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Floral oil production in a family dominated by pollen flowers: The case of Macairea radula (Melastomataceae)

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Abstract:	The Melastomataceae family, the largest radiation of pollen flowers, has been reported to offer floral oils exclusively in the Olisbeoideae subfamily. However, species from other clades such as Macairea radula (Marcetieae, Melastomatoideae) exhibit staminal glands that secrete oil-like viscous substances whose chemical composition and function are still unknown. We used anatomical sections and histochemical tests to characterize these staminal glands and their exudate. We also used GC-MS to characterize the chemical composition of the oil from the flowers and the scopae of visiting oil bees (Centris aenea). The staminal glands consist of glandular emergences with a multiseriate stalk and a conspicuous multicellular secretory head. Histochemical tests revealed the presence of lipids and phenolic compounds inside the glandular head cells. Although histologically different from trichomes, these glands are morphologically similar to trichomatic elaiophores. GC-MS confirmed the non-volatile lipidic nature of the staminal gland secretion, which consists of a mix of medium to long chain alkanes and nutritious fatty acids. Therefore, M. radula staminal glands produce oils similar in composition to the oils produced as bee reward by other angiosperm flowers. Some of these compounds were also found in the oils extracted from visiting bee scopae, suggesting that the oils produced by the staminal glands can be collected by bees. In addition, or alternatively, these oils could promote better adhesion of pollen to the bee's body. Oil production by staminal glands of M. radula may attract oil-collecting bees more consistently, ultimately contributing to the plants' reproductive success.
Suggested Reviewers:	Elisabeth E. A. Dantas Tölke, PhD USP IB: Universidade de Sao Paulo Instituto de Biociencias elisabeth.tolke@gmail.com Elisabeth is a botanist with experience in the area of anatomy of reproductive organs, secretory structures and has great experience in different analysis techniques (light and electronic microscopy, CG-MS and histochemistry).
	Isabel Cristina Machado, PhD Professor, Universidade Federal de Pernambuco Departamento de Botânica imachado@ufpe.br Isabel has great experience in the area of floral resources and plant-pollinator interaction, especially plants that offer oils and oil-collecting bees.
	Andrea A. Cocucci, PhD Professor, Instituto Multidisciplinario de Biología Vegetal, Universidad Nacional de Córdoba, CONICET, Córdoba, Argentina aacocucci@imbiv.unc.edu.ar Andrea has experience in the field of evolutionary ecology studies with special reference to floral biology and pollination. In addition, he has participated in important works aimed at oil-secreting plants

Opposed Reviewers:	
Response to Reviewers:	Reviewer #1 - We thank Reviewer#1 for yet another careful review of our manuscript. We now strive harder to reduce the text and focus our conclusion on our concrete findings. We followed your suggestion and the text was entirely revised by an English native speaker. Finally, the additional suggestions on the attached file were accepted in the manuscript file and can be seen in red highlighting. Some comments that needed to be answered are found in the Review Renponse file .

Floral oil production in a family dominated by pollen flowers: The case of *Macairea radula* (Melastomataceae)

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Running title: Floral oil in *Macairea* (Melastomataceae)

Abstract

The Melastomataceae family, the largest radiation of pollen flowers, has been reported to offer floral oils exclusively in the Olisbeoideae subfamily. However, species from other clades such as *Macairea radula* (Marcetieae, Melastomatoideae) exhibit staminal glands that secrete oil-like viscous substances whose chemical composition and function are still unknown. We used anatomical sections and histochemical tests to characterize these staminal glands and their exudate. We also used GC-MS to characterize the chemical composition of the oil from the flowers and the scopae of visiting oil bees (*Centris aenea*). The staminal glands consist of glandular emergences with a multiseriate stalk and a conspicuous multicellular secretory head. Histochemical tests revealed the presence of lipids and phenolic compounds inside the glandular head cells. Although histologically different from trichomes, these glands are morphologically similar to trichomatic elaiophores. GC-MS confirmed the non-volatile lipidic nature of the staminal gland secretion, which consists of a mix of medium to long chain alkanes and nutritious fatty acids. Therefore, *M. radula* staminal glands produce oils similar in composition to the oils produced as bee reward by other angiosperm flowers. Some of these compounds were also found in the oils extracted from visiting bee scopae, suggesting that the oils produced by the staminal glands can be collected by bees. In addition, or alternatively, these oils could promote better adhesion of pollen to the bee's body. Oil production by staminal glands of *M. radula* may attract oil-collecting bees more consistently, ultimately contributing to the plants' reproductive success.

Keywords

elaiophores; gas chromatography; oil-flowers; pollen dilemma; staminal glands

1. Introduction

Floral resources modulate plant-pollinator interactions (Fowler et al., 2016) and the diversity of these resources reflects the variety of interactions involving flowers and animals (Ollerton et al., 2011). Usually, plants offer pollen and nectar as a floral resource to their pollinators. Pollen and nectar are most frequently available together, although different combinations of floral resources can also be found in nature (Simpson and Neff, 1981). Plants within 14 unrelated angiosperm families are known to offer floral oils as the main or ancillary resource actively used by their pollinators (Buchmann, 1987; Alves-dos-Santos et al., 2007; Renner and Schaefer, 2010; Neff and Simpson, 2017; Possobom and Machado, 2017). In such oil-flowers, the lipidic components can be secreted in different parts of the flower by secretory epidermal cells (epithelial elaiophores) or glandular trichomes (trichomatous elaiophores). The secreted floral oils consist of highly energetic compounds, mainly non-volatile fatty acids and/or mono- or diglycerides (Vogel, 1974; Simpson and Neff, 1981; Buchmann, 1987).

The main pollinators of oil-flowers, oil-collecting bees, bear morphological and behavioural adaptations such as specialized bristles, and rubbing or scraping behaviour, respectively (Buchmann, 1987; Alves-Dos-Santos et al., 2007; Renner and Schaefer, 2010; Possobom and Machado, 2017). When visiting oil-flowers, they actively collect floral oils and use them together with pollen as food for their larvae or for insulating brood cells (Vogel, 1974; Simpson and Neff, 1981; Buchmann, 1987; Possobom and Machado, 2017). Floral oils also have an adhesive function that helps pollen grains stick to the pollinator's body and prevents them from being easily lost or wiped off for pollinator consumption (Vogel, 1981; Gates, 1982; Moyano et al., 2003). Therefore, from the bees' perspective, floral oils supply nutritional and nest materials and, from the plant's perspective, an additional oil supply may reduce the costs of excessive pollen loss during removal and transport or even due to direct consumption by bees (Westerkamp, 1996; Harder and Routley, 2006; Lunau, 2015).

An additional offer of floral oils may be especially important in plants producing mainly pollen as a reward for bee visitors, such as the vast majority of Melastomataceae species (Renner 1989). To our knowledge, this plant family encompasses few but well-known representatives of oil-flowers restricted to the subfamily Olisbeoideae (Buchmann and Buchmann, 1981; Buchmann, 1987; Renner, 1989). Plants within Olisbeoideae produce oil in specialized glands located on the dorsal surface of the anther connectives and this trait is one of the synapomorphies of the clade, the first to diverge within Melastomataceae (Clausing and Renner, 2001; Stone, 2006). However, staminal glandular trichomes are reported for different lineages within the family, especially in the morphological description of taxonomical studies (Renner, 1989; Guimarães et al., 1999; Fracasso and Sazima, 2004; Martins 2009). To the best of our knowledge, the composition of the secretion from glandular trichomes can be quite different among different species of melastomes and nothing is known about the composition of the secretion from the staminal glands of this family outside the subfamily Olisbeoideae (Eyde and Teeri, 1967).

Most melastome flowers have poricidal anthers which require interactions with bees able to apply mechanical vibrations on their stamens to effectively remove pollen, a process that characterizes the buzz pollination syndrome observed in these plants (Buchmann, 1983; Renner, 1989). Interestingly, some buzz-pollinating bees can also actively collect floral oils when visiting oil-flowers. This is the case for all bee species in the Centridini tribe, and some bee species within the Meliponini tribe (Alves-Dos-Santos et al., 2007; Cardinal et al., 2018). *Macairea radula* (Bonpl.) DC. have staminal glands located in the filament (Silva and Romero, 2008; Bacci et al., 2016) whose secretion and functions have not yet been clarified. Moreover, this species is mainly visited by *Centris aenea*, an oil-collecting bee also able to perform buzz pollination. The objectives of the present study were to (I) describe the anatomical structure of such staminal glands of *Macairea radula*, and II) to identify the main compounds secreted by these staminal glands and those found in the scopae of their main floral visitor, *Centris aenea*.

2. Materials and Methods

2.1. Study sites and species

Data collection was performed between August 2017 and November 2018 in Fazenda Águas de Santo Antônio (20°25' S 46°40' W; 844 m a.s.l; Delfinópolis municipality), an area comprising fragments of swamps of the Cerrado (Brazilian savanna) with a large population of *M. radula*. The climate is Cwa type with a mean temperature of 21 °C and about 1,709 mm of annual precipitation (IBGE 2004). A voucher specimen of *M. radula* from this area was deposited in the Herbarium Uberlandense collection (HUFU00037777).

Macairea radula (Fig. 1A-B) is a shrub species whose flowers are organized in thyrsoid inflorescences and exhibit eight alternately dimorphic stamens with falciform poricidal anthers in two whorls (Silvia and Romero, 2008; Bacci et al., 2016). Although pollen is the main floral resource collected by pollinators (Oliveira et al., 2020, 2021 *in review*.), glandular structures previously described as trichomes are found on the ventral surface of the stamen filaments, as well as on the lower portion of the style and ovary apex (Fig. 1A) (Silva and Romero, 2008; Bacci et al., 2016). The presence of lipids in the secretion of such staminal glandular structures (hereafter called staminal glands) had already been indicated by previous histochemical tests (LCO, unpublished data). However, its exact chemical composition and biological function are still unknown.

Centris aenea bees are commonly seen visiting *M. radula* flowers (Oliveira personal observation). Such bees are characterized as effective pollinators since they grab and vibrate all floral sex organs and the stigma contacts their bodies during floral visits (Fig. 1B, Oliveira et al., 2020, 2021 *in review*). *Centris aenea* are oil-collecting bees like all other *Centris* species (Machado, 2004). This oil-collecting bee species usually cleans its body in the ventral region of the abdomen after a few visits, transferring masses of pollen and, apparently, oil, to their scopae (personal observation). Since the vibration behaviour of bees may be involved in the exudation of oil by the friction and rupture of staminal glands in *M. radula*, we chose *C. aenea* for the analysis of the oil extract from the scopae.

2.2. Structural characterization of the staminal glands and histolocalization of their content

We carried out the structural characterization of the staminal glands by anatomy and surface examination and analysed the nature of the secretion by histochemical tests. We collected stamens from previously bagged floral buds and from open visited flowers of eight individuals in the studied area. The samples were fixed in Buffered Neutral

Formalin for approximately 72 hours, washed in distilled water, dehydrated in an ethanol series, and stored in 70% ethanol (Lillie, 1965).

Filaments were dehydrated in an ethanol series and embedded in Leica plastic resin (Gerrits, 1991). We obtained longitudinal anatomical sections approximately 5 µm thick with a rotary microtome and adhered them to glass slides. Part of the slides were stained with 0.05% toluidine blue in acetate buffer, pH 4.4, (O'Brien and Feder, 1968) for about five minutes and prepared with distilled water at the time of observation. In the remaining slides, we characterized the nature of the secretion present in the glands using the following tests: Sudan Black B and Sudan Red IV to verify the presence of total lipids (Pearse, 1980); Nadi Reagent to detect terpenoids (David and Carde, 1964); Periodic Acid Schiff's (PAS) to detect polysaccharides (O'Brien and Feder, 1968), Ferric trichloride to detect phenolic compounds (Johansen, 1940), Ponceau Xylidine to detect proteins (Vidal, 1970), and Lugol to detect starch (Jensen, 1962). We also performed the tests with Sudan Black B, Sudan Red IV and Nadi Reagent on fresh material for better visualization of the exudate. Observations and photographs were obtained with an Olympus BX51 photomicroscope equipped with an Olympus DP70 digital camera.

For surface examination by scanning electron microscopy (SEM), we dehydrated previously fixed stamens in an ethanol series and an ethanol: acetone series (1: 0. 1: 1, 0: 1), submitted them to the critical point in a Leica CPD 300 equipment, mounted them on aluminium supports with double-sided carbon adhesive tape, and then metallized them with 75 nm (nanometers) palladium gold for 90 seconds in a Leica EM SCD 050 metallizer. Next, we examined the samples with a Zeiss EVO / MA10 scanning electron microscope.

2.3. Chemical characterization

We collected stamen filaments of non-visited flowers from eight individuals as well as the scopae of three *C. aenea* bees captured while flying after they visited *M. radula* flowers from the same population. Flowers used for the chemical characterization were previously bagged with nylon mesh bags at the bud stage. We stored filaments and scopae in separate glass jars containing dichloromethane solvent (2 ml) for 5 minutes to extract their oil content. Subsequently, we filtered these samples using filter paper and reduced the content under a compressed air flow. The remaining aliquots were stored in a freezer (-20º C) until analysis. In the laboratory, we used a gas chromatography instrument coupled to a mass spectrometer (GC-MS QP2010 Shimadzu) according to methodological standards (adapted from Nunes et al., 2017).

We injected 1 µl of the extract directly into the gas chromatography apparatus with the injection chamber at 250 °C in *splitless* mode. The separation of the compounds was performed in a 30 m × 0.25 mm DB-5MS capillary column (J & W Agilent) , film thickness 0.25 µm, with He (79.7 kPa) as the transport gas at a flow rate of 1.3 ml min-1. Initially, the GC column temperature was 60 °C, linearly increasing by 3 °C per minute to 240 °C for 60 minutes. We analysed the chromatographic data using the GC-MS Solution software (Shimadzu) and we identified the floral oils by comparing the ion mass spectrum and the Kovats retention index with data from the NIST platforms (https://webbook.nist.gov/chemistry/) and PubChem (https://pubchem.ncbi.nlm.nih.gov/). The relative amount (%) of chemical compounds in the sample was obtained by normalizing the areas of total ion spikes (Total Ion Count) in the chromatogram.

2.4. Statistical analysis of the chemical profiles

Looking for evidence that the *C. aenea* oil-collecting bees visiting *M. radula* flowers were collecting oils from the staminal glands, we compared the chemical composition of staminal gland extracts with the composition of the oil extracted from the scopae of the bees. As the dataset of the relative amounts (%) of the identified chemical compounds for plants and bees contains mostly zeros (*i.e.*, chemicals completely absent in certain samples, but present in others), we first performed Hellinger transformation. This transformation relativises the amount of each compound by the row totals and subsequently calculates the square root of each element in the matrix (Legendre and Gallagher, 2001; Legendre and Legendre, 1998). To graphically represent the dissimilarities between the chemical profiles of the staminal glands and bee scopae extracts, we performed a hierarchical clustering analysis (*mcquitty* method using Euclidean distances) using the PVCLUST package in R (Suzuki et al., 2006). We calculated the Percentage Similarity Index (*PS*), which varies from 0 for completely different to 1 for identical chemical profiles, to estimate the relative amount of chemicals shared by staminal glands and bee scopae. To calculate the *PS*, we created another dataset of the mean relative amount (%) of the identified chemical compounds with two rows (one for staminal gland samples and the other for bee scopae samples) and the compound names as columns. Again, we performed Hellinger transformation (Legendre and Gallagher, 2001; Legendre and Legendre, 1998) and calculated the Bray-Curtis distance index (or percentage difference, PD) between extracts using the *vegdist* function (*bray* method) from the VEGAN statistical package (version, 2.5-7, Oksanen et al., 2020). Finally, we calculated the *PS* using the formula $PS = 1 – PD$ (Legendre and Legendre, 1998). All statistical analyses were performed using the environment *R* statistical software, version 4.0.2 (R Development Core Team, 2020).

3. Results

3.1. Structural characterization of staminal glands and histolocalization of their content

The staminal glands of *M. radula* are widely present and distributed in the ventral portion of the filaments (Fig. 1A and 2A-D). The filament has a uniseriate epidermis covered with a thin cuticle layer, followed by parenchymatous tissue consisting of a few layers of cells surrounding a central vascular bundle (Fig. 3A). The filament parenchymatous tissue is continuous and seems to protrude towards the gland's peduncle, indicating the presence of fundamental tissue in such structure (Fig. 3A-B). These glands are structurally characterized as glandular emergences consisting of a multiseriate peduncle and a conspicuous and globular multicellular secretory head, covered with a thin cuticle (Fig. 2C-D and 3A-C). In visited flowers, we could see the ruptured cuticle in the distal region of the gland (Fig. 2D). We histochemically detected the presence of total lipids (Fig. 4A-C) and phenolic compounds (Fig. 4D) in the gland secretion, which accumulates in a subcuticular space (Fig. 4B-C). In addition, we observed lugol-stained starch granules in the parenchymatic tissue of the filament underlying the glands (Fig. 4D)

3.2. Chemical characterization

Extracts from both the staminal glands and the bee scopae showed a lipidic composition mainly indicating the presence of fatty acids and their derivatives (fatty alcohols, fatty aldehydes, alkanes and alkenes, alcohols, terpenoids, esters, and ketones;

Table 1 and Supplementary Material). Three out of the eight plant samples collected did not show identifiable amounts of any organic compounds in the gland tissue extracts. We identified 167 compounds by chromatographic analysis, 72 from the filament extracts and 95 from bee scopae extracts (Table 1 and Supplementary Material - Table 1). Among the compounds identified in the filament extracts, octadecane showed the greater relative abundance (10.35%), followed by nonadecane (9.27%), methyl 2-hydroxyhexadecanoate (a hexadecanoic acid derivative) (8.31%), octadecanoic acid (stearic acid – 7.28%), heptadecane, and icosane (6.91%). On the other hand, the compounds with greatest relative abundances in bee scopae extracts were two unidentified substances eluted within 47.06 min (13.09%) and 57.65 min (5.13%) (Table 1), respectively. Compounds such as 2-methyloctadecane, methyl 2-hydroxyhexadecanoate, 2-methylnonadecane, 2,6,10,14-tetramethylheptadecane octadecane, heptadecane, hexadecane, 8-heptylpentadecane, 2-methylicosane, 2,6,10,15-tetramethylheptadecane and a non-identified substance eluted within 33.34, 47.41 and 51.86 min were found in different percentages in both extracts (Table 1).

The chemical profiles of the oils collected in three out of five flower filaments were more similar to two profiles collected in the bee scopae, while the other two flower filament samples did not share any of their oil components with bees or with other filament samples (Supplementary Material – Fig.1A). The *PS* was only 0.1. In other words, considering the mean relative abundance of each chemical compound, extracts from bee scopae and staminal glands share 6.2 % of their chemical composition (Supplementary Material – Fig.1B).

4. Discussion

Our results showed a morphological similarity between the staminal glands of *M. radula* and the trichomatic elaiophores characterized for oil-flowers (Vogel, 1974; Simpson and Neff, 1981; Buchmann, 1987; Machado et al., 2002). However, these staminal glands correspond to glandular emergences, differing from trichomes by the presence of fundamental tissue. Although the floral oils of these staminal glands are not actively collected by visitors, they are likely to be incidentally carried by *Centris aenea*, the most effective pollinator of this plant. Some oil components from the flower staminal glands are identical to the ones retrieved from the bee scopae. Thus, some of the floral oils of *M. radula* may be inadvertently collected by *C. aenea* bees during the vibrations performed on flowers given their highly frequent visits to the flowers and regular contact with the staminal gland surface (Oliveira personal observation). Since the pollen of *M. radula* flowers is actively collected by *C. aenea* bees using floral vibrations, we hypothesize that the oil from staminal glands would help with pollen adhesion to different parts of the bee body, reducing pollen wastage and consequently optimizing the plant male sexual function.

Within Olisbeoideae, records of oil production are associated with epithelial elaiophores, which are also common in other oil-flowered families such as Malpighiaceae, Krameriaceae, and some Orchidaceae (Vogel, 1974; Buchmann and Buchamann, 1981; Simpson and Neff, 1981; Buchmann, 1987). *M. radula* staminal glands are morphologically similar to the trichomatic elaiophores, but histologically differ from these trichomes by the presence of fundamental tissue. The occurrence of a large amount of starch granules found in filament parenchyma cells near the staminal glands may be related to their energy and maintenance demands. As these polysaccharides are commonly associated with energy storage in several plant organs, these starch granules may act as carbon and energy sources, supplying the lipid secretion process (Zeeman et al., 2010).

The studied species is characterized by oil secretion in stamen filament areas densely covered by the glands, which are also common in species of Iridaceae, Cucurbitaceae, Plantaginaceae and Solanaceae (Vogel, 1974; Simpson and Neff, 1981; Buchmann, 1987). These floral oils may enhance pollen attachment to the bee's body, reducing the costs of pollen loss during its removal and/or transport (Westerkamp, 1996; Lunau et al., 2015). They may also improve the efficiency of pollen transfer similarly to the sticky secretion from the androecium glands of a Malpighiaceae species (Possobom et al., 2010). In fact, the better adhesion of pollen grains by oil secretion has been considered to be one of the roles of the floral oils produced in *Mouriri* species (Melastomataceae-Olisbeoideae) (Buchmann, 1987; Oliveira, 2016) and in staminal glands of Cucurbitaceae, Lamiaceae and Malpighiaceae (Vogel, 1981; Gates, 1982; Moyano et al., 2003). Moreover, the presence of phenolic compounds in the exudate may confer antioxidant and antimicrobial properties (Vinson et al., 1996, 2006). The presence of floral oils in, addition to the pollen commonly offered by *M. radula*, besides the heteranthery and the presence of dimorphic stamens in this species (one set of feeding and one set of pollinating stamens), can play an important role by reducing pollen wastage in this species. It could also represent an adaptation within the largest radiation of pollen flowers, i.e., the one of melastome flowers.

Centris bees have been recurrently reported in the literature to bear adaptations and stereotyped behaviours for oil collection, such as scraping their specialized fore and mid legs on the elaiophores of oil-flowers; however, in *M. radula* they did not exhibit any of these behaviours (Machado, 2004; Oliveira et al., 2020, 2021 *in review*). These bees actively extract pollen grains by vibrations applied to stamens and possibly incidentally impregnating their bodies with oils. Generally, the secretion produced by glandular structures is spontaneously released through micropores in the cuticle or due to active rupture of the cuticle by abiotic and biotic stressors (Ascensão et al.,1999). Differently from unvisited flowers of *M. radula*, visited flowers show ruptures on the cuticle of the gland's head. It is likely that the vibration behaviour promotes the rupture of the cuticle and the leakage of lipid secretion, which would be allocated to the bee's body. Floral oils disassociated from bee active collection could represent a precursor stage in the evolution of exclusive pollination by oil-collecting bees. It may also allow some plants to take advantage of the presence of pollen-seeking oil bees in a diverse plant-bee community.

Our results show that fatty acids and alkanes were the most common compounds in the extracts of stamen filaments and bee scopae. Fatty acids vary in saturation and in the length of their chains (12-20 carbons) while the alkanes range from medium to long chains (16-23 carbon). Fatty acids such as octadecanoic (stearic acid) and hexadecanoic (in the form of hexadecanoic acid, 2-hydroxy-, methyl ester) acids, and tetradecanoic methyl ester (myristic acid), eicosanoic and oleic acids, and the long chain alkanes nonadecane, heptadecane and heneicosane have been found in other oil-flowers (Vogel, 1974; Buchmann, 1987; Vinson et al., 1997; Possobom and Machado, 2017). For example, octadecanoic, hexadecanoic and tetradecanoic acids found in the extracts of *M. radula* staminal glands are also found in Malpighiaceae and *Mouriri* (Olisbeiodeae, Melastomataceae). In addition, eicosanic acid and the same previous acids in their acetoxy forms are also found in Krameriaceae, Scrophularaceae and Primulaceae (Possobom and Machado, 2017), suggesting that the composition of the non-volatile oil secretion of *M. radula* staminal glands is somewhat similar to that of the secretions of other oil-flowers. Interestingly, these oils are all accessible to bee pollinators during their visits to flowers and some of their components were found in the bee scopae analysed here. One of the bee's scopae samples contained a high proportion of diacetin (Table 1). Diacetin is a compound representing an exclusive communication channel between oil-flowers and oil bees and appears to be a strong indicator of pollination by bees that seek nutritive oils in flowers (Schäffler et al. 2015). However, what we have so far is not sufficient for us to state that these oils play a role as resources and have feeding and/or nest building functions.

Our results show that only a minority (6.2%) of the oil compounds found in the scopae of *Centris aenea* are also found in *M. radula* staminal glands. The presence of diacetin and other diverse compounds in the bee samples and the complete absence of some major compounds in the bee scopae in the staminal gland samples support the notion that *M. radula* does not offer oil as a resource to its pollinating bees. Such exclusive compounds should have been detected after visits to other oil-flower species since *Centris* bees are generalist visitors and can collect oil from a wide variety of oil producing flowers in the local community (Machado, 2004). Therefore, the presence of oils in *M. radula* may not have a direct bee feeding function. The staminal oil secretions could perform an adhesive function by preventing excessive pollen loss and enhancing the safe journey of pollen to other conspecific flowers, which would improve/refine the heterantherous system of this plant and consequently its reproductive success. The presence of oil in the bee scopae mixed with pollen masses could also help the bees in packing and carrying the pollen to their nests, eventually making these flowers more attractive to bees that usually mix pollen and oils in their scopae. Further research on the oils collected by bees in *M. radula*, the oils produced by other flowers within the same plant-pollinator community, and the oil content in the nests of these bees will allow a better understanding of the importance and use of *M. radula* oils by their oil-collecting bee pollinators. Although the supply of floral oils is still poorly understood in Melastomataceae, our results extend the occurrence of oil production beyond the Olisbeoideae and provide new facts contributing to the e understanding of questions such as how widespread floral oil production is and which flower structures are associated with it in this family.

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Tables

Table 1 – Commom and major compounds, considering a threshold above 5% of the relative abundance of the absolute values of each individual sample, present in extracts of *Macairea radula* filaments and *Centris aenea* bee scopae resulting from chemical analysis by gas chromatography. RA - relative abundance (%), RT- retention time, MW- molecular weight, MF- molecular formula.

Compound	CAS register number	RA		RT	MF	MW	Function
		Macairea radula	*Centris aenea*				
-	-	11.11	6.31	-	-	-	**Fatty acids and derivatives (%)**
-	-	4.16	10.52	-	-	-	**Fatty alcohols (%)**
-	-	1.38	3.16	-	-	-	**Fatty aldehydes (%)**
-	-	2.77	2.1	-	-	-	**Terpenoids (%)**
-	-	31.94	9.47	-	-	-	**Alkanes (%)**
-	-	1.38	3.15	-	-	-	**Alkenes (%)**
-	-	5.55	6.31	-	-	-	**Ester (%)**
-	-	0	5.26	-	-	-	**Ketones (%)**
-	-	0	1.05	-	-	-	**Epoxide (%)**
Total (n)	-	72	95	-	-	-	-
not identified	-	0.00 ± 0.00	13.09 ± 0.29	47.06	-	-	-
not identified	-	0.00 ± 0.00	5.13 ± 0.07	57.65	-	-	-
Octadecane	593-45-3	10.35 ± 1.22	4.97 ± 0.10	40.82	254.5	C18H38	alkane
Nonadecane	629-92-5	9.27 ± 1.09	0.00	44.64	268.5	C19H40	alkane
methyl 2-hydroxyhexadecanoate	16742-51-1	8.31 ± 0.98	0.28 ± 0.08	35.59	256.42	C16H32O2	hydroxymethylated fatty acids
octadecanoic acid (stearic acid)	57-11-4	7.28 ± 1.86	0.00	36.75	284.5	C18H36O2	fatty acid
heptadecane	629-78-7	6.91 ± 0.81	1.66 ± 0.08	36.71	240.5	C17H36	alkane
icosane (eicosane)	112-95-8	6.91 ± 0.81	0.00	48.21	282.5	C20H42	alkane
hexadecane	544-76-3	4.28 ± 0.50	0.12 ± 0.05	32.29	226.44	C16H34	alkane
not identified	-	2.46 ± 0.29	0.41 ± 0.12	33.64	-	-	-
8-heptylpentadecane	-	2.15 ± 0.25	0.24 ± 0.09	53.02	310.6	C22H46	alkane
2-methyloctadecane	1560-88-9	0.89 ± 0.06	0.32 ± 0.07	41.96	268.5	C19H40	alkane

Compound	**CAS register number**	**RA**		**RT**	**MF**	**MW**	**Function**
		Macairea radula	***Centris aenea***				
2-methylicosane	52845-08-6	0.82 ± 0.10	0.27 ± 0.01	49.82	296.6	C21H44	alkane
not identified	-	0.37 ± 0.04	1.56 ± 0.07	51.86	-	-	-
2-methylnonadecane	1560-86-7	0.21 ± 0.02	1.04 ± 0.07	46.39	282.5	C20H42	alkane
not identified	-	0.15 ± 0.02	0.35 ± 0.06	47.41	-	-	-
2,6,10,14-tetramethylheptadecane	18344-37-1	0.14 ± 0.02	1.65 ± 0.40	42.73	296.6	C21H44	terpenoid
2,6,10,15-tetramethylheptadecane	54833-48-6	0.10 ± 0.01	3.97 ± 0.10	24.66	296.6	C21H44	alkane
Percentage (%)	-	100	100	-	-	-	-

Figure Captions

Figure 1. *Macairea radula* flower in the studied population, Delfinópolis- MG: (A) dimorphic stamens distributed between the antepetalous (four smaller ones) and antesepalous (four major ones) whorls, all of them exhibiting staminal glands distributed in the ventral portion of the filament; (B) *Centris aenea* visiting *M. radula* flower. White bars indicate 1 cm.

Figure 2. Scanning electron microscopy of the staminal glands of *Macairea radula* flowers. (A) and (B) show the high density of staminal glands on the ventral surface of the filament, (C) the intact surface of the secretory head of these glands at the floral bud stage and (D) the ruptured cuticle of the secretory head of the staminal glands in a visited flower.

Figure 3. Anatomical sections of *Macairea radula* filaments stained with Toluidine showing the structure of the staminal gland. P: parenchyma, VF: vascular bundles, EP: epidermal layer, black arrows: projection of parenchymal tissue to the peduncle of the gland, and white arrows: droplets of oil.

Figure 4. Histochemical tests on the staminal glands of *Macairea radula* flowers. Arrows indicating coloured compounds: A: Sudan Red IV staining red lipid secretions, B: lipid secretion stained with Sudan IV covering the gland surface, C: Sudan Black B staining black lipid secretions, D: Ferric Trichloride staining brown phenolic compounds in oil droplets, E: Lugol staining starch granules purple.

Fig. 1

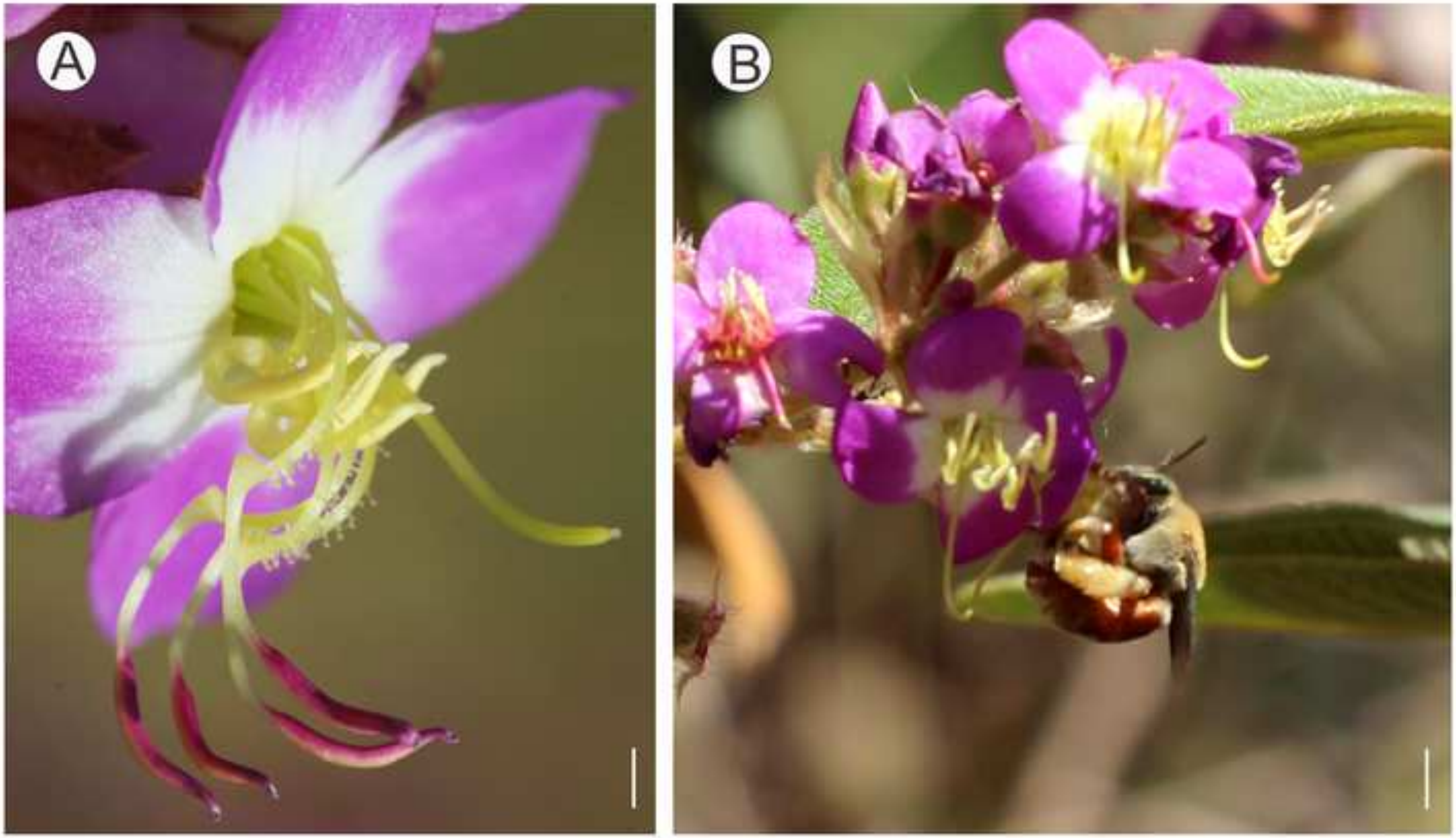

Fig. 2

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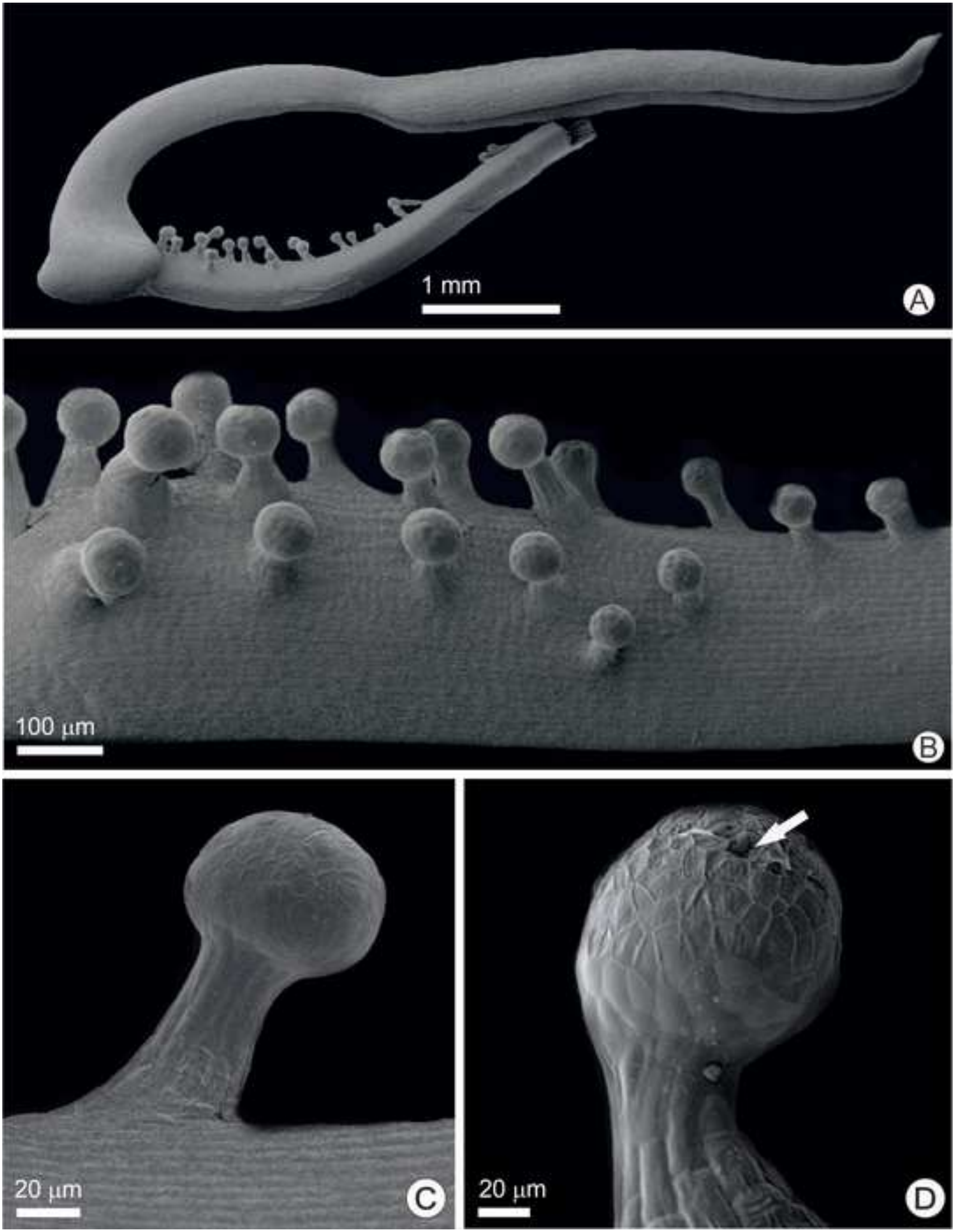

Fig. 3

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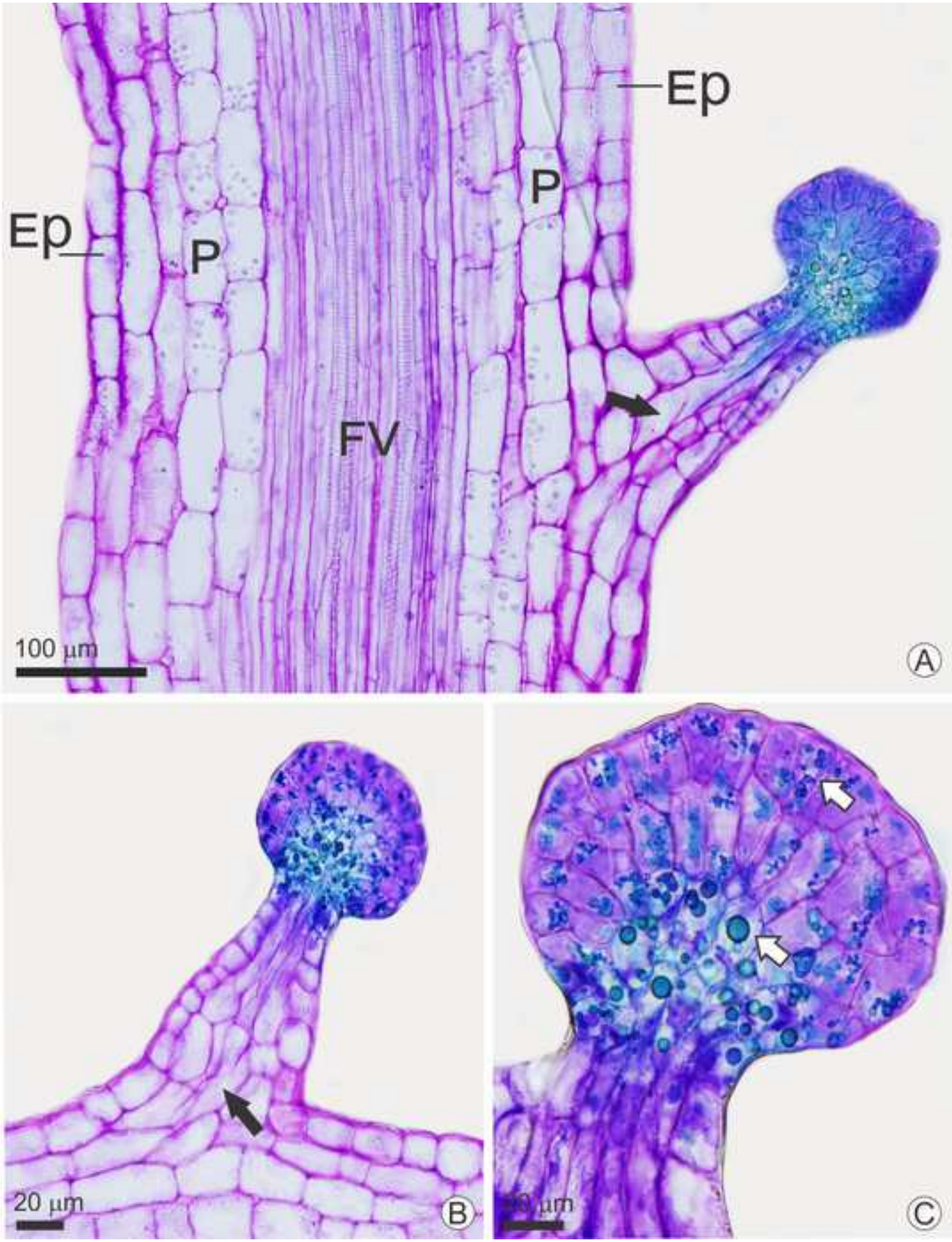

Fig. 4

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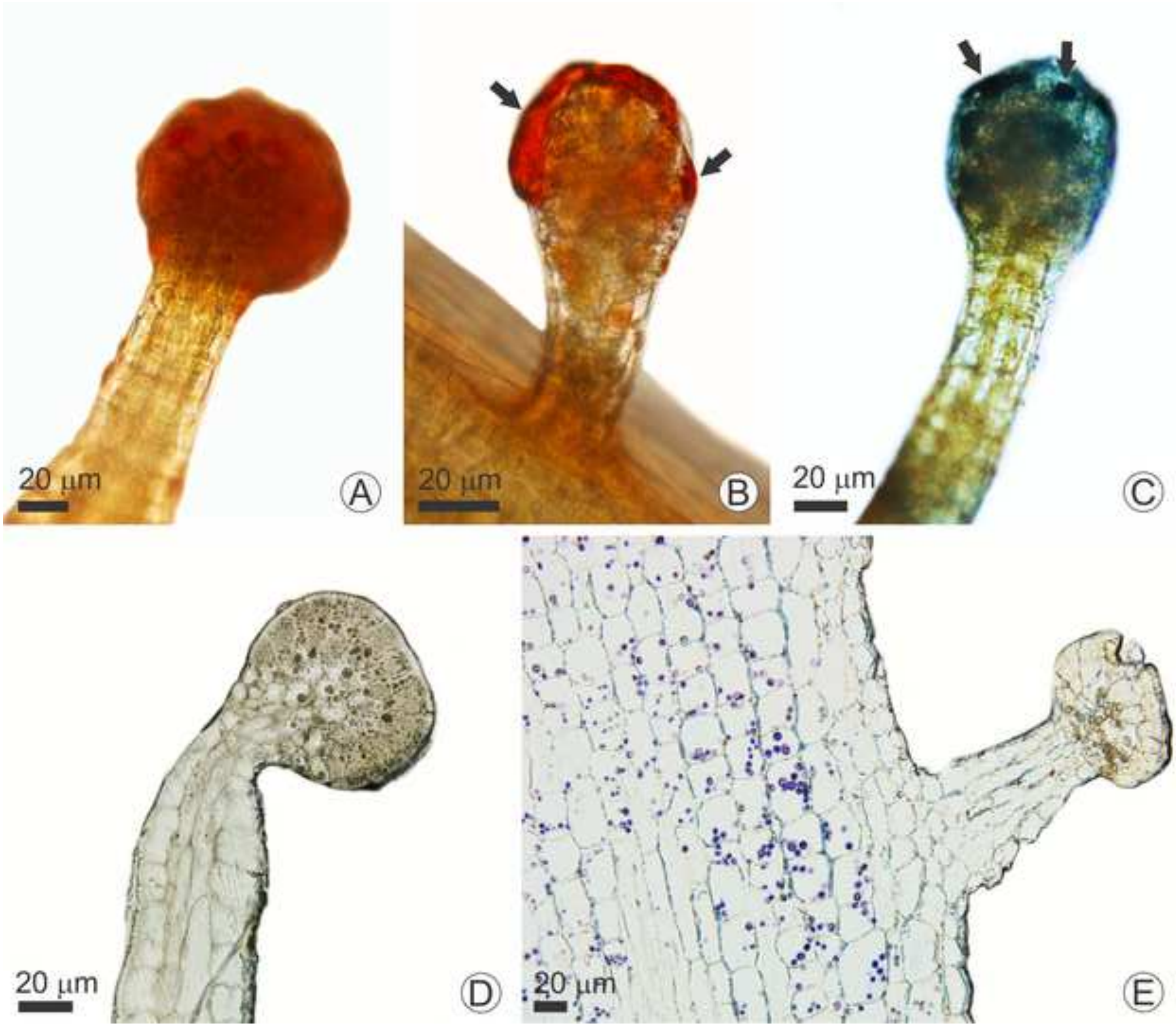

Credit Author Statement

Larissa Chagas de Oliveira: Data curation, Investigation, Writing - Original Draft; **Carlos Eduardo Pereira Nunes:** Methodology, Software, Validation, Writing - Review & Editing; **Vinícius Lourenço Garcia de Brito:** Writing - Review & Editing, Validation, Resources, Funding acquisition, **Ana Paula Souza Caetano:** Methodology, Validation, Supervision, Writing - Review & Editing

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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