Foliar ontogeny in *Gymnostoma deplancheanum* and its evolutionary and ecological significance for scleromorphy and xeromorphy in Casuarinaceae (Fagales)

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Abstract

Key message The phylogenetically basal genus of the Casuarinaceae, *Gymnostoma*, from relatively mesic environments, shows morphological and anatomical structures that are precursors to xeromorphic modifications in the derived genera *Casuarina* and *Allocasuarina*.

Abstract *Gymnostoma* is the basal genus of the Casuarinaceae with a long evolutionary history and a morphology that has changed little over many millions of years. From a wide distribution in the Tertiary of the southern hemisphere, it is now restricted to islands in the Pacific Ocean, the Malesian region and one small area of northeastern Queensland where it occurs in mesic climates, often on poor soils. The unique vegetative morphology it shares with other more derived genera in the family appears to be xeromorphic. Its distribution combined with the fossil evidence that early Tertiary *Gymnostoma* occurred with other taxa whose morphology indicated they grew in mesic environments implies that the reduction in the photosynthetic organs was not specifically related to growing in xeric environments. It may be related to evolutionary adaptation to growing on nutrient poor substrates that may also suffer from seasonal water deficit. The foliage reduction then served as a pre-adaptation for derived species to help them cope with the aridity that developed on the Australian continent through the later part of the Tertiary. The fusion of the leaves to the stem to form phyllichnia was a precursor which enabled the development of specific adaptations in the derived genera *Casuarina* and *Allocasuarina* to improve water conservation, such as stomata restricted to furrows between the phyllichnia and proliferation of structural sclerenchyma that helps prevent cell collapse under drought conditions.

Keywords *Gymnostoma deplancheanum* · Leaf reduction · Morphology · Anatomy · Scleromorphy · Xeromorphy

Introduction

The Casuarinaceae (Fagales) is an eudicot angiospermous family of subtropical to temperate trees and shrubs with four genera, *Gymnostoma*, *Allocasuarina*, *Casuarina* and *Ceuthostoma* (Torrey and Berg 1988; Dilcher et al. 1990; Kubitzki et al. 1993; Steane et al. 2003), which are characterized by an evergreen habit and strong leaf reduction (Zamaloa et al. 2006) and obscure affinities to other angiosperms (Hill and Brodribb 2001). The Casuarinaceae is widely regarded as a group of highly xeromorphic trees and shrubs (Rao 1972; Heywood 1978; Ladd 1988; Hanelt 2000; Schütt et al. 2002). The characteristic strongly reduced shoot morphology is regarded as an expression of high specialization (Moseley 1948; Heywood 1982; Torrey and Berg 1988; Duhoux et al. 1996; Hanelt 2000; Dörken and Parsons 2017). Within living Casuarinaceae,

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Allocasuarina is suggested as the most derived and most specialized genus (Torrey and Berg 1988; Wilson and Johnson 1989; Turnbull 1990; Johnson and Wilson 1989; Maggia and Bousquet 1994; Duhoux et al. 1996) and the vegetative morphology of *Allocasuarina* is nearly identical to *Casuarina* (Torrey and Berg 1988; Guerin 2004). *Gymnostoma*, the most basal genus within Casuarinaceae (Torrey and Berg 1988; Johnson and Wilson 1993; Sogo et al. 2001; Steane et al. 2003) is regarded as reflecting several ancestral features (Christophel 1980) and represents the least specialized genus (Flores 1977; Duhoux et al. 1996; Dörken and Parsons 2017). Its strongly reduced vegetative morphology might reflect a scleromorphic adaptation (Dörken and Parsons 2017; Dörken et al. 2018). Several of the primitive scleromorphic features of *Gymnostoma* are also developed in seedlings of *Casuarina* and *Allocasuarina*, which are superseded by derived xeromorphic ones in adults (Dörken and Parsons 2017; Dörken et al. 2018). Throughout its entire lifespan, *Gymnostoma*, as the least xerophytic genus in the family, lacks specific xeromorphic features, which are developed, however, in all other Casuarinaceae (Dörken and Parsons 2017; Dörken et al. 2018).

For the Casuarinaceae, most of the available data about foliar features describe material from adults (e.g. Barlow 1983; Wilson and Johnson 1989; Dilcher et al. 1990; Johnson and Wilson 1993). There are few data about the foliar change from seedlings to adult (e.g. Hwang and Conran 2000; Dörken and Parsons 2017; Dörken et al. 2018). However, data about the juvenile foliage, in particular all structural changes occurring during the foliar shift from seedlings to adults, are useful to help elucidate the evolutionary history and the ecology of *Gymnostoma*, and also the evolution of scleromorphy and xeromorphy within the family. Thus, in this study the foliar change, including all leaf types occurring in the different ontogenetic stages, will be investigated in *G. deplancheanum* (Miq.) L.A.S. Johnson, which is one of the eight New Caledonian *Gymnostoma* species for all of which there is limited information about their vegetative morphology (e.g. Solereder 1908; Dilcher et al. 1990) and anatomical data are limited (Flores 1977). This morpho-anatomical study on *G. deplancheanum* will provide more detailed information and interpretation of the transition from juvenile to adult foliage and allow comparison with the more derived taxa.

Materials and methods

Materials

Samaras of *G. deplancheanum* (Miq.) L.A.S. Johnson were collected by one of the authors (PL) in southern,

New Caledonia (approximately 22°11'S, 166°43'E). They were germinated and grown in a temperate glasshouse with long day conditions in the Botanic Garden Konstanz (Germany). 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 was collected by PL from a single site from seedlings, a seed-producing tree and from juveniles close by, all growing on nutrient-poor lateritized clayey soil, as well as by VD, from the material grown in Konstanz. Voucher specimens 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 ethanol 70% + 5 ml acetic acid 96% + 5 ml formaldehyde solution 37%) before being stored in 70% ethanol. The anatomy was studied from serial sections using the classical paraffin technique and subsequent astrablue/safranin staining (Gerlach 1984). For SEM analysis, the FAA-material was dehydrated in formaldehyde dimethyl acetal (FDA) for 24 h (Gersterberger and Leins 1978) and later critical point dried. Sputter coating was done with a Sputter Coater SCD 50 Bal-tec (Balzers). The specimens were examined with an Auriga Zeiss TM. 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).

Special terms

The unique, strongly reduced vegetative morphology of Casuarinaceae is described by several special terms that are explained below and illustrated in Fig. 1.

Article Unit of a segmented shoot axis (Fig. 1).

Articulate branchlet A segmented shoot axis consisting of several articles.

Cladodes/phylloclades In previous studies, the articulate branchlets of adults of Casuarinaceae have been described as “cylindric cladodes”, “cladodes” or “phylloclades” (e.g. Rao 1972; Zimpfer et al. 2004; Niinemets et al. 2005; Arena 2008; Taylor et al. 2009; Warrier et al. 2013; Hill et al. 2018). Dörken and Parsons (2017) explained in detail why the use of these terms is difficult, because both refer to flattened, leaf-like structures which originated from strongly modified short shoot axes (Jurzitza 1987; Natho et al. 1990; Weiler and Nover 2008; Bresinsky et al. 2008). Thus, we avoid these terms for describing the articulate branchlets of Casuarinaceae.

Fig. 1 Schematic drawing of a segment in an articulate branchlet of *Gymnostoma*, explaining the special terms used in this study

Furrows/depressions In *Gymnostoma*, the articulate branchlets are cylindrical and consist of longitudinal ridges separated by shallow furrows where stomata are freely exposed. These shallow furrows are called depressions in this paper. This special term is needed, because in all other Casuarinaceae these furrows are variably closely appressed, encrypting the stomata that line the side of the furrow and in many species there are trichomes at the base of the furrow (Fig. 1). Thus, distinct structural differences between *Gymnostoma* and the other Casuarinaceae exist.

Phyllichnia Each article is made up of four elongated leaf bases fused to the shoot axis called phyllichnia. This is the surface expression of the leaf tissue. Each phyllichnium forms a longitudinal ridge and ends with a free leaf tip. The phyllichnium consists of a basal vascular bundle surmounted by the chlorenchyma, variable amounts of sclerenchyma and the epidermis.

Leaf tip In the literature, the terms “tooth” or “teeth” are quite frequently used to describe the free apical parts of the phyllichnia surrounding the subsequent node. In our descriptions here, we refer to the free apical parts of the phyllichnia as leaf tips rather than teeth (for a detailed discussion see Dörken et al. 2018).

Results

Cotyledons

The hypocotyl is well developed. The two opposite, flattened cotyledons are 0.8–1.2 mm long and 0.5–1.1 mm

wide with a distinct petiole. The morphologically adaxial side is light exposed and the morphologically abaxial side is shaded (Fig. 2a). The leaves are amphistomatic; however, most stomata are on the abaxial surface (Fig. 3a, b) where they lack a definite distribution pattern. They are non-encrypted and not covered by waxes (Fig. 3c). They are somewhat raised above the epidermis and well exposed to the airflow (Fig. 3a–c). The epidermis and the stomata are covered with a weakly developed cuticle. The epidermal cells of the upper surface are about twice the size of those of the lower surface. The mesophyll is only weakly dimorphic, with palisade parenchyma located towards the light-exposed adaxial side and spongy parenchyma towards the shaded abaxial surface. Sclerenchyma and collenchyma are absent. There is one collateral vascular bundle supplying each cotyledon. When entering the lamina, the vascular bundle strand branches several times. There is no vascular bundle sheath (Fig. 2b).

Primary leaves

The epicotyl is strongly reduced. The primary leaves have no petiole and are strongly reduced and scale like (Fig. 2c). They are about 0.5–0.8 mm long and 0.2–0.3 mm wide and are strongly adpressed to the shoot axis, so that the adaxial surface is shaded and the abaxial surface light exposed (Fig. 2c). The lamina is free and not fused to the shoot tissue. Epidermal cells of both sides are similar in size and shape. The mesophyll is monomorphic. Sclerenchyma and collenchyma are absent (Fig. 2d). The leaves are amphistomatic (Fig. 3d, e), but most stomata are on the adaxial surface (Fig. 3d). Stomata lack a definite distribution pattern. They are more or less in the same plane as the epidermis (Fig. 3f). There is a single, weakly developed unbranched vascular bundle supplying the leaf (Fig. 2d), ending in the lower third so the distal parts of the primary leaves must be supplied by cell to cell diffusion. In all other features, these leaves are similar to the cotyledons (Fig. 2b).

First phyllichnia

The first subsequent leaves are phyllichnia and are arranged in whorls of four per node. They are 2–3 mm long and 0.2–0.4 mm wide (Fig. 2e). Their adaxial surface is strongly fused to the cortical tissue of the shoot axis (Fig. 2f) and so is typical of the Casuarinaceae phyllichnia structure. Only the acute leaf tips, which soon wither, remain free (Fig. 2e). They surround the subsequent node (Fig. 2e). Thus, the shoot axis is completely surrounded by leaf tissue, forming an assimilating chlorenchyma. A clear cell layer separating leaf and cortical shoot tissues, and a foot layer which is distinctly developed in *Allocasuarina*, is indistinct though there

◀**Fig. 2** *Gymnostoma deplancheanum*, morphology and anatomy of seedlings; **a, b** cotyledons; **a** overview of a seedling showing two petiolate cotyledons; **b** cross-section as marked in **a**; **c, d** primary leaf; **d** cross-section as marked in **c**; **e, f** first phyllichnia; **f** cross-section as marked in **e** (*C* cuticle, *CH* chlorenchyma, *CO* collenchyma, *COR* shoot cortex, *E* epidermis, *M* mesophyll, *PI* pith, *PP* palisade parenchyma, *RC* respiratory chamber, *S* stoma, *SP* spongy parenchyma, *VB* vascular bundle)

is a discontinuous layer of cells with red-staining polyphenol contents (Fig. 2f). Each article has four longitudinal, vertical depressions between the phyllichnia. The depressions are shallow and lack trichomes or papillae (Fig. 2d–f). The non-encrypted stomata are strictly limited to the depression (Fig. 3g) where they are arranged in two vertical,

Fig. 3 *Gymnostoma deplancheanum*, stomatal details of a seedling; **a–c** cotyledons; they are amphistomatic, majority of stomata abaxial; **a** adaxial surface with few stomata; **b** abaxial with the majority of stomata; **c** single stoma; somewhat exerted and freely exposed to the airflow; **d–f** primary leaves; they are amphistomatic, majority of stomata adaxial; **d** adaxial, several stomata are developed (arrow);

e abaxial, only few stomata are developed (arrow); **f** single stoma in more or the less the same plane as the epidermis; **g–i** first phyllichnia; **g** two stomatal bands within a vertical, shallow depression; **h** within a stomatal band the vertical stomatal rows are separated by one row of epidermal cells (arrows); **i** stomatal cells are strongly wax covered

◀**Fig. 4** *Gymnostoma deplancheanum*, morphology and anatomy of juvenile articulate branchlets. **a** Overview of an article; four phyllichnia are developed per node which are fused to each other and to the shoot axis; **b** detail of a phyllichnium; **c** overview of a stomatal band consisting of three rows of stomata; **d** stomatal detail; the stomatal cells are covered with a thick cuticle; a single row of epidermal cells (arrows) separates the stomatal rows within a stomatal band; epidermal cells separating adjacent stomata within a vertical row are usually absent; **e, f** cross-section as marked in **a**; **e** lower third of an article; **f** region of the distal leaf tips (*C* cuticle, *CH* chlorenchyma, *CO* collenchyma, *COR* shoot cortex, *E* epidermis, *LT* leaf tip, *M* mesophyll, *PP* palisade parenchyma, *S* stoma, *SC* sclerenchyma, *SP* spongy parenchyma, *VB* vascular bundle, *X* xylem)

longitudinal bands (Fig. 3g). Each band consists of two vertical stomatal files (Fig. 3g, h). There is one file of epidermal cells separating one stomatal file from the other but there are no cells separating the stomata in each file (Fig. 3h, i). Within each file about 60 stomata per mm are developed. The stomata are not deeply sunken in the epidermis, but are more or less in the same plane (Fig. 2f). The stomatal pore is perpendicular to the midline of the depression. There is no well-developed respiratory chamber below the stomata (Fig. 2f). The stomatal and the epidermal cells within the depressions are covered by a thick cuticle (Fig. 3f). At the ridge of the phyllichnia, a collenchymatic layer is developed below the epidermis, consisting of one to two rows of very thick-walled cells (Fig. 2f). There is no further collenchyma and sclerenchymatic tissues are lacking. Each phyllichnium is supplied by one collateral, unbranched vascular bundle (Fig. 2f), which ends shortly below the leaf tip and lacks a sheath.

Juvenile phyllichnia

The juvenile phyllichnia are 3–4 mm long and 0.4–0.6 mm wide (Fig. 4e, f) and strongly fused to each other and to the shoot axis (Fig. 4e, f). The cortical shoot tissue consists of densely packed roundish cells, lacking intercellular spaces. The chlorenchymatic tissue is dimorphic. It consists of palisade parenchyma-like cells located towards the light-exposed parts of the shoot axis, and spongy parenchyma which is characterized by large intercellular spaces located towards the cortical tissue of the shoot axis (Fig. 4e). A cluster of sclerenchymatic cells also occurs below the vascular bundle strand of each phyllichnium (Fig. 4e). Sclerenchymatic cell clusters are developed in the shoot cortex (Fig. 4f). Within each shallow depression, there is a single stomatal band consisting of three to four stomatal files (Fig. 4c). Within a stomatal band, one file of epidermal cells separates the stomatal files (Fig. 4d). Rarely, there is a single cell developed separating adjacent stomata from each other. In all other morpho-anatomical features, the juvenile phyllichnia are similar to the first

ones developed subsequent to the primary leaves (Figs. 2e, f, 3d–f).

Mature phyllichnia

The phyllichnia are 9–13 mm long and 0.8–1.2 mm wide (Figs. 5a, 6a), and are fused as before (Fig. 6c, d). The chlorenchyma, which surrounds the shoot cortex, appears densely packed in transverse section but longitudinal sections show there is considerable intercellular space particularly below the outer layer of palisade. Calcium oxalate druses occur in the intercellular spaces (Fig. 7c, d). In basal parts of an article, the four vertical, longitudinal depressions are shallow (Fig. 6c) and are more or less completely absent distally (Fig. 6d), so that the shoot axis is quadrangular in shape (Fig. 5c, d). Within each depression, the stomata are arranged in two longitudinal, vertical bands. Each band consists of 4–6 (7) files of stomata (Figs. 5d, 6c, 7c). Only at the midline, which marks the center of each depression are a few short, one-celled trichomes developed (Fig. 5c). The epidermis within the depressions (Fig. 7a, b) and the stomatal cells are covered with a thick cuticle (Fig. 7b, c). Between the epidermis and the chlorenchyma, there is a collenchymatic layer, consisting of one to four rows of cells with strongly thickened walls (Fig. 6c, d). Below each foliar collateral vascular bundle strand, a strongly lignified sclerenchyma is developed (Figs. 6f, 7f). The stem bundle is also surrounded by large clusters of sclerenchymatic cells, rich in lignin (Figs. 6c, d, 7e). As in the juvenile phyllichnia there are no clearly identifiable foot layer cells. In all other morpho-anatomical features, the mature phyllichnia are similar to the juvenile ones (Fig. 4).

Mature shoot axis

The central tissues of each article consist of a cortex of equidimensional parenchyma cells that underlies the phyllichnia and extends almost to the surface of the article at the depression between the phyllichnia. The vascular stele consists (unusually) of eight (rather than four) vascular strands separated by parenchyma cells surrounding a pith that becomes sclerenchymatic with age. Each pair of stem vascular strands alternates with each phyllichnium bundle.

Discussion

The foliar shift from seedlings to adults

The general anatomy and morphology of *Gymnostoma deplancheanum* (cited as *Casuarina deplancheana* var.

◀**Fig. 5** *Gymnostoma deplancheanum*, morphology and anatomy of mature articulate branchlets I. **a** overview of an article; the four phyllichnia are fused to each other and also to the shoot axis; their tips surround the subsequent node; **b** detail of the leaf tips; **c** only at the midline of each shallow depression some trichomes are developed; **d** within the shallow depression stomata are arranged in two bands, each consisting of several rows of stomata; **e, f** shoot apex; **e** SEM; **f** longitudinal section

genuina) and some other *Gymnostoma* species have been described, with excellent line drawings, by Poisson (1874). In our work, we found that *G. deplancheanum* is characterized by a dramatic change in its foliage from seedlings to adults. While the petiolate cotyledons are still foliar (Fig. 2a), the non-petiolate primary leaves are strongly reduced tiny scales (Fig. 2c). The cotyledonary petioles are a unique feature of *Gymnostoma*, being absent in all subsequent leaf types and completely absent in all other Casuarinaceae (Hwang and Conran 2000). Both cotyledons and primary leaves are decussate (Fig. 2a, c). All subsequent leaf types form the typical Casuarinaceae phyllichnia. In mature individuals, the depressions in the articulate branchlets are less distinct than those developed in the juvenile stage (Figs. 2f, 4e). Thus, even in its earliest ontogenetic stages *G. deplancheanum* has a strongly reduced vegetative morphology. The foliar change affects not only the size and shape of leaves and phyllichnia but also their anatomy. Mature phyllichnia are more sclerenchymatic (Fig. 6d), than juvenile ones (Figs. 2f, 4e, f). In addition, there are obvious changes in the arrangement of stomata. In cotyledons, stomata are amphistomatic, with the majority abaxial (Fig. 3a–c). Primary leaves are also amphistomatic, with the majority, however, adaxial (Fig. 3d–f). In cotyledons and primary leaves, stomata lack an organised distribution pattern and are irregularly developed all over the laminar surface. In cotyledons, the stomata are exerted above the epidermis surface and not covered with waxes, while in primary leaves they are more or less in the same plane as the epidermal cells as is also the case for those developed on the phyllichnia later. However, on the phyllichnia they are strongly wax covered. Later, when articulate branchlets show the typical phyllichnia structure of adults, stomata are exclusively located within the longitudinal shallow depressions (Figs. 2e, 3g, f, 4c, d, 5d). The number of stomatal files per depression increases with maturity so that in adults two stomatal bands are developed, each consisting of up to 6 (– 7) stomatal files (Fig. 5d) each originating from one side of a phyllichnium. The number of eight stomatal bands per article as developed in *G. deplancheanum* is quite similar to nearly all other *Gymnostoma* species, except the New Caledonian taxa *G. glaucescens* (Schltr.) L.A.S. Johnson and *G. webbianum* (Miq.) L.A.S. Johnson and *G. papuanum* (S. Moore)

L.A.S. Johnson from Papua New Guinea which have only four stomatal bands (Dilcher et al. 1990).

A comparable early foliar shift, lacking transitional leaves, is also described for other Casuarinaceae genera (Hwang and Conran 2000; Dörken and Parsons 2017; Dörken et al. 2018). However, there are morpho-anatomical differences between *G. deplancheanum* and the *Casuarina*/*Allocasuarina* pair. In all *Gymnostoma* species, there are four phyllichnia per node when adult (Dilcher et al. 1990) as there are for the first leaves of all seedlings in the other genera in the family. However, in all *Casuarina* and most *Allocasuarina* species the number of phyllichnia per node is higher in adults (Fig. 8b, d), in some taxa up to 16 (Torrey and Berg 1988; Natho et al. 1990). The four phyllichnia arrangement is found in some *Allocasuarina* species from several of the Wilson and Johnson (1989) sections, e.g. *A. distyla* (Vent.) L.A.S. Johnson (Williams and Metcalf 1985) or *Allocasuarina* species of section *Oxypitys* (e.g. *A. pinaster* (C.A. Gardner) L.A.S. Johnson, *A. acuarina* (F. Muell.) L.A.S. Johnson; Fig. 8c). Furthermore, in most *Casuarina* and *Allocasuarina* species the longitudinal, vertical, intercostal furrows developed on mature articulate branchlets are deep, nearly closed and in most taxa strongly pubescent (Fig. 8b–d) (Torrey and Berg 1988; Dilcher et al. 1990; Dörken and Parsons 2017; Dörken et al. 2018). In *Gymnostoma*, the depressions between the phyllichnia are shallow and non-pubescent, except for a very few single one-celled trichomes (Figs. 5c, 6d), although in *G. chamaecyparis* Poisson (1874) illustrates short trichomes in the intercostal furrow (Plate VII). While there is some variation between species of *Gymnostoma* in the density of trichomes, they always occur at the junction of phyllichnia and are never as dense as on species in the other genera. Furthermore, the articles of many *Casuarina* and *Allocasuarina* show a strongly developed, mostly T-shaped sclerenchyma in transverse section (Fig. 8b–d), often dividing the phyllichnia into two halves (Johnson and Wilson 1993; Dörken and Parsons 2017), a feature lacking in *G. deplancheanum*. Camera lucida illustrations in Flores (1977) for four species of *Gymnostoma* show variable amounts of sclerenchyma depending on the species. Our specimen has considerably less sclerenchyma under the epidermis than is illustrated for the same species in Flores' diagram and in fact looks more similar to the TS of *G. poissonianum* (Schltr.) L.A.S. Johnson. The arrangement of the vascular bundles in the stele of our specimen consists of eight vascular strands, whereas the Flores' illustration only shows four for the species she examined. Flores also noted and used line drawings to illustrate tracheary cells in transfusion tissue in the four species she examined. However, in our sections these cells were not observed though they are present in *Allocasuarina* (Dörken

◀**Fig. 6** *Gymnostoma deplancheanum*, morphology and anatomy of mature articulate branchlets II. **a** overview of an entire article; **b** longitudinal section as marked in **a**; **c**, **d** cross-sections as marked in **a**; **e**, **f** detail of the leaf tips; **e** overview; **f** cross-section as marked in **e** (*C* cuticle, *CH* chlorenchyma, *CO* collenchyma, *COR* shoot cortex, *E* epidermis, *M* mesophyll, *P* phloem, *S* stoma, *SC* sclerenchyma, *VB* vascular bundle, *X* xylem)

et al. 2018). A further difference between our specimen and *Casuarina/Allocasuarina* is in the lack of a clearly defined foot layer in *Gymnostoma*. There is no clearly recognisable cell layer demarcation between the chlorenchyma mesophyll and the underlying parenchyma cells.

Despite the fact that the number of stomatal bands is quite homogenous among the different *Gymnostoma* species, the number of stomatal files per band varies interspecifically. Within the majority of *Gymnostoma* species, the number varies between 2 and 5 files per band; *G. deplancheanum* has 4–6 (–7) per band. The number of stomatal files per band increases with age (Fig. 5d). In *G. deplancheanum*, the stomatal files within a stomatal band are separated from each other by one file of epidermis cells, a feature similar to *G. nobile* (Whitmore) L.A.S. Johnson (Borneo) and *G. webbianum* (New Caledonia). In most other taxa, the number of epidermal cells separating the stomatal files from each other is higher, e.g. in the New Caledonian *G. leucodon* (Poiss.) L.A.S. Johnson it is up to 5 (Dilcher et al. 1990). There are considerable differences in the exposure of stomata between *Gymnostoma* and the other genera. In *Gymnostoma*, the stomata are non-encrypted and freely exposed (as indicated by the generic name) and in some species the stomatal cells are covered in wax platelets while in others there are no waxes. In contrast in most *Casuarina* and *Allocasuarina* species, the stomatal bands line the closely appressed edges of furrows that usually also contain dense trichomes attached to the base of the furrow (Flores 1977; Torrey and Berg 1988; Dilcher et al. 1990; Dörken and Parsons 2017; Dörken et al. 2018).

Scleromorphy vs. xeromorphy

The overall vegetative morphology of *Gymnostoma* and the other genera of Casuarinaceae is very similar with considerable unique reduction in the photosynthetic organs. The first representatives of the family appear in the Eocene in southeastern Australia and are assigned to *Gymnostoma* (Christophel 1980). Confirmed representatives of *Casuarina/Allocasuarina* do not appear until later in the fossil record (Guerin 2004). Nevertheless, Hill (1994) considered that the three main genera may have been coincident but in different habitats for much of the time since the evolution of the family. However, *Gymnostoma* has been

considered primitive (Dilcher et al. 1990) and is placed at the base of the phylogram by Steane et al. (2003). There has been considerable discussion of whether this type of morphology is the result of adaptation to low-nutrient soils (developing scleromorphy) or xeric climatic conditions (developing xeromorphy, Hill 1994). There is difficulty in assessing the scleromorphism of the Casuarinaceae as the term sclerophyll was originally coined to describe shrubs and trees with generally small, tough or leathery leaves (Seddon 1974). Studies of leaves of plants from various parts of the world (Read and Sanson 2003) and in particular leaves from plants from New Caledonian maquis (nutrient poor soils, mesic climate) and dry forest (higher nutrient soils, lower rainfall) have shown that various sclerophyllous leaf characteristics can be related to low soil nutrient levels (Read et al. 2006) as they will benefit the plant by allowing longer tissue retention and hence an efficient use of scarce nutrients. At the end of the discussion in a review of sclerophylly, Salleo and Nardini (2000) suggested that “sclerophylly developed during the Tertiary, when environmental humidity was much higher than at the present time” although they gave no reason for why this might encourage such development. In the early Tertiary of Gondwana, climatic conditions are considered to have been warm and humid, and this is when evidence of sclerophyll vegetation began to appear (Carpenter et al. 2015), including in the Proteaceae, Myrtaceae and *Gymnostoma*. The development of nutrient poor soils under a tropical leaching climate may have been an impetus for the evolution of sclerophylly (Hill et al. 2018). Currently of the seven species of *Gymnostoma* on New Caledonia, six occur on ultramafic soils that are notoriously nutrient poor to relatively toxic (Read et al. 2006) while only one is not on ultramafic soil [Jaffre et al. (1994) in McCoy 1998], and *Gymnostoma* from other locations are also often on ultramafic soils.

While there are correlations between biophysical characteristics and leaf nutrient levels, the sclerophyll morphology may be a combination of properties that confer protection in stressful environments as well as nonadaptive mechanical properties that are a consequence of being in a stressful environment (Read et al. 2006). It is not possible to apply tests that could be done on leaves to the photosynthetic organs of the Casuarinaceae as they are so different from leaves. Features, however, that contribute to the assessment of leaves as sclerophyllous are their generally high proportion of neutral detergent fiber and leaf anatomical characteristics such as the thickness of cuticle, upper palisade, and lower epidermis (Edwards et al. 2000). All Casuarinaceae are likely to have high proportions of fiber, although it would be difficult to separate the fiber of the phyllicnia from that of the stem. Amounts

◀**Fig. 7** *Gymnostoma deplancheanum*, cellular details of mature articulate branchlets. **a** Epidermis with a thick cuticle; between the epidermis and the chlorenchyma strongly developed collenchymatic clusters are formed; **b** detail of a stomatal band taken from a cross-section; stomata covered with a thick cuticle; below the stomata a small respiratory chamber is developed; **c** outer shoot surface in the region of a vertical stomatal band; detail taken from a longitudinal section; chlorenchyma below the epidermis with large intercellular spaces and calcium oxalate druses; **d** chlorenchyma with calcium oxalate druses; **e** outer part of the concentric stem bundle; towards the shoot cortex large sclerenchymatic clusters are formed; **f** collateral vascular bundle of a phyllichnium, with a large cluster of highly sclerenchymatic cells adjacent to the phloem (*C* cuticle, *CH* chlorenchyma, *CO* collenchyma, *COR* shoot cortex, *COX* calcium oxalate druses, *E* epidermis, *IS* intercellular spaces, *P* phloem, *RC* respiratory chamber, *S* stoma, *SC* sclerenchyma, *X* xylem)

of sclerenchymatous tissue in a photosynthetic organ will also contribute to the assessment of how sclerophyllous a leaf appears. *G. deplancheanum* has a very thick cuticle, relatively dense mesophyll, collenchyma developed between the epidermis and the mesophyll and pericyclic fibers associated with the vascular bundles, so does have characteristics similar to the taxa judged as sclerophyllous and examined by Read and Sanson (2003) and Read et al. (2006). Line drawings of *G. deplancheanum* in Flores (1977) show even more sclerenchyma tissue than in our specimen. Comparison of branchlet transverse section line diagrams of *Gymnostoma* species (Figs 18–21 in Flores 1977, plates VI, VII in Poisson 1874) with similar sections of *Casuarina* and *Allocasuarina* (Figs 35–40 in Macklin 1927, Figs 7–17 Flores 1977) shows that, in general, the *Gymnostoma* species have less sclerenchyma than in the stems of the other genera, but some species such as *G. leucodon* that grows in gallery woodland in southern New Caledonia do have T-shaped sclerenchyma similar to that found in the other genera (Jaffre et al. 1994).

While sclerenchyma may not be directly related to water use efficiency, there are also structures that can be associated with water conservation such as extensive development of epicuticular waxes over stomatal cells and a relatively dense mesophyll. The main change identified as contributing to increased xeromorphy in the family is the restriction of the stomata to grooves formed by the appressed sides of the phyllichnia in *Casuarina*/*Allocasuarina* compared with the fully exposed stomata of *Gymnostoma*. Hill (1990) considered that change as a good illustration of modifications

evolved due to climatic shift to more arid conditions on the Australian continent through the Tertiary. The genus *Gymnostoma* probably appeared in the early Eocene and was widespread in the Oligocene with *Casuarina* and *Allocasuarina* becoming dominant in the Miocene (Hill 1994) so that the physical modification to the foliage of the latter genera may have been driven by adaptation to a drying climate. This would ensure they were successful as aridity increased across the continent while *Gymnostoma* disappeared from Australia, except for a single very restricted species occurring only on stream sides in the far north east wet forests of Queensland (Wilson and Johnson 1989).

While restriction of the stomata to deep grooves is certainly a feature that would reduce water loss from the foliage, the waxes on the stomata of *Gymnostoma* will also decrease water loss. From the distribution of *Gymnostoma* it can be concluded that growing in a high rainfall environment is essential for the genus. In addition to *Gymnostoma*, *Casuarina* species are also native to New Caledonia. However, the *Casuarina* species in New Caledonia occur in different habitats to *Gymnostoma* and tend to be on the lower rainfall areas (rain shadow) to the west of the main north–south massif. Effects of rainfall seasonality, soil depth and texture also need to be considered in such comparisons. While the production of sclerenchyma may be a result of development under stressful low-nutrient conditions, this may also preadapt the plant to better drought tolerance (de Micco and Aronne 2012). Thus, as the area of Australia with an arid climate became more extensive through the Tertiary, there was evolution of *Casuarina* and *Allocasuarina* species to occupy the poor soils in increasing areas of aridity. *C. equisetifolia* L. that occurs on often arid coastal sand has more sclerenchyma than the riparian *C. cunninghamiana* Miq. (Dörken and Parsons 2017). Similarly, *Allocasuarina* species from arid climates tend to have more sclerenchymatic tissues than species from more humid areas (Dörken et al. 2018). The template of sclerenchyma can be modified by species depending on the climatic conditions under which they grow so greater structural rigidity can prevent cell/tissue collapse under conditions of water stress.

Our investigated specimens of *Gymnostoma* seem to be less sclerenchymatic than most *Casuarina* and *Allocasuarina* species. It seems that the amount of sclerenchymatic tissues can show some intraspecific variation, because our

Fig. 8 Schematic line drawings of idealized cross-sections for the different types of articulate branchlets occurring in adult Casuarinaceae; drawings represent the situation in the middle of an article (data for *Casuarina* taken from Dörken and Parsons (2017) and *Allocasuarina* from Dörken et al. (2018); **a** *Gymnostoma deplancheanum*; **b** *Casuarina equisetifolia*; **c** *Allocasuarina acuarina*; **d** *Allocasuarina campestris*. Within the ancestral genus *Gymnostoma* only four phyllichnia are developed per node; in the derived, genera *Casuarina*

and *Allocasuarina* the number of phyllichnia per article is usually higher; *Allocasuarina* articles with four phyllichnia as illustrated for *A. acuarina* (**c**) are uncommon. In *Gymnostoma* the longitudinal shallow depressions are non-pubescent, in *Casuarina* and *Allocasuarina* the longitudinal furrows are deep and have dense hairs attached at the base of the furrow; (grey = cuticle, bright blue = transfusion tissue, bright green = epidermis, brown = shoot tissue, dark blue = phloem, red = xylem; dark green = chlorenchyma, orange = sclerenchyma)

specimens of *G. deplancheanum* are significantly less sclerenchymatic than the *G. deplancheanum* specimen illustrated in, e.g. Flores (1977). In comparison with some *Allocasuarina*, our investigated specimen of *G. deplancheanum* has much poorer (more distant) connection between the chlorenchyma and vascular tissue, and a longer distance between the stomata and the bulk of the photosynthetic cells (Dörken et al. 2018). This would make water transport from the xylem to the sites of evaporation on the palisade cells less efficient

than in *Allocasuarina* and is another indication that the species is not well adapted to dry conditions despite its very reduced vegetative morphology. There are few groups that contain species from very humid environments that look morphologically very similar to species from very arid environments. For instance, Proteaceae species from rain forest have leaves that are very different from closely related taxa from arid environments (e.g. Jordan et al. 2013). Fossil evidence clearly indicates that over geological time climate

change has had a very strong influence on current taxon distribution (e.g. *Nothofagus*, *Beauprea*, Hill 1994). The current phytogeography of *Gymnostoma* combined with the palaeobotanical evidence emphasizes the fact that species distributions may be strongly related to a particular climatic niche so that when climate change becomes too extreme the taxon will either become extinct or become a relic in a place where the climate is suitable. However, this does not necessarily mean the taxon has reached a dead end. The majority of *Gymnostoma* species occur on New Caledonia and it is likely that the genus may have undergone radiation on the island along with many other taxa such as *Araucaria* (Gaudeul et al. 2012) and *Beauprea* (He et al. 2016) as just two examples.

Author contribution statement Plant material was collected by PL and the investigations of the material were performed by VD. The study was designed and the manuscript was written by all three authors.

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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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