

# Extrafloral nectaries in Combretaceae: morphology, anatomy and taxonomic significance

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## ABSTRACT

Extrafloral nectaries (EFNs) in members of the Combretaceae are nectaries not involved with pollination and occurring on vegetative structures; they are believed to attract ants to protect plants against herbivory by other insects. In the Combretaceae EFNs are reported in species of *Terminalia* L. and *Pteleopsis* Engl., putative EFNs in *Meiostemon* Exell & Stace and *Quisqualis* L., and an absence of EFNs in *Combretum* Loeffl. and *Lumnitzera* Willd. EFNs in the family are generally spherical in shape and may be raised, level with the surface or somewhat concave. They are similar in the *Terminalia* and *Pteleopsis* species where they display varying degrees of internal zonation and are composed of small cells; those species observed in the field were all found to have functional EFNs. In *Meiostemon tetrandrum* (Exell) Exell & Stace, *Quisqualis indica* L., *Q. littorea* (Engl.) Exell and *Q. parviflora* Gerrard ex Sond., apparent EFNs lack internal zonation and are composed of enlarged cells; confirmation is required as to whether these are functional. The formation of EFNs appears to be highly flexible. They are usually essentially associated with new growth but their occurrence is sporadic and they do not appear on every leaf or every branch of a plant. The distribution of EFNs on leaves, when present, is of taxonomic significance to separate species of *Pteleopsis* and *Terminalia*; otherwise the presence or absence and distribution of EFNs are too variable and sporadic in occurrence to be of taxonomic significance at the species level. Indiscriminate use of the terms gland and domatium instead of EFN, and possible confusion with damage caused by other organisms, has probably contributed to many of these structures not previously being recorded as EFNs. Floral and extrafloral nectar samples of *T. phanerophlebia* Engl. & Diels differed in sugar composition.

## INTRODUCTION

The Combretaceae is a pantropical family of 20 genera and 500 species (Mabberley 2000). The largest genera are *Combretum* Loeffl. (250 tropical species) and *Terminalia* L. (150 tropical species).

The great structural diversity of EFNs and their occurrence in various plant taxa have been documented in several reviews (e.g. Delpino 1886–1889; Zimmermann 1932; Schnell *et al.* 1963; Bentley 1977; Elias 1983; Fahn 1988). They have only been reported in two families of the Myrtales, viz. the Melastomataceae where they appear to be poorly known and the Combretaceae (Elias 1983). There are relatively few references to EFNs in the Combretaceae and, as far as we have ascertained, there are no reports on the efficacy of EFNs in the family in attracting ants to protect the plants against herbivory. In an extensive study of extrafloral glands in several groups of tropical plants, Schnell *et al.* (1963) make no mention of the family. The Combretaceae is one of 55 families listed by Metcalfe & Chalk (1979) as having EFNs.

Only a limited number of anatomical studies of EFNs have been carried out on members of the Combretaceae. These have been largely confined to various species of *Terminalia* and to *Laguncularia racemosa* (L.) C.F.Gaertn. (Von Höhnell 1882; Zimmermann 1932; Biebl & Kinzel 1965; Belin-Depoux 1978, 1989, 1993; Ramakrishna & Rajashekara 1981). Mention has been made of the possible

occurrence of EFNs in species of *Meiostemon* Exell & Stace, *Pteleopsis* Engl. and *Quisqualis* L. (Tilney 2002).

Foliar nectaries were suspected by Elias (1983) to occur in many additional species of Combretaceae which our observations in the field and on herbarium material have confirmed. Furthermore, it seems that the terms EFNs (or simply glands) and domatia (small structures including pits, hair tufts, etc. associated with veins, often found on leaves of woody dicotyledons and known to harbour mites) have sometimes been used indiscriminately in the family, especially in the early literature, and that possibly some confusion also exists with galls or other markings caused by animal damage. For these reasons, and to investigate their structure more fully, a morphological and anatomical study of mainly EFNs, but including some domatia and galls, was undertaken. The purpose of this paper is therefore to document the occurrence of EFNs in the Combretaceae, to give an account of their morphology and anatomy, and to report on the taxonomic significance of the distribution of EFNs on leaves. In addition, a comparison was made between the sugar composition of secretions of floral and extrafloral nectaries of a single species to ascertain whether or not the same type of insect is likely to visit both kinds of nectaries.

## MATERIALS AND METHODS

All specimens of every species of the southern African genera of Combretaceae as well as all those of more northerly species available at PRE and PRU were studied for the presence and distribution of EFNs, including approximately 130 *Combretum* spp. and *Lumnitzera*. All the southern African species with EFNs were used for the anatomical study as well as selected

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TABLE 1.—Specimens used in the anatomical study of Combretaceae extrafloral nectaries, domatia and galls, with an indication of EFN morphology and predominant shape of the cells of the secretory tissue

| Species and section                                   | Morphology     |                | Voucher specimen(s)                              |
|---|----------------|----------------|--|
|   | EFN            | Cell shape     |  |
| <i>Meiostemon tetrandrum</i> (Exell) Exell & Stace    | raised         | a-elongated    | CP 29 (JRAU) EFN                                 |
| <i>Pteleopsis</i>                                     |                |                |  |
| <i>anisoptera</i> (Welw. ex M.A.Lawson) Engl. & Diels | raised         | ± isodiametric | CP 20 (JRAU) EFN                                 |
| <i>myrtifolia</i> (M.A.Lawson) Engl. & Diels          | surface/sunken | ± isodiametric | PRU3822 (PRU) EFN; Tilney 233 (JRAU) EFN         |
| <i>Quisqualis</i>                                     |                |                |  |
| <i>indica</i> L.                                      | raised         | a-elongated    | PRU3064 (PRU) EFN                                |
| <i>littorea</i> (Engl.) Exell                         | raised         | p-elongated    | Tilney 230 (JRAU) EFN                            |
| <i>parviflora</i> Gerrard ex Sond.                    | raised         | a-elongated    | Abbott 7839 (PRU) EFN                            |
| <i>Terminalia</i>                                     |                |                |  |
| Section uncertain                                     |                |                |  |
| <i>catappa</i> L.                                     | surface        | ± isodiametric | Tilney 256 (JRAU) EFN & domatium                 |
| Section <i>Abbreviatae</i>                            |                |                |  |
| <i>prunioides</i> M.A.Lawson                          | raised         | ± isodiametric | CP 9 (JRAU) EFN; Tilney 212, 232, 254 (JRAU) EFN |
| <i>randii</i> Baker f.                                | surface        | ± isodiametric | CP 22 (JRAU) EFN                                 |
| <i>stuhlmannii</i> Engl.                              | surface        | ± isodiametric | CP 26 (JRAU) EFN                                 |
| Section <i>Platycarpae</i>                            |                |                |  |
| <i>gazensis</i> Baker f.                              | raised         | ± isodiametric | CP 8 (JRAU) EFN                                  |
| <i>mollis</i> Lawson                                  | raised         | ± isodiametric | CP 24 (JRAU) EFN; Milne-Redhead 2729 (K) galls   |
| <i>phanerophlebia</i> Engl. & Diels                   | raised         | ± isodiametric | Tilney 219 (JRAU) EFN                            |
| <i>stenostachya</i> Engl. & Diels                     | raised         | ± isodiametric | CP 25 (JRAU) EFN                                 |
| Section <i>Psidioides</i>                             |                |                |  |
| <i>brachystemma</i> Welw. ex Hiern                    | raised         | ± isodiametric | Tilney 231 (JRAU) EFN                            |
| <i>sericea</i> Burch. ex DC.                          | raised         | ± isodiametric | Tilney 250 (JRAU) EFN                            |
| <i>trichopoda</i> Diels                               | raised         | ± isodiametric | CP 23 (JRAU) EFN                                 |

a-elongated, anticlinally elongated; p-elongated, periclinally elongated; secr., secretory; CP, M. Coates Palgrave.

examples of other species. Single specimens of *Terminalia catappa* L. and *T. mollis* Lawson with domatia and galls respectively were also included for comparison. The sources of this material and authorities for species names are given in Table 1. Fresh material was preserved in FAA; dried material was first rehydrated in distilled water and then placed in FAA. Leaf samples with EFNs of several *Meiostemon*, *Pteleopsis*, *Quisqualis* and *Terminalia* species were examined with Cambridge Stereoscan 240 and Jeol JSM 5600 scanning electron microscopes after being coated with platinum and gold respectively. Transverse sections of leaf portions bearing EFNs were prepared using a freezing or Reichert sliding microtome and staining with alcian blue and safranin, or by embedding in GMA, sectioning with an ultramicrotome and staining according to the periodic acid-Schiff/toluidine blue method (Feder & O'Brien 1968). Photographs were taken with a Leitz diaphan microscope using Pan F film ASA 50 and Agfa APX 25 film. Slides are housed at JRAU. Field observations were made over a number of years on the EFNs of members of the Combretaceae whenever possible.

Nectar samples were taken from the EFNs of a tree of *T. phanerophlebia* growing in the grounds of the University of Pretoria on 13 November 2001. Samples were also taken from the flowers of the same tree on 9 November 2002. They were collected as spots on Whatman no. 1 filter paper and air-dried. The nectar was then eluted with distilled water ( $3 \times 15 \mu\text{l}$ ), using a centrifuge. HPLC was performed isocratically at a flow rate of  $2.5 \text{ ml min}^{-1}$  on a 'Waters Sugarpack' column, with acetonitrile-water (87:13) as eluent. The use of a refractive index detector allowed the accurate calculation of the sugar composition, using peak height and  $8 \text{ mg ml}^{-1}$  of fructose, glucose and sucrose as external standards.

## RESULTS

To date, EFNs, as well as 'glands of unknown function' (see below), have been found in *Laguncularia* C.F.Gaertn., *Meiostemon*, *Pteleopsis*, *Quisqualis* and *Terminalia* but not in *Combretum* and *Lumnitzera*. With the *Meiostemon* and *Quisqualis* species, the glands present are assumed in this paper to be EFNs but field studies are needed to confirm this and also to ascertain whether or not they are functional. In *Lumnitzera racemosa* Willd., at least two types of secretory structures of unknown function are present on the leaves (Tilney 2002). Since this plant is a mangrove, other types of secretory structures, e.g. salt glands, may well be involved.

Ants and other insects were observed at one or more times during the period November to early January on the extrafloral glands of the young newly-formed leaves of *Terminalia brachystemma* Welw. ex Hiern, *T. mollis*, *T. phanerophlebia* (Figure 1A, B), *T. prunioides* M.A.Lawson and *T. sericea* Burch. ex DC. On many of the leaves of *T. phanerophlebia* a white deposit, presumably sugar crystals left after evaporation of water, was visible on the glands on a very hot day (Figure 1C). Crystals were also seen on *T. sericea* in association with the EFNs in the SEM study (Figure 1D). Ants were observed on *Pteleopsis myrtifolia* (M.A.Lawson) Engl. & Diels in November and January.

### Distribution

In all the taxa with EFNs studied, these glands do not appear on every leaf of every branch, thus limiting their taxonomic significance. They are usually most conspicuous, and probably only functional or at least optimally functional, on new growth. When they become non-

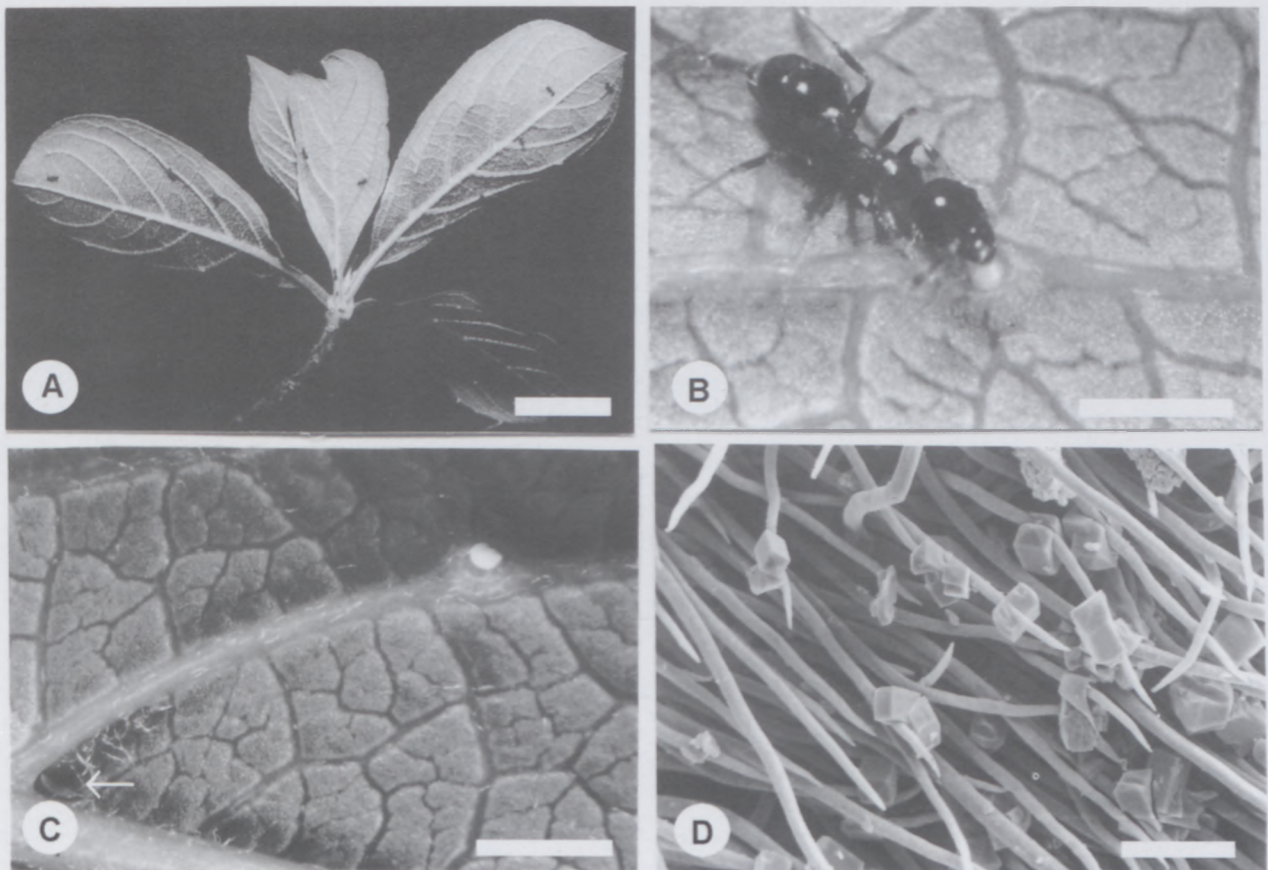


FIGURE 1.—EFNs of *Terminalia* species. A–C, *T. phanerophlebia*: A, leaves with ants, indicating presence of functional EFNs; B, ant on active EFN; C, EFN with white sugary deposit visible on hot days following evaporation; mite (arrowed) can be seen in domatium. D, SEM of portion of *T. sericea* EFN with crystals. Scale bars: A, 20 mm; B, C, 1 mm; D, 50  $\mu$ m.

secretory in older leaves, there appears to be some shrinkage frequently making them more difficult to detect, especially in herbarium specimens.

The typical positions of the EFNs on the leaves in species of *Laguncularia*, *Meiostemon*, *Pteleopsis*, *Quisqualis* and *Terminalia* are shown in Figure 2. In

*Laguncularia racemosa* glands, whose structure and function needs to be ascertained, were also included because of their apparent similarity in herbarium material to certain known EFNs. In *T. prunioides* a pair of EFNs may occasionally be present on the petiole itself rather than at the base of the leaf blade but are then never conspicuous.

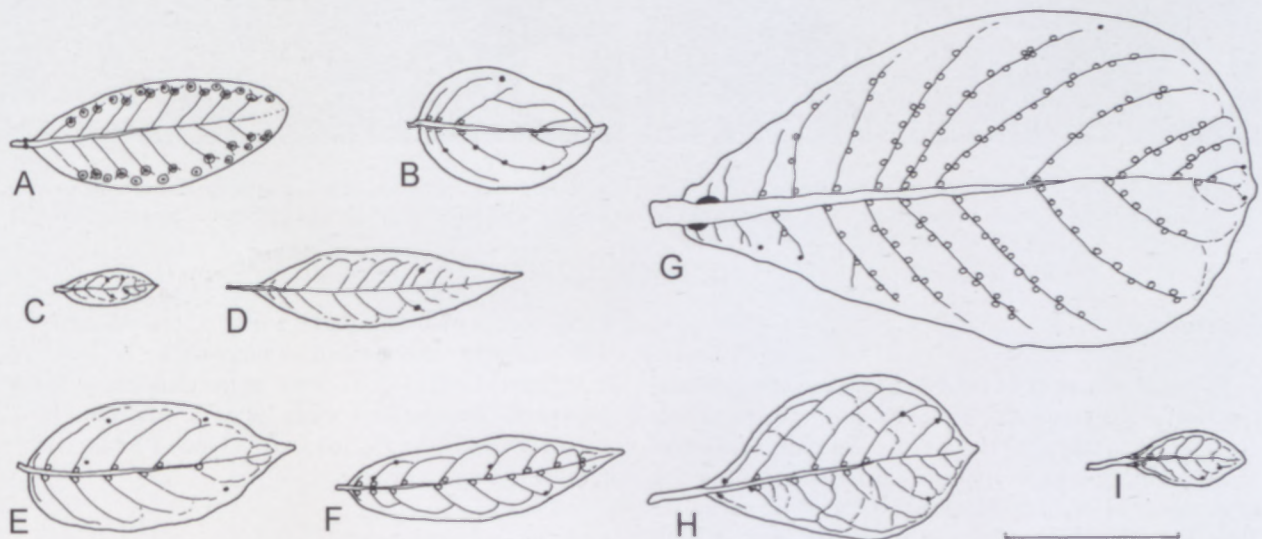


FIGURE 2.—Abaxial surfaces of leaves in taxa of Combretaceae showing variation in position of EFNs and related structures. A, *Laguncularia racemosa*; B, *Meiostemon tetrandrum*; C, *Pteleopsis anisoptera*; D, *P. myrtifolia*; E, *Quisqualis indica*; F, *Q. parviflora*; G, *Terminalia catappa*; H, *T. phanerophlebia*; I, *T. prunioides*. EFNs are shown as black dots, domatia as circles and glands of unknown function as circles with dots. Scale bar: 50 mm.

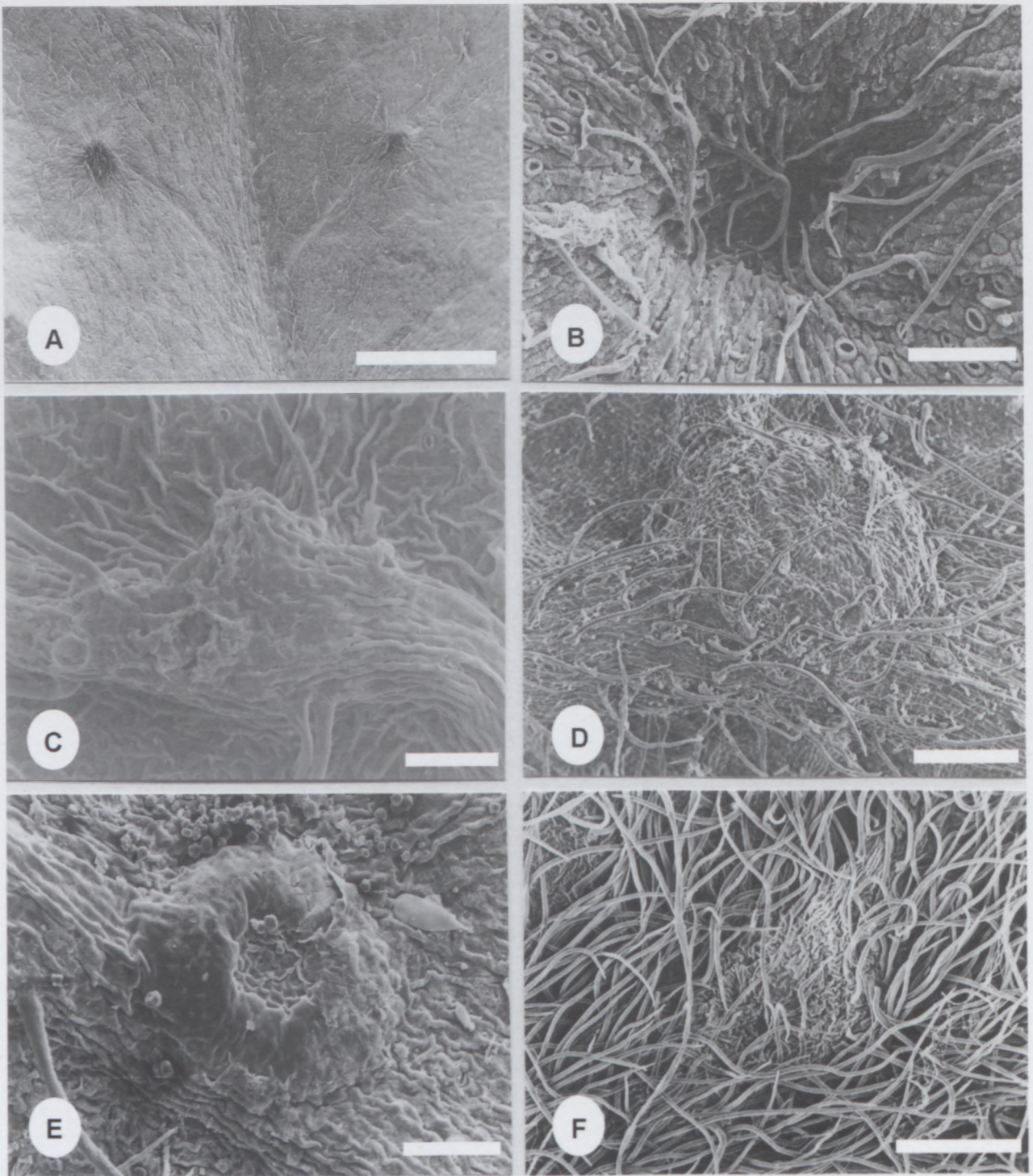


FIGURE 3.—SEM of morphology of EFNs in members of Combretaceae. A, B, *Pteleopsis myrtifolia*, surface or somewhat concave EFNs. C–E, raised EFNs: C, *Quisqualis parviflora*; D, *Terminalia phanerophlebia*; E, *P. anisoptera*. F, surface/raised EFN of *T. gazensis*. Scale bars: A, 1 mm; B, E, 100 µm; C, 50 µm; D, F, 200 µm.

### Morphology

EFNs of members of the Combretaceae are generally spherical in shape and may be raised, level with the surface or somewhat concave (Figure 3). Species studied of *Meiostemon*, *Quisqualis* and some of *Terminalia* (especially members of sections *Platycarpae* and *Psidioides*—see Table 1) and *Pteleopsis anisoptera* (Lawson) Engl. & Diels usually fit into the ‘Hochnektarien’ category of Zimmermann (1932)—referred to as ‘elevated’ by Elias (1983). The other species studied of *Terminalia* (generally belonging to section *Abbreviatae*—see Table 1) and *Pteleopsis*

*myrtifolia*, would be more suitably placed into the ‘Flachnektarien’ category of Zimmermann (1932)—‘surface’ gland of Elias (1983). Some of the glands may be slit-shaped in *Terminalia* species (e.g. *T. sericea*). In *T. prunioides* the EFNs are frequently a reddish colour but are nevertheless inconspicuous.

### Anatomy of EFNs in *t/s*

In species of *Terminalia* the nectaries are more or less spherical structures (Figure 4A–F). Internal zonation varies in degree but usually becomes more marked with

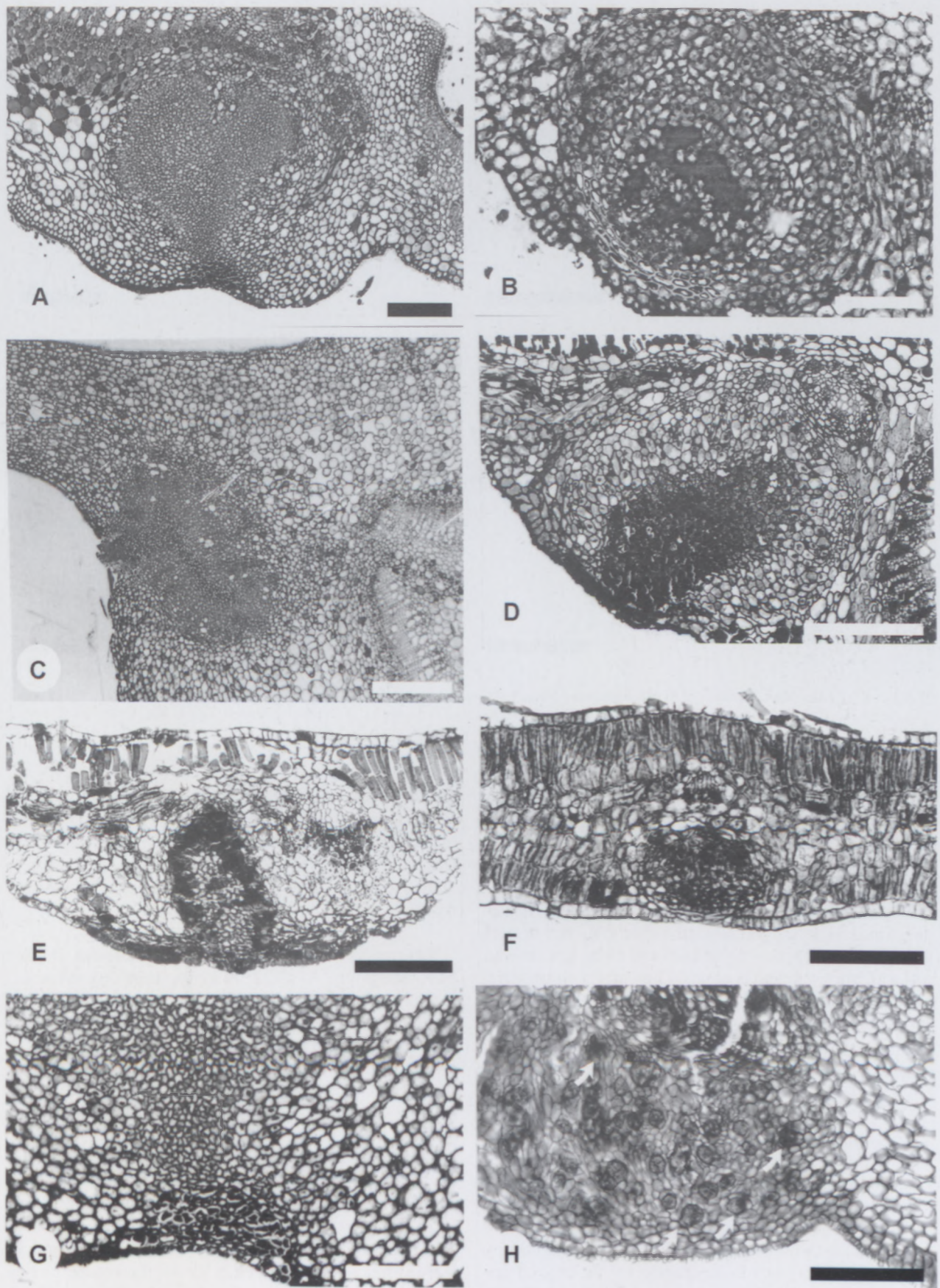


FIGURE 4.—*Terminalia* species: *Us* of EFNs showing varying degrees of internal zonation. A, *T. mollis*; B, *T. prunioides*; C, *T. catappa*; D, *T. brachystemma*; E, *T. trichopoda*; F, *T. randii*. G, *T. mollis* with outer portion of EFN showing epidermal layers and adjacent cells rich in tanniniferous substances; H, peripheral portion of EFN of *T. stenostachya* with abundant crystals (some arrowed). Scale bars: A, D–H, 200  $\mu$ m; B, 100  $\mu$ m; C, 400  $\mu$ m.

age. The nectariferous tissue may be a rounded, oval, heart-shaped or irregular mass of small, tightly-packed,

variously shaped, but predominantly  $\pm$  isodiametric cells with thin walls and dense cytoplasm, which may become

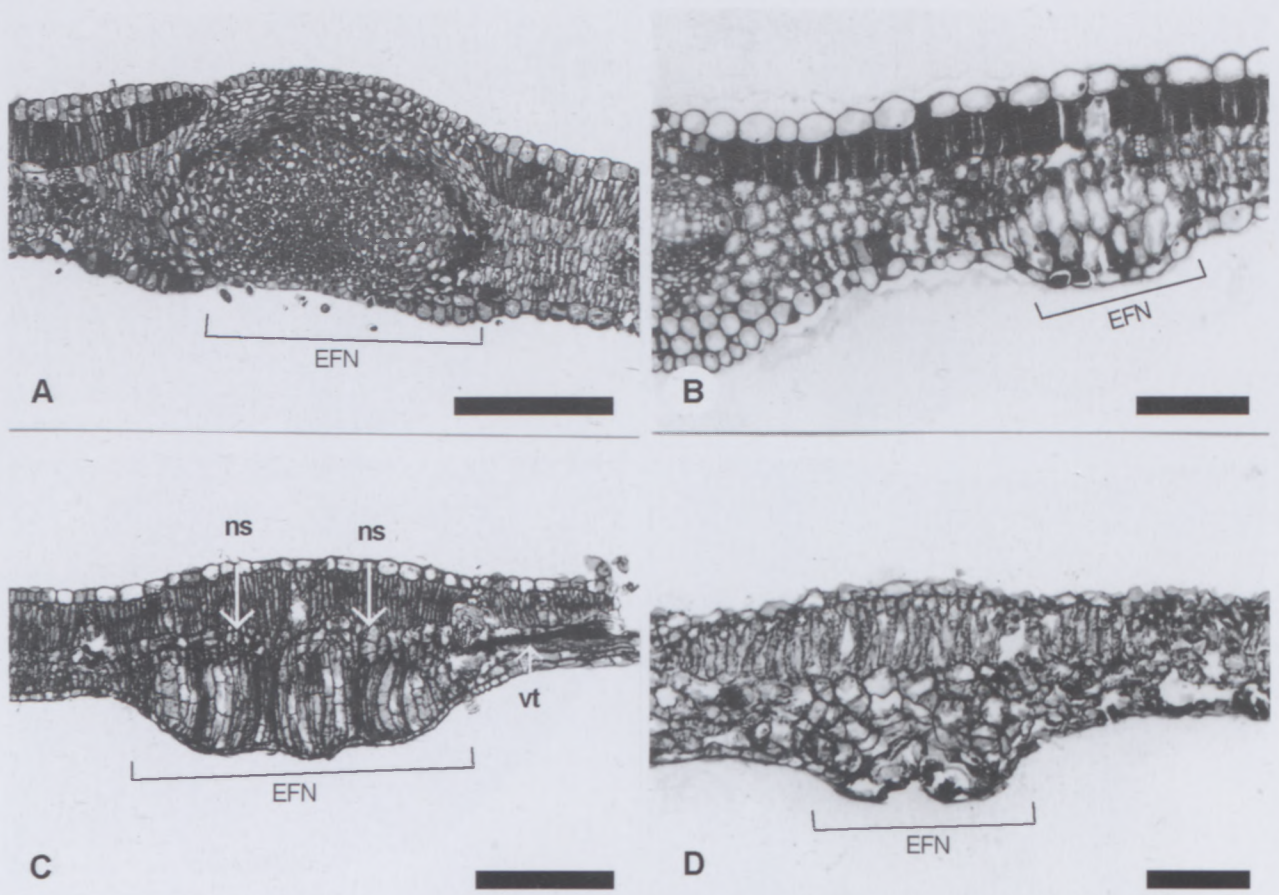


FIGURE 5.—Combretaceae taxa: *t/s* of EFNs or putative EFNs. A, *Pteleopsis myrtifolia*; B, EFNs of *Meiostemon tetrandrum* composed of broad cells; C, *Quisqualis indica* with cells distinctly aligned; D, *Q. littorea*; it is not clear whether structures such as this represent scar tissue. ns, nectary sheath; vt, vascular tissue. Scale bars: A, C, 200  $\mu$ m; B, D, 100  $\mu$ m.

tanniferous with age (particularly in *Terminalia* section *Psidioides*; see Table 1). This is sometimes surrounded by a region of slightly larger parenchymatous cells with sparser cytoplasm but numerous calcium oxalate crystals (Figure 4H). Vascular tissue is associated with the nectariferous tissue and the phloem usually appears well developed. The epidermal cells overlying the nectary may or may not resemble those found elsewhere in size, cell wall thickness and contents. They may well be secretory when young but frequently become thick-walled and tanniferous with age, as do a few layers of adjacent cells, which in species such as *T. catappa* and *T. mollis* (Figure 4G) form a distinctive area. Stomata and a nectary sheath are absent.

*Pteleopsis anisoptera* and *P. myrtifolia* EFNs are similar to those of *Terminalia* species and consist of a roundish to discoid mass of small, thin-walled, variously shaped cells lacking visible intercellular spaces (Figure 5A). The contents of the cells comprising the nectariferous tissue are densely granular towards the base becoming non-granular towards the outside, i.e. the abaxial surface. The surface of the gland is frequently level with the rest of the epidermis but occasionally concave. Stomata are absent in this region. The epidermis overlying the gland is initially much less tanniferous than the rest of the epidermis. The epidermal cells are very similar to the underlying cells and may also be secretory. With age, the epidermal cell walls, together with those of a few layers of adjacent glandular cells, become lignified and the cell

interiors become filled with darkly-staining tannins. Vascular tissue is associated with the base of the nectary but does not appear to differ from tissue elsewhere in the leaf. No nectary sheath is present but druses, some very large, are found in the parenchymatous tissue in the vicinity of the gland.

*Quisqualis indica* EFNs are quite different (Figure 5C), with groups of about six to ten superimposed layers of broad, elongated cells, separated by groups of narrow, columnar cells in close proximity to, but not obviously connected to, the vascular tissue. The nectariferous tissue is separated from adjacent tissue by a nectary sheath composed of one or two layers of more rounded cells. The epidermis does not appear to be secretory. Stomata may be present. Druses (calcium oxalate) of varying sizes are present in the nectariferous tissue and sheath. The vascular tissue close to the nectariferous tissue sometimes appears to be better developed than elsewhere. The glands in *Q. littorea* are much smaller and are composed of broad cells only, most of which are not elongated anticlinally (Figure 5D). The cell walls of the epidermis and underlying layers stain more intensely (suberised?). A nectary sheath is absent. In *Q. parviflora* the mesophyll, including the palisade, in localized areas tends to undergo divisions resulting in fairly large cells. Intercellular spaces are absent and there is no clear cell alignment.

In *Meiostemon tetrandrum*, the EFNs are composed of about two layers of relatively large, anticlinally elon-

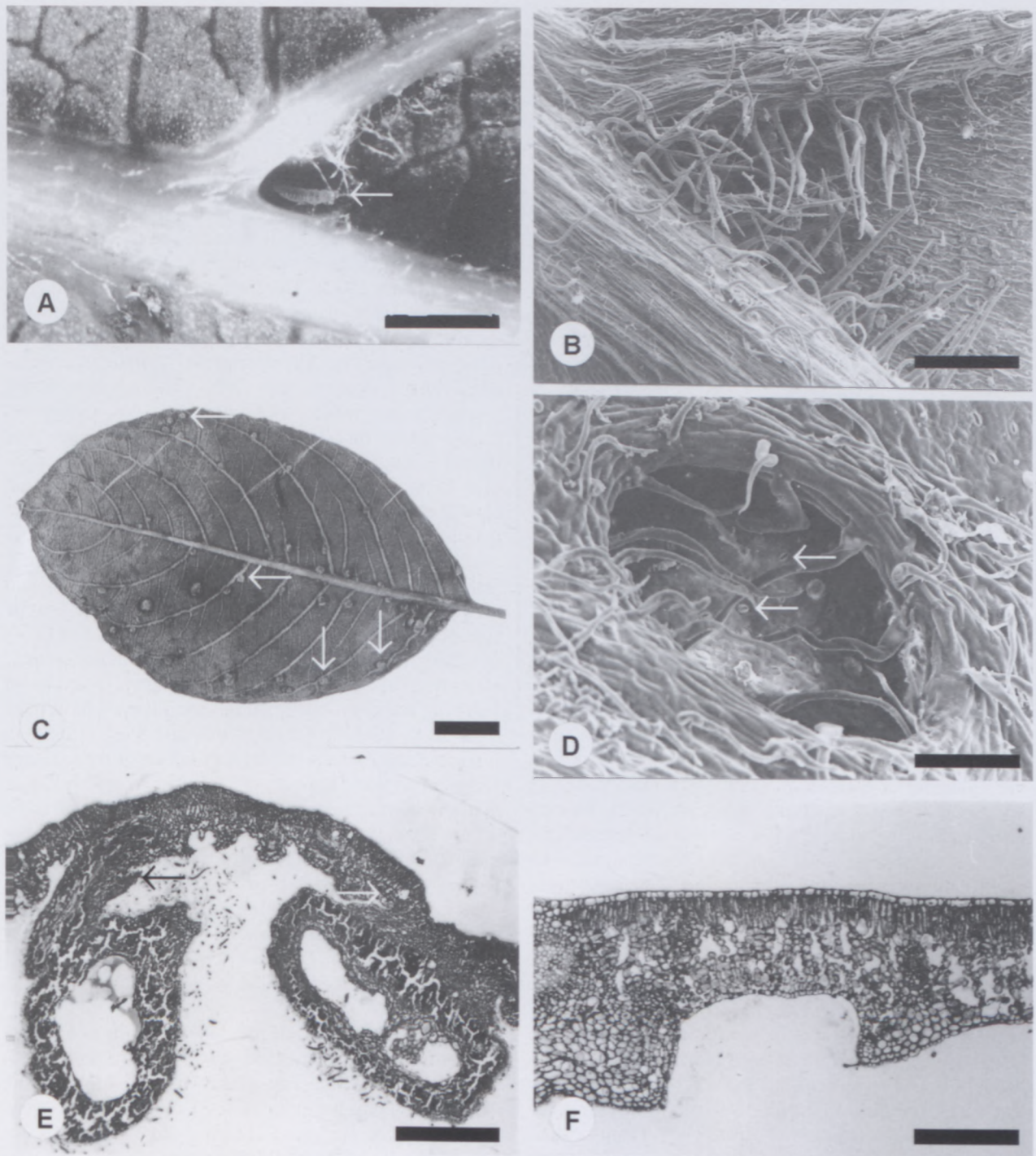


FIGURE 6.—Combreteaceae taxa: domatia and galls. A, domatium of *Terminalia phanerophlebia* with mite (arrowed); B, SEM of hairy domatium of *Quisqualis littorea*; C, leaf of *T. mollis*, showing galls (some arrowed) in positions frequently occupied by domatia and EFNs; D, SEM of pit domatium of *T. catappa*, with stomata (some arrowed) clearly visible; E, galls with stalks (arrowed) and rich in tanniferous substances in t/s of leaf of *T. mollis*; F, pit domatium in t/s of leaf of *T. catappa*. Scale bars: A, E, 500  $\mu\text{m}$ ; B, F, 250  $\mu\text{m}$ ; C, 20 mm; D, 100  $\mu\text{m}$ .

gated cells (Figure 5B). Druses are uncommon. A nectary sheath is absent. Stomata were observed. The epidermis, which appears to be non-secretory, becomes highly tanniferous with age as do the cells in contact with it.

*Domatia and galls*

The typical distribution of domatia in representative taxa under study is shown in Figure 2. The appearance (SEM) of domatia in *Terminalia phanerophlebia*, *Quisqualis littorea* and *T. catappa* is illustrated in Figure 6A, B and D respectively. The latter is also shown in t/s in

Figure 6F. The distribution of galls in *T. mollis* and their structure in t/s is depicted in Figure 6C and E respectively.

*Nectar*

The composition of the nectar produced by the EFNs of *Terminalia phanerophlebia* was 35% fructose, 34% glucose and 31% sucrose with no additional peaks indicative of other sugars being present. This differed markedly from that of the floral nectar which was 65% fructose, 21% glucose and 14% sucrose (Figure 7).

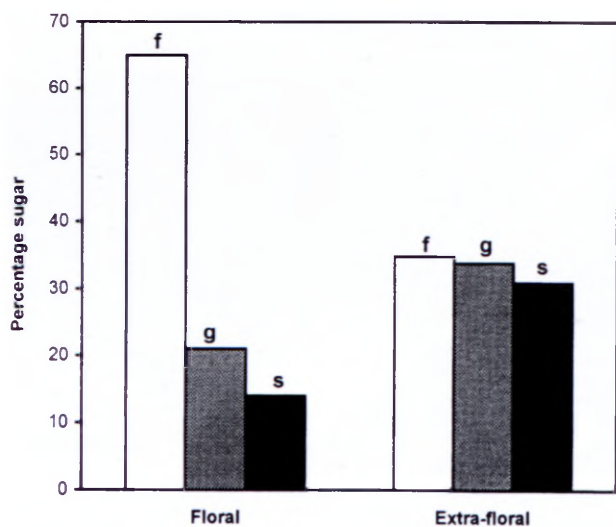


FIGURE 7.—Comparison of sugar composition between floral and extra-floral nectar of *Terminalia phanerophlebia*. f, fructose; g, glucose; s, sucrose.

#### DISCUSSION

Our field observations on five *Terminalia* species (*T. brachystemma*, *T. mollis*, *T. phanerophlebia*, *T. prunioides* and *T. sericea*) confirmed ant and other insect activity on the extrafloral glands in the growing season. Later observations on some of these species revealed no such activity indicating that EFNs may be solely (or optimally) functional on young growth. This is in line with the statement of Elias *et al.* (1975) that foliar nectaries are generally active when the leaves are young. Bentley (1977) cites the studies of a number of researchers, working on families other than the Combretaceae, who also found that EFNs are active on the younger portions of plants, usually being mature and active long before the associated organ is fully developed. Galetto & Bernardello (1992), referring to the work of Hölldobler & Wilson (1990), state: 'There is evidence that plants time their secretions in a way that enhances the protective role of the nectaries'. Jolivet (1996) points out that sugars are generally more concentrated in EFNs than in the phloem partly due to evaporation. In his earlier work on *Clerodendrum fragrans* Willd. (Jolivet 1985), he noted that evaporation could transform the liquid into crystallized sugars on the nectary surface as was found on *Terminalia phanerophlebia* in the present study (Figure 1C). It is noteworthy that when EFNs are observed in nature, they usually appear dry which may create the false impression that they are non-secretory. A tactile stimulation is not necessary to trigger the release of the secretion as EFNs of *T. phanerophlebia* leaves without ants, placed by us in closed containers with a high humidity, produced droplets within minutes. This liquid gradually dissolved any white deposit (sugar crystals) that was present. The EFNs of *T. amazonica* are also active as Schupp & Feener (1991) noted the presence of ants. Ants were also observed by us on *Pteleopsis myrtifolia* during the growing season. No field observations were made by us at this time of year on *Meiostemon tetrandrum*, *P. anisoptera*, *Quisqualis littorea* or on *Q. parviflora*. *Meiostemon*, *Pteleopsis* and *Terminalia* species are deciduous (frequently tardily so); *Quisqualis* species are evergreen. It would thus be informative to see

in the case of *Q. parviflora* at what times it is frequented by ants, if the EFNs are functional. No ant or other insect activity was observed at any time of the year on the glands of *Q. indica*.

#### Distribution

No EFNs were observed in any of the *Combretum* species examined and there do not appear to be any reports of such structures in members of the genus in the literature except on the petiole in *C. argenteum* Bertol. (Zimmermann 1932). The listing of this species is thus considered doubtful and may well be based on confusion with domatia as the latter do occur in members of the genus *Combretum*. The mangrove, *Lumnitzera racemosa*, also appears to lack EFNs. This species, which resembles the other strict combretaceous mangrove, *Laguncularia racemosa*, very closely in leaf anatomy (Biebl & Kinzel 1965; Tilney 2002), thus lacks the petiolar EFNs typical of the latter species. Von Höhnell (1882) refers to two pairs of glands at the apex of the petiole of *Laguncularia racemosa*; the lower pair on small 'warts' but the upper pair not prominent. Belin-Depoux (1993) does not make a size distinction and refers to one to three small prominences on the adaxial side of the petiole of this species. The presence of EFNs in a mangrove seems surprising and observations on their efficacy would be very informative. Glands of unknown function, and only functional in young leaves, have been reported by Biebl & Kinzel (1965) and Stace (1965a) in *Laguncularia racemosa*. The distribution of these glands forms a characteristic pattern (Figure 2A) which is particularly conspicuous in older leaves, and appears to be of taxonomic value. Tomlinson (1986) suggests that these may function temporarily as hydathodes in young leaves, secreting water or mucilage. However, the anatomical structure is similar to what we found in some of the EFNs of the southern African taxa (notably *Terminalia* spp. e.g. *T. trichopoda* Diels) and we recommend that their function be investigated further.

The present study confirmed the presence of EFNs in *Terminalia* but their distribution differs from most previous reports in other species of the genus. In the Combretaceae paired petiolar nectaries, sometimes simply referred to as 'glands', have been reported in unnamed species of *Terminalia*, *Conocarpus* L., *Anogeisus* Wall. and *Laguncularia* by Bentham & Hooker as cited by Solereder (1908); *T. brasiliensis* (authority not indicated), *C. procumbens* L. and *L. racemosa* (Zimmermann 1932); *T. mucronata* Craib & Hutchinson (Belin-Depoux 1978); an unnamed *Terminalia* species (Exell 1978); *T. arjuna* Wight & Arn. (Ramakrishna & Rajashekhara 1981), and in *T. argentea* Mart. & Zucc. and also *T. brasiliensis* Camb. (Oliveira & Leitao-Filho 1987). According to Ramakrishna & Rajashekhara (1981), the EFNs of *T. arjuna* have a prominent stalk which was not observed in any of the species in the present study. In *T. paniculata* Roth, Von Höhnell (1882) describes the glands as large 'warts' ('Warzen'), over 1 mm in height and breadth, occurring on the underside of the lamina on both sides of the base of the midrib. Liben (1965) refers to EFNs being present on the lamina of *T. griffithsiana* Liben, and Van Wyk *et al.* (2000) and Coates Palgrave (2002) on the lamina of certain southern African



*Terminalia* species. In the present study, in some species of *Terminalia*, such as *T. catappa*, glands were found to be next to the midrib near the petiole insertion but were not confined to this position. In such cases, those at the base of the midrib tended to be the largest and most prominent. In other species their distribution appeared completely sporadic without any fixed pattern.

Repeated observations on living material of the indigenous species, *Terminalia phanerophlebia*, revealed occasional glistening areas on some of the veins. These areas were not associated with any visible proliferation of leaf tissue as is found in conventional EFNs. However, it appeared as if a nectar-like substance was being secreted. This raises the question as to whether nectar secretion could be independent of the presence of distinct glands. In the Combretaceae this requires further investigation since non-structural nectaries, which cannot be recognized macroscopically and which exude nectar through stomata, have been reported in many plants of other families (Fahn 1979).

So-called 'inconspicuous bullae', two or three in number and positioned 'on or near laterals' are mentioned in connection with *Pteleopsis anisoptera* but not with *P. myrtifolia* by Carr (1988). These 'bullae' we have identified as EFNs (Figure 3E). When present, we observed 2–6 of these glands per leaf, usually in pairs (Figure 2C). In *P. myrtifolia*, EFNs may also occur but there is almost invariably only a single pair on the lamina (Van Wyk & Van Wyk 1997; Coates Palgrave 2002) in the characteristic position shown in Figures 2D and 3A. When present, these EFNs are a useful taxonomic character.

In the indigenous species *Quisqualis parviflora*, the EFNs are very small and they often appear to be close to one another. The alien, *Q. indica*, has EFNs which are similarly very small but are visible as purple spots which could easily be dismissed as being insect damage. The EFNs appeared to be non-secretory and no ants were observed on the plant. This raises the possibility that feeding activity of ants may be necessary to induce the secretion of nectar in this species. This would provide an effective mechanism to economize on the secretion of nectar, an obviously expensive commodity for the plant to produce. If this is the case, there is the possibility of a substance being secreted to attract ants but these aspects also need further study *in situ*.

It is thus noteworthy that in the case of taxa such as *Pteleopsis anisoptera*, *P. myrtifolia* and *Laguncularia racemosa* the position of the EFNs is genetically fixed but in other taxa (e.g. *Terminalia phanerophlebia*) there is a more flexible system with their formation appearing to be opportunistic. In all cases, however, plants apparently have some control over the formation of these structures (their presence or absence) as well as their functioning.

### Morphology

Zimmermann (1932) reported two types of EFNs on the underside of the leaf in two different species of *Terminalia*, viz. 'Hochnektarien' (raised nectaries) in *T. paniculata*, and 'Flachnektarien' (nectaries flush with the

surface) in *T. catappa*. Pascal *et al.* (2000) prefer the term 'nectary with an apical depression' rather than 'elevated' EFN to describe the stalkless pit or cup-shaped nectaries of some legumes which resemble externally those of some *Pteleopsis* and *Terminalia* species (notably members of the section *Platycarpae*). In particular the scanning electron micrograph of the secretory structure of the legume *Inga feuillei* DC. resembles that of the *Terminalia* species studied and of *Pteleopsis anisoptera*. In the present study, although the glands of the *Meiostemon* and *Quisqualis* species (Figure 3C) are similarly raised to those of the *Terminalia* species (Figure 3D) and *Pteleopsis anisoptera* (Figure 3E), they differ markedly in size and appearance.

### Anatomy

Combretaceous EFNs possess a specially differentiated nectariferous tissue; thus they belong to the structural type (Fahn 1979). However, as was mentioned earlier in connection with *Terminalia phanerophlebia*, there is the possibility of the non-structural type also occurring. The EFNs of the species of *Terminalia* and *Pteleopsis* studied are very similar particularly in having nectariferous tissue composed of very small cells but differ from those of *Quisqualis* and *Meiostemon* which are similar to one another and have secretory tissue in the form of anticlinally elongated palisade-like cells. The latter two genera—as well as the others—do not, however, have elongated epidermal cells which are typical of epidermal glands (Schnell *et al.* 1963; Haberlandt 1965; Wilkinson 1979). The EFNs of the Combretaceae usually have large numbers of calcium oxalate crystals associated with them (Figure 4H) as is reported frequently in various taxonomic groups of plants (Wilkinson 1979). *Pteleopsis myrtifolia* EFNs have the same subepidermal ring of lignified and tanniferous cells as in *T. catappa* and *T. mollis* (Figure 4G). The EFNs of *T. catappa* as described by Zimmermann (1932) and shown in our sections, show a similarity with those of *T. mollis* (Figure 4A, C). Those of *T. mucronata* (Belin-Depoux 1989) also resemble the EFNs particularly of members of the section *Platycarpae*. The 'nectary sheath' of Zimmermann (1932) appears to correspond to the 'median zone' of Belin-Depoux (1989). In *Laguncularia racemosa*, EFNs also consist of differentiated regions (Von Höhnelt 1882; Biebl & Kinzel 1965), and Belin-Depoux (1993) similarly identifies three histological zones in longitudinal sections. Von Höhnelt (1882) reports a similar structure in *T. paniculata* to that in *L. racemosa* apart from an absence of suberisation in the outer sheath of the former.

### EFNs vs. domatia vs. galls

Confusion and difficulties in distinguishing between domatia and glands (EFNs) in many taxa, at least in herbarium material, have been recognized for many years and pointed out by authors such as Jacobs (1966), Wilkinson (1979) and Brouwer (1985). Stace (1965a) mentions the possibility of the mites which inhabit domatia enlarging the domatia, thus making identification of the structures even more difficult. Mites were observed in the domatia of *Terminalia phanerophlebia* (Figure 6A). All the major types of domatia are recorded by Stace (1965b) in the Combretaceae where they are

important diagnostic features in several genera. He makes no mention of EFNs in the family as such but records gland-like structures, found not very frequently, in the domatia of *Buchenavia parvifolia* Ducke and *T. archboldiana* Exell (Stace 1965a). We suspect that many structures thought to be domatia are, in fact, glandular and would more accurately be described as EFNs. If some of these structures are actually EFNs the sugar, in addition to the shelter, could possibly contribute to the high degree of insect infestation and damage in many leaves. Bentley (1977) cites examples where an increase in the rate of secretion caused by sucking insects is positively correlated with increased infestation levels and it would be informative to ascertain whether this is applicable in members of the Combretaceae.

The fact that EFNs and domatia may occupy similar positions on the leaf blade not only results in misinterpretation but it may also indicate that the structures are very closely related in their ontogeny, even to the extent of being interchangeable. The relative abundance of the one or other may well be due to the developmental stage of the leaf. However, Wilkinson (1979) states: 'Since EFNs sometimes occur in the same type of situation as domatia, i.e. in the vein axils on the abaxial surfaces of leaves, the difference of presence or absence of glands is probably of doubtful significance'.

Although EFNs and domatia may be located in similar positions, in our experience it is usually possible to distinguish between them with low magnification. Anatomically the distinction is very clear as can be seen in *Terminalia catappa* (Figures 4C; 6F). Brouwer (1985) gives a comparison between the structure of 'undoubted' domatia and EFNs. In our study of the domatia of *T. catappa* no tanniniferous substances were visible in the vicinity of the domatia and no narrow columnar epidermal cells or hypodermis were evident (Figure 6F). Brouwer (1985) also states that lysigenous cavities in *Lumnitzera racemosa* have been misinterpreted as domatia but no suggestion is made as to their nature.

Jacobs (1966) cites the work of authors who report the presence of galls in the typical position of domatia. This appears to be the result of gall-forming mites preferentially attacking the positions normally occupied by the domatia. Despite similarities (position, shape, the presence often of hairs and of mites), however, galls are generally irregular in extension and cell structure, and the cells 'disturbed', whereas domatia are regular in extension and cell structure, and the cells intact (Jacobs 1966). Furthermore he quotes Lundstroem's (1887) statement that in domatia a palisade layer is present and tannin absent, the opposite of which occurs in galls. Brouwer (1985) and Belin-Depoux (1989) both record the presence of tannins (as well as calcium oxalate crystals) in EFNs. If domatia may 'increase the liability of nerve axils to action on the part of gall-mites' (Jacobs 1966), it seems likely that EFNs would be even more effective in this regard. In the present study galls were frequently observed in the position of EFNs (Figure 6C). Transverse sections of the galls of *Terminalia mollis* (Figure 6E) show that they differ markedly in structure from the EFNs (Figure 4A). The galls are stalked (and therefore more raised) unlike the EFNs. They are also unique in

having one or two large central cavities. Tannins are abundant in the surrounding cells but calcium oxalate crystals are essentially absent. In South African Combretaceae some of the most complex galls are caused by members of the Lepidoptera rather than by mites (Scholtz 1978). Three types of Lepidopteran galls are recorded on the stems and shoots of *Terminalia sericea* and one type in the fruits of *Combretum molle* R.Br. ex G.Don in South Africa. It is evident from Figure 6E that the galls in this specimen are not mite-induced but probably Lepidopteran galls.

#### Nectar

Galetto & Bernardello (1992), reporting on an analysis of the chemical composition of the extrafloral nectar of about 60 plant species, found that three or more sugars were usually present, as is generally the case in floral nectar where sucrose, glucose and fructose are the most common (e.g. Fahn 1988). In *Turnera ulmifolia* L. (Turneraceae), the floral nectar is sucrose-dominant, whereas the foliar nectar is a balanced solution of sucrose, glucose and fructose (Elias *et al.* 1975). Pate *et al.* (1985) noted that in the cowpea [*Vigna unguiculata* (L.) Walp.], the sucrose: glucose: fructose weight ratios of nectar were similar in the two structurally dissimilar types of EFNs but differed markedly in balance between sugars and other organic solutes. Fiala & Maschwitz (1991), in studies of *Macaranga* Thouars (Euphorbiaceae), found that the secretion of the marginal glands of myrmecophytic species lacked sugar unlike that of the non-myrmecophytic species. Freitas *et al.* (2001) studied the composition of nectar produced by floral and EFNs at different stages of development in *Croton sacropetalus* Müll.Arg. (Euphorbiaceae). The sugars in the nectar of all nectary types were mainly monosaccharides with a predominance of glucose over fructose. The only exception was the post-floral nectar (nectar produced after anthesis during fruit development) that mostly had sucrose and a greater proportion of fructose than glucose.

Hardly anything is known about the movement of nectar during the secretory process. For example, since intercellular spaces are essentially lacking, what role does symplastic transport play? It is also not clear how the nectar moves from the nectariferous tissue to the leaf surface. The permeability of the cuticle needs to be investigated and also, in those species where they are present, the possible role of stomata. This requires further study of live material.

In the present study the sugar composition was investigated only in the floral and extra-floral nectar of *Terminalia phanerophlebia*. The EFNs are very active in this species (Figure 1A). In both cases the nectar is composed of only three sugars, fructose, glucose and sucrose (Figure 7). The markedly different ratios of these sugars obtained in floral and extrafloral nectar indicate that different insects would probably be attracted. Our own casual observations suggest that the balanced-sugar nectar is presumably preferred by ants and the fructose-dominant nectar by the pollinators (especially flies) but this aspect obviously requires further study. The very high fructose level of the floral nectar is unusual; fructose-dominant nectar has so far only being found in Aster-

aceae, the genus *Crassula* (Crassulaceae) and in *Welwitschia* (B-E. van Wyk pers. comm.). Bernardello *et al.* (1994) found that the floral nectar sugar composition was remarkably consistent in the different stages throughout the lifetime of the flowers of *Combretum fruticosum* (Loefl.) Stuntz. The nectar contained a very small amount of sucrose (less than 0.6%) and a predominance of hexoses. Fructose and glucose were in a relative ratio of  $\pm 1 : 3.5$  respectively. This thus differs markedly from the sugar composition of both the floral and extrafloral nectar in *Terminalia phanerophlebia*. Further studies are obviously needed to determine whether the unusual sugar composition found in the floral nectar of *T. phanerophlebia* is characteristic of other related taxa and the extent to which the sugar composition of foliar nectar may be of taxonomic significance.

#### CONCLUSIONS

Where EFNs are known to be present, they are only functional, or optimally functional, on young leaves. They can be very small (as in most southern African Combretaceae) and easily overlooked unless specifically searched for. In many taxa their occurrence on a plant is sporadic, thus limiting their taxonomic significance. When present, the EFNs in *Pteleopsis myrtifolia* occur as single pairs in a characteristic position on the lamina which readily enables their identification. In *P. anisoptera*, although the EFNs also tend to be arranged in pairs, many of the gland-bearing leaves have two or three pairs. Glands (EFNs?) on the leaves of *Laguncularia racemosa* form a characteristic pattern. In *Terminalia catappa* there are usually two prominent EFNs, one on either side of the midrib at the base of the leaf, whereas in other species (e.g. *T. phanerophlebia*) they are not necessarily prominent in this position. All southern African species of *Terminalia* except occasionally *T. prunioides* appear to lack paired petiolar glands (not to be confused with paired glands at the junction between petiole and lamina). In this latter species where they were observed on the petiole, they were never conspicuous. Apparent structural EFNs occur in *Meiostemon* and *Quisqualis* species, but it is not known whether they are functional. Ants were, however, seen on young leaves of five *Terminalia* species and were also observed on a plant of *Pteleopsis myrtifolia*. EFNs are absent in *Combretum* and probably also in *Lumnitzera racemosa*.

The macroscopic distinction between EFNs, domatia and galls is often not clear. Structures, previously regarded as domatia, especially on the lamina of *Terminalia* species, may well be EFNs. Since EFNs (and galls) are frequently in the position of domatia, it seems that it is not essential to differentiate between them and that they, just like domatia, can be used for taxonomic purposes. Microscopically they can be readily distinguished. The known microscopic structure of the EFNs of the *Terminalia* and *Pteleopsis* species shows a basic similarity but with somewhat varying degrees of zonation. The structure of the putative EFNs of *Meiostemon tetrandrum* and the *Quisqualis* species studied, on the other hand, differs in cell size, arrangement and zonation from those of the *Terminalia* and *Pteleopsis* species.

In *Terminalia phanerophlebia*, the difference in sugar composition between the nectar of foliar and floral nectaries may have the effect of attracting different insects.

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