

## RESEARCH PAPER

# Morphological patterns of extrafloral nectaries in woody plant species of the Brazilian *cerrado*

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## Keywords

Ant-plant mutualism; *cerrado*; extrafloral nectary; nectary anatomy; savanna.

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## ABSTRACT

Extrafloral nectaries are nectar-secreting structures that are especially common among the woody flora of the Brazilian *cerrado*, a savanna-like vegetation. In this study, we provide morphological and anatomical descriptions of extrafloral nectaries (EFNs) occurring on vegetative and reproductive organs of several plant species from the *cerrado*, and discuss their function and ecological relevance. We describe the morphology and anatomy of EFNs of 40 species belonging to 15 woody families using scanning electron microscopy and light microscopy. We categorise EFNs following a structural-topographical classification, and characterise the vascularised and complex nectaries, amorphous nectaries and secretory trichomes. Fabaceae, Bignoniaceae, Malpighiaceae and Vochysiaceae were the plant families with the majority of species having EFNs. Ten species possess more than one morphotype of gland structure. Observations and experimental field studies in the *cerrado* support the anti-herbivore role of EFN-gathering ants in this habitat. Additional morphological studies of EFNs-bearing plants, including other growth forms (e.g. herbs and lianas), are being undertaken and will hopefully cast further light on the ecological relevance of these glands in the *cerrado*, especially with respect to their attractiveness to multiple visitors.

## INTRODUCTION

Extrafloral nectaries (EFNs) occur in 93 flowering plant and five fern families and can be abundant among the flora of many habitats worldwide, especially in the tropics (Bentley 1977; Koptur 1992; Oliveira & Freitas 2004). For instance, plants bearing such glands account for 33% of the species (woody and climbing plants) in the rain forest of Barro Colorado Island in Panama (Schupp & Feener 1991), and for 12% of the woody species in a West Malaysian rain forest (Fiala & Linsenmair 1995). In different vegetation types in the Brazilian Amazon, 18–53% of the woody species possess EFNs, comprising up to 50% of the local individuals (Morellato & Oliveira 1991). These nectar-secreting glands are structurally diverse and occur on virtually all aboveground plant organs; they are especially common on the leaf blade, petiole, young stems, stipules and on reproductive structures such as

buds, calyx, inflorescence axis, flower peduncles and fruits (Elias 1983; Oliveira & Leitão-Filho 1987; Koptur 1992; Díaz-Castelazo *et al.* 2004, 2005).

Contrary to floral nectaries that attract and reward vertebrate and invertebrate pollinators, EFNs are especially attractive to ants and a variety of other nectar-foraging insects such as wasps, bees, flies and beetles, and more rarely spiders and mites (e.g. Oliveira & Brandão 1991; Koptur 1992; Pemberton & Lee 1996; Kawano *et al.* 1999; Ruhren & Handel 1999; Cuautle & Rico-Gray 2003). Numerous experimental field studies have shown that aggression towards herbivores by EFN-gathering insects, most especially ants, can positively affect plant fitness by decreasing herbivore damage (e.g. Bentley 1977; Koptur 1984; Rico-Gray & Thien 1989; Oliveira *et al.* 1999; Cuautle & Rico-Gray 2003; Rudgers & Gardener 2004; Wäckers & Bonifay 2004). Plant protection, however, is not universal and ant visitation to EFNs may afford no appar-

ent benefit to plants (O'Dowd & Catchpole 1983; Rashbrook *et al.* 1992). Field experiments performed in the Brazilian *cerrado*, a savanna-like vegetation, show that ants visiting EFNs may prey on and/or attack insect herbivores on plants and significantly reduce herbivore damage to leaves, buds or flowers. Ant-derived benefits to plants, however, may vary with the species of visiting ant, with the defensive tactics of associated herbivores, as well as with the plant species (Costa *et al.* 1992; Del-Claro *et al.* 1996; Freitas & Oliveira 1996; Oliveira 1997; Oliveira & Freitas 2004).

The ecological importance of the EFNs has motivated many authors to study the morphology, distribution and frequency of these structures in different plant taxa (*e.g.* Zimmerman 1932; Bentley 1977; Metcalfe & Chalk 1979; Elias 1983; Oliveira & Leitão-Filho 1987; Oliveira & Oliveira-Filho 1991; Morellato & Oliveira 1994; McDade & Turner 1997), as well as the composition of their nectar and its role in insect attraction (Koptur 1992). While there is increasing understanding of the evolutionary significance of EFNs in tropical communities, ecological studies of ant-plant mutualisms have until recently considered only the distribution of these structures among plant organs, neglecting their morphology. According to Díaz-Castelazo *et al.* (2005), the effect of EFN attributes such as gland structure, distribution and secretion on preference by ants and in the structure of the associated ant community needs further investigation.

Floristic surveys carried out by Oliveira & Leitão-Filho (1987) in five areas of *cerrado* in Southeast Brazil revealed that 34 woody species from 15 families have EFNs, accounting for up to 20% of the species within each local woody flora. Plant species had EFNs associated with vegetative (leaves, stems) and/or reproductive organs (bud or flower). The Leguminosae, Bignoniaceae and Vochysiaceae were the families most frequently having such glands (see also Oliveira & Oliveira-Filho 1991). Anatomical data of EFNs for *cerrado* plant species are practically non-existent (Paiva *et al.* 2001; Paiva & Machado 2006a,b,c), although general morphological information on the glands of certain genera is available (Elias 1983; Lersten & Brubaker 1987; Koptur 1992; Lersten & Curtis 1994; Pascal *et al.* 2000; Díaz-Castelazo *et al.* 2005).

In the current paper we describe the morphology and anatomy of the EFNs present in 40 woody species from the Brazilian *cerrado*, and discuss their function and ecological relevance. We categorise the glands following the structural and topographical system originally proposed by Zimmerman (1932) and Elias (1983).

## MATERIALS AND METHODS

The Brazilian *cerrado* covers nearly 2 million km<sup>2</sup>, representing ca. 22% of the country's land surface (Oliveira & Marquis 2002). The biome is extremely variable in physiognomy and ranges from open grassland to forest with a discontinuous grass layer. A continuum of savanna formations lies between these two extremes and spans the

entire range of woody plant density; the whole biome is collectively referred to as the *cerrados* (Oliveira-Filho & Ratter 2002). Material was collected in different areas of *cerrado sensu stricto* (*i.e.* dense scrub of shrubs and trees) in the state of São Paulo, Southeast Brazil. Voucher specimens are deposited in the herbarium of the Universidade Estadual de Campinas (UEC), and the herbarium of the Universidade Estadual Paulista, Instituto de Biociências, Botucatu (BOTU).

We fixed the samples in FAA 50 (Johansen 1940) and preserved in 70% alcohol. For scanning electron microscopy (SEM) studies, the samples were dehydrated with a graded ethanol series; critical-point dried using CO<sub>2</sub>, gold-coated and examined using a Philips 515 SEM at 20 kV. For light microscopy, the material was dehydrated with a graded ethanol series, infiltrated and embedded in glycol-methacrylate historesin, as per standard methods. The sections (6–8 µm thick) were stained with toluidine blue (O'Brien *et al.* 1964). The following histochemical tests were performed: PAS reaction for neutral polysaccharides; ruthenium red for pectins and mucilage (Jensen 1962); Sudan IV for lipids; and 10% aqueous ferric chloride to detect phenolic compounds (Johansen 1940). The nectaries were characterised following the structural and topographical classification originally proposed by Zimmerman (1932) and Elias (1983), and the vascularised and complex nectaries, amorphous nectaries and secretory trichomes were also characterised. In addition to the EFN, we also examined all species for the presence of other secretory structures such as glands, glandular trichomes and secretory spaces. These structures were characterised by their structural-topographical features. Families were organised according to the APGII classification for the families of the flowering plants (Angiosperm Phylogeny Group 2003).

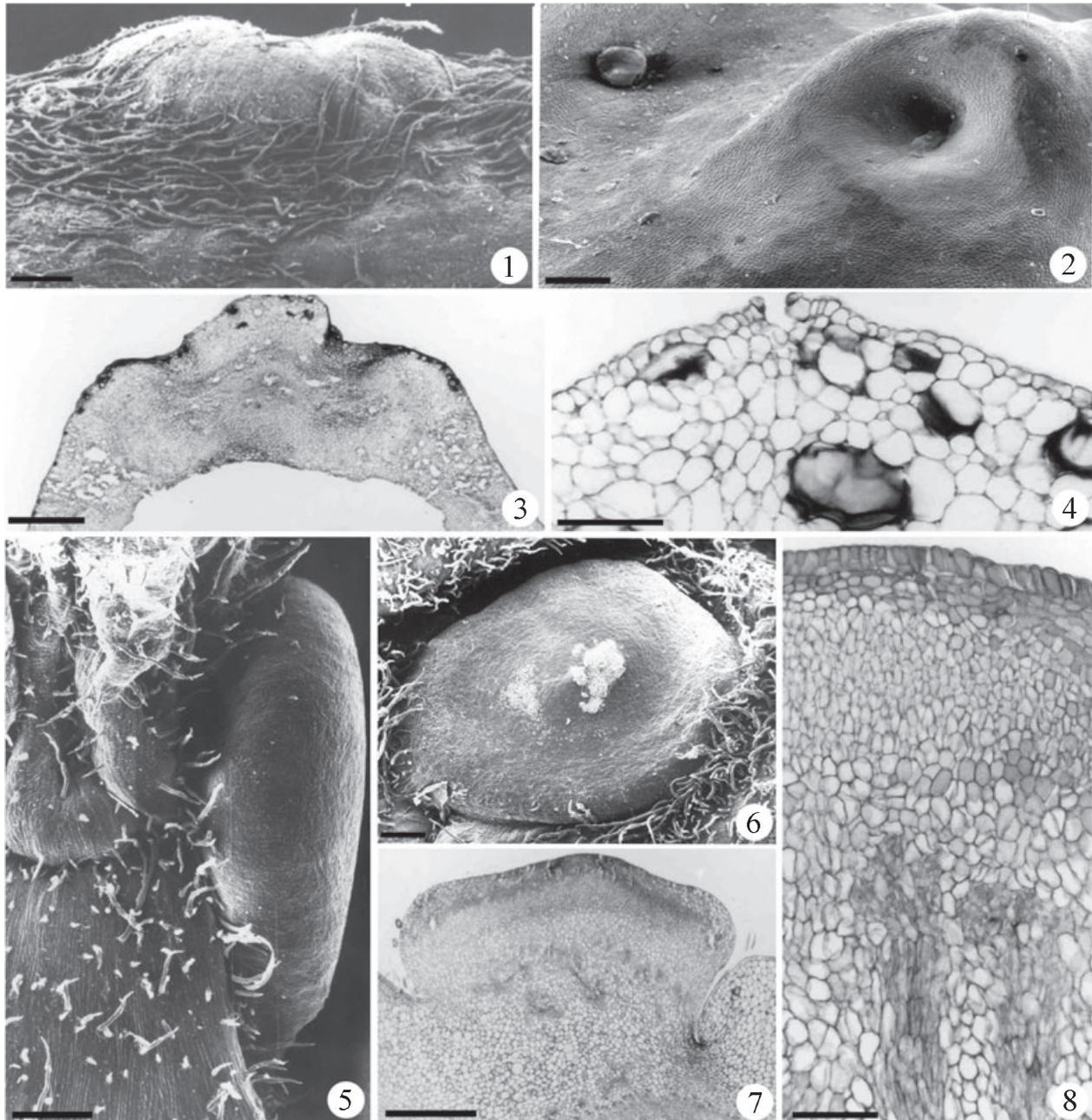
## RESULTS AND DISCUSSION

### Descriptions of extrafloral nectaries (EFNs)

#### *Rosids*

Combretaceae (Myrtales): *Terminalia argentea* and *T. brasiliensis* have slightly elevated nectaries on both sides of the distal part of the petiole; the nectaries are surrounded by non-secretory trichomes (Fig. 1). Stomata with wide pores occur scattered on the nectary surface. In longitudinal section, the nectary has a one-layered epidermis covered by a thick cuticle and a multilayered subepidermal region of compactly arranged parenchymatous cells vascularised by phloem elements. Phenolic compounds, detected by ferric chloride treatment inside the vacuole and druse inclusions, are abundant in the cells of the subepidermal region.

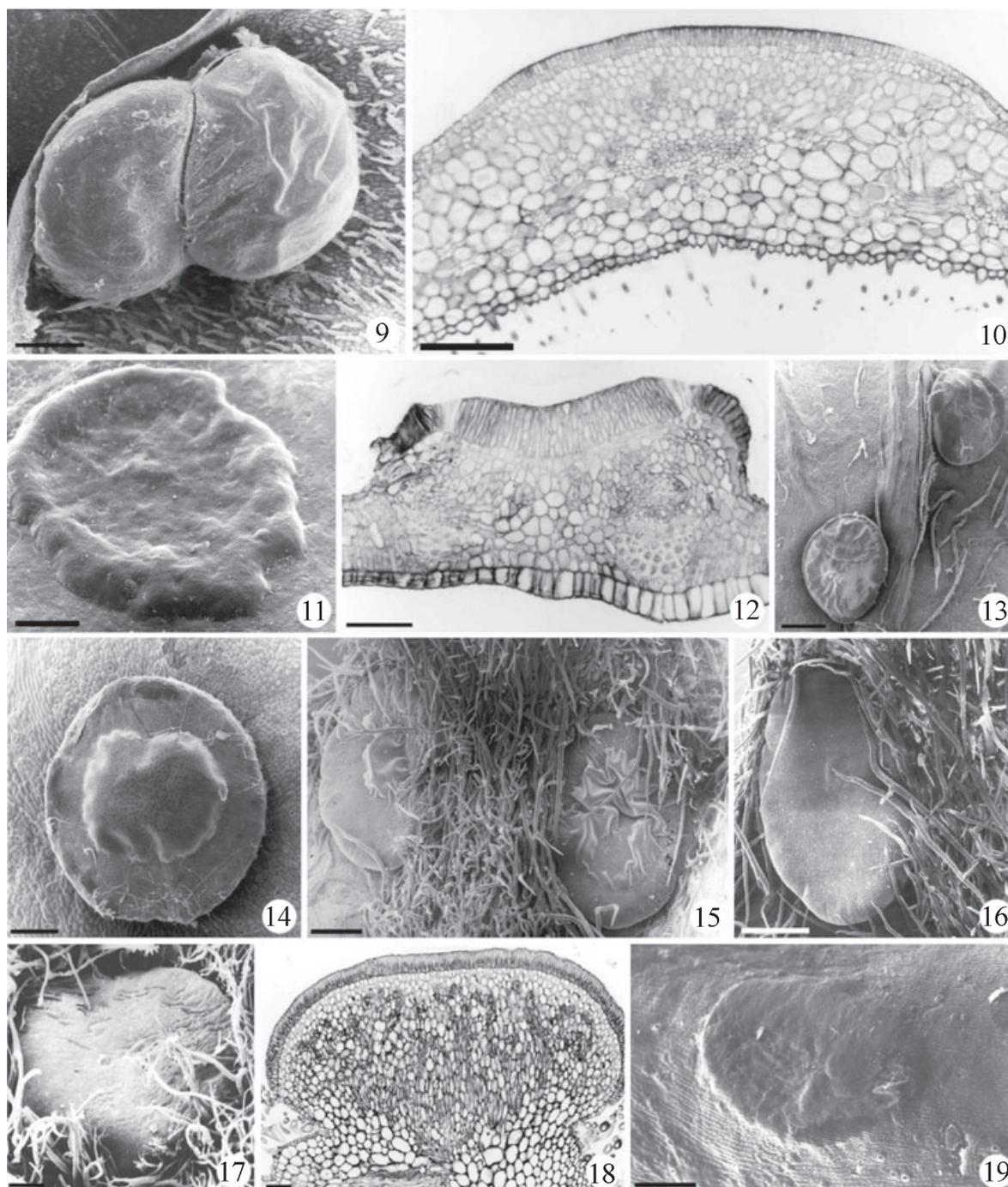
Lythraceae (Myrtales): *Lafoensia paccari* has a pit nectary with a prominent border and a central cavity extending to the surface (Fig. 2). This nectary is located at the apex of the leaf blade and is surrounded by several small scattered glands. In cross section, the pit nectary is



**Figs. 1–8.** EFN morphotypes. 1. Elevated nectary in *Terminalia argentea*. 2–4. Pit nectary in *Lajoensia paccari*. 2. Notice small glands at the nectary base. 3. Nectary transverse section. 4. Stomata at the nectary apices and idioblasts of mucilage in the parenchyma tissue. 5–8. *Qualea grandiflora* nectaries. 5. Lateral view of one of the paired elevated nectaries at the nodal position. 6. Frontal view of one of the paired nectaries showing secretion discharge through a central pore. 7. Petiole longitudinal section showing one of the paired elevated nectaries. 8. Detail of the nectary showing one distinctive subepidermal region formed by parenchyma cells compactly arranged, and phloem clusters in the parenchyma tissue. Scale bar: 1 = 120  $\mu\text{m}$ , 2 = 140  $\mu\text{m}$ , 3 = 100  $\mu\text{m}$ , 4 = 30  $\mu\text{m}$ , 5 = 300  $\mu\text{m}$ , 6 = 220  $\mu\text{m}$ , 7 = 100  $\mu\text{m}$ , 8 = 30  $\mu\text{m}$ .

covered by a one-layered epidermis and a thin cuticle. Its subepidermal region is formed by parenchymatous cells, some of them with mucilaginous contents, as revealed by toluidine blue staining and confirmed by the ruthenium red treatment, and is devoid of conducting elements. The

stomata are projected and their pore is apparently always open (Figs 3–4). Pit EFNs are reported for some Eurosidiae II- Malvales (Malvaceae–Malvoideae and Tiliidae) and for some core eudicots of Polygonaceae (Caryophyllales) and Euasterids I- Lamiales (Oleaceae) (see Zimmerman



**Figs. 9–19.** EFN morphotypes. 9–10. *Caryocar brasiliense* nectary. 9. Paired elevated nectaries on the adaxial side of the sepal. 10. Transverse section of the sepal showing the uni-layered adaxial epidermis formed by secretory palisade cells and parenchyma tissue vascularised by phloem. 11–12. Flattened nectary of *Licania humilis*. 11. Single circular, flattened nectary showing irregular surface due to nectar accumulation beneath the cuticle. 12. Cross section of the leaf blade showing the nectary with a secretory epidermis on the midvein. 13–18. Elevated nectaries of Malpighiaceae species. 13. Discoid nectaries of *Banisteriopsis stellaris* on the abaxial leaf surface. 14. Nectary on the leaf adaxial surface of the same species, notice a central elevation due to nectar accumulation beneath the cuticle. 15. *Banisteriopsis campestris*, notice subsessile nectaries with swollen cuticle and numerous non-glandular trichomes. 16. Nectary of the same species showing smooth cuticle. 17. Nectary of *Peixotoa reticulata*; note pores on the cuticle. 18. Longitudinal section of the *P. reticulata* nectary showing epidermal palisade cells and an inner parenchyma region heavily vascularised. 19. Flattened nectary of *Ouratea spectabilis*, notice giant stomata near the nectary. Scale bar: 9 = 140  $\mu\text{m}$ , 10 = 100  $\mu\text{m}$ , 11 = 500  $\mu\text{m}$ , 12 = 100  $\mu\text{m}$ , 13 = 200  $\mu\text{m}$ , 14 = 130  $\mu\text{m}$ , 15 = 220  $\mu\text{m}$ , 16 = 210  $\mu\text{m}$ , 17 = 200  $\mu\text{m}$ , 18 = 100  $\mu\text{m}$ , 19 = 200  $\mu\text{m}$ .

**Table 1.** Taxonomic distribution, location and morphotype of extrafloral nectaries (EFNs) in selected woody species of *cerrado* in Southeast Brazil.

taxa	EFN location	morphotype
<b>ROSIDS</b>		
<b>Combretaceae (Myrtales)</b>		
<i>Terminalia argentea</i> Mart. and Zucc.	Petiole	Elevated
<i>Terminalia brasiliensis</i> Camb.	Petiole	Elevated
<b>Lythraceae (Myrtales)</b>		
<i>Lafoensia pacari</i> St. Hil.	Leaf blade	Pit; elevated
<b>Vochysiaceae (Myrtales)</b>		
<i>Qualea dichotoma</i> (Mart.) Warm.	Stem, petiole, peduncle	Elevated
<i>Qualea grandiflora</i> Mart.	Stem, petiole, peduncle	Elevated
<i>Qualea multiflora</i> Mart.	Stem, petiole, peduncle	Elevated
<i>Qualea parviflora</i> Mart.	Stem, petiole, peduncle	Elevated
<b>EUROSIDS I</b>		
<b>Caryocaraceae (Malpighiales)</b>		
<i>Caryocar brasiliense</i> Camb.	Shoot tips, calyx	Elevated; formless
<b>Chrysobalanaceae (Malpighiales)</b>		
<i>Licania humilis</i> Cham. and Schl.	Leaf blade	Flattened
<b>Malpighiaceae (Malpighiales)</b>		
<i>Heteropteris acutifolia</i> Juss.	Leaf blade	Elevated
<i>Heteropteris byrsonimifolia</i> Juss.	Leaf blade	Elevated
<i>Banisteriopsis stellaris</i> Juss.	Leaf blade	Elevated
<i>Banisteriopsis campestris</i> Juss.	Leaf blade	Elevated
<i>Peixotoa reticulata</i> Juss.	Leaf blade, petiole	Elevated
<b>Ochnaceae (Malpighiales)</b>		
<i>Ouratea spectabilis</i> (Mart.) Engl.	Cataphylls (stipules)	Flattened
<i>Ouratea castanaefolia</i> (DC.) Engl.	Cataphylls (stipules)	Flattened
<b>Fabaceae (Fabales)</b>		
<i>Anadenanthera falcata</i> (Bent.) Speg.	Rachis	Elevated
<i>Bauhinia rufa</i> (Bong.) Steud.	Intra stipules, petiole	Glandular trichomes
<i>Cassia rugosa</i> D. Don.	Rachis	Elevated
<i>Cassia speciosa</i> Schrad.	Rachis	Elevated
<i>Hymenaea stigonocarpa</i>	Leaf blade	Embedded
<i>Stryphnodendron adstringens</i> (Mart.) Cov.	Rachis	Elevated
<i>Stryphnodendron polyphyllum</i> Mart.	Rachis	Elevated
<i>Stryphnodendron obovatum</i> Benth.	Rachis	Elevated
<i>Plathymenia reticulata</i> Benth.	Stem	Elevated
<i>Enterolobium gummiferum</i> (Mart.) Speg.	Rachis	Elevated
<b>Rosaceae (Rosales)</b>		
<i>Prunus sellowii</i>	Leaf blade	Formless; glandular trichomes
<b>EUROSIDS II</b>		
<b>Malvaceae (Malvales)</b>		
<i>Eriotheca gracilipes</i> (Schum.) Robyns	Leaf blade, petiole	Hollow
<b>ASTERIDS</b>		
<b>Myrsinaceae (Ericales)</b>		
<i>Rapanea guianensis</i> Aubl.	Leaf blade	Glandular trichomes
<i>Rapanea lancifolia</i> Mez.	Leaf blade	Glandular trichomes
<b>EUASTERIDS</b>		
<b>Bignoniaceae (Lamiales)</b>		
<i>Arrabidaea brachipoda</i> (DC.) Bur.	Leaf blade	Scale-like trichomes; peltate trichomes
<i>Cybistax antisiphilitica</i> Mart.	Leaf blade	Scale-like trichomes; peltate trichomes
<i>Tabebuia ochracea</i> (Cham.) Standl.	Leaf blade	Scale-like trichomes; peltate trichomes
<i>Tabebuia caraiba</i> (Mart.) Bur.	Leaf blade	Scale-like trichomes; peltate trichomes
<i>Zeyheria montana</i> (Vell.) Hohene	Leaf blade	Scale-like trichomes; peltate trichomes
<i>Zeyheria tuberculosa</i> Mart.	Leaf blade	Scale-like trichomes; peltate trichomes
<b>Verbenaceae (Lamiales)</b>		
<i>Aegiphila lhotzkiana</i> Cham.	Leaf blade	Flattened; glandular trichomes
<i>Aegiphila sellowiana</i> Cham.	Leaf blade	Flattened; glandular trichomes

Table 1. Continued.

taxa	EFN location	morphotype
<b>Rubiaceae (Gentianales)</b>		
<i>Tocoyena brasiliensis</i> Mart.	Leaf blade, calyx	Elevated; pearl glands
<i>Tocoyena formosa</i> (C. and S.) K. Schum.	Leaf blade, calyx	Elevated; pearl glands

1932) but seems to be restricted to Lythraceae within Myrtales.

Vochysiaceae (Myrtales): as described by Sajo & Rudall (2002), the nectary is oval and elevated in *Qualea* and is located on both sides of the young stem nodes (Fig. 5), at the base of the leaf petioles and/or of the flower peduncle. Minute non-secretory trichomes are distributed around the nectaries. The secretion is shed outside by a pore located on the central region of the nectary (Fig. 6). In longitudinal section (Figs 7–8), the cells of the one-layered epidermis are irregular in size and shape and covered by a thin cuticle (Fig. 8). Below the epidermis, the 3–4 layers of parenchymatous cells are compactly arranged and cover the 4–5 layers of small cells containing phenolic compounds and lipids. Only phloem cells, seen as clusters of sieve members and companion cells, irrigate the nectary (Fig. 8). In *Qualea* the EFNs may act indirectly as defensive structures as, according to field observations of Oliveira *et al.* (1987), EFN-gathering ants, particularly the aggressive *Camponotus*, attack caterpillars feeding on the leaves. In *Qualea grandiflora*, ant-excluded trees suffer higher levels of leaf damage by lepidopteran caterpillars than the ant-visited plants (Costa *et al.* 1992) and in *Qualea multiflora*, the ants visiting EFNs reduce the damage by grazing herbivores on leaves, buds and flowers, increasing fruit production by 40% (Del-Claro *et al.* 1996).

#### Eurosids I

Caryocaraceae (Malpighiales): the EFNs in *Caryocar brasiliense* consist of paired, elevated oval glands with smooth surfaces (Fig. 9), and located on the adaxial side of the sepals. Cross sections of the sepals (Fig. 10) show that both epidermis and subglandular tissues are secreting regions; the adaxial epidermis is formed by palisade cells, and the subparenchyma tissue is formed by cells compactly arranged and vascularised by phloem only. In *Caryocar brasiliense* the EFNs are actively visited by more than 30 ant species during both day and night (Oliveira & Brandão 1991). Ant-exclusion experiments revealed that ant visitation to these EFNs decreased the infestation levels by four or five of the principal herbivores associated with this plant (Oliveira 1997; see also Oliveira & Freitas 2004).

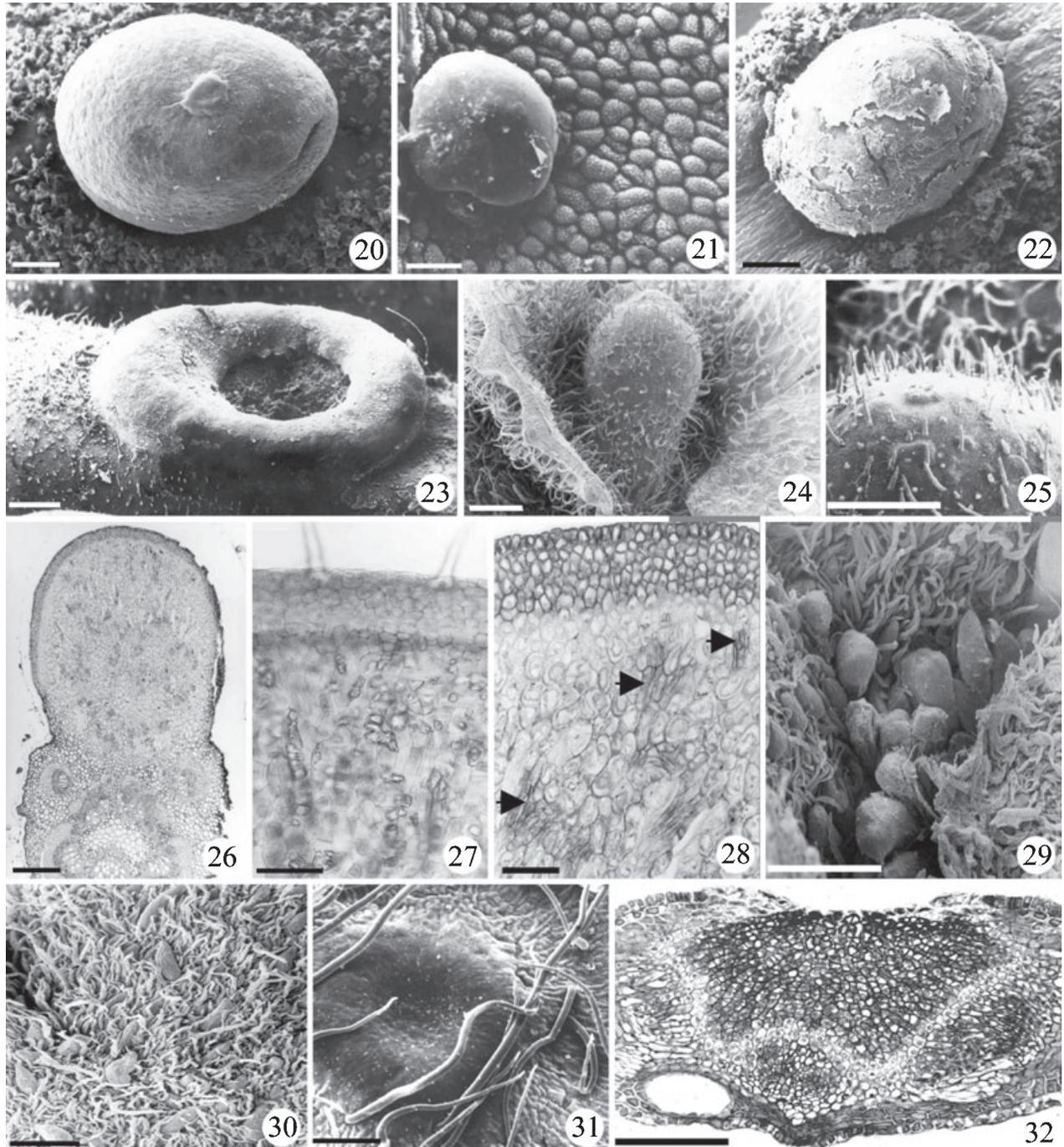
Chrysobalanaceae (Malpighiales): in *Licania humilis* the EFNs occur on the adaxial leaf surface over the midvein. They are circular and flattened with an elevated border (Fig. 11). In cross section (Fig. 12), the one or two-layered epidermis is formed by cells arranged in a palisade. Two

or three layers of irregular parenchymatous cells with thin walls form the subglandular tissue. Both the epidermis and the subglandular tissues are secretory regions (Blüthgen & Reifenrath 2003; Díaz-Castelazo *et al.* 2005). According to Zimmerman (1932), the flattened nectaries are closely pressed against the fundamental tissue of the organs so that its glandular surface is scarcely above or just beneath the surface of the surrounding tissue.

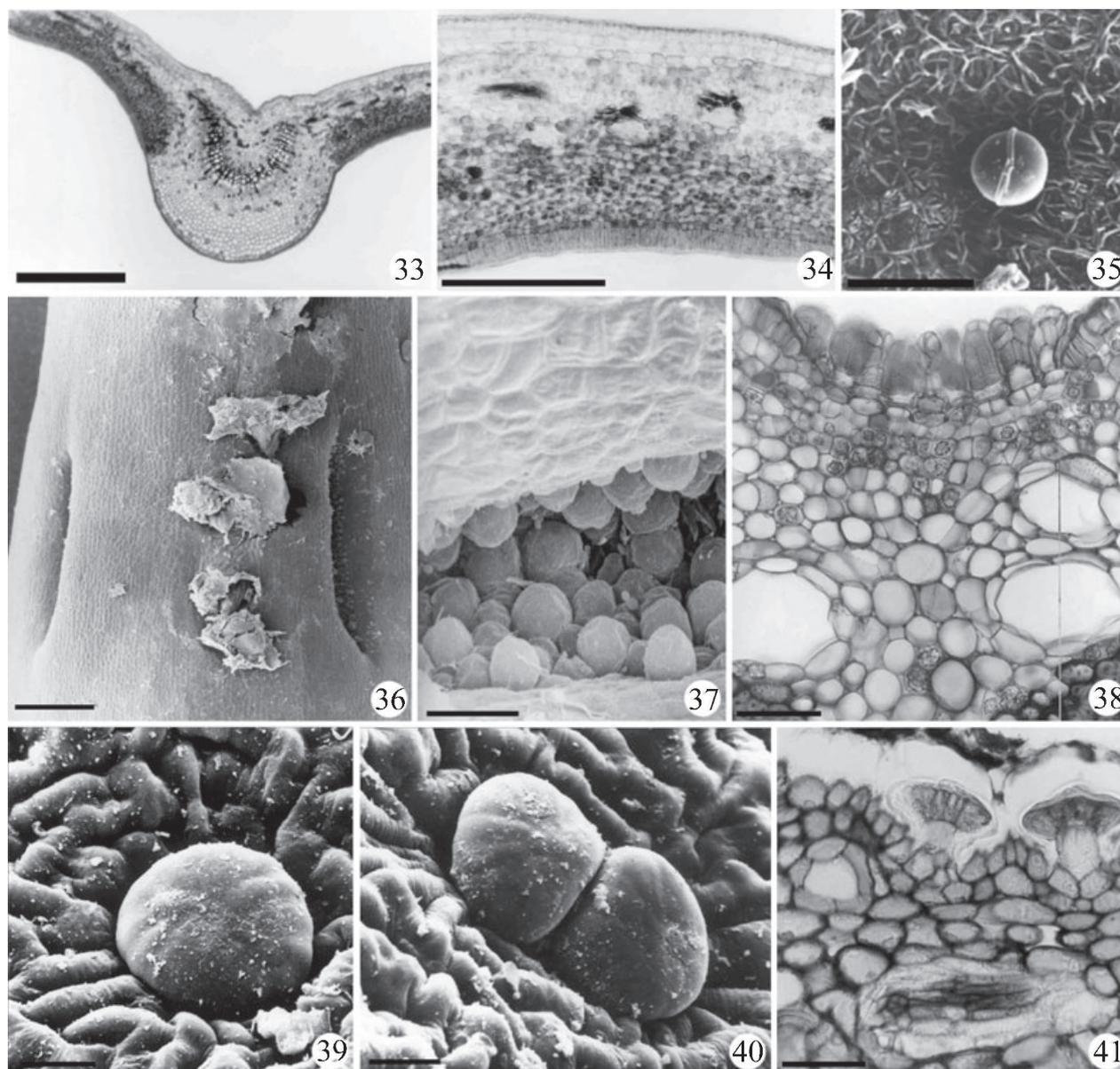
Malpighiaceae (Malpighiales): in all studied species (Table 1) the discoid (Figs 13–16) and elevated nectaries (Figs 17–18) are located on the petiole and on the abaxial leaf surface. They are sessile or short-stalked and consist of a palisade secretory epidermis covered by a thick cuticle plus a subepidermal parenchyma region heavily vascularised by phloem and xylem (Fig. 18). In some nectaries the cuticle is distended (Fig. 13) or wrinkled (Fig. 14), suggesting storage of nectar in the subcuticular space before its release. Cuticle pores through which the nectar could be released were observed with the SEM (Fig. 17). Nectaries similar to this are common in other members of the same family (N. Attala & S.R. Machado, unpublished results).

Ochnaceae (Malpighiales): both *Ouratea spectabilis* and *O. castanaefolia* have rounded or oval flattened nectaries (Fig. 19) on the abaxial surface of the cataphylls. In *O. spectabilis* the EFN is visited by around 26 ant species that significantly reduce the infestation levels and the leaf damage by lepidopteran caterpillars (S.O. Ferreira, unpublished results). In leaves of *Ouratea hexasperma*, species of ants (around 24) attack simulated herbivores on leaves (Oliveira *et al.* 1995).

Fabaceae (Fabales): The EFNs of *Stryphnodendron polyphyllum* (Fig. 20), *S. obovatum* (Fig. 21), *S. adstringens* (Fig. 22) and *Anadenanthera falcata* (Fig. 23) are located on or near the leaf rachis. They are of the elevated type, round or oval, subsessile and with a smooth surface. In *Anadenanthera falcata* (Fig. 23) the nectary is discoid with a central depression. In *Cassia rugosa* and *C. speciosa* the nectary is elevated, clavate, covered by non-glandular trichomes (Fig. 24), and possesses a central depression (Fig. 25). In longitudinal section (Figs 26–28), these nectaries have three regions: the one-layered epidermis, formed by cells of irregular shapes and different sizes, a subepidermal region with 5–6 layers of compactly arranged parenchyma cells, and an inner multilayered region of thick walled cells that often bear small oxalate crystals (druses and prismatic types, Fig. 27). The inner region is irrigated by many phloem conducting elements (Fig. 28) that derive from the vascular bundles of the rachis (Fig. 26).



**Figs. 20–32.** Nectaries of Fabaceae species. 20. *Stryphnodendron polyphyllum*. 21. *S. obovatum*. 22. *S. adstringens*. 23. *Anadenanthera falcata*. 24. *Cassia rugosa*. 25. Detail of the apical region of nectary of the same species. 26. Longitudinal section of the *Cassia rugosa* rachis showing the elevated nectary with an inner parenchyma region densely vascularised. 27. Detail showing non-glandular trichomes, a distinctive subepidermal region formed by densely arranged parenchyma cells, and an inner parenchyma region with numerous small crystals. 28. Detail of the same nectary showing phloem elements (arrows) in the inner nectary region. 29. Inter-petiolar colleters of *Bauhinia rufa*. 30. Petiolar colleters and non-secretory trichomes of the same species. 31. Discoid nectary of *Hymenaea stigonocarpa* surrounded by long non-secretory trichomes; note the concave nectary surface. 32. Longitudinal section of leaf blade of the same species showing embedded nectary in the leaf mesophyll; notice the small and densely arranged secretory cells delimited by a sheath of parenchyma cells. Scale bar: 20 = 270  $\mu\text{m}$ , 21 = 40  $\mu\text{m}$ , 22 = 400  $\mu\text{m}$ , 23 = 330  $\mu\text{m}$ , 24 = 600  $\mu\text{m}$ , 25 = 420  $\mu\text{m}$ ; 26 = 200  $\mu\text{m}$ , 27 = 100  $\mu\text{m}$ , 28 = 60  $\mu\text{m}$ , 29 = 150  $\mu\text{m}$ , 30 = 200  $\mu\text{m}$ , 31 = 100  $\mu\text{m}$ , 32 = 100  $\mu\text{m}$ .



**Figs. 33–41.** EFN morphotypes. 33–35. Nectary of *Prunus sellowii*. 33. Leaf cross section showing formless nectary seen as a dark region. 34. Detail showing abaxial epidermis cells arranged like-palisade and glandular parenchyma tissue. 35. Glandular peltate trichome on the abaxial leaf surface. 36–38. Nectary of *Eriotheca gracilipe*. 36. Paired hollow nectaries in petiole base. 37. Furrow lined with glandular trichomes. 38. Cross section of the petiole showing nectary with elevated border, bottom furrow lined with glandular multicellular trichomes, and subepidermal parenchyma cells with druses. Notice secretory spaces of mucilage immersed in the parenchyma tissue. 39–41. *Rapanea guianensis* nectaries. 39. Solitary nectary. 40. Paired nectaries. 41. Section of the leaf blade showing stalked secretory trichomes with a multicellular head; note cuticle distension due to nectar accumulation under them. Scale bar: 33 = 220  $\mu\text{m}$ , 34 = 50  $\mu\text{m}$ , 35 = 20  $\mu\text{m}$ , 36 = 270  $\mu\text{m}$ , 37 = 30  $\mu\text{m}$ , 38 = 50  $\mu\text{m}$ ; 39 = 150  $\mu\text{m}$ , 40 = 170  $\mu\text{m}$ , 41 = 50  $\mu\text{m}$ .

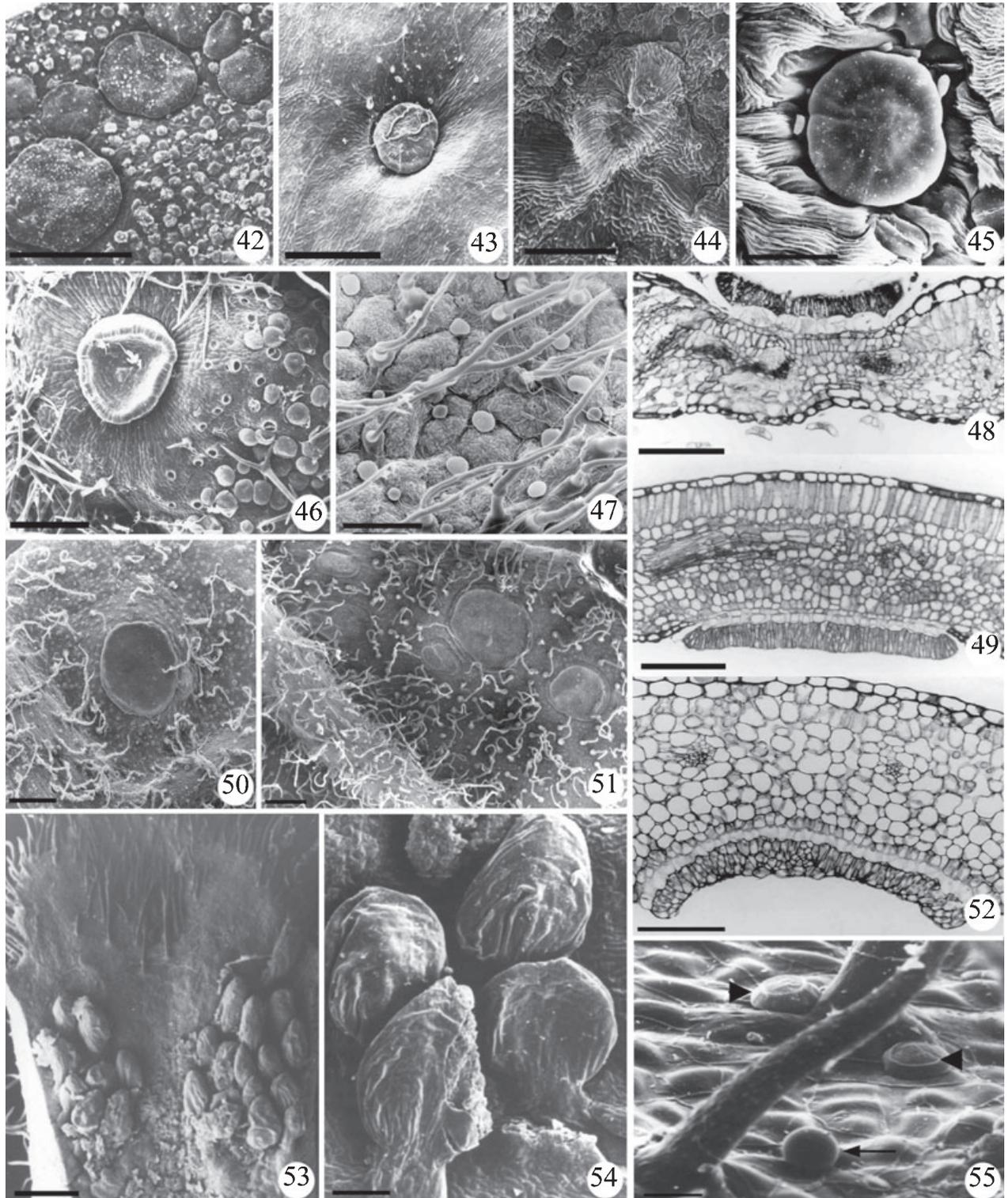
In *Bauhinia rufa* the EFNs are either grouped secretory trichomes surrounded by many non-glandular trichomes located between the stipules (Fig. 29) or scattered secretory trichomes surrounded by non-secretory trichomes (Fig. 30) at the petiole. In *Hymenaea stigonocarpa* the nectary is a disc-shaped gland deeply pressed on the leaf

surface and surrounded by long, non-secretory trichomes (Fig. 31). Cross sections of these nectaries revealed small compactly arranged cells that are completely embedded in the leaf mesophyll (Fig. 32).

Elevated nectaries on the leaf rachis base are very common in Fabaceae. This EFN morphotype is morphologi-

cally and anatomically well known and has been reported for many leguminous plants (Elias 1983; Pascal *et al.* 2000; Paiva *et al.* 2001; Díaz-Castelazo *et al.* 2005). *Bauhinia rufa* is an exception, since it has only glandular tric-

homes that presumably are colleter. Glandular trichomes of variable morphology on the rachis, usually concentrated at the interjugal position, were described in *Delonix*, *Dimorphandra*, *Adenanthera*, *Leucaena* and *Mimosa*,



where they sometimes co-occur with nectaries (Pascal *et al.* 2000). Based on the morphology, topography and aspect of the secretion, we classified *Bauhinia* trichomes as colleters. Colleters are multicellular structures consisting of a short stalk and a large head that occur in young organs of numerous plant families. The secretion is a viscous fluid of carbohydrates, mucilages, proteins, lipophilic substances and phenolic compounds (Thomas *et al.* 1989; Paiva & Machado 2006a,b). They had been interpreted as a protective structure against desiccation in young differentiating leaves or stipules (Thomas 1991). The colleters vary in morphology and anatomy and have received different names, such as squamellae (Ramayya & Bahadur 1968), pectinate glands, shaggy hairs (Solereider 1908; Metcalfe & Chalk 1979), nectarhodes, stipular glands, glandular trichomes, extrafloral nectary, resin glands and glandular emergences (see Thomas 1991).

Nectar-secreting trichomes have been described on leaves (rachis, stipules or stipels), calyx and peduncles of several legume species (Pascal *et al.* 2000; Díaz-Castelazo *et al.* 2005). Embedded nectaries, such as those of *Hymenaea stigonocarpa* (Paiva & Machado 2006c and this paper), represent another type of EFN according to Elias (1983), in addition to Zimmerman's (1932) types. The first record of this EFN type was for the legume *Leonardoxa africana* (Elias 1980), which present a discoid nectary embedded in leaf mesophyll, with a pore opening to the abaxial surface. According to Elias (1980), these nectaries occur near the leaf base and are part of a mutualistic association with ants.

Rosaceae (Rosales): The EFNs of *Prunus sellowii* could not be identified on the leaf surface with the SEM. However, cross sections of the leaf blade revealed a dark green region (Fig. 33) formed by one-layered palisade secretory epidermis covered by a thin cuticle on the abaxial surface (Fig. 34). Below the epidermis, there is a multilayered parenchyma of compactly arranged cells that is vascularised by phloem and xylem. This type, classified as a formless nectary, is an amorphous nectary that lacks structural specialisation but is capable of secreting rich nectar; it is recognisable on the living plants by the presence of an exudate and by a distinct colour on the secretion site (Elias 1983). The leaf surface of the same species is

covered with numerous non-glandular trichomes and glandular peltate hairs (Fig. 35). As ant visitations to the EFNs of *Prunus serotina* significantly reduce caterpillar survivorship (Tilman 1978), this author suggested that the extra-floral nectar production is timed to maximise the predation of the caterpillars by the ants.

#### Eurosids II

Malvaceae–Bombacoidae (Malvales): the only studied species, *Eriotheca gracilipes*, has paired lateral nectaries at the petiole base besides numerous scattered scale-like secretory glands, and nectaries over the veins on the abaxial surfaces of the leaves. The nectaries are longitudinal furrows (Fig. 36) on the organ surface limited by an elevated rim, lined with numerous multicellular secretory trichomes (Fig. 37). In cross section, the rim is formed by one or two layers of epidermal palisade cells covered by a thick cuticle; the parenchyma tissue is non-vascularised and formed by several layers of compactly arranged cells with many druses (Fig. 38). Secretory spaces of mucilage are immersed in the parenchyma tissue. This type of EFN is classified as a hollow nectary, according to Zimmerman (1932), and is also common in the Malvaceae–Malvoideae (Rocha *et al.* 2002).

#### Asterids

Myrsinaceae (Ericales): Both in *Rapanea guianensis* and in *R. lancifolia* the glands are elevated and can be isolated (Fig. 39) or paired (Fig. 40). In longitudinal section (Fig. 41), each nectary is a secretory trichome formed by several (2–6) basal cells, a unicellular stalk, and a multicellular head of anticlinally developed secretory cells, arranged on a disc covered by a thick cuticle, with the secretion stored beneath. Indeed, nectar-secreting trichomes are well-documented within the Eurosids I (Fabaceae) and Euasterids (Apocynaceae, Asteraceae) (see Oliveira & Leitão-Filho 1987; Díaz-Castelazo *et al.* 2005).

#### Euasterids I

Bignoniaceae (Lamiales): the EFNs take the form of flattened dishes (Figs 42–44), a dish with a secretory centre and a more elevated loose cupule (Fig. 45) or a dish with a low rim (Fig. 46), besides peltate glandular trichomes

**Figs. 42–55.** EFN morphotypes. 42–49. Nectaries on the leaf surface of Bignoniaceae species. 42. Aggregation of discoid nectaries on the abaxial leaf surface of *Arrabidaea brachipoda*; note glandular trichomes besides EFNs. 43. Single nectary on the adaxial leaf surface of the same species. 44. Aggregation of nectaries on the abaxial leaf surface in *Cybistax antisiphilitica*. 45. Detail of one nectary of the same species. 46. *Tabebuia ochracea*; note a high density of glandular peltate trichomes surrounding the nectary over an elevation. 47. Peltate secretory trichomes on the abaxial leaf surface in *Zeyheria tuberculosa*. 48. Cross section of *Arrabidaea brachipoda* leaf blade showing scale-like nectary on the adaxial leaf surface. 49. Cross section of *Cybistax antisiphilitica* leaf blade showing extensive nectaries on the abaxial leaf surface. 50–52. Nectaries of Verbenaceae species. 50. *Aegiphila sellowiana* nectary on the leaf abaxial surface; few non-secretory trichomes and numerous peltate secretory trichomes surround the single nectary that is localised over an elevation. 51. Aggregation of nectaries in *Aegiphila lhotzkiana* leaf blade. 52. Longitudinal section of *Aegiphila sellowiana* nectary showing the nectary composed of a multilayered secretory epidermis, an intermediary cell layer and parenchyma tissue formed by cells compactly arranged. 53–55. *Tocoyena formosa*. 53. Colleters on the inner side of the sepals. 54. Detail showing colleters; notice the cuticle disruption. 55. Discoid glands (head arrows) and pearl gland (arrow) on the adaxial leaf surface of the same species. Scale bar: 42 = 300 µm, 43 = 210 µm, 44 = 260 µm, 47 = 20 µm, 46 = 270 µm, 47 = 100 µm, 48 = 50 µm, 49 = 50 µm, 50 = 500 µm, 51 = 500 µm, 52 = 30 µm, 53 = 250 µm, 54 = 70 µm, 55 = 20 µm.

(Fig. 47). These nectaries are named scale-like nectaries and occur clustered (Fig. 42) or scattered (Fig. 43) on the leaf surface, distinctly raised above the leaf surface (Figs 42, 46), or sunken on the surface (Fig. 44), being more frequent close to the midvein. Originally described by Zimmerman (1932), they are considered as squamiform elongations of the epidermis as seen in *Arrabidaea brachiopoda* (Fig. 48) and have been extensively studied in Bignoniaceae (Elias & Gelband 1976; Elias 1983; Rivera 2000; Díaz-Castelazo *et al.* 2005). As in *Cydistax antisiphilitica*, the EFNs in longitudinal section have an extensive upper secretory region, formed by one layer of epidermal palisade cells covered by a thin cuticle, and an underlying region with one layer of isodiametric cells with suberized anticlinal walls connected to the leaf parenchyma (Fig. 49). According to Elias & Gelband (1976) and Elias (1983), scale-like nectaries are easily accessed by ants and secrete enough nectar to support large populations of these insects. Besides the EFNs, peltate secretory trichomes (Figs 42, 46) occur in all studied species. Similar trichomes on the ovary of *Zeyheria montana*, a Bignoniaceae species, produce terpenes and alkaloids and are not associated with the nectar production (Machado *et al.* 2006).

Verbenaceae (Lamiales): in the two studied species, the nectary is oval or round with a flattened surface and prominent borders (Figs 50–52) on the abaxial leaf surface. In longitudinal section (Fig. 52), the nectary has a concave surface and is formed by a palisade epidermis with one to three cell layers plus one layer of thick-walled cells with suberized anticlinal walls, and an inner parenchymatous region vascularised by phloem and xylem. NEFs with similar structure were described for *Cytherexylum mirianthum*, a Verbenaceae species from forest vegetation (S.R. Machado, unpublished results). Besides the EFNs, scattered secretory trichomes (Figs 50–51) occur in all studied species.

Rubiaceae (Gentianales): *Tocoyena brasiliensis* and *T. formosa* have a cluster of glands on the adaxial surface at the base of the sepals (Figs 53–54). Based on arrangement, morphology and secretion aspects, we classified these glands as colleters. Such secretory structures are typical of Rubiaceae species (Thomas 1991). On the adaxial surface of the leaves (Fig. 55) there are sessile, discoid glands with prominent borders (elevated nectaries) plus spherical glands, which we have named ‘pearl glands’. Pearl glands were described for the unrelated clade of Magnoliids in *Piper regnellii* (Silva & Machado 1999), and the Eurosid Malvaceae *Hibiscus pernambucensis* (J.F. Rocha & S.R. Machado, unpublished results), and are associated with mucilage and protein secretion. Both studies also provided strong evidence of an association between the pearl glands and ants.

#### General patterns of EFNs in the Brazilian *cerrado*

The taxonomic distribution, location and morphology of EFNs in selected woody species of *cerrado* are shown in Table 1. For 33 of the 40 species studied (82.5%), EFNs

were associated with vegetative plant organs (*e.g.* leaves, stipules), whereas only seven species (17.5%) had EFNs located on both vegetative and reproductive structures (*e.g.* calyx, flower peduncle). No species had glands associated exclusively with reproductive organs. The leaf blade was by far the most common location of EFNs, occurring on 22 of the 40 species studied (55%). Elevated nectaries were the most frequent morphotype (22 species), followed by different types of trichomes (13 species).

The elevated nectary predominated in the Rosids (Combretaceae, Lythraceae and Vochysiaceae) and Eurosids I (Caryocaraceae, Fabaceae) clades, although in the Eurosids I – Chrysobalanaceae it is flattened (Table 1). In Rosaceae that also belongs to the Eurosids I clade, the nectary is amorphous and detectable only when sectioned; while in the Eurosids I – Ochnaceae the nectary is flattened like those of Chrysobalanaceae (Table 1). Besides the elevated morphotype, the Rosids–Lythraceae also has a particular type of nectary, named pit nectaries and described for taxonomically unrelated families, such as Oleaceae (Euasterids I) and Polygonaceae (a Core Eudicots – Caryophyllales). Within the Eurosids I – Fabaceae some representatives have glandular trichomes or embedded nectaries, instead of the more frequent ‘elevated’ type found in the leaf rachis. As Fabaceae is one of the largest and most widespread families of the flowering plants, it could be expected that its secreting structures will also be highly variable. Finally, in the Eurosid II family Malvaceae (Bombacoide) the nectary is of the hollow type.

Within the Euasterids clade the nectary morphology was quite variable, being flattened in Verbenaceae and elevated in Rubiaceae. The latter family also has pearl glands on the adaxial surface of the leaves, not described before. In Bignoniaceae there are peltate trichomes and scale-like nectaries that are frequent in the family and considered an advanced form of ant attraction (Elias & Gelband 1976; Elias 1983) (Table 1). In the Asterids family Myrsinaceae the nectary is a simple glandular trichome and is described here for the first time.

#### CONCLUSIONS

Regardless of the fact that nectar secretion and some other attributes of EFNs have not been treated in this work, available information showed that EFN morphology and size (nectary length and width) are related to the nectar volume and the vascular supply, which determine secretion rates (Rudgers 2004). Elevated and hollow EFNs possess a more complex structure and vascular supply, secreting greater amount of nectar (Díaz-Castelazo *et al.* 2005). On the other hand, from a functional perspective, large numbers of small secretory units (*i.e.* trichomes), with lower structural costs, can be as effective as large, morphologically complex glands (Díaz-Castelazo *et al.* 2005). Structurally, colleters can be trichomes, emergences or glands (Thomas 1991), and are often confused with nectar-secreting structures.

Although the EFNs are usually considered as nectar-secreting structures, the composition of their exudates and consequently their functions can only be determined by chemical analysis. For example, the EFNs of *Citharexylum mirianthum* (Verbenaceae) produce lipids (S.R. Machado, unpublished results) and those of *Passiflora foetida* (Passifloraceae) also secrete lipids rather than sugars; so they are not 'nectaries' *sensu stricto* (Roth 1968; Durkee *et al.* 1984).

The distribution of the EFN morphotypes across different taxa described in the present study did not indicate any taxonomic association, even though some morphotypes were restricted to a particular group, such as the scale-like trichomes and the pearl glands of the Euasterids–Rubiaceae, the hollow and the pit nectary of some Rosids–Lythraceae, and the amorphous nectary of the Eurosids–Rosaceae (Table 1). The occurrence of the same EFN morphotype in unrelated groups, such as Vochysiaceae and Rubiaceae, may indicate an adaptive convergence related to similar selective forces operating when the plants first invaded *cerrado* areas. In this context, the insect community associated with EFNs may have acted as selective agents on gland and nectar traits (Díaz-Castelazo *et al.* 2005). Although ants are the main visitors of EFNs, a variety of other arthropod groups (*e.g.* wasps, bees, flies, beetles, spiders, mites) also regularly visit these liquid food sources (see Koptur 1992; Pemberton & Lee 1996; Ruhren & Handel 1999; Rudgers 2004), and the relative effect of these groups on the origin and evolution of nectaries and on plant fitness has only more recently been comparatively analysed (Cuautle & Rico-Gray 2003; Rico-Gray & Oliveira 2007). Observations and experimental field studies in the *cerrado* support an anti-herbivore role of EFN-gathering ants in this habitat (Oliveira & Freitas 2004, and included references). Additional morphological studies of EFN-bearing plants, including other growth forms (*e.g.* herbs and lianas), are being undertaken and will hopefully cast further light on the ecological relevance of these glands in *cerrado*, especially with respect to their attractiveness to multiple visitors.

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