The Wood Anatomy of *Callitris* Vent. (Cupressaceae): an SEM Study

A thesis submitted for the degree of Doctor of Philosophy of The Australian National University

Roger David Heady
April 1997
DECLARATION

Except where specific acknowledgment is given, the research in this thesis is my original work.

Roger David Heady
Acknowledgements

I acknowledge the overall supervision of this research by Dr Phillip Evans, Senior Lecturer in Forestry, ANU, who introduced me to the science of Wood Anatomy, and has been my mentor in this exciting field since I was first made a convert. During the six years of the study, Dr Evans constantly provided me with encouragement, ideas, and feedback on progress. Without his assistance, this study would never have been completed.

I also acknowledge the invaluable specialist help given to me in this study by my three course advisers:

Ross Cunningham of the Statistical Consulting Unit, ANU, for advice and assistance in the design of experiments, the processing of data, and patient explanations of results.

Dr Sally Stowe, Facility Coordinator of the ANU Electron Microscope Unit, who gave me help and advice in EM and light microscopy.

David Jones, Senior Research Scientist at the Australian National Botanic Gardens, gave me advice on taxonomy, assistance with the location of several species, and obtained some of my wood samples of Callitris canescens, Callitris endlicheri, Callitris glaucophylla and Callitris verrucosa.

The ANU's Electron Microscope Unit provided me with virtually unlimited use of the SEM, as well as facilities for my sample preparation, photographic processing, and computing needs.

The Forestry Department of the Australian National University provided administrative support and funding for the travel and equipment requirements of this research, as well as some samples from the wood collection.

Christine Donnelly of the ANU's Statistical Consulting Unit programmed much of my data to produce statistically accurate and meaningful results.

Peter Beutel, Senior Technical Officer of the Forestry Department, ANU, provided me with excellent companionship on several field trips. Peter's phenomenal strength in operating the wood corer, and his map reading, planning, and organisational skills, enabled us to consistently find the required trees and to bring home the necessary pieces of wood.

Dr Simon Barry of the ANU's Statistical Consulting Unit provided me with advice and assistance in statistical data processing for a period of six months.

I am indebted to Dr Ken Hill of the Royal Botanic Gardens, Sydney, for his advice on the taxonomy of Callitris.
I thank the CSIRO Forestry and Forest Products Laboratory, Melbourne, the NSW Forestry Commission Laboratories, Sydney, and the Service des Forêts Patrimoine Naturel, Noumea, New Caledonia, for providing several wood samples from their collections.

I am also indebted to the following people for obtaining, or assisting me in finding, some of the wood samples used in the research:

Brendan Lepschi and Tarina Lally of the Department of Conservation and Land Management, Kensington, WA, for obtaining all of my samples of Callitris preissii.

Dr Stephen Harris, Senior Botanist of the Parks and Wildlife Service in Tasmania, for information on the location of Callitris oblonga and Callitris rhomboidea in that state.

Dr Ken Groves of Margules Groome Poyry Pty Ltd, and Steve Kitchener of the Queensland Forestry Service, for obtaining samples of Callitris intratropica.

Bernard Suprin of the Service des Forêts Patrimoine Naturel, Noumea, New Caledonia, for samples of Callitris neocaledonica and Callitris sulcata.

Graeme Siemon and Frank Podger of Department of Conservation and Land Management, WA for wood samples of Actinostrobus pyramidalis.

Guy Thomas, manager of the Maiala National Park, Queensland, for permission to obtain wood samples of Callitris macleayana within a National Park.

Dr Penny Gullan, Division of Botany and Zoology, ANU, for a sample of wood of Diselma archeri.

Dr Cranfield and the WA Department of Conservation and Land Management for permission to collect wood samples in the wild in WA.

Rod Holmes, for allowing me to obtain wood samples of Callitris monticola in the Glen Innes National Park, NSW.

I am grateful to Frank Brink of the Electron Microscope Unit, ANU, for assistance with the drawing of the diagrams used in this thesis, and also for his help in the most difficult task of all; sticking the photographs into place.

I thank my darling wife, Yolanda, for her understanding of my need to spend so many hours sitting at an electron microscope. I realise that now I no longer have an excuse for the jungle that has developed in the backyard! I also thank my daughters, Sondi and Asmara, son-in-law Greg, Jason, and grand-daughter Ebany, for their love and patience.

I am indebted to Vincent Craig, of the Department of Chemistry, ANU, for helpful discussion of the physics of water flow in capillaries.

Finally, to the makers of the Cambridge Instruments S360 Scanning Electron Microscope, I send my congratulations. That is truly a wonderful piece of equipment.
Abstract

A comprehensive study of the wood anatomy of all species of the Australasian softwood genus *Callitris* Vent. (Cupressaceae) was carried out using scanning electron microscopy (SEM). The research centred around three anatomical features: callitroid thickening, the warty layer, and rays. Multiple wood samples, obtained from trees growing in their native environments were examined, quantitative measurements of these features were carried out, and data were statistically analysed. This provided information which enabled comparative anatomical, taxonomic, and ecological aspects of callitroid thickening, warts and rays to be described. The anatomy of bordered pits, crossfield pits, and axial parenchyma in all species of *Callitris* was also studied, and a general wood anatomy description of the genus was developed.

Callitroid thickening was found in all (20) species of *Callitris*, including five species in which thickening has been reported to be absent, and two species not previously examined. A supplementary study using light microscopy failed to detect callitroid thickening in most of the species which the literature reported it absent, thus indicating that the superior resolution of SEM over light microscopy was the main factor responsible for its detection by SEM. Callitroid thickening was found to vary in morphology, with 1-4 bars being associated with individual pits, and bars extending either fully or partially across the width of the tracheid inner walls. The proportion of pits with associated callitroid thickening ranged from 1% in rainforest species to 98% in species of dry environments. Callitroid thickening was generally more prevalent in narrow tracheids than in wide ones.

Warty layers consisted of a heterogeneous mixture of two different populations of warts; (i) large, complex warts with nodule-like projections, and (ii) small, hemispherical warts. Pairs of warts were occasionally anastomosed.

The heights of individual rays ranged from approximately 20 µm (one cell), to greater than 600 µm (more than 30 consecutive cells). However, contrary to reports in the literature, rays in *Callitris* were not 'frequently more than 30 cells high', neither were ray tracheids present.

A short study of callitroid thickening and warts in the wood of 22 Cupressaceae species from 12 different genera found thickening in five species, and nodulated warts in seven species in which it had not previously been reported.

In dry-habitat species, callitroid thickening was common, mixed populations of 'small hemispherical' and 'large complex' warts occurred, and rays tended to be short and narrow, whereas in wet-habitat species, callitroid thickening was uncommon, warts were exclusively small and hemispherical, and rays were taller and wider. These ecology-related differences were shown to be fundamentally genetically-controlled; in a
controlled-environment experiment, no significant differences were found between thickening, warts, and rays of trees grown under wet and dry conditions.

Callitroid thickening and warts generally showed little significant variation radially or vertically within a tree stem, or between the earlywood and latewood of a growth ring. However, juvenile wood was characterised by short rays and the absence of callitroid thickening with 3 or 4 bars.

Callitroid thickening and warts were shown to be useful taxonomic features within the genus Callitris.
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Chapter 1

General Introduction

This Chapter presents a general introduction to the thesis. The background to the research is described, and the genus *Callitris* is introduced in a concise literature review of its biology, utilisation, and taxonomy. Aims are defined, and the purpose, scope, and methods of the study are outlined.
1.1 Introduction to the Thesis

1.1.1 Background to the Research

Variability is a fundamental attribute of wood. Manifestations of this variability range from differences which are readily apparent to the unaided human senses, such as wood colour, gross structure, hardness, odour, density, and taste, to those which become noticeable only with the assistance of technology, such as variability in the lengths of tracheids, differences in the shapes of pit apertures between individual wood cells, or lignin content changes within the cell wall. Some morphological variability is conspicuous, such as that apparent in the three basic planes of wood (i.e. the transverse, tangential longitudinal, and radial longitudinal planes); while other variability is much more subtle, often occurring as morphological differences within or between individual cells, or of features within cells. Some of the variability in wood is genetically controlled, and the differences are regular and predictable in their occurrence. Typical are those which occur between the wood structure of species of the order Coniferales, in which vertical transport of liquids within the tree stem occurs in tracheids, and which is a distinctly different system from that of species of the order Dichotyledoneae, in which the same function is generally carried out by vessels. Environmental effects can also be superimposed on the basic structure of wood. Characteristic of these are the variations in wood cell size that are delineated as earlywood and latewood in 'growth rings'. These variations are usually more irregular, due to the vagaries of the environment.

It is the diversity of wood structure that confers variety and flexibility on the use of wood as a resource; certain timbers being favoured for particular purposes, but not for others. It also enables wood to be used as an indicator of taxonomy, and of past climatic conditions (the science of dendrochronology). The lack of uniformity in wood can, however, affect its efficient use as an industrial raw material. For example, variability in the strength of timber has a profound effect on the building industry (Panshin & de Zeeuw 1980). Similarly, wood pulp that has inherent variation in its fibre length or strength is more costly to process than the less heterogeneous variety (Zobel & Buijtenen 1989). To the forester therefore, a reduction in the variability of timber is usually considered desirable, and various silvicultural techniques are used to promote uniformity in wood (Zobel & Jett 1995).

The study of the variability of wood is known as the science of wood anatomy. It began as a purely descriptive science in the mid-seventeenth century when Robert Hooke, in his famous 'Micrographia' gave an account of charcoal and petrified wood (Baas 1982). From this, the field of 'Comparative' wood anatomy developed in which the "descriptive elucidation of wood structure, for its own sake" (Baas 1982) was the major theme. The significance of wood anatomical diversity to taxonomy, separating species, genera, and families, was then recognised and 'Systematic' wood anatomy was
initiated (Brazier 1975). Similarly, from "the realisation that adaptation to ecology is a central theme in wood evolution" (Carlquist 1988) the field of 'Ecological' wood anatomy had its beginnings. Ecological wood anatomy may also be considered 'Functional' wood anatomy "since in describing differences among species or genera, we are usually describing differences in adaptation to ecological features" (Carlquist 1988).

Other branches of the science relate to the evolutionary history of wood structure ('Phylogenetic' wood anatomy) and the effect of wood structure on the strength of timber ('Technological' wood anatomy). The historical development of all of these themes of the science of wood anatomy have been described by Brazier (1975) and Baas (1982).

The softwood genus *Callitris* Vent., commonly known as cypress pine, lends itself to the first three themes of the study of wood anatomy for the following reasons. Firstly, *Callitris* has a very wide geographical distribution, and the natural habitats of its individual species are equally broad-ranging in type, varying from wet sub-tropical forest to temperate woodland and semi-arid inland scrub. The inherent diversity of habitat of the genus is thus of interest from the point of view of relating differences in wood anatomy to characteristics of the natural habitat (i.e. Ecological wood anatomy) and facilitates speculation on the functional, or adaptive advantage of particular wood anatomy features.

Secondly, the general taxonomy of *Callitris* has been the subject of controversy in recent years due to the lack of significant differences in conventional taxonomic indicators (i.e. the shape and size of the leaves and cones of its individual species). Therefore the possibility of using wood anatomy to separate taxa within the genus (i.e. Systematic wood anatomy) is an attractive one.

Thirdly *Callitris* wood is of interest from the point of view of comparative wood anatomy since a certain aspect of its (Comparative) wood anatomy, callitroid (or callitrisoid)\(^1\) thickening, is reported to be virtually unique to the genus. Furthermore, no comprehensive or even completely representative investigation of the wood anatomy of all species in the genus has ever been carried out. Examination of the seven accounts of the general wood anatomy of *Callitris*; Baker & Smith (1910), Patton (1927), Peirce (1937), Phillips (1948), Greguss (1955; 1972), and Venning (1979) has revealed that all of these studies are chronically incomplete. There is no description in any of these reports of the wood anatomy of several of the presently-recognised *Callitris* species, and since the taxonomy of the genus has undergone many changes in the classification and nomenclature of its taxa, most species now have multiple synonyms making it uncertain which *Callitris* species are being referred to by these authors. Many reports

\(^1\) Although the terms 'callitroid thickening' and 'callitrisoid thickening' have both been used in the literature, 'callitroid thickening' appears to be more generally accepted, and for standardisation purposes, will be used in preference to 'callitrisoid thickening' throughout this thesis.
are based on extremely limited sampling (often only one sample) and the sampled wood is often branch or twig material and therefore not typical of the particular species. The usefulness of the descriptions is further reduced by poor statistical methodology. For example, Greguss (1955) quotes only the maximum and minimum values for several anatomical features and gives no means, deviations or sample sizes. Some differences of opinion occur between the various authors. For example Patton (1927) recorded callitroid thickening as present in *Callitris intratropica* Benth. et Hook. whereas Greguss (1955) found none. Similarly Peirce (1937) noted ray tracheids in several *Callitris* species whereas Baker & Smith (1910) and Greguss (1955) reported them to be absent, and Patton (1927) made no comment regarding this feature. Thus, an untidy and confused state exists in regards to the (comparative) wood anatomy of *Callitris*, and a comprehensive study of the anatomy of the entire genus is required.

### 1.1.2 Literature Review of the Biology, Taxonomy, and Utilisation of *Callitris*

The genus *Callitris* consists of monoecious (Dallimore & Jackson 1966; Silba 1986) tall shrubs, or small to medium trees (Elliot & Jones 1980) with generally pyramidal habit (Maiden 1917) and spreading or erect branches (Hart & Price 1990). Foliage is dense, bright green or glaucous (Garden 1956). Juvenile leaves are needle-like in whorls of four, decurrent for only a small portion of their length, triangular in cross section and are usually found only on young plants, but in some species persist even on cone bearing shoots (Garden 1956). Adult leaves are scale-like and triangular, in whorls of three and with bases decurrent and fused to the stem (Hart & Price 1990). Bark is usually greyish and furrowed (Hart & Price 1990). The female cones are woody and globular, usually possessing six scales arranged in one whorl. The alternate scales of the cone are shorter and narrower than the others, which are broad at the base, pointed at the apex, thick and woody, smooth, or warty on the outer surface (Dallimore & Jackson 1966). Cones open out from the top when ripe, forming an open cup in which the seeds lie loosely attached to the scales (Ewart 1930). Seeds are oblong, 2 to 9 on each scale (Dallimore & Jackson 1966), and with 1 to 3 lateral wings (Hart & Price 1990). Male cones are ovoid to cylindrical in shape and are positioned at ends of branchlets (Silba 1986). Stamens occur in whorls of 3-4 (Silba 1986).

The name *Callitris* is derived from the Greek *kallistos* -'beautiful' and *treis* -'three', in allusion to the three-fold arrangement of its leaves and cone scales (Elliot & Jones 1980; Hart & Price 1990). The genus is commonly known as 'cypress pine' and there are a number of local names for individual species, all based on the word 'pine' (Table 1.1) although as Ilic (1994) points out, 'pine' does not in any way refer to the genus *Pinus*.

The genus *Callitris* is of the order Coniferales, and family Cupressaceae. It is currently considered to consist of 20 species, 18 of which are endemic to Australia while two are found only in New Caledonia (Table 1.1). Peirce (1937) assigned *Callitris*
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<td>baileyi</td>
<td>C.T. White</td>
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<td>columellaris</td>
<td>F. Mueller</td>
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<td>drummondii</td>
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<td>Syn. Qld. Flora (1883) 497 (1883)</td>
<td>C. arenosa A. Cunn.</td>
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<td>C. calcarata A. Cunn.</td>
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<td>N. WA; NT; N. QLD</td>
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<td>monticola</td>
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<td>muelleri</td>
<td>(Parlatore) F. Mueller</td>
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<td>C. propinqua R. Br.</td>
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<td>R. Br. ex Bak. et Sm.</td>
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<td>neocaledonica</td>
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<td>Journ. Bot. LII (1914) 239</td>
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<td>E. Tasmania</td>
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<td>oblonga</td>
<td>A. et L.C. Richard</td>
<td>Conif. (1826) 49, t.18, No. 2</td>
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<td>Engl. Jahrb. XXXIX (1907) 16</td>
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</table>

Table 1.1: Listing of *Callitris* species, showing author citation, place of publication, synonyms, common names and geographical distribution.
to the subfamily Thuyoideae, and in which he also placed the genera; *Actinostrobus* (two species), *Tetraclinis* (one species), *Callitropsis* (one species), *Widdringtonia* (five species), *Fitzroya* (one species), *Diselma* (one species), *Thuja* (six species), *Libocedrus* (nine species), and *Fokienia* (three species). Other, more distantly related genera were assigned to subfamily Cupressoideae which contains *Cupressus* (12 species), *Chamaecyparis* (six species), and subfamily Juniperoidae which contains *Arceuthos* (one species) and *Juniperus* (60 species). *Callitris* is usually distinguished from closely-related genera on the basis of the number of scales on the female cones (six scales for *Callitris*, four for *Tetraclinis* and *Widdringtonia*), and by having adult leaves in whorls of three, compared to whorls of four for *Tetraclinis*, and whorls of two for *Widdringtonia* (Dallimore & Jackson 1966). On the basis of leaf and cone morphology, *Callitris* is said to have the greatest similarity with *Actinostrobus* (Hart & Price 1990) which has the same number of cone scales and leaf whorls as *Callitris*. However, *Actinostrobus* can be distinguished by having bracts pressed closely to the lower half of its cone scales, whereas in *Callitris*, cones are not enclosed by bracts (Dallimore & Jackson 1966).

*Callitris* is not a large genus but it has a more extensive geographical distribution than any other genus of Australian conifers (Baker & Smith 1910). The genus is represented in every state of mainland Australia, and there are two *Callitris* species both in Tasmania and in New Caledonia. *Callitris intratropica* which is widespread across northern Australia (Thompson & Johnson 1986) occurs in an "apparently stable mixture with rainforest species" (Kirkpatrick et al. 1987; Harris & Kirkpatrick 1991). Two of the more common species (*C. glaucophylla* and *C. endlicheri*) form a major component of the vegetation in several plant communities of dry inland areas of Australia (Adams 1985). *Callitris glaucophylla* is reported to have escaped from cultivation and has become locally naturalised in sand-pine (*Pinus clausa* Vasey) scrub in several counties of Florida, USA (Hart & Price 1990).

Tree habit-form, and preferred habitat of species of *Callitris*, are inherently linked. The Australian mainland species; *C. macleayana* and the two New Caledonian species (*C. neocaledonica* and *C. sulcata*) occur as medium-sized (i.e. 30 m tall) trees in wet sub-tropical forests. Other species (typically *C. endlicheri*, *C. glaucophylla*, *C. tuberculata*, and *C. verrucosa*) occur as shorter (i.e. to 20 m) trees, mostly in relatively infertile woodland in warm sub-humid to dry climatic zones of the Australian inland. The species *C. canescens*, *C. drummondii*, *C. monticola*, and *C. roei* occur mainly as tall, multistem (i.e. mallee) shrubs in dry to semi-arid habitats (Garden 1956; Dallimore & Jackson 1966).

The generic name 'Callitris' first appeared in 1808, validly published in Ventenat's *Decas Generum Novorum* and "this was referred to in Richard's Mémoires sur les Conifères et les Cycadees published in 1826" (Bullock 1957). In 1825, "Mirbel, of the Paris Herbarium, thinking Ventenat's name of *Callitris* too closely resembled in sound
that of Labillardiere's genus *Calythrix* of the Myrtaceous Group of plants, substituted the name 'Frenela' and published it in *Mem. Mus. Hist. Nat. Paris* 13" (Baker & Smith 1910). The name *Frenela* persisted for many years and was used by Bentham (1863) in his *Flora Australiensis*. Mueller (1882) however used the term 'Callitris' and referred to 'Frenela' as a synonym, as did Bailey (1902). Baker & Smith (1910) recognised the "name 'Frenela' to be an illegitimate nomenclatural synonym of *Callitris*. Bullock (1957) proposed that the lectotype species of the generic name *Callitris* Vent. (1808) be *Callitris rhomboidea* R. Brown ex L. C. Rich. (1826).

Listings of species considered to make up the genus have changed many times over the years. Recent major changes to the taxonomy of the genus *Callitris* were brought about by Garden (1956), Blake (1959), Thompson (1961) and Thompson & Johnson (1986).

The taxonomic characters that have been used to differentiate species within the genus *Callitris* all relate to leaf and cone morphology (Costermans 1981). The principal ones are: (i) the dorsal surface of the leaf (whether rounded or keeled); (ii) the shape of the ends of the cone scales (pointed or rounded); (iii) cone scale separation at the base of the cone (high or low); (iv) cone surface (rough or smooth); and (v) the physical size of the cones (larger or smaller than 1 cm) (Dallimore & Jackson 1966). Species of *Callitris* have also been separated using volatile leaf oils as taxonomic markers (Adams & Simmons 1987).

*Callitris* does not regenerate well, if at all, after fire (Adams 1985) and the genus is also susceptible to grazing by rabbits (Salmon 1990). Several *Callitris* species are now quite uncommon. Dallimore & Jackson (1966) describe *C. muelleri* and *C. roei* as 'rare'. However, no *Callitris* species are included in the CSIRO's listing of 'Rare or Threatened Australian Plants' (ROTAP) (Briggs & Leigh 1988) although *C. oblonga* is classified as "vulnerable" and "occurring in small populations which are mainly restricted to highly specific and localised habitats which are continually being depleted through changes in land use".

The wood of *Callitris* is 'particularly dense and hard for that of a softwood' (Phillips 1948). It tends to have an 'oily feel' and is knotty (Ilic 1994) close-grained, fragrant, and the colour is variable, ranging from dark-brown to light-brown and with distinct heartwood and sapwood (Dallimore & Jackson 1966). Sapwood of *C. glaucophylla* (the main timber species) is pale and wide, heartwood light-yellow to dark-brown (Boland *et al.* 1984). The odour given off by the wood of *Callitris* is generally, (*C. macleayana* being the exception), camphor-like, quite pleasant, somewhat aromatic and characteristic (Baker & Smith 1910).

Several species of *Callitris*, particularly *C. glaucophylla* and *C. intratropica*, produce valuable timber which is prized because its inherent resistance to termite attack eliminates the need for termicide treatment with organo-chlorines and arsenicals (Rudman & Gay 1964; French *et al.* 1979). This toxic and repellant action of the wood
is said to be due to the presence of l-citronellic acid, eudesmol and azulenes and certain other extractives (Yazaki & Hillis 1977; French et al. 1979). Cypress pine is also dense and relatively strong for a conifer. Several timber mills in western NSW and southern Queensland specialise in the timber of white cypress pine (*C. glaucophylla*) which is also renowned for its high decay resistance (Ilic 1994) and its relatively small shrinkage during drying compared to most other timbers (Salmon 1990). It can be used in many circumstances without the need for seasoning (Salmon 1990). It is therefore valued for building purposes, particularly in areas where termites are a problem, and also for flooring, scantling, furniture, and posts and rails (Dallimore & Jackson 1966: Boland et al. 1984). It was (in the late nineteenth century) used for piles and the sheathing of boats because it resisted the attacks of the marine borer *Teredo* (Bailey 1902). Cypress pine timber has recently been exported to the Japanese island of Kyushu, where termites are a serious problem. The marketing agents (Austwood Aust. Pty Ltd) originally used the term 'Australian Pine' to describe the timber but encountered market resistance because 'pine timber', in general, has low termite and fungal-decay resistance. An alternative name 'Goshu Hinoki' - (translated 'Australian Hinoki', where Hinoki is the name of the most popular, durable timber in Japan) will be used in future (M. Kiguchi pers. comm.).

Other economic products of *Callitris* are tannin from the bark and fragrant oils by distillation of shoots, leaves and cones. Resin, similar to 'sandarac of commerce' from wounds in the bark (Dallimore & Jackson 1966) and from stumps and cut logs is composed of a number of diterpenoid acids and is used by the pharmaceutical industry for the coating of pills (Lassak & McCarthy 1990). Twigs of *C. rhomboidea* were once used as an anthelmintic "to expel worms in horses" (Bailey 1909; Lassak & McCarthy 1990). *Callitris glaucophylla* and *C. intratropica* are often planted as shade trees for stock (Elliot & Jones 1980) and *C. rhomboidea* is often grown as an ornamental (Nadolny & Benson 1993).

Descriptions of the wood anatomy of the genus *Callitris* have been made by Baker & Smith (1910), Patton (1927), Peirce (1937), Phillips (1948), and Greguss (1955; 1972). The PhD dissertation on the general taxonomy of *Callitris* by Venning (1979) includes a taxonomic study of the wood (twigs and branches only) of the genus. Other references to the wood anatomy of individual species, or of specific anatomical features of the genus include: Kleeberg (1885), Budkevich (1936), Liese (1957; 1965b), Cronshaw (1961), Wardrop (1964), and Ilic (1994). Diagnostic keys to separate the wood of *Callitris* from other genera are those of Peirce (1937), Barefoot & Hankins (1982), and Wheeler et al. (1985).

1.1.3 Requirements and Considerations

There are two basic techniques available to the modern wood anatomist for the study of wood microstructure: light microscopy (LM) and scanning electron
microscopy (SEM). The light microscope has long been the traditional tool in the field, and as Barefoot & Hankins (1982) point out, it still remains the major instrument of wood anatomy. This is due, in part, to the much lower overall cost of LM and its less stringent specimen preparation requirements. The ability of the light microscope to produce images in their natural colour (an electron beam has no inherent colour information so that images produced by an SEM\(^2\) are always 'black-and-white') is probably another factor in favour of LM, particularly in relation to the use of colour differences to identify wood features. LM is also useful for 'through-the-sample' viewing of thin, stained, sections whereas the SEM always shows only the extreme upper surface topography by reason of the small 'escape depth' of the secondary electrons forming the image. However, since it first became commercially available in 1965, SEM has been increasingly used in the field of wood anatomy. There are probably two major reasons for this. Firstly, SEM images typically have great depth of field, giving a resultant 'three-dimensional' perspective of the sample, whereas the depth of field of LM is poor. Secondly, whereas LM is limited by the wavelength of light to a resolution\(^3\) of 200 to 300 nm (thus restricting the light microscope to a maximum of about 1,000 times magnification), the resolution of an electron microscope is limited by the effective wavelength of an electron accelerated in a vacuum, and this is theoretically 10,000 times shorter than that of light. This permits resolutions in the order of 1 to 10 nm at useful magnifications of from 5 to 50,000 times which is ideally suited for imaging of most wood micro-features of *Callitris*.

One important aim of this study was to respond to the need for a full revision of the wood anatomy of all *Callitris* taxa. It was considered that such a study would be of most benefit if carried out in a quantitative manner, since this would enable statistical analysis and interpretation of differences too subtle to be described in any other way. The practicality of this consideration was enhanced by a further decision to use SEM as the principal research tool, since SEM enables lineal measurements to be made at micron scale, accurately and quickly.

Since it was not feasible to quantify all aspects of the wood anatomy of *Callitris*, it was decided to concentrate efforts on a limited number of micro-morphological features, and to choose those which the literature indicated to be most characteristic of the genus. Three features were chosen: (1) callitroid thickening of the pits - generally accepted to be the most important identifying feature of the genus due to its virtual exclusiveness to *Callitris* (Patton 1927; Peirce 1937; Phillips 1948; Greguss 1972; Venning 1979); (2) extraordinarily large-sized warts in the warty layer lining the

\(^2\) Note that the term 'SEM' is used to indicate both the process (i.e. scanning electron microscopy) and the equipment (i.e. the scanning electron microscope). This is in accordance with common practice in the field of electron microscopy and will be continued throughout this thesis.

\(^3\) Resolution is commonly defined as the minimum distance that two objects can be separated and still be observed as distinct.
tracheids (Wardrop et al. 1959; Liese 1965a); and (3) exceptionally tall rays - frequently more than 30 cells high (Peirce 1937; Wheeler et al. 1985). However, in addition to these features, the wood anatomy of all species in the genus was examined using both SEM and LM.

Because of the confusion that exists with regard to the taxonomy of Callitris, it was decided that the 20 species listed in Table 1.1 would be considered as definitive of the genus for the purposes of the study. This listing accords with the opinion of Hill (pers. comm.), whose classification will be used in a forthcoming edition of the authoritative volume 'Flora of Australia' (George 1994 general editor of the series). The former Australia-wide species C. preissii Miq. and its three sub-species; ssp. murrayensis, ssp. preissii and ssp. verrucosa will now be classed as five distinct but closely related species which are as follows: (i) Callitris tuberculata R. Brown ex Mirbel is formed from the inland Western Australian (WA) population of what was formerly C. preissii ssp. verrucosa; (ii) Callitris murrayensis R. Brown ex Baker et Smith is formed from the population of the Murray River basin area, which was formerly C. preissii subspp. murrayensis; (iii) Callitris gracilis R. Baker is an endemic of the Bylong, New South Wales (NSW) area which was originally classed as a species by Baker (1903), but had since been grouped under C. preissii ssp. preissii. This now reverts to its original status; (iv) Callitris verrucosa (A. Cunn. ex Endl.) Garden is now formed from the populations of the dry inland areas of NSW, Victoria (VIC), and South Australia (SA) which were formerly classed as C. preissii ssp. verrucosa; and (v) Callitris preissii R.T. Baker becomes "a narrow endemic from Rottnest Island and around Perth" WA (Hill pers. comm.) which was formerly grouped under C. preissii ssp. preissii.

Information regarding the Callitris taxa in general, and the New Caledonian species in particular, was also obtained from Garden (1956), Dallimore & Jackson (1966), Laubenfels (1972), Silba (1986), and Thompson & Johnson (1986).

1.2 Aims and Structure of the Thesis

There were three principal aims for the research, each involving a different theme of wood anatomy.

The first aim was to qualitatively and quantitatively describe callitroid thickening, the warty layer, and rays in all (20) species of Callitris. Supplementary to this aim was the recognition of a need to produce a full wood anatomy description of each species based on observations made by SEM but supplemented by LM, that would update and consolidate previous descriptions in the literature, all of which were based on observations made only under LM. These aims relate to 'Comparative' wood anatomy and represent a response to the incomplete and confused state of anatomical descriptions of Callitris in the literature.
The second principal aim was to determine the potential use of inter-specific differences in callitroid thickening, wart, and ray morphology in separating *Callitris* taxa. This aim related to 'Taxonomic' wood anatomy and was a response to difficulties encountered in defining taxa within the genus using classical means, as is evidenced by the many changes in classification of the various species of *Callitris* that have taken place in the past. Two other aspects of relevance to this aim were as follows: (i) there was a need to determine the inherent variability of these wood anatomy features within the tree as a whole, since such information affects the taxonomic value of the features in relation to wood samples from unknown positions within trees, and (ii) there was also a need to separate variability due to environmental influences from the (potentially taxonomically-useful) genetically-controlled differences.

The widespread distribution and large range of habitat types inherent to the various species of the genus provided the opportunity to study 'Ecological' aspects of the wood anatomy of *Callitris*. Thus the third aim was to compare callitroid thickening, warts, and rays in species endemic to contrasting habitats and to relate between-species morphological differences in these features in terms of their (likely) physiological function.

The thesis consists of ten chapters. After these introductory paragraphs, the materials and methods that are common to all sections of the study are described in Chapter 2. Chapters 3, 4, and 5 examine callitroid thickening, the warty layer, and rays respectively in all species of *Callitris*. Callitroid thickening, warts and rays are again the subject of Chapter 6, which deals with the within-tree variation in these features; Chapter 7 is concerned with the genetic and environmental components of their phenotypic expression; and Chapter 8 their potential as taxonomic indicators. Chapter 9 describes the wood anatomy of the genus as a whole, and the final chapter (10) integrates the entire study, presents conclusions, and recommends further research to supplement and enhance the study.

Each chapter is preceded by a concise introductory paragraph and has a self-contained literature review relevant to the particular subject under study. To show wood micro-anatomy in its true three-dimensional perspective, the text is illustrated with many 'micrographs' (i.e. photographs formed from SEM images).
Chapter 2

Materials and Methods

This Chapter describes the requirements, considerations, procedures and techniques of wood sampling, specimen preparation, SEM operation, data acquisition and statistical analysis used throughout the thesis.
2.1 Introduction to Materials and Methods

The experimental methods used in this thesis involved the following:

a) Obtaining a representative and comprehensive sampling of the wood of all *Callitris* taxa.

b) Preparation of wood samples for SEM.

c) SEM imaging of the features of interest, and the acquisition of accurate quantitative information on their morphology.

d) Statistical analysis of the data to enable formation of generalisations regarding these features.

2.2 Sampling

2.2.1 Introduction to Sampling

Throughout the thesis, a 'sample' is defined as a single piece of wood taken from an individual tree or xylarium specimen block. With the exception of the whole sections of tree stem used in Chapter 6, no multiple samples from a single source (tree or xylarium block) were used.

The following criteria were identified as essential to the sampling process:

i) It was crucial that all wood samples were correctly identified as to their species and their source.

ii) A sufficient number of samples were required to constitute a fully comprehensive sampling of the wood of each species.

iii) Each sample had to provide enough material to prepare SEM specimens for all of the experiments since there was little possibility of acquiring further samples of the same type, especially those obtained from trees growing in very remote areas.

iv) It was preferable to obtain samples from trees growing in localities widely separated over their distribution range in order to incorporate the natural variability occurring between populations and habitats, and thereby ensuring representative sampling.

v) It was desirable that the living trees suffered minimal damage as a result of the action of sampling.

vi) Wood from the stems of juvenile trees, or samples of twig or branch were not acceptable as a sample material. Only mature, main-stem wood was required.

vii) In order to avoid the encrustation and masking of wood micro-features by the resinous extractive matter that is typical of heartwood in *Callitris*, the sapwood, and not the heartwood of trees, was the preferred sample material.
2.2.2 Meeting Sampling Requirements

2.2.2.1 Sample Types

Three types of wood sample were used; (a) cores taken from live trees using an increment borer (corer), (b) small pieces of wood sawn from specimen blocks held in various xylaria, and (c) whole sections of tree stem. Almost all of the samples used in the study were of type 'a'.

Core samples were obtained from the trunks of trees, or in the case of those Callitris species with mallee (multistem) habit, from a major stem. A 5.15 mm core diameter, 300 mm length, three thread increment borer (Haglof model 63259) was used to obtain the cores. Each core was taken from a position on the trunk away from branchlets and bent portions of the stem in order to exclude knots or compression wood from the sample (Plate 2.1).

Small pieces of wood were obtained from xylaria located at The Australian National University Department of Forestry; the CSIRO Forestry and Forest Products Laboratory, Melbourne; the NSW Forestry Commission Laboratories, Sydney; and the Service des Forêts Patrimoine Naturel, Noumea, New Caledonia. Wood block samples had the advantage of being relatively large in size compared with cores, thus allowing greater ease of manipulation during cutting and also enabling a larger number of SEM specimens to be prepared (from which the better quality ones could be selected). However, wood block samples had the disadvantage of often consisting solely of heartwood, which was likely to be contaminated with extractives. Furthermore, wood block samples were not available for many of the less common, non-commercial timber species, or for any of the small, multi-stemmed shrub species; C. drummondii; C. canescens; C. monticola and C. roei.

Four sections cut directly across the tree stem were obtained from C. glaucophylla trees growing in NSW State Forests. Sections of stem were also obtained from dead or fallen trees when this was the only sample material available (several specimens of C. monticola were of this type). It was important, however, that 'dead' wood was not weathered. Sections were most useful when there was a need to be able to discriminate between juvenile and mature wood of the tree (as was involved in the 'within-tree' research described in Chapter 6).

2.2.2.2 Number of Samples

In his investigation of wood of the genus Callitris, Peirce (1937) used only one sample, obtained from a particular xylarium specimen block, for each of the species that he examined. Similarly, Greguss (1972) based many of his descriptions of individual Callitris species on only a single wood sample. Such small sample numbers are condemned by Jane (1970) who suggests that as many samples as possible should be used.
Obtaining multiple samples was, however, particularly difficult in the case of some *Callitris* species since many are uncommon, not commercially available as timber, and are found only in National Parks or State Forests where trees are protected by law.

A further problem in this regard was that not all wood samples formed useful SEM specimens due to the presence of spiral grain, knots, fungal infestation, extractive encrustation, and compression wood. Unfortunately these problems were often not apparent until the specimen had been prepared and viewed at high magnification by SEM. In order to compensate for such problems, additional wood samples often had to be obtained.

Owing to time and resource limitations, it was not possible to obtain more than four samples each of the New Caledonian species; *C. neocolaedonica*, and *C. sulcata*, five samples each of *C. baileyi*, *C. drummondii*, *C. gracilis*, *C. monticola*, *C. muelleri*, *C. roei*, and *C. tuberculata*, and seven samples of *C. columellaris*, and *C. murrayensis*. Eight or more samples of the remaining, more common, species were collected. In all, the collection comprised a total of 189 samples of *Callitris* species (Appendix 1).

### 2.2.2.3 Physical Dimensions of Samples

It was possible to obtain cores of either 5 mm or 10 mm diameter using commercially-available increment coring tools. Since the 5 mm diameter cores were of sufficient size for preparation of SEM specimens and produced less damage to the sampled tree than the 10 mm ones, most of the samples obtained from trees were of this size.

In view of the relatively small stem diameters of some species of *Callitris*, a short length of core sample was favoured. However, the physical size of wood samples had to be sufficient to enable preparation of at least one radial longitudinal section (RLS) and one tangential longitudinal section (TLS) specimen and, if possible, allow some additional material for losses incurred during SEM specimen preparation. In terms of 5 mm diameter cores, a one cm length of core was generally sufficient to produce the required specimens.

### 2.2.2.4 Sample Representativeness

Wood anatomy descriptions are usually based on mature wood from the main stems of trees since juvenile wood is not considered to be 'normal' for the species from a taxonomical point of view (Panshin & de Zeeuw 1980). It is commonly known that most branch wood is juvenile in character and often contains compression wood due to its horizontal growth habit (Zobel & Buijtenen 1989). For this reason, Jane (1970) recommends that branch wood should not be used in wood anatomy descriptions. With the minor exception of the study described in Chapter 6, and for which details of the interiors of stems was an objective, a wood sample of the genus *Diselma* for which it was not possible to obtain stem material (Chapter 8), and the experiments described in
Chapter 7 which involved juvenile wood from young trees grown in a glasshouse, only stem wood, from positions low in the trunks of trees of mature appearance, was used in this thesis. However, since core samples were acquired under field conditions, from trees of unknown age, growing wild in their natural environment, the possibility remains that samples, or parts of samples, were juvenile, rather than mature wood.

It was also preferable that the samples were taken from the sapwood and not from the heartwood of the tree, not only from the point of view of reducing the risk of micro-features being obscured by resinous extractives, but also because the sapwood was from the outer, more mature part of the tree. Thus sampling of sapwood reduced the risk of the inadvertent inclusion of juvenile wood in the sample. The outer-most part of the core was always the preferred part of the sample during specimen preparation.

Whenever possible, collection of specimens was carried out from trees growing in localities widely separated over the natural distribution range of the particular species. This was done in order to obtain a representative within-species sampling. For example, samples of *C. rhomboidea* were obtained from trees growing in the South Coast area of NSW as well as from five different locations in Tasmania. Similarly, samples of *C. canescens* were collected from two isolated populations, one occurring in South Australia and the other in Western Australia (Appendix 1). However, such diverse sampling was not always practical, particularly for some of the rarer species. For example, samples of *C. drummondii* could be obtained only from trees growing within a few meters of each other in one of the few remaining isolated populations of this species. Similarly all samples of *C. baileyi* were collected from trees growing within an area of a few hectares on a private property. Where the population was limited in number, samples were taken from trees of the most mature appearance, growing as far apart as possible.

**2.2.2.5 Sampling Restraints**

It was important that living trees suffered minimal damage as a result of the sampling of their wood. This was very important, since many of the species are uncommon, and are protected by law. Therefore to harm them would be not only undesirable from an ethical point of view, but would also be illegal. For this reason, trees could not be felled; samples had to be obtained by non-destructive means. Coring imparts minimal harm to the trees; this has been discussed by Swart (1980).

Coring was carried out mainly on trees growing on roadside verges in non-restricted areas. There were several occasions, however, when it was necessary to obtain permission to core trees growing on private properties, in protected areas and National Parks. This was found to be a very involved and time-consuming process and permission to obtain wood samples by any method likely to be more damaging to live trees, would have been extremely difficult.
Following the removal of each core from the tree, a short portion of fungicide-treated wooden dowel was hammered into the hole to plug it.

2.2.2.6 Sample Identification

Correct identification of species was a major concern. Extreme care was taken, not only to achieve the initial correct taxonomic identification of trees sampled in their natural habitat, but also to maintain positive identification of the samples, specimens and data throughout all phases of the study.

Taxonomic identification of sampled trees was achieved in a variety of ways. Firstly, wood samples were acquired only within areas of known geographical distribution for particular species, as detailed by various authors including Garden (1956), Thompson (1961), and Elliot & Jones (1980). Information obtained from the National Botanic Gardens Herbarium, Canberra (NBGH); the Western Australian Herbarium, and the Botany Branch of the Queensland Department of Primary Industries was also used to locate specimens of several of the less common *Callitris* species. Five species; *C. drummondii*, *C. canescens*, *C. roei*, *C. gracilis*, and *C. muelleri* were identified by sampling the small isolated populations at the precise location of voucher specimens previously collected by personnel from the NBGH.

Descriptions of *Callitris* cone and leaf morphology by Garden (1956), Thompson (1961), and Elliot & Jones (1980) were helpful in identifying the species of trees growing in the wild (Plate 2.2). It was, however, not always possible to sample cone-bearing trees, especially since some species (e.g. *C. columnellaris*) maintain their cones for only a few months of the year (Baird 1953). Herbarium material, in the form of cones and leaves was, whenever possible, obtained from each sampled tree and brought back for verification of taxonomic features (Plates 2.3 and 2.4). Such material was found to remain viable for several weeks when placed in small plastic bags located in a refrigerator. In addition, a number of voucher specimens were lodged with the NBGH. These are listed with their NBGH voucher number in Appendix 1.

Each wood sample was identified immediately on its removal from the tree or xylarium block by a four-letter identification code, and a sample number. For example, 'endl-2' was used to indicate specimen number two of the species *C. endlicheri*, and 'glau-5' the fifth sample of *C. glaucophylla*. This identification remained with the wood sample throughout specimen preparation, data gathering and data handling phases of the study.

2.2.2.7 Sources of Samples

Appendix 1 details the geographic locations of the sites from which all of the wood samples (cores) used in the thesis were obtained. Site information has been added, including mean annual rainfall (Bureau of Meteorology 1988) or Schmid (1995) [for the New Caledonian species] for the nearest climatological station to the site.
Plates 2.1-2.4: Wood Sampling

1 = Corer inserted into the trunk of a tree during the removal of a wood core.
2 = Cones and leaves of tree used for identification of species in the wild.
3 = Cone and leaf material from sampled tree of *C. gracilis* used for verification of species.
4 = Cone and leaf material from sampled tree of *C. oblonga* used for verification of species.
2.3 Wood Specimen Preparation for SEM

2.3.1 Introduction

Wood cores and off-cuts from xylarium sample blocks required appropriate preparation prior to observation by SEM. This section of the thesis describes the processes that were used to convert wood samples into SEM specimens.

Three basic preparation requirements were identified. Firstly, in view of the large number of samples to be prepared (189), a process which produced consistently useful results was essential. Secondly, since core samples provided only a limited amount of wood, the preparation procedure had to be efficient in terms of sample usage. Thirdly, each wood sample had to remain uniquely and appropriately identified throughout the preparatory process.

2.3.2 Considerations, Requirements and Limitations of the Preparation Process

The preparation of accurate planer wood surfaces on wood samples has been described by Greguss (1955), Jane (1970), Meylan & Butterfield (1972), Exley et al. (1974; 1977), and Kucera (1981; 1986). Both Greguss (1955) and Jane (1970) described the application of the sliding microtome (sledge) for producing cut surfaces suitable for LM. Methods of softening wood prior to cutting that were described by these authors included "boiling the wood in water for up to three days" (Greguss 1955); the use of steam; and, for very hard woods, soaking in hydrofluoric acid (Jane 1970). The preparation of wood specimens specifically for SEM was described by Exley et al. (1974), who recommended a 'free-hand' method of cutting specimens: i.e. both the specimen and the blade were held in the hands and the cutting action was made while viewing the specimens under a stereomicroscope. Exley et al. (1977) further developed the method of cutting to produce two prepared surfaces, angled at 45° to each other on the sample. This was to facilitate SEM viewing of two planes of wood at the same time. One of the cutting methods described by Kucera (1981), introduced the idea of using a clamp to hold the wood while it was cut with a hand-held blade (it was claimed that the clamp imposed less reliance on steady hands). Both Exley et al. (1977) and Kucera (1981) recommended the use of steel single-edged razor blades for cutting.

In deciding upon the most appropriate sample preparation method for this study, the following aspects were considered: Firstly, it was noted that all previous authors described methods suitable for samples consisting of relatively large, angular, blocks of wood, rather than small cylindrical samples, of 5 mm in diameter and 10 mm in length from an increment corer, the prime material for this study. For this reason, the sliding microtome (sledge), recommended by Jane (1970), was considered unsuitable, and

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1 For simplicity, hereafter in this thesis, use of the word "sample" will refer to wood prior to any form of preparation whereas wood during and after preparation for SEM will be referred to as a "specimen".
instead, the method of cutting of wood samples by use of a hand-held blade (Exley et al. 1974) was favoured. The cutting of multiple-faced specimens, recommended by Exley et al. (1977), was considered to be too difficult to achieve with consistent accuracy. In any case, the SEM used in this study had provision for the simultaneous insertion of eight specimens into the chamber, thus negating the need to use multiple-faced specimens as a means of saving time on repeated SEM chamber vacuum pump-downs. Instead, only single-faced specimens were used. Wood samples were clamped in a vice and cut using a hardbacked razor blade while viewing the process under a stereomicroscope. This is similar to the cutting method of Kucera (1981). The method of softening wood by boiling it in water (Greguss 1955; Meylan & Butterfield 1972) was considered likely to cause damage to the micro-structures involved in this study. An alternative wood-softening method involving soaking the wood in water at room temperature was substituted after substantial testing of its effects on the ease of cutting of Callitris wood and its (lack of) effect on the wood microstructure. It was found that all Callitris wood specimens, other than those freshly taken from a tree required softening before cutting. Dry or insufficiently softened wood specimens could not be accurately nor cleanly cut.

The specimen preparation requirements for SEM viewing of callitroid thickening (Chapter 3) the warty layer (Chapter 4) and rays (Chapter 5) were identical. Thus only one set of specimens from each wood sample was used for work outlined in Chapters 3, 4, and 5 and for some of that of Chapters 8 and 9. The minimum requirement was for one RLS-faced and one TLS-faced SEM specimen from each wood sample, although several specimens were usually produced and the most successfully-prepared sample, (in relation to the suitability of the cut surface) was chosen. SEM specimens cut in TS were used only for measurements of tracheid width size in growth rings (Chapters 6 and 9) and were not required for all wood samples.

RLS-faced and TLS-faced specimens had to be cut to expose the interior of the tracheid along their true plane, and the cut was required to be part-way into the cell wall so that structures on the opposing lumen wall were made visible but not damaged by the cutting process. It was particularly important that the surfaces were cleanly cut and not frayed by the action of the cutting blade. True-to-the-plane faces of the wood were preferred since oblique surfaces generally present a confused cell pattern to the viewer and are more difficult to interpret (Meylan & Butterfield 1978b). True plane surfaces in SEM images are also aesthetically more pleasing. Similarly, cleanly-cut surfaces generally present a more informative image, are more easily focussed, and are less likely to present artefacts.

Consideration was given to final preparation of wood surfaces by sanding them with fine sand paper. However, tests indicated that such treatment produced excessive scouring of the surfaces even when very fine paper (grit size 500) was used. In addition, the powdered wood produced by the polishing action tended to occlude
tracheid lumens and, in general, results were far less satisfactory than those produced by cutting with a blade. For these reasons, no images of sanded surfaces were used in the study.

The maximum size of prepared SEM samples was dictated by limitations imposed by the size of the SEM sample mounts (stubs), which had a diameter of 12 mm, by the size of the wood samples (cores 5 mm in diameter), and by the need to make efficient use of the available material. The face of most core specimens, after the necessary trimming, measured approximately 3 by 4 mm. A width of 3 mm and a length of 10 mm proved to be the most convenient size for accurate cutting of wood block specimens. Where multiple specimens of a sample were produced, two or three samples of this size could be accommodated on a standard SEM stub.

Some samples were found to contain resinous encrustations on their surfaces which inhibited detailed imaging of microstructures. Jurbergs (1965) referred to a similar problem in wood of slash pine (*Pinus elliottii* Engelm.) and reported that the encrustation 'appeared to be resin droplets which can be removed by hot alcohol extraction'. Although several chemical treatments of this kind were tested none were fully effective in removing encrustations from *Callitris*. Soaking the already-cut specimens in a 15% solution of sodium hypochlorite for twenty minutes (Meylan & Butterfield 1978b) and then washing in many changes of distilled water was the most effective method. Other treatments that were attempted but were less successful were; (a) Six hours of soaking the wood samples in ether; (b) Extraction with ether or a mixture of four parts acetone: one part ethanol: one part toluene in a Soxhlet apparatus for 48 hours; and (c) Soaking the sample in a potassium hydroxide (KOH) solution. In general it was found that encrustation was highly variable in its occurrence within tracheids, and that it was simpler to avoid encrusted areas and to use those less contaminated than to attempt to remove it. Selection of less contaminated areas for imaging was also preferable to chemical extraction in that it avoided the possibility of the treatment influencing the morphology of the micro-feature under investigation.

Specimens had to be inserted into the high vacuum chamber of the SEM without rapid and violent evaporation of moisture from the wood and consequent damage to the prepared surface. It was a basic requirement, therefore, that SEM specimens were dried after the cutting process. Three methods of drying biological specimens are commonly used in electron-microscopy: (i) dehydrating the specimen in a graduated series of alcohol followed by critical-point drying; (ii) freeze drying; and (iii) air-drying. A trial of all methods indicated no noticeable difference between them. Meylan & Butterfield (1978b) reached a similar conclusion in their SEM study of New Zealand woods. It was decided therefore, that the simplest method, air drying, would be used in the preparation of samples.

Since wood is a poor conductor of electricity, wood specimen surfaces had to be rendered fully conductive by coating them with a thin film of metal (gold) in order to
prevent 'charging' distortions of the SEM image, the various manifestations of which are well-known in the field of electron microscopy and have been discussed by Echlin (1981). The gold coating not only provided a conduction path to earth for beam electrons (thus preventing charging) but also had the effect of improving secondary electron emission (hence improving the signal-to-noise ratio) and assisted in conducting heat away from the imaged area (thus reducing electron beam damage).

After initial trials, a preparation sequence involving the following sequential operations: softening, cutting, cleaning, dehydrating, mounting, and coating was chosen as best meeting the above requirements. This sequence was used throughout the study and is discussed in detail below.

2.3.3 Wood Specimen Preparation Procedure

The methods used to prepare wood specimens for SEM are described in paragraphs 1-6 below:

1. Softening. Samples were soaked in distilled water contained in 5 mL plastic weighing cups until they became waterlogged and sank to the bottom of the cup. This process took approximately one to five days to complete, depending on the size of the sample and its condition. The water was changed every day during the soaking process, and the container was kept covered in order to avoid contamination by dust and fungal spores. Wood from xylarium block samples was usually very dry, and tended to float on the surface of the water. To counteract this, very dry samples were kept fully submerged in the water by means of a glass microscope slide laid across the top of the container (Plate 2.5). After soaking in this manner, samples were generally sufficiently soft to proceed to the next stage of specimen preparation; i.e. cutting.

2. Cutting. This was carried out using a method similar to that described by Kucera (1981), but modified to suit the small size of the core samples. The sample was clamped rigidly in a vice fitted into a circular depression at the base of a Nikon SMZ-2T stereo-microscope. Cutting was carried out using a hand-held, hard-backed, single-edged razor blade (Magnusun Injector blades type 500/1 890018).

The technique for cutting samples was as follows: A 3 mm section of the core (or for wood block samples, a piece measuring about 25 mm in the longitudinal axis and 5 mm square at the ends) was cut from the sample. The procedure then varied slightly according to which plane was required. For an RLS faced specimen, a split down the longitudinal axis of the core (parallel to the plane of weakness along the middle lamella) was made, and the face thus exposed was used for final surface preparation. A split of this type was not required for TLS samples which were cut across the already-exposed TLS face at the end of the core (Plate 2.6). The required face (RLS or TLS) was then clamped (by the end that was not going to be viewed) in the vice, with the required surface uppermost and raised above the clamping jaws by about 2 mm. The process was carried out while observing it using a stereomicroscope (Plate 2.7) at a
magnification of 10 to 60 times. The final surface was prepared by slicing a thin section off the exposed surface (Plate 2.8) using a new blade (or a portion of the blade not previously used) to form a flat and true plane. The cut was always made parallel to the longitudinal axis of the wood, since cutting at an angle across tracheids caused chaffing of the cut edges. Only a thin slice was taken off the surface of the sample in order to minimise distortion from compression caused by the blade. A useful indication of cutting in the true RLS plane was the length of rays across the face of the cut; true RLS planes contained rays that traversed the entire length of the exposed face. Finally the unwanted (clamped) part of the wood was trimmed away.

Use of wood samples with excessively sinuous or uneven grain reduced the ability to cut plane surfaces large enough to form useful SEM specimens. This type of sample was invariably discarded.

When cutting in the RLS plane, wood tended to split rather than to cut; the blade tended to take the weakest path along the middle lamella, between cells, instead of cutting through the cell wall. Surfaces formed in this way revealed only a flat cell wall and damaged pits, and the internal features of tracheids were not apparent. This problem was exacerbated by preparatory soaking of the wood samples in water. It was, therefore, necessary to soak wood samples for the absolute minimum period when preparing RLS specimens and to hold the blade at a slightly higher angle during cutting in order to reduce the chance of the wood splitting.

3. Cleaning. After cutting, cytoplasmic debris was removed from cut surfaces by washing the whole specimens in several changes of distilled water.

4. Dehydration. Immediately after cleaning, samples were placed with the prepared surface uppermost, on a filter paper at the base of a Petrie dish, kept covered, and allowed to dry at atmospheric pressure at 22 °C in a jar containing a desiccant (silica gel). After two days of drying, the samples were first mounted (Step 5) and then transferred into a vacuum chamber (Plate 2.10) and left for 8 hours at a vacuum of 10 Pa, for final dehydration.

5. Mounting. The clean-cut, dehydrated sample was glued, with the prepared surface uppermost, to a 12 mm aluminium specimen stub using finger-nail varnish as an adhesive (Plate 2.9). There was sufficient space for up to three specimens to be mounted on each SEM stub, but in order to reduce the chance of incorrect identification, only specimens from the same sample were mounted on any individual stub. All SEM specimen stubs were identified by an abbreviated identification code (e.g. 'endl-5' for C. endlicheri sample number 5) and, for convenience, with the appropriate wood plane (i.e. 'RLS', 'TLS' or 'TS') by labelling the underside of the stub with an indelible marker pen.

6. Coating. A 10 nm (i.e. 100 Å) coating of pure gold was applied to all prepared specimen surfaces. This was carried out in an argon gas sputter coating unit (Polaron Model E5000), (Plate 2.11) using a 20 mA ion current for approximately three minutes.
Plates 2.5 - 2.11: Equipment and materials used for the preparation of wood specimens:

5 = Softening: specimens are soaked in water contained in 5mL specimen cups. Microscope slides (76 x 26 mm) cover the cups.
6 = Cutting: softened core sample is cut in required section (RLS or TLS) by use of blade.
7 = Cutting: sample is clamped in a vice, secured to base of Nikon SMZ-2T stereomicroscope.
8 = Cutting: close-up view of cutting operation.
9 = Mounting: specimen is fixed to 12 mm SEM mounting stub using finger-nail varnish.
10 = Dehydration: vacuum chamber and pump used for dehydrating specimens.
11 = Coating: Polaron E5000 Sputter Coating Unit used for coating specimens with gold.
After coating, electrical contact between stub and specimen was enhanced by brushing silver conducting paint (Balzers 8010 14020) around the specimen-stub interface, well clear of the prepared surface.

2.4 SEM Imaging and Data Acquisition

2.4.1 Introduction

This section details general issues relating to the use of the SEM for the research, and describes some of the procedures and techniques used to produce images and acquire data. Only that which is common to the thesis as a whole is described here. More specific information is contained within the relevant chapters, elsewhere in the thesis.

2.4.2 Requirements and Considerations

The SEM imaging requirement was principally for the visualisation of the shapes and spatial distributions of callitroid thickening, warts, and rays in wood of Callitris. These features of interest were each of greatly different size; callitroid thickening bars being in the order of 20 µm in length and one µm in width, warts being typically 0.5 µm in height and width, and rays 20 to 500 µm in height, but consisting of individual cells, each approximately 20 µm in diameter. Thus SEM images needed to have useful resolution capability over a wide range of magnifications.

Individual warts of the warty layer were of the order of one µm in height. In order to produce an image of a single wart (~10⁻⁶ m), magnified to the full size of the SEM viewing screen (~10⁻¹ m) a magnification of about 10⁻¹/10⁻⁶ = 100,000 times was required. A magnification of this order, with accompanying high resolution, is at the limit of attainment by SEM and demanded optimal specimen preparation techniques and high-resolution operating procedures for the SEM. Complicating this situation was the fact that wood micro-anatomical features are composed of organic material, cellulose, hemicellulose and lignin, which can disintegrate in the heat produced by an SEM electron beam. This problem is compounded by the fact that in the SEM, magnification is achieved by scanning a small area of the sample and presenting the image as a (fixed-sized) larger area (i.e. the area of the viewing screen). It follows, therefore, that as magnification is increased, the power of the electron beam is concentrated into a smaller and smaller area on the specimen. For example, at a magnification of 10,000 times, the power that must be dissipated over the imaged area of the specimen is a thousand-fold more than that at a magnification of 10 times. Thus high magnification imaging can be tolerated by the specimen only if the beam current and/or accelerating voltage are reduced to produce a proportional decrease in electron beam power. However, the weakened-power images thus obtained are 'noisier' than images obtained at a higher electron beam power. Therefore an SEM image-
enhancement technique involving the integration of a number of identical real-time SEM images was necessary at high magnifications in order to provide improved signal-to-noise ratios.

Many thousands of measurements in the micrometre ($10^{-6}$ m) or nanometre ($10^{-9}$ m) range were required and this made it impractical to use the displayed SEM scale-bar as the primary measurement tool. It was therefore necessary to choose between measuring wood micro-features directly on the SEM screen, or transferring the SEM image to a computer, and making measurements using a commercially-available Image Analysis program such as 'Photoshop' (Version 2.5 Adobe Systems Inc.) or 'N.I.H. Image' (U.S. National Institute of Health). Initial tests of each method indicated that while the latter had the advantages of allowing automated recording of data measurements (thereby eliminating the need to transcribe each individual datum), measurements carried out on the SEM screen were more accurate, and allowed optimisation of conditions for each measurement. It was decided to carry-out all linear measuring operations directly on the SEM screen, and to digitise images for computer analysis only for the measurement of areas.

It was essential that the specimen morphology visible in the SEM image related only to that inherent in the specimen, and was not wholly, or partly, an artefact of the viewing process or of damage imparted by the electron beam. This called for SEM procedures to minimise charging and beam-damage artefacts.

Comparisons between micro-features was an essential part of the thesis, particularly in relation to inter-species taxonomy. It was, therefore, important to maintain consistency in the imaging process. In order to achieve this, certain SEM operating procedures were adopted as standard, and were used throughout all phases of the research. Micrographs of the images were usually taken at standard magnifications, to enable visual comparisons of features to be made.

In order to view features within tracheids, specimen manoeuvrability in the SEM was also an important requirement.

This study involved investigations of shape, size and occurrence of micro-features and it was not concerned with their chemical composition or internal structure. Therefore only 'secondary electron' imaging was required (and not other forms of electron microscopy such as TEM, backscattered electron microscopy or cathodoluminescence).

Four different types of SEM were available for use by this study: (i) a Hitachi S2250N; (ii) a JEOL J6400; (iii) a Cambridge Instruments S360 fitted with a high brightness lanthanum hexaboride (LaB$_6$) electron source; and (iv) a Hitachi S4500 field-emission SEM. Of these, the Cambridge Instruments S360 (Plate 2.12), was chosen as the most suitable SEM for the required tasks since it was able to produce the required high resolution images, it had an on-screen point-to-point measuring system,
built-in image-enhancement software, and allowed free manoeuvrability of the specimen.

2.4.3 SEM Procedures and Techniques

Because of the heterogeneous nature of wood specimens and the resultant inconsistency in SEM viewing requirements, it is not practical to detail all SEM operating parameters used in this study. In general, however, each SEM image represented a compromise between the required resolution and depth of field, which in turn are related to the working distance (i.e. the distance between the final lens and the specimen). A short working distance results in high resolution but low depth of field, whereas a long working distance results in a low resolution but high depth of field. Thus, any improvement produced in one of these parameters results in a deterioration in the other. Similarly, a compromise was necessary with regard to electron beam current; a lowered beam current resulted in higher resolution, but 'noisier' images. Thus beam currents were usually set at the lowest value consistent with an acceptable level of image noise.

The general requirements for imaging were achieved by using SEM operational parameters as follows: For high magnification (i.e. greater than 10,000 times) images of relatively small features, or for producing high definition micrographs, a short working distance (6 mm) was used and the electron beam diameter was reduced (10 to 20 pA beam current). For the lower magnification (100 to 10,000 times) images used for data acquisition, a working distance of 18 mm was used and the beam current was set to 100 pA. An electron accelerating voltage (EHT) of 15 kV and the use of a 30 µm final aperture was standard for all magnifications. These SEM operating parameters were based only on experience, and variations from them were sometimes necessary in order to compensate for the variability of wood specimens.

In order to reduce electron beam-induced artefacts in images, such as burning, cracking, and vapour-contamination of surfaces at high magnification, focusing was carried out on an area immediately adjacent to the required structure and the sample moved into position just prior to producing the final image. This procedure was particularly important for the acquisition of artefact-free micrographs at high magnification.

A very dramatic improvement in the depth of field of tilted (i.e. angled) specimens could be gained by use of a 'Dynamic Focus' function. In order to use this facility, the angle of tilt of the specimen surface was entered into the microprocessor-controlled operating system of the SEM. The focussing of the image of the specimen, throughout the complete image scan and for all distances of the sample from the condensing lens was then automatically carried out by the SEM lens control system. This resulted in focussed images over the whole of the image scan, rather than on only a single area at a fixed distance from the lens.
The 'beam-blanking function' (i.e. deliberately driving the beam fully off the electron optical axis) was used to reduce charging and deterioration of the wood specimen while the measurement processes were being carried out on the SEM screen. As soon as the composite image was formed and electronically 'frozen' on the screen, 'beam-blanking' was selected so that the specimen was not subjected to the damaging effect of the beam during the taking of the measurements. This had no effect on either the (frozen) image on the screen or the measuring process. On completion of measurements, beam-blanking was de-selected, and normal imaging resumed on the sample which, because of the repression of the beam by the beam-blanking function, had not deteriorated from its condition prior to the measurement procedure. This was particularly useful in the case of features on the borders of the image, which could only be viewed by moving the sample to bring the borderline features fully into view.

2.4.4 Data Acquisition

A point-to-point measuring facility, built into the SEM, was the prime means of measurement of anatomical features of interest. The measurement facility consisted of two cursors which could each be independently moved to any point on the image displayed on the SEM screen (28 cm x 21 cm) while a digital read-out of the distance between them, in nanometres, was displayed on-screen (Plate 2.13). The readout was continuously updated (at TV rate) for cursor position changes and variation in magnification. It enabled much more rapid and accurate measurements between points on the image than could be achieved by referring to the scale bar on micrographs. The measurement system was checked regularly using two laser-inscribed standards to ensure that accuracy was maintained at all magnifications.

Where information on the frequency or density of a feature was required, data were acquired by counts of the feature, carried out at a standard magnification (thus over a known area of sample).

The requirement for all wood specimens to remain individually identified throughout the SEM viewing process was achieved by applying the sample identification code to each SEM image. A scale-bar, and where applicable, details of special preparation treatment of specimens, plane of wood (TLS or RLS), or growth-ring information were also applied. These image annotations were retained on micrographs of images.

In order to reduce the chances of inadvertently missing the features of interest or counting them twice, an electronic graticule was used to divide the SEM screen into four sections (Plate 2.13). The sub-divided screen areas were viewed consecutively in order to reduce the area that was under observation at any particular time. As a further precaution, features were measured on screen from left to right and from top to bottom in an orderly fashion.
Plate 2.12: The Cambridge Instruments S360 Scanning Electron Microscope used for this study.

Plate 2.13: Image on the SEM screen (28 cm x 21 cm) during a point-to-point measurement operation. At the extreme top of the screen is specimen identification information and a 1 μm scale bar. The screen is divided by an electronic graticule into four quadrants. In the top left quadrant, two cursors (+), which can be individually moved to any position on screen, have been manoeuvred to indicate the top and bottom of a wart. The distance between the cursors (the height of the wart) is indicated in the bottom right-hand corner (422 nm).
Micrographs of selected SEM images were recorded on Kodak 220 Plus X Pan film using a Leica camera back. Stereo-pair micrographs, using $6^\circ$ of specimen tilt difference between images, were produced for selected SEM scans.

Digitised SEM images were used where there was a need to measure areas of wood features. They were formed by converting SEM images into 'TIFF' (Tagged Image File Format) files by use of an SEM image digitiser (ImageSlave, Meeco Holdings Pty Ltd, Sydney) installed on an IBM PC-AT computer. The digitised images were composed of 1024 x 768 pixels of 8 bits each (256 grey levels). Each image required 790 kilobytes of digital storage space.

2.5 Statistical Analysis of Data

2.5.1 Introduction

The data acquisition phases of the research produced a large amount of qualitative and quantitative data representing counts of the occurrence, and measurements of the dimensions, of different microscopic wood features. The processing of these data mainly involved extracting ranges and means (with confidence limits); analyses of variance, and correlations. A range of appropriate statistical techniques were required. The statistical analyses of quantitative wood anatomy data has been discussed by several accounts in the literature including Rendle & Clarke (1934); Burley & Miller (1982) and Ilic & Miller (1994).

2.5.2 Statistical Analysis Requirements and Data Representativeness

It was realised that variation in callitroid thickening, wart, and ray morphology in *Callitris* would be attributable to genetic, environmental and sampling factors. Four levels of variability in wood feature morphology of *Callitris* were anticipated: (i) between-species, (ii) within-species, (iii) within individual trees, and (iv) the individual feature level:

(i) Between-species differences would reflect genetically-determined variation of features in *Callitris*. Since it would be necessary to obtain the wood by random sampling of trees, the data obtained for analysis at this level would also be likely to contain inherent sampling effects.

(ii) At the within-species level, data from wood samples grown in different areas of the natural distribution of a particular species were likely to reflect the environmental variability between individual habitats. In addition, within-species genetic variability between populations and within individual trees as well as random sampling effects would be likely to occur at this level.

(iii) At the individual tree level, data on anatomical features would reflect inherent differences between the planes of the wood (i.e. TLS, RLS and TS) and also differences
between the inner parts of the trunk (juvenile wood) and the outer parts (mature wood). There would also be likely differences between wood from different areas within individual growth rings. Most morphological variability in this category could be distinguished and separated as required by using appropriate sample preparation or specimen imaging techniques. Thus, as noted above, the appropriate plane of wood was cut during sample preparation and viewed during imaging, wood sampling was carried out in a manner selecting only mature wood and avoiding juvenile wood, and information regarding the different areas within growth rings could be selected during imaging.

(iv) The individual feature level would reflect the inherent genetic and environmentally-produced variability of features.

It was realised that analysis of data describing wood feature morphology between samples would result in heterogeneity determined, to a greater or lesser extent, by a combination of all of these individual influences, and in some cases it would not be possible to separate them. Nevertheless it would be necessary for the data to be representative of the appropriate level of consideration for which it was being used.

Variability at the between-species level was of interest from the point of view of its use for the taxonomic separation of species. For this reason, there was a need to know the extent and the representativeness of between-species variability seen in the morphology of wood features within the samples. The determination of within-species variability was, in general, not an aim of the thesis. However, its presence, and an indication of the magnitude of its effects, would be apparent particularly in the differences between mean values of quantitative data for samples and in the sizes of the accompanying confidence intervals.

Where it was necessary to determine within-plane differences in wood features, specimens were prepared accordingly (i.e. cut and viewed in the particular plane of interest). Similarly, earlywood-latewood differences could be determined by comparing data from specific areas within growth rings, as carried out in Chapter 6.

2.5.3 Data Handling and Analytical Methods

Means with 95% confidence intervals were commonly used throughout this thesis to quantitatively describe and compare the variability of wood features. Data were transcribed from their original (hand-written) form onto computer program spreadsheets (Excel 4 for Macintosh, Microsoft Corp.) where data were stored, and could be wholly, or in part, analysed. Certain basic analytical tools, made available within the spreadsheet program, were used for preliminary examination of the data. In some cases, the standard deviation of data was proportional to the mean, indicating log-normally distributed data. Where this was detected, the log-normally distributed data were first changed to logarithmic form by converting each relevant cell of data on the spreadsheet to an equivalent (natural) logarithm value, using Excel's 'LN' function. The logarithmic
data could then be treated as being 'nornally' distributed and means and confidence limits could be formed of this converted data. Back-transforms, from natural logarithm to original, could also be carried out if required using the 'EXP' function. Log-normally distributed data that had been converted to \(\log_e\) form was usually plotted with two Y-axis scales, the scale on the left indicating \(\log_e\), and the scale on the right indicating the back-transformed values.

In cases where data distributions were neither normally nor log-normally distributed, data were first aggregated to species level by calculating means for each specimen and then an analysis of variance (ANOVA) was used to provide estimates of species means and appropriate standard errors. In this way, irrespective of the underlying distribution of the raw data, the specimen means (in accordance with the Central Limit Theorem\(^2\)) were rendered towards normality. This and other more sophisticated analyses of data, particularly regression analysis, canonical variate analysis, analysis of true/false type data, or information derived from models based on raw data, were carried out by the Statistical Consulting Unit at The Australian National University using the Genstat 5 (Release 3.1) program (Lowes Agricultural Trust of the Rothamstead Experimental Station).

Bar charts, box graphs and scatter diagrams are the means of communicating the information produced as a result of statistical analysis. Most graphs were generated by use of the program 'Kaleidagraph' (Version 3.02 Abelbeck Software).

A commonly repeated requirement of the study was to present salient features of the distribution of 50 or more data values of a particular anatomical parameter for each of the 20 species of *Callitris*. Rather than use 20 individual frequency histograms to show these distributions, box graphs were used because 20 boxes (each individual box representing one of each of the species of *Callitris*) placed adjacent to one another in a single graph, constituted a reasonably neat and concise indication of the variability in the distribution of the parameter for all of the different species. Cleveland (1985) states that the strength of the box graph presentation lies in the following three factors: (1) the ability to allow percentiles to be compared effectively; (2) they can be used even when the number of distributions is not small; and (3) by graphing the large and small values, unusual values are not neglected as they often are when a summary of the distribution consists of a sample mean and the sample standard deviation. The latter factor was considered to be particularly important since in this study, the maximum values of several of the parameters (e.g. maximum ray height, and maximum tracheid widths)

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\(^{2}\) The Central Limit Theorem states: "If random samples of \(n\) observations are drawn from a population with finite mean \(\mu\) and standard deviation \(\sigma\), then, when \(n\) is large, the sampling distribution of the sample mean \(\bar{y}\) will be approximately a normal distribution with mean equal to \(\mu\) and standard deviation \(\sigma/\sqrt{n}\). The approximation will become more and more accurate as \(n\) becomes larger and larger" (Mendenhall 1988).
were sometimes of taxonomic interest, equally as much as the mean and median values. All box graphs in this thesis are of the following form. The line inside the box shows the median value of the variable represented by the box. The top and bottom of the box mark the upper and lower quartiles respectively. The lines extending from the top and bottom of the box (whiskers) indicate 1.5 times the inter-quartile range for the edge of the box back to the nearest data point (i.e. the maximum and minimum of the 'usual' data points). Outlying (unusual) data values are plotted as small open circles.

**Callitrold Thickening**

In this Chapter, the occurrence, morphology, frequency, and visual prominence of callitrold thickening is described for all (20) species of *Callitrodox*, homotypic differences in these parameters are assessed, and findings are compared with reports in the literature. The anatomical, histological, and statistical significances of callitrold thickening are also discussed.
Chapter 3

Callitroid Thickening

In this Chapter, the occurrence, morphology, frequency, and visual prominence of callitroid thickening is described for all (20) species of *Callitris*. Inter-species differences in these parameters are assessed, and findings are compared with reports in the literature. The anatomical, taxonomic, and ecological significances of callitroid thickening are also discussed.
3.1 Introduction

3.1.1 Background

Callitroid (or callitrisoid) thickening is the term given to raised bars of tissue situated above and below individual pit apertures in softwood tracheids (Patton 1927; Cronshaw 1961; IAWA Committee on Nomenclature 1964). The bars extend across the cell wall in RLS, normal to the tracheid axis, delimiting a rectangular area with the pit aperture at its centre (Cronshaw 1961). In TLS, the thickenings arch out over the pit aperture into the lumen forming 'awns' (Patton 1927; IAWA Committee on Nomenclature 1964).

The literature indicates that callitroid thickening is regularly developed only in the genus *Callitris* (Phillips 1948; Jane 1970). Similar thickening bars have been reported in 'occasional specimens' of related genera, e.g. *Juniperus* and *Pseudolarix*, and sometimes more distantly related genera such as *Pinus*, but these occurrences are regarded as "rare and inconspicuous" (Phillips 1948). Because of this, callitroid thickening is thought to be the most important identifying diagnostic feature for distinguishing wood of *Callitris* from that of other genera, and use is made of this feature in the taxonomic keys of Peirce (1937), Barefoot & Hankins (1982), and Wheeler et al. (1985).

However, reports in the literature indicate that callitroid thickening does not occur in some species of *Callitris*, and its absence from the wood of *C. intratropica*, *C. macleayana*, *C. muelleri*, *C. oblonga*, *C. rhomboidea*, and *C. sulcata* has been reported by Patton (1927), Budkevich (1936), Phillips (1948), and Greguss (1955; 1972). As a result, callitroid thickening can be used as the defining factor in the formation of a dichotomy of taxa within the genus; one group consisting of species in which thickening occurs, the other group consisting of species in which thickening is reported to be absent. This method of taxonomically differentiating groups of *Callitris* species has been used by Patton (1927) and also Greguss (1955; 1972).

Additionally, in those species in which callitroid thickening is reported to be present, not all pits have associated thickening bars: i.e., the frequency of thickening varies (Patton 1927; Greguss 1972). Three groups of *Callitris* species were differentiated from each other on the basis of the frequency of their thickening by Venning (1979).

The value of callitroid thickening as a taxonomic indicator either of genus or of species is, however, severely diminished by omissions and inconsistencies in the literature. Firstly, two *Callitris* species; *C. monticola* and *C. neocaledonica* have received no mention in previous reports as to whether or not they possess thickening. Similarly, as a result of the many taxonomic and nomenclatural changes within the genus that have occurred in the past, the presence or absence of thickening in the recently defined species is also uncertain. Many of the previous investigations are based on grossly limited sample numbers, while other studies used twig or branch material which is normally considered to be unrepresentative of the wood (mature wood from the trunk is more
favoured). There is also disagreement between authors as to the presence or absence of thickening in several of the species. Finally, although callitroid thickening is reported to vary in its frequency and visual prominence, these parameters have been described in the literature in only a very brief and subjective manner.

As a result of the inconsistencies between previous studies, and the incomplete or unsatisfactory nature of many of them, the diagnostic value of callitroid thickening is curtailed, and a wide-ranging study of this feature in all taxa of the genus would be of benefit from both an anatomical and a taxonomical point of view.

3.1.2 Literature Review of Callitroid Thickening

The first reference to callitroid thickening in the literature was made by Kleeberg (1885). He described the thickening bars as "kleine horizontale Leistchen" (translated from German: 'small horizontal ledges') at the tops and bottoms of pits in *Frenela* (*Frenela* is a synonym of *Callitris*). Kleeberg's pioneering description was overlooked by some later authors. For example, Venning (1979) states that callitroid thickening was first described by Patton (1927). Similarly, no reference is made to Kleeberg by Patton (1927), Dadswell & Eckersley (1935) or Peirce (1937), and although Greguss (1955) refers to callitroid thickening as "bars of Kleeberg", in apparent acknowledgment of the initial description of this feature, Kleeberg's paper is not included in his references.

Patton (1927) included three diagrams with his descriptions of callitroid thickening, copies of which are reproduced below:

*Diagram A:* "In radial section each bordered pit is associated with a more or less rectangular plate of thickening extending across the tracheide [sic] beyond the margins of the pit, but not reaching to the upper or lower margin". *Diagram B:* "in tangential section these bars appear as projecting awns". *Diagram C:* "bands of thickening found on ray cells, connecting medullary rays with the tracheides".

Patton (1927) described callitroid thickening as "structures, very definite and characteristic" and commented:-

"This character, therefore, serves as a useful guide in the classification of the genus."
In his 'Systematic Anatomy Key to the Woods of the Cupressaceae', Peirce (1937) used the presence of callitroid thickening, which he termed; "secondary thickening across the tracheid pit-borders", as being taxonomically indicative of wood of *Callitris* and separating it from wood of *Tetraclinis*, for which he described "bands of secondary thickening absent". The use of callitroid thickening as an indicator of the genus *Callitris* has since become well established, and has been used in taxonomic keys as a discriminating factor for the identification of *Callitris* from softwoods of other genera for a number of decades (Phillips 1948; Jane 1970; Barefoot & Hankins 1982; Wheeler et al. 1985).

On a within-genus basis, reports of callitroid thickening by Kleeberg (1885) and Patton (1927) indicated that it was absent from some species of *Callitris*. Patton (1927) wrote:

"The thickening band is absent from *C. muelleri*, *C. oblonga*, *C. macleayana*, *C. rhomboidea* and *C. tasmanica."

Budkevich (1936) (in Russian), like Patton (1927) found thickening present in *C. robusta* R.Br. (synonym for *C. verrucosa*) and absent in *C. oblonga*.

Some of the photographs of the general wood anatomy of *Callitris* by Baker & Smith (1910) show callitroid thickening to be present in *C. glaucophylla*, [i.e. in Figures 83 and 86 of *C. glauca* (synonym of *C. glaucophylla*)] and *C. endlicheri*, [Figures 137 and 138 of *C. calcarata* (synonym of *C. endlicheri*)] although the authors make no direct reference to it in their text. However, in the case of *C. macleayana* the observation is made that:

"The bordered pits occur on the radial walls and in transverse section are less prominent (in section) than in other species, the free edges scarcely protruding into the lumina of the tracheids."

This reference by Baker & Smith (1910) to 'less prominent' pits in *C. macleayana* suggests that the authors may have been inadvertently referring to the lack of visual enhancement of pits by callitroid thickening in this particular species compared to other *Callitris* species. This possibility is supported by the reports of several later authors; Patton (1927), Phillips (1948), and Greguss (1955; 1972), all of whom claim that *C. macleayana* has 'no callitroid thickening bars'.

Greguss (1955) expanded the species listing further and suggested that the genus *Callitris* could be divided into two groups of taxa on the basis of presence or absence of 'bars of Kleeberg' [callitroid thickening]. In the group with callitroid thickening present were placed *C. glauca* R.Br. (synonym for *C. glaucophylla*), *C. robusta* R. Br (synonym for *C. verrucosa*), and *C. verrucosa* R. Br. In the second group of species (those with callitroid thickening reported absent) were: *C. intratropica* Benth. et Hook., *C. oblonga*, *C. cupressiformis* Vent. (synonym of *C. rhomboidea*), and *C. sulcata*. 
Table 3.1 summarises literature reports regarding the occurrence of callitroid thickening in *Callitris*. Various authors have indicated thickening to be present in the following six species: *C. baileyi*, *C. canescens*, *C. columellaris*, *C. drummondii*, *C. endlicheri*, and *C. glaucophylla*. Species in which thickening is reported to be absent are: *C. muelleri*, *C. oblonga*, *C. rhomboidea*, *C. roei*, and *C. sulcata*. Some omissions and anomalies are evident in the listing. Firstly, *C. intratropica* Benth. *et* Hook. is recorded as having thickening present by Phillips (1948), yet Greguss (1955) records it absent. A second anomaly involves *C. macleayana* in which callitroid thickening is reported absent by all literature reports except that of Venning (1979), who reports it present. The presence or absence of callitroid thickening in the species which are currently termed: *C. gracilis*, *C. murrayensis*, *C. preissii*, *C. tuberculata*, and *C. verrucosa* is unknown since these species have not been reported upon under their current taxonomic representation. Finally, *C. monticola* and *C. neocaledonica* have not previously received any mention in the literature as to whether they possess thickening.

In species in which callitroid thickening occurs, bars are not necessarily present on all of the pits. Variation is reported to exist in the frequency of thickening, and on the basis of this, Venning (1979) was able to classify callitroid thickening in *Callitris* species into three grades of occurrence frequency: (1) common, (2) scarce, and (3) absent. The species classified by Venning (1979) as "commonly having thickening" were: *C. columellaris*, *C. drummondii*, *C. endlicheri*, *C. glaucophylla*, and *C. verrucosa*. In the group with 'scarce' callitroid thickening were: *C. baileyi*, and *C. macleayana*. Venning’s third group, in which thickening was absent, was comprised of *C. muelleri*, *C. oblonga*, *C. rhomboidea* and *C. roei*. Other reports on variation in the frequency of occurrence of callitroid thickening tend to be sketchy and subjective. Thus *C. glaucophylla* is said to have all pits thickened (Patton 1927), while in *C. intratropica* and *C. columellaris* thickened pits are "less frequent" (Phillips 1948) or "sparsely present" (Patton 1927) and the frequency of thickening in *C. canescens* is reported to be "very rare" (Greguss 1972).

Callitroid thickening is also known to vary in its visual prominence or 'strength'. In some species the bars are reported to be "well developed" (Phillips 1948) and "clearly visible" (Greguss 1972) while in other species, thickening is said to be "less strongly developed" (Phillips 1948).

Several authors have examined the morphology of callitroid thickening and from their findings were able to comment upon its structure and formation. Cronshaw (1961) examined *C. glaucophylla* using TEM, and reported that thickening bars and awns were "attached along their length to the radial walls". Wardrop (1964) observed that warts were present on the thickening bars as well as the remainder of the tracheid walls, and concluded from this that the bars were elaborations of the S₃ layer.
Table 3.1: Table showing the occurrence of callitroid thickening in species of *Callitris*, based on five reports: Patton (1927); Phillips (1948); Greguss (1955); Greguss (1972); and Venning (1979). Reports of the presence of callitroid thickening in *C. preissii* Miq. (Greguss 1972); *C. propinquus* R.Br. (Patton 1927); *C. robusta* R.Br. (Patton 1927; Greguss 1955); and *C. verrucosa* R. Br. (Patton 1927; Venning 1979), are not included in the table due to inability to correspond these synonyms with their present species categorisation.
Davis & Ingle (1966) accounted for the formation of callitroid thickening as "extensive eccentric growth of the S3 layer" and reported that whereas the S3 layer normally consists of much less than 30 lamellae, compared to the S2 of 30-150 lamellae, in callitroid thickenings the S3 layer was about twice the thickness of the S2 layer. A 'tapering-off' in the thickness of bars at the tangential wall, as viewed in RLS, is reported by Davis & Ingle (1966) and also by Venning (1979). Davis & Ingle (1966) also reported that callitroid thickening bars tended to be "closer to one another" where they merged with the tangential wall and Kleeberg (1885) described bars as "bow-shaped".

Although callitroid thickening conventionally involves only the inter-tracheid pits, 'callitroid-like' thickening of the crossfield pits between rays and tracheids has been reported in C. verrucosa (under its synonym of C. robusta) by Patton (1927), in C. endlicheri (under its synonym of C. calcarata) by Peirce (1937) and in C. endlicheri and C. glaucophylla by Ilic (1994). Howard & Manwiller (1969) also observed "callitroid-like thickenings on pits connecting longitudinal tracheids with rays" in several species of the southern hard pines (Pinus spp.). However, Venning (1979) reported that no crossfield pit thickening was found in any of the wood samples that she examined.

The terms 'callitroid' thickening and 'callitrisoid' thickening have both been used in the literature to describe the bars present on pits of Callitris. Phillips (1948), Greguss (1955), Cronshaw (1961), Wardrop (1964), Davis & Ingle (1966), Jane (1970), Venning (1979), and Ilic (1994) used the term 'callitroid' thickening, whereas the term 'callitrisoid' thickening was favoured by IAWA Committee on Nomenclature (1964), Meylan & Butterfield (1972), Greguss (1972), Panshin & de Zeeuw (1980), and Wheeler et al. (1985).

The functional or ecological significance of callitroid thickening has not been commented upon in the literature.

3.1.3 Considerations, Objectives, Requirements, and Aims of this Chapter

It was the intention of this Chapter to report on a wide-ranging investigation of callitroid thickening in all species of Callitris. To this effect, four primary objectives were identified:

i) Since the literature indicates that callitroid thickening is present in some species of Callitris, absent in others, and has not been reported upon in some species, an objective was to determine which species had, and which species did not have, callitroid thickening. This called for a comprehensive survey of pits in samples of all species of Callitris, and the recording of the occurrence of thickening.

ii) In those species in which callitroid thickening is reported to be present, the literature indicates that not all pits have associated thickening bars and that the frequency of thickening varies according to species. A quantitative study of the
frequency of callitroid thickening, by comparison of counts of numbers of pits with associated thickening, and pits without thickening, was therefore also an objective.

iii) Variation in the visual prominence of thickening between species of Callitris has previously been commented upon in the literature, but only in a subjective manner. Another objective was, therefore, to quantify the visual prominence of thickening in each Callitris species.

iv) The structural morphology of callitroid thickening has been described previously in the literature in only basic terms, e.g. as 'pairs of thickening bars' and as 'awns'. The fourth objective of this study was to quantify the three-dimensional shape and size of thickening bars, and to determine their between-species and within-species variation (if any). This called for measurement of the physical dimensions of bars; their heights (protrusion into the lumen), their angles to the cell wall, and the distances of separation between the individual bars of each pit.

It was decided that the first three objectives would best be met by using specimens cut in RLS since the literature refers to 'bars' rather than to 'awns' with respect to occurrence, frequency, and visual prominence of thickening, even though bars and awns are simply different manifestations of the same thing. However, the fourth objective necessitated the use of both RLS and TLS specimens since measurements of both bars and awns were required. Combination of the data acquisition tasks for the first and second objectives into a single operation (i.e. the recording of the absence or presence of thickening on all visible pits on each sample) was undertaken.

A comprehensive quantitative study of crossfield pit thickening was not one of the primary objectives of this study. Its presence or absence, and its general morphological characteristics were, however, noted in the course of imaging of all specimens in order to be able to determine qualitatively, the extent of its occurrence and its taxonomic relevance within the genus.

Thus the aims of the study reported on in this Chapter were threefold: (i) to determine the occurrence of callitroid thickening in all species of Callitris; (ii) to describe quantitatively its frequency, visual prominence, and structural morphology within individual species; and (iii) based on the findings, to suggest the taxonomic, and ecological significances of thickening.

3.2 Materials and Methods

3.2.1 Sampling and Preparation

A total of 176 samples, ranging from four each of C. neocaledonica and C. sulcata to 21 samples of C. rhomboidea were used.

TLS and RLS specimens were prepared for SEM viewing using the methods described in Chapter 2.
3.2.2 SEM Viewing and Data Acquisition Procedures

Samples were mechanically rotated within the SEM chamber so that the longitudinal axes of tracheids were always orientated vertically on the screen. This standardisation of orientation facilitated comparison of images.

In order to allow for inherent variability within samples, an effort was made to view as much of the specimen surface as possible and not just the pits within a particular area. This was carried out by viewing the specimen surface (approximately 3 mm wide and 4 mm in length) in a series of ordered traverses from left to right and from top to bottom of the sample and recording the occurrence or absence of thickening on all visible pits. This ensured that data were averaged over the whole specimen, and included pits in both the earlywood and the latewood. It also reduced the possibility of pits being inadvertently counted or measured more than once. Similarly, the sizes of thickening bars were measured in lateral traverses of the samples, but for only one pit per tracheid, and on only one lateral traverse of each sample.

Care was taken to measure only thickening associated with tracheid-to-tracheid bordered pits and not the thickening of crossfield pits between tracheids and rays.

3.3 Results

3.3.1 Occurrence of Thickening

With the exception of six (out of ten) samples of *C. macleayana*, and two (out of four) samples of *C. neocaledonica*, thickening of one or more pits was observed in all 176 samples examined in the study. Plates 3.1-3.24 show micrographs of callitroid thickening manifested as bars (in RLS) or as awns (in TLS) for all species of *Callitris*. Thus callitroid thickening occurred\(^1\) throughout the genus, including those species in which it had previously been reported to be absent (*C. macleayana*, *C. muelleri*, *C. oblonga*, *C. rhomboidea*, *C. roei*, and *C. sulcata*), in those species not previously reported upon in the literature (*C. monticola* and *C. neocaledonica*) and also in the species in which the presence or absence of thickening was uncertain due to recent changes in the taxonomy of *Callitris* (*C. gracilis*, *C. murrayensis*, *C. preissii*, *C. tuberculata*, and *C. verrucosa*). Thickening was also confirmed as present in all species in which it had previously been commented upon in the literature (*C. baileyi*, *C. canescens*, *C. columellaris*, *C. drummondii*, *C. endlicheri*, *C. glaucophylla*, and *C. intratropica*).

Inter-tracheid pits on TLS surfaces were not common in any *Callitris* species, but when they occurred, callitroid thickening was sometimes present (Plate 3.25).

\(^1\) Note that the term 'thickening occurred' is used here to mean that thickening was present on at least some of the pits observed in the multiple samples of each species, and the term is not intended to indicate that thickening was present on all observed pits of all samples of any of the species.
Chapter 3 Callitroid Thickening

Plates 3.1-3.8: Callitroid thickening in *Callitris* species, viewed at various magnifications.

1 = *C. baileyi*; 2 = *C. baileyi* (TLS view); 3 = *C. canescens*; 4 = *C. columellaris*;
5 = *C. drummondii*; 6 = *C. endlicheri*; 7 = *C. glaucophylla*;
8 = *C. glaucophylla* (TLS view).
Plates 3.9-3.16: Callitroid thickening in *Callitris* species, viewed at various magnifications.

9 = *C. gracilis*; 10 = *C. intratropica*; 11 = *C. macleayana*; 12 = *C. monticola*;
13 = *C. muelleri*; 14 = *C. muelleri* (TLS view); 15 = *C. murrayensis*;
16 = *C. neocaledonica*. 
Plates 3.17-3.24: Callitroid thickening in *Callitris* species, viewed at various magnifications.

17 = *C. oblonga*; 18 = *C. preissii*; 19 = *C. rhomboidea*;
20 = *C. rhomboidea* (TLS view); 21 = *C. roei*; 22 = *C. sulcata*; 23 = *C. tuberculata*;
24 = *C. verrucosa*. 
3.3.2 Frequency of Thickening

Callitroid thickening was sometimes universally present on all of the pits visible on the prepared surface of an individual specimen (Plate 3.26) but, as already mentioned, it was absent from eight samples of *C. macleayana* and *C. neocaledonica* (Plate 3.27). However, in general, the proportions of pits with thickening and those with no thickening differed between species, and there was also variability between samples of particular species. Between species, the frequency of callitroid thickening ranged from being present on less than one percent of pits, for *C. macleayana* and *C. neocaledonica*, to 98 percent in *C. glaucophylla* and *C. verrucosa*. In order to determine the probability of thickening being present in the various species, a record was kept of the number of 'thickened and non-thickened pits' in each specimen, and the resulting data were analysed using a logit regression model. From this model, the probability of thickening occurring in each species of *Callitris* was determined (Figure 3.1); it can be seen that several groups of *Callitris* species can be separated on this basis.

![Figure 3.1: Probability of callitroid thickening in species of Callitris. Vertical lines represent 95% confidence intervals.](image)

Those with high probability of thickening (> 0.8) were *C. canescens*, *C. endlicheri*, *C. glaucophylla*, *C. gracilis*, *C. tuberculata* and *C. verrucosa*. Species with moderate probability (0.5 to 0.8) were *C. drummondii*, *C. murrayensis*, *C. preissii* and *C. roei*. The species: *C. baileyi*, *C. columellaris*, *C. intratropica*, *C. monticola*, *C. muelleri*, and *C. rhomboidea* had a probability of 0.1 to 0.4 and the remaining species with the
lowest probability (< 0.1) were *C. macleayana*, *C. neocaledonica*, *C. oblonga*, and *C. sulcata*. Between-sample variation in frequency of thickening is reflected in the confidence intervals associated with this predicted probability. Confidence intervals are not symmetrical about the means because of the non-linear transformation of the probability.

Callitroid thickening tended to occur more often in narrow tracheids than in wide ones. This effect was particularly noticeable at latewood-earlywood boundaries where tracheids of greatly different widths occurred adjacent to each other (Plate 3.28). Callitroid thickening rarely occurred in the widest tracheids of any species. A logit regression model of the effect of tracheid width on the probability of thickening was formed from data acquired by measuring tracheid widths at the locations of pits and noting the presence, or absence, of thickening. The regressions clearly show the relationship between the probability of thickening and tracheid width in *Callitris* species (Figure 3.2).

![Figure 3.2: Predicted probability of the presence of callitroid thickening in tracheids of various widths in species of *Callitris*. The species, in numerical sequence are as follows: 1 = baileyi; 2 = canescens; 3 = columellaris; 4 = drummondii; 5 = endlicheri 6 = glaucophylla; 7 = intratropica; 8 = macleayana; 9 = monticola; 10 = muelleri 11 = murrayensis; 12 = neocaledonica; 13 = oblonga; 14 = preissii; 15 = rhomboidea; 16 = roei; 17 = sulcata; 18 = tuberculata; 19 = verrucosa](image-url)
It can be seen that *C. endlicheri* (5), *C. glaucophylla* (6), *C. murrayensis* (11), *C. tuberculata* (18), and *C. verrucosa* (19) maintained a high probability of thickening in all but their widest tracheids. Thickening in *C. monticola* (9), *C. muelleri* (10), *C. neocaledonica* (12), and *C. oblonga* (13) occurred only in the very narrowest tracheids and the probability of thickening occurring decreased to zero in wider tracheids. In the remaining species, *C. baileyi* (1), *C. canescens* (2), *C. columnellaris* (3), *C. drummondii* (4), *C. preissii* (14), *C. rhomboidea* (15) and *C. roei* (16) the probability of thickening was high in the narrow tracheids and low in wide tracheids. *Callitris gracilis* is not included in Figure 3.2 due to the late acquisition of samples (and hence relevant data) for this species. In general, callitroid thickening did not occur in the widest tracheids of any species, and the maximum tracheid width in which thickening of pits occurred, varied with species. Despite this overall variability within samples, thickening of pits was usually consistent throughout the visible length of individual tracheids.

### 3.3.3 Thickening Morphology

SEM imagery of pits with callitroid thickening revealed that there was both quantitative and qualitative variation in the morphology of thickening, and that this variability occurred throughout the genus *Callitris*. Quantitative variation occurred in the sizes and shapes of thickening bars. Qualitative variation of thickening morphology occurred in relation to the number of bars associated with individual pits. It was possible to classify thickening into four basic 'types' on the basis of number of thickening bars observed, and these were designated as 'Types 1, 2, 3, and 4' (Figure 3.3). The frequency of each basic type of thickening varied between specimens and between species.

#### 3.3.3.1 The Four Basic Types of Thickening Morphology

In 'Type 1' thickening, either one or two bars occurred in association with the pit, but either one bar or both bars did not extend fully across the tracheid lumen (as viewed in RLS). There were several variations on the basic form of Type 1 thickening. Bars were sometimes attached to one side wall but did not extend fully across to the other wall (Plate 3.29); sometimes bars were present only in the region near the pit aperture and did not extend to either wall (Plate 3.30); and sometimes one bar extended fully across the lumen, but the other bar was either entirely lacking (Plate 3.31) or did not extend fully across the lumen (Plate 3.32). Typically, the bars of Type 1 thickening were relatively thin, and visually indistinct compared to the three other types of thickening (below). In TLS, one or two weak awns were sometimes observed in association with a pit (Plate 3.33).
Figure 3.3: The four different 'types' of callitroid thickening.
In 'Type 2' thickening, two bars were present both of which extended completely across the lumen in the RLS (Plate 3.34). In TLS, two awns, one above and one below the aperture were apparent (Plates 3.35, 3.57 and 3.59). Type 2 thickening bars were usually visually distinct in species in which thickening was common but they tended to be consistently weak in species in which thickening was uncommon.

In 'Type 3' thickening, single bars occurred both above and below the pit aperture, but in addition, a third bar crossed over the centre of the pit aperture parallel to the upper and lower bars (Plate 3.37). The third bar was sometimes incomplete in the region of the pit aperture (Plate 3.38). All three bars were usually of similar thickness and height, and were visually distinct. In TLS, three awns were apparent for this type of thickening (Plate 3.39).

'Type 4' thickening consisted of two pairs of bars associated with individual pits. The second pair of bars was located outside and parallel to those adjacent to the pit aperture so that there were two bars above, and two bars below the aperture (Plates 3.40 and 3.58). The outer pair of bars was always thinner and visually indistinct compared to the inner pair. Occasionally the outer pair of bars did not fully extend across the lumen whereas the inner pair of bars were always visually distinct and extended across the complete lumen width. The second set of bars tended to be separated from the first pair by a similar distance to that separating the inner bars. In TLS, the four bars were sometimes apparent as four awns (Plate 3.41). In a variation of this type of thickening, four bars were associated with each pit but the outer pair of bars were joined with the inner ones by branching connections (Plate 3.42).

Deviations from these four standard types of thickening were rare. However, forms of thickening with more than four bars were occasionally noted (Plate 3.43) In some of the wider tracheids in which biseriate pitting occurred, thickening bars were sometimes shared across both pits (Plate 3.44) and occasionally a single bar extended from one side wall to the other wall, angled between pit apertures (Plate 3.45).

A heterogeneous combination of types, together with pits having no associated thickening, was sometimes found within individual specimens (Plate 3.46).

### 3.3.3.2 Proportions of the Four Basic Types of Thickening Within Species

Pits with one of the four basic types of thickening, and pits with no associated callitroid thickening, were present to varying degrees in samples of most species. In order to determine the proportions of each type of thickening, RLS specimens were imaged at approximately 1000 times magnification and the types of thickening associated with all visible pits on the surface of the specimen (typically 100 to 500 pits) were noted. This was carried out for all specimens of all species of *Callitris* and the findings are shown in Table 3.2. It can be seen that, proportionally, Type 1 thickening accounted for almost half (i.e. 49.9%) of all callitroid thickening occurring...
within the genus. The proportion of Type 2 thickening was 47.7%, and Types 3 and 4 formed only small percentages (0.8% and 1.6% respectively) of total thickening.

<table>
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<th>2 Type 2</th>
<th>3 Type 3</th>
<th>4 Type 4</th>
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Table 3.2: Proportions of thickening Types 1, 2, 3, and 4, as percentages of total thickening, in species of Callitris. Columns 1-4 indicate percentages of thickening Types 1 to 4 respectively. Column 5 shows the number of pits, and column 6 the number of specimens, upon which the data is based. The bottom row of the table shows the mean percentage of each type of thickening.

However, these proportions varied greatly between species and an ANOVA of the data confirmed that the distribution of thickening types differed significantly (p < 0.001) between species. Table 3.2 shows that Type 1 thickening tended to be the most prevalent type of thickening in C. columnellaris, C. intratropica, C. macleayana, C. monticola, C. muelleri, C. neocaledonica, C. oblonga, C. rhomboidea and C. sulcata. Type 2 thickening was common in C. drummondii, C. endlicheri, C. glaucophylla, C. gracilis, C. murrayensis, C. tuberculata, and C. verrucosa. Types 3 and 4 thickening tended to occur in species in which Type 2 thickening was more common. Type 4 thickening formed a relatively high proportion of the total thickening in those species with a high probability of thickening, i.e. C. glaucophylla (11.1%) and C. verrucosa (8.1%). However, within samples, thickening was never all of one type and a heterogeneous association of the various thickening types, including pits with no thickening, was usually evident.

Figure 3.4 shows the probability of thickening Type 2 occurring in all pits with thickening (i.e. the probability of occurrence of Type 2 thickening in relation to all other types of thickening) in all Callitris species. It can be seen that species with high
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The probability of Type 2 thickening were *C. drummondii, C. endlicheri, C. glaucophylla, C. gracilis, C. murrayensis, C. tuberculata*, and *C. verrucosa*. Thus, the probability of Type 1 thickening in these species is very low, particularly in the two species which have high proportions of thickening types 3 and 4 (*C. glaucophylla* and *C. verrucosa*). Figure 3.4 also shows that species with low probability of Type 2 thickening were *C. columellaris, C. intratropica, C. macleayana, C. neocaledonica*, and *C. sulcata*. The large confidence intervals for *C. macleayana, C. neocaledonica*, and *C. sulcata* are an indication of the low levels of thickening of any type in these species.

![Figure 3.4: Bar chart showing approximate probability of occurrence of Type 2 thickening (in all pits with thickening) in species of Callitris. 95% confidence intervals are indicated.](image)

3.3.3.3 A Quantitative Study of Thickening Morphology

In order to quantify the morphology of each of the four types of callitroid thickening, the following six parameters were measured: (1) the widths of individual bars; (2) the heights of bars (i.e. the distance of protrusion of awns into the lumen space); (3) bar angles (in relation to the surface of the tracheid inner wall); (4) lumen widths (i.e. distances across the lumen at points directly straddling the pits); (5) the distance of separation between pairs of bars at their centres and at their left and right extremities and (6) the thicknesses of the 'upper' and 'lower' bars at their centres and at their left and right extremities (as seen in RLS). The 'type' of thickening associated with each pit was also recorded. The points of measurement of these parameters are depicted in Figure 3.5. Thickening bar width, height and angle were measured (from awns) in TLS. The lumen width measurements were used only as a reference for relating the other
parameters to lumen width. The angle of thickening bars (to the wall of the tracheid) was measured by using a protractor held against the TLS image on the SEM screen. Thickening bar lengths, bar separations and bar thicknesses were measured in RLS. Means and standard deviations of these measurements were calculated.

Figure 3.5: Measurement parameters of callitroid thickening morphology.

Callitroid thickening bars tended to be rectangular in cross section (Plate 3.47), with a mean height of 1.8 µm (s.d. = 0.8, n = 302) and width of 1.2 µm (s.d. = 0.5; n = 302). There was species variation in bar size: in *C. glaucophylla* mean bar height and width were 2.3 and 1.3 µm respectively whereas in *C. macleayana* the equivalent parameters were 0.9 and 0.8 µm. Bar widths varied to some extent with 'type' of thickening. Type 1 bars were the narrowest, with an average width of 1.4 µm (s.d. = 0.5, n = 153) while Type 4 bars tended to be the widest; their mean width being 2.0 µm (s.d. = 0.7, n = 213). The bars were angled inwards towards the pit aperture with an average angle of 71°.

The distance between thickening bars was generally greater in wide tracheids than in narrow ones. The average distance separating type 2 thickening bars was 6.3 µm (s.d. = 1.58, n = 865). The two outer bars of Type 3 thickening were spaced 11.7 µm apart (s.d. = 2.18, n = 147). In general, bars curved around the pit aperture so that they were closer together at their ends than at their centres. The effect, which has been commented upon previously (Davis & Ingle 1966), was more pronounced in species possessing wide tracheids (e.g. *C. columellaris*, *C. intratropica*, and *C. murrayensis*). In very wide tracheids the ends of bars were often closer together than the diameter of the pit aperture so that the overall shape of the pair of bars when viewed in RLS was 'elliptical' (Plate 3.48).
Plates 3.25-3.32: Callitroid thickening in *Callitris* species viewed at various magnifications:

25 = Callitroid thickening of pits on TLS surfaces.
26 = Presence of thickening on all visible pits in this specimen of *C. endlicheri*.
27 = Thickening absent from all visible pits in this specimen of *C. macleayana*.
28 = Reduced occurrence of thickening in wide tracheids.
29-32 = Various forms of Type 1 callitroid thickening.
Plates 3.33-3.40: Callitroid thickening in *Callitris* species viewed at various magnifications:

33 = Type 1 callitroid thickening (TLS view). 34 = Type 2 callitroid thickening.
35 = Type 2 callitroid thickening (TLS view). 36 = Type 2 callitroid thickening viewed
at 55°. 37 = Type 3 callitroid thickening. 38 = Type 3 callitroid thickening with
incomplete centre bar.
39 = Type 3 callitroid thickening (TLS view). 40 = Type 4 callitroid thickening.
Plates 3.41-3.48: Callitroid thickening in *Callitris* species viewed at various magnifications:

41 = Type 4 callitroid thickening (TLS view).
42 = Type 4 callitroid thickening with 'branched' bars.
43 = Callitroid thickening with multiple bars.
44 - 45 = Forms of thickening associated with biseriate pits.
46 = Types 2, 3, and 4 thickening occurring in close proximity in a specimen.
47 = Thickening bars rectangular in cross-section. 48 = Elliptical shape of thickening.
Measurement of the thickness of bars at their centres and at their left and right extremities (carried out in RLS) indicated that bars tended to be of constant thickness throughout their length.

3.3.3.4 The Morphology of Awns

The depth of field of the SEM was sufficient for awns to be seen (in TLS) as both the cut sections, as well as the portions of the bars which usually extended onto the TLS lumen surface (Plate 3.49). Occasionally, awns from a single pit extended across the full width of the lumen wall, and anastomosing occurred between awns of pits on opposite sides of the lumen; the upper awn of one pit joining with the lower awn of the adjacent pit, as shown in Plate 3.50.

A warty layer usually covered the outer surface area of awns, but warts were usually reduced in size on the sides adjacent to the pit aperture (Plate 3.51).

3.3.4 Visual Prominence of Thickening

Callitroid thickening varied in its visual prominence and individual pits in all species could be subjectively classified as having either visually indistinct (Plate 3.52) or distinct (Plate 3.53) thickening. Thickening in C. canescens, C. columellaris, C. drummondii, C. endlicheri, C. glaucophylla, C. murrayensis, C. preissii, C. roei, C. tuberculata, and C. verrucosa was almost always distinct whereas thickening in C. baileyi, C. intratropica, C. macleayana, C. monticola, C. muelleri, C. neocaledonica, C. oblonga, C. rhomboidea, and C. sulcata was usually indistinct.

Four factors likely to influence visual prominence of callitroid thickening were identified. Firstly, distinct thickening bars tended to be taller (i.e. were raised higher above the tertiary wall layer) compared with indistinct ones. Secondly, the angle to which bars were inclined to the tracheid wall was usually closer to a right angle in distinct thickening. The bars of distinct thickening also tended to be thick whereas bars of indistinct thickening were usually thin. Finally, bars of distinct thickening (Plate 3.53) usually extended completely across the tracheid lumen (i.e. was of type 2, 3, or 4) compared to indistinct thickening in which the bars were generally of 'Type 1' (Plate 3.52). Bar thickness and bar length had greater effect on visual prominence than bar height and bar angle.

The visual distinctiveness of callitroid thickening, based on canonical variate analysis of bar thickness and bar length, was modelled. Data from this model is plotted in Figure 3.6. It can be seen that thickening was most indistinct in C. macleayana, C. neocaledonica, and C. sulcata, and that species with the most distinct thickening were C. glaucophylla, C. tuberculata and C. verrucosa. Species with indistinct thickening tended to have larger confidence intervals indicating that the distinctiveness of their thickening was more variable than that in other species.
3.3.5 Crossfield pit Thickening

Crossfield pit thickening was present to some extent in all species of Callitris, but was more common, and more distinct, in those species which had a high occurrence of bordered pit thickening (i.e. in C. canescens, C. columellaris, C. drummondii, C. endlicheri, C. glaucophylla, C. murrayensis, C. preissii, C. roei, C. tuberculata, and C. verrucosa). Plate 3.54 shows typical crossfield pit thickening in RLS, and Plate 3.55 gives a TLS view. Crossfield pit thickening occurred more often where rays crossed narrow tracheids than where they crossed wide tracheids (Plate 3.56). Crossfield pit thickening occurred only on the tracheid side of crossfield pits (not on the ray side). Owing to its more concentrated spatial arrangement, thickening of crossfield pits was sometimes more distinct than thickening of bordered pits, especially in species with a low frequency of bordered pit callitroid thickening.
Plates 3.49-3.56: Callitroid thickening in *Callitris* species viewed at various magnifications:

49 = Tracheids viewed longitudinally at 55° to show thickening bars extending onto TLS lumen wall. 50 = Anastomosing of adjacent awns.

51 = Warts covering surface of thickening bar. 52 = Indistinct thickening.

53 = Distinct thickening. 54 = Crossfield pit thickening.

55 = Crossfield pit thickening (TLS view).

56 = Comparison of crossfield pit thickening in earlywood and latewood.
Plate 3.57 (Stereo pair) Aspirated pit pair with callitroid thickening (TLS).

Plate 3.58 (Stereo pair) RLS view of callitroid thickening.

Plate 3.59 (Stereo pair) Callitroid thickening (awns) viewed in TLS.
3.4 Discussion

3.4.1 Comparison with the Literature

There are two major differences between the findings of this study and previous accounts of callitroid thickening in the literature. Firstly, callitroid thickening was shown to occur to some degree in all species of *Callitris* whereas previous reports (Patton 1927; Phillips 1948; Greguss 1955; Venning 1979) indicated that it was absent in certain species (Table 3.1). Secondly, whereas in all previous reports callitroid thickening was described as two (full) bars, this study showed that two bar (Type 2 thickening) accounted for less than half (47.7%) of total thickening (Table 3.2) and 53.3% of callitroid thickening involved three or four thickening bars associated with the each pit (i.e. Types 3 and 4 thickening) or bars only partially extending across the RLS lumen wall (i.e. Type 1 thickening).

In reference to the first finding, the question arises as to why callitroid thickening was found to occur in all species here, whereas previous studies reported it to be absent in several of the *Callitris* species. The following factors are of importance in distinguishing callitroid thickening:

i) Thickening may have been consistently reported to be absent in some species because previous studies used light microscopy, which has inferior resolution to the SEM used here. This lack of resolution could have been particularly significant in relation to the reporting of 'type 1' thickening which is less distinct than the other types of thickening, but constitutes a significant proportion (i.e. 49.9%) of callitroid thickening. The fact that thickening of Type 1 was not previously reported in the literature also supports this contention. In order to further investigate this possibility, wood sections of each species were prepared for LM (see Chapter 9) and examined for callitroid thickening under the light microscope. Thickening was observed by LM in *C. canescens*, *C. drummondii*, *C. endlicheri*, *C. glaucophylla*, *C. gracilis*, *C. intratropica*, *C. monticola*, *C. murrayensis*, *C. preissii*, *C. rhomboidea*, *C. roei*, *C. tuberculata*, and *C. verrucosa*. No thickening was found in *C. baileyi*, *C. columnellaris*, *C. macleayana*, *C. muelleri*, *C. neocaledonica*, *C. oblonga*, and *C. sulcata*. Thus, in reference to Figure 3.6, light microscopy appears to be ineffective in detecting thickening at approximately -6 on the 'index of visual prominence' scale; species with an index lower (i.e. more negative) than -6 (i.e. *C. macleayana*, *C. muelleri*, *C. neocaledonica*, *C. oblonga*, and *C. sulcata*) are those for which thickening was not detected by LM. Most of these species are also those reported to be 'without thickening' in the literature (*C. neocaledonica* had not previously been examined). Species with an index of visual prominence higher (more positive) than -6 include all those for which thickening has been previously reported. Thus it is feasible that there is a limit below which callitroid thickening is not readily observable by light microscopy, and that this 'visibility limit' occurs at approximately -6 on the 'index of visual prominence' scale (Figure 3.6). This limit does not apply to SEM.
ii) Low frequency of thickening; the number of samples should be sufficient for a thickened pit to be encountered. For example, in the case of *C. macleayana*, this study found thickening in fewer than half the samples examined (i.e. thickening occurred in only four out of ten samples). Thus if only one sample was examined then, based on the frequency of thickening found in *C. macleayana* in this study, then it is quite likely that no thickening would be observed.

iii) The number of pits viewed within individual samples; in this study, only one in 200 pits in *C. macleayana* was found to be thickened. It follows, therefore, that large numbers of pits would need to be viewed in order to record thickening in this (and several other) species.

iv) Representativeness of the wood samples; twig and branch material is not fully representative of the true wood anatomy of a species, and it invariably contains compression wood. Previous studies often used such material, whereas in this study, only stem wood was used. Thus, for example, while *C. roei* was found to have abundant and distinct thickening here, it was also noticeable that compression wood of this species possessed no callitroid thickening. It therefore seems possible that non-representative juvenile wood or the presence of compression wood in the twig wood material examined by Venning (1979) was the reason why she failed to find callitroid thickening in *C. roei*. Similarly, in the study of *C. morrisonii* (synonym of *C. canescens*) by Greguss (1955) in which the sample material was "a single bough 1 cm thick", callitroid thickening was reported to be "very rare", whereas in this Chapter, in which ten samples of main stem wood were used, thickening was found to occur in 86% of pits.

v) Within-sample variability; this study found callitroid thickening to be more frequent in narrow than in wide tracheids (Figure 3.2). It is possible that previous light microscope studies of callitroid thickening may not have been sufficiently attentive to very narrow tracheids. This may be the reason why Phillips (1948) found callitroid thickening in *C. intratropica* but Greguss (1955) failed to detect it. However, it is not likely to be responsible for the failure of Patton (1927) to detect thickening in *C. muelleri* and *C. rhomboidea*, and for Venning (1979) to report thickening "absent" in *C. roei* since these species have thickening in their (relatively) 'wide' tracheids as well as their 'narrow' ones.

The second major difference between reports in the literature and the findings here relates to the morphology of thickening. This study showed that two bar (Type 2) thickening (the only style referred to in the literature) was not the only type. It accounted for only approximately half of all thickening found (Table 3.2) and a significant proportion of callitroid thickening involved one, three, or four bars associated with each pit. The question therefore arises as to how these features were missed by previous studies. Firstly, thickening of Type 1 may not have been previously described because of the restricted resolution of light microscopy used in...
past studies, particularly since thickening Type 1 is usually indistinct. Secondly, the second pair of bars (furthest out from the pit aperture) that are present in Type 4 callitroid thickening, are always more indistinct than the inner pair. This indistinct nature of such thickening may have been sufficient to prevent them from being noticed during previous studies which relied upon light microscopy.

The claim that bars tend to taper off in thickness towards the tangential wall (Davis & Ingle 1966) was not supported by the findings of this study; bars tended to be of equal thickness throughout their length. The observation of Panshin & de Zeeuw (1980) that bars are slightly curved into "parenthesis shape" was, however, confirmed; pairs of bars were usually closer together at their ends than at their centres. This effect was much more noticeable in wide tracheids than in narrow ones.

A warty layer was observed on the thickening bars, including those of Type 1, 3 and 4 (Plates 3.29, 3.37 and 3.40 respectively). This suggests that bars in all four types of thickening are elaborations of the S3 layer, as has previously been suggested (for Type 2 thickening only) by Cronshaw (1961) and Wardrop (1964).

3.4.2 Taxonomic and Nomenclatorial Implications

Some revision of the comparative and systematic wood anatomy of *Callitris* is necessary as a result of findings reported in this Chapter. Firstly, since callitroid thickening has been shown to be universal in all species of *Callitris*, the method of differentiating groups of taxa on the basis of 'occurrence' or 'non-occurrence' of callitroid thickening in their wood, as suggested by Greguss (1955), can no longer be valid when using SEM. Instead, a proportionate scale of the 'frequency' of thickening (i.e. the proportion of pits with and without thickening) would be more useful as a means of identifying *Callitris* species.

Two findings relating to the systematic wood anatomy of *Callitris* can also be suggested: firstly, when searching for thickening in species in which the frequency of its occurrence is very low (e.g. in *C. macleayana, C. neocaledonica, C. oblonga,* and *C. sulcata*), advantage should be taken of the finding that callitroid thickening occurs more often in narrow tracheids than in the wide ones. Similarly, this study has pointed out the need for comprehensive examination of pits in order to locate Type 1 thickening.

It is also now known that callitroid thickening can involve one, two, three, or four bars, and pits with bars extending only partially across the width of the lumen. This fact has not been previously reported in the literature, even though the occurrence of such thickening is not uncommon (for example Type 4 thickening represents 1.4% of thickening occurrences). Thus the description of the basic structure of callitroid thickening as "a pair of bars crossing the pit horizontally above and below the pit aperture" (Ilic 1994) and as being "pairs of bars" extending across the (full) width of the tracheid lumen (IAWA Committee on Nomenclature 1964), needs to be re-examined in the light of the results of this Chapter. Similarly, it can now be reported...
that the outer bars of 'Type 3' and 'Type 4' thickening are situated above and below the pit border, and are not 'within the area of the circular border around the pit' as was previously suggested for 'Type 2' thickening by Patton (1927) and Cronshaw (1961).

The quantitative investigation of the 'visual prominence' of callitroid thickening indicated that this parameter was likely to be relevant when using LM for the identification of several *Callitris* species which have a history of commercial use: *C. endlicheri* and *C. glaucophylla* (having distinct thickening) and *C. intratropica*, *C. macleayana* and *C. sulcata* (which have indistinct thickening).

The finding that callitroid thickening is unlikely to be detected in *C. baileyi*, *C. columellaris*, *C. macleayana*, *C. muelleri*, *C. oblonga* and *C. sulcata* by LM, but can be detected by SEM, also indicates a need for a modified taxonomic approach when using SEM.

Callitroid thickening of crossfield pits was found to occur in all species of *Callitris* and, like bordered pit thickening, was more prevalent in species from dry habitats than those endemic to wet habitats. A general observation, that was made during this study, was that the thickening bars of crossfield pits, being more concentrated, were often more distinct and easier to locate than inter-tracheid bordered pit thickening, particularly when the general level of callitroid thickening was low. It is therefore suggested that crossfield pit thickening may be a useful taxonomic indicator in such situations.

### 3.4.3 Ecological Significance

The ecological significance of callitroid thickening, and its adaptive value to the living tree has not previously been discussed in the literature, and is, therefore, still a matter of conjecture. A clue to its functional role may lie in the finding here, that callitroid thickening is more prevalent in *Callitris* species native to dry habitats than in those from wet environments. This is readily apparent in Figure 3.1. It can be seen that species with low probability of thickening: *C. macleayana*, *C. oblonga*, *C. neocaledonica*, and *C. sulcata* are those native to high rainfall habitats (or in the case of *C. oblonga*, from Tasmanian river flood plains). Species with high probability of thickening *C. endlicheri*, *C. glaucophylla*, *C. tuberculata*, and *C. verrucosa* are endemic to much drier areas of inland Australia. This suggests, therefore, that the presence of callitroid thickening is of some adaptive significance to trees growing in arid environments where water availability may limit growth.

A hypothesis for the functional significance of callitroid thickening is suggested, in which the bars act to strengthen tracheid walls against physiologically adverse conditions brought on by acute shortage of water (or air embolism) within tracheids. In this hypothesis, callitroid thickening bars act as bracing supports on either side of pit apertures to prevent collapse of the walls of tracheids in their region of perforation, where they are likely to be weakest. A similar function has been suggested for
trabeculae in softwoods (Grosser 1986); for helical thickening (Carlquist 1988); and for thickening of pits in the transfusion tracheids of leaves of Cupressaceae (Gadek & Quinn 1988). In this hypothesis, Type 3 and Type 4 callitroid thickening would provide increased support in the form of three and four bars respectively. The finding that thickening is more prevalent in dry habitat species where the chances of embolism are greater, where narrow tracheids must withstand stronger capillary pressures than in wide tracheids, and where pit apertures in narrow tracheids represent a relatively larger proportion of the cell wall that is weakened than in wide tracheids, (assuming that pit apertures are of equal size in both narrow and wide tracheids) provides further support for this hypothesis.
Chapter 4

The Warty Layer

This Chapter describes the morphology of the warty layer on the inner walls of tracheids in Callitris. The shapes and sizes of individual warts in all of the 20 species of the genus are determined, detailed quantitative descriptions are produced, and inter-species comparisons made. Anatomical, taxonomic and ecological implications of the variability in wart morphology are discussed.
4.1 Introduction

4.1.1. Background

The warty layer can be described as an amorphous, membrane-like layer of material (Parham & Baird 1974) which lines the internal surfaces of mature longitudinal tracheids of many softwoods (Liese 1957), the vessel elements and fibres of hardwood species (Baird et al. 1974a) and the interiors of pit chambers (Robards 1965). Surface and cross-sectional views of the layer show it to consist of encrusted globules which protrude from the cell wall, giving the appearance of 'warts' (Parham & Baird 1974).

The small size of individual warts of the warty layer places them close to, or slightly below, the resolution threshold of the light microscope (Jurbergs 1965). Although an early wood anatomy study of a seraya (Parashorea plicata Brandis) using light microscopy (Bailey 1933) commented on the "mats of fine texture filling entire pit cavities and projecting into the lumen of the cell", the actual wart-like morphology of the layer was not reported until 1951, after TEM studies by Kobayashi & Utsumi (1951) and by Liese (1951). Thus, as pointed out by Liese (1965a) the warty layer is "one of the very few basic wood cell wall components, the existence of which has been established only by electron microscopy".

The universality of the warty layer in wood is now well known (Harada et al. 1958; Ohtani 1979). Of several hundred softwood and hardwood species from about 160 families that were investigated by Liese (1965a) it was found that most had a distinct warty layer, and the presence of warts has now been documented for essentially all conifers (Harada & Côté 1985). The nature of warts in softwoods has been reviewed by Harada (1953) and by Liese (1965a) and in hardwoods by Ohtani (1979) and Ohtani et al. (1983). Warts have also been observed in several bamboo species (Parameswaran & Liese 1977). The warty layer is found in earlywood and latewood, sapwood and heartwood, stem, branch-wood, and roots of trees and also in the vascular bundles of certain herbaceous plants (Liese 1965a). Because of this universality, the warty layer can be regarded as a general morphological feature of woody plant cells (Liese 1965a).

The warty layer of *Callitris* is distinctive in having numerous, bigger warts (Liese 1957; 1965a). This large size makes them easier to see, and was the reason for the interest in *Callitris* and its close relative *Actinostrobus*, which also has large warts, during early studies of the warty layer using transmission electron microscopy (TEM) and metal-shadowing techniques (Liese & Johann 1954; Liese 1957; Wardrop & Davies 1962). Since the warty layer is a distinctive feature of *Callitris* and no comprehensive study of its occurrence and form has ever been undertaken, it was considered important to examine the morphology of the warty layer of all species in the genus. Recent technological improvements to SEM resolving power were particularly important in the decision to undertake this study since, in accordance with the taxonomic aims of this
thesis (Chapter 1), examination of the morphology of warts at resolutions hitherto unattainable would be necessary in order to distinguish differences (if any) between warts of individual *Callitris* species.

### 4.1.2 Literature Review

#### 4.1.2.1 Wart and Warty Layer Morphology

In softwoods, the morphology of individual warts of the warty layer has been variously described as "spherical" (Cronshaw 1965; Jurbergs 1965; Liese 1965a), "rounded cone" (Harada & Côté 1985), "conical" (Robards 1965; Ohtani & Fujikawa 1971), and "complex" (Ohtani & Fujikawa 1971).

Warts in hardwoods are sometimes termed 'vestures'. Ohtani et al. (1984) reported that there is no difference between vestures and warts in either morphology, origins or chemical composition, and suggested that the terms 'warts' and 'warty layer' should be abandoned and replaced by the terms 'vestures' and 'vestured layer'. Ohtani (1994) termed vestures as being "larger, more complex warts".

A study of warts (vestures) in vessel elements and fibres of 203 New Zealand hardwood species by Ohtani et al. (1983) concluded that warts could be classified on the basis of shape into two main types: unbranched and branched. The unbranched warts were described as hemispherical, conical, capillary, or rod-like and were found in all the species possessing warts, whereas the authors found complicated branched warts in only some of the warted species, and where they did occur, all intermediates in shape and size between simple unbranched warts and complicated branched ones were found.

Wart 'size' has been reported as a basal diameter measurement: 0.05 µm to 0.5 µm (Jurbergs 1965; Liese 1965a; Robards 1965; Harada & Côté 1985) and also as a height measurement (i.e. depth of protrusion into the lumen): 0.1 µm to 1.0 µm (Liese 1965a). In a study by Liese (1965a) warts were reported to be "never homogeneous in size and when big warts occurred, then numerous smaller ones were also present".

The frequency of warts is reported to vary between neighbouring cells and can range from "a densely packed arrangement, where one wart is situated beside the next, to such a sparsity that the cell wall seems to be almost without warts" (Liese 1965a). In some cells "the underlying S₃ is clearly visible; in others, the warts completely mask the S₃ layer" (Koch 1972).

The distribution of warts is mostly regular and is independent of the frequency. Local crowding of warts is rare (Liese 1965a). Kuo & Manwiller (1986) found that the distribution patterns of warts closely followed the micro-fibrillar orientation of the S₃ layer. They suggested that warts were localised thickenings of the ends of micro-fibrillar bundles.
4.1.2.2, Functional, Taxonomic and Phylogenetic Aspects of Warts

Little has been written regarding the significance of the warty layer to the function of the living tree. It seems likely however, that the warty layer acts as a terminal lamella of the cell wall and as such, must affect its diffusion properties (Liese 1965a). Similarly, the rough surface of the cell wall imparted by warts possibly influences the flow of water in the conducting sapwood (Liese 1965a).

The adhesion and fixation of chemicals in wood are also likely to be affected by the warty layer. Excessive drying of wood could change the properties of the warty layer, thus affecting the penetration of solutions into the cell wall (Koch 1972).

Differences in the shape, size, and distribution frequency (density) of warts in various species of softwoods and hardwoods has enabled warty layer morphology to be used as an indicator of phylogenetic trend. In a study of angiosperm wood, Parham & Baird (1974) reported that species with more advanced vessel types rarely displayed a warty layer and suggested that the warty layer is a primitive structure which, with increasing evolution, becomes lost.

The taxonomic value of warts is limited by non-uniformity, both between and within species (Jurbergs 1965; Ohtani et al. 1983) and there are differences of opinion between various authors as to their value. Ohtani & Fujikawa (1971), studied 16 coniferous species and reported that variability between warts within an annual ring was characteristic, and could be used to differentiate species. Ohtani (1979) and Ohtani et al. (1983) also commented on the taxonomic usefulness of warts. Liese (1965a), on the other hand, urged caution, and pointed out that warts are occasionally sparse in certain portions of tracheids and the pattern of frequency and distribution may vary between species and large differences may exist even between neighbouring cells. Liese (1965a) however, acknowledged that a distinction was possible between genera such as *Picea* with very few warts and *Abies* with frequent warts.

Chemically, the warty layer is believed to consist of a combination of lignin and carbohydrate (Dunning 1969) and it has been found to be very resistant to chemical treatment. Liese (1965a) stated that fungi of the brown rot group do not appear able to digest the warts nor the covering membrane. Warts can survive the standard pulping processes and their presence has been noted in finished chemical pulp fibres (Jurbergs 1965). Warts can, however, be removed from the S₃ layer by treatment with hydrogen peroxide and glacial acetic acid (Robards 1965). This indicates that warts do not have a cellulose component (Scurfield & Silva 1969).

The formation of the warty layer has been the subject of study and speculation by numerous authors (Wardrop & Davies 1962; Cronshaw 1965; Baird et al. 1974b). Liese (1965a) suggested that the warty layer was formed from fragments of dead protoplasmic material trapped between remnants of the plasma membrane and the tonoplast. However Cronshaw (1965) and Baird et al. (1974) argued that warts develop external to the plasma membrane during a late stage of cell wall formation and prior to
degeneration. It has also been shown that the mechanism of their formation is different from that of the primary or secondary wall (Takiya et al. 1976).

4.1.2.3 Warts in *Callitris*

Wardrop (1959) noted that warts in *C. glauca* R.Br. (synonym of *C. glaucophylla*) were "particularly large". Liese (1965a) commented that *Callitris* was a species with "numerous bigger warts" and reported that their occurrence in five different species was "strong". Wardrop (1964) reported the presence of warts on callitroid thickening bars. Using optical microscopy, Ilic (1994) noted that the warty layer of *C. glaucophylla* was "distinct and coarse" whereas warts in *C. endlicheri* were "finer, more numerous, and hence more difficult to observe". Under SEM, Ilic (1994) found warts of *C. glaucophylla* to be "typically smooth in outline resembling gently undulating hills, measuring ca 1 µm at the base" and those in *C. endlicheri* "substantially narrower" and with "irregular outline".

4.1.3 Aims, Requirements and Considerations of this Chapter

The aims reported on in this Chapter in accord with the overall aims of the thesis were: (i) to make a quantitative high-resolution SEM investigation of wart and warty layer morphology in all species of *Callitris*; (ii) to determine whether wart morphology can be used taxonomically to identify species of the genus; and (iii) to compare warts in species endemic to contrasting habitats and relate morphological differences (if any) to likely physiological function.

The prime requirement during data acquisition was to measure the size and shape of a statistically representative number of individual warts in samples of each species. This involved high-magnification (30,000 to 50,000 times) SEM imaging in order to be able to distinguish the morphology of individual warts and to permit accurate measurement of their heights and widths. Magnifications of this order, and with accompanying high resolution, are at the upper limit of the capability of conventional SEM. The difficulty of the task was compounded by the delicate, organic nature of warts which is not conducive to absorbing the power of an electron beam, and also by the inaccessibility of the warty layer on the inner walls of tracheids which made free specimen manoeuvrability inside the SEM chamber a prime necessity.

A second major requirement was to determine the frequency (density) and arrangement of warts within the warty layer. This involved the imaging of whole sections of tracheid wall so that patterns of wart distribution could be observed and numbers of warts per unit area could be counted.

Metal-shadowed specimens, viewed using TEM, were used in early studies of warts (Liese & Johann 1954; Liese 1957; Wardrop & Davies 1962; Tsoumis 1965) prior to the commercial availability of the SEM in 1965. However the lack of
manoeuvrability of such specimens, strict limits to sample dimensions, and the inherent low depth of field of TEM, precluded its use here.

Time constraints limited the measurement of warts to only two areas (individually measuring 3 µm by 4 µm) in each sample. It was important, therefore, that these areas were clearly defined so that they were representative, characteristic, and consistent between samples and species. Several criteria were used to select areas for imaging and wart measurement. Firstly, only wide (earlywood) and not narrow (latewood) tracheids were selected because the wider tracheids afforded more ready imaging of their interiors and also because some samples, particularly those of rainforest habitat (C. neocaledonica and C. sulcata) contained little or no latewood. Secondly, only areas clear of extractive deposits and cutting debris were chosen in order to avoid features or parts of features being hidden from view. Thirdly, positions in the approximate centre of the RLS or TLS wall, away from pits, were chosen because it was easier to manoeuvre and image the centre than the sides of tracheids. Furthermore, central positions in the tracheid were preferred because literature reports indicated that warts in the centres of softwood tracheids were more typical in size of the whole warty layer than warts at the 'corners' of tracheids where larger and more densely arranged warts occur (Ohtani & Fujikawa 1971; Baird et al. 1974). Subject to the above restrictions, individual tracheids, and areas within tracheids, were selected for imaging at random.

4.1.4. Special Note Regarding Journal Article

The research described in this Chapter formed the basis for a paper that has been published in the Journal of the International Association of Wood Anatomists (Appendix 2).

The number of species (16) examined in the IAWA paper differs from the number referred to in this thesis. The reason for this is that at the time of submission of the paper to the IAWA Journal, relevant literature indicated that Callitris was comprised of the 16 species listed in Table 1 of the paper (Appendix 2). Note that the species C. preissii Miq. was said to consist of three sub-species; subspp. verrucosa, subssp. murrayensis and subssp. preissii and also that the species referred to here as 'C. gracilis' was considered to be part of 'C. preissii'. This taxonomic grouping was referred to in descriptions by Garden (1956) and Dallimore & Jackson (1966). Subsequent to the submission of the paper in March 1994, the taxonomy of the genus was revised, and the new classification is shown in Table 1.1 of Chapter 1.

Furthermore, additional wood samples of various species were examined here which were not available at the time of writing of the journal article. In this thesis, data from these additional samples has supplemented data obtained from the original samples.
4.2 Materials and Methods

4.2.1 Sampling and Preparation

The standard procedures used to sample and prepare TLS-faced and RLS-faced specimens (described in Sections 2.2 and 2.3 of this thesis) were used.

4.2.2 SEM Viewing and Data Acquisition

Several different techniques for imaging tracheid walls were tested in order to find one which facilitated measurement of the sizes of individual warts. Direct imaging of warts exposed in longitudinally-sectioned tracheids (i.e. effectively viewing warts 'from directly above') allowed wart basal diameters, but not their heights, to be measured. Alternatively, tilting the specimen to an angle of 90° and viewing warts at the cut (TS) surface allowed warts in each tracheid to be effectively viewed 'from the side' and facilitated the measurement of both wart height and wart width. However, this method enabled only a few warts in the immediate foreground of the cut TS face of the tracheid to be observed, and most warts in the background were either partly or fully obscured by those in front. Viewing warts in longitudinally-sectioned tracheids obliquely (i.e. 'tilting' the specimen to an angle of 55°) proved to be an acceptable compromise. At this angle, and at a magnification of 30,000 times, the number of warts obscuring other warts was minimal. In this configuration, the image consisted of a rectangular area of tracheid wall measuring 4 µm x 3 µm and which typically contained 20 to 80 warts. The small amount of 'foreshortening' of the vertical components of features in images caused by the inclined viewing angle was corrected, where necessary, by applying a compensation factor to all data. However, since all vertical components were equally affected, and most data were used comparatively, this was rarely necessary.

An integrated image, consisting of 50 real-time scans, was produced by the noise reduction electronics of the SEM to provide stable images for the measurement of warts. Wart height and wart width measurements were performed directly on the SEM screen using the point-to-point measurement facility described in Chapter 2.

Special operating practices for SEM viewing were used in order to reduce artefacts in the image caused by burning, cracking, and charging of surfaces at high magnification. Focusing was carried out on an area immediately adjacent to the required surface, and the sample was moved into position just prior to forming the desired image. Beam-blanking (described in Chapter 2) was used to reduce charging and deterioration of the sample while the measuring process was carried out.

To reduce the occurrence of 'charging' artefacts in SEM images, wood specimens were coated with gold (in the manner described in Chapter 2). However, this was the source of a further artefact, the well-known 'stippling' of specimen surfaces when viewed at high magnification (Echlin 1981). This occurs because during the sputter application of the coating, gold tends to nucleate into nodules of the order of 10 nm in
size, which is within the resolution capability of SEM, and becomes visible as stippling of the sample surface at magnifications in excess of 30,000 times (Echlin 1981). However, since the gold nucleations were smaller than the smallest warts by a factor of 10 they were not of major concern. A positive effect of the nucleations was that they were useful for optimising focussing and astigmatism adjustments at very high magnification.

Wart frequency (density) data for each specimen were derived from counts of the numbers of warts observed within the image at a magnification of 30,000 times (corresponding to an area of 4 x 3 µm on the specimen). For warts which were not fully contained within the imaged area but overlapped the borders, the following rule was applied:

\[
\text{Warts were included in the count if they straddled the top or right-hand borders of the image but not if they straddled the bottom or left-hand borders.}
\]

Data acquisition was carried out, in the manner described above, on two individual specimens of each wood sample (one specimen cut in RLS, the other in TLS). Data were recorded, by hand, onto individual data sheets. A video print of each SEM image was attached to the data sheets as a visual summary of the warts corresponding to the data.

4.3 Results

4.3.1 Wart Morphology

Warts were complex, asymmetrical and heterogeneous (Plates 4.1 - 4.20) and their visual appearance was further complicated by the occurrence of 'nodules' on the exterior surfaces of the larger warts of some of the species, for example Plates 4.2 and 4.3. These nodules were hemispherical or tubular in shape, their diameter (approx. 60-100 nm) was always smaller than the diameter of the main trunk of the wart, and they occurred at varying positions on the wart surface. The nodules of \emph{C. endlicheri} tended to protrude further from the main body of the wart than nodules in other species, in extreme cases appearing 'branch-like' (Plate 4.10). However, nodules did not extend more than 250 nm from warts of any species. Two aspects of nodularity were identified: (i) not all warts were nodulated and there was species-dependent variation in the proportion of warts which had nodules and those which did not; and (ii) the 'degree' of nodularity of individual warts varied in that some warts had only one nodule whereas others had multiple nodules (14 nodules were counted on a particular wart in a sample of \emph{C. verrucosa} whereas rarely more than a single nodule could be seen on individual warts of \emph{C. columnellaris}).
Plates 4.1-4.8: Warts in *Callitris* species:

1 = *C. baileyi*; 2 = *C. columellaris*; 3 = *C. glaucophylla*; 4 = *C. gracilis*;
5 = *C. intratropica*; 6 = *C. monticola*; 7 = *C. muelleri*; 8 = *C. murrayensis*.

Tracheid axis is vertical and viewing angle is 55°. Magnification = 30,000 times.
Plates 4.9-4.10: Warts in *Callitris* species: 9 = *C. drummondii*; 10 = *C. endlicheri*.
The arrow indicates a branch-like nodular protrusion.
Tracheid axis is vertical and viewing angle is $55^\circ$. Magnification = 64,000 times.
Plates 4.11-4.12: Warts in *Callitris* species: 11 = *C. macleayana*; 12 = *C. canescens*.
Tracheid axis is vertical and viewing angle is 55°. Magnification = 64,000 times.

Tracheid axis is vertical and viewing angle is 55°. Magnification = 30,000 times.
In order to quantify wart nodularity, a count was made of the number of nodules on 50 individual large-sized warts in five specimens of each species (ignoring those species which did not have nodular warts (i.e. *C. intratropica*, *C. macleayana*, *C. neocaledonica* and *C. sulcata*). In each tracheid, five large warts were examined and the number of nodules on each was recorded. This procedure was then repeated in an adjacent tracheid until a total of 50 warts had been counted. In all, a total of 4250 warts were examined. A magnification of the order of 28,000 to 65,000 times and the highest attainable resolution was used. It was, of course, possible to observe only one side of each wart, although nodules protruding from the sides of warts could also be seen and were included in the count. Thus, in this study, wart nodularity is a comparative, rather than an absolute, measurement. A variance component analysis model was formed from the nodularity data, and from this model, an index of nodularity was derived for all *Callitris* species (disregarding species without large warts). Figure 4.1 shows the nodularity index for each species. Warts in *C. canescens*, *C. glaucophylla*, *C. oblonga*, and *C. verrucosa* had greater nodularity than all other species except *C. endlicheri*, *C. rhomboidea* and *C. tubeculata*. Overall, the difference in nodularity between species was shown by ANOVA to be 'highly significant' (p < 0.001).

![Figure 4.1: An index of the nodularity of warts in species of *Callitris*. 95% confidence intervals are shown.](image)

Although there was considerable variation in wart morphology, both between and within *Callitris* species, there was sufficient homogeneity in the general 'form' of the largest warts of some species to enable them to be distinguished from warts of other
species in the genus and this is evident in Plates 4.1 to 4.20. In *C. canescens* (Plate 4.12), *C. glaucophylla* (Plate 4.3), *C. preissii* (Plate 4.15), *C. tuberculata* (Plate 4.19), and *C. verrucosa* (Plate 4.20) large warts tended to be squat and conical in shape. The large warts of *C. drummondii* were usually tall, upright and rectangular to triangular (Plate 4.9). Warts of *C. endlicheri* (Plate 4.10) were typically tall, with an enlarged, pedestal-like base and a narrow, tube-like upper part that was often bent over at the top. Warts in *C. glaucophylla* (Plate 4.3) and *C. verrucosa* (Plate 4.20) were usually large, whereas those of *C. muelleri* (Plate 4.7) and *C. oblonga* (Plate 4.14) were typically much smaller. In *C. intratropica* (Plate 4.5), *C. macleayana* (Plate 4.11), *C. neocaledonica* (Plate 4.13) and *C. sulcata* (Plate 4.18), warts were invariably relatively small and hemispherical.

Wart height and wart width measurements were used to calculate wart size (i.e. height multiplied by width), and wart shape (i.e. height divided by width). For each species, these parameters reflected variation at three levels; specimen (tree) level, section (RLS or TLS) level, and the individual wart level. It was required that analysis of variance models for these data should include both fixed and random effects; fixed effects for species and for section type, and random effects for specimens, sections, and for residual error. Following initial analysis of these data it was apparent that there was considerable heterogeneity in wart characteristic at all three levels. Not only were there apparent differences in the means of the distributions, but also between variances and skewness properties. In fact for some species there was clear multi-modality, suggesting mixed wart populations. It was not possible to identify factors which may have produced this heterogeneity. An analysis of variance (ANOVA) ignoring this multi-level structure was inappropriate since the assumptions which underlie this method were clearly not satisfied (i.e. the variances were not constant and the data were not normal). In any case, for data exhibiting distributions of this type, assumptions relating to differences among means would not be sensible. Therefore data were first aggregated to the specimen level by calculating means for each specimen, and then ANOVA of specimen means were used to provide estimates of species means and appropriate standard errors. In this way, irrespective of the underlying distribution of the raw data, the specimen means (in accordance with the Central Limit Theorem) were rendered towards normality and could be used to calculate species means (Chapter 2).

The smoothed histograms of wart size (Fig. 4.2), which were produced from wart height and width data and adjusted for specimen differences, show strong evidence of two distinct populations of warts. Sixteen of the (20) species of *Callitris* have bimodal distributions, each with a small-sized and a large-sized wart population peak, and the peaks overlap each other, thus indicating that the two populations were not completely distinct from each other. Comparison of the distributions also indicates that, in general, the bigger-sized wart populations (i.e. indicated by peaks on the right side of the distribution curves) have greater variation in size and include more warts than the small-
Figure 4.2:
Smoothed histograms of wart size in *Callitris* species.

The horizontal axes indicate wart size [log (height multiplied by width)].

The vertical axes are scaled relative frequencies (i.e. area under each curve = 1).
sized wart populations (peaks on the left) in all species with bimodal distributions. The unimodal distributions of *C. intratropica*, *C. macleayana*, *C. neocaledonica*, and *C. sulcata* suggest that each of these species had only a small-sized wart population.

There were highly significant (p < 0.001) differences in wart size and wart shape between species, as shown in Figure 4.3.

![Figure 4.3: Relationship between wart size and wart shape in Callitris species. 95% confidence intervals are shown.](image)

Three distinct groups of species are apparent. A group of 16 species: *C. baileyi*, *C. canescens*, *C. columellaris*, *C. drummondii*, *C. endlicheri*, *C. glaucophylla*, *C. gracilis*, *C. menticola*, *C. muelleri*, *C. murrayensis*, *C. oblonga*, *C. preissii*, *C. rhomboidea*, *C. roei*, *C. tuberculata*, and *C. verrucosa*, had, in general, relatively large-sized warts with width approximately equal to height (i.e. the logarithm of the mean height/width ratio of the warts was close to zero). Warts of *C. muelleri* were significantly smaller than all other species in this group except *C. menticola*. *Callitris intratropica*, *C. macleayana*, and *C. neocaledonica* formed a group in which warts can be described as 'small' and having a greater width than height (i.e. the logarithm of the mean height/width ratio of the warts is strongly negative). Finally, *C. sulcata* forms a lone grouping, similar to the latter three species in having warts with mean width greater than height, but being significantly larger in size.
Warts were, in general, discrete entities. Even in species where they were densely packed within the tracheid, warts never blended or coalesced together, in fact the regularity in spacing of warts was a feature of the warty layer in *Callitris*. However, pairs of large warts were occasionally joined at the top, forming arch-like structures (Plates 4.21-24). There was no regularity in the direction of the joining; warts combined with other warts in apparently random directions. This effect was observed only between the largest warts, was most common in the tall warts of *C. endlicheri*, and was present in all species except those without a 'large-sized' wart population, i.e. *C. intratropica, C. macleayana, C. neocaledonica* and *C. sulcata*.

4.3.2 Distribution of Warts

Warts tended to occur in distinct, evenly spaced 'lines' or 'rows' on the tracheid walls (Stereo Plates 4.25). The lines on which warts formed sometimes appeared to be raised above the surrounding surface in the form of a spiral ridge and appeared to be aligned in a helical 'Z' spiral at 60° to 90°.

Wart distribution frequencies (densities) ranged from a mean of 0.8 warts/µm² (for *C. macleayana*) to 6.0 warts/µm² (for *C. oblonga*). The mean frequency for each species is shown in Figure 4.4.

![Bar chart showing frequencies of distribution of warts in *Callitris* species. The lower horizontal axis indicates the square root of the frequency, in warts/µm². The upper horizontal axis, which is not a linear scale, indicates wart frequency (warts/µm²). 95% confidence intervals are indicated.](image-url)
Data regarding the frequencies of a total of 129 samples, representing all 20 species of *Callitris* were formed into a variance components model and an ANOVA was carried out on the modelled values. This analysis indicated that there was a highly significant difference (p < 0.001) in wart frequencies between species.

### 4.3.3 Comparison of warts in TLS and RLS

Comparison of wart morphology data obtained from RLS surfaces compared with those from TLS surfaces, indicated that there was no significant difference in size, shape, nodularity, or frequency of distribution between warts observed in the two planes in any *Callitris* species.
Plates 4.21-4.24: Anastomosed warts in *C. preissii* and *C. endlicheri*:
21-23: Viewed at 55° longitudinally into tracheid.
24: Viewed from 'directly above'.

Plate 4.25 (Stereo pair) Distribution of warts in *C. drummondii*. 
4.4 Discussion

4.4.1. Anatomical Observations and Comparison with the Literature

The warty layer of *Callitris* possessed three morphological traits that have been described in hardwoods, but have not previously been reported for softwoods: (1) presence of nodules on warts, (2) anastomosing, and (3) two distinct sizes of warts. The 'nodulated' warts observed in 16 of the 20 *Callitris* species resemble the 'branched' vestures (warts) of hardwoods, (Ohtani & Ishida 1976; Vliet 1978; Ohtani 1979; Ohtani et al. 1983; Harada & Côté 1985; Castro 1988) although in *Callitris*, the 'branches' were usually reduced in size to a bud-like, nodule form. The presence of nodules on warts in softwoods has not been commented upon previously, although a re-examination of certain micrographs of softwood warty layers in previous reports (Liese 1965a; Kuo & Manwiller 1986) revealed their presence. Branched warts in hardwoods are commonly associated with pit apertures (Ishida & Ohtani 1970; Vliet 1978; Cassens 1980; Ohtani et al. 1984a) whereas in areas surrounding pit apertures in *Callitris*, only small, hemispherical, non-nodulated warts were found; large nodulated (branched) warts occurred only on tertiary wall surfaces remote from pits. Anastomosis of warts in *Callitris* appears similar to the anastomosed vestures of certain hardwoods (Ohtani 1979; Ohtani et al. 1983) and between vestures occurring on helical thickening (Ohtani et al. 1984b). However, in *Callitris*, anastomosis was always simply between pairs of warts at their tops, and complex networks or conglomerates of joined wart-like structures, such as those commonly described for hardwoods (Scurfield & Silva 1970; Vliet 1978; Cassens 1980) were not observed. The occurrence of mixed populations of two 'types' of warts of different sizes, as indicated by the bimodal size-distribution curves (Figure 4.2) is similar to that recorded in Japanese dicotyledonous woods (Ohtani 1979), and in New Zealand (NZ) hardwoods (Ohtani et al. 1983). Where branched warts occurred in *Callitris*, a range of morphological intermediates between the large complex branched ones and the small, simple, hemispherically-shaped ones were found. This is similar to variation in size and shape of warts in the vessel elements of NZ hardwoods (Ohtani et al. 1983). The significance of these hardwood-like traits for wart morphology in *Callitris* is unknown, but it seems likely that the branched, anastomosed and mixed-type wart populations in *Callitris* are simply further evidence that warts and vestures are synonymous, as proposed by Scurfield & Silva (1970) and Ohtani et al. (1984a).

The sizes of warts in various hardwood and softwood species, as reported in the literature (Wardrop 1964; Ohtani 1979; Ohtani et al. 1983; Harada & Côté 1985) were measurements of the widths (diameters) of warts across their base (as is apparent by viewing directly down onto the surface of the inner cell wall). Thus, the 'wart size' distribution graphs of Liese (1957) and Ohtani & Fujikawa (1971) are formed only from wart basal diameter measurements. In this Chapter, the combined height and width measurements of individual warts (apparent by viewing warts from the side at a
55° angle) give a more informative indication of the size of warts than that previously obtained since both the width and height measurements are involved. Thus the 'wart size' distribution curves in Figure 4.2 describe wart size more fully. The wart height measurement is particularly important with regard to the 'small hemispherical' wart population in which height was always less than diameter. Thus referring to 'size' only in terms of basal diameter would give a false impression of size. Measurement of both height and width also provides a crude means of quantifying wart 'shape' (i.e. height divided by width).

The largest wart height measurement recorded in this study was 1.2 µm (for C. drummondii) and the largest wart width was 1.4 µm (for C. canescens). This can be compared with warts up to 0.85 µm in diameter in some Japanese coniferous woods (Ohtani & Fujikawa 1971), warts 0.1 to 0.5 in diameter in Pinus spp. (Jurbergs 1965), and heights of 0.5 to 1 µm in "some [coniferous] species" (Liese 1965a). Thus compared to warts reported in other softwood species, wart size in Callitris is very large and this concurs with the report of Wardrop et al. (1959). However, if the branched, anastomosed and aggregated vestures of certain hardwoods are included in the comparison then hardwood warts (vestures) are clearly larger. Vestures up to 3 µm in height and 5 µm in diameter have been reported in Japanese dicotyledonous woods (Ohtani & Ishida 1976). In addition, photomicrographs of vestures (Ishida & Ohtani 1970; Scurfield & Silva 1970; Vliet 1978; Ohtani 1979; Castro 1988) clearly show them to be much larger than warts in Callitris.

A noticeable feature of the warty layer of Callitris was the regularity and equality in wart distribution patterns. The rows of warts around the tracheid wall (Plate 4.25), were similar to those reported in red pine (Pinus resinosa Ait.) by Kuo & Manwiller (1986). The lines on which warts formed occasionally appeared to be slightly raised above the S3 layer surface in the form of a low ridge, especially in cell corners. This provides support for the concept that warts are associated with the underlying microfibrils (Kuo & Manwiller 1986). The 'Z' spiral direction of the wart rows is in agreement with the general microfibril direction reported in tracheids, fibres and vessels of NZ woods by Meylan & Butterfield (1978a).

The frequencies of warts in Callitris ranged from 1.0 warts/µm² for C. macleayana to 5.4 warts/µm² for C. muelleri. Since this is the first report of wart frequency in Callitris there are no comparable figures. However, Ohtani & Fujikawa (1971) reported wart frequencies in Japanese softwoods ranging from 1.0 warts/µm² for Japanese white pine (Pinus pentaphylla Mayr.) to 23 warts/µm² for Chinese juniper (Juniperus chinensis L.). Warts in Callitris were never so numerous that they 'completely masked the S3 layer' as reported in the southern pines (Pinus spp.) by Koch (1972).

Although the sizes of warts varied considerably within and between species, the nodules on warts were much less variable. Nodules were typically 80 nm in diameter.
and protruded about 90 nm from the surface of the wart and there was little variation from this size between species. This is very similar to the size of 'small warts', which ranged in height from about 60 to 110 nm; were always wider than tall; and were of similar size for all species. A possible explanation for this similarity of size is that 'nodules' on warts and 'small, non-nodulated warts' are one and the same. This would imply that the membrane overlying large warts and the tertiary wall layers contains the structures that are manifest as nodules on the former, and small warts on the latter. A similar occurrence has been suggested for hardwoods; Schmid (1965) reported that "warts occur on vestures in the pit membranes of hardwoods" and Tsoumis (1968) suggested that "warts and vestures often coexist, and the former may be superimposed on the latter". Further research is required, however, to substantiate this suggestion in regard to the warts and nodules of Callitris (see Chapter 10).

4.4.2 Taxonomic Implications

In the past, aspects of warty layer morphology, such as the presence/absence of warts, wart distribution density, wart shape and average diameter measurements, were used with varying success as a means of systematic classification of families, genera and species (Frey-Wyssling et al. 1955; Liese 1963; 1965a; Ohtani & Fujikawa 1971; Parameswaren & Liese 1977; Ohtani 1979; Ohtani et al. 1983). This study has shown the value of wart morphology characteristics for identification purposes within the Callitris genus. The 'form' of the biggest warts of the warty layer was sufficiently homogeneous within specimens to enable identification of two individual species, C. endlicheri and C. drummondii. In addition, several groups of species could be distinguished by the general shape of their warts; i.e. typically squat, conical, nodular warts in C. canescens, C. glaucophylla, C. tuberculata and C. verrucosa, small complex warts in C. monticola and C. muelleri, and small, rounded and hemispherical warts in C. macleayana, C. neocaledonica, and C. sulcata. The latter three species were also distinguishable from all others by the lack of nodules on their warts and also by having warts with larger mean width than mean height (Figure 4.3). Callitris sulcata could be further distinguished from C. intratropica, C. macleayana and C. neocaledonica by the larger size of its warts (Figure 4.3). Chapter 8 describes how wart morphology can be used to separate C. endlicheri from C. glaucophylla; two species whose wood is difficult to separate using the standard list of wood anatomical features (Ilic 1994). However, it is not known whether wart morphology is primarily a genetically-fixed trait or if it is mainly influenced by environmental factors. While this question remains unresolved the true taxonomic significance of wart morphology in Callitris (and possibly in other genera) is uncertain. Chapter 7 of this thesis describes an attempt to separate the genetic and environmental components of the within-species variation of warts in three species of Callitris.
4.4.3 Ecological Significance

The wide variety of habitats occupied by the various species of *Callitris* presents an opportunity to correlate differences in wart morphology to the environmental conditions of the habitat. In this study, it was found that species endemic to dry and semi-arid habitats, such as *C. canescens*, *C. drummondii*, *C. glaucophylla* and *C. preissii*, had warty layers with mixed proportions of small hemispherical warts, and warts with complex shape, variable size and nodules, whereas the species with only small hemispherical warts in their warty layer (*C. intratropica*, *C. macleayana*, *C. neocaledonica* and *C. sulcata*) are native to areas within or on the borders of tropical rainforest, or, in the case of *C. intratropica*, occur in wet-tropical regions of northern Australia.

Although it might seem obvious that a structure as intricate as the warty layer of *Callitris* must have a functional purpose in the physiological workings of living trees, details of that role are still very much a matter of conjecture. It is, therefore, useful to discuss the structure and morphology of the warty layer and to speculate on its functional significance. A clue to the functional and adaptive value of warts has been provided by the association between water availability in habitat, and the size of warts. The question that may be posed is: why are warts in dry-habitat species large, of complex shape and nodulated, whereas those of wet-habitat species are small and hemispherical and non-nodulated? The association suggests that there is an adaptive relationship between wart morphology and availability of water in the native habitat. Three hypotheses that may explain this association can be suggested:

Hypothesis (1). The capillary action within tracheids occurs more efficiently when the surface area of the lumen walls is increased. Since large complex warts provide more surface area than small hemispherical ones, warty layer differences between the dry-habitat and the wet-habitat *Callitris* species can be explained in terms of assisting efficient conduction of water in the former. In this hypothesis, nodulation occurs to increase the surface area of the wart. A further postulation is that, in mesic species, large nodulated warts in tracheids would create an unnecessary frictional restriction to the higher inherent rates of water flow and would therefore be a disadvantage in evolutionary terms. Hence the small hemispherical warts found in these species.

Hypothesis (2). Carlquist (1982) suggested that warts may have the effect of facilitating bonding of water molecules to the cell surface and so permitting water columns to withstand higher tensions without breaking. A broken water column causes the tracheid to fill with air, at which point it ceases to conduct (Pickard 1981). Thus large, complex warts may be considered to be an adaptive feature, of value in drought conditions by restricting the formation of air bubbles (embolisms) in the water columns. This idea is, however, not supported by Defay *et al.* (1966) who showed that rupture of the liquid column was more likely to occur when the inner surface of a capillary tube was rough.
Hypothesis (3). In this hypothesis, the shape of warts in dry-habitat species is conducive to the maintenance of high relative humidity in tracheids in which the water column has already broken. Unless the air adjacent to the cell wall in a cell which has developed an embolism maintains a high relative humidity then water will migrate from the cell wall to the lumen via normal diffusion processes (Siau 1971). Therefore, maintenance of high humidity in the air space prevents the drying out of cell walls and this would ultimately prevent water loss from adjacent, water-filled tracheids. Clearly, this would be of benefit to trees growing in environments in which water is limiting. In this hypothesis, the concave surfaces in large complex warts function to form minute reservoirs of liquid within the air-filled tracheid thereby increasing the relative humidity of the air within the tracheid. Nodulation of warts and the multiplicity of shapes and sizes of warts in dry-habitat species would enhance this effect. The basic action of the water - air moisture equilibrium is well-known in capillary theory as Kelvin's Equation (Defay et al. 1966). The hemispherical shape of warts of wet-habitat species (which experience far less danger of drying out) provides no such action.
Chapter 5

Rays

A comprehensive study of four parameters of the morphology of rays in the wood of *Callitris* is described. Quantitative measurements are used to derive values for ray height, ray width, ray frequency, and ray volume. Based on values for these parameters, comparisons are made of ray morphology in all species of *Callitris*, and the findings are compared with those of the literature. Taxonomic and ecological significances of morphological variability are discussed.
5.1 Introduction

5.1.1 Background

Xylem rays are sheets of one or more layers of parenchyma cells which extend radially across the grain, so forming the radial component of wood (Greguss 1955; Carlquist 1988; Lev-Yadun & Aloni 1995). Although raylessness is a characteristic of a few hardwoods (Carlquist 1988), rays are universal in conifers (Greguss 1955).

Ray tissue comprises only about 7% of the total volume of softwood tissue (Panshin & de Zeeuw 1980) and 6-28% of hardwoods (Carlquist 1988) but it is important physiologically in that it controls the secretion of deposits during heartwood formation (Bamber 1980), provides transverse pathways for the flow of sap, nutrients and metabolites (Petric & Scukanec 1975; Bamber & Burley 1983) and acts as a store for reserve carbohydrates (Greguss 1955). Rays also function in regeneration and in the compartmentalisation of damage after wounding (Lev-Yadun & Aloni 1995) and in the radial transport of gases within wood (Back 1969).

The physical and commercial properties of timber are affected by rays. Ray tissue shrinks and swells less radially than the longitudinal tissue and therefore restrains the radial shrinkage or swelling of wood (Petric & Scukanec 1975). Showy figure in RLS is related to the size and distribution of the rays in the wood (Zobel & Buijtenen 1989). Thus, large volumes of ray tissue are a useful characteristic for radially-sliced veneer. However, as pulp for paper manufacture, rays cells are undesirable, tending to be short and thin-walled, and thereby contributing little to the strength properties of the finished product (Zobel & Buijtenen 1989). Thus a reduced proportion of ray parenchyma relative to axial tracheid (in softwoods) is considered desirable where the timber is intended specifically for pulpwood production.

The variability of ray morphology within and between species is well-known, and differences in ray morphology have been used taxonomically (Peirce 1937; Phillips 1948; Greguss 1955; Greguss 1972; Barefoot & Hankins 1982; Wheeler et al. 1985; Schweingruber 1990; Roig 1992). Rays vary in relation to the occurrence of ray tracheids and included resin ducts, and in the morphology of such features. However, it is mainly the quantitative variability in size, shape, structure and arrangement of ray cells that is most useful diagnostically for the identification of wood (Greguss 1955).

Quantitative differences in ray morphology have been previously described in the literature, mainly in the form of four basic parameters; ray height, ray width, ray frequency and ray volume (Phillips 1948; Greguss 1955; Carlquist 1988). The first three parameters have been used for the purpose of taxonomic differentiation (Greguss 1955). The latter parameter, ray volume, is more difficult to determine, and is referred to in the literature to a much lesser extent, usually in relation to the influence of rays on pulping or shrinkage.

The morphology of rays in Callitris, expressed partly in terms of these parameters, has been described by Baker & Smith (1910), Patton (1927), Peirce (1937), and Greguss
(1955; 1972). However, there are several issues raised in these previous descriptions that require further study. The first concerns ray height. Peirce (1937) reported rays in Callitris to be "1-36 cells high", and this was quoted by Wheeler et al. (1985) in suggesting that Callitris was one of the few softwoods with "rays frequently more than 30 cells high". However, Barefoot & Hankins (1982) expressed concern regarding the diagnostic use of "tall rays over 30 cells high" for Callitris, and in their own studies, were "unable to confirm that rays in the genus were frequently more than 30 cells in height".

Descriptions of the rays of several Callitris species are also required; those of C. monticola and C. neocaledonica have not previously been described in the literature, and rays in all of the recently-defined species: C. gracilis, C. murrayensis, C. preissii, C. tuberculata, and C. verrucosa have not been described under their current taxonomic classifications. In addition, a review of literature indicates that previous accounts of ray morphology in Callitris are far from satisfactory. Several out-of-date synonyms are used in these studies, and the sample data upon which many of these reports are based are inadequate, being obtained from a single wood specimen or from branch or twig material.

The presence of ray tracheids in Callitris also requires confirmation. Ray tracheids were reported as being present by Peirce (1937), whereas both Baker & Smith (1910) and Greguss (1955; 1972) reported them to be absent.

This Chapter responds to these omissions and anomalies of the literature.

5.1.2. Literature Review of Parameters Used to Describe Ray Morphology

The most commonly-used quantitative descriptive parameter for ray morphology is 'ray height'. Ray height can be expressed in two ways: (1) as the measured distance, in μm, from top to bottom of the ray; or (2) as a count of the number of ray cells included over the same length (Carlquist 1988).

Because of the great variability in the heights of individual rays within any given area, descriptions of ray height are normally expressed in terms of the mean height and the range of heights in a one mm² area of the wood. For example, mean ray height and range of the heights of rays per mm² were reported by Roig (1992) in describing the wood anatomy of three South American species of Cupressaceae.

In softwoods, rays are considered to be exceptionally 'tall' if they are 30 or more cells high. For this feature to be included in taxonomic keys there must be an average of more than two tall rays per mm² in TLS (Wheeler et al. 1985) or more than one ray per mm width of a TLS section (Phillips 1948; Barefoot & Hankins 1982). Rays in softwoods were classified by Phillips (1948) in his identification key of softwoods, into two groups; i.e.: (1) tall rays (frequently more than 30 cells high); and (2) short rays (rarely more than 15 cells high). Both classes applied to a one mm width of tangential section. This classification was also adopted by Barefoot & Hankins (1982) and by
Wheeler (1985). However, Schweingruber (1990) classified tall rays of European softwoods as those greater than 10 cells in height, and low rays as being less than 10 cells in height.

Carlquist (1988) cautions that ray height differs appreciably in quantitative respects according to the maturity of the wood sample and Phillips (1948) says that rays in the first 20-30 rings of most species are usually low in height so that only fully adult wood should be taken into account when using this feature.

The parameter 'ray width' is defined as the horizontal axis of the ray as seen in a tangential section as it occurs within the tree. Phillips (1948) suggests that the predominant shape of the middle cells of the taller rays in earlywood should be used when comparing the widths of rays and Carlquist (1988) says that ray width is measured at its widest point. Ray width can be expressed either in microns (µm) or in terms of numbers of cells (Carlquist 1988). However, since rays in softwoods are generally uniseriate (i.e. are only one cell wide) the latter means of measurement is usually confined to hardwoods, although biseriate rays have been reported in California redwood (*Sequoia sempervirens* Endl.) and Monterey cypress (*Cupressus macrocarpa* Hartev.) by Phillips (1948) and in the *Diselma, Fitzroya, Thuopsis*, and *Libocedrus* genera by Peirce (1937) and partially-biseriate rays (biseriate over one-third or more of their length) are of regular occurrence in several [softwood] species (Phillips 1948).

Ray frequency (density) can be determined by counting the number of rays that intersect an imaginary line running tangentially across a transection (Carlquist 1988) or by counting the number of rays in an area of one mm² (Roig 1992). Ray frequencies ranging from 20 to 500 rays per mm² have been reported for the wood of gymnosperms (Greguss 1955). Although usually quoted in anatomical descriptions of wood, ray frequency is considered to be an unreliable taxonomic differentiating feature because of its variability (Carlquist 1988) and is generally not used as a feature in taxonomic keys.

The proportion of ray tissue relative to the total wood tissue is termed the 'ray volume' (Petric & Scukanec 1975). Ray volume is not used as a taxonomic indicator. Ray area (and hence ray volume) has traditionally been determined by accurately weighing a photograph of a TLS section of wood, cutting out only the rays from the photograph, weighing the excised pieces, and comparing their weight to the total weight of the photograph (Myer 1922). The more modern method of determining ray area is by analysis of digitised images of rays. Ray tissue typically forms from 6% to 28% of the volume of wood of hardwoods (Carlquist 1988). Softwoods in general are reported by Panshin & de Zeeuw (1980) to have ray volumes of 7.1%, and Koch (1972) reported ray volumes ranging from 7.6% in loblolly pine (*Pinus taeda* L.) to 11%, in slash pine (*Pinus elliottii* Engelm.).
5.1.3. Literature Review of Rays in *Callitris*

Rays in *Callitris* were reported by Patton (1927) to be "variable in height and elliptical". Patton (1927) also described the ray heights of five individual species of *Callitris* on the basis of number of ray cells as follows: "rays in *C. intratropica* are on average 7 to 25 cells high (maximum 33); *C. muelleri* 2 to 4 cells (max. 10); *C. rhomboidea* 3 to 7 cells (max. 13); *C. oblonga* 1 to 4 cells high; and in *C. endlicheri* (as its synonym, *C. calcarata*) 5 to 14 cells high".

Peirce (1937) reported the rays of *Callitris* to be "uniseriate, or occasionally biseriate in part, 1-36 cells in height (mean height 15-18 µm), and with normal ray tracheids present".

Greguss (1955) reported on the cell number and height and width measurement of rays of several species of *Callitris* as follows: rays in *C. glaucophylla* (as *C. glauca*) were on average 1 to 8 cells high (maximum 10), height 14 to 24 µm, width 8 to 12 µm; in *C. verrucosa* R. Br. 6 to 8 cells high (maximum 14), height 17 to 22 µm, width 5 to 13 µm; in *C. sulcata* 1 to 10 cells high (maximum 15), height 13 to 26 µm, width 4 to 18 µm; in *C. oblonga* 3 to 5 cells high (maximum 15), height 11 to 17 µm (maximum 24), width 6 to 13 µm; and in *C. intratropica* Benth. et Hook. 1 to 4 cells high (maximum 10), height 15 to 37 µm, width 4 to 18 µm. Greguss (1972) further added to this listing, describing *C. drummondii* as having rays on average 5 to 6 cells high, height 14 to 26 µm, width 13 to 14 µm; and *C. canescens* (as *C. morrisonii* R. Baker) with rays on average 2 to 4 cells high and height 14 to 15 µm (ray width was not reported for this species).

Ray and ray cell frequency for several *Callitris* species was reported by Greguss (1955) as follows; *C. verrucosa* R. Br., 60 to 65 rays and 260 to 265 ray cells per mm²; *C. sulcata*, 5 to 20 rays and 260 to 265 ray cells per mm²; and *C. oblonga*, 110 to 120 rays and 410 to 420 ray cells per mm².

5.1.4. Aims and Requirements of this Study

The aims of this Chapter were fourfold: (i) to quantitatively describe ray height, ray width, ray frequency, and ray volume in all (20) species of *Callitris*; (ii) to make interspecies comparisons of ray morphologies; (iii) to compare results with those of previous studies, and, in particular, to investigate the validity of the report by Peirce (1937) of the occurrence of exceptionally tall rays (greater than 30 cells high) and the presence of ray tracheids in *Callitris*; and (iv) based on the findings, to suggest the taxonomic and ecological (functional) significances of variability in ray morphology.

In order to carry out these aims, quantitative data regarding the heights and widths of individual rays were required. It was also necessary to determine the number of rays and ray cells per unit area, and to measure the volumes of ray tissue relative to other tissues in all specimens. Samples cut in TLS were required for SEM viewing since TLS sections allow convenient measurement of ray height, width, frequency and area.
5.2 Materials and Methods

5.2.1 Sample Preparation

For this study, sampling of wood, and the preparation of TLS-faced specimens for SEM imaging, was carried out as described in Chapter 2. Four samples of *C. neocaledonica* and *C. sulcata*, five samples of *C. baileyi*, *C. drummondii*, *C. gracilis*, *C. monticola*, *C. muelleri*, *C. roei*, and *C. tuberculata*, seven samples of *C. columellaris* and *C. murrayensis*, and eight samples of all other *Callitris* species were examined.

It was found that the cut ends of rays in TLS specimens occasionally exuded resinous extractives when the wood samples were subjected to the vacuum of the SEM. However, the imaging distortions caused by these secretions were sporadic and localised, so that degraded areas could be avoided, and only unaffected areas used for imaging. Thus no special cleaning of specimens was necessary.

5.2.2 Data Acquisition Procedures

For the acquisition of ray height data, SEM images of TLS-faced specimens at 100 times magnification were used. Two measurements were carried out on each individual ray; (1) the ray was measured (in μm) from tip-to-tip using the on-SEM-screen point-to-point measuring facility, and (2) a count was made of the number of cells in the ray. This process was repeated for all rays visible in the image (which represented an area of approximately one mm$^2$ on the specimen surface). In order to ensure that individual rays were not missed, and were not measured more than once, the SEM screen was divided into four sections using an electronic graticule (Chapter 2), and rays in each section were measured consecutively. For rays which were not fully contained within the imaged area, but were partly obscured by the image border, the following rule was applied:

*Rays were included in the count if they straddled the top or right-hand side of the image but not if they straddled the bottom or left-hand side of the image.*

Thus, in order to measure the heights of rays within the sample area, all rays that were fully included in the 100 times magnification image were measured first, then the sample was moved slightly downwards and to the left in order to accommodate imaging and measurement of rays that were originally not completely in view but were cut off by the top and right-hand sides of the image. This ensured that the counts of the number of rays within the image (and hence the calculated ray frequency values) were accurate.

The mean ray width for each specimen was obtained from 10 measurements, recorded at a standard magnification of 1000 times. Each ray width measurement was taken at the centre of a randomly-selected ray and included both the lumen diameter and the cell wall thicknesses on either side of the lumen but did not include the crossfield pit 'callitroid' thickening awns protruding from the side wall into the lumen (where these
occurred). The mean ray width for each species was calculated from the average of specimen means.

In order to determine the occurrence of biseriate rays in *Callitris*, a TLS image representing an area of approximately one mm\(^2\) on the specimen surface was observed, and a record was made of the numbers of uniseriate, partly-biseriate or fully biseriate rays present in the image. This procedure was repeated in 4-8 specimens for each species.

Ray frequency data were acquired by counting the number of rays contained within a 100-times-magnification SEM image of each specimen. For convenience, this was carried out at the same time as ray height was measured. At a magnification of 100 times, the area of specimen surface on screen was 1.21 x 0.905 mm\(^2\) (i.e. 1.085 mm\(^2\)). Ray frequency (rays/mm\(^2\)) for each sample was derived from the numbers of rays observed in the imaged area, divided by the area of the image.

Ray volume (as a percentage of total volume) was determined by first converting SEM images of TLS surfaces at 100 times magnification into digital form by use of an image digitiser (ImageSlave, Meeco Pty Ltd.). Digitised images were then viewed by use of an image analysis program (NIH Image 1.58, US National Institute of Heath shareware) operating on a Macintosh LC475 computer. Contrast was reduced, so that there were no fully white areas in the image. The image was viewed at four times its original size. The shapes of all rays in each image were then outlined by use of the 'freehand tool' and the outlined area was 'filled' to full white colour using the 'Fill' application of the image analysis program. The sum of the selected (filled) ray areas relative to the total area was obtained using the density-slice function. Ray area was then equated to ray volume by assuming that the ratio of ray volume to total volume was equal to the ratio of ray area to total area. This assumption is explained by Myer (1922) as follows:-

"Since rays are continuous and vary little in size as they extend in a radial direction, the volume of a block may be considered as a series of tangential sections in which ray numbers, and consequently ray area, remains constant. It follows therefore that the rays of any cut are reasonably representative of all sections and therefore it may be assumed the ray area of the tangential section per unit area varies directly as ray volume per unit volume."

Ray volumes were determined for five samples of each species (four only for *C. neocaledonica* and *C. sulcata*). From these sample values, the mean ray volume for each species was calculated.
5.3 Results

5.3.1 Ray Height

Figure 5.1, and Plates 5.1 and 5.2, indicate the wide range in ray height that occurred both within, and between, species of Callitris. For example, ray height in *C. drummondii*, ranged from 20 µm to 210 µm, and in *C. macleayana*, from 25 µm to 630 µm. Rays tended to be short (median height approximately 70 µm and maximum height typically less than 200 µm) in *C. canescens*, *C. drummondii*, *C. monticola*, *C. muelleri*, *C. roei* and *C. tuberculata*, whereas in *C. columellaris*, *C. intratropica*, *C. macleayana* and *C. neocalydonica*, rays were relatively tall (median height approximately 120 µm, maximum height greater than 300 µm). The minimum heights of rays for all species were approximately the same (20-30 µm) indicating that rays containing only one cell, measuring approximately 20 µm in height, were universal within the genus.

![Boxplots showing heights of rays (measured in µm) in all species of Callitris. A total of 5977 ray heights (approx. 300 for each species) are represented.](image)

Figure 5.1 also indicates that ray heights for individual species were not normally distributed; there were skews in their distributions such that the horizontal lines indicating the medians were displaced from the centres of the boxplots of most species, and the 'whiskers' extending from the tops and bottoms of individual boxes (indicating the maximums and minimums of the 'usual' ray height values) generally extended further into the 'taller values' of ray height than into the 'shorter values'.

Because of this non-normality (positive skewness) in the distributions of ray heights within species, calculation of mean ray height was carried out on the logarithm
of each ray height datum for all rays in each specimen. Thus the data were transformed by taking logarithms of ray heights and calculating the mean ray heights for specimens and species as means of the transformed data (Chapter 2). Figure 5.2 shows mean (logarithmic) ray heights (in µm) for all *Callitris* species.

![Mean logarithms of ray heights (µm) in *Callitris* species.](image)

**Figure 5.2:** Mean logarithms of ray heights (µm) in *Callitris* species. The Y-axis on the right indicates back-transformed log. values and the scale is not linear. 95% confidence intervals are indicated.

It can be seen that although mean ray height differed between species, no particular species could be separated, with 95% confidence, from all of the other species. A similar situation occurs when the logarithm of mean ray height is expressed in terms of a count of the number of cells in the rays as shown in Figure 5.3. Although Figures 5.2 and 5.3 are similar, there are differences between them in relation to which species have the tallest rays. When ray height is expressed in µm, the four species with the tallest rays are, in descending order of height; *C. macleayana*, *C. intratropica*, *C. columellaris* and *C. oblonga*. In contrast, when ray height is expressed as a cell count the species with the tallest rays were (in descending order) *C. intratropica*, *C. columellaris*, *C. macleayana* and *C. oblonga*.

Rays with a minimum height of only one cell (Plate 5.3) occurred in all species of the genus but they were more common in species with generally short rays.
Plates 5.1 & 5.2: Comparison of rays in (1) = *C. drummondii* and (2) = *C. macleayana*. The magnification of both micrographs is the same (approximately 115x).
Figure 5.3: Mean logarithms of number of cells in rays of Callitris species. The 95% confidence intervals are shown. The Y-axis on the right indicates back-transformed log. values and the scale is not linear.

Figure 5.4 shows the ray heights (expressed in terms of numbers of cells) in the two tallest rays found in one mm$^2$ areas of 4-8 samples of Callitris species. The largest inter-species differences occur between C. monticola (3-7 cells) and C. columnellaris (13-33 cells).

5.3.2. Ray Width and Ray Seriation

Figure 5.5 shows ray widths measured in all species of Callitris. It can be seen that ray widths were greatest in C. macleayana, C. neocaledonica and C. sulcata being in excess of 25 µm, and were smallest for C. gracilis and C. tuberculata, for which mean ray widths were less than 19 µm.

No fully biseriate rays were found, although partially biseriate rays were recorded in 18 of the 20 species. Table 5.1 shows the occurrence of partially-biseriate rays as percentages of total ray number for each species. It can be seen that the proportion of partially biseriate rays ranged from none (in C. neocaledonica and C. monticola) to 5.1% (in C. endlicheri).

Where partial biseriation occurred, the biseriate cells within the ray were usually narrower than the uniseriate ones so that the combined widths of the biseriate cells matched the width of the remaining uniseriate cells. Thus, partially-biseriate rays were typically of similar widths to uniseriate ones (Plate 5.4).
Figure 5.4: Box plots showing heights (numbers of cells) of the two tallest rays found in *Callitris* species. The box for each species represents data from 4 to 8 wood samples, and in each sample, an area of 1 mm² of TLS surface was examined.

Figure 5.5: Boxplots showing ray widths (µm) in species of *Callitris*. Individual boxplots represent the mean ray width of 10 rays in each specimen for 4-8 specimens of each species.
### Table 5.1: Occurrence of partially biseriate rays in species of Callitris.

The information contained in individual columns is as follows:
- Col. 2: Number of specimens observed.
- Col. 3: Number of rays observed.
- Col. 4: Number of specimens in which partially biseriate rays occurred.
- Col. 5: Number of rays that were partially biseriate.
- Col. 6: Percentage of all rays that were partially biseriate.

<table>
<thead>
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<th>Callitris species</th>
<th>Number of specimens observed</th>
<th>Number of rays observed</th>
<th>Specimens with part. bis. rays</th>
<th>Number of part bis. rays</th>
<th>% of total rays part biseriate</th>
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#### 5.3.3 Ray Frequency

Ray frequencies (rays/mm²) for all Callitris species are shown in Figure 5.6. It can be seen that, in general, *C. intratropica*, *C. macleayana*, *C. neocaledonica*, and *C. sulcata* had the lowest ray frequency (median frequency less than 30 rays mm²). *Callitris baileyi*, *C. canescens*, *C. drummondii*, *C. murrayensis*, *C. tuberculata*, and *C. verrucosa* had median frequencies typically more than 50 rays mm², and in the remaining species, ray frequencies were 30-50 rays mm².

#### 5.3.4 Ray Volume

Mean ray volume in *Callitris* varied between 4.8 and 10.3%, and the range of ray volume for each species is shown in Figure 5.7. Ray volume was smallest in *C. canescens*, *C. drummondii*, *C. muelleri* (Plate 5.5), *C. roei*, and *C. tuberculata*, and was largest in *C. columnellaris*, *C. intratropica*, and *C. oblonga* (Plate 5.6).
Figure 5.6: Ray frequencies (rays/mm²) in species of *Callitris*. Individual boxes show the frequencies (Y-axis) in one mm² areas of 4-8 specimens of each species.

Figure 5.7: Ranges of ray volumes (percent of total volume) in species of *Callitris*. 
5.3.5 Relationships Between Parameters

Figure 5.8 shows that an inverse relationship occurs between the ray heights and the ray frequencies of *Callitris* species. It can be seen that species with tall rays have low ray frequencies whereas species with short rays tend to have high ray frequencies. In this respect, two groups of species can be discerned. In the first group are *C. columellaris*, *C. intratropica*, *C. macleayana*, *C. neocaledonica* and *C. sulcata* which have tall, infrequent rays. All remaining species belong in the second group in which shorter rays occur at a higher frequency.

The relationship of ray height to ray width was determined by measuring both of these parameters for 25 individual rays at random in one specimen of each *Callitris* species. Figure 5.9 is a scatter diagram showing their relationship.

It can be seen that ray cells were generally 'square' in shape; with their heights equal to their widths (to within approximately 10%) in all species except *C. baileyi*, *C. monticola* and *C. neocaledonica* in which ray cells tended to be more 'upright', (i.e. taller than wide).

The same ray height and ray width data used previously (but only for *C. baileyi*; *C. columellaris*; *C. macleayana* and *C. verrucosa*) was plotted in scatter diagrams in order to show the relationships of ray height to width (Figure 5.10). This shows that, in general, tall rays were wider than short ones.

5.3.6 Qualitative Observations

Rays were wholly parenchymatous; ray tracheids were absent from all species. This observation is based on the fact that none of the cells in the rays (and particularly the cells at the margins of rays) had bordered pits (Plate 5.7); the pits in rays were always of the crossfield type [Phillips (1948) distinguishes ray tracheids from ray parenchyma on this basis].

The internal surfaces of ray cells were smooth; there was no visible warty layer (Plate 5.8).

The cells at the margins of rays were typically elongated vertically, whereas cells in the centres of rays tended to be more circular (Plates 5.9 and 5.10).
Plates 5.3-5.10: Rays in *Callitris* species viewed at various magnifications:

3 = Ray consisting of only one cell. 4 = Partially-biseriate ray is indicated by arrows.
5 & 6 = TLS images of rays indicating the difference in ray volume between *C. muelleri* and *C. oblonga*. *Callitris muelleri* had ray volumes of 4.0-5.6% whereas in *C. oblonga* ray volumes of 9.0-11.2% occurred.
7 = RLS view of rays in *C. intratropica*. 8 = Internal surface of ray; note absence of warts. 9 & 10 = Elongation of cells at margins of rays.
Figure 5.8: Relationship between mean log. of ray height (measured in µm) versus mean log. of ray frequency in (rays/square mm) for all species of *Callitris*. 95% confidence intervals are indicated.

Figure 5.9: Mean cell height plotted against mean cell width (both in µm). The oblique line indicates ray cell height = ray cell width (i.e. 'square' or circular cells). Species on the left side of the line have cells which are vertically longer than wide (i.e. are upright or erect).
5.4 Discussion

5.4.1 Ray Morphology - Comparison with the Literature

The findings of this Chapter tend to contradict two long-standing views regarding the anatomy of rays in *Callitris*. Firstly, Figure 5.4 shows that rays of greater than 30 cells in height were recorded on only two occasions (one each in *C. columellaris* and *C. intratropica*) out of a total of 5160 rays measured. Thus it can be concluded that 'tall rays' are rare in *Callitris*, and are not common to all species as indicated by the report of Peirce (1937). Secondly, ray tracheids were absent from all species; which also contradicts Peirce (1937) but accords with Baker & Smith (1910) and Greguss (1955).

Rays in 13 of the 20 *Callitris* species could, in fact, be classified as being 'short' using the classification scheme of Phillips (1948) since the two tallest rays in one mm² areas of all *Callitris* species except *C. columellaris*, *C. intratropica*, and *C. oblonga* were less than 15 cells high and in *C. endlicheri*, *C. glaucophylla*, *C. macleayana* and *C. murrayensis* the two tallest rays could be classified as 'short' in some of the samples but not in others (Figure 5.4).

Table 5.2 compares values reported by several authors for ranges and mean heights of rays in some species of *Callitris* with values obtained here. In general, with the exception of the absence of tall rays in most species the major difference that can be
discerned is that ray heights found by this study were, on average, larger than those reported in the literature with regard to both the mean and range. For example, in *C. endlicheri*, the mean ray height of 5.5 cells and range of 1-23 cells contrasts strongly with the mean ray height of 1.7 cells and range of 1-10 cells reported by Venning (1979). Most of these differences can be explained by the fact that previous reports used juvenile wood or branch material rather than mature wood. It is well known that ray height is inherently low in juvenile wood (Phillips 1948).

<table>
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<th>Callitris spp.</th>
<th>Synonym used previously</th>
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<th>Venning (1979)</th>
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<td></td>
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Table 5.2: Ranges and mean heights (mean number of cells) of rays in species of *Callitris* as reported in the literature by Baker & Smith (1910); Greguss (1955; 1972) and Venning (1979) compared with the findings of this Chapter.

The inverse relationship between mean ray height and mean ray frequency in species of *Callitris* (Figure 5.8) is similar to that observed by Roig (1992) in individual trees of Chilean cedar (*Austrocedrus chilensis* Florin et Boutleje). The explanation offered by Roig (1992) for this relationship was that "the tree needs to maintain a certain proportion of ray parenchyma cells, and thus balances a decrease in ray height with an increase in ray frequency".

The elongated cells at the margins of rays, accords with Phillips (1948) who reported this as applying generally to most softwoods. A consequence of this was that the cells of very short rays (consisting of only one or two cells) were elongated by approximately 4 µm. This effect is apparent in Figure 5.9 which shows that species with generally short rays (*C. canescens, C. drummondii, C. muelleri, and C. monticola*) had 'upright' cells with cell height greater than width.
There are no previous reports of ray volumes for species of *Callitris*. However, the wide variation in ray volume found in this study (4% to 11.3%) accords with the figures of 7.6% to 11% found in southern pines (*Pinus* spp.) by Koch (1972).

### 5.4.2 Taxonomic Significances

Although none of the morphological parameters of rays was useful in taxonomically separating individual *Callitris* species, mean ray height was effective in separating species with rays greater than 10 cells in height from those with a mean of less than 10 cells (Figure 5.3). Also, on the basis of their wider rays, *C. macleayana*, *C. neocaledonica* and *C. sulcata* could be separated from all other species (Fig 5.5). Ray frequency and ray volume have little value in differentiating species, and minimum ray height was not a useful taxonomic factor; rays of one cell (approximately 20 µm) in height occurred in all species of *Callitris* (Figure 5.1).

The term 'ray height' as it exists in the literature, can be measured either as numbers of cells in a (uniseriate) ray or as the measured distance from the ray's tip to tip. Thus although Figures 5.2 and 5.3 both portray 'mean ray height' under the definition of Carlquist (1988), the mean ray height expressed as a measurement in µm (Fig. 5.2) indicates that *C. macleayana* has the tallest rays of all 20 species whereas mean ray height expressed as a count of the numbers of cells in the rays (Fig.5.3) indicates that *C. intratropica*, *C. columellaris* and *C. oblonga* all have taller rays than *C. macleayana*. The reason for this discrepancy lies in the differences in size of individual cells comprising the rays; cells in rays of *C. macleayana* tend to be much larger than those of the other three species.

### 5.4.3 Ecological Significances

There were some general differences between the ray morphology of *Callitris* species native to wet habitats and those native to dry habitats. Typically, the wet habitat species (i.e. *C. macleayana*, *C. neocaledonica* and *C. sulcata*) had tall, wide rays, at low frequency of occurrence and a large ray volume. Dry-habitat species (e.g. *C. canescens*, *C. drummondii*, *C. monticola*, *C. roei*, and *C. tuberculata*) tended to have short, narrow rays at high frequencies, and a small ray volume. These differences are apparent to varying degrees in Figures 5.1 through 5.8. For example, in terms of ray height, width, frequency, and volume, *C. drummondii* (native to dry areas of Western Australia) has a mean height of 60 µm, mean width of 18 µm, ray frequency of 57 rays/mm² and a ray volume of 6% whereas the equivalent parameters for *C. macleayana* (endemic to Queensland rainforest margins) are: mean height of 180 µm, mean width of 27 µm, ray frequency of 15 rays/mm² and a ray volume of 8%. Thus, in general terms, the ecological strategy for dry-habitat *Callitris* species is to have a lesser amount of ray tissue with narrow cells congregated into short rays occurring at high frequency. In contrast, the strategy for wet-habitat species is a greater amount of ray tissue consisting...
of larger cells congregated into tall rays, but at lower frequencies of occurrence. Species endemic to habitats which are neither particularly wet or dry were found to have (in general) ray morphological characters midway between those of the very dry and very wet types. These habitat-related differences distinguished *C. macleayana*, *C. neocaldonica*, and *C. sulcata* from all other species of the genus. The reasons for this morphological dichotomy are not known.

**Within-tree Variability**

A study of within-tree variability in Callicoma species, worts, and reeds in a single tree of *Callicoma psittacophylla* is described. The study focused on: (i) variation occurring radially and vertically within the whole stem, (ii) variation between the earlywood and latewood within a growth ring, and (iii) variation within and between trunks.
Within-tree Variability

A study of within-tree variability in callitroid thickening, warts, and rays in a single tree of *Callitris glaucophylla* is described. The study relates to: (i) variation occurring radially and vertically within the whole stem; (ii) variation between the earlywood and latewood within a growth ring; and (iii) variation within individual tracheids.
6.1 Introduction

6.1.1 Background

Within the trunk of a mature conifer tree there are three sources of variation in the micro-morphological features of the wood: (i) differences between juvenile and mature wood; (ii) differences between earlywood and latewood within growth rings; and (iii) differences within individual tracheids. These influences are universal in conifers, and are widely reported in the literature (Stern & Greene 1958; de Zeeuw 1965; Jane 1970; Zobel & Buijtenen 1989).

The first of these areas of within-tree variability, differences between juvenile wood and mature wood, are a function of the age of the cambium at the time of wood formation. Juvenile wood is formed close to the pith, and the process continues (in the excurrent [monopodial] growth of conifers) throughout the earliest formed parts of the trunk into the terminal leader. Thus juvenile conifer wood occurs as a 'central cylindrical column' within the trunk (Panshin & de Zeeuw 1980) extending radially from the pith for 10-25 years of growth, throughout the vertical length of the stem. Mature wood forms around this cylinder, outwards to the cambium and is produced over the remainder of the life of the tree. It follows that mature wood usually forms a much greater proportion of the whole trunk of a mature tree than juvenile wood. Mature wood therefore possesses characteristics which are considered 'normal' for wood of the species (Panshin & de Zeeuw 1980) and these characteristics can be different, both quantitatively and qualitatively, from those of juvenile wood (Phillips 1948; Sterne & Greene 1958).

A second area of within-tree variability results from differences in growth that are related to environmental (usually seasonal) influences during the formation of the wood (Panshin & de Zeeuw 1980). These changes in growth produce the earlywood and latewood, that together form 'growth rings' within conifers. Earlywood tracheids tend to be larger in radial diameter and to have thinner walls than those of latewood (Creber & Challoner 1990) but there can be "variation in cell composition and density of growth increment in different species, in individual trees of the same species and at different heights within a given tree" (Panshin & de Zeeuw 1980). These quantitative differences between earlywood and latewood can be the greatest source of variability in wood properties within species (Zobel & Buijtenen 1989).

At the microscopic level, a third area of within-tree variability in wood anatomy occurs within individual wood cells. An example of variability in this category is the difference in wart size that occurs between warts close to, and those remote from, pit apertures (Liese 1965a; Baird et al. 1974).
Differences between the heartwood and sapwood of trees, which are manifest mainly in terms of wood colour (Phillips 1948), are reported to be related only to the degree of extractive deposition within the wood and not generally to variability of the microstructural features of wood anatomy (Panshin & de Zeeuw 1980).

Because of the effects of within-tree variability, wood anatomy descriptions and taxonomy keys, such as those of Phillips (1948) and Schweingruber (1990), are always based on mature wood from the trunk. For the same reasons, most descriptions of wood are based on earlywood only, and in the case of very small features such as warts, all observations are made in similar positions within each individual tracheid to avoid within-tracheid variability. Thus, in Chapter 4, only mature wood from the outer regions of trunks was sampled, thereby avoiding possible variation in wart morphology caused by differences between juvenile and mature wood. Furthermore, inherent variability within individual growth rings (if any) was integrated into a mean value for the specimen because the data were taken at random from many different areas of the prepared surface. Similarly, measurements were made only of warts situated in the centres of tracheids in order to avoid those in the 'cell corners' which, in other genera, are reported to be larger than warts situated centrally on the RLS and TLS walls (Ohtani & Fujikawa 1971; Baird et al. 1974a; Verhoff & Knigge 1976).

In practice, however, such specific selection of the sampling site within a particular tree may not be possible. The sample may be from an unknown position within the trunk, or only branch or twig material may be available. It then becomes advantageous to have a knowledge of the nature of the variability within the whole tree, particularly that of the juvenile and mature wood. Knowledge of the micro-morphology of stem and non-stem wood may also be necessary in order to more readily interpret some previous wood anatomy descriptions, such as that of Venning (1979) in which only twig material was used. Such information is also essential when dealing with prehistoric wood or charcoal.

Other than that reported to be inherent to conifers in general, within-tree variability in the wood anatomy of Callitris is not described in the literature. It is not known whether callitroid thickening, warts and rays differ between juvenile and mature wood and earlywood and latewood, or if their morphology remains the same throughout the tree. Thus, in order to present a broader and more balanced description of the wood anatomy of the genus, and to provide information of taxonomic value in relation to wood samples from unknown positions within trees, a need was identified to determine the within-tree differences in these features. This Chapter describes variation in callitroid thickening, warts, and rays in a single tree of C. glaucophylla at three different levels: (i) within the trunk as a whole, (ii) within growth rings and, (iii) within individual tracheids.
6.1.2 Aim, Objectives, Considerations and Requirements

The aim of this Chapter was to examine the within-tree variability of callitroid thickening, warts, and rays in relation to: (i) differences occurring radially and vertically within the whole stem, and in particular, those related to the occurrence of juvenile and mature wood; (ii) differences between earlywood and latewood within a single growth ring; and (iii) differences in morphology [of warts only] relating to spatial position within individual tracheids.

Since this was an investigation of variability in callitroid thickening, warts and rays on three vastly different scales of size (i.e. within the whole tree trunk, within an individual growth ring, and within individual tracheids), it was impractical to achieve the aims in a single study. Therefore the Chapter consists of three separate investigations.

There was a need to obtain wood samples from precisely located radial positions and vertical heights within the stem. This requirement precluded the taking of samples from trees by use of an increment corer. Instead, complete sections of trunk from various stem heights were necessary. Thus, unlike all other wood sampling described in the thesis, trees had to be felled for the work described in this Chapter. Callitris glaucophylla was chosen as the representative species for this study since it is relatively common, and is currently the principal species of Callitris exploited for timber production (Salmon 1990).

Wood sample material for all three parts of the within-tree study was obtained from four C. glaucophylla trees, all growing within a one square kilometre area of the Gillenbah State Forest near Narrandera, NSW, at latitude 34° 48' South, longitude 146° 30' East. The trees were from a pure stand in flat, grassy woodland, with red sandy soil and at an elevation of approximately 145 m. Narrandera has a mean yearly rainfall of 441 mm. Most rainfall occurs in the winter months and there are, on average, 71 'raindays' (i.e. >0.2 mm rainfall in the day) per year. All four trees were of similar height (8-12 metres) and basal diameter (18-24 cm). All were of mature appearance (Plate 6.1). They were given the names: glau-21, glau-22, glau-23, and glau-24 to identify them.

The trees were felled at a position as close as possible to ground level (approximately 10 cm from their bases). Stem cross-sections, three cm in thickness, were taken from the base of each trunk. Sections of the same thickness were also taken from a point just below the crown, about 7 to 11 metres up each trunk and from approximately midway between the top and bottom sampling positions (Plates 6.2 & 6.3). Figure 6.1 shows the dimensions of the trunk, the position of the heartwood-sapwood interface, and growth ring information, in each wood section, for all four trees.

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1The term 'tree', and not 'sample' will be used to denote the basic source of the wood used in the research described in this Chapter. This is in order to differentiate the multiple sections of trunk used in this research, from the individual (core) samples used throughout the remainder of the thesis. The term 'specimen' continues to indicate wood prepared for SEM viewing.
Plate 6.1: Tree glau-22 (*C. glaucophylla*) used in this study of within-tree variability.

Plate 6.2: Sections being cut from the trunk of tree glau-22.
Figure 6.1: Measurements of heartwood diameter and trunk diameter (in cm) correlated with growth ring information at the tops, middles and bases of trees; glau-21, glau-22, glau-23 and glau-24 (C. glaucophylla)
6.2 Variation Occurring Radially and Vertically Within the Stem

6.2.1 Introduction

It was the aim of this part of the study to assess variability in callitroid thickening, wart, and ray morphology occurring radially and vertically within the whole stem and to determine whether differences in these features (if any) could be used as indicators of juvenile and mature wood.

Because time was restricted, it was decided that only a single tree of the four that were sampled would be examined in detail. This was to allow selection of the most suitable tree for study, and also to enable cross-checking of findings (if required).

The number of sampling points to be taken radially and vertically within the stems had to be considered in relation to the time and resources available, and the desire to obtain information consistent with the findings of earlier Chapters. A feasibility study was carried out to determine the time required to sample at a particular location within the tree stem, to prepare specimens, form SEM images, and acquire and analyse the data. Based on the information provided by this study, it was decided that it would be most appropriate to sample the wood sections at radial locations five growth rings apart, from the pith to the outermost sapwood at three vertical locations within the trunk; base, middle, and top.

6.2.2 Literature Review of Within-tree Variability Occurring Radially and Vertically Within the Stem

The most commonly reported aspect of within-tree variation in wood morphology is tracheid length. Knowledge of this parameter dates back more than a century to the pioneering work of Sanio (quoted in de Zeeuw 1965), whose research on the differences in tracheid length occurring in various parts of trees of Scots pine (Pinus sylvestris L.) is still of relevance today (de Zeeuw 1965). Sanio showed that a definite pattern of cell length change occurs radially from pith to bark. The shortest cells occur near the pith and there is an increase in their length outwards to the bark that follows an approximate logarithmic curve, with the longest cells being formed at tree maturity (de Zeeuw 1965). These early findings have been upheld by more recent investigators (Taylor 1968).

Phillips (1948) reported that "wood from near the pith consists of smaller elements, often has more resinous tissue and sometimes spiral thickenings and other features not found in adult wood". Rays in the first 20-30 rings (i.e. in juvenile wood) of most species are usually low (Phillips 1948). In juvenile wood, ray type, may also be different from that of mature wood (Stern & Greene 1958).
Scaramuzzi (quoted in de Zeeuw 1965) measured cell wall thickness and its relationship with the lumen diameter in a hybrid poplar (Populus x euramericana cv. 'T214). His study revealed a distinct increase in the wall thickness to lumen diameter ratio outwards from the pith.

The deposition of extractives in heartwood, and the physical differences (colour, density, and smell) that accompany these accumulations, differentiate heartwood from sapwood (Phillips 1948; Panshin & de Zeeuw 1980). Other than the "accelerated formation of tyloses in vessels of hardwoods" (Panshin & de Zeeuw 1980) there are no reported differences between the morphology of wood micro-anatomical features in heartwood and sapwood. Jane (1970) wrote; "histologically, heartwood and sapwood elements are identical".

Verhoff & Knigge (1976) found 'no significant relationship between the sizes and frequencies of warts and the age of the wood within a 92 year old stem of silver fir (Abies alba Mill.).

Lee & Wang (1996) examined latewood tracheid length versus ring number in three trees of Japanese cedar (Cryptomeria japonica Don) and statistically defined the point at which juvenile wood formation ceased and mature wood formation began. This was found to be at the 23rd growth ring, where the tracheid mean length variance component reached zero percent.

6.2.3 Specimen Preparation and Imaging

Transverse faces of the stem cross-sections were finely sanded so that growth rings were clearly visible. Strips of wood, extending across the complete radius of the disc, from bark through to, and including the central core, and approximately 3 cm in width were cut from each section (Plate 6.4). These strips were then sanded, initially using 150-mesh sandpaper and finishing with 500-mesh sandpaper. This highlighted the growth rings. Rings were further distinguished by dampening the prepared surface with water. Every fifth ring from the pith was then marked with pencil and numbered.

After close examination of the growth rings, and preparation of samples from several representative parts of all of the trees, it was decided that the prime source of data for the study would be glau-22 since it appeared to be free of compression and decayed wood, and its grain was straight in the top, middle and base sections of the trunk. It was also decided that data regarding callitroid thickening, warts and rays at selected sampling points in the other three trees (glau-21; glau-23 and glau-24) would be used only to verify some of the findings obtained from glau-22.
Plate 6.3: Sections, 3 cm thick, cut from the top, middle, and base of the trunk of tree glau-22.

Plate 6.4: A strip cut from the base section of glau-22 after completion of fine sanding. From pith (left) to bark (right), 94 growth rings were identified.
The radial strips of wood from glau-22 were sawn into small blocks, each block containing five growth-rings. Owing to the taper of the trunk, this provided 19 specimens from the basal cross-section, 14 from the middle section, and 7 from the top section. TLS and RLS specimens were prepared for SEM from these blocks as described in Chapter 2. All specimens, images and data were positively identified with tree number, stem cross-section (base, middle or top) and tree-ring number\(^2\) (rings from pith) throughout all stages of the study.

Samples from the centre regions of the mature trunk were heartwood. Initially, it was not known how encrustation by extractives and waxes, commonly present in heartwood of *Callitris*, would affect the visibility of anatomical features and whether special cleaning procedures would be required. Eventually it was found that heartwood specimens in TLS commonly exuded extractives from the severed ends of rays when subjected to the hard vacuum of the SEM. This caused severe 'charging' in localised areas of such surfaces. This was not a problem for high magnification images (such as those used for warts and thickening) since the degraded areas could be avoided, and data could be taken from unaffected areas. However, for the low magnification (x100) images, such areas could often not be avoided. It was therefore necessary to immerse cut TLS surfaces in a 15% solution of sodium hypochlorite for five minutes followed by several washings in distilled water (Meylan & Butterfield 1978b) in order to clean them and avoid this problem. All TLS surfaces used for viewing rays at relatively low magnification (including those of the relatively uncontaminated sapwood) were treated in this manner. The cleaned specimens were not used for the study of warts or callitroid thickening in order to avoid possible size or shape artefacts caused by the cleaning process, and which would be more likely to be apparent at high magnification.

The interiors of heartwood tracheids in RLS were also often encrusted with extractives, making it difficult to observe details of callitroid thickening and warts. The encrustation occurred in a variety of forms. However, it was found that while many tracheid walls were obscured by this encrustation, other tracheids, sometimes even those adjacent to fully encrusted ones, were uncontaminated (Plate 6.5) and the latter could be used for the study of within-tracheid features in the normal manner. Thus no special cleaning of RLS specimens was required.

The viewing and measurement of callitroid thickening, warts and rays was carried out as described in Chapters 3, 4, and 5 respectively.

\(^2\) For simplicity, the 'ring number' annotations made on the figures in this Chapter indicate the upper range of the rings included in the specimen. Thus rings 0 to 5 are indicated by 'S' and rings 5 to 10 are indicated by '10' and so on.
6.2.4 Pith to Bark Variation in Callitroid Thickening

On each (RLS) specimen, all visible pits were examined, and the absence of thickening, or its type, (i.e Type 1, 2, 3, or 4) was recorded for each individual pit. Each specimen contained five growth-rings and there were approximately 100 to 250 pits visible on each specimen. A total of 3541 pits, representing 19 specimens in the base section, 2494 pits from 14 specimens in the middle section and 1306 pits from seven specimens in the top section, were examined. Figure 6.2 shows the proportions of pits in which callitroid thickening (of any type) was present in specimens cut from the top, middle, and base of the tree. It can be seen that callitroid thickening of pits occurred at similar frequency (85% to 100%) radially, (from pith to bark) and vertically, (base, middle and top section) throughout the stem of tree glau-22. A regression analysis carried out on these thickening occurrence data confirmed no significant difference in frequency of thickening between sampling points, either radially or vertically, within the stem.

Figure 6.3 shows the proportions of thickening Types 1, 2, and 3 (as percentages of all thickening) at each radial sampling point in the top, middle and base sections of the trunk. Although thickening Type 1 varied between approximately 5 and 20% of the total thickening, a perceptible increase in such thickening occurred from pith to bark at all three vertical levels.

Figure 6.2: Occurrence of callitroid thickening at five-ring intervals from pith to bark at the base, middle and top of the tree. The X-axis indicates rings from pith; the Y-axis indicates proportion of pits thickened.
Figure 6.3: Occurrence of callitroid thickening Types 1, 2 and 3 in the top, middle and base sections of glau-22. The Y-axes show the percentage of total thickening. The X-axes indicate rings from pith.
Type 2 thickening shows an equivalent decrease in proportion from pith to bark (approximately equal to the increase in Type 1 since the proportions of Types 3 and 4 were relatively small). Type 3 thickening was much less common; i.e. a maximum of 3% of all thickening was of this type. However, it was absent from pith to ring 25, and sporadically present from ring 25 to the bark. A similar though less definite situation also occurred for type 4 thickening. It can be inferred, therefore, that in the juvenile wood, thickening Types 1, 3, and 4 are less frequent and Type 2 thickening is more frequent.

In general, these results indicate that callitroid thickening did not vary in its frequency of occurrence either radially or vertically throughout the trunk of this tree. It follows, therefore, that juvenile wood cannot be differentiated from mature wood in regard to this parameter. However, if thickening is classed according to its type (i.e. 1, 2, 3 or 4) then the occurrence of thickening type 3 is clearly indicative of mature, rather than juvenile wood. Variability in thickening Types 1, 2, and 4 is of minor significance.

6.2.5 Pith to Bark Variation in the Warty Layer

The warty layer throughout the stem was comprised of a heterogeneous mixture of two populations of warts; the first population being small and hemispherical; the second population large and nodulated. Descriptions of the two populations of warts making up the warty layer in Callitris were detailed in Chapter 4. In general, there was little difference in wart morphology between vertical and radial regions within the stem.

Further indication of the size distribution of warts at each radial point can be gained from Figure 6.4 which shows that although there were differences in the medians and ranges of wart heights from pith to bark, the differences were random, and there were no general trends in this parameter.

Similarly Figure 6.5 shows that the mean logarithm of wart size varied equally about a value of approximately 5.5 throughout all radial positions. In order to test the statistical significance of this observation, a regression analysis was carried out on the wart size data. This analysis indicated that while there was some evidence of differences relating to distance from pith and height within the trunk, these effects were not significant at the 5% level.

These results indicate that wart size did not vary significantly either radially or vertically throughout the trunk, in accord with Verhoff and Knigge (1976), and suggest that juvenile wood cannot be differentiated from mature wood in C. glaucophylla on the basis of warty layer morphology.
Figure 6.4: Boxplots indicating wart heights at five-ring intervals from pith to bark at the base of tree glau-22 (C. glaucophylla). Each boxplot represents 30-50 warts.

Figure 6.5: Mean log. wart size (\{log.\[ heights multiplied by widths\]}), of all warts in a one mm² area of inner tracheid surface, at five-ring intervals, from pith to bark, at the base, middle, and top of tree glau-22 (C. glaucophylla). The X-axis indicates rings from the pith. The Y-axis indicates mean log. of wart size.
6.2.6 Pith to Bark Variation in Rays

The heights and frequencies of all rays in one mm$^2$ areas of (TLS) samples were measured and the median ray height at each sampling region was calculated. Figure 6.6 plots these median values and it can be seen that rays tended to be short (i.e. less than 100 µm in height) in the region from the pith radially outwards to Ring 20. In contrast, rays in samples more than 20 rings from the pith were generally greater than 100 µm in height. The difference is also apparent in Plates 6.6 and 6.7. Figure 6.7 shows the relationship of ray height and ray frequency to radial position within the trunk. This indicates a grouping of ray height and frequency values for Rings 5, 10 and 15, and a different grouping for rays beyond the 20th ring. This effect is apparent at the base, middle and top of the trunk. A regression analysis indicated significant ($p < 0.001$) differences in the heights and frequencies of rays from samples obtained from the first 15 rings and those from Ring 20 to the bark. This accords with the observation of Phillips (1948) that (for softwoods in general) shorter rays occur in the inner, juvenile wood than in the outer, mature regions of the tree stem.

![Figure 6.6: Median ray height at five-ring intervals, from pith to bark, at the base, middle and top of tree glau-22 (C. glaucophylla). The X-axis indicates radial position within the trunk (rings from pith to bark). The Y-axis indicates median ray height (in µm).](image-url)
Figure 6.7: Scatter diagrams showing mean ray height ($\mu$m) versus ray density (rays/mm$^2$) at various distances from pith to bark, at the base, middle and top of tree glau-22.
Figure 6.8 shows no general differences between the ray volumes of the inner or outer regions at any of the three vertical heights in the tree. This observation accords with Taylor (1968) who investigated the variation in ray volume in yellow poplar (Liriodendron tulipifera L.) trees from pith to bark at two vertical heights (breast height and 20 feet) and reported no significant differences in ray volumes radially, or at these two heights.

The observation that ray volume remains relatively constant from pith to bark may be considered as further evidence of the physiological necessity for the tree to maintain a constant proportion of ray parenchyma cells within its wood (in addition to the evidence of the inverse relationship between ray height and ray density as detailed in Section 5.4.1 of this thesis). Thus the amount of ray tissue in juvenile wood remains similar to that of mature wood although the heights and frequencies of the rays in each region are quite different.

These results indicate that rays are smaller in height and occur at greater frequencies in wood close to the pith than in wood in outer regions of the stem. This suggests that, in this tree, the interface between juvenile wood and mature wood occurred in the region between the 15th and 20th growth ring from the pith.

![Ray volume graph](image-url)

**Figure 6.8:** Ray volume (as a percentage of total volume of wood) at five-ring intervals from pith to bark at the base, middle and top of tree glau-22 (C. glaucophylla). X-axis indicates radial position within the trunk (rings from pith to bark). Y-axis indicates percent ray volume.
6.2.7 Pith to bark Variation: General Comments

Figures 6.2-6.6 indicate that the measured parameters of callitroid thickening, warts and rays on either side of the sapwood/heartwood interfaces (ring 73 in the base section, ring 54 in the mid section and ring 17 in the top section) were the same. Thus the only differences between heartwood and sapwood that were found by this study were those of degree of encrustation by resinous extractives. This accords with the view that sapwood and heartwood differ only in the degree of extractive deposition and not in wood micro-morphology (Jane 1970; Panshin & de Zeeuw 1980).

The finding that some tracheids in heartwood appeared to be completely free of amorphous extractive deposits while others were partly or totally encrusted with them is similar to the report of Koch (1972) who found that the occurrence of resin-filled tracheids in heartwood of various species of southern pine (Pinus spp.) was "sporadic".

Variation in ray morphology with tree height indicated that juvenile characteristics continued in the first-formed 15 rings of the stem even after the tree reached maturity. This confirms that Callitris, in common with other conifers, exhibits excurrent (monopodial) growth; i.e., possesses stems that continue through the crown and end in a terminal leader, and that juvenile wood characteristics continue in this terminal leader throughout 15 rings (years) of growth.

The usefulness of callitroid thickening and warts as taxonomic characters is enhanced as a result of the finding that these characters do not vary greatly between juvenile and mature wood. The study has also demonstrated the value of ray height as an indicator of the occurrence of juvenile wood in C. glaucophylla.

6.3 Variation Occurring Between the Earlywood and Latewood of a Growth Ring

6.3.1 Introduction

In this part of the study of within-tree variation, the same morphological features considered in the previous section, i.e. callitroid thickening, warts and rays, are again investigated, but this time on a much smaller scale of reference; i.e. within an individual growth ring. Wood from rings 91-92 of tree glau-22 was used for the investigation because it was sapwood and was free of encrustation by extractives, the earlywood and latewood were visually prominent, and the wood had very straight grain which facilitated specimen preparation.

6.3.2 Literature Review of Variability Between Earlywood and Latewood

The most manifest difference between earlywood and latewood in softwoods is that "earlywood tracheids tend to be larger in radial diameter and to have thinner walls than those of the latewood" (Panshin & de Zeeuw 1980; Creber & Challoner 1990).

Differences in tracheid length have also been described within individual growth rings in both the radial and vertical planes of stems of softwoods (Zobel & Buijtenen......
Minimum tracheid length occurs in the first portions of the earlywood, and there is a gradual increase in length radially through to the latewood, where maximum tracheid length occurs. Differences in tracheid length within a ring can be as large as 0.5 mm (de Zeeuw 1965).

Wart diameters in coniferous woods are generally smaller in earlywood than in latewood (Baird et al. 1974). Differences in the basal diameters of warts, ranging from 50 to 450 nm in the earlywood to 50 to 850 nm in the latewood of 16 coniferous woods were reported by Ohtani & Fujikawa (1971). In the same study, wart frequencies ranged between 100 and 2300 per 100 µm² across an annual ring, and frequencies were lowest in the last-formed latewood. Verhoff & Knigge (1976) found that the size of warts was reduced in the middle earlywood compared to all other regions of the growth ring in silver fir (A. alba).

Phillips (1948) reported spiral thickening present only in earlywood and not in latewood of Douglas fir (Pseudotsuga menziesii [Mirb.] Franco).

Differences in the number of pits on radial cell walls in black spruce (Picea mariana Mill.) earlywood and latewood were reported by Koran (1974). Earlywood tracheids were found to contain, on average, 53 pits on each radial wall, whereas in latewood, a mean of 15 pits occurred. In addition, the diameters of pits in earlywood were found to average 16.4 µm compared with 6.1 µm in latewood.

6.3.3 Separation of Earlywood and Latewood

It was necessary to clearly and unambiguously differentiate earlywood and latewood in order to select representative tracheids for study. The definition of latewood according to Mork (1928) is:

'latewood cells are those in which double the cell wall thickness is greater than the lumens diameter'

This definition is the most commonly quoted criterion for separating earlywood and latewood (Koran 1974; Zobel & Buijtenen 1989; Creber & Chaloner 1990). However, Denne (1989) has pointed out an ambiguity regarding its interpretation. This ambiguity concerns whether "the cell wall thickness" refers to (a) 'the common cell wall between two adjacent lumina', or to (b) 'the wall thickness of an individual cell'. Because of this, Denne (1989) recommends that the parameters used in the calculation of latewood should always be precisely stated.

In this study, measurements were made of wall thicknesses and respective lumen diameters of 50 tracheids in the latewood region adjacent to the earlywood/latewood interface of Ring 92 in the Base section of glau-22 (C. glaucophylla). On analysis of this data it was found that 49 of the 50 tracheids which appeared to possess the visual
characteristics of latewood were classified as 'latewood' by Mork's criteria when cell wall thickness was defined as 'the common cell wall between two adjacent lumina' i.e. by 'interpretation (a)' referred to above. On the other hand, when 'interpretation (b)' (the wall thickness of an individual cell) was used as the cell wall thickness parameter, 30 of the 50 (visually apparent) latewood cells were not classed as 'latewood' by Mork's definition. Therefore 'interpretation b' was rejected, and in this thesis, where Mork's definition was used to identify latewood, 'cell wall thickness' was defined as 'the common cell wall between two adjacent lumina'.

6.3.4 Within-Growth Ring Variation in Callitroid Thickening

The earlywood-latewood interface of Ring 92 in the base section of tree glau-22 (Plate 6.8) was examined in five different regions. In each region, 100 pits in both the earlywood and latewood were examined, and a record was made of the occurrence and type of thickening (i.e. Type 1, 2, 3, or 4) or its absence. This procedure was repeated in each of the five regions. Figure 6.9 shows the frequencies of occurrence of the various types of callitroid thickening in earlywood and latewood.

![Figure 6.9: Bar chart showing types of thickening occurring in 500 bordered pits in each of the earlywood and latewood areas of a single growth ring (ring 92) in tree glau-22 (C. glaucophylla). The Y-axis indicates percentage of total pits. The X-axis indicates type of thickening (i.e. type 1, 2, 3, or 4) or absence of thickening (none).](image)

It can be seen that in earlywood, callitroid thickening was absent from 5% of pits whereas in latewood, all pits were thickened. In earlywood, Type 1 and 2 thickening
occurred in 35% and 55% of pits respectively, whereas in latewood, only 4% of thickening was of Type 1 and 88% was of Type 2. These results indicate that absence of callitroid thickening, and thickening of Type 1, is more common in earlywood, whereas a high proportion of thickened pits (mainly of Type 2) is typical of latewood.

6.3.5 Within-Growth Ring Variation in Warts

Plate 6.9 shows adjacent latewood and earlywood tracheids in Ring 91 of the Base section of glau-22. It can be seen that although the widths of the tracheids in the earlywood (on left side) and latewood (on right side) are different, the sizes and frequencies of warts in each tracheid are similar. In order to quantify this observation, all warts in individual 12 µm² areas of tracheid on both sides of the earlywood - latewood interface were measured for height and width. The parameter value 'log mean wart size' (i.e. \( \log_e [\text{wart height multiplied by width}] \)) was calculated for each wart, and wart density values (in warts/µm²) were also derived. The process was repeated in earlywood and latewood at five positions along the earlywood - latewood interface of Ring 92. It was found that there was little difference between the sizes and densities of warts in the earlywood and those of latewood within the same growth ring (Figure 6.10).

![Boxplots showing wart heights at five different points in earlywood and latewood of glau-22 (Ring 92).](image)

6.3.6 Within-Growth Ring Variation in Rays

In extending radially outwards from the pith to the cambium, rays pass through each growth-ring in succession. Thus, it follows that when viewed in TLS (in which rays are cut transversely) there will be no difference in their individual morphology or in
their spatial arrangement between the earlywood and latewood of any particular ring. This was confirmed by observation of earlywood and latewood sections cut in Ring 91 in the base section of glau-22. Ray morphology in the earlywood and the latewood was indistinguishable; there was no evidence that "ray cells in wood tend to be taller in earlywood than in the denser latewood", as suggested by Phillips (1948).

### 6.4 Variation Occurring in the Warty Layer Within Individual Tracheids

#### 6.4.1 Within Tracheid Variation: Introduction

Three aspects of variability in the warty layer within tracheids of softwoods have been commented upon in the literature: (i) Warts in pit cavities are reported to be smaller than those lining the inner walls of tracheids; (ii) warts in the 'corners' of tracheids are reported to be larger and to occur at higher frequency than those located centrally on the TLS and RLS walls; and (iii) warts are reported to be smaller within the area bounded by callitroid thickening bars than those outside of the bars. In this part of the study of within-tree variation in wood anatomy of *Callitris*, an investigation was carried out to determine variation in the morphology of the warty layer within individual tracheids in tree glau-22 (*C. glaucophylla*).

#### 6.4.2 Literature Review of Variability in Warts Within Tracheids

The presence of warts within pit chambers (i.e. on the inner wall of the pit border) is reported to be common in softwoods (Liese 1965a; Ohtani *et al.* 1984) and they have been specifically noted in *Fitzroya* (Roig 1992) and in *Libocedrus* (Meylan & Butterfield 1978b). Warts in pit borders are reported to be generally small and not so variable as in tracheids (Liese 1965a).

Warts in the 'corners' of softwood tracheids are generally larger and more concentrated in frequency than those more centrally situated on the RLS and TLS cell wall surfaces. This has been quantitatively described in the Japanese conifers: todomatsu (*Abies sachalinensis* Mast.); sugi (*Cryptomeria japonica* D. Don.); akamatsu (*Pinus densiflora* Sieb et Zucc.); and himekomatsu (*Pinus pentaphylla* Mayr.) by Ohtani & Fujikawa (1971). Similar observations were also made by Baird *et al.* (1974a) for balsam fir (*Abies balsameae* [L.] Mill.) and by Verhoff & Knigge (1976) for silver fir (*A. alba*).

In *Callitris*, warts are reported to occur only on the outer surfaces and tops of callitroid thickenings, and to be much reduced in size, or absent, inside the area bounded by the thickening (Cronshaw 1961; Wardrop 1964; Davis & Ingle 1966; Ilic 1994).

#### 6.4.3 Within-tracheid Variation: Warts in Pit Borders

During preparation of RLS samples there were many occasions when cuts through the middle lamellae of two contiguous tracheids separated the junctions of the two $S_1$
layers, and (unintentionally) laid bare pit chambers in which the inner pit border surface of a particular pit pair could be observed. This presented an opportunity to inspect the warty layer present on the inner pit border surface. In some cases, an exposed pit border happened, by chance, to be immediately adjacent to an exposed tracheid interior, so that the warty layer on the inner tracheid wall and the warty layer within the pit border were visible simultaneously in the SEM image (Plate 6.10). This gave opportunity for warts on the pit border interior to be quantitatively compared with warts lining the walls of adjacent tracheids.

An investigation was carried out on samples from the base section of tree glau-22, Rings 90-94. Warts in pit borders and in adjacent tracheids were imaged at 30,000 times magnification (Plates 6.11 & 6.12) and their basal widths were measured (conducted on an *ad hoc* basis; advantage being taken of exposed pit border interiors and adjacent tracheid warty layers wherever, by chance, they occurred). Observations were made on five different pit border interiors and their respective adjacent tracheid wall linings. Figure 6.11 shows that warts in the pit borders ranged from approximately 85 nm to 180 nm in diameter with median of 140 nm whereas warts in the adjacent tracheids ranged from 120 nm to 440 nm in diameter with median of 170 nm. The smaller size and reduced variability of warts in pit borders compared to those on the tracheid walls accords with the comment of Liese (1965a) who stated that warts in pit borders [of softwoods in general] are "smaller and less variable than warts in tracheids".

![Figure 6.11](image_url)

**Figure 6.11:** Boxplots indicating width of warts measured in tracheids (trac) and in pit borders (pit) at five different locations in sapwood of glau-22 (*C. glaucophylla*).
Plate 6.5 Three adjacent tracheids of heartwood (rings 50-55 in Base section of glau-22); the two on the left showing severe encrustation by extractives and were unsuitable for study whereas the tracheid on the right was uncontaminated and could be used as required.

Plates 6.6-6.7: Comparison of rays in Base section of glau-22. 6 = Rings 5-10; 7 = Rings 90-94.

Plates 6.8-6.9: Comparison of thickening (8) and warts (9) at earlywood/latewood interface.

Plate 6.10: Exposed pit border (marked at left) and tracheid inner wall (marked at right).

Plates 6.11 & 12: Comparison of the sizes of warts in the above pit border (11) and tracheid (12).
6.4.4 Within-tracheid Variation: Warts in Cell Corners

An examination of the RLS/TLS interfaces in several tracheids in the sapwood of glau-22 (C. glaucophylla) failed to find any visible differences between the sizes of warts on the radial and tangential walls or on the interface between them. There was no difference in wart morphology at the RLS/TLS boundaries whether the interface was diffuse or distinct (Plates 6.13-16) or between the RLS/TLS boundaries of earlywood and latewood. Thus, although there are reports of larger warts at higher frequencies on the RLS-TLS interfaces than in the more central regions of the cell wall in some other softwoods (Baird et al. 1974a; Ohtani & Fujikawa 1971; Verhoff & Knigge 1976) this was not observed here for C. glaucophylla.

6.4.5 Within-tracheid Variation: Warts On and Between Callitroid Thickening Bars

An examination of warts on callitroid thickening bars found that whereas large nodular warts occurred on the outer surfaces and tops of callitroid thickening bars (Plate 6.17) warts were much reduced in size or absent from the rectangular areas of the cell wall enclosed by the bars and immediately adjacent to the pit (Plate 6.18). Measurements were made of the diameters of warts situated within the area surrounded by the thickening (Plate 6.19) and also those in areas of tracheid wall located outside the thickening (Plate 6.20). Figure 6.12 shows the wart width distributions.

Figure 6.12: Boxplots showing the width (in nm) of warts on the tracheid wall between thickening bars and those outside the area delimited by the bars.
Plates 6.13-16: Images of warts in cell corners.
Plate 6.17: Warts on outer surface of callitroid thickening bar.
Plate 6.18: View of warts in the area between callitroid thickening bars.
Plates 6.19-20: Comparison of sizes of warts between thickening bars (19) and outside bars (20).
It can be seen that within the thickening bars, widths ranged from 100 to 180 nm with a median of 125 nm. Outside of the bars, the equivalent parameters ranged from 120 to 1000 nm with a median of 140 nm. It was concluded that warts are reduced in size or are absent from the areas bounded by callitroid thickening bars, confirming the findings of Cronshaw (1961); Wardrop (1964); and Davis & Ingle (1966).
Chapter 7

Environmental Effects

The experiment described in this Chapter examines whether the differences in callitroid thickening, wart and ray morphology found in *Callitris* species endemic to wet habitats and those of dry habitats (Chapters 3, 4 and 5) are related to water availability or are permanent adaptive features under genetic control. Trees of three different *Callitris* species were grown in either very wet, or very dry conditions, in an otherwise identical environment. The morphology of thickening, warts and rays in the wood grown under both extremes of water availability was measured and the data compared, using statistical techniques. The findings are discussed in relation to their impact on the taxonomic significance of wood anatomical features in *Callitris*.
7.1 Introduction
7.1.1 Background

Although most wood morphological features are generally "strongly inherited" (Zobel & Buijtenen 1989), some are sensitive indicators of the influence of abiotic factors such as the availability of soil nutrients, water, or light (Baas 1982). Phenotypes with a strong environmental component are not useful for taxonomic and diagnostic purposes (Carlquist 1988), mainly because of the wider variability in their character, which reflects too strongly the vagaries of the environment in which they grew. Thus, in order to decide whether a wood feature is useful as a taxonomic indicator, the environmental and genetic components of its phenotypic variability must be distinguished from each other, and their proportional influences determined. In practice, however, this is usually a very difficult task to achieve and has rarely been done for most wood anatomical features.

Chapters 3, 4 and 5 showed that differences occurred in the frequency of callitroid thickening, wart morphology, and the heights and frequencies of rays between species of Callitris, and that these phenotypic characteristics differed most prominently between species native to dry habitats compared with those endemic to wet environmental conditions. Thus, pits with a high proportion of callitroid thickening, large, complex, nodulated warts, and short rays were shown to be typical of dry-habitat species, while the low occurrence of callitroid thickening, small, hemispherical warts, and tall rays were more characteristic of wet-habitat species. However, the descriptions of species in these earlier Chapters were based on observations made of wood specimens grown under natural environmental conditions. Therefore no attempt was made to determine whether these phenotypic differences were inherent to the particular species in which they occurred, or were the result of growth variation due to differences in water availability between the respective environments in which the trees were grown. Knowledge of the relative influences of 'genes and environment' on callitroid thickening, wart and ray morphology would be desirable before any practical taxonomic applications of the differences in these features could be considered.

In her review of Morphology of warts in the tracheids of cypress pine (Callitris Vent.) (Heady et al. 1994 -Appendix 2), Wheeler, stated that:

"The authors are dealing with a quantitative rather than a qualitative trait, and they do a good job in tying the expression of wart morphology to ecological factors. While I have no doubt that the ability to form warts in Callitris is genetically fixed, is there any evidence that features such as size, nodulation, etc, are? Perhaps under water stress, the duration of cell wall deposition is increased thus leading to larger warts and joining of warts in any species should it grow in arid conditions. If such be the case then wart morphology would have no true taxonomic significance. This question could best be determined by growing different species side by side under identical conditions (often easier said than done)".
Wheeler went on to say that this was a common, unstated problem in many wood anatomy studies. However, her criticism was accepted, and based on her final suggestion regarding "growing different species side-by-side under identical conditions", an experiment was undertaken to determine the effect of water availability on the morphology of warts in the wood of *Callitris*. Trees were grown in a controlled-environment in which soil water was varied, being either very wet, or very dry. All other environmental factors were kept identical for all of the trees. Wood of trees grown under wet conditions was compared with wood of the same species formed in trees grown under dry conditions. Conclusions could then be drawn as to whether the differences in wood anatomy that were noted in previous chapters were due to the availability of soil moisture, or due to 'genetically fixed' factors. It was also decided to examine not only the micro-morphology of the warty layer, as suggested by Wheeler, but also callitroid thickening and rays, since, as mentioned above, the anatomy of these features also varied between the *Callitris* species endemic to dry and wet habitats.

It was realised that a severe limitation on the proposed experiment was the length of time available to grow the trees from which wood samples would be obtained. Thus the period of growth of the trees under the different experimental conditions was limited by circumstances to approximately 15 months. Based on the finding described in Chapter 6, (i.e. that the transition from juvenile wood to mature wood occurred in *Callitris* after 20-25 years, a study of mature wood was not possible. Subsequent descriptions therefore only involve juvenile wood grown under two different extremes of soil moisture availability.

### 7.1.2. Literature Review of Environmental Effects on Wood Morphology

The effects of drought on the development of secondary xylem has been discussed by Creber & Chaloner (1990). They point out that for xylem initials to differentiate and expand to full-sized tracheids, an adequate supply of water is required, and that if this is not forthcoming, narrower tracheids result within the growth ring for that year. Creber & Chaloner (1990) further state that if the water shortages are followed by a resumption in water availability, large cells are once again formed, and the band of small cells within the ring is a 'false ring'.

In a study of the effects of water supply on the development of wood, Larson (1963) subjected five-year-old red pine (*Pinus resinosa* Ait.) trees to artificial drought periods of varying duration during their growing season. These drought conditions were produced by withholding water from trees grown under otherwise 'normal' glasshouse conditions. A single drought period (of three weeks) was found to result in a zone of narrow-diameter latewood tracheids and the formation of a false growth ring. Two drought periods produced two false rings. Larson (1963) was therefore able to correlate the formation of these false rings with decreased needle elongation and auxin synthesis,
and concluded that the influence of drought was directly on the terminal meristems, and
only indirectly on tracheid diameter through the intermediate action of the auxin.

Zahner et al. (1964) investigated the effect of artificial drought periods on the
earlywood and latewood features of six 20-year-old P. resinosa trees. Three trees were
irrigated, and three were subjected to artificial drought, for a period of one year. The
growth ring produced during drought was then analysed for its gross anatomical features
and it was shown that low internal moisture stress resulted in prolonged formation of
earlywood tracheids whereas drought conditions resulted in "rapid transition to
flattened latewood tracheids and cessation of growth". It was concluded by these
authors that the anatomy of the latewood portion of the growth ring was strongly
influenced by water deficit.

Nicholls (1971) studied the effect of supplementary soil moisture on wood
characteristics of radiata pine (Pinus radiata D. Don.) growing in a plantation at Mt
Crawford, 40 km north-east of Adelaide, South Australia, an area in which trees may be
expected to be under some moisture stress during summer. Supplementary water was
applied at the rate of 25 mm per week during summer and early autumn. The relief of
water stress during the driest months of the year resulted in increases in stem diameters,
and the proportion of thick walled (latewood) cells was increased from approximately
20% to 30%. Average tracheid length was not affected by supplementary watering.

Several studies (Lange 1965; Pearman 1971; Ash 1983) have found a correlation
between growth ring width in Callitris and water (rainfall) availability. In an examination
of growth rings in trees of C. macleayana, Ash (1983) concluded that a correlation of
0.74 occurred between annual growth ring width, and the duration of the wet season in
their rainforest habitat, the Atherton Tablelands of Queensland. Lange (1965)
investigated the occurrence of false growth rings in C. glaucophylla (using its synonym
of C. columellaris) in trees from a very low rainfall area near Woomera, South Australia.
He found that trees sometimes produced more than one ring per year, and in other
years, none, depending upon the occurrence of rainfall in the region. Pearman (1971)
examined C. preissii, growing in Perth (Western Australia) and found some correlation
between rainfall and tree ring occurrence.

7.1.3. Aims, Considerations and Requirements

The aim of this part of the study was to determine the influence of water
availability in the growing environment of Callitris trees on the morphology of callitroid
thickening, warts, and rays of their wood.

There were three major tasks involved in the experiment. The first was to grow a
number of genetically-similar Callitris trees under wet or dry conditions with all other
environmental conditions kept identical. The second task was to examine and measure
the morphology of callitroid thickening, warts and rays in the wood grown under wet
and dry conditions. Finally, it was necessary to analyse data statistically, and relate
morphological differences in the wood features (if any) to water availability during growth.

The choice of species for the experiment was influenced by the availability and viability of seedling trees since it was important to have the longest possible growing period for the experiment. A study of all Callitris species was not practical; there were no available sources of seed for many species, and seed of some species failed to germinate within a ten-week period after sowing. It was considered essential, however, to grow a species with wet-habitat morphology (i.e. low occurrence of thickening, small hemispherical warts and relatively tall rays, i.e. C. intratropica, C. macleayana, C. neocaledonica and C. sulcata, and also a representative of species with the dry-habitat morphology (i.e. high frequency of thickening, large nodulated warts, and short rays), most prominent in C. endlicheri, C. glaucophylla, C. tuberculata and C. verrucosa). Callitris glaucophylla was included in the experiment since it is a dry-habitat species and the typical morphology of juvenile wood in this species was already known (Chapter 6). Because of the rapid germination of their seeds, two other species were selected; C. intratropica representing a 'wet-environment' species, and C. endlicheri representing an additional 'dry-environment' species.

Genetic factors, likely to cause variability in the resulting phenotypes, were minimised as much as possible. All trees for each particular species were from the same seed-stock. Seed of C. endlicheri was obtained from five cones taken from a single tree growing near Canberra, Australian Capital Territory (ACT); that of C. intratropica was obtained from three cones collected from a single tree growing near Palmerston, Northern Territory (NT). After germination, seedlings of similar size, and vitality, were selected. Those of excessively strong or weak appearance, or those with stems that were not straight and upright, were not used in the experiment. In the case of C. glaucophylla, one-year-old tube-stock seedlings of similar appearance were obtained from the Government Nursery in Forbes, NSW.

The 'controlled-environment', in which each tree was grown, was a pot, on a bench-top, in a glasshouse. Soil type, and freedom for root growth (i.e. pot size and amount of soil in the pot) was the same for all trees of each species. The temperature in the glasshouse was maintained at 20 °C. Possible environmental differences between the two groups (dry and wet) caused by their individual positions on the greenhouse bench, i.e. differences in light availability, ventilation or shading by trees in adjacent pots, were minimised by randomisation and replication of pot positions in a manner recommended in general terms by Clarke (1980) and more specifically, by the Statistical Consulting Unit of The Australian National University, Canberra. This involved dividing the bench space into areas, and locating one pot for each treatment method (i.e. a wet and a dry pair) for each of the three species in each area, and replicating this pattern of arrangement subsequently on the bench to involve all pots (Figure 7.1). The number of plants of each species had to be sufficient for several repetitions of wet/dry pairs, but
was limited by the amount of space available within the greenhouse (a bench measuring 4.3 m by 0.7 m). This afforded space for nine wet/dry pair repetitions; i.e. nine trees of each of the three species to be grown under wet conditions, and nine trees of each species to be grown under dry conditions (54 trees in toto).

![Figure 7.1](image)

**Figure 7.1:** Arrangement of trees on glasshouse bench showing the randomisation and replication of pot positions used in the experiment. Each cell represents the position of a pot on the bench. The alphanumeric coded information is as follows:

- **Species of Callitris:** G = glaucophylla; E = endlicheri; I = intratropica
- **Specimen Number:** (1 to 9)
- **Treatment Type:** W = wet; D = dry

It was decided that trees grown under wet conditions would be maintained with unlimited water availability in continuously damp soil and those grown under dry conditions would be grown in relatively dry soil for an identical period. Both the wet and the dry conditions were intended to be sufficiently drastic to affect the phenotypes (i.e. influence wood morphology), but it was equally important that conditions were not so stressful as to kill the trees. It was realised that restricting the amount of water being given to immature *Callitris* seedlings without risk of them dying is difficult; if water-stress becomes too acute, then the tree will never recover, regardless of how much water the tree is then given (D. Jones pers. com.). Thus the degree of water-stress endured by the trees would be impossible to quantify in a meaningful way, and it would be possible only to compare trees grown in relatively dry conditions with those grown in relatively wet conditions. Furthermore, no attempt would be made to produce the 'natural' water conditions for any of the trees since these conditions were not known and it was unlikely that, even if they were known, they could have been successfully emulated, in a practical sense, throughout the growing period.

Prior to setting-up the experiment, there was concern as to whether wood from small tree stems less than two years old (5 mm to 10 mm in diameter) could be cut and prepared for SEM imaging using the techniques developed (in Chapter 2) for large wood specimens or cores. Of particular concern was the ability to obtain specimens with true TLS surfaces in view of the high curvature of tangential surfaces in two-year-old stems. Also, it was not known whether trees at the end of the two-year growing period would be large enough to provide sufficient material for preparation of SEM specimens. A preliminary study was therefore undertaken to investigate the validity of these
concerns. Several one-year-old tube-stock seedlings of *C. glaucophylla* were used to prepare SEM specimens. Results showed that sample preparation, SEM imaging, and acquisition of the necessary data was feasible in a reliable and reproducible manner. In addition, it was shown (Chapter 6), that micro-anatomical variation could be successfully quantified in wood from the innermost rings (i.e. 0-5 rings from the pith) in *C. glaucophylla*.

This study was essentially an experiment in which the wood anatomy of trees after growth under two different 'treatments' (i.e. two different conditions of water availability) was compared. Hence it follows that an essential part of the experimental design was to determine whether these treatments affected tree growth and wood anatomical features in general. If, for example, there were no differences in the callitroid thickening and wart and ray morphology of trees grown under 'dry' and 'wet' conditions, then it would be essential to show that the results were not simply a manifestation of insufficient differences in water availability for the two treatments. For this reason, it was decided to demonstrate the effect of water availability on the morphology of wood by examining stem widths and tracheid diameters in trees subjected to the two treatments. The former parameter was chosen because an effect of wet and dry treatments on stem size was noted during the routine stem measurements that were taken during the experiment. Tracheid diameter was chosen because the effect of water availability on tracheid diameter in *Callitris* trees growing in arid environments has already been reported in the literature (Lange 1965; Pearman 1971; Ash 1983). Thus at the conclusion of the growing period, transverse (TS) sections of stems of all of the trees involved in the experiment were prepared and imaged, stem diameters were measured, and the number of tracheids present in a standard area of TS surface was determined. The acquired data were then statistically analysed to determine differences between the wood subjected to the two types of treatment.

**7.2 Materials and Methods**

**7.2.1. Germination and Growth of Trees**

Seed of both *C. intratropica* and *C. endlicheri* was sown in loamy soil contained in seedling punnets measuring 13 x 7 x 4 cm. Germination occurred within three weeks. After one month's growth the seedlings were pricked-out individually into long-bottomed pots measuring 5 x 5 x 12.5 cm (Plate 7.1). After a further six weeks of growth, 18 trees of each species, all of similar height, stem diameter, and general appearance, were selected for use in the experiment. These seedling trees (Plate 7.2) were transplanted into the pots that were used throughout the remainder of the experiment. 'Propagation pots' of 19 cm diameter and 19 cm depth were used for *C. intratropica* and 'Forestry pots' 15 cm square at the top and of 24 cm depth were used for *C. endlicheri*. The 'tubestock' tree seedlings of *C. glaucophylla* (Plate 7.3) were transplanted into pots of the same size and shape as those used for *C. intratropica*. Soil
Figures 7.1 – 7.3:

1= Newly germinated *Callitris* seedlings pricked out into 5 x 5 x 12.5 cm pots.

2= Tree of *C. endlicheri* ten weeks after germination.

3= Tubestock tree of *C. glaucophylla* prior to start of experiment.
used in the pots was standard potting mix (a sterilised mixture of one third each of peat, sand and soil). No fertiliser was added. The transplanted trees were allowed to consolidate for a few weeks before being subjected to experimental conditions in Glasshouse 16 at the rear of the Research School of Biological Sciences at The Australian National University, Canberra, ACT (Plates 7.4 and 7.5).

A water-proof, plastic tray was placed under each individual pot. The purpose of these trays was to prevent water movement between the drainage holes at the bases of the pots. Also, since the trays of the over-watered trees tended to remain continuously filled with water, they also effectively increased the availability of water for these trees. Trees kept under wet conditions were watered at least twice in one day for two days per week, three days per week in the summer. Watering was carried out from a handheld hose, the water being sprayed directly onto the soil in the pots (not on the foliage). Trees kept under dry conditions were checked daily and watered only when appearing overly water-stressed. The amount of water applied to trees varied according to tree appearance, and was not recorded. Trees were monitored daily for signs of acute stress and the watering for any individual tree was adjusted accordingly. An indication of the amount of moisture in the pots could be achieved by weighing them, but because of the practical difficulty of carrying this out on a daily basis, it was considered that monitoring tree appearance was a more practical indicator of water availability. In under-watered trees, stress was indicated by slight wilting of the leaves and stems, and by a change in the normal leaf colour of the species to a lighter shade of green, particularly leaves close to the stem and in the lower parts of the tree (this was, however, difficult to discern for the glaucous leaves of C. glaucophylla). Over-watered trees of all three species showed no signs of being stressed (by too much water) at any stage during growth. Moss was observed to cover the soil surface in pots of over-watered trees, but was absent from pots of trees kept under dry conditions (Plate 7.6).

Tree stem diameters, and tree heights (soil level to top of tree foliage) were recorded at regular intervals during growth. Stem diameters were measured using digital callipers and tree heights by means of a ruler. These measurements were used only to monitor tree growth, and the information gained was not subjected to detailed analysis. After six months growth, the leaves and branches growing around the lower parts of the boles of trees were removed to a height approximately 3 cm above ground level. This was undertaken to avoid the formation of tracks and needle traces in the region of the stem that was to form the wood sample after the growing period. This was particularly important for C. endlicheri, the seedlings of which tended to retain needles to ground level.
Figures 7.4 - 7.6:
4= View of the glasshouse in which trees were grown.
5= Trees growing in pots on bench-top in the glasshouse.
6= Trees in late stage of growth.
At the time of harvesting, all trees were alive. Trees of *C. endlicheri* and *C. glaucophylla* had fully adult leaves whereas *C. intratropica* trees still retained a major proportion of juvenile leaves. None of the trees had reached a sexually reproductive stage.

### 7.2.2 Specimen Preparation and SEM Imaging

Since wood that has been freshly taken from a tree is far easier to cut and prepare for SEM than dry wood samples, specimens were prepared immediately after removal from the tree. Cutting and preparation of all specimens took approximately four weeks to complete and paired wet/dry trees were progressively harvested over this period. The growing periods for individual pairs of trees ranged from 455 to 480 days. Photographs of some of the pairs of trees of each species at the completion of the growing period are shown in Plates 7.7 to 7.15.

Specimen preparation consisted firstly of removing a section of stem, approximately four cm in length from the lower bole of each tree, just above the soil line. The stem section was then sawn transversely (cross-cut) into samples approximately 8 mm in length. It was preferable to saw the stems rather than cut them with a blade in order to reduce distortion or compression of the wood specimen. The bark layer was removed and discarded, and the samples were immediately immersed in water in order to retain their softness for the cutting operation. TLS surfaces were cut by forming a flat surface approximately 2 mm wide at a tangent to the outer circumference of the stem. RLS surfaces were cut by first splitting the centre of the stem, and shaving off a very thin slice of the split surface to form a smooth cut surface on the remainder.

Imaging was restricted to areas of the outer parts of the stem for both TLS and RLS sections. This was essential in the case of *C. glaucophylla* trees which were one-year-old tubestock at the start of the experiment and therefore required observations to exclude the centre of the stem (representing wood growth prior to the controlled conditions). Viewing the outer parts of the stem also ensured that the most recently-formed wood was used. Areas of sample in which needle traces were apparent (Plates 7.16 and 7.17) were avoided during the data acquisition process.

The stem area of each tree was determined by viewing a transverse section of stem in the SEM, and measuring two diameters, at right angles to each other. This process was repeated for all trees involved in the study. Stem areas for all trees were then calculated.
Photographs of *C. endlicheri* trees (wet/dry pairs) E8, E9, and E6 at the end of the growing period. The labelling on the pots indicates specimen number and treatment type: W = wet; D = dry.

**Plates 7.7-7.9**
Plates 7.10-7.12

Photographs of *C. glaucophylla* trees (wet/dry pairs) G5, G9, and G3 at the end of the growing period. The labelling on the pots indicates specimen number and treatment type:

\[ W = \text{wet}; \quad D = \text{dry}. \]
Plates 7.13-7.15

Photographs of *C. intratropica* trees (wet/dry pairs) 12, 15, and 18 at the end of the growing period. The labelling on the pots indicates specimen number and treatment type: 
W = wet; D = dry.
Each TS specimen was then viewed at a position close to its outer perimeter, at a magnification of 1000 times. This resulted in a standard area 121 x 90.5 µm (i.e. 0.011 mm²) being imaged. A count was then made of the number of tracheids present in the image. Tracheids which were not fully contained within the borders of the image were included in the count if they straddled the upper or right-hand border, and not included in the count if they straddled the lower or left-hand borders. Ray cells in the imaged area were avoided by moving to a slightly different imaging position if they were originally in view. This procedure was then repeated at three other positions, all at right angles to each other, at a position near the outer edge of the TS face of each tree, for all trees involved in the experiment.

Quantitative data on callitroid thickening, warts and rays in trees grown under wet and dry conditions was obtained in a manner similar to that described in Chapters 3, 4, and 5. Data regarding callitroid thickening was acquired by viewing each specimen in RLS, and recording the occurrence of thickening, and its type (i.e 1, 2, 3, or 4), on all visible pits (approximately 100-250 per specimen).

The methods used to analyse wart morphology were the same as those used in Chapter 4; i.e. data were acquired by observing tracheids in RLS, at 55° tilt, and at 30,000 times magnification. In order to reduce the possible influence of the size of tracheids on wart morphology, when selecting sites for imaging, tracheids of similar lumen width were selected for each wet/dry pair. The heights and widths (at base) of each individual wart for all warts in each image were recorded. From these measurements, wart size values (i.e. \( \log_e \) height multiplied by \( \log_e \) width) were calculated.

Measurement of ray height was carried out by viewing 1.08 mm² areas (corresponding to the SEM image at a magnification of 100 times) in TLS-faced specimens representing the outermost regions of the stems. In the imaged areas, 90 to 130 rays typically occurred in each specimen. The height of each ray was determined in the form of a count of the number of cells in the TLS view of the ray. These data acquisition procedures were repeated for all of the 54 trees involved in the experiment.

### 7.3. Results

#### 7.3.1 Comparison of Stem Areas and Tracheid Sizes

The boxplots of Figure 7.2 represent the stem areas of the nine 'wet' and the nine 'dry' trees of each species. It can be seen that stem areas tended to be greater in the trees grown in wet conditions than those grown in dry conditions. For example, stem areas of the trees of *C. endlicheri* that were grown under wet conditions ranged from 62 to 113 mm² and the median was 84 mm², whereas for trees grown under dry conditions the stem areas ranged from 34 to 82 mm² and the median was 58 mm². Plates 7.18 and 7.19 show this trend in the wet/dry pair E6 (*C. endlicheri*). The stem area data were compared by ANOVA. Results indicated that there was a highly significant difference
between the stem areas of trees grown in wet and those grown in dry conditions.

![Stem area boxplots](image)

**Figure 7.2:** Boxplots showing stem areas of *C. endlicheri*, *C. glaucophylla*, and *C. intratropica* trees grown under wet and dry conditions.

Tracheids of 'wet treatment' trees tended to be homogeneous in size and shape and to occur in ordered 'rows' whereas tracheids of 'dry treatment' trees were typically heterogeneous in size and shape and were much less ordered in their distribution. The difference is apparent in Plates 7.20 - 7.23. In Figure 7.3, the boxplots show numbers of tracheids counted in four 0.011 mm² areas of tracheid stem in each of the nine 'wet' and nine 'dry' trees for the different species. There tended to be more tracheids present in trees grown under dry conditions than in those grown under wet conditions, i.e. tracheids tended to be smaller so that there were more of them per given area when grown under dry than wet conditions. For example, for the nine trees of *C. endlicheri* that were grown under wet conditions, the number of tracheids counted (per 0.011 mm²) ranged between 158 and 194 and the median was 169, whereas for trees grown under dry conditions, the number of tracheids in areas of the same size ranged between 165 and 236 and the median was 198. Similar observations were made for *C. glaucophylla* and *C. intratropica*. There were also species differences; in *C. intratropica* there were fewer tracheids per given area than for *C. endlicheri* and *C. glaucophylla*. Statistical analysis confirmed that, in all three species, there was a highly significant difference (p < 0.001) between the numbers of tracheids per mm² in trees grown in a wet environment and those grown in a dry environment.
Plates 7.16 & 7.17 = Needle traces in wood samples, as seen in TLS (16) and RLS (17).
Plates 18 & 19 = Transverse sections of stems of wet/dry pair E6 (C. endlicheri):
18 = wet treatment, 19 = dry treatment.
Plates 20 to 23 = Transverse sections, all at 1000x magnification, showing tracheid sizes in
wood of sample G7 (C. glaucophylla) grown in wet and dry conditions:
20 = wet treatment, 21 = dry treatment.
22 = wet treatment, 23 = dry treatment.
7.3.2 Comparison of the Occurrence of Callitroid Thickening

Table 7.1 shows the frequencies of pits with callitroid thickening (Type 1 and Type 2, and pits with no thickening) for trees grown under wet and dry conditions (Type 3 and Type 4 thickening was not recorded for any of the trees involved in the study).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2 Pits exam.</th>
<th>3 Pits exam.</th>
<th>4 % thickening</th>
<th>5 % thickening</th>
<th>6 % type 1</th>
<th>7 % type 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>species</td>
<td></td>
<td>wet</td>
<td>dry</td>
<td>wet</td>
<td>dry</td>
<td>wet</td>
<td>dry</td>
</tr>
<tr>
<td>endlicheri</td>
<td>1390</td>
<td>1294</td>
<td>61.5</td>
<td>66.9</td>
<td>35.5</td>
<td>17.4</td>
<td></td>
</tr>
<tr>
<td>glaucophylla</td>
<td>1305</td>
<td>1322</td>
<td>63.6</td>
<td>63.7</td>
<td>27.1</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>intratropica</td>
<td>1532</td>
<td>1518</td>
<td>0</td>
<td>1.3</td>
<td>-</td>
<td>68.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.1: Occurrence of callitroid thickening in *C. endlicheri*, *C. glaucophylla* and *C. intratropica* trees grown in either very wet or very dry soil moisture conditions. Columns 2 and 3 indicate total numbers of pits examined in each species for each type of treatment (i.e. either wet or dry). Columns 4 and 5 show the percentages of pits on which thickening occurred for wet and dry treatments. Columns 6 and 7 show the percentages of all thickening that was of 'type 1' for wet and dry treatments.

In *C. endlicheri*, the frequency of callitroid thickening was slightly higher in the trees grown under dry conditions, 66.9%, compared with 61.5% in trees grown in wet conditions. In the trees grown under dry conditions, Type 1 callitroid thickening
occurred in 17.4% of the pits compared with a value of 35.5% for pits of trees grown in wet conditions.

For *C. glaucophylla*, callitroid thickening occurred at a frequency of approximately 63% for trees grown under both wet and dry conditions. Plate 7.23 shows occurrence of thickening in Tree G8 grown under wet conditions. Where thickening of pits occurred, 27% was of Type 1 in trees were grown under wet conditions whereas 24% was of Type 1 in trees grown under dry conditions.

Table 7.1 also shows that of 1,532 pits observed in the 9 samples of *C. intratropica* grown under wet conditions, none had associated thickening bars. Of 1,518 pits in the 9 samples of the same species grown under dry conditions, isolated occurrences of thickening in five of the nine trees were found, and this constituted 1.3% of total pits. Where thickening occurred, it was only weakly visible and it was Type 1 thickening in 68.1% of pits.

Statistical analysis of thickening data for *C. endlicheri* and *C. glaucophylla* indicated that there was no significant difference between the frequencies of occurrence of thickening in trees grown in wet or in dry conditions in either species.

### 7.3.3 Comparison of Warty Layer Morphology

Warts in all three species had shapes similar to those found in mature wood, as described in Chapter 4; nodules occurred on warts in *C. endlicheri* (Plate 7.24) and *C. glaucophylla* (Plate 7.25) and only small, hemispherical warts were found in *C. intratropica* (Plate 7.26). These basic shapes were retained in all three species regardless of treatment type (wet or dry).

A variance analysis of wart size (log. [height multiplied by width]) indicated there was no significant difference in the wart morphology in wood from trees grown in wet or dry conditions in any of the three species, although there was a highly significant ($p < 0.001$) difference between species.

### 7.3.4 Comparison of Ray Morphology

Plates 7.27 to 7.30 show TLS surfaces of wood of *C. glaucophylla*, and *C. intratropica* grown under both wet and dry conditions. No effect of treatment on ray height is apparent in these plates. It can be seen that rays are short (typically 1-3 cells) and that their frequency (density) is high. This conforms with the finding in Chapter 6 that rays in juvenile wood are shorter and occur at higher frequencies than in mature wood. Figure 7.4 shows frequency histograms of ray heights (numbers of cells) in wood grown under wet and dry conditions.

The ray height data were compared by variance components analysis. This indicated that there were no significant differences between the ray heights of trees grown in wet conditions and those grown in dry conditions. There were, however, large between-species differences.
Figure 7.4: Frequency histograms showing numbers of cells in rays of *C. endlicheri*, *C. glaucophylla*, and *C. intratropica* grown under wet and dry conditions.
Plates 7.23 - 7.30: Various aspects of the morphology of wood grown in either wet or dry conditions:

23 = Callitroid thickening in sample G8 (C. glaucophylla) grown in wet conditions.
24 = Warts in sample E1 (C. endlicheri) grown in wet conditions.
25 = Warts in sample G5 (C. glaucophylla) grown in wet conditions.
26 = Warts in sample I5 (C. intratropica) grown in dry conditions.
27 & 28 = Rays in sample G5 (C. glaucophylla) grown in wet (27) and dry (28) conditions.
29 & 30 = Rays in sample I3 (C. intratropica) grown in wet (29) and dry (30) conditions.
7.4. Discussion and Conclusions

The suggestion of Wheeler (outlined in the introduction to this Chapter) that "larger warts and joining of warts [may occur] in any species should it grow in arid conditions" was not supported by the results of this study. There was no significant difference between the sizes of warts in (juvenile) wood of *C. intratropica* trees grown in dry or wet conditions. In addition, there was no significant reduction in wart size in wood of the dry-habitat species *C. endlicheri* and *C. glaucophylla* grown in very wet conditions compared to those from trees grown in dry conditions. Thus, the results suggest that wart morphology is genetically controlled, and is not under the environmental influence of moisture availability. It follows, therefore, that this study supports the use of wart morphology as a feature for the taxonomic differentiation of *Callitris* species.

In general, despite some small differences which were noted, a similar conclusion can be reached with regard to the frequency of callitroid thickening of pits. Plate 7.23 shows strong thickening present in wood of *C. glaucophylla* grown in very wet conditions, and statistical analysis indicated that there was no significant difference in thickening of woods of the dry-habitat species *C. endlicheri* and *C. glaucophylla* grown under wet conditions and those grown in dry conditions. It was not possible to include *C. intratropica* in the analysis because of the very low levels of thickening found in this species. However, since in Chapter 3 it was shown that thickening frequency in narrow tracheids (i.e. latewood) was higher than in wide tracheids (i.e. earlywood), and in Section 7.3.1 it was shown that narrower tracheids occurred in trees given 'dry' treatment, it is possible that the small differences in callitroid thickening that occurred between the *C. intratropica* trees of wet and dry treatments is a result of the differences in their tracheid widths.

Results suggested that ray height was not affected by water availability since no significant difference between ray heights of wood grown under wet and dry conditions was found.

Measurements of stem areas and counts of tracheid numbers per unit area (i.e. tracheid size) indicated that these parameters were significantly affected (p < 0.001) by water availability. It can be argued therefore, that the two different 'treatments' of the experiment (i.e. the two conditions of water availability) were sufficiently intense to affect certain aspects of the wood anatomy of the trees and it can be therefore further argued that the lack of a significant difference between the wet-environment and dry-environment trees in respect to callitroid thickening frequency, wart size, and ray height and frequency was not the result of insufficient or inadequate treatment.

These findings refer only to juvenile wood and the extent to which they relate to the morphology of mature wood is still a matter of conjecture. It is possible that the resilience of juvenile trees to water restriction or over-supply is of adaptive benefit to the species in a manner that does not affect the mature tree. For example, root
penetration into the soil is much less for juvenile trees, therefore it is likely that the shallower-rooted juvenile trees would need to be more resilient to flooding or drought of the upper layers of the soil. Further experimentation, of the type undertaken in this study can be suggested, but based on experience here, it would be increasingly difficult to maintain the glasshouse experimental conditions over longer periods; tree crowns become too large for the allocated space, root systems too large for pots, and it becomes more difficult to maintain water-stress conditions for larger trees without risk of mortality. Plantation-grown trees, subjected to control of the water by irrigation or protection from rainfall, could be a viable alternative. For example, in the study by Nicholls (1971), trees of *P. radiata* in which natural rainfall was supplemented by irrigation showed a significant increase in their tracheid widths over those which were not irrigated. Similarly, Nicholls & Waring (1977) achieved decreased growth ring width by reducing the availability of water to plantation-grown trees. This partial droughting was carried out by constructing sloping platforms over the plots and covering them with bituminous sheeting (water intercepted by the sheeting was directed away from the plots by guttering). A similar field experiment, using *Callitris*, and with subsequent comparison of callitroid thickening, warts and rays would be a useful addition to the experiment described here. Such an experiment could be extended over a much longer period of time than is possible in controlled environment conditions in a glasshouse, and would therefore allow mature, rather than juvenile wood to be examined.

It can be concluded that, in general, the degree of water availability in the growing environment of juvenile *Callitris* trees has minimal effects on the morphology of callitroid thickening, warts, and rays in their wood. This study therefore gives some indication that the general dichotomy that exists in the features between *Callitris* species native to wet environments compared to those endemic to dry conditions, is primarily genetic, and not environmentally determined.
Chapter 8

Taxonomic Resolution

In this Chapter, a three-part study examining aspects of the application of callitroid thickening, wart and ray morphology to the taxonomy of *Callitris* is described. In the first part, these wood features are used in an attempt to taxonomically separate three *Callitris* species that are reported to be difficult to distinguish from each other; *C. columnellaris*, *C. glaucophylla* and *C. intratropica*. The second part involves a survey of the occurrence of callitroid thickening and large, nodulated warts in wood of several genera closely related to *Callitris*. In the third part, the woods of *C. endlicheri* and *C. glaucophylla* are distinguished by means of differences in their wart morphology.
8.1. Introduction

8.1.1 Background to the Objectives

In Chapters 3, 4, 5, and 7, some of the between-species differences in the frequency of callitroid thickening and the morphology of warts and rays appeared to be sufficiently consistent to be of possible use in the taxonomic separation of species of *Callitris*. For example, in *C. glaucophylla*, callitroid thickening always had a high frequency of occurrence, large and nodulated warts were present in the warty layers of all specimens, and median ray height was 14 cells whereas in *C. intratropica* the thickening had a much lower frequency of occurrence, warts were small and hemispherical, and median ray height was 18 cells. In general, this characteristic morphology was maintained throughout all samples of species and, as shown in Chapter 6 (for *C. glaucophylla*), varied to only a minor degree between juvenile and mature wood and between earlywood and latewood. Furthermore, Chapter 7 showed that callitroid thickening and wart and ray morphology was largely unaffected when trees were grown under conditions of differing moisture availability, suggesting that between-species differences in such anatomical features are under genetic, rather than environmental, control. While the species-dependent variability in wood anatomy was of interest from a functional and adaptive point of view, it was thought that such differences could also be used taxonomically to differentiate *Callitris* species not readily separated by other means.

Identification of *Callitris* species using wood micro-features would be of great taxonomic value since some species within the genus are very difficult to separate from each other by comparison of key features of their leaves and cones, a problem that has been fully discussed by Venning (1979). It would also be of use for the taxonomic identification of unknown wood or timber samples, and, since wood features tend to be persistent and not prone to deterioration due to ageing (Barefoot & Hankins 1982; Smith *et al.* 1995), the possibility of using callitroid thickening, warts, and rays in the generic and specific identification of archaeological wood or charcoal, is a very real and attractive one.

This Chapter consists of three different studies, each involving the application of earlier findings regarding callitroid thickening, wart, or ray morphology for taxonomic purposes. The first application involves three *Callitris* species which have been the subject of much taxonomic controversy over a period of several decades due to the inability of 'conventional' (i.e. cone and leaf morphology) taxonomic techniques to distinguish them. The three species; *C. columellaris*, *C. glaucophylla*, and *C. intratropica*, were originally combined as a single taxon (*C. columellaris*) by Blake (1959) but were later designated as distinct species by Thompson & Johnson (1986). The study described here attempts to separate these taxa on the basis of their wood
morphology in order to support or counter the conclusions of Thompson & Johnson (1986).

The second study makes a brief survey of callitroid thickening and large nodulated warts in 23 different species of 12 genera closely related to Callitris in order to gain an indication of the occurrence of these features in the family Cupressaceae. Such knowledge would be useful as an indication of the exclusiveness of callitroid thickening and large nodulated warts to Callitris, and hence their likely effectiveness as intergeneric taxonomic features.

The third study involves C. endlicheri and C. glaucophylla, which are difficult to separate from each other, not with respect to conventional taxonomic features, but using their woods, which are said to have "no distinguishing features" (Ilic 1994). An attempt is made to separate the woods of these two species on the basis of their individual wart morphology.

8.2 Separation of Callitris Taxa on the Basis of Callitroid Thickening, Wart, and Ray Morphology

8.2.1 Introduction

There has been a long history of disagreement, controversy and change regarding the general taxonomy of Callitris. This is evident from Table 8.1 which shows the various listings of Callitris taxa published over the past century. It can be seen that each list is different from the other with respect to number of taxa included in the genus, some of their nomenclatures, and their classification as species, sub-species and varieties. The list of Bentham (1863) recognised nine species and seven varieties; that of Baker & Smith (1910) 17 species; and that of Garden (1956) 16 species and three sub-species. As a result of the many revisions in classification and changes in nomenclature, most species of Callitris now have multiple synonyms.

Several reasons for the confusion can be proposed. Firstly, precise taxonomic classification of certain Callitris species based on herbarium material is often extremely difficult, due to inter-specific similarities in the morphology of cones and leaves. This difficulty has been remarked upon by various authors including Venning (1979), Thompson & Johnson (1986), and Adams & Simmons (1987). Species such as C. intratropica may exist purely on the grounds of geographical isolation (Thompson & Johnson 1986). Also, the various species of Callitris are widespread over a range of habitats and climatic types, and this universality gives rise to localised variation within taxa (Costermans 1981). Venning (1979) investigated the use of a range of taxonomic characters for separating Callitris taxa including: karyotype analysis, wood anatomy, pollen morphology, starch gel electrophoresis of seed proteins, and shape of mature and juvenile leaves. She concluded that the differentiation process was "complex", and
would represent "an on-going challenge for systematics in years to come". Adams & Simmons (1987) used volatile leaf oils as taxonomic markers for Victorian species of *Callitris* in a further effort to overcome taxonomic problems.

<table>
<thead>
<tr>
<th>Bentham (1863)</th>
<th>Baker &amp; Smith (1910)</th>
<th>Garden (1956)</th>
<th>This thesis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. robusta</em> var. <em>verrucosa</em></td>
<td><em>C. tuberculata</em> R. Br.</td>
<td></td>
<td><em>C. tuberculata</em> R. Br. ex Mirbel</td>
</tr>
<tr>
<td><em>F. robusta</em> var. <em>verrucosa</em></td>
<td><em>C. verrucosa</em> R. Br.</td>
<td><em>C. preissii</em> (Miq.) ssp. <em>verrucosa</em> (A. Cunn. ex Endl.) Garden</td>
<td><em>C. verrucosa</em> (A. Cunn. ex Endl.) Garden</td>
</tr>
<tr>
<td><em>F. robusta</em> var. <em>verrucosa</em></td>
<td><em>C. propinqua</em> R. Br.</td>
<td><em>C. preissii</em> (Miq.) ssp. <em>murrayensis</em> Garden</td>
<td><em>C. murrayensis</em> R. Br. ex Baker &amp; Smith</td>
</tr>
<tr>
<td><em>F. robusta</em> var. <em>verrucosa</em></td>
<td><em>C. glauca</em> R. Br.</td>
<td><em>C. hugeli</em> (Carr.) Franco</td>
<td><em>C. glaucophylla</em> Thompson et Johnson</td>
</tr>
<tr>
<td><em>F. robusta</em> var. <em>microcarpa</em></td>
<td><em>C. arenosa</em> A. Cunn.</td>
<td><em>C. columellaris</em> (F. Muell.)</td>
<td><em>C. columellaris</em> (F. Muell.)</td>
</tr>
<tr>
<td><em>F. robusta</em> var. <em>microcarpa</em></td>
<td><em>C. intratropica</em> Bent &amp; Hook.</td>
<td><em>C. intratropica</em> Bak. et Smith.</td>
<td><em>C. intratropica</em> Bak. et Smith.</td>
</tr>
<tr>
<td><em>F. endlicheri</em> Parl.</td>
<td><em>C. calcarata</em> R. Br.</td>
<td><em>C. endlicheri</em> (Parl.) Garden</td>
<td><em>C. endlicheri</em> (Parl.)</td>
</tr>
<tr>
<td><em>F. rhomboidea</em> var. Tasmanica</td>
<td><em>C. tasmanica</em> Baker &amp; Smith.</td>
<td><em>C. tasmanica</em> Baker &amp; Smith</td>
<td><em>C. tasmanica</em> Baker &amp; Smith</td>
</tr>
<tr>
<td><em>F. parlatorei</em> F. v. M.</td>
<td></td>
<td><em>C. baileyi</em> T. White</td>
<td><em>C. baileyi</em> T. White</td>
</tr>
</tbody>
</table>

Table 8.1: Listings of *Callitris* species as reported by Bentham (1863), Baker & Smith (1910) and Garden (1956) and as used in this thesis. The information for this table was compiled using information from Patton (1926) and Garden (1956).

The species *C. columellaris* (*C. arenosa* is a synonym), *C. glaucophylla*, (synonyms include *C. glauca* and *C. hugelii*) and *C. intratropica*, are particularly difficult to distinguish from each other and have been the subject of much controversy with respect to their status as individual species. In 1959 these three species which Garden (1956) had previously listed as *C. columellaris*, *C. hugelii*, and *C. intratropica*, were united as a single species; *C. columellaris* F. Muell., by Blake (1959), who argued...
that there were no distinctive morphological characters to differentiate them:-

"I can find no difference whatever in the shape and stoutness of the twigs, shape and size of the leaves, or in the male amenta".

This taxonomic grouping was generally accepted (Willis 1962; Blombery 1967; Yazaki & Hillis 1977; French et al. 1979; Clayton-Greene 1983; Yazaki 1983) and persisted until Thompson & Johnson (1986) formed polygraphs from five cone characters and discriminated three distinct species. *Callitris hugelii* (synonym *C. glauca*) was renamed *C. glaucophylla*, and the other two taxa were reinstated as *C. intratropica* and *C. columellaris*:

"The distinction between *C. glaucophylla* and its two close relatives, (*C. columellaris* and *C. intratropica*), is difficult to define from herbarium material although this does not preclude the recognition of the three taxa as species." (Thompson & Johnson 1986).

The classification of these three species has been accepted by recent authors including Salmon (1990), Ilic (1994) and Walsh & Entwisle (1994).

In the studies described in Chapters 3, 4, and 5 of this thesis, differences were found between the morphology of callitroid thickening, warts and rays in some *Callitris* taxa including *C. columellaris*, *C. glaucophylla* and *C. intratropica*. It was thought that such differences might be of taxonomic value in the separation of these three species.

8.2.2 Aims and Considerations

The aim of this section was to examine whether it was possible to taxonomically separate *C. columellaris*, *C. glaucophylla* and *C. intratropica* on the basis of differences in the morphology of callitroid thickening, warts, and rays in their wood.

No sampling, preparation, imaging, or data acquisition was required for this study; comparison of the wood morphologies of the three species was based on relevant data acquired during the research described in Chapters 3, 4, and 5.

8.2.3 Results and Discussion

In Chapter 3, a logit regression model of the probability of occurrence of callitroid thickening in all species of *Callitris* was constructed. The respective probabilities of thickening for *C. columellaris*, *C. glaucophylla*, and *C. intratropica* calculated from the model are reproduced in Figure 8.1. It can be seen that there are large differences in the probability of callitroid thickening occurring in these species; in *C. columellaris* it is 0.48, in *C. glaucophylla* 0.98, and in *C. intratropica* 0.22. The non-overlap of the 95%
confidence intervals for each of these mean values indicates that species differences are statistically significant \((p = 0.01)\).

![Figure 8.1: Probability of callitroid thickening occurring in \(C.\) \textit{columellaris}, \(C.\) \textit{glaucophylla}, and \(C.\) \textit{intratropica}. 95% confidence intervals are shown.](image)

Similarly, in the model of wart morphology in all \textit{Callitris} species that was formed in Chapter 4, quantitative values for wart size and shape in \(C.\) \textit{columellaris}, \(C.\) \textit{glaucophylla}, and \(C.\) \textit{intratropica} were determined. Wart size in this model was defined as: the logarithm of [height multiplied by width of wart]; and wart shape was defined as the logarithm of [height divided by width of wart]. The wart size/shape values for \(C.\) \textit{columellaris}, \(C.\) \textit{glaucophylla} and \(C.\) \textit{intratropica} are reproduced in Figure 8.2. It can be seen that no overlap occurs between the 95% confidence intervals for wart size or shape, again indicating that species differences are significant \((p = 0.01)\).

![Figure 8.2: Wart size/shape values for \(C.\) \textit{columellaris}, \(C.\) \textit{glaucophylla}, and \(C.\) \textit{intratropica}.](image)

Values for mean ray height in \(C.\) \textit{columellaris}, \(C.\) \textit{glaucophylla}, and \(C.\) \textit{intratropica} that were determined in Chapter 5, are reproduced in Figure 8.3. It can be seen that although \(C.\) \textit{glaucophylla} can be separated from both \(C.\) \textit{columellaris} and \(C.\) \textit{intratropica}, the latter two species cannot be separated from each other; i.e. their confidence intervals overlap, and this prevents separation of these two species using ray height.

![Figure 8.3: Mean ray height values for \(C.\) \textit{columellaris}, \(C.\) \textit{glaucophylla}, and \(C.\) \textit{intratropica}.](image)

From these findings, two general conclusions can be inferred. Firstly, the significant differences \((p = 0.01)\) in the callitroid thickening frequency and wart size between the three taxa lend support to the assertion of Thompson & Johnson (1986) that they are three different species. Secondly, whereas callitroid thickening frequency and wart morphology are useful taxonomic indicators for the separation of these three species, ray height cannot discriminate between them.
Figure 8.2: Relationship between wart size (log. [height multiplied by width]) and wart shape (log. [height divided by width]) in *C. columellaris*, *C. glaucophylla* and *C. intratropica*. 95% confidence intervals are shown.

Figure 8.3: Ray heights in *C. columellaris*, *C. glaucophylla*, and *C. intratropica*. 95% confidence intervals are shown.
8.3 Callitroid Thickening and Warts in Genera Closely Related to Callitris

8.3.1 Introduction

The family Cupressaceae consists of three subfamilies; (i) Thujoideae; (ii) Cupressoideae; and (iii) Juniperoideae (Phillips 1948). As a member of subfamily Thujoideae, Callitris is most closely related to Actinostrobus, Callitropsis, Diselma, Fitzroya, Fokienia, Libocedrus, Tetraclinis, Thuja, Thujopsis, and Widdringtonia. Genera more distantly related to Callitris are Chamaecyparis and Cupressus of the Cupressoideae sub-family, and still more distantly related, are Arceuthos and Juniperus of the Juniperoideae (Peirce 1937; Phillips 1948).

Although callitroid thickening is "the most important differentiating feature of the genus Callitris" (Venning 1978) it is not exclusive to the genus and its occurrence has been reported in three other genera of the Cupressaceae; Actinostrobus (Kleeberg 1885; Phillips 1948); Juniperus (Barefoot & Hankins 1982); and (as "callitroid pits") in Tetraclinis (Schweingruber 1990). Callitroid thickening is reported to be absent from Callitropsis and Widdringtonia (Phillips 1948). Outside of the Cupressaceae, callitroid thickening has also been reported in golden larch (Pseudolarix amabilis Rehd., family Pinaceae) by Phillips (1948); in rimu (Dacrydium cupressinum Soland., family Podocarpaceae) by Butterfield & Meylan (1980) and in the family Pinaceae: pond pine (Pinus serotina Michx.), pitch pine (P. rigida Mill.), shortleaf pine (P. echinata Mill.), slash pine (P. elliottii Engelm.), and loblolly pine (P. taeda L.) by Howard & Manwiller (1969).

The occurrence of a warty layer in tracheids of various species of the Cupressaceae has been sporadically reported in the literature. Meylan & Butterfield (1978b) reported that the tracheid walls of pahautea (Libocedrus bidwillii Hook. f.) were "sometimes heavily warted, the warty layer also lining the pit chambers". Kocon (1986) found warts ranging in diameter from 50 to 450 nm in juniper (Juniperus communis L.). Schweingruber (1990) described warts in tracheids of thuja (Tetraclinis articulata [Vahl.] Masters) as being "fine, and visible only at magnifications of 400 times or higher". Roig (1992) stated that a warty layer was present in both tracheids and pit borders of alerce (Fitzroya cupressoides [Mol.] Johnston), but made no comment on the size or shape of individual warts. Wardrop & Davies (1962) reported "very large warts" in Actinostrobus pyramidalis Miq., and Liese (1965a) described warts in African cypress (Widdringtonia dracomontana Stapf.) as being "bigger than 0.5 µm [in diameter at the base]". However, it is not known whether large warts, with complex shape and with nodules, as described in Chapter 4, occur in other genera of the Cupressaceae. Such knowledge would be useful from a taxonomic point of view since, if exclusive to Callitris, they would be a very important generic taxonomic feature.

The aim of this study was to report on the occurrence of callitroid thickening and large, nodulated warts in the woods of species closely related to Callitris. It was not
intended for the study to be properly representative of any genera, but simply to give an indication of these features within the Cupressaceae as a basis for further, more detailed, studies.

8.3.2 Materials and Methods

Wood samples from a total of 22 different species, as shown in Table 8.2, were studied. Samples from live trees were obtained for *A. pyramidalis* (complete sections of stem) and *D. archeri* (a small piece of twig, 0.7 mm in diameter). All other samples were derived from off-cuts of sapwood from wood sample blocks held at the Department of Forestry at The Australian National University, Canberra. Representatives of the Cupressaceae genera, *Fokienia* or *Arceuthos* were not available.

RLS specimens were prepared for SEM as described in Chapter 2 of this thesis. Data on the occurrence of callitroid thickening was acquired by observing all visible pits on specimen surfaces and recording the absence or presence of thickening. Particular attention was given to pits in the latewood of all species since it was shown in Chapter 3 that callitroid thickening (in *Callitris*) occurred more frequently in narrow tracheids than in wide ones. Where thickening was present, its type (i.e. 1, 2, 3, or 4, as defined in Chapter 3) was recorded.

Warty layers in specimens were viewed, and data regarding wart height and width were obtained, as described in Chapter 4. The occurrence of nodules on wart surfaces was also recorded.

8.3.3 Results and Discussion

The results of the study are summarised in Table 8.2. Callitroid thickening was observed in the following species; *A. pyramidalis* (Plate 8.1), *C. araucarioides* (Plate 8.2), *C. sempervirens* (Plate 8.3), *D. archeri* (Plate 8.4), *J. procera* (Plates 8.5 and 8.6), and *T. articulata* (Plate 8.7). The thickening observed in these species was morphologically similar to that observed in *Callitris* (Chapter 3); thickening of 'Types 1 and 2' was distinguished, and there were isolated occurrences of thickening 'Type 3' thickening (in *C. sempervirens* Plate 8.8). Crossfield pit thickening was observed in *C. sempervirens* and in *A. pyramidalis* (Plate 8.9). Thickening in these genera tended to occur more frequently in association with narrow rather than wide tracheids (Plates 8.3 and 8.6), as was shown in Chapter 3 for *Callitris*. Thickening was absent from the other genera (*Chamaecyparis*, *Fitzroya*, *Libocedrus*, *Thuja*, *Thujopsis*, and *Widdringtonia*), the species of which are listed in Table 8.2.
<table>
<thead>
<tr>
<th>Genera</th>
<th>Species</th>
<th>Origin &amp; Habitat</th>
<th>Sample Id.</th>
<th>Thickening</th>
<th>Wart Morph.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinostrobus</td>
<td><em>pyramidalis</em> Miq.</td>
<td>Western Australia</td>
<td>Acti-1,2</td>
<td>PRESENT</td>
<td>Lge. Nodulated</td>
</tr>
<tr>
<td>Callitropsis</td>
<td><em>araucarioides</em> Comp.</td>
<td>New Caledonia</td>
<td>Calli-1-4</td>
<td>PRESENT</td>
<td>Lge. Nodulated</td>
</tr>
<tr>
<td>Chamaecyparis</td>
<td><em>lawsoniana</em> Murr.</td>
<td>USA California to Oregon</td>
<td>12/4 (5.1)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Chamaecyparis</td>
<td><em>nootkatensis</em> Lamb.</td>
<td>USA Alaska to Oregon</td>
<td>12/4 (5.2)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Cupressus</td>
<td><em>funebris</em> Endl.</td>
<td>China</td>
<td>E1/116 (6.6)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Cupressus</td>
<td><em>obtusa</em> K.Koch</td>
<td>Japan</td>
<td>E1/116 (6.2)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Cupressus</td>
<td>* sempervirens* L.</td>
<td>S. Europe</td>
<td>E1/116 (6.5)</td>
<td>PRESENT</td>
<td>Small</td>
</tr>
<tr>
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<td><em>torulosa</em> Don</td>
<td>W. Himalaya, China</td>
<td>E1/116 (6.1)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Diselma</td>
<td><em>archeri</em> Hook. f.</td>
<td>Tasmania</td>
<td>Dise-1</td>
<td>PRESENT</td>
<td>Lge. Nodulated</td>
</tr>
<tr>
<td>Fitzroya</td>
<td><em>cupressoides</em> (Mol.) Johnston</td>
<td>S. America peat bogs</td>
<td>Fitz-1</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Juniperus</td>
<td><em>communis</em> L.</td>
<td>Scotland Chalk Limestone</td>
<td>12/34 (7.2)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Juniperus</td>
<td><em>foetidissima</em> Wild.</td>
<td>Greece</td>
<td>12/34 (7.5)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Juniperus</td>
<td><em>procera</em> Hochst.</td>
<td>East Africa</td>
<td>12/34 (7.4)</td>
<td>PRESENT</td>
<td>Small</td>
</tr>
<tr>
<td>Juniperus</td>
<td><em>scopulorum</em> Sarg.</td>
<td>USA Dry rocky ridges</td>
<td>12/34 (7.1)</td>
<td>No</td>
<td>Lge. Nodulated</td>
</tr>
<tr>
<td>Libocedrus</td>
<td><em>bidwillii</em> Hook. f.</td>
<td>New Zealand</td>
<td>E1/116 (2.2)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Libocedrus</td>
<td><em>decurrens</em> Torr.</td>
<td>California</td>
<td>E1/116 (2.1)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Tetraclinis</td>
<td><em>articulata</em> (Vahl) Mast.</td>
<td>N. Africa, Algeria</td>
<td>tetr-1,2</td>
<td>PRESENT</td>
<td>Lge. Nodulated</td>
</tr>
<tr>
<td>Thuja</td>
<td><em>occidentalis</em> L.</td>
<td>USA</td>
<td>12/4 (3.1)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Thuja</td>
<td><em>plicata</em> D. Don</td>
<td>Alaska to California</td>
<td>12/4 (3.2)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Thuja</td>
<td><em>dolaboroides</em> Sieb. et Zucc.</td>
<td>Japan</td>
<td>E1/120 (4.1)</td>
<td>No</td>
<td>Small</td>
</tr>
<tr>
<td>Widdringtonia</td>
<td><em>juniperoides</em> Endl.</td>
<td>South Africa</td>
<td>widd-1</td>
<td>No</td>
<td>Small</td>
</tr>
</tbody>
</table>

**Table 8.2:** Occurrence of callitroid thickening and large, nodulated warts in some species related to *Callitris.*
Plates 8.1-8.8: Callitroid thickening in species other than Callitris: 1 = Actinostrobus pyramidalis; 2 = Callitopsis araucariodes; 3 = Cupressus sempervirens; 4 = Diselma archeri; 5 = Juniperus procera; 6 = Juniperus procera (showing occurrence of thickening only in narrow tracheids); 7 = Tecticornis articulata; 8 = Type 3 thickening in Cupressus sempervirens.
The presence of callitroid thickening in the genera *Diselma* and *Cupressus* has not previously been reported in the literature, and for *Callitropsis*, callitroid thickening was previously reported to be absent (Phillips 1948). This study also represents the first report of thickening in *A. pyramidalis* and *J. procera* (previous reports of thickening in both *Actinostrobus* and *Juniperus* did not involve these particular species). It is also the first report of the presence of crossfield pit thickening in a genus other than *Callitris*. Thus, this study has shown that callitroid thickening is somewhat less exclusive to the genus *Callitris* than has been previously suggested.

Table 8.2 shows that large, nodulated warts occurred in tracheids of *A. pyramidalis* (Plate 8.10), *C. araucarioides* (Plate 8.11), *D. archeri* (Plate 8.12), *J. foetidissima* (Plate 8.13), *J. scopulorum* (Plate 8.14), *J. virginiana* (Plate 8.15), and *T. articulata* (Plate 8.16). The shapes and sizes of the warts in these species were variable, both within and between species. Warts in *A. pyramidalis* (Plate 8.10) were larger than those in any species of *Callitris*. In warty layers where large nodulated warts occurred, small hemispherical warts were also always present, in a manner analogous to *Callitris* (Chapter 4). Other than the reporting of "very large warts" in *Actinostrobus* by Wardrop & Davies (1962), this represents the first report of large warts in these genera and the first to acknowledge the occurrence of nodules on their surfaces. It also shows that the presence of large, nodulated warts is not a taxonomic keying factor exclusive to *Callitris*; i.e. the results indicate that large nodulated warts may be of more general occurrence within the Cupressaceae.

In Chapters 3 and 4 it was shown that the presence of large nodulated warts and frequent, distinct callitroid thickening tended to occur together in species endemic to dry habitats. Therefore the occurrence of both callitroid thickening and large nodulated warts in *A. pyramidalis*; *C. araucarioides*; *D. archeri*; and *T. articulata* (Table 8.2) was of special interest. While both *A. pyramidalis* and *T. articulata* are dry-habitat species, *C. araucarioides* is endemic to wet tropical forests of New Caledonia and *D. archeri* is found in a region of Tasmania which cannot be classed as 'dry'. It is possible that occurrence of callitroid thickening and large nodulated warts in *C. araucarioides* and *D. archeri* are due to a closer taxonomic relationship between these species and *Callitris*. The results, therefore, indicate a need for caution when discussing the ecological significance of wood anatomical features. Clearly more comprehensive studies are required to understand the taxonomic and ecologic significance of callitroid thickening and large nodulated warts in wood of the Cupressoideae.

These findings support the assertion of Phillips (1948) that the classification of subfamilies in the Cupressaceae (i.e. Thujoideae; Cupressoideae; and Juniperoideae), does not appear to coincide with any classification based on the anatomy of the wood.
Plates 8.9: Crossfield pit thickening in *Actinostrobus pyramidalis.*

Plates 8.10-8.16: Large nodulated warts in species other than *Callitris:*

10 = *Actinostrobus pyramidalis* 11 = *Callitropsis araucariodes*; 12 = *Diselma archeri;*
13 = *Juniperus foetidissima*; 14 = *Juniperus scopulorum*; 15 = *Juniperus virginiana;*
16 = *Tetraclinis articulata.*
8.4 Separation of the Woods of *C. endlicheri* and *C. glaucophylla* on the Basis of Wart Shape

8.4.1 Introduction

The wood of *C. endlicheri* is very similar anatomically to that of *C. glaucophylla* and it is very difficult to distinguish them from each other. In a recent anatomical study using light microscopy, Ilic (1994) found "no simple clear-cut discriminative features between the two species". The properties and commercial uses of the woods of each species are, however, very different. The wood of *C. glaucophylla* (commonly known as white cypress pine) is considered to be a superior timber to that of *C. endlicheri* (black cypress pine) in that it has greater resistance to termites and decay (Wallis 1963; French et al. 1979; Yazaki 1983) and the bole is generally straighter and of better form (Ilic 1994). As a result of this, white cypress is commonly used out-of-doors for fenceposts, poles and weatherboards (Swain 1928; Bootle 1985; Salmon 1990) whereas black cypress is rarely favoured for external use. Occasionally there are reports of the premature failure of white cypress pine in ground contact. Such reports may arise because of the inadvertent (or illegal) substitution of white cypress pine by black cypress pine (Johnson and Ilic, pers. comm.). However, in the absence of a method of discriminating between the woods of the two species, it is not possible to check the veracity of such reports. Both species are common, and are widely distributed in inland areas of New South Wales and to a lesser extent, in Queensland and Victoria. In some areas their distributions overlap. A means of separating the wood of *C. endlicheri* and *C. glaucophylla* would have useful practical applications and would be relevant to both the systematic and technological themes of the science of wood anatomy.

In Chapters 3, 4, and 5 of this thesis, callitroid thickening, wart, and ray morphology were described for all *Callitris* species. Although only minor differences were found between the frequency of callitroid thickening, wart size, and mean ray heights for *C. endlicheri* and *C. glaucophylla*, it was noted that the shapes of the largest warts of the two species were, in general, different from each other. Warts of *C. endlicheri* tended to have a tubular upper-part often bent-over at the top, and a wide, pedestal-like, base (Plate 8.17) whereas equivalent warts in *C. glaucophylla* were generally conical and upright in shape (Plate 8.18). It was noted, however, that these differences in the shape of warts applied mainly to the largest, nodulated warts of warty layers, and were by no means homogeneous or uniform in character. In fact the warty layers of the two species consisted of a heterogeneous assortment of warts of a range of sizes and shapes, and both included a population of small hemispherical warts that were seemingly identical. Nevertheless the distinction between the largest warts appeared to be sufficiently common to allow separation of species.
Plate 8.17: Characteristic morphology of large nodulated warts in *C. endlicheri*.

Plate 8.18: Characteristic morphology of large nodulated warts in *C. glaucophylla*.
Thus, the aim of this study was to determine whether it was possible to distinguish between the woods of *C. endlicheri* and *C. glaucophylla* on the basis of differences in the shape of their large, nodular warts.

### 8.4.2 Considerations, and Requirements

Of major consideration was the development of an appropriate means of quantifying differences (if any) in wart shape between *C. endlicheri* and *C. glaucophylla*. Measurement of the heights and basal widths of warts, as used in the research of Chapter 4, was inappropriate because warts of *C. endlicheri* and *C. glaucophylla* tended to have similar heights and basal widths. Instead, in order to most accurately capture the tubular shape of *C. endlicheri* as opposed to the conical, pyramidal shape of *C. glaucophylla*, it was decided to measure wart width at positions one-third and two-thirds from the top of the wart. The difference between the values resulted in a parameter for 'shape' in the middle region of the wart.

A second parameter for wart shape that appeared to be useful in differentiating these species was that large warts in *C. endlicheri* were typically bent-over at the top whereas large warts in *C. glaucophylla* were characteristically upright. Data for the quantification of this parameter was acquired by recording a '1' if the wart was bent-over at the top and a '0' if the wart was upright. Warts were defined as 'bent-over' only if the angle of the top part of the wart was different from the angle at the base of the wart. Warts which were continuously inclined from the base upwards were not categorised as being 'bent-over'.

### 8.4.3 Materials and Methods

Eight wood specimens each, of *C. endlicheri* and *C. glaucophylla* were used in this study. No sampling or specimen preparation was required; the specimens used were the same ones that were collected and prepared for the study of warts described in Chapter 4. Warts were viewed and measurements were carried out in similar manner to that described in Chapter 4, but at a magnification best suited to accurate measurement of wart width (typically 30,000 to 70,000 times).

The following parameters were recorded for the two tallest warts per image:

- **W1** = width (nm) at a point approximately one-third of the distance from the top to the base of the wart.
- **W2** = width (nm) at a point approximately two-thirds of the distance from the top to the base of the wart.
- **BT** = ('1' recorded if wart was bent-over at top, '0' if wart was not bent-over).

The above three measurements were recorded for two warts in 25 adjacent tracheids (i.e. 50 warts were measured in each specimen) for each of the eight wood specimens in each species.
For each individual wart, the two width measurements (W2 and W1) were subtracted from each other in order to form an indication of wart 'shape'. This was repeated for all 800 measurements (400 each for C. endlicheri and C. glaucophylla).

**8.4.4 Results and Discussion**

Figure 8.4 shows boxplots of wart shape ([W2 - W1]) for C. endlicheri and C. glaucophylla.

![Boxplots comparing the 'shape' (lower minus upper width measurements) of warts of C. endlicheri with those of C. glaucophylla.](image)

It can be seen that although there was a similar range of shapes of warts in both species, the median values (indicated by the horizontal line within each individual box) were different from each other; in C. endlicheri the median value is at approximately 80 nm whereas in C. glaucophylla the median value is 120 nm. This indicates that there was generally a smaller difference between the upper (W1) and the lower (W2) width measurements for warts in C. endlicheri compared with those of C. glaucophylla; i.e. large warts in C. endlicheri tended to be 'tubular' in shape whereas those in C. glaucophylla were generally more 'pyramidal'. A comparison of wart shape in the two species is shown in Figure 8.5, and an ANOVA indicated that the wart shape of C. glaucophylla was significantly different (p < 0.05) from that of C. endlicheri.

The occurrence of warts bent-over at their tops (based on a model formed from parameter BT) in the two species is shown in Figure 8.6. Regression analysis indicated a highly significant (p < 0.001) difference between the proportions of 'bent-over warts' in wood of the two species.
Figure 8.5: Bar chart showing wart shape (log.\([W2-W1]\)) in *C. endlicheri* and *C. glaucophylla*. 95% confidence intervals are indicated.

Figure 8.6: Bar chart showing predicted probability of warts bent-over at the top in *C. endlicheri* and *C. glaucophylla*. 95% confidence intervals are indicated.

It can therefore be concluded that the woods of *C. endlicheri* and *C. glaucophylla* can be mutually distinguished on the basis of the shape of their warts. The findings of this study also show that wart shape in these two species is of taxonomic significance and suggests that further studies of this parameter in other genera possessing large
nodulated warts (Section 8.3) may be of value. The finding of a means of separating the wood of these species is also of practical use from the point of view of technological wood anatomy, as discussed in the introduction to this section of the Chapter.

It should be pointed out that this finding does not conflict with past reports referring to the lack of taxonomic significance in the **warty layer** in wood in general. Thus the comment of Panshin & de Zeeuw (1980), i.e. "they [warts] possess little taxonomic significance other than to separate the hard pines, which have them, from the soft pines, which do not show them" relates to absence or presence of the warty layer as a whole, and not to the **morphology of individual warts**, referred to here. Similarly the suggestion of Liese (1965a), i.e. that warts show "too much variability" so that "the warty layer is of little value for the systematic classification of families, genera and species", was generally supported by the finding here and in Chapter 4; i.e. in general, wart morphology in the warty layers of *Callitris* is highly variable. In fact, in order to separate the woods of *C. endlicheri* and *C. glaucophylla* it was essential to concentrate on two particular aspects of the wart morphology, i.e. the differences in the shapes of the mid-portions and the bending-over at the tops of only the largest warts, and to resort to high resolution SEM at greater than 30,000 times magnification followed by sophisticated statistical analysis. Thus, although the separation of *C. endlicheri* and *C. glaucophylla* by use of wart morphology was shown to be possible by this study, the method was not without complication, requiring a demanding technique and complex equipment.
Chapter 9

Wood Anatomy Description of *Callitris*.

This Chapter describes the microscopic wood anatomy of all species of *Callitris*. It is based on quantitative measurements made using SEM, supplemented by a qualitative LM study. Some information regarding callitroid thickening, wart, and ray anatomy from Chapters 3, 4, and 5 is incorporated into the descriptions.
9.1 Introduction

9.1.1 Background to this Wood Anatomy Study

Several descriptions of the wood anatomy of *Callitris* have been made in the past; the most comprehensive are those of Patton (1927), Peirce (1937), Phillips (1948), and Greguss (1955; 1972). The value of these reports is, however, diminished by a number of common factors. Firstly, the descriptions are principally those of species used for timber production, and the wood anatomy of several of the less common taxa: e.g. *C. baileyi*, *C. neocaledonica*, *C. monticola*, and *C. roei* are ignored. Most reports are based on limited wood sample numbers, and are poorly representative, i.e. are descriptions of branch rather than stem wood. All descriptions used LM, and did not benefit from the high resolution of SEM imaging. The quantitative information presented by Patton (1927) and Greguss (1955) is in textual form, and is not tabulated or graphed, making it difficult for the reader to make comparisons between species. Finally, most of the species nomenclatures used in past descriptions are synonyms, which are no longer in use, due to the many reclassifications that have occurred. Thus it is sometimes difficult to be sure which taxa are being referred to, especially those which have a long history of change, e.g., *C. glaucophylla*, *C. columellaris*, *C. preissii* and *C. verrucosa*.

Three aspects of the wood anatomy of *Callitris* which were reported by Patton (1927), Peirce (1937), Phillips (1948), and Greguss (1955) are not supported by findings in this thesis; (i) callitroid thickening was found to occur in all species of the genus rather than being absent in some of them; (ii) individual rays exceeding 30 cells in height were rare in *Callitris* and did not commonly occur in all species as was previously suggested, and (iii) ray tracheids were absent from all species in the genus. These amendments require inclusion into the wood anatomy description of *Callitris*.

Furthermore, the occurrence of the four different styles of callitroid thickening, and the findings regarding the morphology of warts in the warty layer of *Callitris*, which were described in Chapters 3 and 4, can now be considered an essential part of the general wood anatomy of the genus.

This Chapter describes the microscopic wood anatomy of all species of *Callitris*, revising, supplementing, and where necessary, amending, previous descriptions.

9.1.2 Literature Review of the Wood Anatomy of *Callitris*

An early description of the anatomical features of *Callitris* (under its generic synonym, *Frenela*) was made by Kleeberg (1885) who first described callitroid thickening bars on pits.
Kleeberg's report appears to have gone un-noticed by Baker & Smith (1910) who state:

"Very little, if anything, appears to have been done to investigate the anatomical structure of the timber of Australian Callitris."

Baker & Smith (1910) went on to briefly describe several features of the wood anatomy of four species of *Callitris*, remarking on "black-coloured axial parenchyma cells" and "uniseriate ray parenchyma" as the outstanding characters. They also commented that the "double rows of pitted cells" [i.e. biseriate pitting], which they observed in *C. macroayana*, were "of rare occurrence in Callitris". However, Baker & Smith (1910) overlooked callitroid thickening as a feature of the wood anatomy of *Callitris* even though it is visible in several of their photographs.

An anatomical study of Australian coniferous timbers by Patton (1927) included comments on the general anatomy of *Callitris*. Growth rings were described as "more or less distinct, but never strongly marked, and with spring wood passing gradually into the summer wood"; cells were "thick walled, almost circular, and arranged in radial rows" and "bordered pits uniseriate, at times incompletely biseriate". Rays were reported to be uniseriate and of variable height, to have "horizontal walls parallel", and to be "strongly resinous". Ray heights (in terms of numbers of cells) and occurrence of callitroid thickening in several *Callitris* species were described by Patton (1927) as follows: *C. arenosa* A. Cunn. (synonym for *C. columnellaris*) was described as having rays 7 to 25 cells high (max 33) and to have [callitroid] thickening present; *C. muelleri* was described as having "the thickening band [i.e. callitroid thickening] absent" and "very short rays from 1 to 4 cells high"; *C. oblonga" short rays from 1 to 4 cells high" and no callitroid thickening; in *C. rhomboidea* rays were reported to be "usually from 3 to 7 cells high, maximum 13"; and *C. robusta* R. Br. (synonym for *C. verrucosa*), was reported as having callitroid thickening "commonly present" and ray height "4 to 10 cells". Little indication was given by Patton (1927) of the geographical sources, or of the number of samples used in the study, so it is difficult to determine which of the presently-recognised taxa were being referenced, or the representativeness of his study.

Dadswell & Eckersley (1935) described the physical properties of wood of *C. glauca* (synonym of *C. glaucophylla*) as "brown in colour, moderately light in weight, brittle, and somewhat greasy", and "often showing white needle crystals on the surface". The 'burning splinter test' for this species was described as:- "match size splinters burn to a full white ash". Dadswell & Eckersley (1935) also reported the following anatomical characteristics for *C. glauca*: growth rings "distinct"; callitroid thickening present; tracheids "of varying shapes and sizes, in fairly regular radial arrangement"; bordered pits with "round to somewhat orbicular orifices". In addition: "crossfield pits 2-4, bordered, with slit-like or lenticular orifices; rays "5-8 per mm,
barely visible to the naked eye, mostly uniseriate" and "up to 30 cells high, parenchyma common, resinous, diffuse or contiguous to rays".

The description of callitroid thickening by Budkevich (1936) [in Russian] is similar to that of Patton (1927) and involves the same species (C. oblonga and C. robusta R. Br.).

Peirce (1937) described the wood of Callitris in terms of its rounded tracheids, uniseriate pitting, tall (1-36 cells high) and occasionally partially biseriate rays, and abundant parenchyma cells. The descriptions were based on single wood specimens of six different Callitris species: C. arenosa (synonym for C. columellaris), C. calcarata (synonym for C. endlicheri), C. glauca (synonym for C. glaucophylla), C. intratropica, C. rhomboidea, and C. robusta (synonym for C. verrucosa) which were obtained from the collection of the Yale xylarium.

Phillips (1948) includes a generic anatomical description of wood of Callitris in his monograph on softwood anatomy. Callitroid thickening is referred to as being "regularly developed in all species of Callitris of commercial importance except C. macleayana". Phillips (1948) also noted "abundant parenchyma", and "rays with cupressoid crossfield pits and thin, unpitted horizontal walls" in C. macleayana, and Callitris was listed as one of the genera having regular or sporadic occurrence of "zonate parenchyma".

The wood anatomy of seven species of Callitris; C. glauca (synonym for C. glaucophylla), C. robusta (synonym for C. verrucosa), C. verrucosa R.Br., C. cupressiformis (synonym for C. rhomboidea), C. sulcata, C. oblonga, and C. intratropica Benth. et Hook., was described by Greguss (1955). Of these species, callitroid thickening was reported to be present in C. glauca and C. verrucosa, and to be absent in the others. Ray heights and frequencies and numbers of pits in crossfields were described in all of these species except C. glauca, for which ray frequency figures were not reported.

Greguss (1955) classified the rays of Callitris as being 'primitive' in that they are homogeneous, their tangential and radial cell walls are thin and smooth, their crossfield pits are poorly defined, and they lack both resin ducts and ray tracheids. Rays with this simple structure also characterise the primitive Cycadaceae, Ginkgoaceae, Araucariaceae, and Podocarpaceae (Greguss 1955). The presence of ray cells with smooth thin walls was also used by Greguss (1955) to classify Callitris among the more 'primitive' genera of the Cupressaceae: Actinostrobus, Callitropsis, Tetroclinis, and Widdringtonia; as distinct from the more advanced of the family with pitted ray cell walls and bead-like thickenings: Cupressus, Diselma, Fitzroya, and Juniperus.

In a subsequent study, Greguss (1972) gave a full description of the anatomy of two species: C. morisonii (synonym of C. canescens) and C. drummondii. The wood sample used by Greguss to describe C. morisonii was obtained from "a single sample of bough, 1 cm thick and 3 to 4 years old" and that used to describe C. drummondii was also poorly representative being "sections cut of a thickish bough or trunk".
Ilic (1994) reported that the wood of *C. endlicheri* is almost identical to that of *C. glaucophylla*, and gave a full description of their (mutual) anatomical characteristics. Tracheids (in *C. endlicheri* and *C. glaucophylla*) were reported to be "generally rounded, polygonal to irregular in cross-section, and arranged in fairly regular rows". Ilic (1994) also described crossfield pits in these two species as "cupressoid, 2-4 apertures, slit-like to lenticular" and, "[axial] parenchyma abundant to fairly sparse, resin-filled, loosely zonate to diffuse, sometimes contiguous to rays, and end walls smooth and entire".

A carbon-replica TEM micrograph of warts in compression wood of *C. calcarata* (synonym of *C. endlicheri*) by Liese (1965a) showed warts to be as numerous in the cavities as in the tops of the ribs.

The wood anatomy descriptions of *Callitris* by Venning (1979) were based on twig samples and not the more representative mature stem-wood. It was reported that the gradation from late to earlywood was "diffuse" in *C. baileyi*, *C. columellaris*, and *C. endlicheri", "sharp" in *C. canescens*, *C. drummondii*, *C. macleayana*, *C. oblonga*, *C. roei*, and *C. rhomboidea*, and that parenchyma, indentures, and ray tracheids were absent from all of these species. The descriptions by Venning (1979) were distinctive in that they include the only account in the literature (of some of the features) of the wood anatomy of *C. roei*. Furthermore, callitroid thickening was reported as being "present" in *C. macleayana* whereas it is reported to be absent in all other accounts.

### 9.1.3 Aims and Considerations

The aim of this study was to quantitatively and qualitatively describe the microscopic wood anatomy of *Callitris* using SEM, supplemented by LM.

It was realised that the small size and tubular shape of the core samples used in this thesis (as described in Chapter 2) would make it difficult to examine the large-scale anatomical features of the wood, such as growth-ring distinctiveness and the conspicuousness of latewood, since these features require observation of at least one full growth ring and this is often not possible for very small samples. In addition, since the core samples were sapwood only, features such as wood colour, odour, taste, and greasiness, which are necessarily based on observations of heartwood, could not be described. Also, in order to relate more appropriately to the general theme of the thesis, it was decided to limit the study to anatomical features, and not to include any physical characteristics, such as density and hardness, which are often used for wood identification (Phillips 1948).

It was also necessary to choose the most effective method of presenting the anatomical information, since details of 19 different features in all 20 species of *Callitris* were required. Textual presentation, as used by Greguss (1955; 1972), was not favoured because it would be too cumbersome. Instead it was decided that anatomical information would be presented in tabular form since this would not only convey much of the essential detail in a concise manner, but it would also allow inter-species comparisons,
thereby being of more practical use to wood anatomists. The descriptions of callitroid thickening, the warty layer and rays are concise reviews, mainly in tabular form, of the findings of Chapters 3, 4, and 5. Anatomical descriptions of bordered pits, crossfield pits, tracheids, and axial parenchyma cells are based on qualitative information and quantitative data obtained using both SEM and LM.

Although compression wood is not part of the 'natural' wood anatomy of any genus, "its universal occurrence in practically all coniferous forest trees" (Tinell 1986) and its sporadic presence in wood samples collected for this thesis (as discussed in Chapter 2) warrants a need for its recognition in Callitris, and it is therefore included here as an 'anatomical feature'.

Species descriptions using SEM are based on observations of 4-8 samples, and those involving LM, on a minimum of two samples.

9.1.4 Review of the Characteristics used to Describe Features of Wood Anatomy

The following wood anatomy features are those which are commonly used to describe and differentiate softwood species. The information defining them is derived mainly from the monograph on softwood identification by Phillips (1948). Only those relevant to the Cupressaceae and not involving heartwood or the physical features of wood are included. These features, which will subsequently be used in Table 9.1, are as follows:

1) Axial parenchyma present. In TS, cells with dark-coloured contents (using LM) were visible. In RLS and TLS, cells totally filled with an amorphous substance, and with horizontal cell walls, were apparent to both LM and SEM.

2) Parenchyma abundant. "In the portion of the growth ring (seen in TS) containing most parenchyma, five or more parenchyma cells per mm$^2$ were apparent" (quoted from Phillips 1948).

3) Parenchyma zonate. There was aggregation of parenchyma cells in limited tangential zones extending within a growth ring (seen in TS).

4) Growth rings indistinct. There was "no marked contrast between the final row of latewood tracheids and the succeeding row of earlywood tracheids in TS at magnification of 50 times or more" (quoted from Phillips 1948).

5) Latewood conspicuous. Latewood was "dense, sharply defined from the earlywood of the same ring, and of an average width not less than one-quarter the width of the growth ring" (Phillips 1948).

6) Latewood cells thick-walled. There was occurrence of "thick-walled latewood tracheids in which the lumina were correspondingly reduced so that the last few layers of tracheids had their tangential walls thicker than the width of the lumina" (Phillips 1948).

7) Ray tracheids present. Ray tracheids can be distinguished from ray parenchyma (particularly the cells at the margins of rays) by the presence of bordered pits
whereas ray parenchyma cells have only crossfield pits. Phillips (1948) says "in order to distinguish ray tracheids from ordinary parenchymatous marginal cells, it is essential to observe the fully bordered pit-pairs in sectional view".

8) **Horizontal walls thin.** In RLS, horizontal ray "walls appeared to be thinner than the adjacent vertical tracheid walls above or below the ray" (Phillips 1948).

9) **Horizontal walls smooth.** In TS the horizontal ray cell walls can be either smooth or pitted (Phillips 1948).

10) **Indentures present.** An 'indenture', refers to the depression at the corners of ray cells and, as seen in RLS, is "a pit-like hollow in the horizontal wall in which the ends of the vertical walls stand" (Phillips 1948).

11) **Biseriate rays frequent.** This parameter is defined using the terminology of (Phillips 1948) as positive only if "one-third or more of the sizeable rays (five or more cells high) are partly or completely biseriate".

12) **Tall rays.** More than two rays per mm$^2$ in TLS exceed 30 cells in height.

13) **Short rays.** Less than two rays per mm$^2$ in TLS exceed 15 cells in height.

14) **Bordered pits multiseriate.** "This refers to the extensive occurrence of multiseriate pitting throughout the length of the tracheids of earlywood and not to occasional small groups of opposite pits" (Phillips 1948). This character was only to be used positively.

15) **Torus present.** The pit membrane has a centrally thickened torus.

16) **Crossfield pits 'cupressoid'.** "Apertures in the earlywood are included within the limits of the border and are rather narrower than the border" (Phillips 1948).

17) **Resin ducts present.** Resin ducts can be 'normal' or 'traumatic'.

18) **Callitroid thickening (SEM).** Callitroid thickening was observed using SEM.

19) **Callitroid thickening (LM).** Callitroid thickening was observed using LM.

### 9.2 Materials and Methods

There was no requirement for wood sampling to be carried out specifically for this Chapter since use was made of remaining wood sample material and previously-prepared SEM specimens from the research described earlier in Chapters 3, 4, and 5. However, because of their fragility, the margos of bordered pits, required special fixation and dehydration to preserve their integrity before high magnification examination by SEM. The procedure was as follows: Freshly-cut SEM specimens were fixed in 2.5% gluteraldehyde (made up in 0.1M PO$_4$ buffer and 0.15M sucrose at pH 7.2) for 2 hours. They were then washed in buffer for two periods, each of three minutes; post-fixed in 1% osmium tetroxide (OsO$_4$) solution (made up in washing buffer) for 2 hours; washed four times for 5 minutes, and then dehydrated sequentially in 30%, 50%, 70%, and finally 100% alcohol, for 10 minutes. The specimens were then air dried at 22°C over a period of two days.
Use was also made of a cryogenic preparation and specimen viewing system (Oxford Instruments Ltd type CT-1500), attached to the SEM, which allowed preparation and viewing of specimens at liquid nitrogen temperature (-196 °C), thus enabling wood specimens to be viewed without any preparatory drying. This was particularly useful for the imaging of fragile pit margo strands.

Because of the small size of the wood core samples, the sledge microtome, the standard instrument for preparing light microscopy sections (Jane 1970), was not used here. Instead, it was found that small shavings of wood, cut from core samples using a hand-held blade, produced acceptable sections for LM.

Sample preparation for LM examination consisted of four sequential steps: (1) softening; (2) cutting; (3) staining; and (4) mounting. Wood core samples were softened by soaking them in water for two days immediately prior to cutting. The wet (waterlogged) samples were then clamped in a vice and thin (1-2 mm² square and 20-50 µm thick) sections, i.e. approximately one cell thickness, were shaved off using a hand-held razor blade (Magnusun Injector blades type 500/1 890018). One section in each of the TS, TLS, and RLS planes was cut for each sample. The sections were then placed in safranin solution (50% safranin/50% alcohol) for 30 minutes in order to stain the secondary wall red. Samples were then immersed in 50% ethyl alcohol (50% ethyl alcohol/50% water) for 20 minutes, then in 75% ethyl alcohol (75% ethyl alcohol/25% water) for 20 minutes, then in 100% ethyl alcohol for 20 minutes, and finally in xylene for five minutes. A drop of DPX mountant (Agar Aids Pty. Ltd.) was then placed on a glass microscope slide, and the stained wood sections were taken directly from the xylene and immersed in the mountant. A coverslip was placed over mountant and sample. These prepared slides were allowed to set for 12 hours on a warm (40 °C) surface before use.

Light microscopy of the prepared slides was carried out using a Zeiss Axioskop microscope with halogen transmitted illumination and a blue filter. Selected wood features were photographed on 35 mm colour film (Kodak Gold III, ASA 100).

9.3 Wood Anatomy Description of Callitris.

9.3.1 General

Table 9.1 shows the occurrence of the wood features which were defined in Section 9.1.4. It can be seen that certain features: (1) axial parenchyma, (2) zonate parenchyma, (9) smooth horizontal ray walls, (15) a torus, and (16) cupressoid crossfield pits were common to all species of Callitris. Other features: (6) thick-walled latewood, (7) ray tracheids, (8) thin ray horizontal walls, (10) indentures, (11) frequent biseriate rays, (12) tall rays, (14) multiseriate bordered pits, and (17) resin ducts, were universally absent. For all other features, differences occurred between Callitris species.
Table 9.1: Wood anatomy features of *Callitris* as determined by a combined SEM/LM study.  

The features are based on those used by Phillips (1948) and each is fully described in Section 9.1.4.  

<table>
<thead>
<tr>
<th>Feature</th>
<th>1 ba</th>
<th>2 can</th>
<th>3 col</th>
<th>4 dru</th>
<th>5 end</th>
<th>6 gla</th>
<th>7 gra</th>
<th>8 int</th>
<th>9 mac</th>
<th>10 mue</th>
<th>11 mur</th>
<th>12 neo</th>
<th>13 obl</th>
<th>14 pre</th>
<th>15 rho</th>
<th>16 roe</th>
<th>17 sul</th>
<th>18 tub</th>
<th>19 ver</th>
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<td>++</td>
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<tr>
<td>(2) Parenchyma zonate</td>
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<td>++</td>
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<td>X</td>
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<td>(12) Tall rays</td>
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<td>(13) Short rays</td>
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<td>(14) Bordered pits multiseriate</td>
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<td>(15) Torus present</td>
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<td>(16) Crossfield pits cupressoid</td>
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<td>(17) Resin ducts present</td>
<td>X</td>
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<tr>
<td>(18) Calitroid thickening (SEM)</td>
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<tr>
<td>(19) Calitroid thickening (LM)</td>
<td>X</td>
<td>++</td>
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</tbody>
</table>

The full listing is as follows: 1. bai = baileyi; 2. can = canescens; 3. col = columellaris; 4. dru = drummondii; 5. end = endlicheri; 6. gla = glaucophylla; 7. gra = gracilis; 8. int = intratropica; 9. mac = macleaya; 10. mue = mueleri; 11. mur = murrayensis; 12. neo = neocaledonica; 13. obl = oblonga; 14. pre = preissii; 15. rho = rhomboidea; 16. roe = roei; 17. sul = sulcata; 18. tub = tuberculata; 20. ver = verrucosa.
9.3.2 Axial Parenchyma

Axial (longitudinal) parenchyma cells occurred in all species of *Callitris* but they were more commonly present in some species (e.g. *C. drummondii* and *C. rhomboidea*) than in others (e.g. *C. macleayana* and *C. oblonga*). Under SEM they appeared as isolated longitudinal cells, totally filled with an amorphous substance (Plates 9.1 & 9.2). Using LM, they could be discerned by the dark colour of their contents, and in TLS and RLS appeared as isolated longitudinal cells approximately 200-400 µm in length and of similar width (approximately 10-50 µm) to the surrounding tracheids (Plates 9.3-9.5). Individual parenchyma cells were separated by horizontal end-walls (Plates 9.2, 9.3, & 9.4,) which were smooth, unpitted, and approximately 2 µm thick. In TS, (Plate 9.5) individual parenchyma cells were always isolated from each other, but tended to cluster in varying abundance in tangential zones within the growth ring.

9.3.3 Bordered pits

Complementary pairs of bordered pits occurred on the radial (RLS) walls of contiguous tracheids (Plate 9.6). Each individual pit had a dome-shaped pit border which extended from the cell wall into the lumen space (Plate 9.7). The connected cavities of each bordered pit were separated by a margo and a torus (Plates 9.8 and 9.10).

The pit margo was composed of radially-oriented fibrils with a central torus which was 'convex-lens' shaped, smooth, and had no apparent perforations (Plate 9.6 and 9.8). Pit margos appeared to consist of many layers of interspersed fibrils which extended into the side of the torus. Individual fibres of the margo were approximately 50 nm in diameter (Plates 9.12-9.13). Most pits seen in SEM were aspirated, possibly as a "result of preparatory drying" (Petty 1972).

Bordered pit apertures were distinctly circular in shape, with a mean diameter for the genera of 3.5 µm, and for individual species ranging from 2.5 µm (in *C. tuberculata*) to 5.1 µm (in *C. neocaledonica*) as shown in Table 9.2. The mean (outer) diameters of bordered pits in *Callitris* ranged from 11.6 µm (for *C. tuberculata*) to 20.2 µm (for *C. neocaledonica*) (Table 9.2). An oval-shaped opening depression, measuring from 3.9 to 9.0 µm in the major axes and 2.8 to 5.2 µm in the minor axes, occurred at the top of the pit dome (Table 9.2 columns 6 and 7). The pit dome was raised approximately 1.5 µm above the wall surface (Plate 9.7). The insides of bordered pit chambers were usually lined with warts which ranged in width from 100-175 nm (Plate 9.9).

The bordered pits of all species were typically uniseriate. Occasionally opposite pitting occurred in some species, particularly those in which very wide tracheids were common; i.e. *C. macleayana*; *C. neocaledonica*; and *C. sulcata* but in such species, pitting could not be termed multiseriate. In these species also, occasional pitting of tangential (TLS) walls occurred. Pitting of tangential walls was rare in all other species of *Callitris*. SEM examination suggested that there were fewer bordered pits in latewood than in
earlywood although this could not be confirmed due to the inability to cut tracheids through their entire length during specimen preparation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Aperture</th>
<th>Range Aperture</th>
<th>Mean Outer</th>
<th>Range Outer</th>
<th>Mean opening</th>
<th>Range opening</th>
</tr>
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<td>2.8 - 3.6</td>
<td>13.7</td>
<td>11.8 - 15.5</td>
<td>7.6/4.2</td>
<td>6.6/4.3 - 8.6/5.1</td>
</tr>
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<td>canescens</td>
<td>2.7</td>
<td>2.1 - 3.4</td>
<td>14.0</td>
<td>11.8 - 16.3</td>
<td>5.6/3.3</td>
<td>4.8/2.5 - 6.3/4.0</td>
</tr>
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<td>4.9</td>
<td>4.2 - 5.2</td>
<td>19.3</td>
<td>16.6 - 22.2</td>
<td>9.0/4.8</td>
<td>8.2/3.3 - 9.5/5.8</td>
</tr>
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<td>2.6 - 3.3</td>
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<td>12.6 - 13.6</td>
<td>6.2/3.7</td>
<td>4.8/2.3 - 7.0/3.8</td>
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<td>6.2/5.9 - 9.4/5.5</td>
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<td>15.1 - 16.3</td>
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<td>6.0/3.3 - 7.5/3.0</td>
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<td>14.1 - 19.1</td>
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<td>13.1</td>
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<td>17.8 - 19.5</td>
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<td>11.6</td>
<td>9.8 - 13.4</td>
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<td>3.5 - 4.6</td>
<td>17.5</td>
<td>16.7 - 18.2</td>
<td>8.4/4.9</td>
<td>3.5/4.9 - 4.6/4.9</td>
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</tbody>
</table>

Table 9.2: Measurements of bordered pits in species of *Callitris*:
- Column 2 = Pit aperture, mean diameter (µm).
- Column 3 = Pit aperture, range of diameters (µm).
- Column 4 = Mean diameter of bordered pits (µm).
- Column 5 = Range diameters of bordered pits (µm).
- Column 6 = Outer opening of pit, mean length/width (µm).
- Column 7 = Range of openings length/widths (µm).

### 9.3.4 Callitroid Thickening

Callitroid thickening in *Callitris* consisted of raised bars of tissue situated adjacent to bordered pit apertures in the inner walls of tracheids. Since bordered pits are almost exclusive to radial walls of tracheids, callitroid thickening can be seen in full, only in RLS. In TLS, the cut sections of bars and the portions of the bars which have extended fully across the radial wall and onto the tangential wall (commonly known as awns), were visible.

Under SEM, callitroid thickening was universal throughout the genus (Feature 18 of Table 1) but its frequency was variable, and dependent on species. Typically, thickening occurred in less than 1% of pits in *C. neocaledonica* whereas in *C. verrucosa*, 98% of pits possessed thickening. The frequency of thickening in all species of *Callitris* is shown in Table 9.3. Callitroid thickening occurred in both earlywood and latewood, but was typically present at a higher frequency in narrow than in wide tracheids. In *C. macleayana*, *C. neocaledonica*, *C. oblonga*, *C. rhomboidea*, and *C. sulcata*, which often have very wide tracheids, callitroid thickening was extremely rare; its occurrence being limited to the very narrowest tracheids of these species.
Plates 9.1 - 9.5 Axial parenchyma cells in Callitris spp.
1 = SEM image (RLS) showing two parenchyma cells in C. verrucosa.
2 = Close-up view of end wall of a parenchyma cell.
3 = LM view of parenchyma in C. rhomboidea.
4 = LM close-up view of end walls of parenchyma cells.
5 = LM transverse view showing parenchyma (with conspicuous dark-coloured contents) zonate in latewood.
Plates 9.6 - 9.13: Bordered pits in *Callitris*:

6 = TLS view of pit pair showing non-aspirated membrane centrally located in pit cavity.
7 = SEM view of tracheid interior at 70° tilt angle showing raised domes of bordered pits.
8 = Dome removed, revealing torus and margo.
9 = Pit membrane removed, revealing interior wall of pit border covered with small warts.
10 & 11 = Pit membrane showing central torus and radially-oriented fibrils.
12 = View of fibrils showing characteristic 'string of beads' appearance.
13 = Side-on view of the membrane with the torus in the top right of image.
Plate 9.14 (Stereo pair) Bordered pit in *C. endlicheri*. The pit border has been removed to expose the membrane.
Four different styles of callitroid thickening: 'Types 1, 2, 3, and 4' (as defined in Chapter 3) occurred in *Callitris*. The most common type of thickening was 'Type 2' which accounted for approximately 50% of all callitroid thickening. Callitroid thickening Types 3 and 4 typically occurred in only 1% and 2% respectively, of all pits possessing thickening.

The bars of callitroid thickening tended to be rectangular in cross section ranging in thickness from 1.3 to 1.9 µm and height from 1.8 to 2.2 µm. The distance between bars was usually greater at their centres than at their ends; i.e. bars tended to curve around the pit aperture. The average distance between thickening bars was 6.3 µm. Bars were typically inclined inwards towards the pit aperture at angles ranging from 40° to 90°.

The visual prominence of thickening, seen as bars in RLS, and as awns in TLS, varied with species. In general, 'Type 2' thickening was visually more prominent than 'Type 1' thickening.

Anastomosing sometimes occurred between the awns of adjacent pits.

A search for callitroid thickening, carried out using LM (Feature 19 of Table 1), revealed its presence in 13 of the 20 Callitris species (Plates 9.15-9.28). Thickening was not observed (using LM) in *C. baileyi, C. columellaris, C. macleayana, C. muelleri, C. neocaledonica, C. oblonga,* or *C. sulcata.*
Plates 9.15-9.28: Callitroid thickening in various Callitris species as observed using LM:
15 = C. canescens; 16 = C. drummondii; 17 = C. endlicheri; 18 = C. glaucophylla;
19 = C. gracilis; 20 = C. intratropica. Scale: 1 cm = 25 µm.
Plates 9.21-9.26: Callitroid thickening in various Callitris species as observed using LM:

21 = C. monticola; 22 = C. murrayensis; 23 = C. preissii; 24 = C. rhomboidea;
25 = C. roei; 26 = C. tuberculata. Scale: 1 cm = 25 µm.
Scale: 1 cm = 25 µm.
9.3.5 Compression wood

Compression wood in *Callitris* had four characteristic features which (Timell 1986) describes as typical of compression wood in softwoods. These features were (i) checks (slits) occurring in the tracheid walls and across pit orifices (Plates 9.29 and 9.30); (ii) rib-like striations in the tracheid walls (Plates 9.29 and 9.30); (iii) rounded cell shape (Plate 9.31); and (iv) warty layer either reduced or absent (Plate 9.31). In addition, it was noted that callitroid thickening was much reduced in occurrence, or absent from compression wood.

9.3.6 Crossfield Pits

Crossfield pits, which provide a connection between ray parenchyma cells and longitudinal tracheids, were most easily viewed in RLS at a magnification of 100 - 500 times.

The number of pits within crossfields varied with species. Table 9.4 shows that species with wide tracheids (*C. macleayana; C. neocaledonica* and *C. sulcata*) tended to have the most pits within their crossfields.

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<th>1 Species</th>
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<th>3 max pits</th>
<th>4 Mean aperture</th>
<th>5 Min aperture</th>
<th>6 Max aperture</th>
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<td>9.0 - 11.9</td>
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Table 9.4: Crossfield pits in species of *Callitris*:

Column 2 = Median number of pits per crossfield.
Column 3 = Maximum number of pits per crossfield.
Column 4 = Pit aperture, mean diameter (µm).
Column 5 = Pit aperture, maximum diameter (µm).
Column 6 = Pit aperture, minimum diameter (µm).
Column 7 = Mean outer diameter of pits (µm).
Column 8 = Range of outer diameters of pits (µm).
Crossfields on the outer extremes of rays tended to have more pits than those involving the inner ray cells. Also wide crossfields in earlywood tended to have more pits than those in latewood.

Crossfield pit apertures were invariably oval in shape; and conformed to the definition of 'cupressoid' by Phillips (1948), in that "pit apertures in earlywood are included within the limits of the border and are rather narrower than the border". The major/minor axes of the aperture ranged from a mean of 3.1/1.8 µm (for C. canescens) to a mean of 6.9/3.6 µm (for C. neocaledonica). The mean aperture dimensions for all species of Callitris are shown in Table 9.4. The alignment of the crossfield pit apertures was oblique in a 'Z' spiral at 60° to 90° (Plate 9.33), and followed the alignment of warts in the warty layer. Pits were usually regularly arranged, with pairs of pits vertically superposed within the crossfields.

The outer diameters of crossfield pits varied with species, and tended to be large (mean greater than 11µm) in C. murrayensis, C. neocaledonica and C. sulcata and small (mean less than 8µm) in C. canescens, C. intratropica and C. tuberculata.

'Callitroid thickening' of crossfield pits (Plate 9.34) occurred in all species of Callitris but its frequency was variable, and depended on species. Thickening occurred only on the tracheid side, and not on the ray side, of the crossfield pits and occurred more often in crossfields within narrow tracheids than wide tracheids. In TLS, crossfield thickening awns were apparent in most species; and were particularly prevalent in C. glaucophylla, C. tuberculata and C. verrucosa.

9.3.7 Rays

Individual rays in the wood of Callitris could be seen with the naked eye in TLS. At a magnification of 50 or more, the cells comprising the ray could also be distinguished. The heights, widths, volumes and frequencies of rays within wood are most conveniently measured in TLS.

The mean height of rays in Callitris was 85 µm and mean ray heights for individual species ranged from 57 µm (C. monticola) to 177 µm (C. macleayana) as shown in Table 9.5. Maximum ray height (i.e. the maximum height recorded for an individual ray contained within a 1 mm² area of TLS surface) ranged from 131 µm for C. monticola, to 635 µm for C. macleayana.

Mean ray height in terms of numbers of cells within rays for individual species ranged from 2.6 cells (for C. monticola) to 8.6 cells (for C. columellaris) as listed in Table 9.5. Mean ray height for the genus is 4 cells. Rays taller than 30 cells (i.e. approximately 600 µm) were rare, and were limited to C. columellaris and C. intratropica. Maximum ray height in terms of numbers of cells ranged from 7 for C. monticola to 33 for C. columellaris. Rays of one cell in height were present in all species, although they occurred more frequently in species endemic to dry habitats (i.e. C. canescens, C. tuberculata, and
C. verrucosa) than in species endemic to wet habitats (i.e. C. macleayana, C. neocaledonica, and C. sulcata).

The inner cells of tall rays were typically 'square' with a mean ray cell length and width of approximately 20 µm, whereas cells at the margins of rays, and those forming very short rays (i.e. of 1-3 cells in height) tended to be elongated in length (approx. 25-45 µm) and correspondingly narrower in width. Ray cells in C. macleayana, C. neocaledonica, and C. sulcata were typically taller and wider (mean cell size 25 µm) compared to ray cells of all other Callitris species (mean cell size approx. 20 µm).

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<tr>
<th>Species</th>
<th>Mean (µm)</th>
<th>Max (µm)</th>
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Table 9.5 Mean and maximum heights of rays in Callitris species expressed in terms of µm (Columns 2 and 3), and in terms of number of cells (Columns 4 and 5).

Ray frequencies ranged from a mean of 18 (C. macleayana) to 57 rays per mm² (C. canescens and C. drummondii). Mean ray frequency values for all species of Callitris are shown in Table 9.6.

The percentage of ray tissue (i.e. ray volume) varied from 4% to 11.3%, the value being somewhat dependent on species (Table 9.6).

Ray widths varied between species. Table 9.6 shows that C. macleayana; C. neocaledonica and C. sulcata had rays of the order of 25 µm wide whereas in all other Callitris species rays were of the order of 20 µm wide. Rays are almost always uniseriate, although partial biseriation occurred in some species, being most common in C. endlicheri, in which, on average, 3.8% of the rays were biseriate (Table 9.6). Partial biseriation occurred in the central regions of the rays and not in the cells at the margins of the rays.

Rays in Callitris were wholly parenchymatous; ray tracheids were absent from all species. Ray cells in sapwood commonly contained starch granules.
Table 9.6: Values for various ray parameters in species of Callitris:
Column 2 = Mean ray frequency (rays/mm²).
Column 3 = Ray frequency range (rays/mm²).
Column 4 = Ray volume (%).
Column 5 = Ray width (µm).
Column 6 = Occurrence of partially biseriate rays (% of total rays).

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<td>27.5</td>
<td>25-31</td>
<td>6.8</td>
<td>25.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Tuberculata</td>
<td>55.6</td>
<td>40-85</td>
<td>5.1</td>
<td>17.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Verrucosa</td>
<td>52.3</td>
<td>41-68</td>
<td>8.8</td>
<td>19.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

9.3.8 Tracheids

The bulk of the woody tissue of Callitris consisted of axial tracheids (Plate 9.35) which were typically arranged in orderly radial rows (Plate 9.36).

Tracheid width was variable, and the range of tracheid diameters was dependent, to some extent, on species. Tracheid widths ranged from a mean of 15.2 µm for *C. tuberculata*, to a mean of 50.1 µm for *C. neocaledonica* (Table 9.7). The narrowest (latewood) tracheids in *C. roei* were typically 10 µm in diameter whereas the equivalent cells in *C. neocaledonica* were of the order of 25 µm in diameter.

Tracheid lumina tended to be rounded in earlywood. In latewood, tracheids had a 'squashed' or 'radially flattened' appearance. There was also a difference between earlywood and latewood cells in relation to the sapwood cell wall colour (not apparent to SEM imaging) in that latewood cells tended to be darker in colour than earlywood cells. There was little difference between earlywood and latewood cell wall thickness within individual growth rings.

The distinctiveness of growth rings varied between species. Rings were relatively prominent in *C. oblonga* and *C. roei*, and were least prominent in the tropical species *C. macleayana, C. neocaledonica* and *C. sulcata*. Growth rings were 'indistinct in relation to Characteristic 4 of Table 1 in all species except *C. canescens, C. endlicheri* and *C.
oblunga. Latewood was conspicuous only in C. glaucophylla, C. monticola and C. oblonga. In other species the latewood was less than one quarter of the width of the growth ring. Latewood was never thick-walled.

<table>
<thead>
<tr>
<th>Species</th>
<th>mean (µm)</th>
<th>min (µm)</th>
<th>max (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>baileyi</td>
<td>29.6</td>
<td>23.1</td>
<td>35.8</td>
</tr>
<tr>
<td>canescens</td>
<td>18.5</td>
<td>8.2</td>
<td>25.4</td>
</tr>
<tr>
<td>columellaris</td>
<td>20.2</td>
<td>10.9</td>
<td>27.5</td>
</tr>
<tr>
<td>drummondii</td>
<td>18.1</td>
<td>6.7</td>
<td>29.4</td>
</tr>
<tr>
<td>endlicheri</td>
<td>20.1</td>
<td>12.6</td>
<td>26.8</td>
</tr>
<tr>
<td>glaucophylla</td>
<td>19.2</td>
<td>9.5</td>
<td>28.6</td>
</tr>
<tr>
<td>gracilis</td>
<td>18.2</td>
<td>11.2</td>
<td>25.5</td>
</tr>
<tr>
<td>intratropica</td>
<td>21.6</td>
<td>15.8</td>
<td>28.2</td>
</tr>
<tr>
<td>macleayanana</td>
<td>47.1</td>
<td>30.6</td>
<td>58.5</td>
</tr>
<tr>
<td>monticola</td>
<td>15.4</td>
<td>9.7</td>
<td>28.6</td>
</tr>
<tr>
<td>muelleri</td>
<td>21.1</td>
<td>9.8</td>
<td>33.4</td>
</tr>
<tr>
<td>murrayensis</td>
<td>24.8</td>
<td>19.1</td>
<td>39.0</td>
</tr>
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<td>neocaldenica</td>
<td>50.1</td>
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<td>73.0</td>
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<tr>
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<td>29.6</td>
</tr>
<tr>
<td>preissii</td>
<td>21.7</td>
<td>15.3</td>
<td>30.5</td>
</tr>
<tr>
<td>rhomboidea</td>
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<td>10.1</td>
<td>31.5</td>
</tr>
<tr>
<td>roei</td>
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<td>28.5</td>
</tr>
<tr>
<td>sulcata</td>
<td>46.2</td>
<td>18.2</td>
<td>59.3</td>
</tr>
<tr>
<td>tuberculata</td>
<td>15.2</td>
<td>7.4</td>
<td>21.1</td>
</tr>
<tr>
<td>verrucosa</td>
<td>23.2</td>
<td>10.9</td>
<td>38.1</td>
</tr>
</tbody>
</table>

Table 9.7: Table showing mean tracheid widths (column 2), minimum tracheid widths (column 3) and maximum tracheid widths (column 4) observed in species of Callitris. All values are in µm.

9.3.9 Warty Layer

A warty layer was present on the inner walls of tracheids in all species of Callitris and was clearly visible to SEM. The warty layer was apparent to LM only as a slight 'stippling' of the walls of the lumen, as occurs in Plate 9.27. Warts appeared regularly aligned in 'rows' which were equally spaced around the tracheid wall in a helical 'Z' spiral at 60° to 90°. Warts were either very small or were absent from the regions close to the aperture of bordered pits. Warts were present on the outsides of callitroid thickening bars, but were absent from the area adjacent to the aperture which is delineated by the bars.

The mean frequencies of warts in the warty layers of individual Callitris species ranged from 0.8 to 6.1 warts/µm² (Table 9.8). Mean wart frequency for the genus was 3.1 warts/µm².

The warty layers of Callitris were formed from a heterogeneous association of two different populations of wart. The first population consisted of small, hemispherical warts; individuals wart being 60-150 nm in height and typically 100-200 nm wide at their base. These warts were present in all (20) species of Callitris. The second type of wart was large, ranging from 150-1500 nm (i.e. 0.15-1.5 µm) in both height and width, of
highly variable shape, and often with 1-8 nodules on its surface. In *C. intratropica*, *C. macleayana*, *C. neocaledonica*, *C. sulcata* only the small wart population was present. In all other species, a heterogeneous mixture of the small hemispherical and large nodulated warts occurred.

Occasional anastomosing of two adjacent warts occurred in all species in which large nodulated warts were present.

<table>
<thead>
<tr>
<th>Species</th>
<th>Morphology</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>baileyi</em></td>
<td>Mixed populations</td>
<td>2.8</td>
</tr>
<tr>
<td><em>canescens</em></td>
<td>Mixed populations</td>
<td>2.1</td>
</tr>
<tr>
<td><em>columellaris</em></td>
<td>Mixed populations</td>
<td>3.1</td>
</tr>
<tr>
<td><em>drummondii</em></td>
<td>Mixed populations</td>
<td>2.2</td>
</tr>
<tr>
<td><em>endlicheri</em></td>
<td>Mixed populations</td>
<td>2.5</td>
</tr>
<tr>
<td><em>glaucophylla</em></td>
<td>Mixed populations</td>
<td>2.1</td>
</tr>
<tr>
<td><em>gracilis</em></td>
<td>Mixed populations</td>
<td>3.2</td>
</tr>
<tr>
<td><em>intratropica</em></td>
<td>Small, hemispherical</td>
<td>2.1</td>
</tr>
<tr>
<td><em>macleayana</em></td>
<td>Small, hemispherical</td>
<td>0.7</td>
</tr>
<tr>
<td><em>monticola</em></td>
<td>Mixed populations</td>
<td>4.1</td>
</tr>
<tr>
<td><em>muelleri</em></td>
<td>Mixed populations</td>
<td>5.2</td>
</tr>
<tr>
<td><em>murrayensis</em></td>
<td>Mixed populations</td>
<td>2.8</td>
</tr>
<tr>
<td><em>neocaledonica</em></td>
<td>Small, hemispherical</td>
<td>1.2</td>
</tr>
<tr>
<td><em>oblonga</em></td>
<td>Mixed populations</td>
<td>6.0</td>
</tr>
<tr>
<td><em>preissii</em></td>
<td>Mixed populations</td>
<td>4.3</td>
</tr>
<tr>
<td><em>rhomboidea</em></td>
<td>Mixed populations</td>
<td>4.5</td>
</tr>
<tr>
<td><em>roei</em></td>
<td>Mixed populations</td>
<td>2.7</td>
</tr>
<tr>
<td><em>sulcata</em></td>
<td>Small, hemispherical</td>
<td>2.1</td>
</tr>
<tr>
<td><em>tuberculata</em></td>
<td>Mixed populations</td>
<td>3.4</td>
</tr>
<tr>
<td><em>verrucosa</em></td>
<td>Mixed populations</td>
<td>3.0</td>
</tr>
</tbody>
</table>

**Table 9.8:** The warty layer of *Callitris*. Column 2 indicates warty layer morphology (i.e. small, hemispherical warts only or a mixture of large, nodulated and small, hemispherical warts). Column 3 indicates approximate wart frequency/mm².
Plates 9.29 - 9.32: Features of compression wood in *Callitris*:
29 = Checks occurring in tracheid walls and across pit orifices (SEM view); 30 = LM view.
31 = Rib-like striations in tracheid wall (TS view); 32 = RLS view.

Plates 9.33 - 9.34 Features of crossfield pits in *Callitris*:
33 = RLS view of ray in *C. muelleri* showing cupressoid shape of crossfield pits (SEM).
34 = RLS view of ray in *C. verrucosa* showing crossfield pit thickening.

Plates 9.35 - 9.36 Features of tracheids in *Callitris*:
35 = TS view of separated tracheids in *C. muelleri*. 36 = Tracheids arranged in radial files.
Chapter 10

Conclusions and Recommendations

This Chapter discusses the main findings of the study and attempts to integrate them in order to form some overall conclusions. Recommendations for further research are also made.
10.1 Introduction

In this final Chapter of the thesis, some of the principal findings of the research are reviewed and discussed in relation to the aims. An attempt is made to correlate and integrate them, to highlight their interrelationships on a broader scale, and where possible, to suggest further implications. The discussions lead to proposals for further research which, although beyond the scope of this thesis, are suggested as a means of further elucidating or supplementing some of the findings.

Since there were three basic aims to the thesis involving the; (i) anatomical, (ii) taxonomical, and (iii) ecological themes of wood anatomy, discussion of the findings will be grouped accordingly.

10.2 Discussion of Findings Relating to Comparative Anatomical Descriptions

Since a major objective of the thesis was to describe callitroid thickening, warts, and rays in all *Callitris* species, it is appropriate to consider here, how effectively this was achieved in relation to studies in the past, all of which used LM rather than SEM as used here. The greater resolution of SEM seems likely to have been critical to the finding that there are different 'types' of callitroid thickening whereas previously only one type (i.e. two thickening bars extending fully across the lumen) had been reported. In Chapter 9, a study of wood samples using LM was able to resolve only 'Type 2' thickening and not the other thickening types (i.e. Types 1, 3 and 4). It can be concluded therefore, that SEM is essential for the resolution of Types 1, 3, and 4 callitroid thickening, and the use of LM by previous studies is the reason for their failure to detect these other morphological forms of thickening. It is also the most likely reason for reports of the absence of callitroid thickening in *C. macleayana, C. neocaledonica, C. oblonga* and *C. sulcata* since thickening in these species is almost always of Type 1.

An important finding regarding the warty layer was that nodules occur on the surfaces of large warts of most species of *Callitris*; an observation which has not previously been reported in the literature for any softwood. Since the diameters of these nodules was in the order of 100 nm, well below the resolving power of LM, it is without question that the use of SEM was essential to this finding. A similar conclusion can be made with regard to three other findings i.e.: (i) individual warts of particular species have shapes which are characteristic of the species and are different from those of other species; (ii) anastomosis sometimes occurs between adjacent warts within the warty layer and (iii) two populations of warts, one small and hemispherical and the other large and nodulated, occur as a heterogeneous mixture in the warty layers of most *Callitris* species. In Chapter 4 it was pointed out that 'small hemispherical warts', and the 'nodules on larger, complex warts' are very similar in their sizes and shapes and it was suggested that a possible explanation for this similarity is that 'nodules' on warts and 'small hemispherical warts' are one and the same. It is suggested that further
research (using TEM) be carried out to determine if the membrane overlying large warts and the tertiary wall layers contain structures that are manifest as nodules on the former and small warts on the latter.

The individual cells of rays are within the resolution range of both LM and SEM so that accurate assessment of 'ray height' (in terms of the number of cells in the ray) could be carried out equally well using either LM or SEM. However, in Chapter 5 it was shown that because of between-species differences in the sizes of ray cells, the measurement of 'ray height' in terms of the number of cells does not provide an accurate basis for interspecific comparisons. For example, it was shown that *C. macleayana*, which had the tallest (measured) rays of all *Callitris* species, was classed, when using counts of cells, as having lower ray height than *C. columellaris* and *C. intratropica* because its ray cells were generally larger than those of the other species. Because of this anomaly, it is concluded that 'ray height' in *Callitris* is better expressed as a 'lineal measurement' (i.e. distance from tip to tip of the ray) and in this respect the accuracy and speed of the SEM on-screen measurement was far superior to that which may be achieved using LM.

This study has demonstrated the applicability of SEM imaging to the study of wood anatomy, and particularly to investigation of the morphology of callitroid thickening, warts, and rays. However, several other factors contributed to the applicability of the general study method to the aims. Firstly, the almost exclusive use of core samples, taken from trees growing in their native environment, ensured that a far more representative number of samples could be studied than would have been the case if samples had been obtained from wood blocks held in xylaria collections. Core sampling also ensured that sapwood was obtained, and this was preferable to heartwood for SEM since in most, although not all, tracheids of heartwood, the cell wall features were hidden from view by amorphous extractive material (as described in Chapter 6). The small amount of wood material provided by core samples necessitated the development of preparation techniques which were efficient in terms of sample usage and consistent in the production of useful specimens (Chapter 2).

SEM was also shown to be useful in describing some features of the general wood anatomy of *Callitris*; the morphology of bordered and crossfield pits were successfully quantified. SEM proved to be of less use in anatomical descriptions requiring larger areas of view (e.g. growth rings) and features prominent as a result of their colour (e.g. axial parenchyma).

Just as it seems likely that investigations described in the literature failed to detect callitroid thickening in *C. oblonga* and several other species due to inadequate resolution of LM, so it is possible that there are some wood features that were not detected during this investigation due to inadequate resolution of the SEM used to view the samples. A feature in this category was noted during the study of bordered pits described in Chapter 9. It consisted of bead-like structures on the filamentous strands of the pit
membrane (Plates 9.12 and 9.13 in Chapter 9). These structures were common to both of the species investigated (C. endlicheri and C. preissii). Their nature and adaptive function is unknown. Photographs in Liese (1965b) show similar features on a pit membrane of Scots pine (P. sylvestris). Further investigation of these structures was outside the scope of the thesis and beyond the capability of the SEM to further resolve (they were smaller than 50 nm in diameter and the filaments of the margo were too easily destroyed by heat from the electron beam). It is possible the structures are an artefact of the gold coating process used during sample preparation. Alternatively, they may be a structure of fundamental importance to wood anatomy. It is recommended therefore that an ultra-high resolution SEM (FESEM) study be carried out to investigate this structure, and that the investigation should be combined with cryogenic and low electron accelerating voltage techniques in order to be able to reduce electron beam damage of the margo during observation and to minimise the development of coating artefacts.

10.3 Discussion of Findings Relating to Taxonomy

The second aim of the thesis was to investigate the potential use of inter-species differences in callitroid thickening, warts and rays in separating Callitris taxa. Of major interest in respect of this aim was the finding that callitroid thickening is universal within the genus, indicating that the use of ‘presence or absence of thickening’, which is commonly advocated in the literature as a means of differentiating between Callitris species (Phillips 1948; Greguss 1955; 1972) is not valid for SEM studies. However Chapter 9 indicated that because of the reduced resolution of light microscopy, the method is still generally appropriate for LM. In Chapter 5 it was shown that the proportion of pits with and without thickening bars (i.e. the frequency of callitroid thickening) varied between species, and that this was a useful taxonomic parameter for studies using SEM.

Chapter 4 demonstrated that the ‘shapes’ of the biggest warts in the warty layers of Callitris were sufficiently characteristic to distinguish certain species. Both C. drummondi and C. endlicheri could be individually distinguished on this basis. Several groups of species could also be discerned. For example, the warts of C. macleayana and C. neocaledonica were always small, hemispherical, and without nodules, and while they could not be separated from one another, their morphology distinguished them from all other Callitris species. In Chapter 8 it was also shown that wart morphology could be used to separate the woods of C. endlicheri and C. glaucophylla which are reported to have no other distinguishing features (Ilic 1994). It was concluded from these findings that wart morphology can be used as an indicator of Callitris species. The taxonomic use of this factor is different from all previous studies in the literature which used aspects of ‘warty layer morphology’, such as presence/absence of warts, or warty layer distribution frequencies.
Prior to this study, the most important taxonomic indicator afforded by ray morphology in *Callitris* was that 'rays in the genus are commonly greater than 30 cells in height' (Peirce 1937; Wheeler *et al.* 1985). Chapter 5 however, failed to substantiate this claim, and concluded that rays of this height were not common in the genus, and occurred in only two of the (20) species. In addition, ray tracheids in *Callitris*, reported by Peirce (1937) were not found in any of the species. Two factors limiting the taxonomic value of rays in wood of *Callitris* were found. Firstly, it was shown (Chapter 6), as others have found within softwoods in general, that ray height varied significantly between juvenile and mature wood, hence reducing the diagnostic value of rays in wood samples from unknown positions within trees. Secondly, mean ray height was shown to be of limited value in separating species because of the wide range of ray heights within samples. It was concluded that the most useful taxonomic feature of rays in *Callitris* was the mean height of the two tallest rays in one square mm across several different areas.

In Chapter 8, the frequency of callitroid thickening and the morphology of warts were both demonstrated to have taxonomic application in the mutual separation of *C. columellaris*, *C. glaucophylla* and *C. intratropica*; species that are well-known to be difficult to discern from each other using normal (i.e. cone and leaf) taxonomic features. However, these species could not be differentiated on the basis of ray height. Thus a general conclusion is that whereas the occurrence and frequency of callitroid thickening and wart morphology are useful taxonomic features for distinguishing between *Callitris* species, ray height is of less value.

Callitroid thickening types 3 and 4 were found to occur only rarely in juvenile wood (Chapter 6). It was also shown that there was negligible variation in the frequency of thickening between juvenile and mature wood in a specimen of *C. glaucophylla*. This indicates that callitroid thickening is a reliable taxonomic character in samples from trees of unknown age. Thickening was also shown to occur in both earlywood and latewood, but its frequency was greater in narrow tracheids than in wide ones. The greater frequency of thickening in narrow tracheids was particularly useful in species such as *C. macleayana*, and *C. neocaledonica* where thickening was rare. It can be concluded from this finding that the narrower tracheids should be checked in preference to the wide tracheids when searching for callitroid thickening in species where the thickening frequency is low.

In Chapter 7 a controlled-environment experiment was carried out to determine differences in callitroid thickening, warts and rays in *C. endlicheri*, *C. glaucophylla* and *C. intratropica* trees grown under different conditions of water availability. It was found that no significant differences occurred in callitroid thickening, warts, and rays in wood grown under the two types of treatment and from this it was inferred that differences in these features between dry and wet-habitat species are genetically and not environmentally determined. However, as pointed out in Chapter 7, this experiment
was severely limited by the length of time available (15 months) for growing the trees under the controlled conditions. The results therefore involved only juvenile wood, not mature wood. It is suggested that further research be carried out by using mature trees growing in controlled conditions. Thus the investigation could be of the style described by Zahner et al. (1964) who subjected *P. resinosa* trees to wet conditions by irrigation equivalent to 5 cm of rainfall per week and to drought conditions by restricting root spread by means of a trench and constructing a rainproof shed over the roots within the trenched area. In such a study the effect of water on the tree need not be over the whole life of the tree but rather over a shorter period (Zahner et al. 1964 used one growing season). Non-destructive sampling (e.g. coring) could then be used to allow changes in the anatomy of the wood formed under different conditions of moisture availability to be monitored.

Callitroid thickening was also found to occur in several other Cupressaceae genera (*Actinostrobus*, *Callitropsis*, *Diselma*, and *Tetraclinis*) including two genera in which it had not previously been reported (*Diselma* and *Cupressus*) and one genus in which it had previously been reported to be absent (*Callitropsis*) suggesting that callitroid thickening is less exclusive to the genus *Callitris* than has previously been thought. This may somewhat limit the value of callitroid thickening as a taxonomic feature.

Further studies of the use of wart morphology to separate species of *Callitris* as well as species of related genera would be useful to demonstrate the value of warts as a taxonomic feature. During the course of this study, certain populations of *Callitris* were not used as sample sources due to the uncertainty of their species. It is suggested that studies of warts in wood of trees of these populations be carried out in conjunction with a taxonomic study of their cone and leaf morphology in order to further determine the taxonomic potential of warts. These populations are as follows: (1) The population of *Callitris* which occurs in the Willis area near the Jacobs River Bridge, Lower Snowy River, Kosciusko National Park, 50 km south of Jindabyne, NSW. Pulsford (1991) describes this population as *C. glaucophylla* and Jones (pers. comm.) believes them to be *C. murrayensis*. (2) The population of *Callitris* that grows on the roadside near the Ferries-McDonald Conservation Park, 18 km southwest of Murray Bridge, South Australia. There is some uncertainty as to whether this population is *C. drummondii* or *C. canescens* (Jones pers. comm.). (3) Nadolny & Benson (1993) report that *C. oblonga* has two distinct areas of distribution: (i) northeastern Tasmania and (ii) the Northern and Southern Tablelands of NSW (only the Tasmanian population was involved in this thesis). The female cone of the NSW populations is generally smaller than that of the Tasmanian form (Nadolny & Benson 1993) and it is claimed that this may be sufficient to consider the two populations as separate taxa (Briggs and Leigh 1988) or as separate species (Harris quoted in Nadolny & Benson 1993).

The study of crossfield pit 'callitroid' thickening was not an aim of this thesis, although during the present study, records were kept of its occurrence and qualitative
morphology in all species of *Callitris*. It was universal to the genus (Chapter 3), and it was also recorded in *Actinostrobus pyramidalis* and *Cupressus sempervirens* (Chapter 8). Crossfield pit thickening was noted to be more prevalent in species with a high occurrence of bordered pit callitroid thickening. A further general observation was that owing to the more concentrated spatial arrangement of crossfield pit thickening within ray crossfields, it was sometimes visually more apparent than the bordered pit callitroid thickening, especially in species with a low frequency of both types of thickening. For this reason it may be of use taxonomically, and it is recommended that a study of the taxonomic potential of 'callitroid' thickening on crossfield pits in *Callitris* or in other genera be carried out.

10.4 Discussion of Findings Relating to Ecology (Function)

The third major aim of the thesis was to correlate the differences in callitroid thickening, wart, and ray morphology of individual *Callitris* species with the type of habitat to which the particular species are endemic (i.e. to investigate ecological aspects of the wood anatomy of *Callitris*).

The important finding of the thesis in relation to this aim was the distinct dichotomy which occurs between the morphology of callitroid thickening, warts, and rays of *Callitris* species native to dry and those endemic to wet habitats. Dry-habitat species were found to have, in general, high frequencies of callitroid thickening, large warts of complex shape, and short rays occurring at relatively high frequency. In wet-habitat species, callitroid thickening was less commonly present on pits, warts were generally small and hemispherical, and rays were taller and occurred at a lower frequency. Questions can therefore be raised regarding the adaptive significance of these habitat-related differences. For example, are they a means of increasing the efficiency of water conduction in greatly different conditions of water availability, or are they safety mechanisms, reducing the chance of tracheids being disabled by air embolisms formed under water stress? A hypothesis was proposed (Chapter 3) in which callitroid thickening acted as 'structural supports' to prevent collapse of cells in the region of the pit aperture where the cell wall is weakest. Three hypotheses regarding the functional significance of warts were proposed in Chapter 4, two of them suggesting that the role of the warty layer was to improve the efficiency of vertical water transfer within the tree and one hypothesis suggesting that warts could reduce seepage (and hence wastage) of water into cells in which embolism had already occurred by maintaining the air in the disabled cell at 100% relative humidity. Rays of dry-habitat species were typically short but occurred at high frequency whereas those of wet-habitat species were generally taller but occurred at lower frequency. Thus, as pointed out in Chapter 5, in both dry and wet-habitat species the number of ray cells present in the wood was similar, but the spatial arrangement of these cells was different.
The findings of Chapter 8 gave indication of the occurrence of large nodulated warts and callitroid thickening in several species of genera closely related to Callitris. However, because of the very limited sampling used in the study it was not possible to determine if there was a correlation between the occurrence of these features and habitat type as has been shown to occur for Callitris (discussed above). Thus, there is a need to determine whether the dichotomy that exists in the wood morphology of dry and wet-habitat species of Callitris extends into other genera, i.e. is the occurrence of large nodulated warts and high occurrence of callitroid thickening in wet-habitat species exclusive to Callitris or is it a more fundamental finding applicable to more than one genus of softwood? The findings of Chapter 8 indicated that A. pyramidalis exhibited the typical 'dry habitat' morphology. However, since the genus Actinostrobus contains only two species, both of which are of the 'dry-habitat' type, this cannot be further investigated in relation to the results obtained with Callitris. A more appropriate genus to study would be one which, like Callitris, has multiple wet-habitat and dry-habitat species. The genera selected for such research would ideally contain multiple species, although not more than could be conveniently handled, and the species would be sufficiently common for sampling to be possible. The genera recommended for further research are: Juniperus which has 60 species and Cupressus which has 12 species (Li 1953). In Chapter 8 it was found that of the five species of Juniperus examined, three species (J. foetidissima, J. scopulorum, and J. virginiana) had large nodulated warts and one species, (J. procera) had callitroid thickening. Similarly, of four species of Cupressus examined, no large warts occurred but callitroid thickening was found in one species (C. sempervirens).
References


Bullock, A.A. 1957. The typification of the generic name *Callitris*. Taxon. 6: 227-228.


References


Location: Between Rosewood and Grandchester, Queensland. On the private property of Mr Hugh Arthur at end of Coulsen's Road. Latitude: 27° 40' S. Longitude 152° 32' E.
Site: Partially cleared hillside of mixed Eucalypts and grassland. The mean annual rainfall for Ipswich is 877 mm. Five samples collected from an area of approximately 1 km² by RH and PB on 18/11/1991.
Appendix 1 Sources of Samples

2: *C. canescens* (Parl.) S.T. Blake, comb. nov.

**Site (1):** On roadside verge approximately 30km from Lake Grace on road to Newdegate, Western Australia. 33° 10' S. 119° 30' E. The mean annual rainfall for Lake Grace is 353 mm. Trees approximately 3 m high. Foliage silvery-green. Five samples collected in an area approximately 300 m square by RH 07/02/1992.

**Site (2):** 30 km S. of Port Lincoln (Cape Wiles) South Australia. 34° 48' S. 135° 52' E. The mean annual rainfall for Port Lincoln is 489 mm. Three samples collected by DJ Aug 1995. (Nat. Bot. Gardens voucher 14117).
3: *C. columellaris* F. Mueller

Location: On road between the Pacific Highway and Bribie Island, QLD. Latitude: 27° 04' S. Longitude 153° 12' E. Trees (10-25m tall) on roadside verges, 1 to 10 km from the bridge to the island. The mean annual rainfall for Caloundra is 1573 mm. Seven samples collected by RH and PB on 20/11/1991.
4: *C. drummondii* (Parlatore) F. Muell.

**Location:** Approximately 15 km from Ravensthorpe on road from Ravensthorpe to Hopetoun (near Kindup) WA. Latitude: 33° 41'S. Longitude 120° 11' E.

**Site:** The mean annual rainfall for Ravensthorpe is 419 mm. Trees on roadside verge.

Five samples collected from an area of approximately one square km on both sides of the road by RH on 07/02/1992.
Appendix 1

Sources of Samples

5: *C. endlicheri* (Parl) F.M. Bailey

**Site (1)** Areas approximately 20 km north of Temora, NSW. 34° 27' S. 147° 32' E. Five cores collected by DJ and CBH 22/10/1992. (NBG vouchers 10464, 10462, 10484, 10436, 10442). The mean annual rainfall for Temora is 529 mm.

**Site (2)** From roadside 20.2 km north of Rylstone, NSW. 32° 39' S. 149° 58' E. Four cores collected by RH and PB 09/02/1995.

**Site (3)** Roadside near Kandos, NSW. 32° 52' S. 149° 58' E. Two cores collected by RH and PB 09/02/1995.

**Site (4)** Roadside 26 km west of Cowra, NSW. 33° 45' S. 148° 31' E. Two cores collected by RH and PB 10/02/1995.
6: *C. glaucophylla* Thompson & Johnson

**Site (1)** Roadside verges near Coonabarabran, NSW. 30° 30' S. 149° 30' E. Five cores collected by RH & PB 22/11/91. Mean annual rainfall for Coonabarabran: 735 mm.

**Site (2)** 20 km west of Rankins Springs, NSW. 33° 51' S. 146° 16' E. Two cores collected by DJ and CBH 23/10/1992. (NBG vouchers 10467, 10463).

**Site (3)** Roadside near Tamworth, NSW. 31° 05' S. 150° 56' E. Four cores collected by RH & PB 10/11/92.

**Site (4)** 100 m off Newell Highway, Gillenbah State Forest, Narrantera, NSW. 34° 48' S. 146° 30' E. Four samples collected by RH, PB & JC 22/02/94.

**Site (5)** Roadside 52 km north west of Cowra, NSW. 30° 30' S. 149° 30' E. One core collected by RH & PB 10/02/1995.

**Site (6)** Roadside 62 km east of Forbes, NSW. 33° 23' S. 148° 25' E. One core collected by RH & PB 10/02/1995.

**Site (7)** Roadside 5 km east of Cudal, NSW. 33° 26' S. 148° 23' E. Two cores collected by RH & PB 10/02/1995.

**Site (8)** Roadside 1 km west of Eugowra, NSW. 33° 26' S. 148° 23' E. One core collected by RH & PB 10/02/1995.

**Site (9)** Wilpena Pound SA. 31° 34' S. 138° 35' E. Four cores collected by DJ 21/08/95. (NBG voucher 10498).
7: *C. gracilis* R. Baker

**Location:** Kerrabee area off Honesuckle Creek, approximately 16 Km east of Bylong NSW. Latitude 32° 24' S. longitude: 150° 16' E.

**Site description:** About 50 trees of *C. gracilis* grow in mixed Eucalypt grassland, 100 m from road, below the bluff on the northern side of the road. The mean annual rainfall for Gulgong is 642 mm.
8: *C. intratropica* R.T. Bak. & H.G. Smith

**Site (1)** From roadside verge 11.8 km from Mareeba, QLD. 16° 59' S. 145° 30' E. One sample collected by AS on 22/01/92. Mean annual rainfall for Mareeba is 945 mm.

**Site (2)** Litchfield area, Northern Territory. 13° 29' S. 130° 42' E. Samples collected by KG and SK in July 1992. The mean annual rainfall for Darwin is 1535 mm.

**Site (3)** North-west corner of Atherton Tablelands, Queensland. 17° 16' S. 145° 29' E. Samples collected by KG and SK in July 1992.

**Site (4)** Central area of Groote Eylandt, 17 km west of Umbakumba, Northern Territory. 13° 49' S. 136° 38' E. Samples collected by KG and SK in July 1992.
9: *C. macleayana* (F. Mueller) F. Muell.

Site (1) Cypress Grove, in Maiala National Park, QLD. 27° 20' S. 152° 48' E. The mean annual rainfall for Mt Glorious is 1655 mm.

Site (2) Off Browns Road, outside Maiala National Park, QLD. 27° 20' S. 152° 48' E.

Site (3) Windsor Tablelands, between Cooktown & Cairns, QLD. 16° 12' S to 16° 16' S. 145° 04' to 145° 06' E. (Collector JA). Mean annual rainfall for Cairns: 2032 mm.

Site (4) Windsor Tablelands, between Cooktown and Cairns, QLD. 16° 12' S to 16° 16' S. 145° 04' to 145° 06' E. (Collector TA 1994).

Samples from sites 1 & 2 collected by RH and PB on 21/11/1991.
**10: C. monticola** J. Garden

**Location:** Near Waratah Trig. Gibraltar Range National Park, between Glen Innes, and Grafton NSW. 29° 29' S. 152° 20' E. The mean annual rainfall for Glenn Innes is 847 mm. Five samples collected by RH and PB on 09/11/1992.
11: *C. muelleri* (Parlatore) F.Mueller

**Location:** 1.1 km along fire trail 4EBW, off Gloworm Tunnel Road, 2 km north-east of Lithgow, NSW. Latitude: 33° 20' S. Longitude 150° 15' E.

The mean annual rainfall for Lithgow is 863 mm. Five samples collected from an area of approximately 200 m square by RH and PE on 23/09/1992.
12: *C. murrayensis* R.Br. ex Bak. & Sm.

**Location:** Bordering Princes Highway 8 km south of Tailem Bend, South Australia. Latitude 35° 15' S. Longitude 139° 28' E.

**Site description:** Sandy soil, approximately 2 km from River Murray. The mean annual rainfall for Tailem Bend is 383 mm. Seven samples collected by RH on 24/04/95.
13: *C. neocaledonica* Dümmer

**Location:** Montagne des Sources, New Caledonia. Latitude: 22° 10' S. Longitude 166° 30' E. Altitude 1050 m.

The mean annual rainfall for the region is 1500-3000 mm (Schmid 1995).

**Tree descriptions:** Height 3-5m; diameter 30-35 cm. Three samples collected by BS on 03/05/1991.
14: *C. oblonga* A. & L.C. Richard

**Site (1)** Floodplain of St Pauls River, off Royal George Road, 4 km south of Avoca, Tasmania. 41° 49' S. 147° 44' E.

**Site (2)** Off Plains Road, close to St Pauls River, Royal George, Tasmania. 41° 50' S. 147° 54' E.

**Site (3)** In floodplain of Apsley River, off Coles Bay Road, 9 km south of Bicheno, TAS. 41° 55' S. 148° 18' E. The mean annual rainfall for Bicheno is 691 mm.

**All Sites:** Riverine vegetation, in poorly drained areas or river floodplain.

All samples collected by RH, PE and MK on 14/07/1994.
15: *C. preissii* R.T. Baker

**Site (1)** Woodman Point, Munster (south Fremantle) WA. 32° 08' S. 115° 44' E. The mean annual rainfall for Fremantle is 771 mm. Five samples collected by BL and TL 02/07/1995. (NBG voucher 1782).

**Site (2)** Merrifield Road, Mullaloo, Perth, WA. 31° 51' S. 115° 44' E. Two samples collected by BL and TL 02/07/1995. (National Botanic Gardens voucher 1906).

**Site (3)** Bournemouth Road, Trigg, Perth, WA. 31° 53' S. 115° 45' E. Five samples collected by BL and TL 02/07/1995. (National Botanic Gardens voucher 1911).

**Site (4)** Ord/St Ellen Street, Fremantle, WA. 32° 02' S. 115° 45' E. Three samples collected by BL and TL 02/07/1995. (National Botanic Gardens voucher 1914).

**Site (1)** Burrewarra Point, 15 km south of Batemans Bay, NSW. 35° 50' S. 150° 14' E. Site: scrub 500 m from beach. Collected by RH and PE 30/09/1992.

**Site (2)** Off Old Coach Road, 9 km north of Cranbrook, close to junction Swan River and West Swan Creek, Tas. 41° 54' S. 148° 04' E. Site: below road, next to river but above river floodplain.

**Site (3)** 1 km north of Bicheno, Tasmania. 41° 53' S. 148° 18' E. Site: well-drained sandy soil 2 km from beach. The mean annual rainfall for Bicheno is 691 mm.

**Site (4)** Sherbourne Road, 6 km north of Cranbrook, Tasmania. 41° 58' S. 148° 08' E.

**Site (5)** Mayfield Bay, 9 km S. of Swansea, TAS. 42° 15' S. 148° 00' E. On steep, well-drained hillside, 100 m from beach. Mean annual rainfall for Swansea is 614 mm.

Samples from sites 2-5 collected by RH, PE and MK 14/07/1994.
17: *C. roei* (Endlicher) F. Muell.

Site (1) Roadside verges, 3 to 30 km east of Lake Grace on the road to Newdegate, WA. 33° 10' S. 118° 28' E. The mean annual rainfall for Lake Grace is 353 mm. Four samples collected by RH on 07/02/1992.

Site (2) Roadside verges, Newdegate to Lake King Road, WA. 33° 10' S. 119° 46' E. Two samples collected by RH on 07/02/1992.
18: *C. sulcata* (Parl.) Schlechter

**Location:** Banks of Dumbea du Nord, New Caledonia. Latitude 22° 15' S. Longitude 166° 25' E.

The mean annual rainfall for the region is 1500-3000 mm (Schmid 1995). Three samples (each of two cores) collected by PE and BS on 07/03/1991.
19: *C. tuberculata* R. Brown ex Mirbel

**Location:** Roadside verges, 15 to 32 km east of Lake Grace on the road to Newdegate, WA. Latitude: 33° 06' S. Longitude: 118° 25' E.

The mean annual rainfall for Lake Grace is 353 mm.

Five samples collected by RH on 07/02/1992.
Appendix 1: Sources of Samples

20: *C. verrucosa* (A. Cunn. ex Endl.) J. Garden

**Site (1)** Off road to Rankins Springs, 31.5 km north of Griffith, NSW. Latitude 34° 17' S. 146° 02' E. DJ & CBH on 24/10/1992. (NBG vouchers 10482 & 10483).

**Site (2)** Near Wilmington, SA. 32° 39' S. 138° 06' E. Two cores collected by DJ Aug 1995. (NBG voucher 14144). Mean annual rainfall for Port Augusta is 242 mm.

**Site (3)** The area of Tooligie, SA. 33° 52' S. 135° 42' E. Four cores collected by DJ Aug 1995. (NBG voucher 14141).

**Site (4)** 44 km east of Euston, NSW. 34° 35' S. 143° 20' E. Mean annual rainfall for Euston is 312 mm. Four cores collected by DJ Aug 1995. (NBG voucher 14085).

**Site (5)** 12.6 km east of Euston, near Lake Bereemba, NSW. 34° 35' S. 142° 50' E. DJ August 1995. (NBG voucher 14084).
### Institution and Name Abbreviations:

**Institution Abbreviations:**

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<th>Abbreviation</th>
<th>Full Name</th>
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<tr>
<td>ANUB</td>
<td>Dept. Botany &amp; Zoology, ANU, Canberra ACT</td>
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<td>ANUF</td>
<td>Forestry Department, ANU, Canberra ACT</td>
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<tr>
<td>CALM</td>
<td>Cons. and Land Management, PO Box 104, Como, WA 6152</td>
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<td>CSIRO</td>
<td>Division of Forest Products, CSIRO, Clayton, Vic</td>
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<td>JAPA</td>
<td>Forestry &amp; Forest Products Research Labs, Ibaraki, Japan</td>
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<td>MARG</td>
<td>Margules Groome Poyry Pty Ltd, 3 Franklin St, Manuka, ACT</td>
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<td>NBGH</td>
<td>National Botanic Gardens Herbarium, Canberra ACT</td>
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<td>NEWCAL</td>
<td>Service des Forets Naturel, Noumea, New Caledonia</td>
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<td>QFS</td>
<td>Queensland Forestry Service</td>
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**Name Abbreviations:**

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<td>Brendan Lepschi CALM</td>
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<td>BS</td>
<td>Bernard Suprin NEWCAL</td>
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<td>TQ</td>
<td>T.K. Qin ANUB</td>
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</tbody>
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Letter of acceptance, reviewer's comments, and a copy of the Paper "Morphology of Warts in Tracheids of Cypress Pine (Callitris Vent.)", published in the IAWA Journal:


North Carolina State University
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Dr. R.D. Heady
Department of Forestry
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Canberra, ACT, 0200
AUSTRALIA

February 7, 1994

Dear Dr. Heady:

I'm pleased to tell you your paper "Morphology of Warts in the Tracheids of Cypress Pine (Callitris Vent.) is accepted for publication in the IAWA Journal, pending minor revision. Enclosed are 1) annotated copies of your manuscript, 2) copies of the reviews, and 3) your diskette. Both reviewers felt the paper was interesting and well written, and they had only a few comments.

Regarding the minor comments of reviewer 2, I don't think that you need to change rays to cross fields, and I'm not sure about changing medullary ray to primary ray. Please respond to the numbered comments of reviewer 1.

I've made some editorial notations in green ink. Please: 1) throughout the ms. use '&' rather than 'and' for multiple-author citations given within parentheses; 2) citations in parentheses do not need to be set off by commas, 3) double-space the ms. throughout, including the summary and references, 4) do not place an extra line between paragraphs, 5) indent the first line of a paragraph, 6) number the pages, 7) begin sentences with full generic name, rather than abbreviation.

Please send me the revised manuscript and diskette, rather than sending it to Leiden. I look forward to seeing the final version of your very nicely done paper.

Thank you.

Sincerely yours,

Elisabeth Wheeler
MORPHOLOGY OF WARTS IN THE TRACHEIDS OF CYPRESS PINE (CALLITRIS VENT.)

by

R.D. Heady¹, R.B. Cunningham², C.F. Donnelly² and P.D. Evans¹

¹Department of Forestry, and ²Department of Statistics, The Australian National University, Canberra, ACT, 0200, Australia

Summary

A high-resolution SEM examination of the warty layer in tracheids of the Australasian softwood genus Callitris Vent. has revealed that warts in 12 of the 16 species have variable, complex morphology and node-like projections, giving them a ‘nodulated’ or ‘branched’ appearance similar to those described for certain hardwoods. Pairs of warts were occasionally anastomosed. Warts could be categorised into two types; large and nodulated, or small and hemispherical. In the four Callitris species native to high rainfall environments, warts were invariably of the latter type and were morphologically distinct from the mixed populations of small hemispherical and large nodulated warts found in species from dry habitats. This suggests that large nodulated warts are of adaptive value in water-stress conditions. Wart morphology was useful as an indicator of Callitris species although intra-specific variation limited the accuracy of diagnosis.

Key words: Warty layer, wart morphology, vestures, Callitris Vent., SEM, taxonomy, nodules, tracheids.

Introduction

Numerous accounts of the morphology of warts in softwood tracheids and hardwood vessels and fibres are reported in the literature. In certain softwoods, wart shape has been described as being “spherical” (Cronshaw 1965; Jurbergs 1965; Liese 1965; Ohtani & Fujikawa 1971) and in others as “rounded cone” (Harada & Côté 1985), “conical”, or “complex” (Ohtani & Fujikawa 1971). In hardwoods, warts and vestures, which are considered to be synonymous (Côté & Day 1962; Ohtani et al. 1984), are reported to be “knob-like” (Van Vliet 1978) or “branched, unbranched, hemispherical, conical, capillary, or rod-like” (Ishida & Ohtani 1970; Ohtani 1979; Ohtani et al. 1983; Harada & Côté 1985). The size of individual warts in warty layers shows variation both within and between species of softwoods (Liese 1963; Ohtani & Fujikawa 1971) and species of hardwoods (Ohtani 1979; Ohtani et al. 1983). In softwoods, warts are usually larger in the corners than in the centres of tracheids (Baird et al. 1974; Verhoff & Knigge 1976; Harada & Côté 1985) and there is variation in wart size between earlywood and latewood (Ohtani & Fujikawa 1971; Verhoff & Knigge 1976).

Differences in the shape and size of warts between various softwood and hardwood species have been used with rather limited success both taxonomically (Liese 1963; Ohtani & Fujikawa 1971; Ohtani 1979; Ohtani et al. 1983) and phylogenetically (Parham & Baird 1974).

The Australasian softwood genus, Callitris Vent. (Cupressaceae) commonly known as cypress pine, consists of 16 species (Garden 1956; Dallimore & Jackson 1966; Thompson & Johnson 1986), 14 of which are endemic to Australia while two are found only in New Caledonia. Apart from C. macleayana and the two New Caledonian species (C. neocaledonica and C. sulcata) which are found in or on the margins of rain forests, Callitris occurs mainly as woodland species on relatively infertile sites in warm sub-humid to semi-arid climatic zones. The species range from tall shrubs to medium-sized trees. Wood of Callitris is generally distinguished from that of other conifers by the existence of “callitrisoid (callitroid) thickening” in the area of its...
Table 1. Listing of species, showing number of specimens examined in this study, habitat type, and sample collection area. Australian states and territories are abbreviated as follows: Australian Capital Territory = ACT, New South Wales = NSW, Northern Territory = NT, Queensland = Qld, South Australia = SA, Western Australia = WA.

<table>
<thead>
<tr>
<th>Callitris species</th>
<th>Samples</th>
<th>Habitat type (Sample collection area)</th>
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<tbody>
<tr>
<td>C. bailey C.T. White</td>
<td>5</td>
<td>Warm subhumid forest (southern Qld)</td>
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<tr>
<td>C. canescens (Parl.) S.T. Blake</td>
<td>5</td>
<td>Rocky semi-arid scrubland (southern WA)</td>
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<td>C. columellaris F. Muell.</td>
<td>7</td>
<td>Sub-humid coastal plains (southern Qld)</td>
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<td>C. drummondii (Parl.) F. Muell.</td>
<td>5</td>
<td>Sandy semi-arid scrubland (southern WA)</td>
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<td>C. endlicheri (Parl.) F.M. Bailey</td>
<td>12</td>
<td>Cool infertile woodland (ACT, NSW)</td>
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<td>C. glaucophylla Thompson &amp; Johnson</td>
<td>15</td>
<td>Temperate woodland (central NSW)</td>
</tr>
<tr>
<td>C. intratropica R.T. Baker &amp; H.G. Smith</td>
<td>8</td>
<td>Wet-tropical woodland (WA, Qld, NT)</td>
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<td>C. macleayana F. Muell.</td>
<td>7</td>
<td>Tropical rain forest (southern Qld)</td>
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<tr>
<td>C. monticola J. Garden</td>
<td>6</td>
<td>Sub-tropical rocky outcrops (NSW, Qld)</td>
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<td>C. muelleri (Parl.) F. Muell.</td>
<td>6</td>
<td>Mixed dry sclerophyll forest (NSW)</td>
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<td>C. neocaledonica Dummer</td>
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<td>Tropical rain forest (New Caledonia)</td>
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<td>C. oblonga L. Richard</td>
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<td>Temperate river flats (southern NSW)</td>
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<td>C. preissii Miq.</td>
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<td>Temperate woodland (NSW, WA)</td>
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<td>Temperate coastal sands (southern NSW)</td>
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<td>Infertile semi-arid scrubland (inland WA)</td>
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<td>C. sulcata (Parl.) Schlechter</td>
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<td>Tropical rain forest (New Caledonia)</td>
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pits (Patton 1927; Peirce 1937; Phillips 1948; Greguss 1972). Lack of callitrisoid thickening separates C. macleayana from all other “commercial timber species of Callitris” (Phillips 1948). Ray height was also used by Patton (1927) to separate wood of C. intratropica from C. muelleri, C. rhomboidea, and C. oblonga. Callitris is known to have ‘strong occurrence’ of a warty layer in five species and is referred to as a genus with “numerous bigger warts” (Liese 1957, 1965), but there are no reports in the literature regarding the occurrence, form, or taxonomic significance of warts in all of the species in the genus.

The aim of the present study is two-fold; firstly to examine the morphology of warts in all species of Callitris by use of high-resolution scanning electron microscopy; and secondly to determine whether features of warty layer morphology can be used taxonomically to identify Callitris species.

Materials and Methods

Wood samples of the 16 Callitris species were obtained mainly from 5 mm diameter increment cores, or sections taken from the main stems of trees or shrubs, of unknown age, growing wild and within the known distribution range of each species (Table 1). In addition, some samples were cut from wood specimen blocks obtained from reference collections held at the Forestry Department of the Australian National University, Canberra; the NSW Forestry Commission Laboratories, Beecroft, Sydney; the CSIRO Division of Forest Products, Clayton, Melbourne; and the Service des Forêts Patrimoine Naturel, Noumea, New Caledonia. For some species, as many as 15 wood samples were examined, while for other less common ones, only four or five samples could be obtained (Table 1). In all, a total of 116 wood samples were studied. Samples were considered at the species level only. Thus for the purposes of this study, the three subspecies of Callitris preissii Miq.; i.e. subsp. verrucosa, preissii and murrayensis (Garden 1956; Dallimore & Jackson 1966) were combined as C. preissii.

Dry wood samples were softened by soaking in distilled water at 20°C for four days until saturated. Soaking time was reduced for sapwood samples cut from fresh, green cores.
For the cutting of plane surfaces, wood samples were clamped in a small vice and viewed under a stereomicroscope at low magnification (Kucera 1981). A hand-held, single-edged razor blade (Magnuson Injector type) was then used to cut a clean and accurate face measuring approximately 5 by 3 mm in either the radial longitudinal (RLS) or tangential longitudinal (TLS) plane. After surface cutting, cytoplasmic debris was removed from prepared surfaces by washing samples in several changes of distilled water. Samples were dried at atmospheric pressure over silica gel at 22°C for two days and under vacuum (10^-4 Pa) for eight hours. The dried samples were then attached to aluminium stubs using nylon fingernail varnish as an adhesive and sputter coated with a 10 nm layer of gold.

A Cambridge Instruments S360 scanning electron microscope (SEM) fitted with a high-brightness lanthanum hexaboride (LaB6) electron source was used to examine and measure the size of individual warts of each species and for the production of micrographs. The electron optics system of the SEM was optimised for high resolution, but with sufficient depth of field to enable all of the selected image to be focused. This involved the use of a 30 µm diameter final aperture, a working distance of approximately 8 mm, electron beam current of 5 pA and an accelerating voltage of 10–20 kV.

In order to measure wart height and width, prepared specimens were tilted at an angle of 55° and the tracheids were viewed longitudinally at a magnification of 30,000 times. In this particular configuration it was possible to clearly image a rectangular area of the tracheid wall measuring 4 x 3 µm. Areas at the approximate centre of the tracheid, clear of pits, rays, debris and wax were selected for imaging, and earlywood, rather than latewood, tracheids were preferred, although it was often difficult to distinguish between them. Samples showing features of reaction wood were always rejected. Composites, consisting of 50 real-time SEM scans, were formed in order to provide clear, noise-reduced images. The height of each wart was then measured from a point at its outermost tip, perpendicularly to a point along its base, using the movable cursors of an on-SEM-screen point-to-point measurement facility. Wart width was measured in a similar fashion, but with the cursors positioned at opposite sides of each wart, along its baseline. For each of the 116 wood specimens, height and width measurements were obtained for all warts located within the imaged area (12 µm^2) of RLS tracheid wall. The measurement process was then repeated on equalized-sized areas of tracheid wall, using surfaces cut in the TLS plane from the same wood specimens. In total, height and width measurements were made on 7,434 individual warts representing all 16 of the Callitris species. In addition, wart density data (warts/µm^2) were derived from counts of the numbers of warts observed in imaged areas of each specimen.

High-resolution micrographs were formed by viewing the tracheids longitudinally at a magnification of 50,000 times. Slow-scan (160 second) SEM images were then recorded directly onto Kodak PXP 220 film. The slightly 'stippled' texture of surfaces visible in these images is an artefact of the gold-sputtering process used in the preparation of specimens.

The measured variables, wart height and wart width, were used to derive wart size (i.e. height multiplied by width), and wart shape (i.e. height divided by width). For each species, these parameters reflected variation at three levels; specimen (tree) level, section (RLS or TLS) level, and the individual wart level. It was required that analysis of variance models for these data should include both fixed and random effects; fixed effects for species and for section type, and random effects for specimens, sections, and for residual error. Following initial analysis of data, it was apparent that there was considerable heterogeneity in wart characteristics at all three levels. Not only were there apparent differences in the means of the distributions, but also between variances and skewness properties. In fact, for most species there was clear multimodality suggesting mixed wart populations. It was not possible to identify factors which may have produced this heterogeneity. An analysis of variance ignoring the multi-level structure was inappropriate since the assumptions which underlie this method were clearly not satisfied; the variances were not constant and the data were not normal. In any case, for data exhibiting distributions of
Figs. 1 & 2. Scanning electron micrographs of warts in *Callitris* species. Tracheid axis is vertical and viewing angle is 55° - 1: *C. baileyi*. - 2: *C. canescens*. - Scale bars = 500 nm; magnification = $\times$ 50,000.
Figs. 3 & 4. Scanning electron micrographs of warts in *Callitris* species. Tracheid axis is vertical and viewing angle is 55°. – 3: *C. columellaris*. – 4: *C. drummondii*. – Scale bars = 500 nm; magnification = × 50,000.
Figs. 5 & 6. Scanning electron micrographs of warts in *Callitris* species. Tracheid axis is vertical and viewing angle is 55°. – 5: *C. endlicheri*. The arrow indicates a 'branch-like' nodular protrusion. – 6: *C. glaucophylla*. – Scale bars = 500 nm; magnification = × 50,000.
Figs. 7–11. Scanning electron micrographs of warts in Callitris species. Tracheid axis is vertical and viewing angle is 55°. – 7: C. intratropica. – 8: C. macleayana. – 9: C. muelleri. – 10: C. neocalydonica. – 11: C. oblonga. – Scale bars = 500 nm; magnification of Fig. 7 = × 50,000, of Figs. 8–11 = × 25,000.
Figs. 12–16. Scanning electron micrographs of warts in *Callitris* species. Tracheid axis is vertical and viewing angle is 55°. – 12: *C. monticola*. – 13: *C. preissii*. – 14: *C. rhomboidea*. – 15: *C. roei*. – 16: *C. sulcata*. – Scale bars = 500 nm; magnification of Fig. 12 = × 50,000, of Figs. 13–16 = × 25,000.
this type, assumptions relating to differences among means would not be sensible. Therefore data were first aggregated to specimen level by calculating means for each specimen, and then analysis of variance was used to provide estimates of species means and appropriate standard errors. In this way, irrespective of the underlying distribution of the raw data, the specimen means (by the Central Limit Theorem) were rendered towards normality. Wart density and wart nodularity values for species were derived in a similar way.

Results
Warts were complex, asymmetrical and heterogeneous and their visual appearance was further complicated by the occurrence of 'nodules', on the exterior surfaces of some of the larger warts (Figs. 1–16). These nodules were hemispherical or tubular in shape, their diameter (approx. 60–100 nm) was always smaller than the diameter of the main trunk of the wart, and they occurred at varying positions on the wart surface. The nodules of Callitris endlicheri tended to protrude further from the main body of the wart than nodules in other species, in extreme cases appearing 'branch-like' (Fig. 5). However, nodules did not extend more than 250 nm from warts of any species. The number of nodules on individual warts varied within and between species and this is apparent in Figures 1–16. Warts of C. canescens (Fig. 2) tended to have many nodules (e.g. as many as nine nodules were visible on one side of a wart), while rarely more than a single nodule could be seen on individual warts of C. columellaris (Fig. 3). Not all warts were nodulated and there was species-dependent variation in the proportion of warts that had nodules and those that did not. In order to determine nodularity, a total of 100 of the largest warts on five samples of each species (four samples only for C. neocaledonica and C. sulcata) were examined and the number of nodulated warts was counted. Degree of nodularity (i.e. the probability of any particular wart possessing nodules) for each species was calculated from specimen means and is shown in Figure 17. Overall, the difference in nodularity between species was highly significant (p < 0.001). Four species (C. intratropica; C. macleayana; C. neocaledonica; and C. sulcata) had warts upon which no nodules were observed, and warts in C. canescens, C. oblonga, and C. rhomboidea clearly had greater nodularity than all other species except C. glaucophylla, C. monticola and C. preissii.

Although there was considerable variation in wart morphology, both between and within Callitris species, there was sufficient homogeneity in the general 'form' of the largest warts of some species to enable them to be distinguished from warts of other species in the genus. In C. canescens, C. glaucophylla, and C. preissii, (Figs. 2, 6 and 13, respectively) large warts tended to be squat, conical in shape and nodular. The large warts of C. drummondii were usually tall, upright and tooth-like in appearance (Fig. 4). Warts of C. endlicheri were typically tall, with an enlarged, pedestal-like base and a narrow, tube-like upper part.

[Bar graph showing nodularity of Callitris species]
Fig. 18. Smoothed histograms of wart size for each Callitris species. The horizontal axes indicate wart size \( \text{log. (height multiplied by width)} \). The vertical axes are scaled relative frequencies (i.e. the area under each curve = 1).
that was often bent over at the top (Fig. 5). The largest warts of *C. muelleri* (Fig. 9) and *C. monticola* (Fig. 12) tended to be smaller than all other *Callitris* species except those of *C. intratropica, C. macleayana, C. neocaledonica*, and *C. sulcata* (Figs. 7, 8, 10, and 16), in which warts were invariably relatively small and hemispherical.

The smoothed histograms of wart size (Fig. 18), which are scaled for relative wart frequencies, show strong evidence of two distinct populations of warts. Twelve of the species have bimodal distributions, each with a small-sized and a large-sized wart population peak, and the peaks overlap each other, thus indicating that the two populations were not completely separate. Comparison of the bimodal distributions also indicates that, in general, the bigger-sized wart populations (i.e. indicated by peaks on the right side of the distribution curves) had greater variation in size and included more warts than the small-sized wart populations (peaks on the left) in all species with bimodal distributions. The unimodal distributions of *C. intratropica, C. macleayana, C. neocaledonica*, and *C. sulcata* indicate that each of these species had only a small-sized wart population.

There were highly significant (p < 0.001) differences between species in wart size and wart shape, as shown in Figure 19. Three distinct groups of species are apparent. A group of 12 species: *C. baileyi, C. canescens, C. columnellaris, C. drummondii, C. endlicheri, C. glaucophylla, C. mонтicola, C. muelleri, C. oblonga, C. preissii, C. rhomboidea, and C. roei* had, in general, relatively large-sized warts with width approximately equal to height (i.e. the logarithm of the mean height/width ratio of the warts was close to zero). Warts of *C. muelleri* were significantly smaller than all other species in this group except *C. monticola*. Another group is formed by *C. intratropica, C. macleayana*, and *C. neocaledonica* in which warts can be described as ‘small’ and having a greater width than height (i.e. the logarithm of the mean height/width ratio of the warts is strongly negative). Finally, *C. sulcata* forms a lone grouping, similar to the latter three species in having warts with mean width greater than height, but being significantly larger in size.

Wart distribution densities ranged from a mean of 1.0 warts/µm² (for *C. macleayana*) to 5.4 warts/µm² (for *C. muelleri*) and mean density comparisons for species are shown.
in Figure 20. There was a highly significant (p < 0.001) difference in wart density between species. Callitris monticola, C. muelleri, and C. oblonga had higher densities than nine other species and C. intratropical, C. macleayana, C. neocaledonica, had clearly lower densities than all other species except C. drummondii and C. sulcata.

Warts were, in general, discrete entities. However, pairs of large warts were occasionally joined at the top, forming arch-like structures (Figs. 21–24) similar to the anastomosed vestures of certain hardwoods (Ohtani 1979; Ohtani et al. 1983). There was no regularity in the direction of the joining; warts combined with other warts in apparently random directions. Anastomosing was more common in the corners of tracheids where warts usually tended to be taller and more densely packed together. The effect was observed only between the largest warts, was most common in the tall warts of C. endlicheri, and was present in all species except those without a ‘large-sized’ wart population, i.e. C. intratropical, C. macleayana, C. neocaledonica and C. sulcata.

No significant difference in size, shape, nodularity, or density of distribution was found between wart populations on the RLS faces and those on the TLS faces in specimens of any of the species.

Warts tended to occur evenly spaced in distinct ‘lines’ or ‘rows’ on the tracheid walls (Figs. 25–26).

Discussion

The warty layer of Callitris showed three morphological traits that have been reported in hardwoods but have not previously been described for softwoods: (1) presence of nodules on warts, (2) anastomosing, and (3) two distinct sizes of warts. The ‘nodulated’ warts observed in 12 of the 16 Callitris species resemble the ‘branched’ vestures (warts) of hardwoods, (Ohtani & Ishida 1976; Van Vliet 1978; Ohtani 1979; Ohtani et al. 1983; Harada & Côté 1985; Castro 1988) although in Callitris, the ‘branches’ were usually reduced in size to a bud-like, nodule form. The presence of nodules on softwood warts has not been commented upon previously, although a re-
Fig. 26. Stereo pair of tracheid wall in *Callitris canescens* showing alignment of warts in 'rows'. Tracheid axis is horizontal. Scale bar = 50 µm.

examination of certain micrographs of softwood warty layers in previous reports (Liese 1965; Kuo & Manwiller 1986) revealed their presence. Branched warts in hardwoods are commonly associated with pit apertures (Ishida & Ohtani 1970; Van Vliet 1978; Cassens 1980; Ohtani et al. 1984) whereas areas surrounding pit apertures in *Callitris* had only small, hemispherical, non-nodulated warts, and large nodulated (branched) warts were found only on lumen wall surfaces remote from pits (Fig. 25). Anastomosing of warts was always simply between pairs of warts at their tops, and complex networks or conglomerates of joined structures such as those commonly described for hardwoods (Scurfield & Silva 1970; Van Vliet 1978; Cassens 1980) were not observed in *Callitris*. The occurrence of mixed populations of two 'types' of warts of different sizes, as indicated by the bimodal size-distribution curves (Fig. 18) is similar to that recorded in Japanese dicotyledonous woods (Ohtani 1979), and in New Zealand (NZ) hardwoods (Ohtani et al. 1983). Where branched warts occurred in *Callitris*, a range of morphological intermediates between the large complex branched ones and the small, simple, hemispherically-shaped ones was found and this is similar to variation in size and shape of vestures in the vessel elements of NZ hardwoods (Ohtani et al. 1983). The significance of these hardwood-like traits in *Callitris* wart morphology is unknown but it seems likely that the branched, anastomosed and mixed-type wart populations in *Callitris* are simply further evidence that warts and vestures are synonymous, as proposed by Ohtani et al. (1984).

The sizes of warts in various hardwood and softwood species, as reported in the literature (Wardrop 1964; Ohtani 1979; Ohtani et al. 1983; Harada & Côté 1985) were simply the widths (diameters) of warts across their base (as is apparent by viewing directly down onto the top of the lumen surface). In this study, the combined height and width measurements of each individual wart (apparent by viewing warts from the side), give a better indication of the size of warts than that previously obtained and also provide a crude means of quantifying 'shape' (i.e. height/width). The largest wart height measurement recorded in *Callitris* by this study was 1.2 µm (for *C. drummondii*) and the largest wart width was 1.4 µm (for
C. canescens). This can be compared with warts up to 0.85 μm in diameter in Japanese coniferous woods (Ohtani & Fujikawa 1971) and warts up to 1.1 μm in diameter in NZ hardwoods (Ohtani et al. 1983). Thus in general, wart size in Callitris is very large. However, if the branched, anastomosed and aggregated vestures of certain hardwoods are included in the comparison then hardwood warts (vestures) are clearly larger. Vestures up to 3 μm in height and 5 μm in diameter have been reported in Japanese dicotyledonous woods (Ohtani & Ishida 1976). In addition, photomicrographs of vestures (Ishida & Ohtani 1970; Scurfeld & Silva 1970; Van Vliet 1978; Ohtani 1979; Castro 1988) clearly show them to be much larger than the warts in Callitris.

A noticeable feature of the warty layer of Callitris was the regularity and equality in wart distribution patterns. The rows of warts around the tracheid wall (Fig. 26) were similar to those reported in red pine (Pinus resinosa Ait.) by Kuo and Manwiller (1986), and were usually aligned in a helical 'Z' spiral at 60°–90° (the helical direction appears reversed in Figure 26 because the micrographs record the view from the lumen side of the cell wall). The lines on which warts formed occasionally appeared to be slightly raised above the S3 layer surface in the form of a low ridge, especially in cell corners. This provides support for the concept that warts are associated with the underlying microfibrils (Kuo & Manwiller 1986).

The wide range of habitat-type occupied by the various species of Callitris presents an opportunity to relate species differences in wart morphology to ecological factors. In this study, it was found that species endemic to dry to semi-arid habitats such as C. canescens, C. drummondii, C. glaucophylla and C. preissii (Table 1) had warty layers with mixed proportions of small hemispherical warts, and warts with complex shape, variable size and nodules, whereas the species with small hemispherical warts only (C. intratropica, C. macleayana, C. neocaledonica and C. sulcata) are native to areas within or on the borders of tropical rain forest, or, in the case of C. intratropica, occur in wet-tropical regions of northern Australia (Table 1). Carlquist (1982) postulated that warts have the effect of increasing the surface area of tracheid walls, hence facilitating bonding of water molecules to the cell surface and so permitting water columns to withstand higher tensions without breaking. Thus warts may be considered to be an adaptive feature, of value in conditions of ecological or physiological drought. Since large complex warts provide more surface area than small hemispherical ones, warty layer differences between the dry-habitat and the wet-habitat Callitris species support this hypothesis.

In the past, aspects of warty layer morphology, such as the presence/absence of warts, wart distribution density, wart shape and average diameter measurements, were used with varying success as a means of systematic classification of families, genera and species (Frey-Wyssling et al. 1955; Liese 1963, 1965; Ohtani & Fujikawa 1971; Ohtani 1979; Ohtani et al. 1983). Recent improvements in SEM technology, particularly in high brightness electron emitters, noise reduction electronics, on-screen measuring facilities and improved specimen manipulation abilities, have enhanced the accuracy and ease of application of wart morphology characteristics for taxonomic purposes. This study has demonstrated that wart morphology within the Callitris genus is useful for identification. The 'form' of the biggest warts of the warty layer was sufficiently homogeneous within specimens to enable identification of two individual species, C. endlicheri and C. drummondii. In addition, several groups of species could be distinguished by the general shape of their warts; i.e. typically squat, conical, nodular warts in C. glaucophylla, C. canescens and C. preissii; small complex warts in C. monticola and C. muelleri and small, rounded and hemispherical warts in C. intratropica, C. macleayana, C. neocaledonica and C. sulcata. The latter four species were also distinguishable from all others by the lack of nodules on their warts (Fig. 17) and also by having warts with larger mean width than mean height (Fig. 19). Callitris sulcata could be further distinguished from C. intratropica, C. macleayana and C. neocaledonica by the larger size of its warts (Fig. 19).
(Garden 1956), but precise taxonomy is sometimes difficult (Thompson & Johnson 1986). As a result, many changes in classification have occurred and most species have many synonyms (Garden 1956). Three Callitris taxa which were originally recognised as separate species, C. glauca R. Br. ex R.T. Baker & H. G. Sm., C. intratropica R.T. Baker & H. G. Sm., and C. columellaris F. Muell., were united as a single species, C. columellaris F. Muell., by Blake (1959), who argued that there were no distinctive morphological characters to differentiate them. This taxonomic grouping persisted until Thompson and Johnson (1986) formed polygraphs from five cone characters and discriminated three distinct species, one of which they named C. glaucophylla, and the other two were reinstated as C. intratropica and C. columellaris. During the present study, differences in wart morphology were observed between the latter three species, thus supporting the classification of Thompson and Johnson (1986). Warts in C. intratropica were invariably small and hemispherical in form (Fig. 7) and were therefore quite distinct from the large, variable, nodulated warts occurring in tracheids of C. columellaris (Fig. 3) and C. glaucophylla (Fig. 6). The distinction is also evident in Figure 18 which shows that C. intratropica has a uni-modal peak, indicating a small-sized wart population only, unlike C. columellaris and C. glaucophylla which have bimodal distributions indicating both small-sized and large-sized wart populations. The form of warts of C. columellaris and C. glaucophylla differed from each other – large warts of C. glaucophylla tended to be squat, conical and complex whereas those of C. columellaris were usually more regular and rectangular. Also, on the basis of wart nodularity (Fig. 17), and density of distribution (Fig. 20), all three species are clearly different from each other.

This study has demonstrated that wart morphology is useful for the identification of Callitris species, but it is not known to what extent wart size, shape, and nodularity are influenced by environmental factors. While these questions remain unresolved, the true taxonomic significance of wart morphology in Callitris is uncertain.

References


Heady, Cunningham, Donnelly & Evans — Warts in Callitris 281


This is a clearly written account accompanied by excellent micrographs. The introduction adequately explains the authors' purpose, and the discussion aptly summarizes their results. This manuscript is acceptable, but I would like the authors to consider/address the following points:

1. The scale bars are of different sizes and appear to be those engraved directly upon the SEM negatives. In some instances these bars are so small as to be practically invisible (for example, Figs. 8, 13, 15, 16, 21, 22). I would like to see scale bars such as that used in Fig. 25 attached to all Figs.

2. Materials and Methods -- Usually it is a good idea to indicate the location of the reference collections along with other pertinent information concerning the specimens. This is only a suggestion.

3. I found Figs. 18 & 20 confusing. In the former I think both axes are logarithmic. If so, it should be stated in the caption. In Fig. 20, if wart distribution densities ranged from 1 to 5.4, why was a log scale necessary for the Y-axis? Also, the numbers as presented in the text do not seem to correspond with their logarithms in Fig. 20. My problem with these figures no doubt results from my mathematical ignorance, but I feel that the figure explanations should be clarified.

4. All figures should be initially cited in the Results section. This might be difficult for Fig. 25, but Fig. 26 can be easily accommodated in this way.

5. The authors are dealing with a quantitative rather than a qualitative trait, and they do a good job in tying the expression of wart morphology to ecological factors. While I have no doubt that the ability to form warts in Callitris is genetically fixed, is there any evidence that features such as size, nodulation, etc. are? Perhaps under water stress, the duration of cell wall deposition is increased thus leading to larger warts and joining of warts in any species should it grow in arid conditions. If such be the case, then wart morphology would have no true taxonomic significance. This question could best be determined by growing different species side by side under identical conditions (often easier said than done). I guess that what I am saying is that the authors should approach their taxonomic conclusions with caution. Perhaps a caveat of some sort is in order in the discussion. I realize that this is a common, unstated problem in many similar studies (mine included), but the great intra- and inter-specific variation in Callitris tends to focus attention on the problem.

6. Minor comments on the manuscript.
Appendix 2

Publication

Comments by Referee 2

This is a well-written paper containing interesting results which merit publication. Especially, the findings about micromorphology of warts using a high-resolution SEM are of considerable interest. I have no serious criticism regarding method, results and interpretation of results. Judging from my experiments, however, I think that more clear SEM micrographs without "stippled" texture were able to be taken if wood samples were coated with platinum.

Minor comments:
1. Page 2, line 8. Medullary ray should read Primary ray.
2. Page 3, line 12.-rays should read cross fields.
3. Page 5, line 4,3 from bottom. warts µm² should read warts/µm².
4. Page 7, line 23. tertiary cell wall should read S³ layer.
5. Caption of Fig. 18. What is the unit in the horizontal axes? What is meaning of figures in the vertical axes?
Dear Dr. Heady, and Dr. Evans,

it was a pleasure to read your fine paper on the wart structure in Callitris. Congratulations for the results and for extending our appreciation of the significance of this peculiar structure. You were kind enough to cite also earlier publications from me, which is always a welcome reason to think back and reconnect with older days. For the completion of your collection of older papers I enclose some papers, in which you may be interested in, in case the wart structure story interests your further. During my stay at the CSIRO 1958 some collaborative work with Dr. Alan Wardrop was also directed to the warts.

kind regards and good wishes

sincerely Yours

[Signature]

Prof. Dr. Walter Liese
Warts and all

Just when I thought,
That a warty layer wart
in wood, was simply globular,
On the SEM screen,
Something not before seen,
On each wart, protrusions, quite nodular.

So warts have warts!
Will future reports
of microscopists, have this to excite 'em,
In next level of resolution,
The ultimate solution,
Warts, on warts, on warts, ad infinitum?

Roger Heady 1997

(With apologies to Dean Swift [On Poetry: A Rhapsody. Page 20. Printed 1733])