for light and scanning electron microscopy. Leaves of *C. sylvestris* were collected and fixed in the field for histochemical tests, revealing only polysaccharides in secretory epidermal cells and outside of these cells. The glands have precocious development and are composed of a non-vascular central axis covered with a palisade-like secretory epidermis. During senescence, the cells of the palisade-like secretory epidermis and central axis appear to lose their typical form, with some cell walls disintegrating. In the mature leaf, an abscission zone at the base of the gland causes detachment of the gland. The placement, anatomical structure, precocious activity and polysaccharide secretion (in *C. sylvestris*) allow the recognition of the glands associated with the marginal teeth in *Casearia* as colleters. Similar studies are needed for other genera in order to clarify how colleters have evolved in Salicaceae and how they are functionally and anatomically related to other teeth types in the family. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, **181**, 682–691

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INTRODUCTION

Casearia Jacq., with almost 180 species, is one of the largest genera of Salicaceae. About 75 species are present in the tropical and subtropical areas of the Americas (Sleumer, 1980) and 21 of these are endemic to Brazil (Marquete, 2010). The genus is organized into six sections [*Crateria* Benth., *Guidonia* (A.DC.) Eichler, *Endoglossum* Sleumer, *Gossypiospermum* Griseb., *Piparea* (Aubl.) Benth. and

Casearia]. Section *Casearia* is subsequently organized into six informal groups (Ilicifoliae, Nitidae, Singulares, Arboreae, Aculeatae and Decandrae; Sleumer, 1980). Traditionally, this genus has been included in Flacourtiaceae, but, based on molecular data, it was transferred to a broadly circumscribed Salicaceae (Chase *et al.*, 2002; APG III, 2009; APG IV, 2016) or a narrowly circumscribed Samydaceae (Wurdack & Davis, 2009; Xi *et al.*, 2012).

Salicaceae *s.l.* are characterized by the absence of cyanogenic glycosides (with the exception of *Banara* Aubl.) and the presence of salicin phenolic

*Corresponding author. E-mail: rmeira@ufv.br

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compounds (Chase *et al.*, 2002), seeds often arillate, the presence or absence of nectariferous discs or glands in the ovary and salicoid teeth on the margin of the leaves of most genera (Judd *et al.*, 2007). Various types of leaf teeth have been reported in Salicaceae, described as nectariferous salicoid or violoid teeth (Alford, 2005). The presence of salicoid teeth was the only morphological synapomorphy proposed for Salicaceae, but it is absent in *Casearia* (Judd *et al.*, 2007) and closely related genera, including *Laetia* Loefl. ex L. and *Ryania* Vahl, which also occur in Brazil (Alford, 2005).

Salicoid teeth are characterized by the presence of a spherical callosity fused to the tooth apex, are non-deciduous and appear as a non-opaque dark seta (Hickey & Wolfe, 1975; Metcalfe & Chalk, 1979; Judd *et al.*, 2007). Several vascular bundles merge and branch towards a salicoid tooth, forming excessive vascularization (Thadeo, Azevedo & Meira, 2014). Violoid teeth have a medial vein running to the apex, where it expands into an opaque glandular termination, without a deciduous apical seta (Hickey & Wolfe, 1975). *Casearia* deviates from this generalization for Salicaceae in having theoid teeth (Judd *et al.*, 2007), in which the medial vein of the tooth runs to the apex, and expands and becomes congested with opaque material, so that the tooth apex is capped by an opaque deciduous seta (Hickey & Wolfe, 1975).

The presence of 'glands' on the apex of leaf margin teeth in *Casearia* has been reported by several authors (Sleumer, 1980; Marquete, 2010; Marquete & Mansano, 2012, 2013). These descriptions were based on fresh samples observed in the field and herbarium samples analysed with a stereomicroscope, and are thus limited to general morphology. The glands present on the leaf base and margin of species of other genera of Salicaceae have been anatomically and/or functionally described and characterized as 'like colleters' in *Dovyalis hebecarpa* (Gardner) Warb. (Wilkinson, 2007), extrafloral nectaries (EFNs) in *Populus grandidentata* Michx. (Curtis & Lersten, 1978), *Populus trichocarpa* Torr. & A.Gray ex Hook. (Escalante-Pérez *et al.*, 2012) and *Prockia crucis* P.Browne ex. L. (Thadeo *et al.*, 2008), and resin glands in *Salix lucida* Muhl. (Curtis & Lersten, 1980) and *Populus deltoides* W.Bartram ex Marshall (Curtis & Lersten, 1974). The theoid teeth of six *Casearia* spp. were anatomically described as non-epithem hydathodes (Thadeo *et al.*, 2014).

Hydathodes, EFNs and colleters are secretory structures that are present on leaf margins in some species (Curtis & Lersten, 1974, 1978; Fahn, 1979; Thadeo *et al.*, 2008; Coutinho, Valente & Meira, 2010; Mercadante-Simões & Paiva, 2013). The main parameters for distinguishing such structures are the morphoanatomical description, the functional role and the chemical nature of the secreted compounds. Hydathodes secrete water exuded by an epidermal pore (Elias & Gelband, 1977; Fahn, 1979). EFNs produce a sugar solution (nectar) exuded by an epidermal pore or secretory epidermis, exhibit great morphoanatomical variation and are distributed widely on plants (Fahn, 1979; Nicolson & Thornburg, 2007). EFNs occur on the rachis, petiole and adaxial face of the leaf (Fahn, 1979) and leaf margin (Thadeo *et al.*, 2008; Rocha *et al.*, 2009; Coutinho *et al.*, 2010). Colleters produce a sticky secretion that functions as a lubricant, protecting meristems and developing organs against desiccation and attack by pathogens (Thomas, 1991; Mayer, Cardoso-Gustavson & Appezzato-Da-Glória, 2011; Mayer, Carmello-Guerreiro & Mazzafera, 2013). Colleters occur on the adaxial surface of young reproductive and (or) vegetative organs in several families of eudicots (Fahn, 1979; Thomas, 1991; Klein *et al.*, 2004; Martins, 2012; Coelho *et al.*, 2013; Dalvi *et al.*, 2014; Lopes-Mattos *et al.*, 2015) and only Orchidaceae among monocots (Leitão & Cortelazzo, 2008; Mayer *et al.*, 2011). Recently, colleters have also been reported at the leaf margins for some species (Paiva, 2012; Mercadante-Simões & Paiva, 2013; Vitarelli *et al.*, 2015; Feio, Riina & Meira, 2016). Therefore, anatomical studies and histochemical tests of *Casearia* are necessary to identify the type of gland that is associated with the teeth and to determine the chemical composition of the secretion, respectively. The aim of this work was to characterize the theoid teeth of *Casearia* anatomically by studying species of all six sections (*sensu* Sleumer, 1980).

MATERIAL AND METHODS

Samples of entire mature and/or young leaves (from the first or second node) for 43 species of all six sections of *Casearia* (*sensu* Sleumer, 1980) were collected from herbarium material (Appendix). The samples were rehydrated (Smith & Smith, 1942), and then dehydrated in an ethanol series and stored in 70% ethanol.

To describe the structure of the glands on the margin of leaf teeth, whole or fragmented leaves were cleared, stained with 50% alcoholic fuchsin, according to Vasco *et al.* (2014), and mounted in glycerin jelly (Johansen, 1940). Fragments of leaf with glands were selected from the leaf margin using a stereomicroscope (Stemi 2000-C®, ©Carl Zeiss Microscopy GmbH, Jena, Germany), embedded in methacrylate resin (Historesin Leica; Leica Microsystems, Heidelberg, Germany) and sectioned using an automatic

rotary microtome with glass knives (Leica RM2265, Deerfield, IL, USA). Longitudinal and cross-sections, 5 μm thick, were stained with toluidine blue at pH 4.7 (O'Brien & Mccully, 1981) and mounted on slides with resin (Permount; Fischer Scientific, Fair Lawn, NJ, USA).

Samples of stem apical meristem and leaves in different stages of development of *Casearia sylvestris* Sw. were collected in the Flona de Paraopeba National Park (Paraopeba, MG, Brazil) to describe secretion and to perform histochemical tests. These samples were fixed in FAA (formaldehyde, acetic acid, 50% ethanol, 1 : 1 : 18 by volume) for 48 h under vacuum and stored in 70% ethanol (Johansen, 1940) prior to being embedded and sectioned as described above. The histochemical tests included xylidine Ponceau for total proteins (O'Brien & Mccully, 1981) and PAS (periodic acid Schiff's reagent) for total polysaccharides (McManus, 1948). Some samples were sectioned on a table microtome (LPC model; Rolemberg and Bhering Trade and Import LTDA, Belo Horizonte, Brazil) and tested with Sudan IV for the detection of total lipids (Pearse, 1980).

Imaging was performed using an Olympus AX70TRF light microscope (Tokyo, Japan) equipped with a U-Photo System and an AxioCam HRc digital camera (Zeiss, Göttingen, Germany).

For micromorphological analysis, samples stored in 70% ethanol were dehydrated in an ethanol series and subjected to critical point drying using CO_2 (CPD 020; Bal-Tec, Balzers, Liechtenstein). The samples were then fixed on stubs and sputter coated with gold (FDU 010; Bal-Tec). Observations were performed using a Leo 1430 VP scanning electron microscope (Zeiss, Cambridge, UK) at the Center for Microscopy and Microanalysis at Universidade Federal de Viçosa (UFV), Minais Gerais, Brazil.

RESULTS

Of the 43 species examined, only *C. eichleriana* Sleumer had leaves with entire margins (Fig. 1A, B). The remaining 42 species had leaves with marginal theoid teeth, as illustrated by *C. mestrensis* Sleumer and *C. altiplanensis* Sleumer (Fig. 1C, D). These theoid teeth may have two portions: a basal non-glandular portion and a glandular apical portion. The basal non-glandular portion may present variable length (Fig. 1C–F), reaching up to twice the length of the glandular portion, or may be absent (Fig. 1G). Variations in the length of the non-glandular portion were noticed among different specimens and among different leaf regions.

In the leaf primordia and young leaves (Fig. 1C), the glandular portion is present, whereas, in mature leaves, a darker region at the non-glandular portion shows the abscission zone of the senescent gland (Fig. 1D–F). As a result of the abscission of the glandular portion, scars can be observed on the marginal tooth (Fig. 1H).

Glands in *C. sylvestris* differentiate precociously, as observed in the leaf primordium. This differentiation occurs prior to the differentiation of the foliar mesophyll. In this phase of development, the leaf primordium still has the leaf margins folded in a way in which the ventral surface remains protected (Fig. 2A–D) and the dorsal surface maintains contact with the leaves of the following node. The fluid secretion produced by glands flows out, lubricating the primordium and protecting against dehydration. As the leaves expand, the edges unroll and expose the leaf margins with the glands (Fig. 2E, F).

In the first stages of development, the differentiating glands exhibit a secretory epidermis surrounding the central axis of non-secretory cells. The secretory epidermis is covered with a thin cuticle and the cells have a conspicuous medially located nucleus, evident nucleolus and dense cytoplasm (Fig. 2G). The polysaccharide nature of the secretion that accumulates in the subcuticular space and the extravasated secretion was confirmed by the PAS test (Fig. 2H). Pores or a ruptured cuticle were not observed. The histochemical test for protein (xylidine Ponceau) was negative and the test for lipids (Sudan IV) was weakly positive (data not shown).

The glands of young and/or fully expanded leaves are non-vascularized and have central axis cells with thin pectocellulosic walls and secretory palisade-like epidermal cells covered by a thin cuticle (Figs 2B, 3A–C). In *C. rupestris* Eichler, druses were observed in the central axis cells (Fig. 3B). The marginal glands can have a short peduncle (Fig. 3D), corresponding to the non-glandular portion, or can be sessile (Fig. 3E, F).

In mature leaves, some of the glands produced secretion (Fig. 3D, E), whereas others were senescent (Fig. 3G, H) and/or absent as a result of abscission (Fig. 1H). The secretory epidermal cells in the senescent glands had a hyaline cytoplasm and were deformed, some with broken walls (Fig. 3B). In the final phase of senescence, which may precede gland abscission, the epidermal and central axis cells were intensely stained green by toluidine blue (Fig. 3C), indicating the presence of phenolic compounds. At the intersection of the glandular and non-glandular portions of the teeth, a tissue of tabular cells corresponds to the abscission zone (Fig. 3C). In senescent glands, the typical form described for the secretory
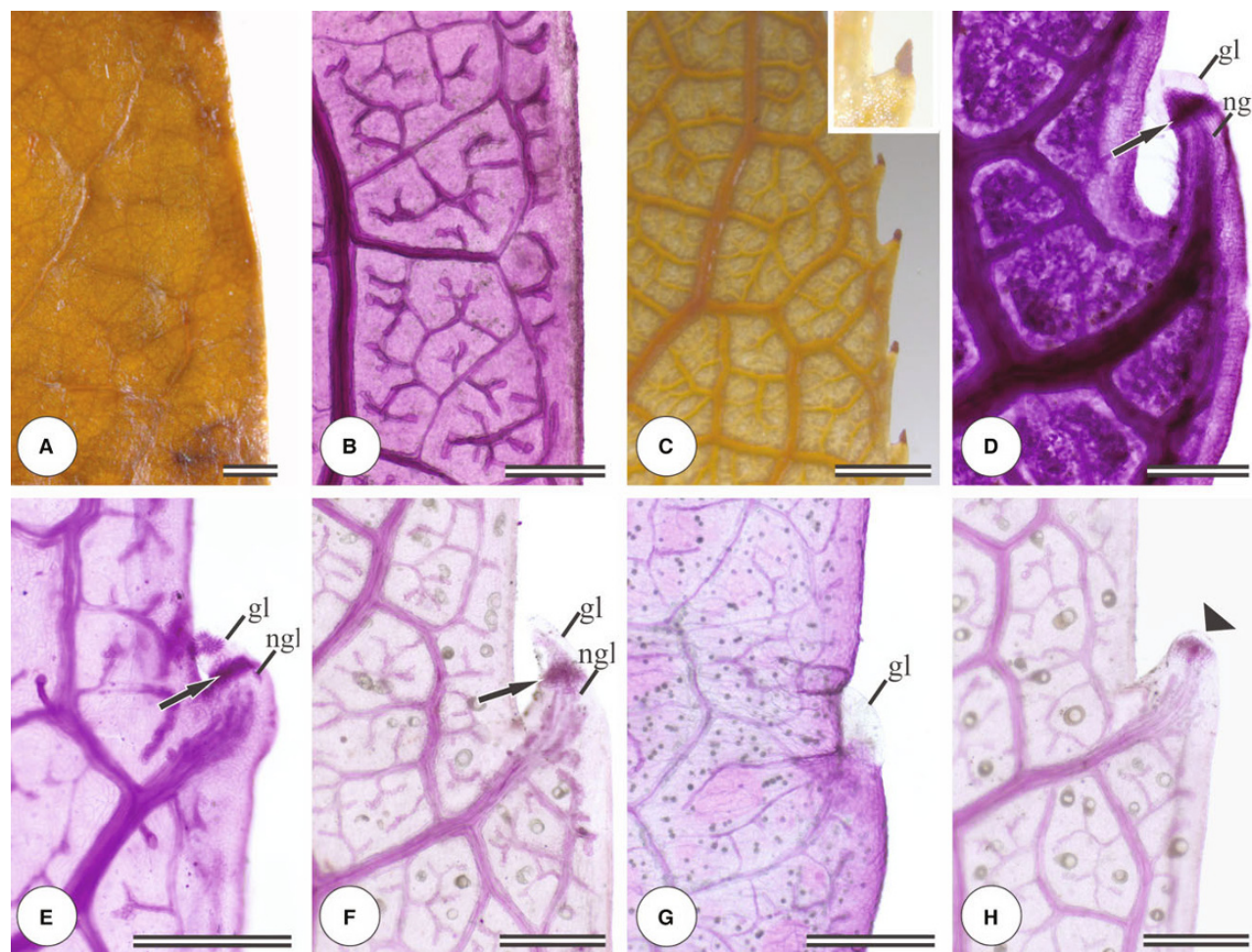
Figure 1. Leaf margins of *Casearia* spp. viewed with a stereomicroscope (A, C) and under light microscopy of cleared samples (B, D–H). A, B, Entire margin of the leaf of *C. eichleriana*. C–G, Glands on the apex of a marginal tooth showing the glandular apical (gl) and non-glandular (ngl) portions. C, Young leaves of *C. mestrensis*. Note the tooth glands. D–G, Senescent glands of *C. altiplanensis* (D), *C. lasiophylla* (E), *C. tremula* (F) and *C. decandra* (G); darker region at the non-glandular portion shows the abscission zone (arrow) of the senescent gland. H, Scars on the marginal tooth formed by the abscission of glands in *C. decandra*. Note the variation in the non-glandular portion (ngl). Bars: A, C, 0.1 cm; B, D–H, 300 μm.

phase is altered, with the surface becoming wrinkled and cells collapsing (Fig. 3G, H).

DISCUSSION

The glands associated with the marginal teeth of leaves of *Casearia* are considered as colleters because of their placement, anatomical characteristics, premature secretory activity and polysaccharide nature, as detected in *C. sylvestris*. The colleters described in this work correspond to the previously described opaque deciduous seta of theoid teeth (Hickey & Wolfe, 1975), as also reported for *Casearia* (Judd *et al.*, 2007). The absence of other previous records of colleters in *Casearia* can probably be explained by samples being limited to fully expanded leaves, as these glands are senescent at this stage and often fall off, leaving just scars that are difficult to interpret. The present study demonstrates the importance of investigating young leaves and the relevance of combining analyses of the chemical nature of secretions with anatomical studies for the correct identification of these marginal glands.

The presence of colleters on leaf margins has been suggested for *Dovyalis hebecarpa*, also Salicaceae, which were called 'colleter-like-glands' (Wilkinson, 2007), because they resembled the 'standard type colleter'. The necessity of studying young leaves to identify marginal glands, as these structures could
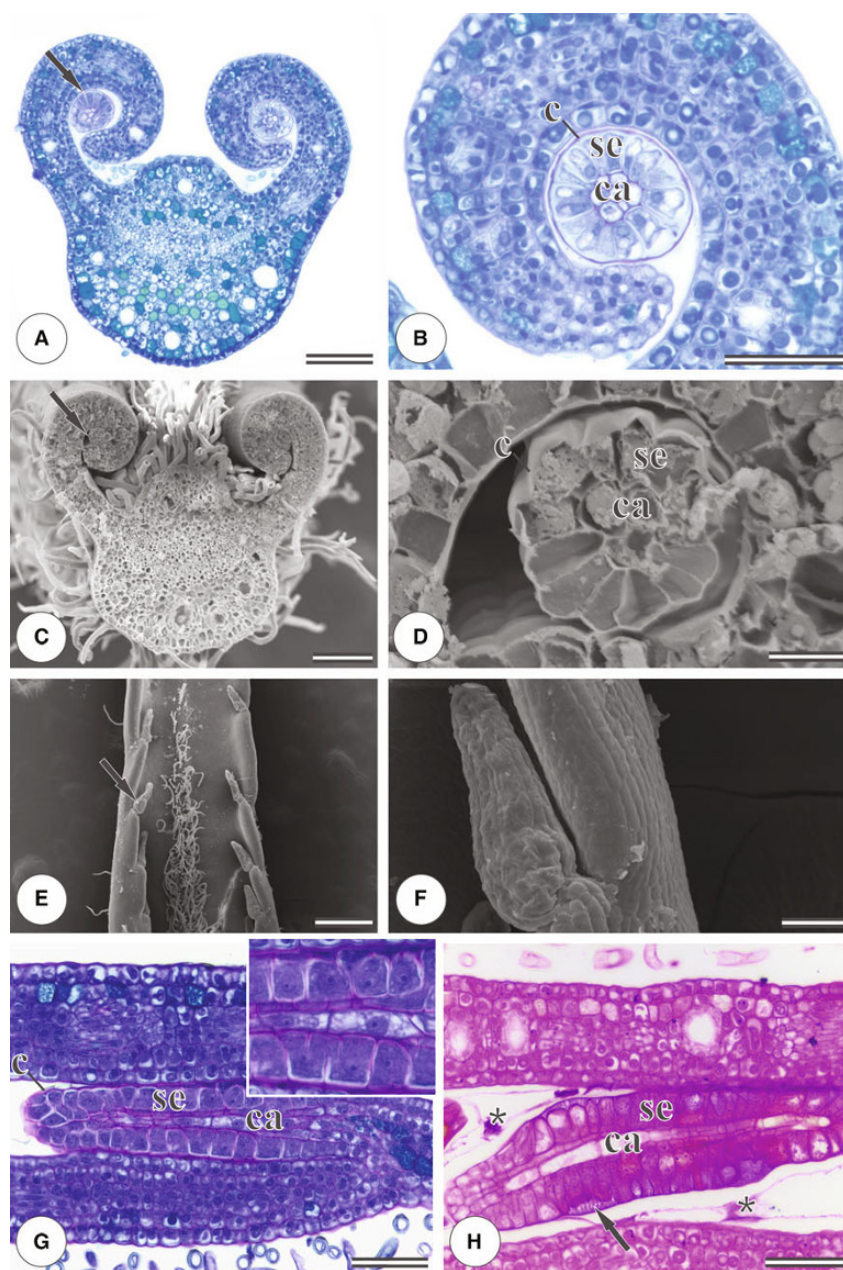
Figure 2. Leaf primordium of *Casearia sylvestris* showing glands under light microscopy (A, B, G and H) and scanning electron microscopy (C–F). Cross-sections (A–D) and longitudinal sections (G, H). A–D, Folded margins showing glands. Note the thin pectocellulosic walls and thin cuticle covering secretory palisade-like epidermal cells. E, F, As the leaves begin to expand, the edges unroll and expose the margin with glands in the apex of teeth. G, Central axis (ca) and secretory epidermal cells (se) of glands with conspicuous nuclei in a medial position. H, Positive reaction to PAS (periodic acid Schiff's reagent) in the secretory epidermis showing secretion in the subcuticular space (arrow) and outside the cell (asterisk). c, cuticle. Bars: A, C, 100 μm; B, F–H, 50 μm; D, 10 μm; E, 300 μm.

necrose or abscise, was reinforced by this author (Wilkinson, 2007). The presence of colleters on the leaf margin has already been recorded for 21 species of *Croton* L., *Astraea lobata* (L.) Klotzch and *Brasiliocroton mamoninha* P.E.Berry & Cordeiro (Euphorbiaceae, Vitarelli *et al.*, 2015), *Croton echinocarpus* Müll. Arg. and *C. urucurana* Baill. (Euphorbiaceae, Feio *et al.*, 2016) and in leaf teeth for some species of *Prunus* L. (Rosaceae, Chin *et al.*, 2013), *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae, Paiva, 2012) and *Tontelea micrantha* (Mart. ex Schult) A.C.Sm. (Celastraceae, Mercadante-Simões & Paiva, 2013).

The leaf teeth of six *Casearia* spp. were described as non-epithem hydathodes (Thadeo *et al.*, 2014). In the present study, we re-analysed these species and were able to verify that, in the young and/or fully expanded leaves, the marginal teeth possessed colleters and not hydathodes. As these colleters are deciduous, previous authors probably observed only the main vein region of the tooth with vasculature exclusively comprising xylem, leading them to interpret the structure as a hydathode. However, a senescent colleter can be seen in an image in Thadeo *et al.* (2014: fig. 1C), which was described as a theoid tooth seta.

Figure 3. Marginal glands in *Casearia* spp. Light microscopy (A–C) and scanning electron microscopy (D–H). A, Non-vascularized gland of *C. aculeata* showing central axis with thin pectocellulosic cell walls, and secretory palisade-like epidermis covered by a thin cuticle. B, Gland in *C. rupestris* with druses (dr) in the central axis; note the collapse of a few of the secretory epidermal cells (black arrow). C, Gland in *C. mestrensis* showing phenolic compounds in central axis (white asterisk) and abscission zone (az). D, Short-stalked gland (white arrow) in *C. luetzelburgii*. E, F, Sessile gland in *C. tremula* and *C. rupestris*, respectively. G, Senescent gland with collapsed cells (black arrow) in *C. mestrensis*. H, Gland surface that has become wrinkled (black arrow) in *C. grandiflora*. az, abscission zone; c, cuticle; ca, central axis; dr, druses; se, secretory epidermis; black asterisk, secretion in glands. Bars: A, B, 50 μm; C, 25 μm; D, 200 μm; E, G and H, 40 μm; F, 60 μm.
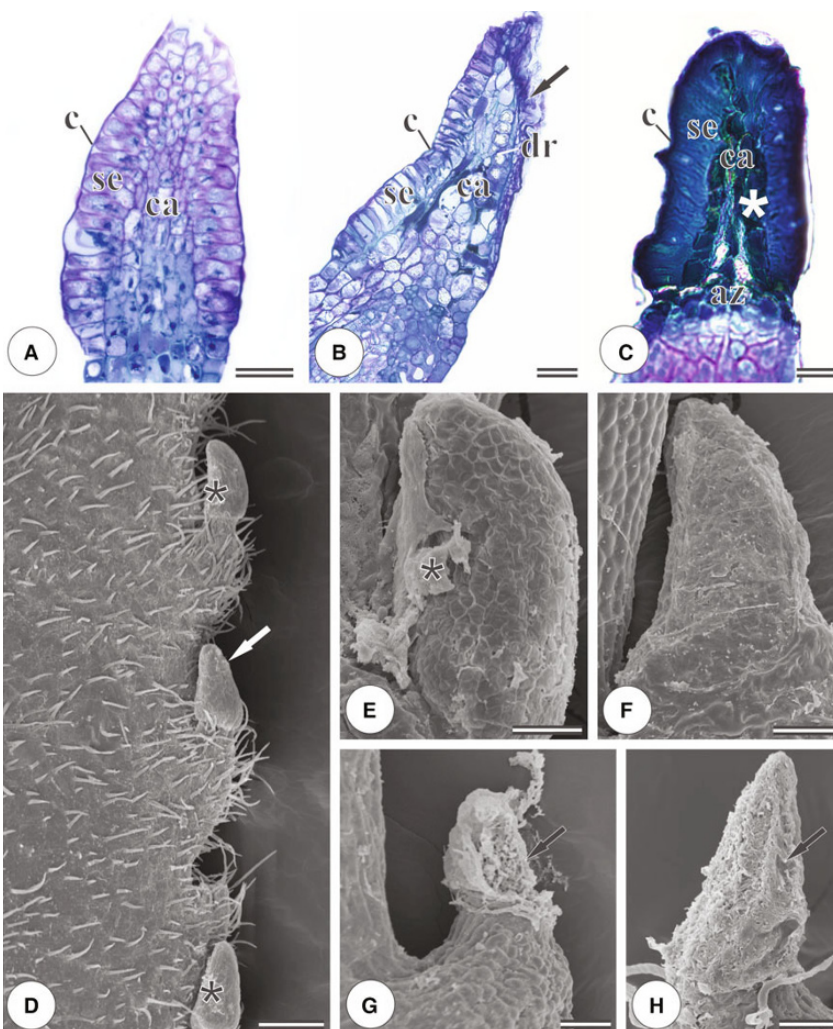
Anatomical studies and chemical analyses of secretion are important tools, especially in combination, for the correct identification and characterization of marginal glands, as demonstrated here. In Salicaceae, for example, there are both resin glands and nectaries in the marginal teeth of *P. grandidentata* and the nectaries differ from the resin glands because they secrete nectar (Curtis & Lersten, 1978).

The colleters of *C. sylvestris* are active in the leaf primordium and the polysaccharide secretion involves the meristematic region and the primordium. These results agree with the known functions of colleter secretion: to help lubricate the vegetative and reproductive meristems and the leaf primordium and to reduce water loss to the environment, protecting the developing organs from dehydration (Thomas, 1991; Mayer *et al.*, 2013).

Colleters in *Casearia* are the standard type according to the classification proposed by Lersten (1974). Such structures have an elongated axis covered by a one-layer palisade-like secretory epidermis. Standard type colleters have been described for other species of Malpighiales (Sheue, Chen & Yang, 2012; Vitarelli *et al.*, 2015). Colleter type and position have been used as taxonomic characters (Thomas, 1991; Klein *et al.*, 2004; Coutinho *et al.*, 2010; Silva *et al.*, 2012; Vitarelli *et al.*, 2015). The present study is the first record of colleters in Salicaceae and their taxonomic potential deserves to be investigated further.

The colleters of all the studied *Casearia* spp. are non-vascularized, indicating that this may be a feature common to the entire genus. Non-vascularized colleters are known from Myrtaceae (Silva *et al.*, 2012), *Chamaecrista* (L.) Moench (Fabaceae, Coutinho, Francino & Meira, 2015), *Caryocar brasiliense* Camb. (Caryocaraceae, Paiva & Machado, 2006) and *Macrocarpaea obtusifolia* (Griseb.) Gilg (Gentianaceae, Dalvi *et al.*, 2014). Vascularized colleters are present in Rubiaceae (Miguel *et al.*, 2006) and Apocynaceae (Martins, 2012). There is no functional

correlation between the presence of vascularization and secretory activity; however, the presence or absence of vascularization can be taxonomically useful (Coutinho *et al.*, 2015).

The presence of calcium oxalate crystals, as seen in *Casearia rupestris* Eichler*,* has also been recorded from *Tontelea micrantha* (Celastraceae, Mercadante-Simões & Paiva, 2013) and *Psychotria nuda* (Cham. & Schltdl.) Wawra (Rubiaceae, Miguel, Moraes & Cunha, 2009). The function of these crystals remains unknown and is controversial; however, several hypotheses have been proposed for these crystals being able to regulate calcium inside the plant, protecting the plant against herbivores and providing plant detoxification (Franceschi & Nakata, 2005).

The cuticle that covers the secretory epidermis of the colleter is smooth and thin, and there is no evidence of pores or other disruptions that are known mechanisms of exudation in colleters, as reported in *Psychotria carthagenensis* Jacq. (Vitarelli & Santos, 2009), *Croton glandulosus* L. (Machado *et al.*, 2015) and *Chamaecrista* spp. (Coutinho *et al.*, 2015).

The morphological features of colleters of *Casearia* at senescence agree with those reported in the literature. The disorganization and collapse of the palisade-like epidermal cells in the central axis were also verified in *Bathysa gymnocarpa* K.Schum. and *B. stipulata* (Vell.) C.Presl. (Miguel *et al.*, 2010), *Alseis pickelii* Pilg. & Schmale (Rubiaceae, Tullii *et al.*, 2013) and *Macrocarpaea obtusifolia* (Dalvi *et al.*, 2014). The formation of an abscission layer was noticed in *Cariniana estrellensis* (Lecythidaceae, Paiva, 2012), *Tontelea micrantha* (Mercadante-Simões & Paiva, 2013), *Dovyalis hebecarpa* (Wilkinson, 2007) and *Macrocarpaea obtusifolia* (Dalvi *et al.*, 2014). Premature activity in colleters is important for the lubrication and protection of young or developing leaves, which then senesce or detach in mature leaves.

The presence of colleters in Malpighiales seems to be common, as they have been reported on the adaxial face of stipules (Paiva & Machado, 2006; Sheue *et al.*, 2012), the margin of leaf primordia and young leaves (Vitarelli *et al.*, 2015) and leaves and the base, margins and apex of stipules and in pistillate and staminate flowers (Feio *et al.*, 2016). The present work demonstrates the presence of colleters in 42 *Casearia* spp. and represents the first record of these structures in Salicaceae. Our data show these structures to be quite common in the genus, and a reinterpretation for the concept of theoid teeth according to Hickey & Wolfe (1975) is presented. The following definition is proposed: theoid teeth occur on leaf margins, are variable in size and are formed of a vascularized non-secretory portion (which can be absent) and a secretory portion which constitutes a colleter. Investigations into the presence of colleters in other genera could address the phylogenetic or functional importance of these structures in Salicaceae.

REFERENCES

Alford MH. 2005. Systematic studies in Flacourtiaceae. PhD Dissertation, Cornell University, Ithaca, NY.

APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161:** 105–121.

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

Chase MW, Zmarzty S, Lledó MD, Wurdack KJ, Swensen SM, Fay MF. 2002. When in doubt, put it in Flacourtiaceae: a molecular phylogenetic analysis based on plastid *rbcL* DNA sequences. *Kew Bulletin* **57:** 141–181.

Chin S-W, Lutz S, Wen J, Potter D. 2013. The bitter and the sweet: inference of homology and evolution of leaf glands in *Prunus* (Rosaceae) through anatomy, micromorphology, and ancestral-character state reconstruction. *International Journal of Plant Sciences* **174:** 27–46.

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.

Coutinho IAC, Francino DMT, Meira RMSA. 2015. New records of colleters in *Chamaecrista* (Leguminosae, Caesalpinioideae *s.l.*): structural diversity, secretion, functional role, and taxonomic importance. *International Journal of Plant Sciences* **176:** 72–85.

Coutinho IAC, Valente VMM, Meira RMSA. 2010. Ontogenetic, anatomical and histochemical study of the extrafloral nectaries of *Sapium biglandulosum* (Euphorbiaceae). *Australian Journal of Botany* **58:** 224–232.

Curtis JD, Lersten NR. 1974. Morphology, seasonal variation, and function of resin glands on buds and leaves of *Populus deltoides* (Salicaceae). *American Journal of Botany* **61:** 835–845.

Curtis JD, Lersten NR. 1978. Heterophylly in *Populus grandidentata* (Salicaceae) with emphasis on resin glands and extrafloral nectaries. *American Journal of Botany* **65:** 1003–1010.

Curtis JD, Lersten NR. 1980. Morphology and anatomy of resin glands in *Salix lucida* (Salicaceae). *American Journal of Botany* **67:** 1289–1296.

Dalvi VC, Cardinelli LS, Meira RMSA, Azevedo AA. 2014. Foliar colleters in *Macrocarpaea obtusifolia* (Gentianaceae): anatomy, ontogeny, and secretion. *Botany-Botanique* **92:** 59–67.

Elias TS, Gelband H. 1977. Morphology, anatomy, and relationship of extrafloral nectaries and hydathodes in two species of *Impatiens* (Balsaminaceae). *Botanical Gazette* **138:** 206–212.

Escalante-Pérez M, Jaborsky M, Lautner S, Fromm J, Müller T, Dittrich M, Kunert M, Boland W, Hedrich R, Ache P. 2012. Poplar extrafloral nectaries: two types, two strategies of indirect defenses against herbivores. *Plant Physiology* **159:** 1176–1191.

Fahn A. 1979. *Secretory tissues in plants*. London: Academic Press.

Feio AC, Riina R, Meira RMSA. 2016. Secretory structures in leaves and flowers of two dragon's blood croton (Euphorbiaceae): new evidence and interpretations. *International Journal of Plant Sciences* **177** [Epub ahead of print].

Franceschi VR, Nakata PA. 2005. Calcium oxalate in plants: formation and function. *Annual Review of Plant Physiology* **56:** 41–71.

Hickey LJ, Wolfe JA. 1975. The bases of angiosperm phylogeny: vegetative morphology. *Annals of the Missouri Botanical Garden* **62:** 538–589.

Johansen DA. 1940. *Plant microtechnique*. New York: McGraw-Hill.

Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ. 2007. *Plant systematics: a phylogenetic approach, 3rd edn*. Sunderland, MA: Sinauer Associates Inc.

Klein DE, Gomes VM, Silva-Neto SJ, Cunha M. 2004. The structure of colleters in several species of *Simira* (Rubiaceae). *Annals of Botany* **94:** 733–740.

Leitão CAE, Cortelazzo AL. 2008. Structural and histochemical characterization of the colleters of *Rodriguezia venusta* (Orchidaceae). *Australian Journal of Botany* **56:** 161–165.

Lersten NR. 1974. Morphology and distribution of colleters and crystals in relation to the taxonomy and bacterial leaf nodule symbiosis of *Psychotria* (Rubiaceae). *American Journal of Botany* **61:** 973–981.

Lopes-Mattos KLB, Otuki SAP, Azevedo AA, Pereira ZV, Meira RMSA. 2015. Colleters in 10 species belonging to three tribes of Rubiaceae: morphoanatomical diversity and potential as useful characters for taxonomy. *Botany-Botanique* **93:** 1–10.

Machado SR, Paleari LM, Paiva EAS, Rodrigues TM. 2015. Colleters on the inflorescence axis of *Croton glandulosus* (Euphorbiaceae): structural and functional characterization. *International Journal of Plant Sciences* **176:** 86–93.

Marquete R. 2010. O gênero Casearia Jacq. no Brasil. PhD Thesis, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro/Escola Nacional de Botânica Tropical, Rio de Janeiro.

Marquete R, Mansano VF. 2012. Taxonomic revision of the *Casearia ulmifolia* complex (Salicaceae). *Novon* **22:** 196–206.

Marquete R, Mansano VF. 2013. A new species of *Casearia* (Salicaceae) from Brazil. *Journal of Systematics and Evolution* **51:** 228–229.

Martins FM. 2012. Leaf and calycine colleters in *Odontadenia lutea* (Apocynaceae – Apocynoideae – Odontadenieae): their structure and histochemistry. *Brazilian Journal of Botany* **35:** 59–69.

Mayer JLS, Cardoso-Gustavson P, Appezzato-Da-Glória B. 2011. Colleters in monocots: new record for Orchidaceae. *Flora* **206:** 185–190.

Mayer JLS, Carmello-Guerreiro SM, Mazzafera P. 2013. A functional role for the colleters of coffee flowers. *Annals of Botany* **5:** 1–13.

McManus JFA. 1948. Histological and histochemical uses of periodic acid. *Stain Technology* **23:** 99–108.

Mercadante-Simões MO, Paiva EAS. 2013. Leaf colleters in *Tontelea micrantha* (Celastraceae, Salacioideae): ecological, morphological and structural aspects. *Academie des Sciences, Comptes Rendus, Biologies* **336:** 400–406.

Metcalfe CR, Chalk L. 1979. *Anatomy of the dicotyledons: systematic anatomy of the leaf and stem with a brief history of the subject, Vol. 1, 2nd edn*. Oxford: Clarendon Press.

Miguel EC, Gomes VM, Oliveira MA, Cunha M. 2006. Colleters in *Bathysa nicholsonii* K. Schum. (Rubiaceae): ultrastructure, secretion protein composition, and antifungal activity. *Plant Biology* **8:** 715–722.

Miguel EC, Moraes DG, Cunha M. 2009. Stipular colleters in *Psychotria nuda* (Cham. & Schltdl.) Wawra (Rubiaceae): micromorphology, anatomy and crystals microanalysis. *Acta Botanica Brasilica* **23:** 1034–1039.

Miguel EC, Oliveira MA, Klein DE, Cunha M. 2010. Ultrastructure of secretory and senescence phase in colleters of *Bathysa gymnocarpa* and *B. stipulata* (Rubiaceae). *Revista Brasileira de Botânica* **33:** 425–436.

Nicolson SW, Thornburg RW. 2007. Nectar chemistry. In: Nicolson SW, Nepi M, Pacini E, eds. *Nectaries and nectar*. Dordrecht: Springer, 215–264.

O'Brien TP, Mccully ME. 1981. *The study of plant structure principles and selected methods*. Melbourne: Termarcarphi Pty.

Paiva EAS. 2012. Colleters in *Cariniana estrellensis* (Lecythidaceae): structure, secretion and evidences for young leaf protection. *The Journal of the Torrey Botanical Society* **139:** 1–8.

Paiva EAS, Machado SR. 2006. Colleters in *Caryocar brasiliense* (Caryocaraceae), ontogenesis, ultrastructure and secretion. *Brazilian Journal of Biology* **66:** 301–308.

Pearse AGE. 1980. *Histochemistry: theoretical and applied, Vol. 2, 4th edn*. Edinburgh: Churchill Livingstone.

Rocha DL, Campos SL, Valente VMM, Francino DMT, Meira RMSA. 2009. Morphoanatomy and development of leaf secretory structures in *Passiflora amethystina* Mikan (Passifloraceae). *Australian Journal of Botany* **57:** 619–626.

Sheue C-R, Chen Y-J, Yang YP. 2012. Stipules and colleters of the mangrove Rhizophoraceae: morphology, structure and comparative significance. *Botanical Studies* **53:** 243–254.

Silva CJ, Barbosa LCA, Marques AE, Baracat-Pereira MC, Pinheiro AL, Meira RMSA. 2012. Anatomical characterization of the foliar colleters in Myrtoideae (Myrtaceae). *Australian Journal of Botany* **60:** 707–717.

Sleumer H. 1980. Flacourtiaceae. In: Rogeron CT, ed. *Flora Neotropica. Monograph 22*. New York: The New York Botanical Garden, 1–499.

Smith FH, Smith EC. 1942. Anatomy of the inferior ovary of *Darbya*. *American Journal of Botany* **29:** 464–471.

Thadeo M, Azevedo AA, Meira RMSA. 2014. Foliar anatomy of Neotropical Salicaceae: potentially useful characters for taxonomy. *Plant Systematics and Evolution* **300:** 2073–2089.

Thadeo M, Cassino MF, Vitarelli NC, Azevedo AA, Araújo JM, Valente VMM, Meira RMSA. 2008. Anatomical and histochemical characterization of extrafloral nectaries of *Prockia crucis* (Salicaceae). *American Journal of Botany* **95:** 1515–1522.

Thomas V. 1991. Structural, functional and phylogenetic aspects of the colleter. *Annals of Botany* **68:** 287–305.

Tullii CF, Miguel EC, Lima NB, Fernandes KVS, Gomes VM, Cunha M. 2013. Characterization of stipular colleters of *Alseis pickelii*. *Botany-Botanique* **91:** 403–413.

Vasco A, Thadeo M, Conover M, Daly DC. 2014. Preparation of samples for leaf architecture studies, a method for mounting cleared leaves. *Applications in Plant Sciences* **9:** 1–4.

Vitarelli NC, Riina R, Caruzo MB, Cordeiro I, Aguilar J-F, Meira RMSA. 2015. Foliar secretory structures in Crotoneae (Euphorbiaceae): diversity, structure, and evolutionary significance. *American Journal of Botany* **102:** 1–15.

Vitarelli NC, Santos M. 2009. Anatomia de estípulas e coléteres de *Psychotria carthagenensis* Jacq. (Rubiaceae). *Acta Botanica Brasilica* **23:** 923–928.

Wilkinson HP. 2007. Leaf teeth in certain Salicaceae and 'Flacourtiaceae'. *Botanical Journal of the Linnean Society* **155:** 241–256.

Wurdack KJ, Davis CC. 2009. Malpighiales phylogenetics: gaining ground on one of the most recalcitrant clades in the angiosperm tree of life. *American Journal of Botany* **96:** 1551–1570.

Xi Z, Ruhfel BR, Schaefer H, Amorim AM, Sugumaran M, Wurdack KJ, Endress PK, Matthews ML, Stevens PF, Mathews S, Davis CC. 2012. Phylogenomics and a posteriori data partitioning resolve the Cretaceous angiosperm radiation Malpighiales. *Proceedings of the National Academy of Sciences of the United States of America* **109:** 17 519–17 524.

APPENDIX List of material used. Species of *Casearia* and voucher information

Section: *Taxon*: Voucher (Herbarium code). Herbarium acronyms: CEPEC, Herbário do Centro de Pesquisa do Cacau; IAC, Instituto Agronômico de Campinas; INPA, Instituto Nacional de Pesquisas da Amazônia; NY, New York Botanical Garden; RB, Jardim Botânico do Rio de Janeiro; UEC, Herbário da Universidade Estadual de Campinas; VIC, Herbário da Universidade Federal de Viçosa. Collections fixed in FAA (formaldehyde, acetic acid and 50% ethanol) are indicated by asterisk (*).

Guidonia: *Casearia spinescens* (Sw.) Griseb.: *Kuhlmann 6446* (RB), *Stannard 826 and Araıs* (NY), *Britton 6875* (NY), *Buntriy 6610* (NY), *Curtiss 750* (NY), *León 5056* (NY).

Endoglossum: *Casearia tremula* (Griseb.) Griseb. ex Wright: *Howard 10581* (NY), *Arnoldo-Broeders 3793* (NY), *Buntriy 5103* (NY), *Dugand 6324* (NY), *Gentry and Cuadros 47607* (NY).

Gossypiospermum: *Casearia luetzelburgii* Sleumer: *Farney 4160 and Gomes* (IAC); *Casearia gossypiosperma* Briq.: *Scardino 1015 et al.* (RB), *Silva and Barbosa 4865* (RB), *Thomas et al. s.n.* (CEPEC), *Duarte and Chiregatto 104* (IAC); *Casearia praecox* Griseb.: *Lack, 5971* (NY), *Stevens 22930* (NY), *Burger and Gentry Jr. 9168* (NY).

Crateria: *Casearia obliqua* Spreng.: *Carvalho s.n.* (CEPEC), *Hoehne s.n.* (CEPEC), *Carvalho s.n.* (RB), *Marquete 4147 et al.* (RB), *Farney 4313 and Gomes* (IAC); *Casearia selloana* Eichler: *Paula 693* (RB), *Macedo 865 and Souza* (IAC); *Casearia sylvestris* Sw.: **Fernandes and Pereira s.n.* (VIC), **Fernandes and Pereira s.n.* (VIC), *Lobão 272 et al.* (RB), *Bovini et al. 802* (RB).

Piparea: *Casearia commersoniana* Cambess.: *Peixoto et al. 955* (RB), *Bovini et al. 1844* (RB), *Peixoto et al. 354* (RB), *Lima et al. 2196* (CEPEC), *Santos and Alves 220* (CEPEC), *Marquete et al. 3404* (IAC); *Casearia javitensis* Kunth: *Lopes 872 et al.* (CEPEC), *Carvalho et al. 1479* (CEPEC), *Kollmann et al. 4079* (RB), *Pereira 49 and Cardoso* (RB), *Silva et al. 8532* (IAC), *Torres et al. 1927* (IAC), *Bernacci et al. 4611* (IAC); *Casearia spruceana* Benth. ex Eichler: *Pires 420* (IAC), *Amaral 718 et al.* (INPA).

Casearia: *Casearia aculeata* Jacq.: *França et al. 3560* (CEPEC), *Pott et al. 8880* (RB), *Heringer et al. 2305* (RB), *Bernacci et al. 790* (IAC), *Bertoni s.n.* (IAC); *Casearia altiplanensis* Sleumer: *Silva et al. 5247* (RB), *Marquete and Mendonça 2848* (RB), *Duarte et al. 185* (VIC); *Casearia arborea* (Rich.) Urb.: *Mori and Funch 13372* (CEPEC), *Thomas et al. s.n.* (CEPEC), *Braga 1226* (RB), *Lobão et al. 1309* (RB), *Filardi et al. 814* (RB), *Torres et al. 1937* (IAC); *Casearia bahiensis* Sleumer: *Fiaschi et al. 2325* (CEPEC), *Mori et al. 12076* (CEPEC), *Folli*

3512 (RB), *Folli 4360* (RB); *Casearia combaymensis* Tuslane: *Granville and Hoeselc s.n.* (NY), *Palacios 1405* (NY), *Mello 3288* (INPA); *Casearia corymbosa* Kunth: *Smith 122* (NY), *Ortiz 436* (NY); *Casearia decandra* Jacq.: *Pereira 1156* (CEPEC), *Forzza et al. 4356* (CEPEC), *Jardim et al. 2849* (CEPEC), *Lobão 1658 et al.* (RB), *Marquete 3377 et al.* (RB), *Torres s.n.* (IAC); *Casearia duckeana* Sleumer: *Albuquerque 67-46* (INPA); *Casearia eichleriana* Sleumer: *Harley et al. 19200* (CEPEC), *Jesus et al. 1311* (CEPEC), *Zappi et al. 2091* (RB), *Martinelli 5827* (RB), *Carvalho et al. 1116* (IAC); *Casearia espiritosantensis* R.Marquete & Mansano: *Siqueira 783* (RB); *Casearia fasciculata* (Ruiz & Pav.) Sleumer: *Krukroff's 5148* (NY), *Prance 30240 et al.* (NY), *Krukoff's 5174* (NY); *Casearia grandiflora* Cambess.: *Thomas et al. s.n.* (CEPEC), *Belém and Mendes 27* (CEPEC), *Forzza 3782 and Mello-Silva* (RB), *Soavedra 417 et al.* (RB), *Bernacci 2248* (IAC), *Árbocz 7203* (IAC), *Marquete et al. 3166* (IAC); *Casearia guianensis* (Aubl.) Urb.: *Croat 14978* (NY), *Riedel s.n.* (NY), *Kerbbride et al. 1684* (NY), *Harris 10367* (NY), *Howard 9705* (NY); *Casearia hirsurta* Sw.: *Breteler 3675* (NY), *Proctor 10183* (NY), *Molina 3384* (NY); *Casearia lasiophylla* Eichler: *Wesenberg 132 et al.* (RB), *Lindeman 5334 and Haas* (RB), *Echternacht 671 and Dornas* (RB), *Fernandes et al. 33439* (UEC), *Alencar and Oliveira 1557* (IAC), *Torres et al. 749* (IAC), *Torres et al. 743* (IAC), *Torres and Figueiredo 409* (IAC); *Casearia manausensis* Sleumer: *Cid et al. 1879* (INPA), *Mota 67 and Coêlho* (INPA), *Coelho 3201* (INPA), *Monteiro 1330 et al.* (INPA), *Ferreira 1435 et al.* (INPA);

Casearia mariquitensis Kunth: *Evans 2575 and Kalmar* (RB), *Pereira 7888 et al.* (RB), *Tozzi et al. s.n.* (UEC), *Bertoni 281* (IAC); *Casearia melliodora* Eichler: *Jardim et al. 1594* (CEPEC), *Paixão et al. 197* (CEPEC), *Macedo 12* (IAC), *Macedo and Lima 470* (IAC); *Casearia mestrensis* Sleumer: *Silva 158* (CEPEC), *Sarmento 644* (RB), *Valente 2328 et al.* (VIC), *Melo 3245 and Franco* (IAC); *Casearia negrensis* Eichler: *Duarte 6557* (RB), *Amaral 100 et al.* (INPA), *Coelho 4121* (INPA), *Amaral 750 et al.* (INPA); *Casearia nigricans* Sleumer: *Vieira 561 et al.* (INPA); *Casearia nitida* L. Jacq.: *Correll 43589* (NY), *Britton 5677 et al.* (NY); *Casearia oblongifolia* Cambess.: *Cavalcante 04* (RB), *Lima et al. 4568* (CEPEC), *Siqueira 74* (RB), *Marquete 1421* (IAC); *Casearia obovalis* Poepp. ex Griseb.: *Cid and Nelson 2810* (RB); *Casearia paranaensis* Sleumer: *Marchiori 501 et al.* (UEC), *Souza 111* (IAC), *Zickel et al. s.n.* (IAC), *Rosi et al. 602* (IAC), *Anunciação et al. 243* (IAC); *Casearia pauciflora* Cambess.: *Braga 3923 et al.* (RB), *Nadruz 2341 et al.* (RB), *Braga et al. 1622* (IAC); *Casearia pitumba* Sleumer: *Amaral 760 et al.* (INPA); *Casearia resinifera* Spruce ex Eichler: *Ducke s.n.* (RB), *Ducke 1447* (NY); *Casearia rufescens* Cambess.: *Pirani 5248 et al.* (RB), *Carvalho and Silva s.n.* (IAC), *Valente 2327 et al.* (VIC); *Casearia rupestris* Eichler: *Pott 12235 et al.* (RB), *Marquete 3170 et al.* (RB), *Mota 2096* (VIC), *Duarte and Silva 90* (VIC), *Árbocz s.n.* (IAC); *Casearia sessiliflora* Cambess.: *Cavalcanti 166 and Sá* (RB), *Cavalcanti 153 et al.* (RB); *Casearia ulmifolia* Vahl ex Vent.: *Alencar et al. 1163* (UEC), *Hatschbach 61927* (CEPEC).