

VESTURED PITS: A DIAGNOSTIC CHARACTER IN THE SECONDARY XYLEM OF MYRTALES

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JANSEN, S., PLETTERS, A., RABAEY, D. & LENS, F. 2008. Vestured pits: a diagnostic character in the secondary xylem of Myrtales. Vestures are small projections from the secondary cell wall associated with tracheary elements of the secondary xylem. They are usually associated with bordered pits and characterize various angiosperm families, including important timber species such as Dipterocarpaceae and *Eucalyptus* trees. The micromorphology and distribution of vestures were studied in 22 species representing all families within the order Myrtales based on light and scanning electron microscopy. Vestures are consistently present near the outer pit aperture of bordered vessel pits, suggesting the synapomorphic character of this feature for the entire order. It is unclear in which geological period this feature originated in the evolution of the pre-Myrtalean lineages. In some species vestures are associated with inner pit apertures, inner vessel walls, simple perforation plates, depressions of the cell wall and bordered pits of tracheids or fibre-tracheids. A compact network of branched vestures almost completely filling the entire pit chamber is the most common vestured pit type in Myrtales, although considerable variation may occur within a wood sample. The micromorphology of vestures seems to some extent correlated with quantitative pit characters. Understanding the exact function of vestured pits with respect to hydraulic efficiency and safety remains a challenge.

Keywords: vestures, wood anatomy, bordered pits, Myrtales, scanning electron microscopy, light microscopy, tracheary elements

JANSEN, S., PLETTERS, A., RABAEY, D. & LENS, F. 2008. Pit vestur: ciri diagnostik dalam xilem sekunder Myrtales. Vestur ialah unjuran kecil daripada dinding sel sekunder yang berkaitan dengan trakea xilem sekunder. Vestur biasanya berkait dengan pit berbirai dan mencirikan pelbagai famili angiosperma termasuk spesies balak yang penting seperti Dipterocarpaceae dan *Eucalyptus*. Mikromorfologi dan taburan vestur dikaji dalam 22 species yang mewakili semua famili dalam order Myrtales berdasarkan mikroskopi cahaya serta mikroskopi elektron pengimbas. Vestur sentiasa hadir berhampiran apertur luar pit vesel berbirai. Ini mencadangkan wujudnya sifat sinapomorfik ciri ini bagi seluruh order Myrtales. Tidak dapat dipastikan bila ciri ini muncul dalam evolusi zuriat pra-Myrtales. Dalam sesetengah spesies, vestur berkait dengan apertur pit dalam, dinding sel dalam, plat perliangan, lekukan dinding sel dan pit berbirai trakeid atau trakeid serabut. Jaringan padat vestur bercabang yang hampir memenuhi seluruh ruangan pit merupakan jenis pit vestur yang paling biasa dalam Myrtales, walaupun mungkin terdapat variasi yang agak banyak dalam sampel kayu. Mikromorfologi vestur nampaknya berkait dengan sifat pit kuantitatif. Memahami fungsi pit vestur dari segi kecekapan dan keselamatan hidrolis masih merupakan satu cabaran.

INTRODUCTION

Pits that show protuberances from the secondary cell wall are defined as vestured pits (Bailey 1933, Jansen *et al.* 1998a). In most cases, vestures are associated with the pit chamber or outer pit aperture but vestures can also be found on inner vessel walls, near perforation plates and grooves or thickenings of the vessel wall. Although the distribution of this feature can hardly be observed under the light microscope when pits are minute (< 4 µm) and/or when vestures are

weakly developed, the taxonomic significance of this character at a high taxonomic level had already been established by Bailey (1933). Groundbreaking scanning electron microscopic (SEM) observations have further elaborated our knowledge of the taxonomic distribution of vestured pits (Côté & Day 1962, Schmid & Machado 1964, Scurfield & Silva 1970, Meylan & Butterfield 1974, Jansen *et al.* 2001). Examples of large monophyletic taxa with vestured pits

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include the order Gentianales and families such as Brassicaceae, Malpighiaceae, Polygonaceae and most Fabaceae (Herendeen 2000, Jansen & Smets 2000, Jansen *et al.* 2001). The order Myrtales also forms a large, natural group that is thought to be entirely characterized by vested pits, although confirmation based on SEM observation is still required for Penaeaceae and Psiloxylaceae. Light microscopic observations have shown vested pits in secondary xylem of both families (Carlquist & Debuhr 1977, Van Vliet & Baas 1984), suggesting that vested pits occur in all Myrtales (Baas *et al.* 2000). Studying the micromorphology of vested pits in this species-rich clade is also justified from a functional point of view, since vested pits are thought to be important to whole plant hydraulic function, especially with respect to increased hydraulic resistance, increased resistance to cavitation and preventing damage to pit membranes through rupture (Zweypfenning 1978, Jansen *et al.* 2003, Choat *et al.* 2004).

Several types of vestures have been proposed based on their size, shape and distribution. Van Vliet (1978) recognized two major types of vestures in the Myrtales according to their point of attachment: (1) vestures are attached to all parts of the pit chamber surface and branch into a compact mass of vestures of equal thickness (type A), and (2) a ring of trunk-like vestures is attached to the opening of the pit canal (type B). Based on the degree of vesture thickness and branching pattern, Van Vliet (1978) distinguished three subtypes within type B. It has been suggested that some vested pit types are characteristic of particular families, subfamilies or tribes, although their major taxonomic significance relies on the presence or absence of the feature (Van Vliet 1978, Jansen *et al.* 1998a, 2001). In addition to taxonomic differences, some authors noticed that the micromorphology of vestures may depend on the type of pitting and on pit characteristics (Meylan & Butterfield 1974, Ohtani & Ishida 1976, Van Vliet 1978, Wu *et al.* 1989). While Jansen *et al.* (2004) did not find any variation in incidence of vested pits by pit size, data from the InsideWood database indicate that vested pits are about twice as common in woods with minute to medium-size pits ($\leq 4\text{--}10\ \mu\text{m}$) compared with woods with large ($\geq 10\ \mu\text{m}$) pits (Wheeler *et al.* 2007). Therefore, it remains questionable to what extent pit structure (e.g. size or shape of pit border, area of pit aperture

and depth of the pit canal) may affect the shape and distribution of vestures.

This paper investigates the distribution and micromorphology of vestures in 22 species representing all Myrtales families according to APG (2003). The aims of this paper are (1) to verify the synapomorphic nature of vested pits within the order, (2) to document the micromorphological variation, and (3) to investigate possible correlations between pit structure and vesture morphology.

MATERIALS AND METHODS

All wood samples studied were collected from the wood collection (Kw) or herbarium of the Royal Botanic Gardens, Kew. A list of the specimens studied is given below, with family classification following the APG (2003). Transverse and longitudinal sections as thin as possible (ca. 10–15 μm) were sectioned with a sliding microtome to observe the bordered pit structure in detail. Preparation of the wood samples was according to standard techniques (Jansen *et al.* 1998b). The micromorphology of vestures was investigated using a field-emission SEM (Hitachi S-4700). Wood anatomical terminology follows the IAWA list (IAWA Committee 1989). Measurements on SEM images were carried out using Carnoy 2.0 and mean values were based on 10 measurements (Schols *et al.* 2002).

Alzateaceae: *Alzatea verticillata* Ruiz & Pav., Peru, San Martin, Zepelacio, Moyobamba, G. Klug 3349, Kew herbarium; **Combretaceae:** *Anogeissus leiocarpa* Guill. & Perr., Sokoto, Moiser, Kw 9106; **Crypteroniaceae:** *Dactylocladus stenostachya* Oliv., Sarawak, Kw 10496; **Heteropyxidaceae:** *Heteropyxis natalensis* Harv., South Africa, Smithsonian Institute 150, Kw 10558; **Lythraceae:** *Duabanga sonneratioides* Buch.-Ham., USA, Plant Introduction Garden, Florida, M. Scott, Kw 10508; *Galpinia transvaalica* N.E.Br., Swaziland, J. Prior 403, Kw 75654; *Punica protopunica* Balf.f., Socotra, A.R. Smith, J. Lavranos 287, Kw 10543; **Melastomataceae:** *Conostegia xalapensis* D.Don., Mexico, Jalisco, H. Iltis, F. Iltis & R. Koepfen 631, Kw 10295; *Dissotis principes* Triana var. *candolleana* (Cogn.) A.Fern. & R.Fern., Swaziland, J. Prior 362, Kw 75589; *Memecylon amabile* Bedd., India, Maharashtra, Edin. Forest Exhib. 1884, Kw 10312; *Mouriri plasschaerti* Pulle, Surinam, G. Stahel, Kw 10373; **Myrtaceae:** *Cleistocalyx ellipticus* (A.C.Sm.) Merr. & Perry, Fiji, Galoa,

Serua, Ram Swamp, RSI 2463, FD 1275, Kw 9389; **Oliniaceae:** *Olinia cymosa* (L.f.) Thunb., South Africa, Cape of Good Hope, collector unknown, Kew herbarium; **Onagraceae:** *Fuchsia excorticata* L.f., New Zealand, J. Edgerley 1839, Kw 11425; **Penaecaceae:** *Endonema lateriflora* (L.f.) Gilg., South Africa, E.Esterhuysen 5064, Kew herbarium; *Glischrocolla formosa* (Thunb.) R.Dahlgren, South Africa, J.P.Rourke 1046, Kew herbarium; *Penaecia cneorum* Meerb. ssp. *gigantea* R.Dahlgren, South Africa, R. Dahlgren & Strid 2925, Kew herbarium; *Sonderothamnus petraeus* (W.F.Barker) R.Dahlgren, South Africa, Dahlgren & Strid 3519, Kew herbarium; **Psiloxylaceae:** *Psiloxylon mauritianum* Baill., Mauritius, Grand Bassin, Coode 4693, Kw 10061; **Rhynchocalycaceae:** *Rhynchocalyx lawsonioides* Oliv., South Africa, Natal, Verdcourt H.1000/73, Bot. Research Institute Pretoria, Kw 10480; **Vochysiaceae:** *Qualea dinizii* Ducke var. *glabrifolia* Meurs, Surinam, G. Stahel, Kw 916; *Vochysia vismiiifolia* Spruce ex Warm., South America, Spruce, Kw 939.

RESULTS

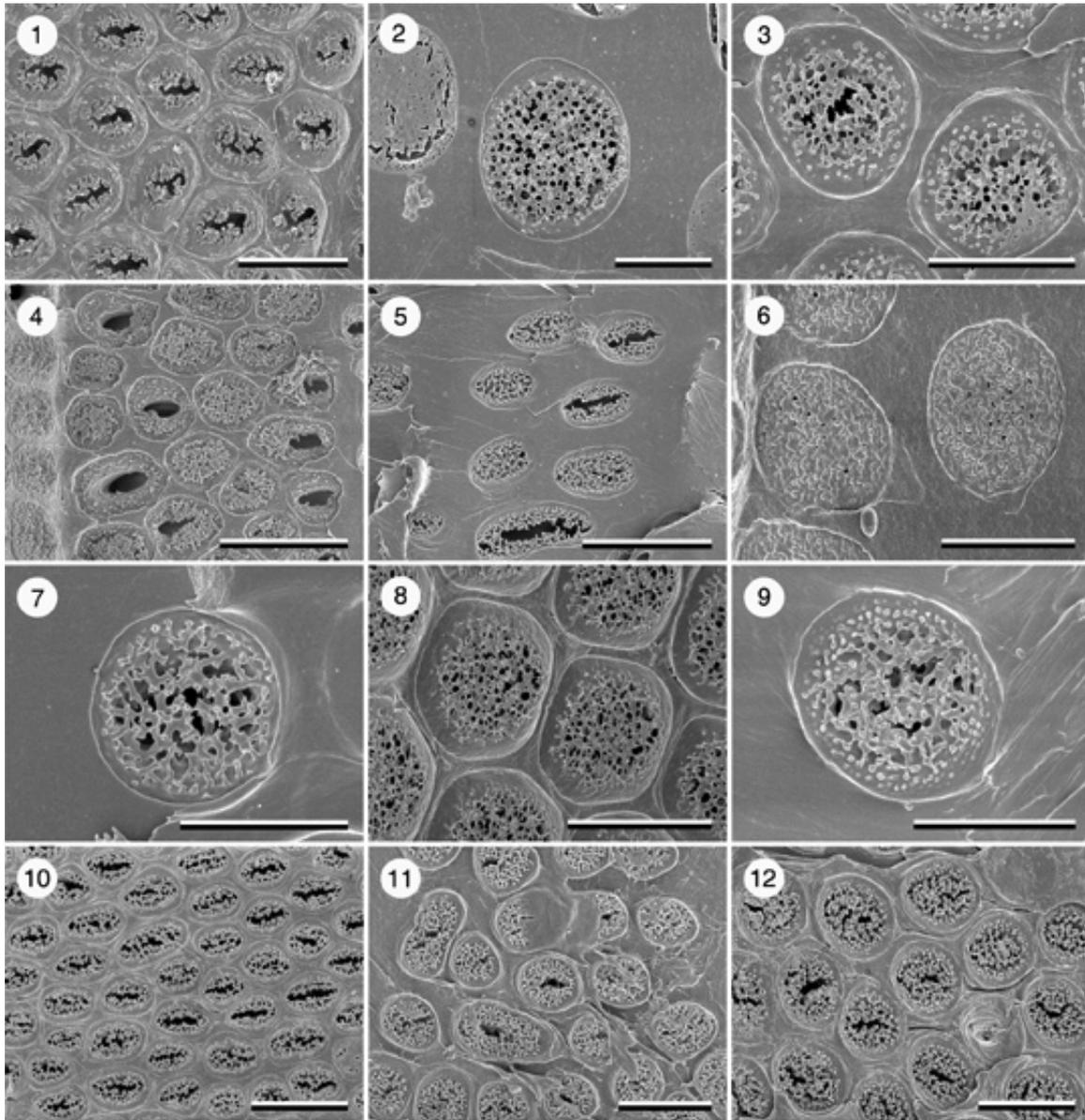
All wood samples studied have vestures associated with the outer pit aperture of vessel pits (Figures 1–16), which show an opposite to alternate arrangement. The micromorphology of the vestures differs considerably, not only between different species and families, but also between pits within a single vessel element. Mean values for the thickness of vestures (diameter of vestures at the broadest point) are around 0.1–0.2 μm . The longest branches of vestures can be up to a few μm in height. The smallest vestures are simple and dot-like (Figure 1) and generally occur on the roof of the pit chamber, i.e. away from the pit aperture, while the branched vestures partly or completely overarch the outer pit aperture (Figures 3, 7 and 9). Remarkably thick and large vestures that are occasionally anastomosed occur in the species Myrtaceae, Vochysiaceae and Psiloxylaceae. In some species (*Alzatea verticellata*, *Memecylon amabile*, several Penaecaceae and *Rhynchocalyx lawsonioides*) the vestures are distributed on all parts of the pit border and correspond to type A according to Van Vliet (1978) (Figures 6, 11 and 16). However, it is not always easy to determine to which parts of the pit border vestures are attached. Also, several species show a combination of vested pit types A and B (Table 1). Species with vestures that are

only weakly branched include *Anogeissus leiocarpa* (Figure 1), *Heteropyxis natalensis*, *Conostegia xalapensis*, *Fuchsia excorticata*, *Qualea dinizii* and *Vochysia vismiiifolia* (Figure 14).

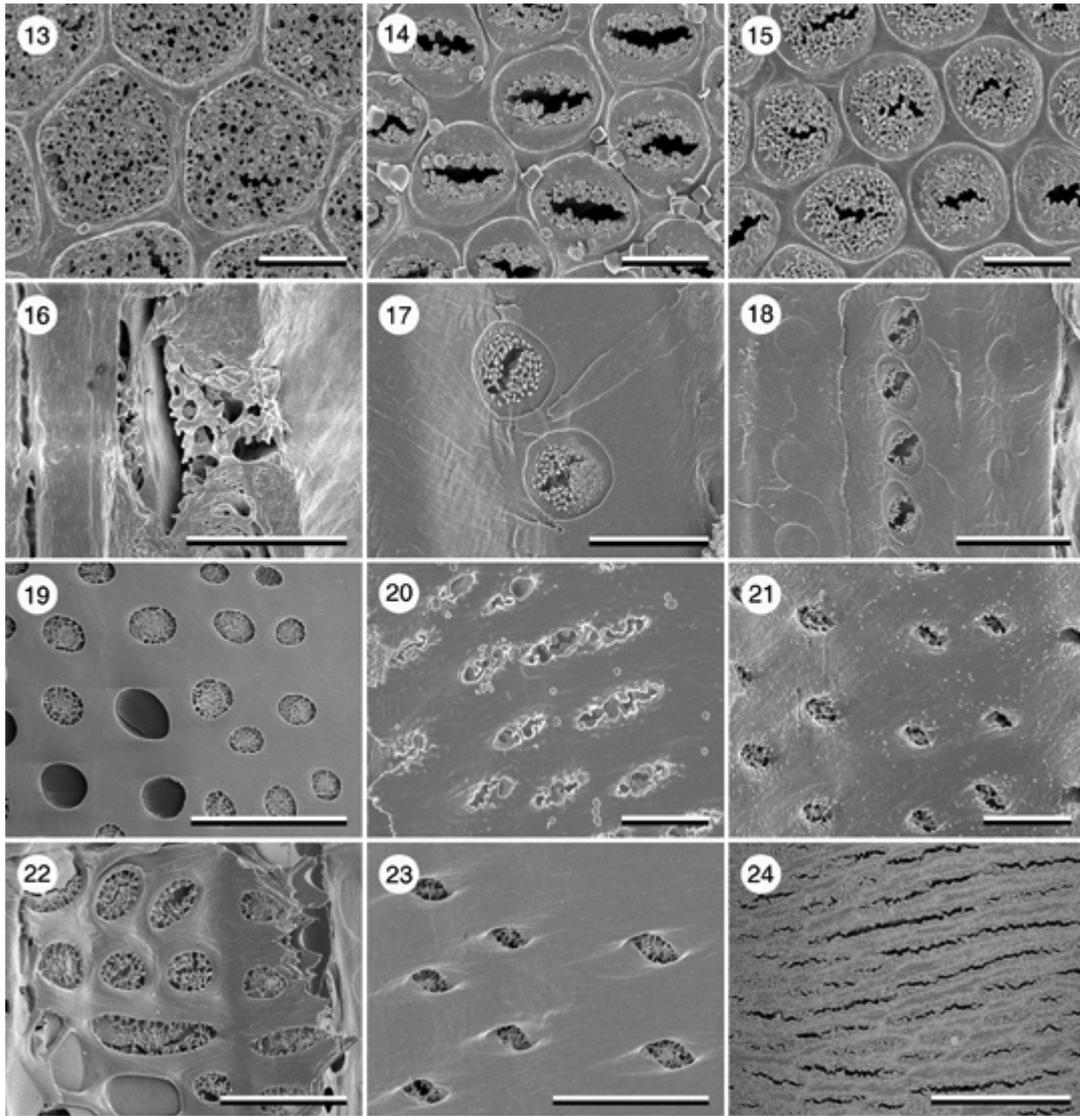
Vestures associated with the pit border are interconnected in most species and form a complex network, which may partially (Figures 1, 10, 14 and 15) or completely (Figures 2–9 and 13) occlude the pit chamber. A more or less central crack or gap in this network is observed in some samples and may reflect the outline of the slightly elongated pit aperture (Figures 5, 10, 14 and 15). Branched vestures are partly or wholly detached from the pit chamber wall due to sample preparation in some pits such as in *Memecylon amabile* (Figure 4). Micromorphological variation of the vestures can be illustrated in *Qualea dinizii*: some vessel pits show a large network of branched vestures (Figure 15), while more weakly developed vestures occur in other vessel pits (Figure 14).

A summary of quantitative vessel pit features is given in Table 1. The vessel pits are on average 5 μm in horizontal diameter. The mean surface area of the total pit border of vessel pits is ca. 20 μm^2 , but varies from 58 μm^2 in *Duabanga sonneratioides* and 43 μm^2 in *Dactylocladus stenostachya* to ca. 9 μm^2 in *Punica protopunica* and *Heteropyxis natalensis*. Although it is not always easy to delimit the border of the outer pit aperture on SEM images, especially when vestures are abundantly present, the outer pit aperture is in most species relatively wide, with 0.44 as the mean ratio of the outer pit aperture area per pit border area. The proportion of the area of the outer pit aperture per area of pit border varies from 0.11 in *Duabanga sonneratioides* to 0.6 and 0.63 in *Dactylocladus stenostachya* and *Dissotis principes* respectively. The surface area of the inner pit aperture varies from 1.1 μm^2 in *Olinia cymosa* to 22.9 μm^2 in *Dactylocladus stenostachya*.

When the total intervessel wall between two neighbouring vessels is thick (e.g. 10–14 μm in *Anogeissus leiocarpa*), there is a clear pit canal, which does not show vestures. In some species, however, the total intervessel wall is thin (ca. 3 μm) and a pit canal is almost lacking (e.g. *Dactylocladus stenostachya* (Figure 19), *Dissotis principes* (Figure 22), *Fuchsia excorticata* (Figure 25)). Vestures associated with the pit border can sometimes be seen through the inner pit aperture (Figure 23). Vestures associated with inner pit apertures (Figures 26 and 27) appear



Figures 1–12 Vestured vessel pits viewed from the outer vessel wall, with the pit membrane removed in most pits. TLS = tangential longitudinal section. RLS = radial longitudinal section. Figure 1. *Anogeissus leiocarpa* (Combretaceae): small vestures partly overarching the outer pit aperture (TLS); Figure 2. *Dactylocladus stenostachya* (Crypteroniaceae): network of branched vestures filling the pit chamber (RLS); Figure 3. *Galpinia transvaalica* (Lythraceae s.l.): branched vestures near the outer pit aperture and small, unbranched vestures near the rim of the pit border (TLS); Figure 4. *Conostegia xalapensis* (Melastomataceae): vestures occluding the pit chamber, vestures were partly removed in some pits during sample preparation (RLS); Figure 5. *Dissotis principis* var. *candolleana* (Melastomataceae): branched vestures filling partly or completely the pit border (RLS); Figure 6. *Memecylon amabile* (Memecylaceae): pit aperture occluded by vestures (TLS); Figure 7. *Mouriri plasschaerti* (Memecylaceae): branched and unbranched vestures (RLS); Figure 8. *Cleistocalyx ellipticus* (Myrtaceae): vestured network overarching outer pit apertures (TLS); Figure 9. *Endonema lateriflora* (Penaeaceae): branched vestures near the outer pit aperture and small, unbranched vestures near the rim of the pit border (TLS); Figure 10. *Psiloxylon mauritianum* (Psiloxylaceae): branched vestures leaving a narrow gap in the pit border of some pits (TLS); Figure 11. *Rhynchoacalyx lawsonioides*: outer pit apertures with distinctly developed vestures (RLS); Figure 12. *Punica protopunica* (Lythraceae s.l.): branched vestures occluding the pit border, with small, dot-like vestures near the rim of the pit border (TLS). Scale bars = 10 μ m in Figures 1, 4, 5, 10; 5 μ m in Figures 2, 6, 8, 11, 12; 4 μ m in Figures 3, 7, 9.



Figures 13–24 Vestured pits viewed from the outer (Figures 13–15, 17–18) and inner (Figures 19–24) pit aperture. Figure 13. *Duabanga sonneratioides* (Lythraceae s.l.): vessel pit border occluded by vestures (TLS); Figure 14. *Qualea dinizii* (Vochysiaceae): small vestures restricted to the outer pit aperture, prismatic crystals from neighbouring parenchyma cells visible (TLS). Figure 15. *Qualea dinizii* (Vochysiaceae): branched vessel networks fill up most of the pit chamber, leaving a small gap in the centre (RLS); Figure 16. *Endonema laterifolia* (Penaeaceae): cross-section through a bordered vessel pit with vestures near the inner and outer aperture (TLS); Figure 17. *Sonderothamnus petraeus* (Penaeaceae): vestured pits in narrow tracheary element (RLS); Figure 18. *Sonderothamnus petraeus* (Penaeaceae): fibre-tracheid with bordered pits showing minute vestures near the outer pit aperture (TLS); Figure 19. *Dactylocladus stenostachya* (Crypteroniaceae): vestures can be seen through most inner pit apertures of the vessel wall (RLS); Figure 20. *Heteropyxis natalensis* (Heteropyxidaceae): narrow and elongated inner pit apertures of a vessel with relatively thick and indistinctly branched vestures (RLS); Figure 21. *Galpinia transvaalica* (Lythraceae s.l.): small, weakly developed vestures randomly distributed on the inner vessel wall, and associated with the inner pit aperture (RLS); Figure 22. *Dissotis principis* (Melastomataceae): vestures associated with most, but not all inner pit apertures of a vessel (RLS); Figure 23. *Mouriri plasschaerti* (Memecylaceae): vestures can be seen through the inner pit apertures, but are absent on the inner vessel wall (RLS); Figure 24. *Cleistocalyx ellipticus* (Myrtaceae): inner vessel wall with elongated inner pit apertures almost completely occluded by vestures, which are also spread out on the inner vessel wall near the inner apertures (TLS). Scale bars = 20 μm in Figures 19, 24; 10 μm in Figures 18, 22, 23; 5 μm in Figures 13, 14, 15, 17, 20, 21; 2 μm in Figure 16.

Table 1 Vestured pit type and vessel pit characteristics of the Myrtales species studied

Species (family)	VPT	PD	PB	OPA	IPA	IWT	IP	IW
<i>Alzatea verticillata</i> (Alzateaceae)	A	8.8 (4.7–15.5)	32.3 (15.9–48.4)	14.6 (5.5–26.8)	10 (3.1–27.5)	?	+	+
<i>Anogeissus leiocarpa</i> (Combretaceae)	B3	6.6 (5.9–7.4)	27.2 (21.2–32.6)	8.7 (7.2–10.3)	4.6 (2.9–6.5)	11.8 (10–14)	?	?
<i>Cleistanthus ellipticus</i> (Myrtaceae)	A-B1	4.8 (4.1–6.6)	21 (16.2–32.3)	11.1 (7.9–17.4)	20.1 (16–24.7)	9 (8–10)	+	±
<i>Conostegia xalapensis</i> (Melastomataceae)	A-B1-B3	4.9 (3.9–7.2)	18.9 (11.5–28.1)	5.5 (3.7–7.9)	5.7 (3.9–8.4)	7.9 (6–11)	+	-
<i>Dactyloctenium stenostachya</i> (Crypteroniaceae)	A-B1	6.9 (5.7–10)	43.3 (33.6–55.4)	26.1 (16.7–33.4)	22.9 (11.2–38.4)	?	-	-
<i>Dissotis principis</i> (Melastomataceae)	B1	5.1 (3.8–7.4)	23.4 (18.4–36.8)	14.9 (6.5–10)	9.4 (3.5–28.6)	3.8 (3–4)	+	-
<i>Duabanga sonneratioides</i> (Lythraceae)	A-B1	8 (7.2–8.7)	58 (50.6–65.2)	6.8 (5.7–7.5)	18.3 (13.7–22.9)	?	-	-
<i>Endonema lateriflora</i> (Penaeaceae)	A	4.8 (4–5.2)	17.2 (10.2–23.4)	4.9 (3.3–6.7)	3.5 (2.4–5.5)	?	+	+
<i>Fuchsia excorticata</i> (Onagraceae)	B3	4.8 (4.1–6.6)	34.1 (20.4–47.6)	19.2 (10.6–28.4)	13.1 (9.5–16.5)	?	-	-
<i>Galpinia transvaalica</i> (Lythraceae)	A-B1	4.4 (3.6–4.9)	14.8 (12.3–17.2)	6.3 (5.8–6.9)	1.7 (1.3–2.8)	9.8 (6–8)	+	+
<i>Glischrocolla formosa</i> (Penaeaceae)	A	4 (3.6–5)	12.1 (9.4–14.1)	?	3.9 (3.3–4.2)	?	+	±
<i>Heteropyxis natalensis</i> (Heteropyxidaceae)	B3	3.3 (2.8–4)	9.2 (7.1–10.5)	2.8 (1.9–3.4)	3.6 (2.5–6.1)	10.4 (9–13)	+	+
<i>Mameylon amabile</i> (Melastomataceae)	A	4.8 (3.4–5.9)	19.6 (15.8–24.5)	9.6 (8.8–11.3)	2.2 (1.4–3)	?	+	-
<i>Mouriri plasschaerti</i> (Melastomataceae)	A-B1	4.3 (3.4–5)	13.8 (8.2–18.5)	6.8 (4.5–8.9)	3.8 (2.8–4.9)	?	±	-
<i>Olinia cymosa</i> (Oliniaceae)	B2-B3	4.3 (3.4–5)	13.7 (9.1–20.2)	2.1 (1.5–2.7)	1.1 (0.7–1.4)	8.8 (7–12)	-	-
<i>Penaea cucurum</i> ssp. <i>gigantea</i> (Penaeaceae)	A	3.3 (3–6.3)	14.7 (8.7–22.1)	6.4 (3.2–10.9)	5 (3.2–7.5)	?	+	-
<i>Psiloxylon mauritianum</i> (Psiloxylaceae)	B1	5.9 (4.9–6.9)	26 (20.7–31.3)	12.6 (8.9–17.2)	5.3 (4.2–6.2)	7 (6–8)	+	±
<i>Punica protopunica</i> (Lythraceae)	A-B1	3.2 (2.9–3.7)	9.7 (7.6–10.9)	4.6 (3.4–5.4)	1.5 (1.2–1.8)	6.6 (5–8)	?	?
<i>Qualea dimizia</i> var. <i>glabrifolia</i> (Vochysiaceae)	A-B1-B3	5.7 (5.2–6.3)	25 (21.8–27.9)	12.4 (10.3–14.3)	6.3 (3.6–9.2)	?	+	+
<i>Rhynchosobolus lausonoides</i> (Rhynchosolaceae)	A	3.6 (2.9–6.1)	10.4 (7.6–19.4)	5.6 (4–10.3)	1.8 (0.8–2.8)	9.2 (8–11)	+	+
<i>Sonderothamnus petraeus</i> (Penaeaceae)	A-B1	3.8 (3.3–4.3)	13.4 (10.6–15.8)	7.2 (3.8–7.5)	4.7 (3.2–9.4)	?	+	±
<i>Vochysia vismifolia</i> (Vochysiaceae)	B1-B2-B3	5.1 (4.5–6.1)	27.5 (21.3–31.7)	12.9 (10.5–16.6)	21.5 (15.7–32.8)	?	+	+

VPT = vestured vessel pit types according to Van Vliet (1978); PD = horizontal pit diameter (μm); PB = surface area of pit border (μm^2); OPA = surface area of outer pit aperture (μm^2); IPA = surface area of inner pit aperture (μm^2); IWT = intervessel wall thickness of two neighbouring vessels (μm); IP = inner pit apertures vestured; IW = inner vessel wall vestured; mean values given for 10 measurements (minimum and maximum values between brackets); + = vestured; - = non-vestured; ± = vestures occasionally present; ? = unknown

to be common in specimens that show small inner pit apertures (e.g. *Heteropyxis natalensis*, *Galpinia transvaalica*), with the exception of *Vochysia vismiifolia*, *Cleistocalyx ellipticus* and *Alzatea verticillata*.

A dense layer of vestures spreading out onto the vessel wall and obscuring the elongated, sometimes coalescent inner pit apertures is found in Myrtaceae and Vochysiaceae, sometimes giving the impression of underlying coalescent inner pit apertures (Figure 24 and 32). Remarkably thick, nodule-like vestures are sporadically observed in *Cleistocalyx ellipticus* (Figures 30–31), and *Vochysia vismiifolia* (Figure 35). In other taxa with slightly vested vessel walls, the vestures are concentrated in the depressions in the vessel wall, for instance around the pit apertures (Figures 20, 21, 28, 29), cell corners (Figure 35), or near the rim of simple perforation plates (Figure 33). Vestures arising from the sides of coalescent inner pit apertures occur in *Cleistocalyx ellipticus* (Figure 32) and *Vochysia vismiifolia* (Figures 35–36). These coalescent inner pit apertures, which can also be interpreted as grooves in the inner vessel wall, are mainly restricted to particular areas of vessel elements, such as cell corners and perforation plates. In addition, vestures are not equally associated with these grooves because they may be distinctly present or almost absent. Vested simple perforation plates are found in *Glischorcolla formosa* and *Sonderothamnus petraeus* (Figure 34) in combination with non-vested perforation plates. The vested perforations are remarkably small in diameter (ca. 10 µm) and are overarched by a prominent border of secondary wall material in a more or less similar way to that found in bordered pits. The perforation plate vestures show a remarkable similarity in morphology to those found in the vessel pits in the same wood. Furthermore, in some perforations the vestures can be seen lining the border on each side of the perforation plate, while in others they line only one of the two borders. The depression between the borders in each perforation plate, however, is always free of vestures.

Besides intervessel pits, vestures are observed in vessel-axial parenchyma and vessel-ray parenchyma pits. In most cases, it is rather difficult to determine the exact nature of the entire pit pair, since only surface structures can be seen under the SEM. In some species, the vessel-ray pits show reduced borders to simple pits, and these are usually non-vested, e.g. in Onagraceae, Melastomataceae,

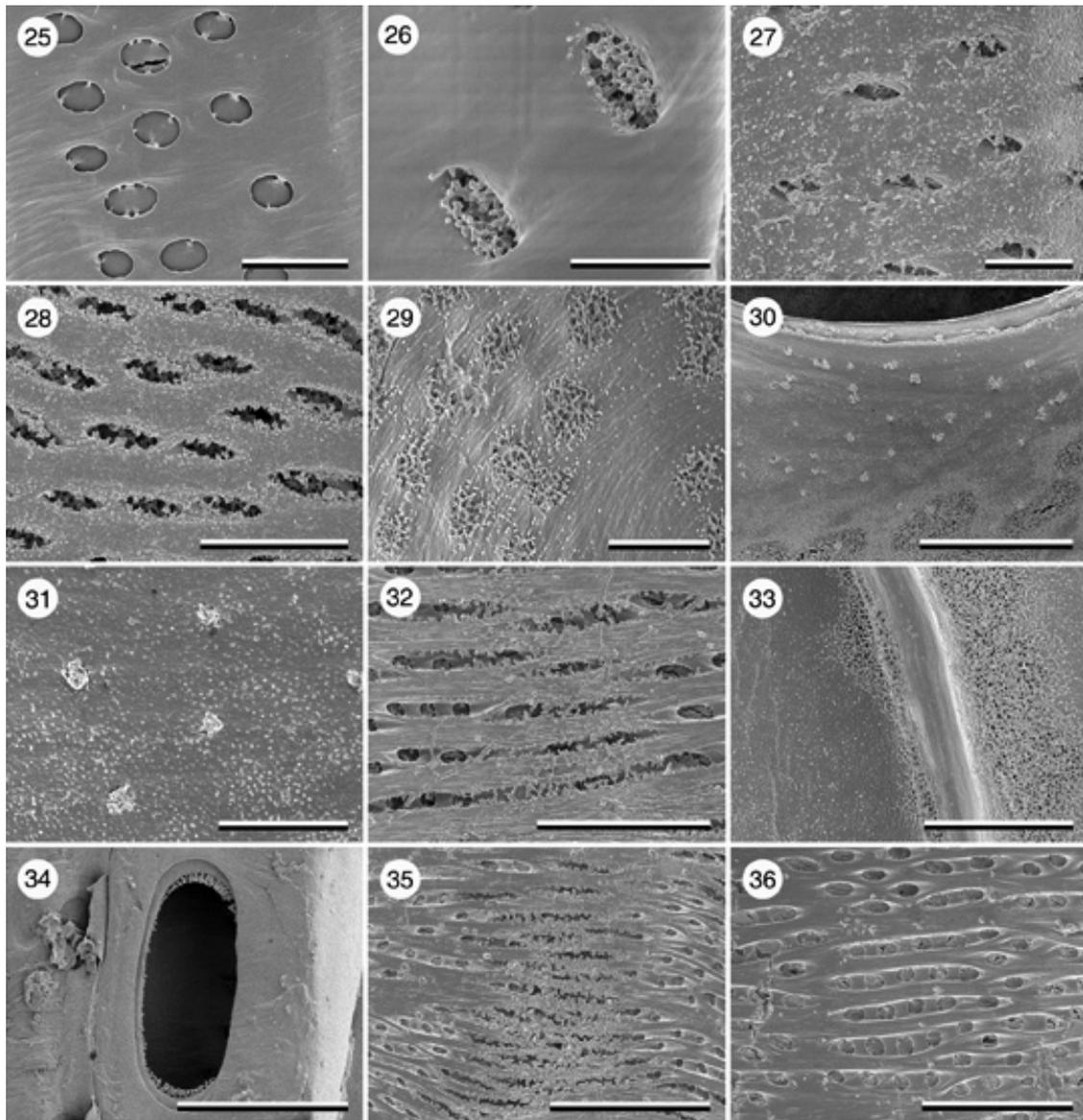
Crypteroniaceae, and Myrtaceae. Interestingly, vestures are absent or very sparsely developed in some vessel-ray pits of *Dactylocladus stenostachya* and *Dissotis principes*, while they are abundant in others (Figures 19 and 22). Large vessel-ray pits are usually non-vested or weakly vested, while smaller pits are more distinctly vested. Vestures associated with pit borders of vessel-ray pits appear to be connected to the pit membrane in *Fuchsia excortica* (Figure 25). Similar observations of intervessel pits with vestures seemingly connected to the pit membrane are observed in *Vochysia vismiifolia* (Figure 36).

Vestures also occur in the bordered pits of fibre-tracheids and tracheids of *Sonderothamnus petraeus* (Figure 17 and 18). The vestures are minute, bead-like in some fibre pits, but more abundant and branched in other pits. In some bordered fibre pits only a few bead-like vestures are present near the pit aperture, while in other pits the vesturing is more abundant and sometimes branched. This variation in vesturing as well as some non-vested fibre pits can be observed in fibre-tracheids in close proximity. Vestures are not observed in association with libriform fibres, axial parenchyma cells and simple pits in the species studied.

DISCUSSION

The nature of vested pits in Myrtales

The presence of vested pits is observed in all families of Myrtales and, as far as we know, illustrated for the first time based on SEM observations in Penaeaceae and Psiloxylaceae. Thus, our results suggest the conservative nature of vested pits for the traditional Myrtales as well as for the Myrtales sensu APG (Van Vliet & Baas 1984, Baas *et al.* 2000, APG 2003). The inclusion of Vochysiaceae, which is transferred from Polygalales to the APG Myrtales, is supported by the distribution of vested pits. Families that were previously placed within Myrtales, but could now be excluded include Anisophylleaceae, Haloragaceae, Lecythidaceae, Rhizophoraceae and Thymelaeaceae. All of these families except Thymelaeaceae show non-vested pits (Orchard 1975, Van Vliet 1976, de Zeeuw 1990, Lens *et al.* 2007). Thymelaeaceae show weakly vested pits in bordered pits of tracheids and fibre-tracheids, although the vessel pits are usually non-vested (Ohtani & Ishida 1976, Jansen *et al.* 2000).



Figures 25–36 Vestures associated with inner vessel walls and perforation plates. Figure 25. *Fuchsia excorticata* (Onagraceae): vessel-ray pitting with few vestures seemingly connected to the pit membrane (TLS); Figure 26. *Penaea cneorum* (Penaeaceae): smooth inner vessel wall and vested inner pit apertures (TLS); Figure 27. *Endonema lateriflora* (Penaeaceae): vested inner vessel wall and inner pit apertures (TLS); Figure 28. *Psiloxylon mauritianum* (Psiloxylaceae): elongated inner pit apertures with well developed vestures and vested inner vessel wall (TLS); Figure 29. *Rhynchocalyx lawsonioides* (Rhynchocalycaceae): vestures occluding inner pit apertures and sparsely spread out on the inner vessel wall (RLS); Figure 30. *Cleistocalyx ellipticus* (Myrtaceae): part of a simple perforation plate, a densely vested inner vessel wall below and remarkably thick, nodule-like vestures near the perforation plate (RLS); Figure 31. *Cleistocalyx ellipticus* (Myrtaceae): detail of minute vestures and large, nodule-like vestures on an inner vessel wall (RLS); Figure 32. *Cleistocalyx ellipticus* (Myrtaceae): coalescent inner pit apertures with a dense distribution of branched vestures (RLS); Figure 33. *Cleistocalyx ellipticus* (Myrtaceae): inner vessel wall with vestures most densely distributed near a simple perforation plate (RLS); Figure 34. *Sonderothamnus petraeus* (Penaeaceae): vestures near the rim of a small, simple perforation plate (TLS); Figure 35. *Vochysia vismifolia* (Vochysiaceae): coalescent inner pit apertures with vestures densely distributed in a small depression of the vessel wall (RLS); Figure 36. *Vochysia vismifolia* (Vochysiaceae): inner vessel wall with coalescent pit apertures and with few weakly developed vestures seemingly connected to the pit membrane (TLS). Scale bars = 50 μm in Figures 35; 40 μm in Figures 36; 20 μm in Figures 30, 32; 10 μm in Figures 25, 28, 33, 34; 5 μm in Figures 26, 27, 29, 31.

Although vested pits may well represent a synapomorphic character for the Myrtales, this can not be determined without commenting on its closely related groups. The position of the Myrtales within the rosids is still unstable according to recent angiosperm phylogenies, and most publications place the order outside both Eurosids I and II (Savolainen *et al.* 2000, Soltis *et al.* 2000, APG 2003, Hilu *et al.* 2003). However, Zhu *et al.* (2007) found some support for a position sister to all other rosids except Geraniales, Vitales and Saxifragales. Among the potential relatives of Myrtales, vested pits have only been recorded in *Alvaradoa amorphoides* but not in *Picramnia ramiflora* (Picramniaceae). They are also lacking in any member of the Vitales, Crossosomatales and Geraniales (Jansen *et al.* 2001). Thus, it is likely that vestures are indeed synapomorphic for Myrtales. However, if Picramniaceae would be sister to the Myrtales, then vestures could be synapomorphic for Myrtales and Picramniaceae. The distribution of vested pits in Picramniaceae needs further observation based on a wider number of genera.

It is suggested that the oldest branches of Myrtales started differentiation between 111 and 107 Ma during the early Albian of the Lower Cretaceous (Wikström *et al.* 2001, Sytsma *et al.* 2004). It could be suggested that vestures originated as a result of ecological adaptation to changes in the habitat or as a functional adaptation to more efficient and safe water transport. Although further experimental research is needed, our current understanding of vested pits is that they increase the resistance to drought-induced cavitation by reducing the probability of air-seeding through pit membranes (Zweypfenning 1978, Jansen *et al.* 2003, Choat *et al.* 2004). Unfortunately, we do not precisely know the ecological preferences or exact distribution of the ancestors of this large and ancient clade. It could be speculated that the origin of vestures at the base of the Myrtales has played a role in the successful speciation of this order, since the order is much larger in number of species than any other presumably related order.

Micromorphological variation of vested pits in Myrtales

Based on our observations and literature data (Van Vliet 1978, Van Vliet & Baas 1984), vested pits of van Vliet's (1978) type A are the

most common type in Myrtales, characterizing Alzateaceae, Crypteroniaceae, Penaeaceae, and Rhynchocalycaceae. Interestingly, these families form a clade together with Oliniaceae based on Stevens (2001 onwards). Type B, on the other hand, is widespread in Onagraceae, Lythraceae and Oliniaceae. The combined occurrence of type A and B is found in Combretaceae, Lythraceae s.l. (including Punicaceae and Sonneratiaceae), Melastomataceae and Myrtaceae. Type B tends to be associated with the evolutionary trend towards herbaceousness, because this type is more common in small trees, shrubs and subshrubs (e.g. Onagraceae). Similarly, vestures of type B were found to be more frequent within Gentianales in subshrubs than in large trees, especially in more herbaceous groups such as Asclepiadaceae and Gentianaceae (Jansen & Smets 2000). A possible explanation is that species with a limited amount of woodiness generally show pit borders with a shallow pit chamber, no pit canal and relatively thin-walled vessels or fibres. Vestures appear to be more weakly developed in thin-walled cells and shallow pit borders. Thus, the morphological variation in vestures encountered could be due to the geometry of the pit border, which in its turn may rely on the thickness of the secondary cell wall.

Due to the large number of intermediate forms, even within a single cell, the different types of vestures show only little diagnostic and taxonomic value. Nevertheless, their location and micromorphology can be regarded as characteristic of a particular group. Examples are the subfamilies Strephonematoideae (type A) and Combretoideae (type B) within Combretaceae (Van Vliet 1978), and a fairly consistent distribution of vested pits of type A in Alzateaceae, Crypteroniaceae, Penaeaceae and Rhynchocalycaceae. Moreover, inner vessel walls that are distinctly vested appear to characterize some Myrtaceae, Vochysiaceae and Psiloxylaceae, which are closely related based on recent phylogenetic insights (Stevens 2001 onwards). Wilson *et al.* (2005) suggested merging Psiloxylaceae and Heteropyxidaceae into Myrtaceae s.l. based on a *matK* phylogeny. The unusual occurrence of 'nodule-like' vestures and vested helical thickenings as observed in Myrtaceae and one sample of Vochysiaceae also reflect a close relationship between groups of this clade. However, these systematic implications are blurred by the variation that can be found

within a single specimen and may need further investigation based on a larger number of species.

In conclusion, the variation in distribution and prominence of vestures together with the variation in the occurrence of different types suggest that the presence of vestures is taxonomically more important at the ordinal level of Myrtales than any additional evidence provided by Van Vliet's vested pit types.

Linking structural features with the distribution and morphology of vested pits

Our quantitative measurements of the morphology of bordered pits suggest that the species studied have a relatively high outer pit aperture area relative to the total area of the pit border. This implies that the area of the pit border is rather limited, and this seems especially to be the case when a branched network of vestures fills the entire pit border. On the other hand, vestures may not fill the entire pit chamber when the size of the pit border becomes too wide to be entirely occluded. This can be illustrated for instance by the distinctly vested pits in bordered vessel pits of Penaeaceae (vested pit type A), while pits of (fibre-)tracheids in the Penaeaceae species studied show a more narrow and elongated outer pit aperture surrounded by small, mainly unbranched vestures. Nevertheless, the idea that vested pits show a higher outer pit aperture area relative to the total area of the pit border should be tested by comparing vested pit species with non-vested pit species. Similarly, the suggestion that inner pit apertures become more frequently vested when these are small and when a pit canal is clearly present would need further investigation in a wider range of taxa.

It has been suggested that vestures are formed during the final stages of cell wall differentiation when the protoplast conglomerates in depressions of the secondary cell wall, since invaginations of the plasma membrane in these areas would result in a retarded withdrawal or breakdown of the cytoplasmic components in maturing cells (Schmid & Machado 1964, personal observation). Excess of lignified wall material may then be deposited onto the cell wall around pit apertures, but also on helical thickenings, rims of perforation plates, or in cell corners (Scurfield & Silva 1970, Ohtani & Ishida 1976,

Ohtani *et al.* 1984). The association of vestures with irregularities on the inner vessel wall (e.g. cell corners, coalescent inner pit apertures, and perforation plates) as illustrated in our observations supports developmental control with respect to the distribution of vestures.

It is unclear to us why vestures are never associated with the rim of large, simple perforation plates, but only with relatively small perforations. Observations of small, simple perforation plates with vestures were also reported by Baas (1977) in *Leptospermum crassipes* (Myrtaceae). Kučera *et al.* (1977) concluded that the observation of vested perforation plates suggests that vestures are deposited prior to the death of the protoplast, which has also been suggested by developmental work (Schmid & Machado 1964, personal observation). As illustrated by Jansen *et al.* (2003, 2004), there is a strong correlation between vested pits and the vessel perforation type, since vested pits are nearly always associated with simple perforation plates. Almost all members of the Myrtales are characterised by simple perforation plates, although there are a few exceptions (Lucas *et al.* 2007). Schmid and Baas (1984) examined in detail the distribution of scalariform perforation plates within Myrtaceae. Multiple perforation plates are thought to be retained in a limited number of Myrtaceae species from cool mesic habitats where there is a lack of strong selective pressure to 'transform' them into simple perforations (Carlquist 2001). As far as we know, the only member of Myrtales with vested scalariform perforation plates was found in *Neomyrtus pedunculata* (Butterfield & Meylan 1974).

There is a clear need to conduct more developmental studies on vestures since more information about their formation may help us to understand their distribution and morphological variation. Further research on the functional significance would also be very welcome and genera such as *Eucalyptus* would be potentially interesting model organisms for investigating the genetic background of vestures.

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