Morpho-anatomical studies on the leaf reduction in *Casuarina*: the ecology of xeromorphy

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Abstract

Key message The foliage characters found in *Casuarina* seedlings may represent the ancestral, scleromorphic ones found in the Casuarinaceae. In the adults studied, these are replaced by derived xeromorphic features.

Abstract The ontogenetic changes in the foliage of two *Casuarina* species were investigated. While the cotyledons are flattened linear structures, all other leaf-types are strongly reduced. Except for the two primary leaves, all subsequent leaves are strongly fused to each other and also to the shoot axis, except for the leaf tips; the shoot axis is completely surrounded by photosynthetic leaf tissue and the branchlet is not made up of cladodes but of extended leaf sheaths which are a novel strategy for achieving reduced photosynthetic area. In seedlings there are four leaves per node, forming four shallow vertical furrows where light-exposed and non-encrypted stomata are developed. These features are also developed in the adult foliage within the strictly scleromorphic genus *Gymnostoma*, clearly the most mesic of the present day genera of Casuarinaceae and very likely to include the ancestral types. Thus, we assume that the *Casuarina*-seedling leaves reflect the ancestral scleromorphic condition. In the adult foliage,

the number of leaves per node is strongly increased, which leads to the formation of several nearly closed vertical furrows on the shoot, where stomata are shaded and strongly encrypted. Thus, the adult foliage shows several xeromorphic features that are absent in the juvenile foliage. Our morpho-anatomical data mapped on ecological and palaeobotanical data show that within Casuarinaceae the foliage shifted from scleromorphic to xeromorphic. Thus, the adult xeromorphic foliage in *Casuarina* is the derived, advanced state.

Keywords Anatomy · *Casuarina* · Leaf · Morphology · Scleromorphy · Xeromorphy

Introduction

This is one of a series of papers dealing with the anatomy, morphology, evolution and ecology of extreme leaf reduction in seed plants, with special emphasis on documenting any changes in the leaf form from seedlings to adults (e.g. Dörken 2013; Dörken and Parsons 2016).

Strong leaf reduction is quite common in gymnosperms (e.g. Farjon 2005, 2010a, b; Eckenwalder 2009; Dörken 2013; Dörken and Parsons 2016), but it can also be found in a wide range of angiosperms (e.g. Rehder 1967; Krüssmann 1976, 1977, 1978; Kubitzki et al. 1993; Kubitzki 2004) and can also be proven for extinct taxa in the fossil record (e.g. Foster and Gifford 1974; Taylor et al. 2009). In evergreen taxa it is often associated with xeric conditions, where it reduces transpiration via the lamina for plants in habitats where water is the main limiting factor (e.g. Thoday 1931; Blum and Arkin 1984; Blum 1996; Bosabalidis and Kofidis 2002; Parsons 2010; Seidling et al. 2012). However, xeric conditions

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especially drought are just some of the forces leading to leaf reduction. In other habitats, e.g. subalpine and alpine ones, leaf reduction can be correlated with stress from both low temperatures and water deficit (e.g. Korner 2003; Parsons 2010). Also, low soil fertility, especially low phosphorus availability, can lead to the formation of strongly reduced leaves (e.g. Loveless 1961, 1962; Beadle 1966; Seddon 1974; Hill and Merrifield 1993; Hill 1998; Salleo and Nardini 2000; Dörken and Parsons 2016) as is typical for several Ericaceae (e.g. Düll and Kutzelnigg 2011; Dörken and Jagel 2014). At first glance, both drought and a lack of nutrients seem to lead to the same leaf structures. However, drought leads to a xeromorphic leaf structure showing, e.g. a thick cuticle, encrypted stomata or dense pubescence, while low soil fertility leads to a scleromorphic one with, e.g. high amounts of sclerenchyma (Dörken and Parsons 2016). In this paper, we strictly follow Hill (1998) using the term xeromorphy to refer to morpho-anatomical responses to water deficit and scleromorphy to nutrient deficient soils especially to low levels of phosphorus. Of course some features like strong leaf reduction can be found in xeromorphic and scleromorphic foliage as well.

In this paper, we turn our attention to the eudicot family Casuarinaceae (Fagales), a small subtropical to temperate group of trees and shrub with four genera, *Gymnostoma*, *Allocasuarina*, *Casuarina* and *Ceuthostoma* (Torrey and Berg 1988; Kubitzki et al. 1993; Steane et al. 2003). All Casuarinaceae are characterized by an evergreen habit and a strong leaf reduction. The family is unusually interesting because its affinities are obscure (Hill and Brodribb 2001); members are easily distinguished from all other angiosperms because of their needle-like articulate branchlets with longitudinal ridges (phyllichnia) separated by furrows and other features (Zamaloa et al. 2006). Due to these unique foliar features we have chosen to work on this family partly because ontogenetic studies about its leaf development do not exist and the exact interpretation of stem and leaf tissue is still open. More importantly, we had access to seed and seedlings of *Casuarina* and were able to compare our data with those of Torrey and Berg (1988) on *Gymnostoma*. This is important because *Gymnostoma* contrasts with *Casuarina* by having the oldest fossils in the family including species with non-xeromorphic characters (Steane et al. 2003). Thus, our aims are to explore the evolution of scleromorphy and xeromorphy by documenting the ontogenetic changes in leaf characters with seedling age in species of *Casuarina* and to compare these data with published data from *Gymnostoma*. Also, we make a detailed examination of the extent of leaf and stem tissue in *Casuarina* branchlets to establish what constitutes leaf reduction in that genus.

Materials and methods

Material

Seeds of *Casuarina cunninghamiana* Miq. were obtained from the Murray Mallee Seedbank, Nyah, Victoria, Australia, and seeds of the ecologically contrasting *Casuarina equisetifolia* L. from the Botanic Garden Konstanz, Germany. They were germinated and grown in a temperate glass house with long day conditions in the Botanic Garden Konstanz. Seeds were sown in a mixture of compost and vermiculite (5:1). The glasshouse temperature ranged from 25 °C (day) to 15 °C (night). Material from adult individuals was received from the Botanic Garden of the Ruhr-University Bochum (Germany) where the trees are cultivated in a temperate house. Voucher specimens of the investigated taxa are lodged at the FRP—Herbarium of the Palmengarten Frankfurt a. M. (Germany).

Methods

Freshly collected material was photographed and then fixed in FAA (100 ml FAA=90 ml 70% ethanol+5 ml acetic acid 96%+5 ml formaldehyde solution 37%) before being stored in 70% ethanol. The leaf anatomy was studied from serial sections using the classical paraffin technique and subsequent astrablue/safranin staining (Gerlach 1984). Macrophotography was accomplished using a digital camera (Canon PowerShot IS2) and microphotography with a digital microscope (Keyence VHX 500F) equipped with a high-precision VH mounting stand with X-Y stage and bright-field illumination (Keyence VH-S5).

Results

Leaf morphology and leaf anatomy of *C. cunninghamiana*

Cotyledons

The hypocotyl is well developed (Fig. 1a). The two small, flattened, bifacial cotyledons are 2.5–4-mm long and 2.0–2.5-mm wide (Fig. 1b). They are amphistomatic and apparently more frequent on the adaxial side. They are irregularly arranged and slightly sunken in the epidermis. The epidermal cells of both sides are similar in size and shape. Those on the leaf margin are up to double the size of the others. The epidermis is covered with a thin cuticle. The mesophyll is distinctly dimorphic. A well-developed palisade parenchyma is orientated towards the light exposed adaxial side of the leaf and consists of 2–3 cell layers. The spongy parenchyma consists of isodiametric cells showing

Fig. 1 *Casuarina cunninghamiana*, leaf development in different ontogenetic stages I: juvenile leaves; **a** seedling; **b** cotyledon (cross section); **c** primary leaf (cross section); **d** young subsequent leaves at a shoot axis (cross axis), *C* cuticle, *CL* chlorenchyma, *CO* col-

lenchyma, *E* epidermis, *M* mesophyll, *PP* palisade parenchyma, *P* phloem, *S* stoma, *SP* spongy parenchyma, *VB* vascular bundle, *X* xylem

large intercellular spaces. The cotyledons are supplied with a single collateral vascular bundle strand that branches several times. There is no distinct bundle sheath (Fig. 1b).

Primary leaves

The epicotyl is strongly reduced (Fig. 1a). The two primary leaves are small, 0.4–0.6-mm long and 0.1–0.2-mm wide (Fig. 1c). The adaxial side is orientated towards the shoot axis and becomes the shaded surface, the former abaxial side the new light-exposed surface. The leaves are amphistomatic, but with stomata mostly arranged on

the adaxial side. They are slightly sunken in the epidermis. The epidermal cells of the light-exposed abaxial side are about double the size of those of the shaded adaxial side (Fig. 1c). Also, the cell walls of the light-exposed epidermal cells are two to three times thicker than the cell walls of shaded epidermal cells. A weakly developed cuticle is present. The mesophyll is monomorphic and consists of several isodiametric cells showing weakly developed intercellular spaces. There is only a single, weakly developed, unbranched collateral vascular bundle strand. There is no distinct bundle sheath (Fig. 1c).

Subsequent juvenile leaves

There are four leaves at each node. They are about 4–10-mm long and 0.2–0.4-mm wide (Fig. 1a). The adaxial surface of subsequent juvenile leaves is orientated towards the shoot axis. The leaves are strongly fused to each other and to the cortical tissue of the complete internode (Fig. 1a). At the subsequent node, the leaves are still fused to each other but no longer to the shoot axis. In the most distal part only the tips are free. Thus the node is surrounded by a closed leaf sheath (Fig. 1a). There is no sharp boundary layer between leaf- and shoot-tissue (Fig. 1d). The stomata which develop are light-exposed and non-encrypted in the four shallow vertical furrows. There are no trichomes covering the stomata; a thin cuticle covers the epidermis. Below the epidermis, a collenchyma consisting of 1–2 layers of small cells with strongly thickened walls occurs, below which is a chlorenchyma consisting of palisade parenchyma-like cells. There is a single unbranched collateral vascular bundle strand supplying the leaf. There is no distinct bundle sheath (Fig. 1d).

Adult leaves

On adult individuals, 6–10 (–11) whorled leaves are inserted per node. These are about 6–15-mm long and 0.2–0.3-mm wide and show a hyaline tip (Fig. 2a). Later the leaf tips become withered and brown. The leaves are strongly fused to each other and also to the cortical tissue for the length of the internode (Figs. 2c, d, 3). Only the leaf tips are free (Figs. 2a, 3a). At the subsequent node, the leaves are still fused to each other but no longer to the shoot axis and surround the node as a closed leaf sheath (Fig. 3). Between leaf- and shoot-tissue no sharp boundary layer is developed (Figs. 2c, d, 3). A weakly developed cuticle is present. Stomata are deeply sunken in the epidermis and only become encrypted in the shaded vertical, nearly closed furrows between the leaves. On the light-exposed surface of the leaves, stomata are absent. Below the epidermis, a collenchyma consisting of 1–2 layers of cells with strongly thickened walls is formed. In the region of the midvein, the collenchyma consists of 4–6 cell layers. The collenchyma cells are about half the size of the epidermal cells. Below the collenchyma, a well-developed palisade parenchyma-like chlorenchyma develops. The leaf is supplied with a single unbranched collateral vascular bundle strand, which ends blindly in distal parts of the leaf shortly below the tip. There is no bundle sheath (Fig. 2b).

Leaf morphology and leaf anatomy of *C. equisetifolia*

Cotyledons

The hypocotyl is well developed (Fig. 4a). The two cotyledons are flattened, bifacial structures, 2.2–2.9-mm long and 0.9–1.2-mm wide (Fig. 4b). They are amphistomatic, with the majority of stomata developed irregularly on the adaxial side. The stomata are slightly sunken in the epidermis. The epidermal cells of both leaf sides are similar in size and shape. The mesophyll is dimorphic, with a distinct palisade parenchyma located towards the light-exposed adaxial side and a spongy parenchyma with huge intercellular spaces towards the shaded abaxial side. The leaf is supplied with a single collateral vascular bundle strand that branches several times. There is no distinct bundle sheath (Fig. 4b).

Primary leaves

The epicotyl is strongly reduced (Fig. 4a). The two primary leaves are about 0.4–0.6-mm long and 0.1–0.2-mm wide (Fig. 4c). They are strongly adpressed to the shoot axis, so that the abaxial side is light-exposed and the adaxial side shaded (Fig. 4a). The primary leaves are amphistomatic, but with stomata mostly arranged on the adaxial side. They are slightly sunken in the epidermis. The epidermal cells of the abaxial surface are about double the size of those of the adaxial side. The cell walls of the shaded epidermal cells are about half or a third of the thickness of those of the light-exposed epidermal cells. The mesophyll is monomorphic and consists of isodiametric cells showing small intercellular spaces. A weakly developed, unbranched collateral vascular bundle is developed. There is no distinct bundle sheath (Fig. 4c).

Subsequent juvenile leaves

In young seedlings, four whorled subsequent leaves are inserted per node (Fig. 4a). They are 5–10-mm long and 0.2–0.4-mm wide (Fig. 4d) and are strongly reduced and fused to each other and to the cortical tissue of the shoot axis over the complete internode (Fig. 4d). Only the leaf tips are free (Fig. 4a). At the subsequent node, the leaves are still fused to each other but no longer to the shoot axis. Thus the node is surrounded by a closed leaf sheath (Fig. 4a). No sharp boundary layer between leaf- and shoot-tissue is developed (Fig. 4d). The stomata which develop are non-encrypted and more or less light-exposed in the four vertical shallow furrows. Trichomes covering the stomata are absent. Below the epidermis, a weakly developed collenchyma can be detected, consisting of one interrupted layer of cells with strongly thickened cell walls. These cells are about half the size of the epidermal cells. Below the collenchyma, a palisade parenchyma-like chlorenchyma is developed. The leaf

Fig. 2 *Casuarina cunninghamiana*, leaf development in different ontogenetic stages II: adult leaves; **a** in distal parts adult leaves are forming a leaf sheath surrounding the subsequent node; **b** cross section as marked in **a**; **c** longitudinal section of a complete internode;

d detail of **c**, between the leaf- and shoot-tissue no sharp boundary layer is developed. *C* cuticle, *CL* chlorenchyma, *CO* collenchyma, *E* epidermis, *P* phloem, *S* stoma, *VB* vascular bundle, *X* xylem

is supplied with one, unbranched, collateral vascular bundle strand. There is no distinct bundle sheath (Fig. 4d).

Adult leaves

6–8 leaves are inserted per node in the branchlets of adult individuals (Fig. 5a). They are 6–13-mm long and 0.2–0.4-mm wide (Fig. 5b). They are strongly fused to each other and to the cortical tissue of the complete internode (Figs. 5b–d, 6b–f). Only the hyaline leaf tips remain free (Fig. 5a). Later they become withered and brown. At the subsequent node, the leaves are still fused to each other but no longer to the shoot axis. They surround the node as a

closed leaf sheath (Fig. 6a). There is no sharp boundary layer between leaf- and shoot-tissue (Figs. 5c, d, 6b–f). The margins of two adjacent leaves form a nearly closed vertical furrow where strongly encrypted stomata are developed (Fig. 5a, b). In the light-exposed parts, stomata are absent. Below the epidermis a well-developed sclerenchyma occurs, consisting of two layers of strongly lignified cells. In the region of the midvein, the sclerenchyma consists of up to seven layers (Fig. 5b). In some leaves, the sclerenchyma is T-shaped and developed from shortly above the vascular bundle to directly below the epidermis. A palisade parenchyma-like chlorenchyma is developed below the sclerenchyma. Due to the T-shape of the sclerenchyma, the

Fig. 3 *Casuarina cunninghamiana*, serial sections of an internode; **a** close up of one internode; **b–f** cross-sections as marked in **a**

Fig. 4 *Casuarina equisetifolia*, leaf development in different ontogenetic stages I: juvenile leaves; **a** seedling; **b** cotyledon (cross section); **c** primary leaf (cross section); **d** young subsequent leaves at the

shoot axis (cross axis). *C* cuticle, *CL* chlorenchyma, *CO* collenchyma, *E* epidermis, *M* mesophyll, *PP* palisade parenchyma, *P* phloem, *S* stoma, *SP* spongy parenchyma, *VB* vascular bundle, *X* xylem

chlorenchyma is divided into two parts, one on either side of the ridge. Each leaf is supplied with a single collateral, unbranched vascular bundle strand. There is no distinct bundle sheath (Fig. 5b).

Discussion

The morpho-anatomical shift from juvenile to adult foliage

A major finding of the results is that the juvenile subsequent leaves and the adult leaves of both species are whorled, strongly reduced and fused to the shoot axis

except for the leaf tips; the shoot axis is surrounded completely by green palisade parenchyma-like leaf tissue which performs virtually all the of the photosynthesis. This is very different to the structure of superficially similar leafless or virtually leafless seed plants like *Ephedra* (Ephedraceae, Gymnospermae), *Cytisus*, *Genista* and *Spartium* (Fabaceae, Angiospermae) where photosynthesis is restricted to green chlorenchymatic stem tissues of the outer stem cortex (Thompson 1912; Voth 1934; Cutler 1939; Inamdar and Bhatt 1971; Freitag and Maier-Stolte 2003; Dörken 2014). A similar situation to that found in *Casuarina* is found in some gymnosperm species, e.g. *Diselma*, *Chamaecyparis*, *Fokienia* and *Thuja* (Cupressaceae), where the shoot axis is also completely surrounded by leaf

Fig. 5 *Casuarina equisetifolia*, leaf development in different ontogenetic stages II: adult leaves; **a** in distal parts adult leaves are forming a leaf sheath surrounding the subsequent node; **b** cross section as marked in **a**; **c** longitudinal section of a complete internode; **d** detail

of **f**, no sharp boundary layer between the leaf- and shoot-tissue is developed. *C* cuticle, *CL* chlorenchyma, *E* epidermis, *P* phloem, *S* stoma, *SC* sclerenchyma, *VB* vascular bundle, *X* xylem

tissue (e.g. Feustel 1921; Krüssmann 1983; Farjon 2005, 2010a, b; Tetzlaff 2005; Eckenwalder 2009; Dörken 2013), albeit with much shorter internodes than *Casuarina* where an extended toothed leaf sheath performs virtually all of the photosynthesis. To our knowledge, this *Casuarina* strategy for achieving reduced photosynthetic area has not been recorded before, except possibly in the case of *Tamarix aphylla* (L.) Karsten (see Fig. 8b of Gaskin 2003).

Our finding that *Casuarina* shoots are clothed by leaf tissue conflicts with the current literature, where *Casuarina* shoots are referred to as “cylindric cladodes” (e.g. Rao 1972; Zimpfer et al. 2004; Niinemets et al. 2005) and

“phylloclades” (Warrier et al. 2013). These terms refer to stem tissue not leaf tissue and so are inappropriate.

All subsequent juvenile leaves and adult leaves of both species have a well-developed collenchyma with strongly thickened cell walls directly below the epidermis except for the adult leaves of *C. equisetifolia*, where that tissue is replaced by a well-developed sclerenchyma with strongly thickened and lignified cell walls. While it appears that *C. equisetifolia* occurs in drier and generally more stressful habitats than *C. cunninghamiana* (see below), the exact reasons for the presence of sclerenchyma in *C. equisetifolia* are not yet known.

Fig. 6 *Casuarina equisetifolia*, serial sections of an internode; **a** close up of one internode; **b–f** cross-sections as marked in **a**

Among extant seed plants, it is not uncommon for the leaf morphology and anatomy of young seedlings to differ markedly from those in adults, as in *Acacia* (Pedley 1986) and *Eucalyptus* (Stebbins 1950). Such observations are often related to the “Rekapitulationstheorie” put forward by Ernst Haeckel in 1866 that ontogeny recapitulates phylogeny, so that, e.g. in phyllodinous *Acacia* species the juvenile pinnate leaves represent the ancestral type of adult foliage (Pedley 1986). In the Casuarinaceae, *Gymnostoma* is clearly the most mesic of the present day genera and is very likely to include the ancestral types. This idea is supported by the fact that in molecular-based phylogenetic family trees, *Gymnostoma* is always placed as basal (Steane et al. 2003). Several significant similarities exist between young *Casuarina* seedlings and adult *Gymnostoma* plants, for example four leaves at a node, shallow furrows between the leaves and non-encrypted stomata that are not covered with trichomes. Thus, the *Casuarina* juvenile features may represent ancestral, scleromorphic *Gymnostoma* adult features which are then replaced by derived xeromorphic features in adult *Casuarina*.

Correlation between leaf structure and environmental conditions

The morpho-anatomical characters of *Casuarina* shoots lead to slow growth, which makes these species poor competitors for light compared to broad-leaved species. Thus, *Casuarina* is often found on sites showing unfavourable conditions for other trees like drought, skeletal, rocky soils or seasonally waterlogged flood plains, marsh margins, deep sand of beach foredunes that includes further stresses such as excessive drainage, soil alkalinity and exposure to wind and salt spray (Ladd 1988). Low fertility of the soils can also be involved (Ladd 1988; Reddell et al. 1997). Like the rest of the family, both *C. equisetifolia* and *C. cunninghamiana* have clustered/ proteoid roots and the nitrogen fixing actinomycete *Frankia* in root nodules, both being adaptations to infertile soils, especially to low levels of nitrogen and phosphorus (Torrey and Berg 1988; Reddell et al. 1986, 1997; Stevens 2015).

Both species are common in wet areas up to 1500-mm mean annual rainfall. While *C. cunninghamiana* is a medium-sized to tall tree 20–30-m high (the largest Australian *Casuarina*), *C. equisetifolia* is a smallish tree 8–16-m high (Boland et al. 2006). Also, *C. cunninghamiana*, being riparian, receives supplementary moisture from flooding and often occur in sheltered areas transitional to rain forests (Ladd 1988). All of this suggests more stressful habitats for *C. equisetifolia*, the stresses including water deficit from excessive drainage on deep sands and wind exposure, as well as exposure to salt spray and alkaline coastal sands (Ladd 1988). All this might lead to the formation of the well-developed sclerenchyma in the adult leaves of *C. equisetifolia*.

Casuarina species, and the whole family of the Casuarinaceae, are widely regarded as a group of plants well adapted to xeric conditions (Rao 1972; Heywood 1978; Ladd 1988; Schütt et al. 2002). Most *Casuarina* species occur in regions with high temperatures and periodic water stress in combination with strong light and so are regarded as highly specialised xerophytes. This idea is supported by the fact that within *Casuarina*, stomata are nearly exclusively encrypted in deep, vertical, nearly closed furrows with a sharp functional boundary layer between the stomatal zone and the chlorenchyma where they are well protected against low humidity and high solar radiation and which may represent a selective advantage in dry climates (Heywood 1978; Steane et al. 2003).

However, in one genus of the Casuarinaceae, the stomata are not strongly encrypted. In *Gymnostoma*, the shoots are more or less quadrangular in cross section with four strongly reduced leaves per node. The four vertical furrows on the shoot are widely opened, and the stomata are non-encrypted and freely exposed to the environmental conditions (Torrey and Berg 1988; Wilson and Johnson 1989; Steane et al. 2003). Furthermore, the stomata of *Gymnostoma* differ from those of the more xeromorphic genera of the Casuarinaceae by a well developed internal opening into large intercellular chambers that can facilitate the gas exchange between the environment and the photosynthetic tissue (Torrey and Berg 1988), a feature lacking in *Casuarina*. By allowing for more furrows per internode, the 6–10 leaves per node in the species studied compared to four in *Gymnostoma* may represent a further xeromorphic development in addition to the stomatal one.

The first Casuarinaceae were possibly rainforest associated taxa occurring in wet climates (Ladd 1988). Thus, the oldest known Casuarinaceae fossils lack xeromorphic features, and stomata were developed in open furrows with few or no trichomes (Steane et al. 2003). From the upper Palaeocene of Australia, branchlets which developed four leaves per node are reported as *Gymnostoma antiquum* and are supposed to reflect the most primitive character states within the Casuarinaceae (Scriven and Hill 1995). Thus, fossil specimens of the Casuarinaceae clearly show that an increased number of leaves per node and also the encryption of stomata are derived features.

The southern Australian climate has significantly changed during the last 35 million years and shifted from ever-wet with mild temperatures to hot dry summers in combination with mild and wet winters. Throughout this period of continental drying, the vegetation has significantly changed as has the morphology of the Casuarinaceae (Hill and Brodribb 2001). Hill (1990) suggested the Casuarinaceae as a group of plants showing perfectly the consequences of a developing aridity in southern Australia during the Cainozoic. *Gymnostoma* is described as

Table 1 Traits which may be related to scleromorphy (S) and xeromorphy (X) in *Gymnostoma* and two species of *Casuarina*

Trait	Taxon		
	<i>Gymnostoma</i>	<i>C. cunninghamiana</i>	<i>C. equisetifolia</i>
Leaves per node/furrows per internode	4 (S)	6–10 (X)	6–8 (X)
Nature of furrow	Open (S)	Nearly closed (X)	Nearly closed (X)
Encryption of stomata	Non-encrypted (S)	Strongly encrypted (X)	Strongly encrypted (X)
Trichomes in furrows	Few or none (S)	Numerous (X)	Numerous (X)

Gymnostoma data from Torrey and Berg (1988) and Steane et al. (2003)

the only Casuarinaceae taxon that could be identified as widespread and common in the uniformly wet climate of the Palaeogene. Later when the southern Australian climate started drying, *Gymnostoma* was no longer so competitive and was largely replaced by the genera *Casuarina* and *Allocasuarina* Hill (1990). Hill and Brodribb (2001) suggest that *Gymnostoma* is adapted to oligotrophic soils, having an evergreen habit, extensive root system and strongly reduced and highly fibrous leaves, thus being scleromorphic. However, those features are also found in *Allocasuarina* and *Casuarina*. Importantly, those genera also have the stomatal and other xeromorphic features, described above which are absent from *Gymnostoma*.

The scleromorphic condition in *Gymnostoma* (Table 1) is the primitive state in the Casuarinaceae. Thus, it follows that more than four leaves per node as developed in *Casuarina* and *Allocasuarina* are a derived feature. The increasing number of leaves per node leads consequently to a strong reduction of the space between the leaves and an increased number of deep and nearly closed furrows in the shoot. This, with the stomatal characters above, are a probable selective advantage in dry climates. It seems likely that the primitive scleromorphic features among the first Casuarinaceae were excellent preconditions to develop resistance against the drying of the climate. The evergreen habit with strongly reduced leaves that are nearly completely fused to the shoot and also the large amount of collenchyma/sclerenchyma within the leaves may represent primitive scleromorphic features which evolved as a response to low soil fertility, that secondarily prevent wilting of branchlets in times of drought and thus may be an important feature allowing *Casuarina* to inhabit extremely xeric sites. The extreme drought resistance of *Casuarina stricta* (now *Allocasuarina verticillata* (Lam.) L.A.S. Johnson) was shown in the field observations of Ashton et al. (1975).

Concluding discussion

In this paper on the Casuarinaceae, using detailed morpho-anatomical work and palaeobotanical and ecological data,

we have been able to document the shift from scleromorphic to xeromorphic features with changing climatic conditions. In addition, for the first time, we have been able to show that the *Casuarina* branchlet is not made up of cladodes but of extended leaf sheaths. These may be a novel strategy for achieving reduced photosynthetic area.

Author contribution statement VD and RP constructed and designed the experiments. RP located the seeds. VD performed the experiments and made the figures. Both authors analyzed the data and wrote the paper.

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Compliance with ethical standards

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

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