



# Strategies for the protection of shoot buds in phanerophyte and geophyte species of *Homalolepis* Turcz. (Simaroubaceae, Sapindales)

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## Abstract

Several structural attributes associated with the buds of shoot apices of angiosperms have been related to protection both at the mechanical and chemical defense levels. Although several characteristics related to the protection of shoot buds are known in different taxa of Sapindales, their occurrence in the neotropical genus *Homalolepis* (Simaroubaceae) are still unknown. *Homalolepis* includes 28 species mostly with extra-Amazonian distribution, occurring mainly in the Brazilian domains of the “Cerrado” and the Atlantic Forest. Its species vary widely in terms of habit, many of which are considered adaptations to the environments in which they occur. In this study, usual light and scanning electron microscopy techniques were applied in order to analyze the morphoanatomical and histochemical characteristics associated with the protection of apical and lateral shoot buds in phanerophyte (*H. arenaria* Devecchi & Pirani (Devecchi & Pirani), *H. glabra* (Engl.) Devecchi & Pirani and *H. insignis* (A.St.-Hil. & Tul.) Devecchi & Pirani) and geophyte (*H. pumila* Devecchi & Pirani, *H. salubris* (Engl.) Devecchi & Pirani and *H. suffruticosa* (Engl.) Devecchi & Pirani) species. Glandular prophylls, cataphylls, suberized layer on the leaf primordium, colleters, non-glandular trichomes, as well as a secretory canal with precocious development in the leaf primordium are among the characteristics that can be considered strategies for the protection of shoot buds in the studied species. Such structures are related to physical (cataphyll and suberized layer) and chemical (prophylls and secretory canal) protection or both (trichomes), against desiccation or attack by herbivores.

**Keywords** Cataphyll · Nectary · Periderm · Prophyll · Secretory canal · Trichome

## 1 Introduction

In domains more susceptible to disturbances (e.g., fire, water scarcity, nutrient deficiency), such as the “Cerrado” (Brazilian savannah), the allocation of plant biomass is substantially large in underground structures (Ottaviani et al. 2020).

In addition to soil resource capture, underground structures have other associated functions, at least two of them notably related to their buds. The first is to provide resistance, with the formation of bud banks that allow resprouting, and the second is related to the protection of buds, not only against damage caused by fire and other abiotic factors, but also against the action of herbivores and parasites (Klimešová et al. 2018).

Although the position below the soil surface provides evident protection to these buds in relation to the aforementioned aspects, the underground environment is mechanically abrasive (Barley and Greacen 1967) and requires that plants use protective strategies against microorganisms (Braga and Dietrich 1987; van der Putten 2003). In the case of the root apical meristem, the root cap and the production of mucilaginous content provide mechanical protection, in addition to the existence of chemical protection and associations with

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competitive microorganisms in roots (van der Putten 2003; Arnaud et al. 2010; Kumar and Iyer-Pascuzzi 2020).

The shoot apical meristem, an important and fragile tissue, is devoid of a cap, so it is common to find other structures that provide mechanical and biological protection. According to Lubbock (1899), the most common strategies for protecting the shoot apex include the expanded base of the older leaf, modified external leaves, stipules (which may be from the leaf itself that is starting to develop or from a different leaf), trichomes, and thorns. In the order Sapindales, a group of malvid eudicotyledons, among the strategies already described, there are excavated petioles with trichomes in *Rhus glabra* L. (Anacardiaceae) (Lubbock 1899), leaf bases in *Acer* (Sapindaceae) (Lubbock 1899), intrapetiolar stipules in *Metrodorea* (Rutaceae) (Cruz et al. 2015), cataphylls covering the apical meristems in *Anacardium humile* A.St.-Hil. (Anacardiaceae) (López-Naranjo and Pernía 1990) and colleters in *Zanthoxylum* (Rutaceae) (Macêdo et al. 2016). In the Simaroubaceae family, there are still few studies on the subject (e.g., Devecchi et al. 2018a,b).

Simaroubaceae (Sapindales) is represented by 117 species in 23 genera distributed mainly in tropical and subtropical regions (Clayton 2011; Devecchi et al. 2018a,b). In Brazil, there are 36 species, 21 out of which are endemic (Devecchi et al. 2020). Recently, Devecchi et al. (2018a), based on morphological data and molecular phylogeny of the family, detected that the genus *Simaba* does not represent a monophyletic group, with its representatives distributed in two clades. One of these clades is composed of the species of *Simaba* sect. *Tenuiflorae* and it is recognized as *Simaba* s.s., with approximately 10 species distributed mainly in the Amazon. The other clade, formed by species previously included in *Simaba* sect. *Grandiflorae* and *Simaba* sect. *Floribundae*, were transferred to *Homalolepis*, a genus that was reestablished to support the species of the two extra-Amazonian sections. In total, 28 species are recognized with predominantly extra-Amazonian distribution, in the “Cerrado” and Atlantic Forest domains.

According to Cavalcante (1983) and Devecchi et al. (2018b), species that currently belong to the genus *Homalolepis* show great variation in the habit (see Fig. 1), including 10–15 cm sub-shrubs such as *H. pumila* Devecchi & Pirani, up to trees that can reach 30 m in height, like *H. paraensis* (Ducke) Devecchi & Pirani. Although the species are often trees or shrubs, such as *H. insignis* (A.St.-Hil. & Tul.) Devecchi & Pirani and *H. glabra* (Engl.) Devecchi & Pirani, with elongated shoots and buds positioned high above the ground (phanerophytes), some species are described as

geophytes, as their shoots consist mainly of a well-developed underground portion as in *H. suffruticosa* (Engl.) Devecchi & Pirani and *H. salubris* (Engl.) Devecchi & Pirani (Cavalcante 1983). This attribute of the geophytic habit has long been interpreted as a likely adaptation to drought and fire, factors that are very common in the “Cerrado” (Rizzini and Heringer 1961; Simon et al. 2009).

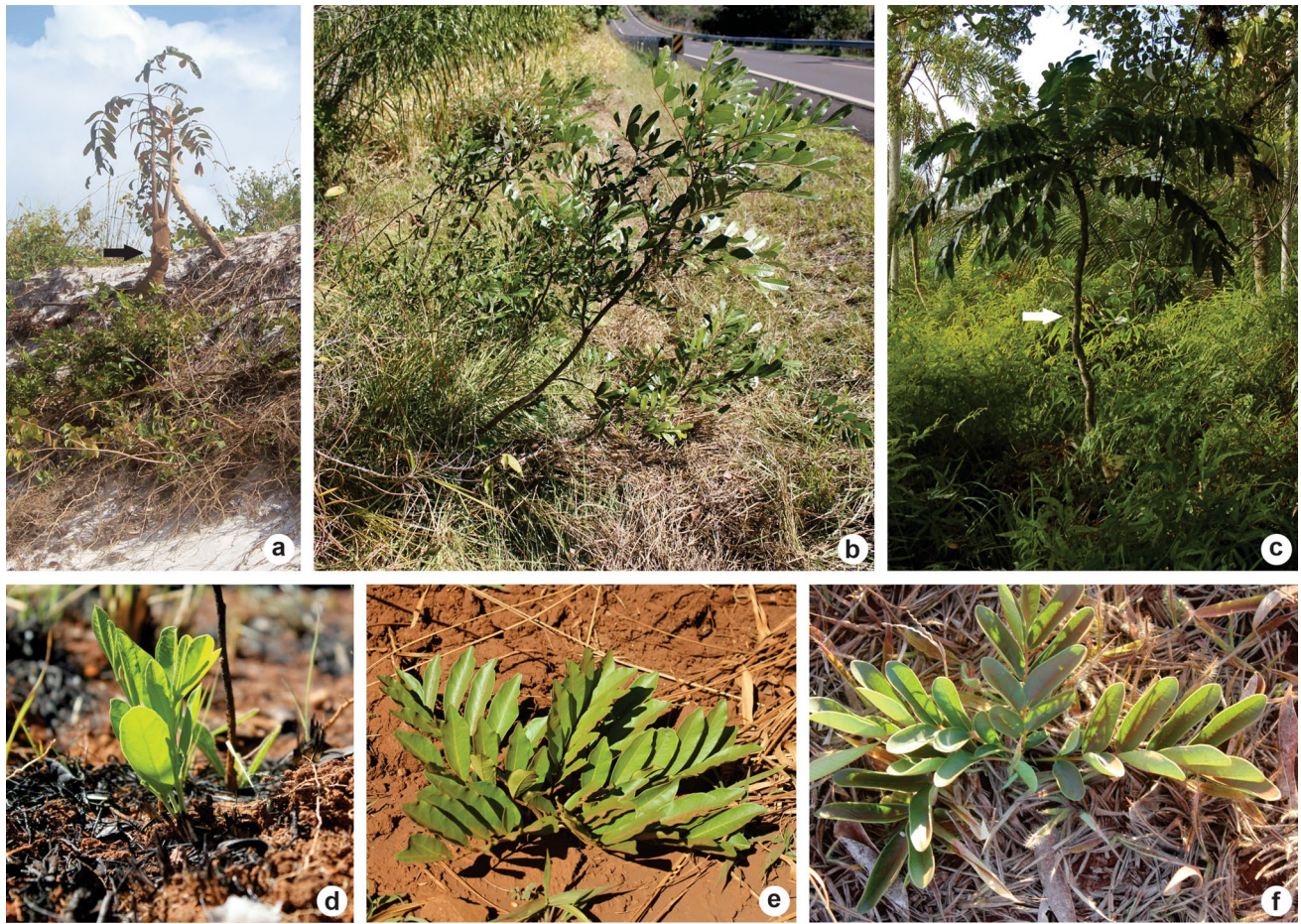
*Homalolepis* represents a good model for the study of bud protection strategies since the genus includes species with aerial and underground stems, which enables an adequate comparative analysis. Thus, we selected six species, three phanerophytes (*H. arenaria* Devecchi & Pirani (Devecchi & Pirani), *H. glabra* and *H. insignis*) and three geophytes (*H. pumila*, *H. salubris* and *H. suffruticosa*), as models for this study. In the infrageneric classification of Devecchi et al. (2018b), three of these species belong to *Homalolepis* sect. *Homalolepis* (*H. glabra*, *H. pumila* and *H. suffruticosa*), while the others belong to *H.* sect. *Grandiflorae*. The six species are endemic to Brazil, except *H. glabra*, which has also been recorded in Paraguay and Argentina (Devecchi et al. 2020).

In this study, we present the morphoanatomical characteristics observed in the vegetative shoot apices of these species that are associated with the protection of apical and lateral shoot buds, also seeking to characterize them in their origin and structure. We also compare these data with the known bud patterns in other species of Sapindales and other groups of geophyte angiosperms.

## 2 Material and methods

One voucher material for each population of each studied species, the phanerophytes *Homalolepis arenaria*, *H. glabra* and *H. insignis*, and the geophytes *H. pumila*, *H. salubris* and *H. suffruticosa* (Fig. 1), was deposited at the Herbarium of the Department of Botany of the University of São Paulo (SPF) (Thiers 2016) (Table 1). Apical regions of the vegetative shoot (Fig. 2) of five individuals from each of the analyzed species were collected and fixed in a solution containing formalin, glacial acetic acid and 50% ethanol (1:1:18, according to Johansen 1940) for 48 h, and then stored in 70% ethanol.

For morphological analysis of the surface, samples were dissected, dehydrated in an ethanol series up to absolute ethanol and subjected to drying to the critical point with liquid CO<sub>2</sub> in a CPD030 device (BAL-TEC AG, Balzers, Liechtenstein). Samples were positioned and adhered to



**Fig. 1** Life form of the studied phanerophyte **a–c** and geophyte **d–f** species of *Homalolepis*. **a** *H. arenaria*. **b** *H. glabra*. **c** *H. insignis*. **d** *H. pumila*. **e** *H. salubris*. **f** *H. suffruticosa*

**Table 1** *Homalolepis* species used in this study. <sup>1</sup>Life forms according to Raunkier (1934)

Life forms <sup>1</sup>	Species	Habitat/Brazilian state	Voucher
Phanerophyte	<i>H. arenaria</i>	Atlantic Forest/Sergipe	Devecchi & Franco 400
	<i>H. glabra</i>	Cerrado/Mato Grosso do Sul	Devecchi et al. 452
	<i>H. insignis</i>	Atlantic Forest/São Paulo	Devecchi et al. 388
Geophyte	<i>H. pumila</i>	Cerrado/Goiás	Devecchi et al. 467
	<i>H. salubris</i>	Cerrado/Mato Grosso do Sul	Devecchi et al. 472
	<i>H. suffruticosa</i>	Cerrado/Mato Grosso do Sul	Devecchi et al. 450



**Fig. 2** Shoot apices of the species studied. **a** *H. arenaria*. **b** *H. glabra*. **c** *H. insignis*. **d** *H. pumila*. **e** *H. salubris*. **f** *H. suffruticosa*

aluminum supports, covered with gold in a SCD050 metalization device (BAL-TEC AG, Balzers, Liechtenstein), and analyzed using a Sigma VP scanning electron microscope (Carl Zeiss, Germany) equipped with a digital image capture system (Robards 1978, modified).

For anatomical analysis, fixed samples were submitted to the paraffin embedding technique using the dehydration and infiltration series with ethanol and tertiary butyl alcohol (Johansen 1940). Serial transverse and longitudinal Sects. (5–10  $\mu\text{m}$  thick) were obtained using the rotary microtome RM 2145 (Leica, Germany) and stained with 1% safranin and 1% Astra blue (in 50% ethanol) (Bukatsch 1972). In order to analyze in more detail some structural and ontogenetic characteristics of the secretory structures

observed in previous studies, shoot apices of *H. glabra* were additionally embedded in 2-hydroxyethyl methacrylate resin (HistoResin Embedding Kit, Leica, Germany), sectioned (3  $\mu\text{m}$  thick) using the rotary microtome RM 2145 (Leica, Germany), and stained with Toluidine blue (CI 52,040) at pH 6.8 in sodium phosphate buffer (O'Brien et al. 1964; Ribeiro and Leitão 2020).

To detect the main classes of structural chemical compounds, as a way to better characterized the observed structures, part of the sections obtained from the samples embedded in paraffin and resin was subjected to the following tests: Periodic Acid-Schiff's reaction (PAS) for carbohydrates (McManus 1948), Ruthenium Red for pectins (Johansen 1940), Tannic Acid and Ferric Chloride for mucilages

(Pizzolato and Lillie 1973), Lugol's reaction for starch grains (Johansen 1940), Aniline Blue Black (CI 20,470) for proteins (Fisher 1968), Sudan Black B (CI 26,150) and Sudan IV (CI 26,105) for lipophilic compounds (Pearse 1985), phloroglucinol and Toluidine Blue O (CI 52,040) for lignin (Johansen 1940; O'Brien et al. 1964; Ribeiro and Leitão 2020), and ferric chloride and Toluidine Blue O (CI 52,040) for phenolic compounds (Johansen 1940; O'Brien et al. 1964; Ribeiro and Leitão 2020). All sections were analyzed and photographed using the DMLB light microscope (Leica, Germany) equipped with a DFC310 FX digital image capture system (Leica, Germany).

To verify the presence of glucose in the aqueous secretion detected in the prophylls, we applied the commercial test strips (glucostrips) directly to the secretion and compared immediately the color observed to the manufacture available pattern.

### 3 Results

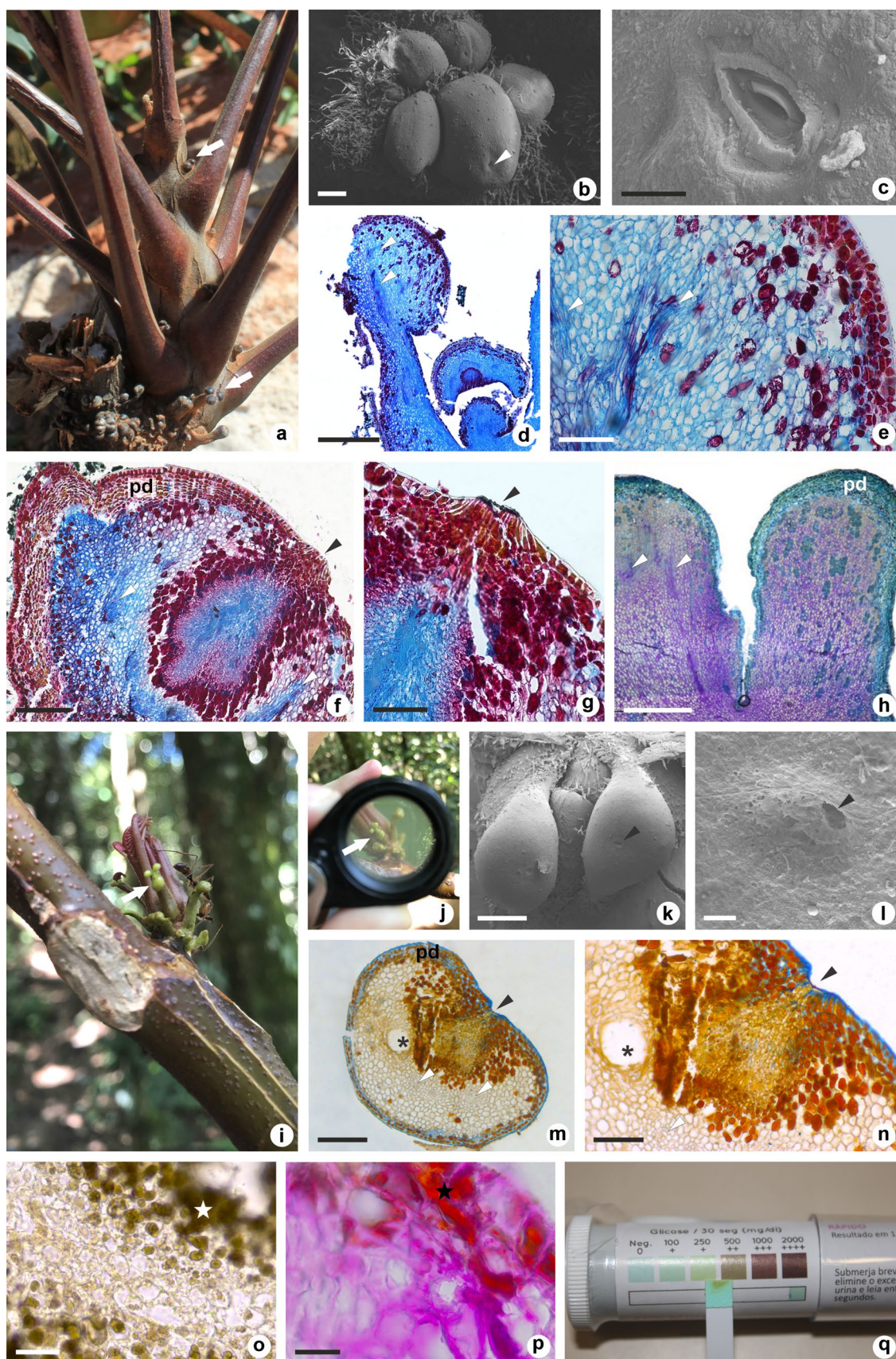
**Reduced prophylls with apical glands** – One of the strategies found are prophylls covering the shoot apices of two phanerophyte species (Fig. 3a–q). In *H. arenaria* and *H. insignis*, reduced prophylls with apical glands were observed in the region of axillary buds (Fig. 3a, b, h–j). The prophylls of *H. arenaria* presented stomata (Fig. 3b, c) and pores (Fig. 3f, g), while only pores were observed in *H. insignis* (Fig. 3k–n). In both species, glands had uniseriate epidermis covered by cuticle even on the pore region (Fig. 3m, n), and collateral bundles were observed in the parenchyma (Fig. 3d–h, m, n). In mature glands, a periderm occurred (Fig. 3f, h, m), except in the pore region, besides many cells with phenolic compounds. In *H. insignis*, histochemical tests revealed the presence of phenolic compounds and carbohydrates inside the parenchymatous cells located mainly close to the pore, in addition to the presence of secretory canals (Fig. 3d–h, m, n). Field observations allow to detect the presence of a transparent secretion and ants on the prophyll region (Fig. 3i, j). Tests for the detection of glucose

in the secretion were applied using a glucostrip and revealed a positive result between 250 and 500 mg/dl (Fig. 3q).

**Cataphylls in the underground shoot system** – In *H. pumila*, *H. salubris* and *H. suffruticosa*, all geophyte species, the presence of leaves modified in cataphylls was identified in the axillary buds (Fig. 4a, d, g). Cataphylls are small and triangular in *H. pumila* (Fig. 4a) and *H. salubris* (Fig. 4d, e), and larger and apically lobed in *H. suffruticosa* (Fig. 4g, h). In all species, the cataphylls presented uniseriate epidermis covering the parenchymatous tissue with several collateral vascular bundles (Fig. 4b, c, f, i). In *H. pumila*, a periderm on the abaxial surface of older dried cataphylls and secretory canals associated with vascular bundles were observed (Fig. 4a).

**Protective layer of suberized subepidermal cells** – Subepidermal layers containing suberin in its walls were observed in cataphylls of the geophyte species *H. salubris* (Fig. 4e, f) and *H. suffruticosa* (Fig. 4i), in the leaf primordium of *H. salubris* (Fig. 4e–f) and *H. insignis* (phanerophyte species, Fig. 4k), and in the leaf base of the geophyte species *H. salubris* (Fig. 4e–f), *H. suffruticosa* (Fig. 4i) and *H. pumila* (Fig. 4l), and in the phanerophyte species *H. glabra* (Fig. 4j) and *H. insignis* (Fig. 4k).

**Trichomes** – The shoot apex of all species was covered by a large amount of non-glandular filiform trichomes (Fig. 5a–x). In *H. glabra*, trichomes originated primarily on the abaxial surface of leaf primordia, from an epidermal cell larger than the adjacent ones, which divided only transversely, creating a multicellular uniseriate trichome (Fig. 5e–j). During development, the trichomes lost their transverse walls except in rare cases (for example *H. glabra*, Fig. 5j, and *H. salubris*, Fig. 5u), acquired a thick and lignified wall (Fig. 5g) and were covered by cuticle except in the inner periclinal wall of the basal cell (Fig. 5i, j), in which pits were also observed (Fig. 5g, h). Phenolic content was observed inside the fully developed trichomes of all species, in addition to pectic content in *H. glabra* (Fig. 5h).



**Fig. 3** Structure of the glandular prophylls of *H. arenaria* **a–h** and *H. insignis* **i–q**. **a** Prophyll location (arrows). **b** Pore location (white arrowhead) on the surface of prophylls. **c** Stomata located on the prophyll. **d, e** Longitudinal sections of the prophyll, with vascular bundles (white arrowhead) consisting of xylem and phloem. **f, g** Longitudinal section of the prophyll on the pore region (black arrowhead), with periderm (pd). **h** Longitudinal section showing a pair of adjacent prophylls, with periderm (pd). **i, j** Prophylls (one of them pointed by the arrow) visited by ant. **k** Prophylls' surface, with pore (black arrowhead). **l** Detail of prophyll pore (black arrowhead). **m, n** Cross sections of the prophyll in pore region (black arrowhead), with a positive reaction to Sudan Black B in the cuticle (blue), vascular bundles (white arrowheads) and secretory canals (\*). **o** Phenolic compounds indicated by the ferric chloride test (dark brown cell content, white star). **p** Carbohydrates indicated by the PAS test, with positive reaction (red cell content, black star). **q** Positive result for glucose (glucostrip). Scale bars: 500  $\mu\text{m}$  in **d, h, k**; 200  $\mu\text{m}$  in **b, f, m**; 100  $\mu\text{m}$  in **e, g, n**; 20  $\mu\text{m}$  in **l, p, o**; 5  $\mu\text{m}$  in **c**. Scanning electron microscopy: **b, c, k, l**

**Colleters** – Colleters originated exclusively from the protoderm were observed on the adaxial surface of the leaf primordia in *H. glabra* (Fig. 6a–k) and *H. salubris* (Fig. 6l–n), and rarely at the margin of the leaf primordia in *H. suffruticosa* (Fig. 6o–q). Colleters were also observed in the basal region of the adaxial face of *H. pumila* cataphylls (Fig. 6r–t). In *H. glabra*, colleters originated asynchronously from a protodermal cell larger than the adjacent ones (Fig. 6c), which divides sequentially into several planes (Fig. 6c–e). The fully developed colleters has a short peduncle and a large head that varies from globose to globose-elongated, both multicellular (Fig. 6f). Histochemical tests carried out indicated the presence of a thin cuticle covering the peripheral cells of the peduncle and head (Fig. 6g), in addition to starch grains (Fig. 6h) and protein content (Fig. 6i) inside cells of the head. The secretion observed occupying the subcuticular and intercellular spaces did not react positively to the tests performed (Fig. 6f, g, j, k), and it was not possible to characterize its chemical nature.

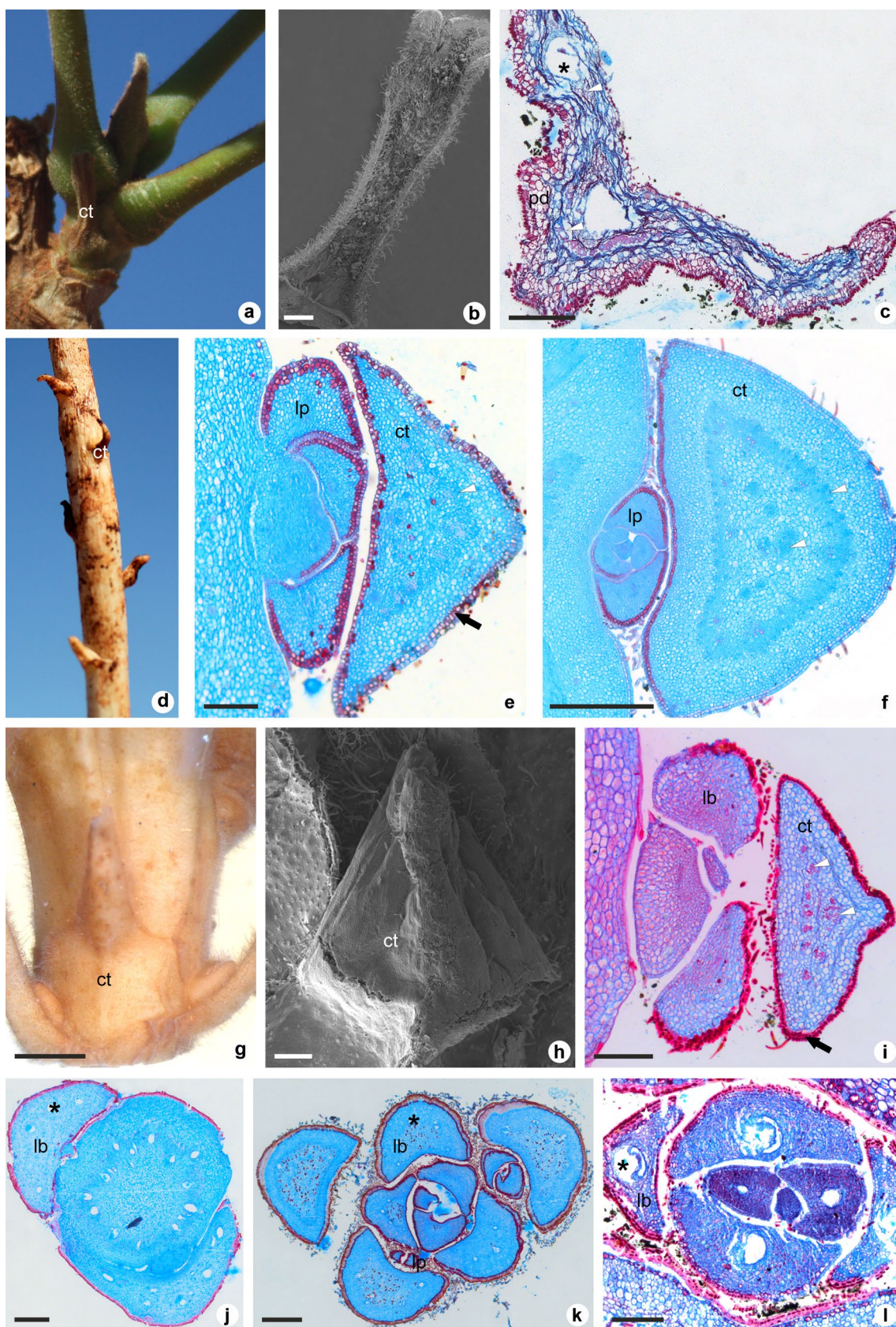
**Secretory canals** – In the leaf primordia of *H. glabra*, *H. pumila* and *H. suffruticosa*, conspicuous secretory canals

associated with vascular tissue were observed (Fig. 7a–r). The canal development was first identified in the central region of the primordia by vascular cells that were larger and had an apparently denser cytoplasm than the adjacent, some of which were binucleated probably related with the mitotic process (Fig. 7b, c). During development, it was possible to observe the presence of spaces in the central region of the canal (Fig. 7d), in addition to the differentiation of phloem and xylem conductive cells adjacent to the canal (Fig. 7e, f). The central cells of the canal increased in size and acquired hyaline cytoplasm, typical characteristics of cell degradation (Fig. 7f–i). With the development of the primordium and the increase in size of the space, the various layers of secretory cells became flattened (Fig. 7j–o). It was interesting to note the occurrence of cells with meristematic characteristics in the apical region of the leaf primordium, in continuity with the canal and in contact with epidermal cells (Fig. 7o).

## 4 Discussion

The reduced prophylls with apical gland seen in *H. arenaria* and *H. insignis* and the cataphylls observed in *H. salubris*, *H. pumila* and *H. suffruticosa* are leaf modifications present in the region of the axillary buds. As expected for leaves of *Homalolepis* and some other Simaroubaceae genera (e.g., Engler 1874; 1931; Clayton 2011; Devecchi et al. 2018a), glandular structures occur in the apical region. Therefore, in these two species, the first pair of leaves generated from the axillary bud does not show complete development, but a differentiation of the apical region with a gland and a reduced leaf base region.

Morphologically, these glands are similar to those commonly found in Passifloraceae and according to the classification for extrafloral glands of *Passiflora* species (Lemos et al. 2017), the apical glands of the prophylls are spheroidal. The in situ detection of carbohydrates corroborates the positive result observed in the test carried out in the field with a glucostrip, which was also observed for the secretion of the leaf apex, indicating its nectariferous nature as reported for





**Fig. 4** Structure of leaf primordia (lp) and cataphylls (ct) of *H. pumila* **a–c**, *H. salubris* **d–f**, *H. suffruticosa* (**g–i**), *H. glabra* (**j**), and *H. insignis* **k**. **a** Position of the cataphyll. **b** Detail of the adaxial surface of the cataphyll, with basal glandular trichomes (black arrowhead) and non-glandular trichomes on both surfaces. **c** Cross section of the cataphyll, with secretory canals (\*) and vascular bundles (white arrowhead). **d** Position of the cataphyll. **e, f** Cross section of the cataphyll, showing the position in relation to the leaf primordia and the suberized subepidermal cells (arrow). **g** Position of the cataphyll. **h** Abaxial surface of the cataphyll, which has a broad base and a narrow apex. **i** Cross section of the cataphyll, showing the position in relation to the leaf primordia and the suberized subepidermal cells (arrow). **j–l** Cross sections of shoot apices showing the protective layer of suberized cells in leaf primordia (lp) and leaf base (lb), in addition to secretory canals (\*). Scale bars: 2 mm in **g**; 500  $\mu$ m in **f, b, j, k**; 200  $\mu$ m in **c, e, h, i, l**. Scanning electron microscopy: **b, h**

most *Homalolepis* species (Devecchi et al. 2018a,b, Devecchi et al. in prep.).

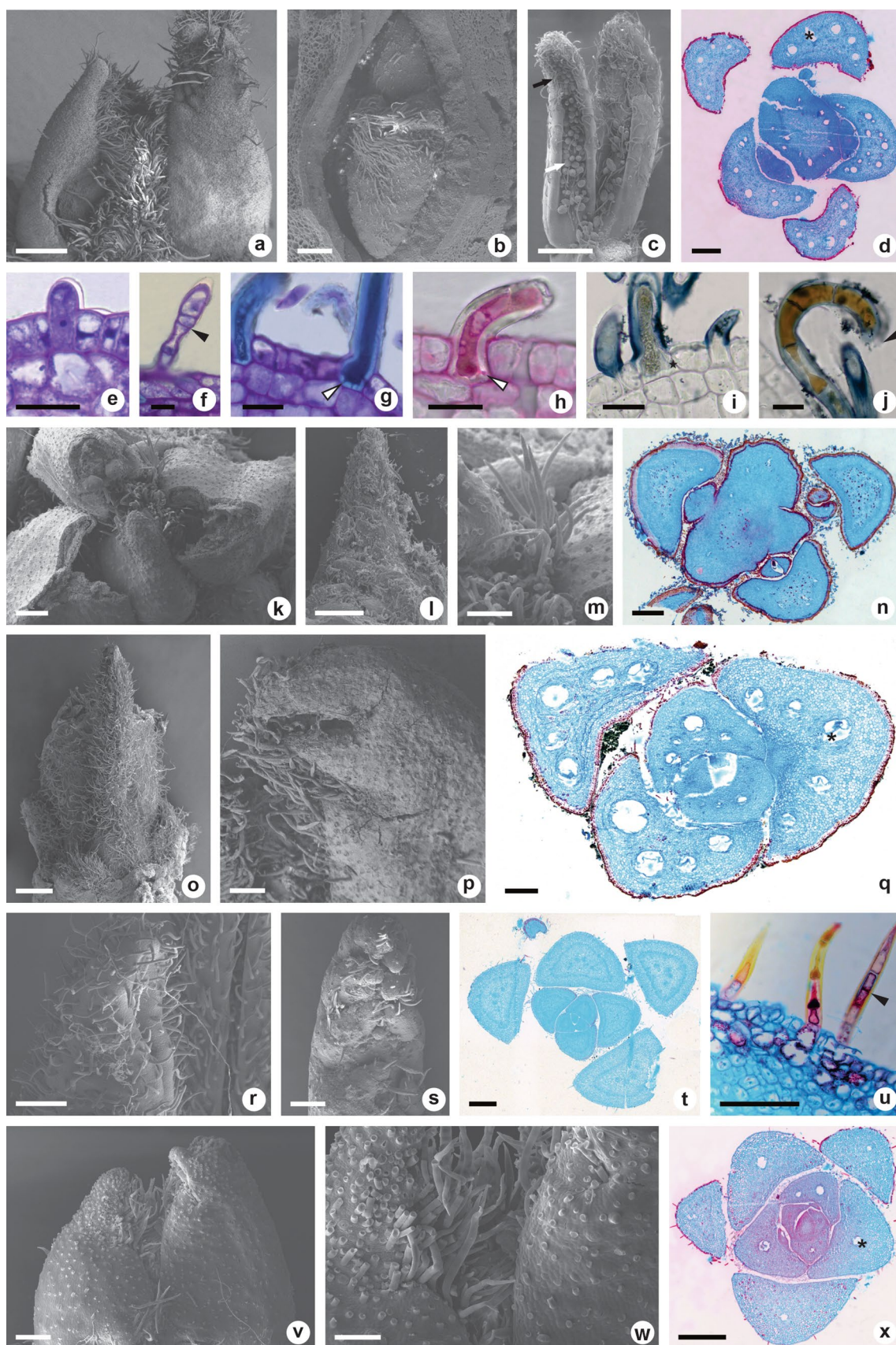
Considering field observations and laboratory results, we can conclude that the apical glands of the reduced prophylls found in *H. insignis* exude sugars. In different plant groups, the presence of exudate from the extrafloral nectary attracts arthropods, mainly ants, which protect the plant against herbivores by eliminating them through bites and stings (Douglas 1983; Suzuki 2004; Heil 2011). In a comparative study of ant-plant-herbivore relationships between the Brazilian “Cerrado” and other domains, Oliveira and Freitas (2004) concluded that the occurrence of rewards from leaves has a very important role in determining a foraging ecology with ants. Future studies may yet clarify whether the presence of these arthropods attracted by prophylls alter the production of secondary compounds that make the plant less palatable, as observed by Douglas (1983) in ferns, or if the number of insects that visit flowers is reduced, interfering with reproduction, as observed by Wagner and Kay (2002).

Structures morphologically similar to the reduced prophylls of *H. insignis* and *H. arenaria* occur in other species of the genus, as *H. pohliana* (Boas) Devecchi & Pirani, *H. trichilioides* (A.St.-Hil.) Devecchi & Pirani, *H. cedron* (Planch.) Devecchi & Pirani, *H. cavalcantei* (W.W. Thomas

Devecchi & Pirani and *H. rigida* Devecchi (pers. obs. Marcelo Devecchi), which have a similar habit, known as palm-like treelets, which emerged in two clades in the phylogeny. The analysis of the presence of reduced prophylls with apical gland based on the phylogeny obtained by Devecchi et al. (2018a) seems to indicate a relationship of the type of habit with the presence of reduced prophylls, and it is also possible to infer that these structures may have evolved at least twice in the family. Studies that map this character can bring new information about its evolution in the group and its ecological role.

Another important feature observed in the leaf primordium, cataphylls and leaf base is the differentiation of subepidermal suberized layers, and an additional protective tissue, the periderm, formed immediately below the suberized hypodermis in *H. pumila*. The periderm is formed from the phellogen, a secondary meristematic tissue, and consists of three parts: phellogen (the cork cambium); phellogen (parenchymatous cells derived centripetally from the phellogen) and phellem, a suberized cell layer produced centrifugally from the phellogen (Esau 1953; Fahn 1982). The protective characteristic of the hypodermis and phellem by the presence of suberin in their walls may consist of an important tissue layer that protects against biotic agents, such as herbivory, and abiotic agents, such as ultraviolet radiation (Kulbat 2016; Campilho et al. 2020) and even resistance to water loss (Braue et al. 1983; Campilho et al. 2020). Since the analyzed specimens of *Homalolepis* are subject to these different biotic and abiotic factors, we can consider that the early development of a protective tissue in the first leaf primordia could be part of the protection strategy in both phanerophyte and geophyte species.

The six species of *Homalolepis* analyzed presented great quantities of filiform non-glandular trichomes covering the apical region of the shoot and both surfaces of leaf primordia, which is described by Lubbock (1899) as part of the protection strategy of the apical shoot meristem. According to the author, the main importance of these trichomes is to enable the occurrence of a region that is very effective at



**Fig. 5** Structure of the shoot apices of *H. arenaria* **a**, *H. glabra* **d–l**, *H. insignis* **m–p**, *H. pumila* (**q–s**), *H. salubris* (**t–w**), and *H. suffruticosa* (**u–w**). **a** Detail of the surface of shoot apex, with a large amount of non-glandular trichomes. **b, c** Detail of the surface of shoot apex, with a large amount of non-glandular (black arrow) and glandular (white arrow) trichomes. **d** Cross section of the shoot apex, showing the organization of leaf primordia. **e, f** Successive stages of development of non-glandular trichomes. **g** Fully developed non-glandular trichome, with a thick and lignified cell wall and phenolic content. **h** Fully developed non-glandular trichome, with a thick cell wall and pectic content. **i, j** Completely developed non-glandular trichomes, with a thick cuticle-coated cell wall (stained in black), except for the basal region (star). **k–m** Detail of shoot apex surface, with a large amount of non-glandular trichomes. **n** Cross section of shoot apex, showing the organization of leaf primordia. **o, p** Detail of shoot apex surface, with a large amount of non-glandular trichomes. **q** Cross section of shoot apex, showing the organization of leaf primordia. **r, s** Detail of shoot apex surface, with a large amount of non-glandular trichomes. **t** Cross section of shoot apex, showing the organization of leaf primordia. **u** Fully developed multicellular non-glandular trichome, with a thick cell wall and phenolic content. **v, w** Detail of shoot apex surface, with a large amount of non-glandular trichomes. **x** Cross section of shoot apex, showing the organization of leaf primordia. Scale bars: 500  $\mu\text{m}$  in **a, c, d, l, n, o, t, x**; 200  $\mu\text{m}$  in **b, k, q, r, s, v**; 100  $\mu\text{m}$  in **m, p, w, u**; 20  $\mu\text{m}$  in **e, f, g, h, i, j**. Symbols: \* = secretory canals;  $\blacktriangleright$  = transverse cell wall;  $\blacktriangleright$  = pits. Scanning electron microscopy: **a–c, k–m, o, p, r, s, v, w, x**

not conducting heat, and thus important against desiccation. In addition, several studies have shown a positive correlation between trichome density and decreased herbivory rates (Agrawal 1999; Handley et al. 2005; De-la-Cruz et al. 2020; Kaur and Kariyat 2020), which is important especially in the early stages of development of plant organs.

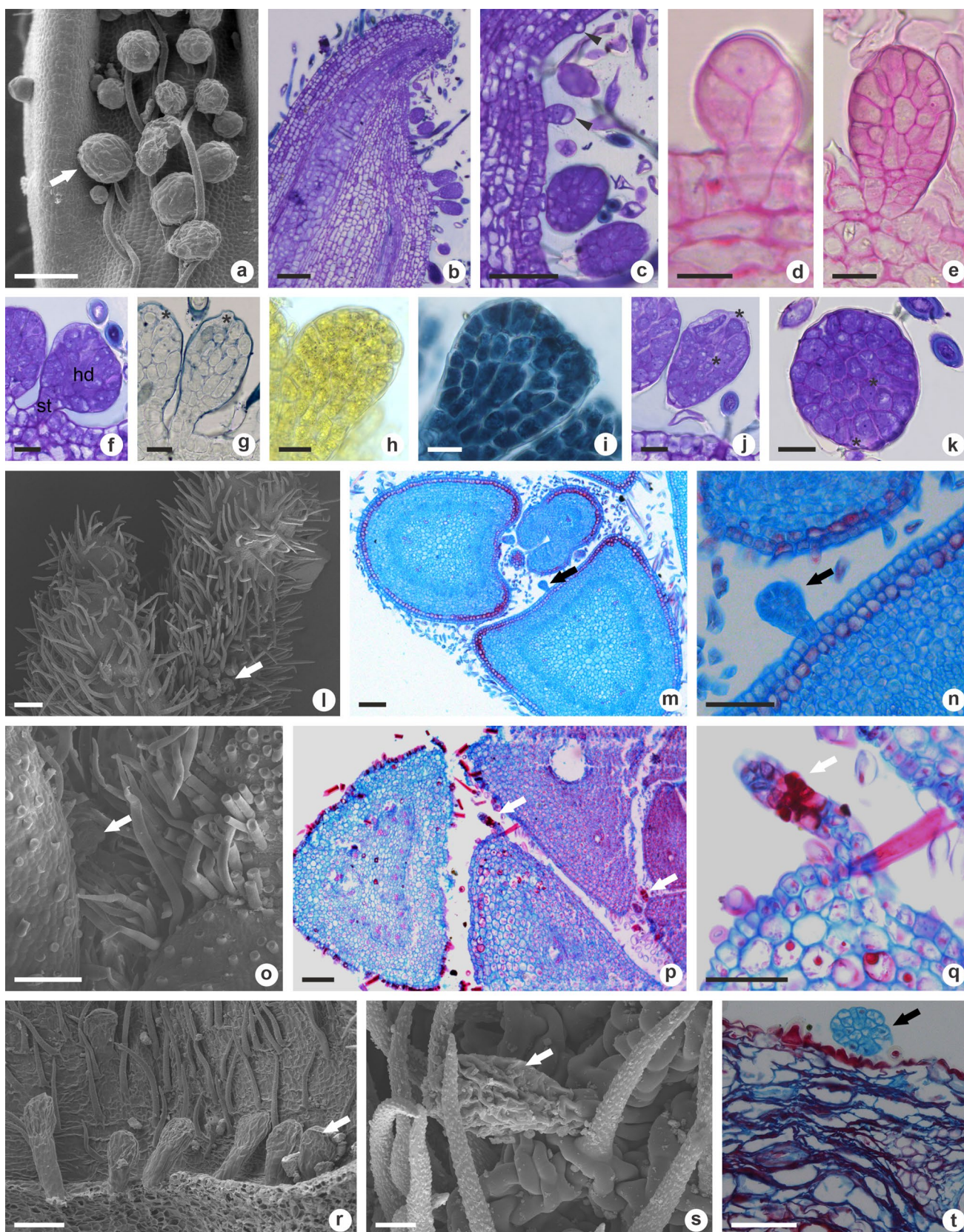
In addition to the physical protection provided by the indumentum, several classes of compounds are effective in chemical protection. Although the presence of chemical compounds inside the trichomes is more commonly described for glandular trichomes, phenolic compounds in non-glandular trichomes such as those observed in *Homalolepis* are reported for some species, associated with protection against ultraviolet radiation and heating, as well as repellents of phytophagous insects (Skaltsa et al. 1994; Karioti et al. 2011; Koudounas et al. 2015). The presence of pectin in the cytoplasm of these trichomes observed in *H. glabra* also may be related to the maintenance of a humid environment in young leaf organs, as described by Lusa et al. (2015) for *Lychnophora diamantinana* Coile & Jones (Asteraceae), since this class of carbohydrate is recognized for its

hygroscopic property. Besides to functioning as a physical barrier, such trichomes can remain biochemically active in the production of chemical compounds even when mature, being able to release the compounds actively, similar to what occurs in glandular trichomes (Lusa et al. 2015; Tozin et al. 2016). Furthermore, the release of compounds could also occur passively, after the trichomes are mechanically damaged, which still needs further investigation.

Another equally interesting character that was observed in phanerophyte (*H. glabra*) and geophytes (*H. pumila*, *H. salubris* and *H. suffruticosa*) species occurring in the “Cerrado” concerns glandular trichomes restricted to the apical region of the shoot and its leaf primordia, in addition to the basal region of the cataphylls. Multicellular secretory structures located mainly in the adaxial region of young vegetative and reproductive organs, specialized in mucilage secretion among other sticky substances and with activity restricted to the early stages of development are classified as colleters (Fahn 1979; Thomas 1991; Klein et al. 2004; Miguel et al. 2006; Paiva 2012; Coutinho et al. 2015).

The record of colleters in Sapindales is currently restricted to few species, including four species of Anacardiaceae (Lacchia et al. 2016), one species of Meliaceae (Tilney et al. 2018)—although Fisher and Rutishauser (1990) reported multicellular structures resembling colleters for another species—and two species of Rutaceae (Macêdo et al. 2016). The protodermal origin of colleters was described for Anacardiaceae (Lacchia et al. 2016) and Rutaceae (Macêdo et al. 2016) besides Simaroubaceae (this study), and broader studies are encouraged to verify if this is a pattern for the order. The number of species bearing colleters is probably underestimated, since due to their protodermal origin, colleters in Sapindales may historically have been interpreted as typical glandular trichomes, but especially because most of the studies were restricted to mature leaves, at which stage of development the colleters are no longer present. Furthermore, more comprehensive histochemical studies are needed to verify the chemical nature of the secretion observed in these structures in *Homalolepis*, since the protective function against desiccation of developing meristematic tissues is closely linked to the compound exuded by the colleters, which may vary within taxonomic groups (Thomas 1991).

The presence of internal secretory structures in leaf primordia, such as the secretory canals of *Homalolepis* sect.



**Fig. 6** Structure of the glandular trichomes of *H. glabra* (a–k), *H. salubris* (l–n), *H. suffruticosa* (o–q), and *H. pumila* (r–t). **a–b** Position of the trichomes, on the adaxial surface of leaf primordia (arrow). **c–f** Successive stages of development of trichomes from leaf primordia (arrowheads) to the fully developed stage, with head (hd) and stalk (st). **g–k** Fully developed trichomes from leaf primordia. **g** Thin cuticle on the surface, with accumulated secretion in subcuticular and intercellular spaces (\*). **h** Starch grains inside the cells. **i** Protein content inside cells of the head. **j–k** Accumulated secretion in subcuticular and intercellular spaces (\*). **l–n** Position of glandular trichomes (arrows) on the adaxial surface of the base of leaf primordia. **o–q** Position of glandular trichomes (arrows) at the intersection between adaxial and abaxial surfaces of the leaf primordium. **r–t** Position of glandular trichomes, on the adaxial surface of the base of cataphylls. Scale bars: 100  $\mu\text{m}$  in **a, b, l, m, o, p, r**; 50  $\mu\text{m}$  in **c, n, q, t**; 20  $\mu\text{m}$  in **e, f, g, h, i, j, k, s**; 10  $\mu\text{m}$  in **d**. Scanning electron microscopy: **a, l, o, r, s**

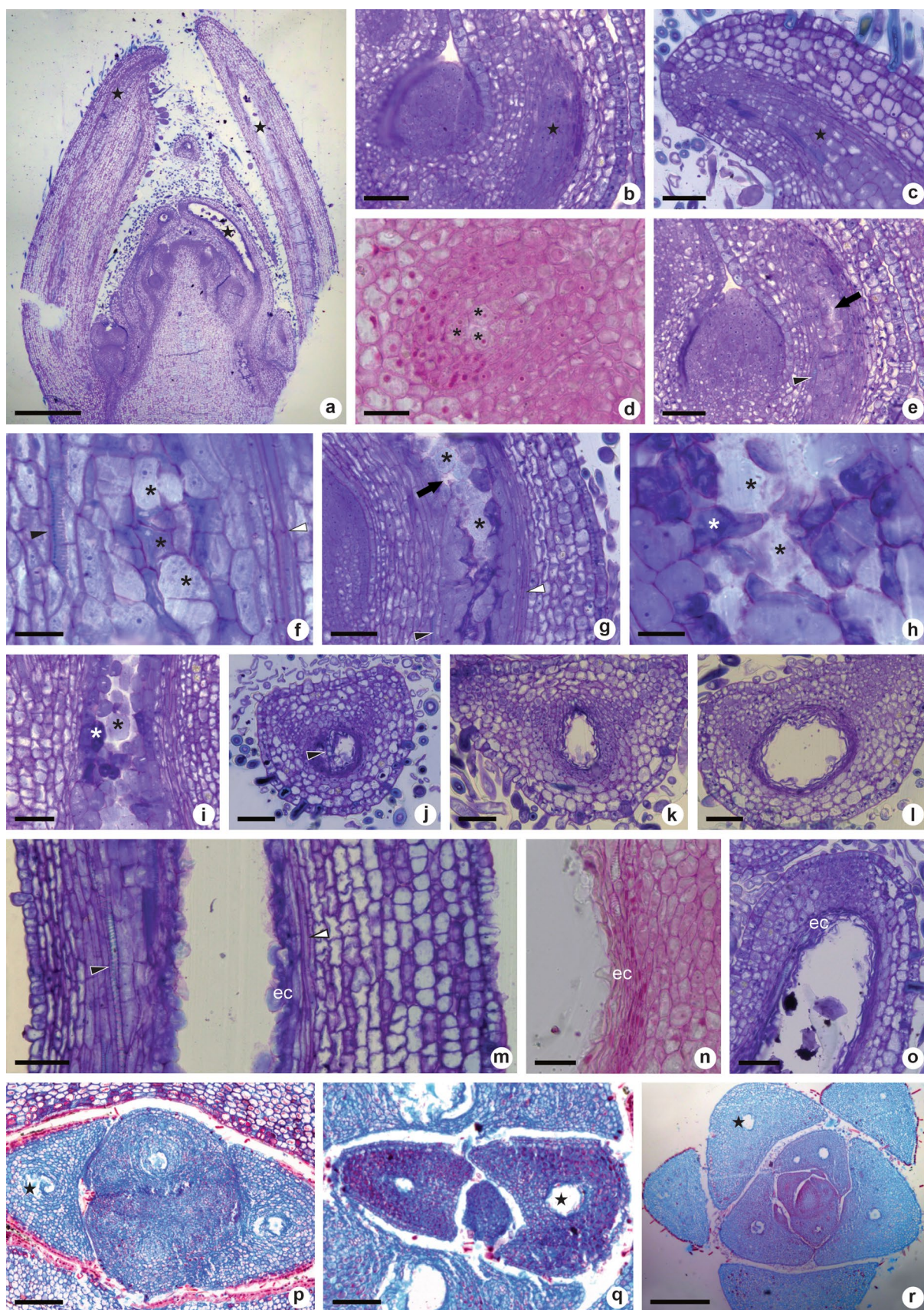
*Homalolepis* species can reinforce the protection against phytophagous insects attack as demonstrated for mature leaves (Dussourd and Denno 1991). Although rarely reported in the literature, the early development of canals still in the leaf primordium stage, in some cases associated with developing vascular tissues, was also observed in *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae, Palermo et al. 2018), a South American species of Sapindales, and in species from other families including Asteraceae (Carlquist 1957) and Leguminosae (Rodrigues et al. 2011).

Secretory canals are relatively common in Simaroubaceae, reported for *Ailanthus*, *Amaroria*, *Brucea*, *Hannoa*, *Homalolepis*, *Mannia* (= *Pierreodendron*), *Odyndea*, *Perriera*, *Picrasma*, *Picrolemma*, *Simaba* and *Simarouba* species, occurring on stem, leaf, inflorescence axis, and flower and fruits parts, often associated with vascular bundles (Boas 1913; Fernando and Quinn 1992; Alves 2015). In Sapindales, secretory canals or ducts occur in

Anacardiaceae + Burseraceae and Simaroubaceae + Meliaceae clades, in vegetative and reproductive organs of many species (Dünisch and Baas 2006; Bachelier 2009; Lacchia and Carmello-Guerreiro 2009; Souza et al. 2016; Palermo et al. 2018; Mashimbye et al. 2020; Tölke et al. 2021), commonly producing resinous exudates (Metcalf and Chalk 1957; Langenheim 2003; Prado and Demarco 2018). Among Simaroubaceae, resin-producing species are described only in the Asian genus *Ailanthus*, with emphasis on *A. altissima* (Mill.) Swingle, used as incense in religious rituals (Langenheim 2003; Shi et al. 2011), but there is no information about the type of structure producing this substance.

The presence of trichomes in all *Homalolepis* species analyzed does not seem to indicate a strategy related to life habit or even to the environment in which these plants occur, since they were observed in the geophyte species occurring in the “Cerrado” (*H. pumila*, *H. salubris* and *H. suffruticosa*), where this condition may be related to protection against desiccation or even to mechanical protection of the apex, which is often found below or at ground level, but also occur in phanerophyte species of the Atlantic Forest (*H. arenaria* and *H. insignis*) and “Cerrado” (*H. glabra*).

Still under an evolutionary context, the comparative analysis between phanerophyte species showed that *H. glabra* has colleters and *H. insignis* does not. This is quite interesting, since from our initial hypothesis, we would expect to find similar strategies related to similar habits. The two lineages detected in the phylogeny of the genus presented by Devecchi et al. (2018a) were described as two sections (Devecchi et al. 2018b), with *H. glabra* in the typical section while *H. insignis* is found in *Homalolepis* sect. *Grandiflorae*. In that case, as these clades diverged a long time ago, it is possible that such a change in the protection strategy of the apical meristem has occurred.



**Fig. 7** Anatomy of the secretory canals of leaf primordia of *H. glabra* (a–o), *H. pumila* (p, q), and *H. suffruticosa* (r). **a** Longitudinal section of shoot apex, showing secretory canals (star). **b, c** Longitudinal sections of leaf primordia with secretory canals in early stages of development, consisting of meristematic cells larger than the adjacent ones (stars). **d** Cross section of a leaf primordium, showing central cells of the secretory canal (\*), larger than the adjacent cells and delimiting a small central space. **e–i** Longitudinal sections of leaf primordia in successive stages of development, showing the increase in size of the central space of the secretory canal (arrow) and the cells in various stages of degeneration (\*). **j–l** Longitudinal sections of leaf primordia, showing the secretory canal in an advanced stage of development. **m–o** Longitudinal sections of the leaf primordia, showing the fully developed secretory canals, with a large central space and the surrounding epithelial cells (ec). **p–r** Cross sections of shoot apex, showing central secretory canals (★) in the leaf primordia. Scale bars: 500 µm in a, r; 200 µm in d, p; 100 µm in q; 20 µm in f, h, n; 50 µm in b, c, e, g, i, j, k, l, m, o. Symbols: ► = conducting cells of the xylem; ▷ = conducting cells of the phloem

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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