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Developmental studies in Casuarina (Casuarinaceae). III. The anatomy of the mature branchlet

by

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Abstract: The branchlet of *Casuarina* has a glabrous (Division Gymnostomae) to pubescent (Division Cryptostomae) epidermis. A sclerenchymatous hypodermis is present which, in several species, is continued into the inner layers of the internodal ridges as T-beam-shaped masses. Photosynthesis is mainly carried out by the mesophyll in the internodal ridges.

The evaluation of characteristics of the vegetative structures supports the accuracy of Poisson's observations. Two main subdivisions, which correspond to Poisson's Cylindricae seu Cryptostomae and Tetragonae seu Gymnostomae, need to be set apart. The two taxa certainly could be represented by separate genera and the term "division", applied by Poisson, is inappropriate and should be dropped.

Several attempts have been made to describe the structure of the mature branchlet of *Casuarina;* those of **Loew** (1865), **Poisson** (1871), **Boodle** and **Worsdell** (1894), **Morini** (1894, 1895), **Cordemoy** (1923), and **Macklin** (1927) have been the more complete. **Loew** (1865) proposed the phyllichnium concept and described the chlorophyllous tissue of the intemodal rib as foliar in nature, and the tracheoidal elements of the ribs as modified parenchyma cells.

Several years later, **Poisson** (1871) reviewed and summarized Loew's work and established the Divisions Cryptostomae (Cylindricae) and Tetragonae (Gymnostomae) based mostly on the phyllichnium concept of Loew. **Boodle** and **Worsdell** (1894) concurred with Loew's ideas; they described the tracheoidal cells (tracheids of Loew) as modified cortical parenchyma and termed it a "transfusion tissue" which linked the xylem of the bundle with the palisade parenchyma of the internodal ribs. They also described an external endodermis which extended around the outer limit of the cortical bundles (leaf traces) and dipped beneath the furrows. A second endodermis external to a "pericyclic layer, one or two cells thick, which is sometimes sclerotic", was reported surrounding the central cylinder of stem bundles.

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Macklin (1927) retained the concepts of foliar phyllichnia, transfusion tissue and two endodermes. She also set apart two branchlet types as represented by *C. stricta* and *C. muelleriana* using the presence or absence of the median T-beam-shaped mass of sclerenchyma of the ridges as a differentiating character.

MATERIAL AND METHODS

The fifteen species and fifty-seven samples (individual trees) of *Casuarina* investigated in this study are listed following **Poisson's** (1871) classification in Table 1, along with their localities of collection, collectors and dates of collection.

Most of the collections were identified to species by Dr. L. A. S. Johnson, Director and Chief Botanist, Royal Botanic Gardens and National Herbarium, Sydney, Australia. Exceptions were the species collected by J. T. Buchholz which were identified by Dr. A. Guillaumin of the Herbarium Museum, Paris, in 1948.

Methods of fixation, softening, dehydration, embedding, sectioning and staining were the same used by **Flores** and **Morrow** (1977). The drawings of longitudinal and transverse sections were made with a Zeiss camera lucida while the photographs were made with an American Optical Microstar 1- research microscope fitted with Polaroid accessories; a 4 x 5 camera back adapter was used to make the photomicrographs using Kodak Ektapan 1462 film.

RESULTS

The mature branchlets of *Casuarina* are of two types and set apart two groups of species. *C. stricta* (Fig. 1) will be used as a model to describe the structure of young stems in the first group which includes the species of **Poisson's** (1871) Division Cryptostomae. This group includes *C. cunninghamiana, C. litorea, C. glauca, C. luehmannii, C. littoralis, C. rigida, C. distyla, C. paludosa, C. nana* and *C. stricta. C. poissoniana* (Fig. 2) will be described as a model for the second group which includes *C. leucodon, C. nodiflora, C. deplancheana* and *C. poissoniana;* the second group is equivalent to **Poisson's** (1871) Division Gymnostomae. Variations among the species in each of the sections are described and summarized in Table 2.

In general, the branchlet structure of *C. stricta* agrees with the previous descriptions given by **Morini** (1894), **Boodle** and **Worsdell** (1894), **Cordemoy** (1923) and **Macklin** (1927), but their descriptions are not complete. *C. stricta* nodes have whorls of nine to twelve leaves. The leaves are gamophyllous, except at their tips, and they encircle the base of the internode above their point of attachment (Fig. 1). The deep furrows, which alternate with the ridges along the internodes, contain many uniseriate branched and unbranched hairs which arise from the epidermal cells in the bases of the furrows (Figs. 1 and 2). Branched hairs are also found on the sides of the laminae of the leaves and in the lower parts of the internodal ridges (Fig. 1). Mature hairs are lignified (as shown by a positive phloroglucin test), and the lumina of their cells are often filled with phenolic substances. The stomata are restricted to the furrows and are of the paracytic type (formerly rubiaceous type of **Solereder,** 1908). They commonly have two parallel subsidiary cells, although in some cases three subsidiary cells are found (Fig. 7b). The lamellae present in the dorsal and ventral walls of the guard cells are thick and frequently lignified, reducing the stomatal aperture (Figs. 7b, c). The stomata are transversely oriented in respect to the longitudinal axes of the branches (Fig. 7b).

Stomatal chambers are small (Fig. 7c). In transection, each internodal ridge of a branchlet has an external convex rounded shape; the epidermal cells of the ridges have thick, pitted walls with heavily cutinized anticlinal walls. The thick external wall contains polyhedric crystals (Fig. 7f) which are distinct when seen in polarized light. A thick cuticle covers the epidermis both in the furrows and over the ridges (Fig. 6). Under the epidermis of the ridges there is a hypodermal T-beam-shaped mass of sclerenchyma cells which intergrades with collenchyma cells with glistening walls (Figs. 3, 5 and 7a). The sclerenchyma consists mainly of fibers accompanied by some pitted sclereids, which are frequently filled with phenolic substances. The arms of the T-beam-shaped rib face the epidermis; the lower part lies in the center of the ridge where it extends to the vicinity of the leaf trace, and often reaches the leaf trace in older internodes. Beneath the hypodermis, and divided by the sclerenchymatous tissue, there are two zones of loosely arranged palisade chlorenchyma (Figs. 3 and 5). The leaf trace is located immediately inside the innermost part of the sclerenchymatous tissue, and is separated from it by only one parenchyma layer of cells (Figs. 3, 5 and 7a). The latter extends to both sides of the ridge, bounding the chlorenchyma and corresponds to the external endodermis of **Boodle** and **Worsdell** (1894); however, the writer was unable to find Casparian strips, or even suberin lamellae, in this layer.

The xylem of each leaf trace consists of a few narrow helical tracheary elements. The phloem is considerably larger in volume and is formed of sieve-tubes and parenchyma; it extends tangentially on either side along the face of the chlorenchyma, giving the bundle a wedge-like shape (Figs. 3, 5 and 7a). Fibers are found associated with the phloem along its outer limit (Figs. 3 and 5). Tracheids are abundant at nodes where the stem bundles divide and anastomose again to form the leaf traces; also, isolated groups of tracheids occur along the lateral margins of the wedge-shaped leaf traces. On both sides of the leaf trace and in the veins of the leaf blades, several thick and simple-pitted walled cells (Figs. 6 and 7b), lacking protoplasmic contents at maturity, are observed. These elements, first observed by **Loew** (1865), resemble some of the tracheoidal idioblasts with simple pits described by **Foster** (1956). They slightly exceed the size of the adjacent mesophyll cells and in older branchlets their walls are rigid and appear birefringent under polarized light. These elements exhibit an affinity for dyes known to stain lignin, such as phloroglucin and safranin. A sheath of lignified elements surrounds the V-shaped leaf trace in mature branchlets.

The cortex contains many parenchyma cells with crystals, phenolic substances or both. As mentioned above, the collateral stem bundles alternate with the leaf traces, occupying the same radii as the furrows; two or three layers of parenchyma cells separate the stem bundles from one another (Fig. 4). A starch sheath delimits the cortex internally in young branchlets and branches; in older stages, Casparian strips appear in this layer. Part of the protophloem is crushed early in development and a mass of fibers, often containing crystals, develops in this region and bounds the phloem externally (Fig. 3). Fibers also occur along the inner side of the bundle delimiting it from the pith. Both the pith and the cortical cell walls are unlignified in young stems, but become lignified in older stems.

The free leaf tips show a similar structure to that of the gamophyllous base of the whorl; in both, a mass of fibers encloses the leaves adaxially and abaxially except at their margins (Fig. 6). In the furrows between the gamophyllous leaf laminae appreciable cell elongation takes place (Fig. 6), increasing the diameter of the leaf sheath. These furrows lose depth distally and finally disappear with the separation of the leaf blades. A scanty chlorenchymatous tissue is found only at the base of the laminae.

TABLE I

Summary of species collected

Species of *Casuarina* L.	Collection date	Collector and* Collection No.		Locality
DIVISION CRYPTOSTOMAE				
C. cunninghamiana Miq.	9-17-1974	C-5594	(P)	23 km WSW of Singleton, Austr.
” ” ”	9-20-1974	C-5646	(S)	Royal Botanic Gardens, Sydney, Austr.
” ” ”	9-16-1974	F-22	(P)	Santa Barbara, Calif., U. S. A.
” ” ”	9-18-1974	F-23	(P)	” ”
” ” ”	9-18-1974	F-24	(P)	” ”
C. distyla Vent.	9-26-1974	C-5653	(S)	Royal National Park, SSE of Sutherland, Austr.
” ” ”	9-19-1974	C-5643	(P)	Cultivated in Carlton, Austr.
” ” ”	9-26-1974	C-5652	(P)	As C-5653
C. glauca Sieber ex Spreng.	9-19-1974	F-25	(P)	Santa Barbara, Calif., U.S.A.
” ” ”	9-13-1974	F-17	(P)	” ”
” ” ”	9-13-1974	F-18	(S)	” ”
” ” ”	9-16-1974	F-19	(P)	” ”
” ” ”	9-16-1974	F-20	(S)	” ”
” ” ”	9-16-1974	F-21	(S)	” ”
C. glauca Sieber ex Spreng.	10-6-1974	C-5737	(P)	3.2 km NE of Raymond Terrace, Austr.
” ” ”	9-18-1974	C-5631	(S)	1.6 km NNW of Hollydeen, Austr.
” ” ”	9-20-1974	C-5649	(S)	As C-5646.
” ” ”	10-6-1974	C-5738	(S)	As C-5737.
” ” ”	9-19-1974	F-26	(P)	Santa Barbara, Calif., U.S.A.
” ” ”	9-13-1974	F-15	(P)	” ”
” ” ”	9-13-1974	F-16	(P)	” ”
C. helmsii Ewart & Gordon		F-27	(?)	U.C.S.B. Greenhouse, Santa Barbara, Calif., U.S.A.

C. *litorea* L., var.** *incana* Benth.	9-20-1974	C-5638	(P)	As C-5646
C. *littoralis* Salisb.	9-16-1974	C-5579	(S)	8 km SW of Wollombi, Austr.
" " "	9-26-1974	C-5650	(P)	As C-5653.
C. *luehmannii* R.T. Baker	9-17-1974	C-5615	(P)	22 km WSW of Singleton, Austr.
" " " "	9-17-1974	C-5616	(S)	" "
C. *nana* Sieber ex Spreng.	10-12-1974	C-5753	(S)	9 km S of the Broidwood Captains Flat Rd. on the Countegamy Rd., Austr.
" " "	10-12-1974	C-5754	(P)	" "
" " "	10-21-1975	C-7279	(P)	Royal National Park, Sydney, Austr.
" " "	10-21-1975	C-7280	(P)	" "
C. *paludosa* Sieber ex Spreng.	10-13-1974	C-5764	(P)	50 km WSW of Eden on the Rockton-Tawamba Rd., Austr.
" " " "	10-13-1974	C-5763	(S)	" "
C. *rigida* Miq.	10-3-1974	C-5687	(S)	Gibraltar Range Ntl. Park, Glen Innes, Austr.
" " "	10-3-1974	C-5688	(P)	" "
C. *stricta* Ait.	9-18-1974	C-5627	(S)	1.1 km NW of the sandy Hollowcastle rocks Rd. on the Wybong Rd., Austr.
" " "	9-18-1974	C-5628	(P)	" "
C. *stricta* Ait.	9-2-1974	F-1	(S)	Santa Barbara Airport. Calif., U. S. A.
" " "	9-2-1974	F-2	(P)	" "
" " "	9-2-1974	F-3	(P)	" "
" " "	9-2-1974	F-4	(S)	" "
" " "	9-2-1974	F-5	(P)	" "
" " "	9-2-1974	F-6	(S)	" "
" " "	9-2-1974	F-7	(P)	" "
" " "	9-2-1974	F-8	(P)	" "
" " "	9-2-1974	F-9	(S)	" "
" " "	9-2-1974	F-10	(S)	" "
" " "	9-4-1974	F-11	(S)	Goleta, Calif., U.S.A.

TABLE 1 (cont.)

Species of *Casuarina* L.	Collection date	Collector and* Collection No.		Locality
C. stricta Ait.	9-4-1974	F-12	(P)	Goleta, Calif.
" " "	9-4-1974	F-13	(P)	Ellwood, Calif., U.S.A.
" " "	9-5-1974	F-14	(S)	" "
DIVISION GYMNOSTOMAE				
C. deplancheana Miq.	9-13-1948	B-1709	(S)	Headwaters of River Pirogues, Plaine des Lacs, New Caledonia
C. leucodon Poiss.	9-13-1947	B-1019	(P)	10 km S of Thio, New Caledonia
C. nodiflora Forst.	11-1-1947	B-1333	(P)	Near Ponerhouen, New Caledonia
" " "	10-20-1947	B-1239	(P)	Along River Thy, N of Saint Louis Mission, New Caledonia
C. *poissoniana* Schltr.	12-9-1974	B-1468	(P)	Plaine des Lacs, route to Yate, New Caledonia
" "	12-10-1947	B-1483	(S)	Along stream, N Dumbea Valley, New Caledonia
" "	10-4-1947	B-1143	(P)	As B-1483

* The (P) written after the collection number denotes a pistillate tree and (S) a staminate tree.

** Fosberg and Sachat (1975) have shown that the name of *C. equisetifolia* (Linn., Amen. Acad., 4: 143, 1859 . . . as *"equisefolia"*, sphalm.) is antedated by five years by *C. litorea* (L., Diss. Stickman, 12, 1754 . . . as *"Casaarina" litorea*, sphalm.). Accordingly the well known name *C. equisetifolia* L., has properly been reduced to synonymy under *C. litorea* L.

*** Collections designated as "B" were made by J.T. Buchholz, University of Illinois, those as "C" were made by R. Coveny, Royal Botanic Gardens and National Herbarium, Sydney, Australia, and those as "F" were collected by the writer. In the specimens collected by the writer, the first date of collection is given; five or six other collections were made from each of many samples during the study on each tree with a minimum of ten pieces being collected from each tree.

C. luehmannii has from nine to twelve leaves at each node. The external faces of the ridges are slightly convex medially (Fig. 8a) and the edges marking the furrows are rounded as in *C. stricta.* The bars of the T-beam-shaped masses of sclerenchyma are very long and are continuous with the sclerenchyma that externally bounds the phloem of the leaf traces (Fig. 8a); a sheath of lignified cells surrounds the leaf trace. The stem bundles are surrounded by sheaths of sclerotic cells which expand on the external side of the phloem, forming short wings of tissue (Fig. 8a). The pith becomes lignified very early. Crystals are present in the pith, the cortex and some chlorenchyma cells. This species exhibits strong similarities to *C. stricta* but the chlorenchyma is more reduced and the tissues are more lignified (Figs. 8a to 8g).

C. helmsii has whorls of four or five leaves. The general structure of the branchlets is similar to that of *C. stricta* and *C. luehmannii*, but the ridges are convex and rounded in their centers. The outer limits of the furrows are narrowed by wing-like projections which reduce the exposure of the furrows to the environment (Figs. 9a to 9g).

C. litorea has from seven to twelve ridges and furrows. The branchlet structure is similar to that of *C. stricta* and agrees with the descriptions given by **Morini** (1894), **Cordemoy** (1923), and **Rao** (1972). A median vertical strip of parenchyma lies embedded in the sclerenchymatous ribs of the ridges (Fig. 10a). The external surfaces of the ridges are nearly flattened. The furrows are narrowed externally and have well-defined cuticles over their outer edges, which reduce the exterior furrow openings (Fig. 10a). Often, tracheoidal cells similar to those of the cortex are observed in the pith (Fig. 10e).

C. cunninghamiana has from six to eight leaves per whorl, seven leaves being the mode. The branchlet cell structure is similar to that of *C. stricta;* however, wing-like projections similar to those of *C. litorea* occur along the outer edges of the furrows and protrude into the furrow apertures (Fig. 11a) diminishing its exposure to the exterior. As found in *C. litorea,* some tracheoidal cells are present in the pith (Figs. 11a to 11b).

C. glauca has from nine to sixteen leaves per whorl; fourteen and fifteen are the most frequent numbers. The organization of tissues in the branchlet is similar to that found in *C. stricta*, but as in *C. litorea* and *C. cunninghamiana,* wing-like projections are present along the outer edges of the furrows. The only variation found in respect to the previously mentioned species is the [illegible]le volume of the sclerenchymatous masses bounding the phloem of the stem [illegible]les (Figs. 12a to 12g).

C. littoralis, with six to eight leaves per whorl, seven being the modal number, differs from the above species because in the distal portion of the leaf whorl the furrows widen, due to cell elongation, and form a rim-like cup between the concrescent margins of two contiguous leaf blades (Fig. 13g). The ridges of the internodes are convex and each forms a median obtuse angle. The inner extensions of the sclerenchyma ribs are nearly lacking (Fig. 13a). The chlorenchyma is continuous below this sclerotic hypodermis and fills the ridge. The leaf traces are similar to those described previously for other species. The sclerenchymatous sheaths bounding the stem bundles form short irregular wings outside of the phloem. The parenchyma layers which separate the stem bundles from one another become lignified quite early, as does the pith; the cortex is, also, frequently found to be lignified. Some multicellular unbranched and branched hairs (Fig. 13d) are present on the adaxial side of the leaf blades and two rows of stomata extend along the median plane of the abaxial sides of the leaves and extend into their free tips (Figs. 13a to 13g).

C. distyla exhibits from six to eight leaves per whorl; most whorls have seven leaves. The branchlet structure is similar to that of *C. littoralis,* but frequently the sclerenchyma ribs extend into the ridges, forming the T-beam-shaped mass, in which the inner part of the T is thinner and diminishes in width toward the center of the stem (Figs. 14a to 14g).

TABLE 2

Summary of features of the mature branchlets

Species *C. deplancheana*	*C. leucodon*	*C. nodiflora*	*C. poissoniana*	*C. nana*	*C. paludosa*	*C. rigida*	*C. distyla*	*C. littoralis*	*C. glauca*	*C. cunninghamiana*	*C. litorea*	*C. helmsii*	*C. luchmannii*	*C. stricta*	Characteristics
—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	Furrows & ridges along the stem
4	4	4	4	4-5	6-8	9-12	6-8	6-8	9-16	6-8	7-12	4-5	9-12	9-12	Leaves per node
+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	Stems quadrangulate in transection
—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	Hairs in the furrows and the ridges
+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	Glabrous stems
+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	Stomata without or with one subsidiary cell
—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	Stomata with 2 or more subsidiary cells
+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	Stomata spread along the stem's surface
—	—	—	—	—	—	+	+	+	—	—	—	—	—	—	Stomata restricted to furrows with some on the free adaxial side
—	—	—	—	+	+	—	—	—	+	+	+	+	+	+	Stomata restricted to the furrows
—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	Haploxylic leaves
+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	Relictual bundles in one or both sides of the mid-vein

—	—	—	—	+	+	+	+	+	—	—	—	—	—	—	Rim-like cup at furrows' end
+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	Epidermal cells hexagonal in surface view
—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	Epidermal cells elongated, almost rectangular in surface view
—	—	—	—	+	+	+	+	+	—	—	—	—	—	—	Epidermal cells round in surface view
—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	Irregular masses of sclerenchyma beneath the epidermis on both sides of the ridges
+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Radially alternating masses of sclerenchyma and chlorenchyma beneath the epidermis
—	+	+	—	—	+	+	+	+	+	+	+	+	+	+	T-beam-shaped masses of sclerenchyma beneath the epidermis, along the ridges
—	—	—	—	+	—	—	—	+	—	—	—	—	—	—	Subepidermal plate of sclerenchyma lacking the central projection
—	+	—	—	—	—	—	—	—	—	—	—	+	+	—	T-beam-shaped mass of sclerenchyma beneath the epidermis reaching the leaf-trace
+	+	+	+	+	+	+	+	—	+	—	—	—	+	—	Wing-like shaped sclerenchyma strands external to the phloem stem bundles
+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	Loosely packed chlorenchyma
—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	Well organized chlorenchyma
—	—	—	—	+	+	+	+	+	—	—	—	—	—	—	Pith sclerified
—	—	—	—	—	—	+	—	—	+	+	+	—	—	—	Tracheoidal cells in the pith
—	—	—	—	—	—	—	—	—	+	+	+	—	—	—	Wing-like corners in the external side of ridges
—	+	+	+	—	—	+	+	+	+	+	+	—	+	+	Angled external side of ridges
—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	Flat external side of ridges
+	—	—	—	+	—	—	—	—	—	—	—	—	—	—	Rounded external side of ridges

C. rigida has whorls of nine to twelve leaves. The branchlet structure is very similar to that of *C. distyla* and *C. littoralis,* however, the furrows are wider and not as deep as those found in these species. Tracheoidal cells occur in the pith, as are found in *C. litorea, C. glauca,* and *C. cunninghamiana* (Fig. 15a to 15g).

C. paludosa has six to eight leaves per whorl, with seven being the most frequent number. This species shows the same basic structure of *C. distyla,* but differs from the latter in that it exhibits flat ridges, almost concave, along the stem when observed in transverse sections. All stomata found were located within the furrows. The pith is sclerified as in *C. littoralis* and *C. distyla* (Figs. 16 a to 16g).

C. nana generally has four leaves per whorl, although five-leaved whorls are sometimes found. The furrows are very deep and filled with hairs. The ridges have convex external faces but are not angled and the edges of the furrows are rounded. The hypodermal sclerenchyma occupies three or four layers beneath the epidermis, but the inner ribs are absent. The ridges are mainly composed of chlorenchymatous tissue. The sclerenchyma bounding the stem bundles forms short wings external to the phloem; the pith is sclerified. The parenchyma layers lying between the stem bundles lignify soon after being formed (Figs. 17a to 17g).

C. poissoniana, the model selected to exemplify the second group of the genus, has quadrangulate stems and tetramerous verticils invariably as do all the species in this group. The four corners (in transverse section) or median ridges are equidistant around the stem, diverging 90° from one another. Furrows are lacking in this group. The branchlets are glabrous and the stomata are sunken and lie in lines along the sides of the ridges and along the edges of the free leaf tips. These lines of stomata may anastomose and then diverge (Fig. 18b).

The guard mother cell produced by the division of a protodermal cell gives rise directly to both guard cells; the daughter cell produced with the mother cell elongates parallel to the stoma. These are the only cells separating the files of stomata and are considered to be subsidiary cells. Frequently, the daughter cell of the protodermal cell divides and both daughter cells differentiate as guard cells; then, the stomata lie side by side along the files. The lamellae present in the dorsal and ventral walls of the guard cells are much thicker and more lignified than those found in the guard cells of the species included in the first group. The stomatal pores are very small and are transversely oriented in respect to the longitudinal axis of the stem (Figs. 18b and 18c); the stomatal chambers are also very small. Stomata develop basipetally in the free abaxial side of leaves and the furrows.

The epidermal cells are of elongated hexagonal shape and rectangular in transection. The anticlinal walls are very thick and heavily cutinized. The thick external walls contain very refractive polyhedric crystals (Fig. 18f) and are covered by a thick cuticle which continues well into the stomatal chambers. Beneath the epidermis, on both sides of the ridges, a short irregular lamina of sclerenchyma is produced. These laminae show slight projections into the ridges. Masses of chlorenchyma fill the ridges. Near the sclerenchymatous laminae, the chlorenchyma forms a loosely packed palisade tissue; the remainder of cells in this tissue are small and isodiametric. The areas between contiguous ridges, represented as furrows in the Cryptostomae, are composed of nonchlorophyllic parenchyma cells in this species. At this level, each chlorenchyma rib is bounded internally by three of four parenchyma cell layers. Immediately under this parenchyma, in line with the median part of a ridge, is found a crescent-shaped leaf trace. The latter has a well-defined xylem and phloem. External to the phloem, a wing is formed due to the development of a large mass of protophloem fibers. As described for the former group, tracheoidal elements, with thick and simple-pitted walls, lacking protoplasts, lie on each side of each leaf trace underneath the chlorenchyma cap. In this species, these cells are very conspicuous and are much larger than their adjacent cells. They extend longitudinally or transversely to the axis, reaching to the chlorenchyma area, and form longitudinal connections. In the leaves, on either side of the leaf vein and intermixed with the tracheoidal elements, a thin lateral leaf vein is formed; the lateral veins originate from the basal part of the median leaf vein. The collateral stem bundles are separated from one another by several parenchymatous layers. A thick mass of sclerenchyma bounds the phloem externally (Fig. 18a); the sclereids of this mass are very thick-walled, especially in the nodal area, where they clearly encircle the stem bundles; at the nodes, the stem bundles form a ring, as they do in the Cryptostomae. Tracheoidal cells are also found in the pith and are mixed in with the sclereids in the stem bundles. The sheath and the free leaf tips have a structure similar to that of the ridges, but the sclerenchyma bounding the phloem of the midvein extends into the sclerotic lamina beneath the adaxial epidermis; also, beneath the epidermis, a layer of fibers which is discontinous in some places, develops abaxially (Figs. 18a to 18f).

C. nodiflora has the same basic branchlet structure found in *C. poissoniana.* It differs from the latter only in the presence of an irregular protrusion of the subepidermal sclerotic lamina into the chlorenchyma along the central outer part of the ridge (Figs. 19a to 19f).

C. leucodon is similar to the two former species; however, in this species the subepidermal mass of sclerenchyma extends to the median leaf trace (Figs. 20a to 20f).

C. deplancheana differs from the preceding three species in the alternation, under the epidermis, of sclerenchymatous and chlorenchymatous tissues around the circumference of the stem. Externally the stomata form lines which internally align with the chlorenchyma groups and are sunken deep within the epidermis. The leaf exhibits long-winged masses of sclerenchyma bounding the phloem externally. The features correspond to those of *C. poissoniana* in this second group but the tracheoidal elements are longer than in that species and their walls are thinner (Figs. 21a to 21f).

DISCUSSION

More recent knowledge in the field of anatomy makes it clear that the "tracheids of the transfusion tissue" (per **Boodle** and **Worsdell,** 1894) are tracheoidal cells as described by **Foster** (1945); some of the tracheoidal cells found in the Casuarinaceae (Division Gymnostomae) are similar to the fusiform and branched types of sclereids (**Foster,** 1945; Plate IV) described for *Trochodendron aralioides* Sieb. and Zucc. The tracheoidal cells are not restricted to the ribs as pointed out by **Loew** (1865), but are also found in the pith (Table 2). The external endodermis (per Boodle and Worsdell) surrounding the chlorenchyma has not been observed and the "probably pericyclic layers" (per Boodle and Worsdell) correspond to the protophloem fibers.

Two features considered earlier as morphological adaptations to drought are the stomata and the trichomes (**Loew,** 1865; **Boodle** and **Worsdell,** 1894; **Morini,** 1894, 1895). Observation of branchlets of species in both Divisions reveals a glabrous or almost glabrous character in the Division Gymnostomae and conspicuous pubescence in the Division Cryptostomae. **Morini** (1895) reported trichomes in *C. chamaecyparis* which constitutes the only known pubescent species of the Division Gymnostomae. The trichomes of the latter species are scarce, unicellular and reduced to a position within the slight furrows found between two contiguous leaves. In the Division Cryptostomae, unicellular and multicellular branched and unbranched hairs are found throughout the different species, some being highly pubescent, as in *C. stricta* and *C. luehmannii,* and some moderately pubescent as in *C. nana* and *C. glauca.* In the Division Cryptostomae, trichomes are mostly restricted to the furrows and the leaf blade margins, although in some species *(C. stricta)* they are abundant on the abaxial rib of the leaves and on the internodal ribs.

The stomata are widely distributed over the plant surface throughout the species in the Gymnostomae; although they are present in the Cryptostomae, they are restricted to the furrows. Exceptions to this rule are *C. littoralis, C. distyla* and *C. rigida* which also show some stomata on the adaxial side of leaves. The reduction in stomatal numbers is apparently correlated with the increase in subsidiary cell production.

Subsidiary cells are defined as cells surrounding the guard cells and clearly differing from the other epidermal cells. By observing the development of the stomata, it becomes evident that the paracytic stomata can be para-mesoperigenous or para-mesogenous (per **Fryns-Claessens** and **Van Cotthem,** 1973); the para-mesogenous type is associated mostly with the species of the Division Gymnostomae, while the para-mesoperigenous type occurs in the species of the Divison Cryptostomae.

Leaf reduction, pubescence, arrangement of leaves in whorls, etc. are characteristics associated with a dry habitat by most authors (**Church,** 1920; **Fahn,** 1964), along with thick cuticle, crystals, and an increase in the number of stomata. Although this research has not been conducted to demonstrate the validity of these opinions for the Casuarinaceae, it is likely that they are morphological adaptations to drought; however, the tendency of these plants seems to be a reduction of the stomata number and not an increase in the number as proposed by **Fahn** (1964). At this point the author has some reservations about the validity of Fahn's statement as a general rule. Probably the number of stomata in the plant is correlated with additional factors not considered before, and a more accurate study of the problem needs to be carried out.

Poisson (1871) divided the Casuarinaceae into two divisions, Casuarinae Cylindricae seu Cryptostomae and Casuarinae Tetragonae seu Gymnostomae, on the basis of **Loew's** (1865) observations of the vegetative structures. The latter division corresponded to the section Nodiflorae established by **Loew** in 1865.

The Casuarinae Cryptostomae have cylindrical branchlets mostly with verticillate leaves, and stem grooves more or less excavate and pilose; staminate inflorescences are simple and pistillate inflorescences are axillary. The Casuarinae Gymnostomae have quadrangulate branches with sparse or falsely verticillate leaves; the staminate inflorescences are compound, occasionally simple, and the pistillate inflorescences are quadrate with exerted bracts. Some species, such as *C. sumatrana, C. deplancheana, C. nodiflora, C. chamaecyparis* and *C. leucodon,* were transferred to this division by **Poisson** (1871).

In **Bentham's** Flora Australiensis (1873) the species were distributed into three sections: section I, Leiopitys Bth.; section II, Trachypitys Bth. and section III, Acanthopitys Miq. The two first sections correspond to Eucasuarina Miq. (**Miquel,** 1868). The division Cylindricae seu Cryptostomae of Poisson (1871) included the three sections proposed by Bentham.

After Poisson and Bentham, no worker attempted a revision of the family for nearly a century. New species were sporadically described and added to the groups, but no revision of those groups was made to determine the natural grouping of the species. As a consequence, the same species was frequently described twice, or even three times, under different names and the number of synonyms within the genus increased. The Index Kewensis (1885-1935) lists sixty species, as valid, of *Casuarina.*

The evaluation of characteristics of the vegetative and reproductive structures corroborate the accuracy of **Poisson's** (1871) observations. Two main subdivisions, which correspond to Poison's divisions Cylindricae seu Cryptostomae and Tetragonae seu Gymnostomae, need to be set apart. **Moseley's** (1948) report on the xylem of *Casuarina* also emphasizes the anatomical difference found between the species of both taxa. The two taxa certainly could be represented by separate genera and the term "division", applied by Poisson, is inappropriate and should be dropped. A new revision of the family which is being carried out by L.A.S. Johnson (personal communication) agrees with these findings.

The first group (= Cryptostomae Poisson) consists of species found mainly in Australia, some of which also extend over tropical Asia to the Mascarene Islands and the Indian Archipelago, and South Pacific Islands (**Bentham,** 1873). The second group (= Gymnostomae Poisson) is formed by species mainly found in New Caledonia, with some species also being distributed in the neighboring islands.

Among the species of each taxa structural variations are found in the reproductive as well as in the vegetative organs; but, as proposed by **Macklin** (1927), the anatomy of the branchlet probably constitutes the most valuable source of constant minor features to recognize and separate species in both divisions.

A larger number of species in the Cryptostomae were examined in this study than in **Macklin's** (1927), who set apart two groups based on branchlet structure. This study indicates that a subdivision of the Cryptostomae into several smaller groups could be more appropriate if a higher number of species is studied for taxonomic purposes.

The fifteen species investigated, from the probable fifty-five forming the family, are not sufficiently significant to determine final taxa within the family. A more exhaustive, statistically sound study of the family is required before proposing probable taxa. For example, *C. nana* has quite a different vegetative and reproductive morphology from the other species in Division Cryptostomae (**Poisson,** 1871).

This species is included by **Bentham** (1873) in his Section Trachypitys. Most of the species grouped in this section have a similar number of divided tubercles in the pistillate cymule on both sides of the central floret (personal observation). A thorough study of the morphological and anatomical characteristics of the species grouped by Bentham into Section Trachypitys, especially *C. humilis, C. microstachya* and *C. drummondiana* might provide enough evidence to group *C. nana* and related species in a subgenus exhibiting quite different branchlet morphology and retaining some of the most primitive floral features of the family.

Regarding the species grouped in the Gymnostomae, information is not complete because of the small proportion of the group studied; the four species included in this study seem to agree sufficiently to remain together as a "division".

Minor groups might be recognized as a greater number of species is investigated. An exhaustive study of the species in this alliance is desirable to complete or modify these preliminary conclusions and information from other sources, such as cytology and phytochemistry, may prove useful in clarifying species relationships.

RESUMEN

El braquiblasto de *Casuarina* presenta una epidermis glabra (Division Gymnostomae) o pubescente (Division Cryptostomae). La hipodermis es esclerenquimática y en muchas especies, se continúa dentro de las capas interiores de las costillas del entrenudo como masas en forma de T. La fotosíntesis es efectuada básicamente por el mesofilo de las costillas internodales.

La evaluación de características de las estructuras vegetativas refuerza la veracidad de las observaciones de Poisson. Dos subdivisiones básicas, que corresponden a las Divisiones Cylindricae seu Cryptostomae y Tetragonae seu Gymnostomae, pueden fácilmente ser reconocidas. Estos dos taxa podrían separarse en géneros diferentes; sin embargo, el término de "división" empleado por Poisson, es inapropiado y debe ser abandonado.

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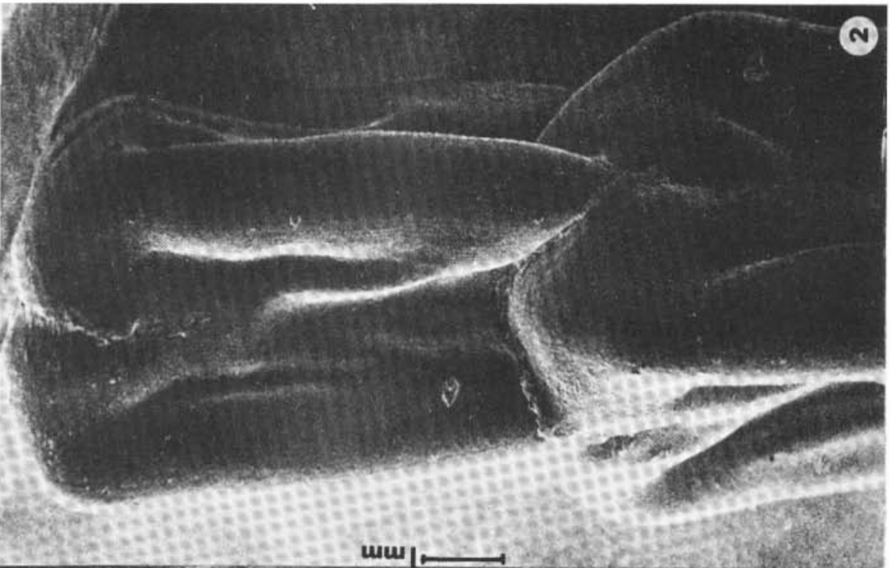
Fig. 1. Scanning microscope photograph of a branchlet vegetative tip of *C. stricta*.

Fig. 2. Scanning microscope photograph of a branchlet vegetative tip of *C. poissoniana*.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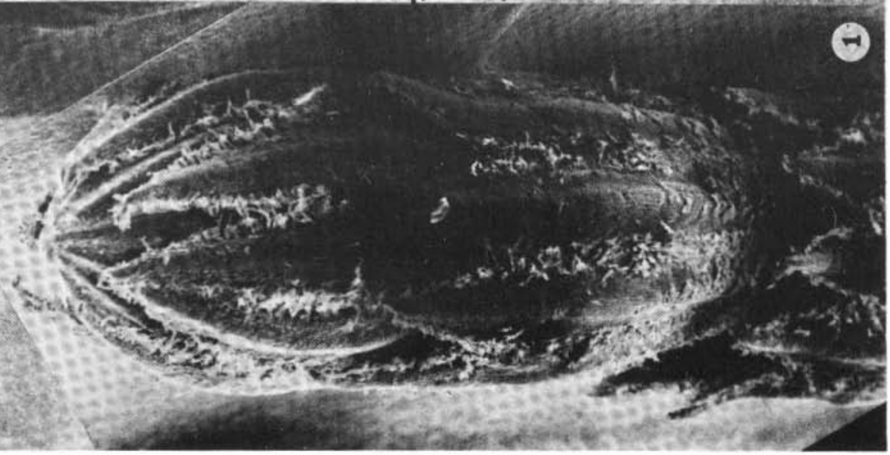
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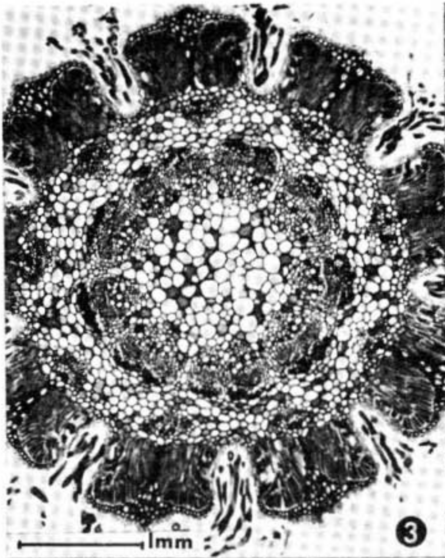
Figs. 3-6. *Casuarina stricta.*

Fig. 3. Distribution of tissues in the internode of a branchlet. Transection.

Fig. 4. Differentiation of a stem bundle of a young branchlet. Transection.

Fig. 5. Transection of an internodal ridge.

Fig. 6. Cell elongation in a furrow between two gamophyllous leaves.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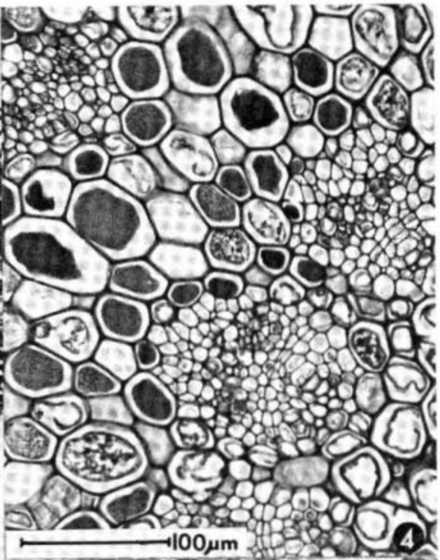
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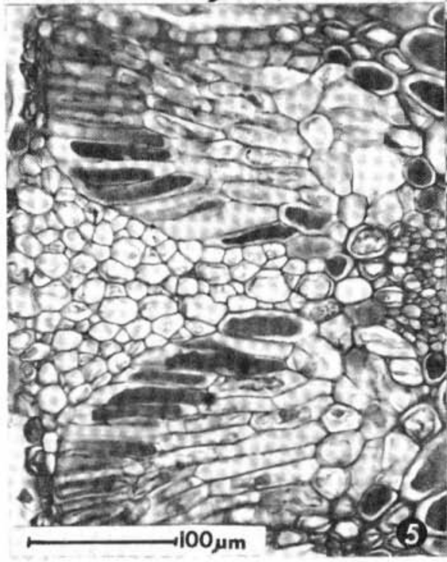
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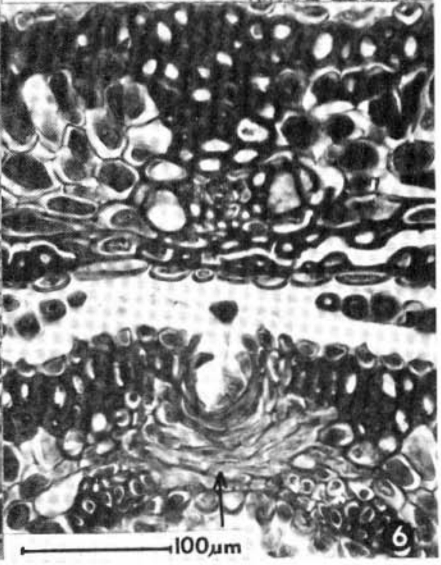
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Figs. 7-12. Camera lucida drawings showing some anatomical features of branchlets in several species of the Casuarinaceae. a, transverse section of the internode; b, external view of stomata; c, stomata as seen in transverse sections; d, hairs; e, tracheoidal cells; f, crystals of epidermal walls; g, leaves.

Fig. 7. *C. stricta*

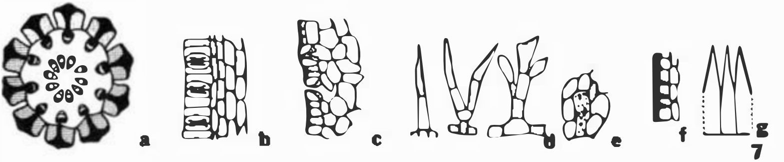
Fig. 8. *C. luehmannii*

Fig. 9. *C. helmsii*

Fig. 10. *C. litorea*

Fig. 11. *C. cunninghamiana*

Fig. 12. *C. glauca*



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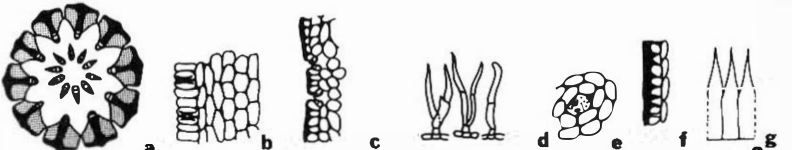

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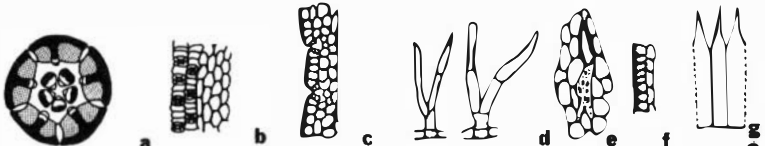

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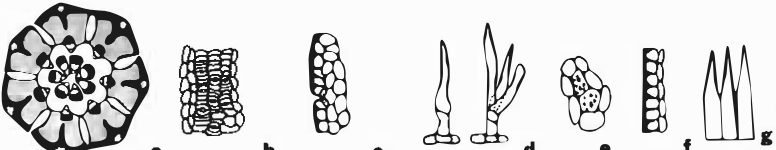

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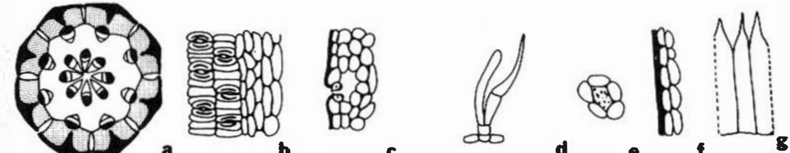

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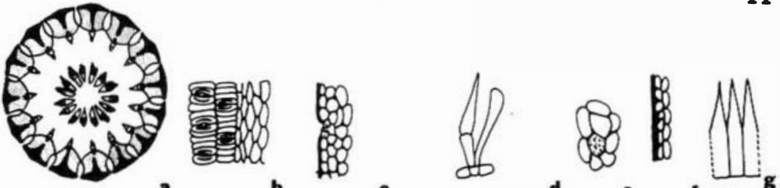

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a ⟝ 1mm

b,c,d,e,f ⟝ 100µm

g ⟝ 1mm

13-17. Camera lucida drawings showing some anatomical features of branchlets in several species of the Casuarinaceae. a, transverse section of internode; b, external view of stomata; c, stomata as seen in transverse sections; d, hairs; e, tracheoidal cells; f, crystals of epidermal walls; g, leaves.

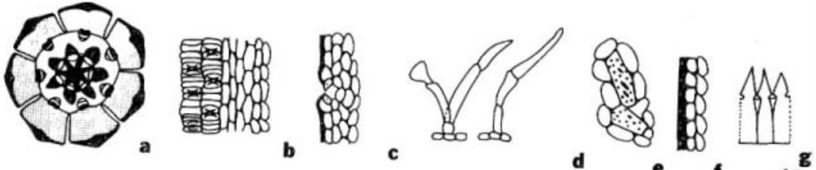
Fig. 13. *C. littoralis*

Fig. 14. *C. distyla*

Fig. 15. *C. rigida*

Fig. 16. *C. paludosa*

Fig. 17. *C. nana*



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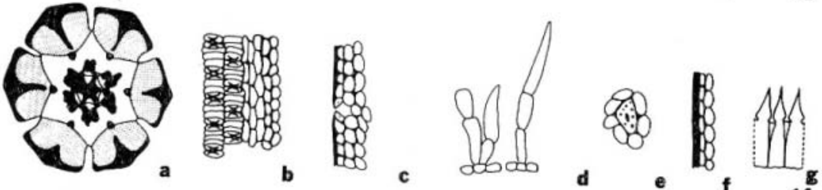

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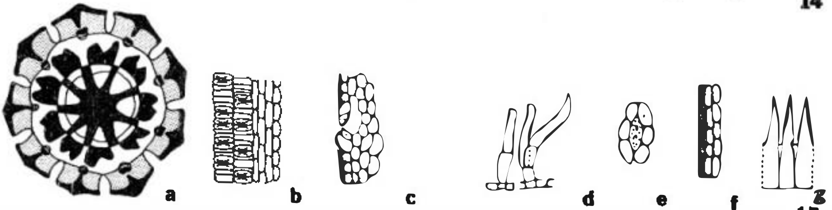

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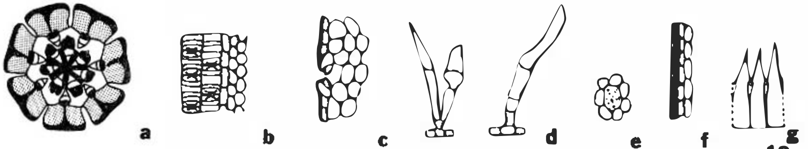

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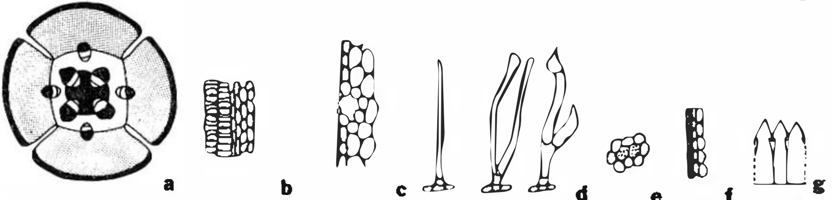

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a 1mm b,c,d,e,f 100µm g 1mm

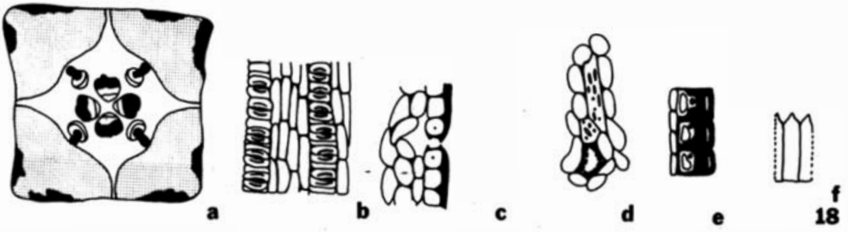
Figs. 18-21. Camera lucida drawings showing some anatomical features of branchlets in several species of the Casuarinaceae (Division Gymnostomae). a, transverse section of internode; b, external view of stomata; c, stomata as seen in transverse sections; d, tracheoidal cells; e, leaves; f, epidermal cells with crystals in the external wall.

Fig. 18. *C. poissoniana*

Fig. 19. *C. nodiflora*

Fig. 20. *C. leucodon*

Fig. 21. *C. deplancheana*



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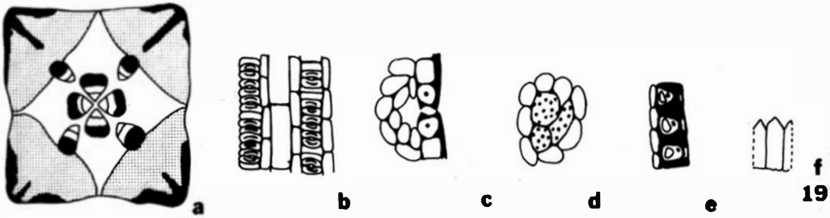

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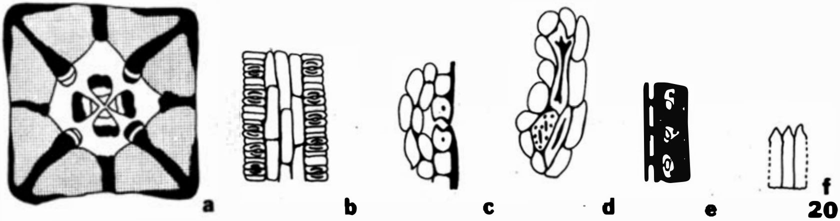

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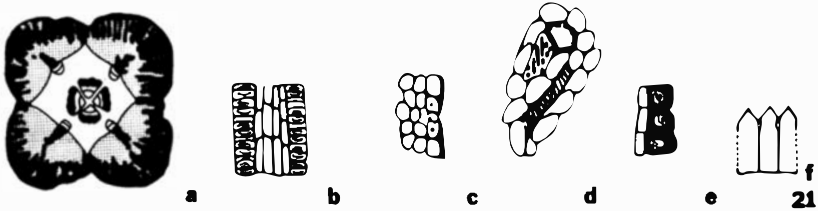

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