SYSTEMATICS AND EVOLUTION OF THE GENUS *ELAEOCARPUS* L. (ELAEOCARPACEAE)

Thesis submitted by

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Systematics and evolution of the genus *Elaeocarpus* (Elaeocarpaceae)

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any University or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

25 November 2021

Janet Gagul **Date**

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STATEMENT ON PUBLICATION OF NAMES

Under article 30 of the International Code of Nomenclature for Algae, Fungi and Plants (Shenzhen Code) (Turland et al., 2018) taxonomic names contained herein are not considered effectively published.

DEDICATION

I dedicate this thesis to:

(1) Late Prof. Robert Johns (14 July 1944 – 21 April 2019), who introduced me to my thesis advisor Prof. Darren Crayn and made me believe in myself. His heart always belonged to Papua New Guinea. He passed away in the late stages of my thesis write up,

(2) My parents (Simakus Gagul and Margaret Kundugl) who had no formal education but believed that I could have one.

STATEMENT OF CONTRIBUTION

Apart from the introduction and the conclusion chapters, the data chapters of this thesis have been prepared as separate manuscripts. Several people have contributed to the thesis in different capacities and these contributions are detailed below.

Chapter 2: This chapter has been published as Gagul, J.N., Simpson, L. & Crayn, D.M. (2018). *Elaeocarpus carbinensis* J.Gagul & Crayn (Elaeocarpaceae), a new species endemic to the Mt Carbine Tableland of northeast Queensland, Australia. *Austrobaileya* 10(2): 247–259. Peter Bannink produced the distribution map, Will Smith provided the botanical illustration and Lalita Simpson assisted with the environmental niche modelling analyses.

Chapter 3: The following people donated Elaeocarpaceae leaf samples and/ or sequences for the molecular analysis: Fabian Brambach, George Weiblen, Shelley James, Yumiko Baba, Michael Lovave, Maurizio Rossetto, Darren Crayn and Ian Cowie. Specimen collection in the field in Papua New Guinea was assisted by family and friends. Mel Harrison assisted with sample preparation and DNA extraction in the laboratory, Mark Coode assisted with plant identification and specialised advice on *Elaeocarpus* and Elaeocarpaceae, Lalita Simpson and Lars Nauheimer assisted in the molecular data analysis, and Darren Crayn helped with the theoretical background.

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A copy of my original data and metadata is stored in a Dropbox folder (another copy will be given to my thesis adviser), and external hard drive which will be deposited at the James Cook University data management repository.

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saw or talked to them.

THESIS ABSTRACT

Despite considerable interest in the evolution of the genus *Elaeocarpus*, the phylogenetic relationships between *Elaeocarpus* species, and particularly New Guinean species, are poorly understood. Taxonomic studies on *Elaeocarpus* have been based largely on morphology, but few have included fruit mesocarp morphology because fruit mesocarps display high morphological variation. Existing classifications based on morphology have not been rigorously tested against molecular datasets. Furthermore, the ontogeny of key taxonomic characters of the fruit and seed, such as mesocarp morphology and endosperm reticulation, has not been investigated. To better understand the evolution of the genus *Elaeocarpus*, I employed a range of techniques and approaches in this thesis, including classical taxonomy and systematics, environmental niche modelling, molecular phylogenetics and phylogenomics, development anatomy of fruit and seed characters, and ancestral state reconstruction.

In Chapter 2, I undertook a detailed investigation of the morphology and environmental niche of a species predicted to be threatened by climate change. The taxon was formally described and named *E. carbinenesis* J.Gagul & Crayn. The conservation outlook for the species was determined using environmental niche modelling analyses using a range of carbon dioxide emission scenarios. The results revealed that by the year 2080, suitable climate for the species will have disappeared from its current range.

In Chapter 3, I reconstructed the phylogeny of *Elaeocarpus* using a multilocus molecular dataset (and whole plastomes for a subset of samples) with substantially

improved sampling of species from New Guinea. Results show that phylogenetic reconstructions based on plastome data are better resolved and better supported than few gene phylogenies.

In Chapter 4, I investigated the anatomy of *Elaeocarpus* fruits at different developmental phases with a focus on endosperm rumination and lignification in seeds. I studied the timing of mesocarp developmental milestones such as differentiation of the two mesocarp layers and lignification, and the onset of endosperm rumination and its progression to maturity. Results showed lignin in pericarp and ovary wall tissues in the earliest stages of development. In contrast, endosperm rumination develops only after fruits have fully expanded, and becomes more pronounced as fruits ripen.

Using the molecular phylogenetics framework from Chapter 3 and an improved understanding of the development of fruit characters gained in Chapter 4, I undertook a detailed survey of mesocarp and seed morphology across the genus and reconstructed the ancestral states and evolution of specific characters (Chapter 5). Results show that the common ancestor of the genus *Elaeocarpus* most likely possessed fibrous mesocarp surface ornamentation, straight embryos, and entire endosperm.

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Chapter 1 – General Introduction

Despite considerable interest in the evolution of the genus, the phylogenetic relationships of *Elaeocarpus* species, and particularly New Guinean species, are poorly understood. Taxonomic studies on *Elaeocarpus* have been based largely on morphology (Coode, 1978, 1984, 2004, 2019). However, a few studies have included fruit mesocarp morphology because fruit mesocarps display high morphological variation (Rozefelds and Christophel, 2000; Dettmann and Clifford, 2002; Liu et al., in press). Existing classifications based on morphology have not been rigorously tested against molecular datasets. Furthermore, the ontogeny of key taxonomic characters of the fruit and seed, such as mesocarp morphology and endosperm reticulation, has not been investigated.

Project aim and objectives

This study aimed to utilise molecular phylogenetics and fruit morphology of both extant and fossil material to investigate the evolution of the genus *Elaeocarpus*. Specifically, I: 1) utilised multi-locus molecular sequences to reconstruct the phylogeny with a focus on species from New Guinea, which are currently under-represented in the molecular dataset, 2) undertook a comprehensive developmental study of fruits, from petal-fall to maturity, 3) determined the evolutionary patterns of fruit morphology in the genus, and 4) assessed the conservation outlook for a species

in Queensland, Australia due to prediction of possible extinction. A glossary of terms, symbols and abbreviations used in this thesis is provided in Appendix 1.1.

The family Elaeocarpaceae

Elaeocarpaceae *sensu lato* Juss. (including Tremandraceae R.Br. ex DC.) is a moderately large family comprising mostly trees and shrubs distributed in tropical and subtropical regions, with a few temperate zone species (Rozefelds and Christophel, 1996a; Coode, 2004; Maynard et al., 2008; Baba and Crayn, 2012) (Fig. 1.1). The family is represented mainly in South America, Australasia and Southeast Asia with outliers in Madagascar and Pacific islands (Crayn et al., 2006); it is absent from continental Africa. Most Elaeocarpaceae are found in rainforests, although a few, especially the Tremandraceous genera (*sensu* Coode, 2004) are typically found in dry areas (Crayn et al., 2006). A detailed account of the family Elaeocarpaceae is provided in Coode (2004).

The family Elaeocarpaceae comprises more than 500 species distributed in 12 genera (*Aceratium* DC., *Aristotelia* L'Her., *Crinodendron* Molina, *Dubouzetia* Brongn. & Gris, *Elaeocarpus* L., *Peripentadenia* L.S.Sm., *Platytheca* Steetz, *Sericolea* Schltr., *Sloanea* L., *Tetratheca* Sm., *Tremandra* R.Br. and *Vallea* L.*f.*) which occur from near sea level to over 3000 m (Maynard et al., 2008). South America supports three genera. Two of these - *Crinodendron* and *Vallea* - are endemic, whereas *Sloanea* is widely distributed (Crayn et al., 2006; Pennington and Wise, 2017). Nine genera occur in Australia, four of which (*Peripentadenia*, *Platytheca, Tetratheca, Tremandra*) are endemic. Australia, therefore, has more genera of Elaeocarpaceae than any other region (Baker et al., 1998). The genera

Elaeocarpus and *Sloanea* are most widely distributed with *Sloanea* comprising about 150 species worldwide (Boeira et al., 2012; Sampaio and Souza, 2016), 127 of which occur in the New World (Pennington and Wise, 2017). The genus *Sericolea* is endemic to New Guinea.

The genus *Elaeocarpus* L.

Elaeocarpus, the speciose genus in Elaeocarpaceae, comprises more than 350 species with a mainly Indo-Pacific distribution (Fig. 1.2). The islands of New Guinea (c. 97 taxa) and Borneo (c. 70 spp.) have the highest number of taxa (Coode, 2004). Australia has 31 accepted taxa (CHAH, 2020; Baba et al., 2020), of which 27 are endemic. Hawaii contains a single endemic species (*E. bifidus* Hook. & Arn.), with two endemic species occurring in Mauritius (*E. bojeri* R.E.Vaughan, *E. integrifolius* Lam.) (Coode, 1987b). The genus is well defined morphologically by the distinct fringed petals and firm fleshy fruits with woody stones. These woody stones (formed from inner mesocarps) are very hard, highly ornamented and vary in size and shape, providing useful characters to differentiate species. Furthermore, a number of fossil mesocarps assigned to *Elaeocarpus* are known, but the relationships of many of these fossil species to extant lineages of *Elaeocarpus* are not fully understood.

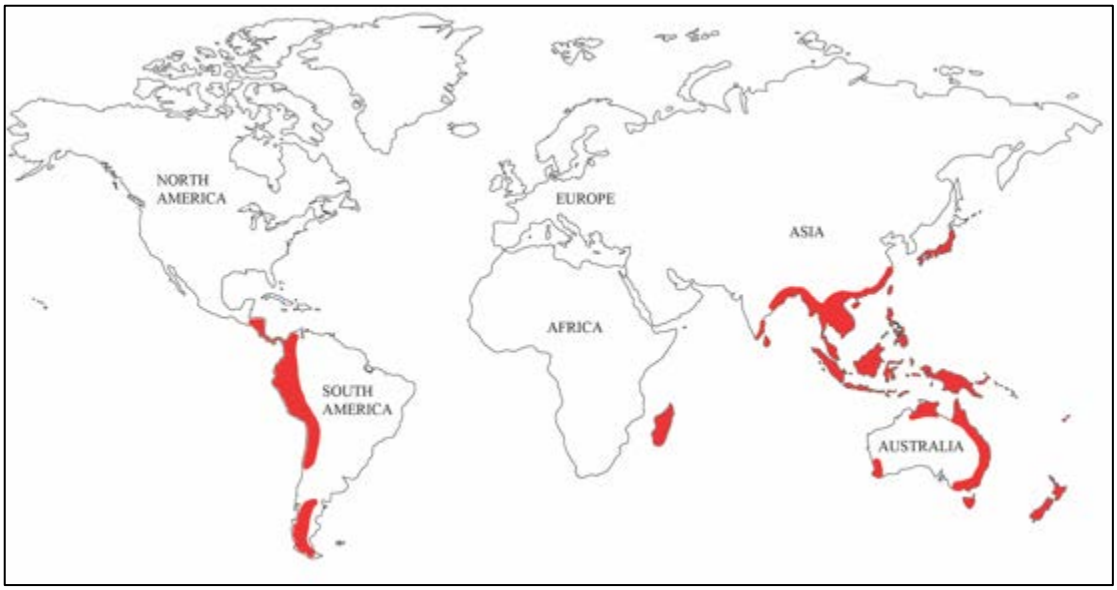


Figure 1.1 Distribution of Elaeocarpaceae worldwide.

There are no representatives of Elaeocarpaceae in North America, Europe and mainland Africa.

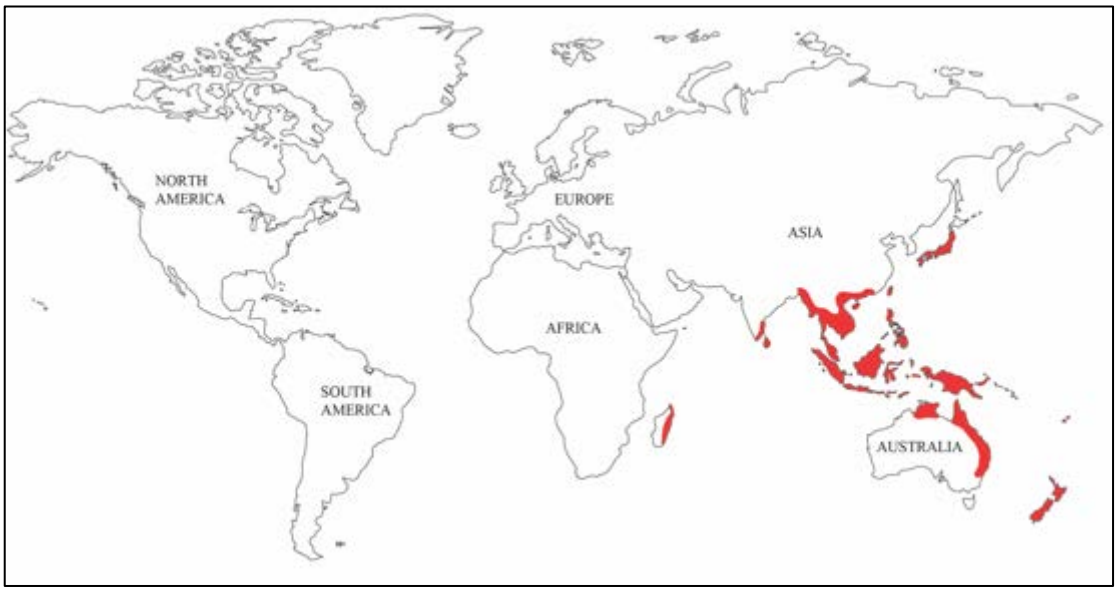


Figure 1.2 Distribution of the genus *Elaeocarpus.*

The genus is centred on the Indo-Pacific with outliers in Madagascar and Hawai'i.

General morphology of *Elaeocarpus*

Elaeocarpus species are mostly shrubs, small or large trees, which can grow up to 45 m tall. Some have a layered canopy with a *Terminalia*-like (sympodial) branching habit, particularly the lowland species that are easily distinguishable among other forest trees (*sensu* Coode, 1978). Roots are sometimes buttressed e.g. in *E. multisectus* Schltr.*, E. ptilanthus* Schltr., *E. sphaericus* (Gaertn.) Ettingsh. and *E. undulatus* Warb. (Coode, 1978), or stilted. The bark is usually smooth, or occasionally fissured (e.g. *E. blepharoceras* Schltr.). Leaves turn scarlet or yellow (e.g. *E. culminicola* Warb. and occasionally *E. arnhemicus* F.Muell.) at senescence. When young, all *Elaeocarpus* leaves are stipulate, becoming mostly exstipulate at maturity, alternate or rarely whorled, clustering toward branchlet tips (e.g. *E. neobritannicus* Coode*,* an endemic New Guinean species restricted to the New Britain islands), occasionally opposite or sub-opposite (e.g. *E. sericoloides* A.C.Sm.). Petioles often have a pulvinus (swelling) at the junction with the leaf lamina. Domatia are usually present as foveoles in most species. Inflorescences are axillary racemes borne on the same year's growth or previous year's growth. Flowers are hairy or glabrous, with 5, rarely 4 petals that are small to large showy, fringed. Stamens eight to at least 20; anthers awned or not. Ovaries are hairy or glabrous, with 2–7 loculi each containing 2–12 ovules (Coode, 1978). Fruits are drupes, usually dull to bright blue at maturity.

Some *Elaeocarpus* characters such as scarlet old leaves, layered canopies, pseudowhorled leaf arrangement, blue fruits, and fruit stones are also found in members of Euphorbiaceae, Rutaceae, Rosaceae, Apocynaceae and Combretaceae.

Examples include: *Terminalia* L. (Combretaceae) with a pseudowhorled leaf arrangement (alternate, and clustered towards branchlet tips giving the appearance of being whorled), with leaves turning red during leaf senescence, a typical character in Elaeocarpaceae (e.g. *E. neobritannicus*). The differences in *E. neobritannicus* are that, the 'barks are greyish and smooth or slightly cracked' rather than 'deeply fissured with yellowish fibrous inner bark (Coode, 1978). The Cassowary plum (*Cerbera floribunda* K.Schum.; Apocynaceae), a native tree from New Guinea and Australia also has blue fruits, which can be confused with *Elaeocarpus*, except that they produce white milky exudate, which *Elaeocarpus* fruits do not. Like *Elaeocarpus* fruits, *Prunus* sp. (Rosaceae) have drupaceous fruits that contain fruit stones. However, *Elaeocarpus* fruit stones are robust and woody, and can survive for longer periods on forest floor before deteriorating whereas the fruit stones of *Prunus* are soft and can deteriorate faster.

Pollen morphology

Pollen grains of Elaeocarpaceae exhibit a wide range of morphological characters (Premathilake and Nilsson, 2001), and vary in size, shape and ornamentation (Shubharani et al., 2013). Coode (2004) reviewed pollen morphology for the genera in the Elaeocarpaceae, but did not study them in detail. In the species rich genus *Elaeocarpus*, few studies have recorded pollen morphology for both extant and fossil species. For extant species, Premathilake and Nilsson (2001) examined pollen from three *Elaeocarpus* species (*E. glandulifer* (Hook.) Mast., *E. montanus* Thwaites and *E. obovatus* G.Don.) from Sri Lanka but found no clear differences in pollen size between the three species, which range from 10–15 x 8–10 µm. However, some differences in the shape of the amb (outline of polar view of a pollen grain)

were observed, with *E. montanus* and *E. obovatus* having a circular, or triangular to trilobate amb, whereas *E. glandulifer* did not. The exine pattern is smooth in all three *Elaeocarpus* species (cf. *E. serratus* L. as reported in Tissot et al., 1994), but a scabrate-perforate sexine pattern occurs in *E. montanus* and *E. obovatus*. Huang (1972) studied the pollen of five species of *Elaeocarpus* from Taiwan and reported them to have smooth, granular to indistinct sexine patterns, and Ikuse (1956) reported two species of *Elaeocarpus* (*E. sylvestris* var. *ellipticus* (Thunb.) H. Hara and *E. photinifolia* Hook. & Arn.) to have finely reticulate pollen. Shubharani et al. (2013) reported a 3-colporate type pollen, prolate to prolate-spheroid exine obscure, and bilateral symmetry in *E. angustifolius* Blume from India (Fig. 1.3).

Based on these studies, the pollen of *Elaeocarpus* species was suggested to be 3-colporate (Premathilake and Nilsson, 2001). However, 2-colporate pollen has also been reported in *Elaeocarpus* (Huang, 1972; Brambach et al., 2016) (Fig. 1.3). These studies are however restricted to India, Sri Lanka, Taiwan and Sulawesi (Ikuse, 1956; Huang, 1972; Tissot et al., 1994; Premathilake and Nilsson, 2001; Coode, 2004; Shubharani et al., 2013; Brambach et al., 2016; Ramasubbu and Irudhyaraj, 2016).

In Australia, Kershaw and Sluiter (1982) have indicated that *Elaeocarpus* pollen is well represented in rainforests in northeastern Queensland at lower elevation (100 m), a claim Coode (1984) refuted based on specimens from Queensland suggesting the genus is more common at mid elevations (800 – 1200 m). However, they have not studied the pollen in detail. Therefore, much pollen research in *Elaeocarpus* remains to be done across a wider range of extant *Elaeocarpus* species, to gain further insights into the pollen morphology in the genus.

For fossil species, paleobotanical studies have reported Elaeocarpaceae, *Elaeocarpus* or *Elaeocarpus*-type pollen from Australia (Luly et al., 1980; Hill and Macphail, 1983; Truswell et al., 1987; Macphail et al., 1994; Blackburn and Sluiter, 1994; Kershaw et al., 1994; Martin, 1998), but these assignments have been questioned (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002). Furthermore, the small, psilate, tricolporate shape of *Elaeocarpus*-type pollen grains are difficult to differentiate from other Elaeocarpaceae due to a lack of detailed studies (Dettmann and Clifford, 2000).

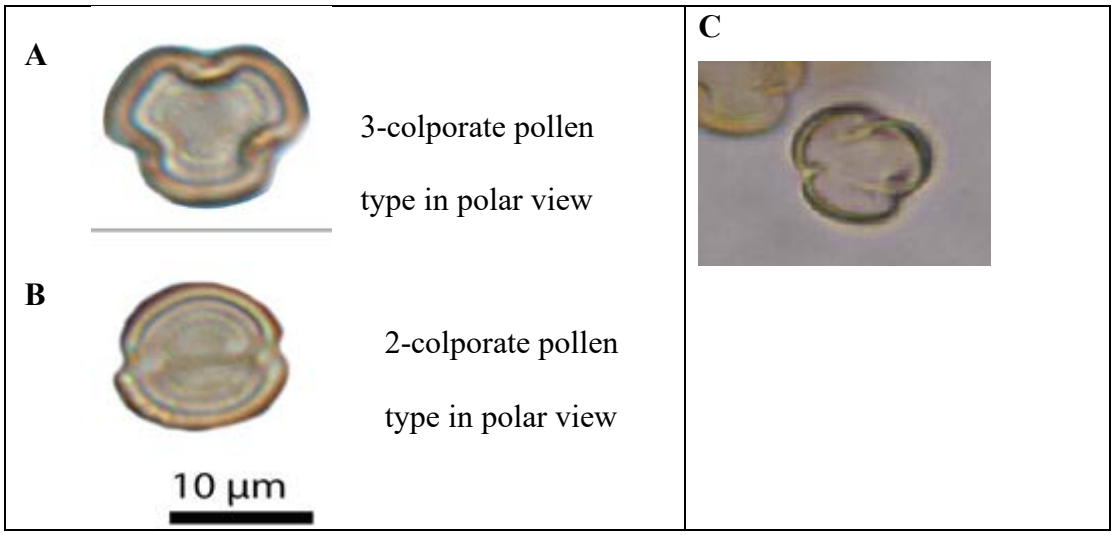


Figure 1.3 Pollen morphology in *Elaeocarpus.*

Three-colporate pollen of *E. firdausii* Brambach, Coode, Biagioni & Culmsee (A) and *E. angustifolius* (C) in polar view, and 2-colporate pollen type in polar view (B). (Brambach et al., 2016; Shubharani et al., 2013).

Fruit morphology

Within Elaeocarpaceae, fruits are either capsules (*Crinodendron*, *Dubouzetia, Peripentadenia*, *Platytheca, Sloanea, Tetratheca*, *Tremandra*, *Vallea*) berries (*Aristotelia, Sericolea*) or drupes (*Aceratium*, *Elaeocarpus*) (Coode, 2004). Berries have an outer skin and inner fleshy mass, containing seeds that have a hardened seed coat whereas in drupes, the seed coat (which is usually papery or membranous) has no protective function - the inner layer of mesocarp (often erroneously referred to as the endocarp, but see below) is hardened to protect the seeds.

Elaeocarpus fruits are small (< 1 cm diam.) to large (4–6 x 3–5 cm) drupes, variously blue in most species, occasionally black (e.g. *E. holopetalus* F.Muell.), brown (e.g. *E. johnsonii* F.Muell., *E. ruminatus* F.Muell.) or red (e.g. *E. grandiflorus* Sm.) at maturity. Iridescent blue fruits are characteristic of some *Elaeocarpus* species (e.g. *E. angustifolius*), due not to pigmentation, but to epidermal microstructure that affects light interference (Lee, 1991). The outer mesocarps (fleshy part of fruit) are succulent or fibrous with a gritty texture, and generally detach cleanly from inner mesocarps (stones) except for species with persistent mesocarp fibres (*E. johnsonii, E. blepharoceras, E. womersleyi* Weibel). Fruit stones (which are formed from inner mesocarps), are usually robust and woody with ornamented surfaces, or with stellate ridges (*E. stellaris* L.S.Sm.; *E. carbinenesis* J.Gagul & Crayn). The fruit stones within *Elaeocarpus* are highly variable and can be used to differentiate species. The number of seeds varies from one to five per fruit and contain embryos that are either curved or straight, and endosperm that is either ruminate or entire (Coode, 1978, 1981, 1984; Baba, 2014; Phoon, 2015).

Fruit and seed development in *Elaeocarpus* and their significance

Few studies have been published on the pattern and timing of fruit stone lignification in angiosperms generally (Tani et al., 2007; Dardick et al., 2010; Hu et al., 2011; Lombardo et al., 2011). Thus, our understanding of the hardening of fruit stones through the formation and lignification of secondary cell walls is limited. Those studies have detected lignin approximately 35–45 days after anthesis (timing may vary among taxa) with hardening and lignification both occurring concurrently. The tissue in which the lignin is first detected is the first to harden. Dardick and Callahan (2014) showed that such processes involve genetic changes that have anatomical effects on fruit development, and discussed how advances have been made in understanding the molecular basis of developmental mechanisms.

Although fruit and seed morphology in *Elaeocarpus* has generally being used in taxonomic and phylogenetic studies (Coode, 1984, 1987; Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002; Baba, 2013; Phoon, 2015) to delimit species, little attention has been given to understanding its anatomical development. Corner (1976) described internal structures of seeds of various angiosperms, including a few *Elaeocarpus* species, but studied only immature fruits of the *Elaeocarpus* species. Therefore, the lack of developmental studies in *Elaeocarpus* has generated confusion and difficulty in describing the internal structures of fruits and seeds in the genus.

Dettmann and Clifford (2000) undertook a detailed study of *Elaeocarpus* fruits, and concluded that the inner mesocarp, rather than the endocarp, is lignified and forms the robust, woody casing for the seeds. However, there are also a few species of *Elaeocarpus* with fibrous mesocarps that require development studies to

understand the taxomic utility of this character within this genus that is largely endowed with fruit stones.

Elaeocarpus fossil records and their importance

Fossils assigned to Elaeocarpaceae include leaf and capsule material from Europe and North America compared to *Sloanea* (Kvaček et al., 2001; Kvaček 2002; Sachse, 2005; Hably 2007; Erdei and Rakosi 2009; Manchester and Kvacek, 2009; Collinson et al., 2010), and fruit stones from Australia and New Zealand compared to *Elaeocarpus* (Burrows 1995, 1997; Rozefelds and Christophel, 1996a, b; Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002).

Elaeocarpus fruit stones, which fossilize readily, can easily be compared to extant fruit stones if good fossil materials are available. A difference in morphological diversity in fossil versus extant *Elaeocarpus* species was noted by Crayn et al. (2006) and confirmed by Rozefelds (pers. comm., 2014). The fossil record provides evidence to suggest that *Elaeocarpus* radiated in Australia (Rozefelds and Christophel, 1996a) but a biogeographical analysis is required to confirm this (Rozefelds and Christophel, 2002). The fossil record also shows *Elaeocarpus* to be at least 30 Mya old (Crayn et al., 2006). Between 11 and 17 fossil species of *Elaeocarpus* (and 30–34 extant species) are known from Australia, which can readily be distinguished based on their mesocarp ornamentation types (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002; Baba and Crayn, 2012). Eight distinct ornamentation types (i.e. baculate, bastionate, echinate, fibrous, granulose, punctate, smooth, verrucate) of *Elaeocarpus* fruit stones are known. Of these, all but one (fibrous) have successfully been compared to fossil species (Rozefelds and Christophel, 1996a, b, 2002;

Dettmann and Clifford, 2000). However, the evolution of *Elaeocarpus* fruit morphology and its value in taxonomy and interpreting the fossil record is poorly understood.

Fossil *Elaeocarpus* leaves have been recorded in early Eocene sediments (Ettingshausen, 1883, 1886, Chapman, 1935; Christophel and Greenwood, 1987; Christophel et al., 1987; O'Dowd et al., 1991; Pole, 1993) but have been confused with some species of Cunoniaceae. There are also records of *Elaeocarpus*-like pollen, which date back to late Eocene (Blackburn and Sluiter, 1994; Kershaw et al., 1994, Macphail et al., 1994) but assigning fossils to that era is doubtful (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002).

A few studies of pollen and leaf morphology of *Elaeocarpus* using fossils from deposits outside of Australia have been carried out, in India (Prakash and Dayal, 1964); New Zealand (Pole, 1993); Easter Island (Horrocks and Wozniak, 2008); and Papua New Guinea (Horrocks et al., 2008).

Ethnobotany of *Elaeocarpus*

Ethnobotany is the study of plants and their uses (Rao and Hajra, 1986). Plants provide food, medicine, fiber for clothing, raw materials for construction of houses, tools, weapons and musical instruments (Krauss, 1993; Balick, 2009; Gagul, 2009). Plants are also used to warrant success in life or to destroy enemies. Traditional medicinal plants continue to play a vital role in remote areas where access to medical services is restricted. With the current demand and increase in the use of traditional

medicines, documenting information on the indigenous knowledge of medicinal plants is critical to support pharmacological study and potential drug discovery.

Species of *Elaeocarpus* have cultural, economic, medical, spiritual and ritual uses in many societies. Much of the ethnobotanical work done on *Elaeocarpus* has focused on biological activity and medicinal applications (Umar et al., 2013), with few studies of other aspects of ethobotany conducted. Studies of species on the Indian subcontinent have documented pharmacological and medicinal properties with diverse health benefits (Kumar et al., 2014; Mahomoodally and Sookhy, 2018) including potential treatments for diabetes, cancer, and other infectious diseases (Umar et al., 2013; Mahomoodally and Sookhy, 2018). However, the ethnobotany of the genus has been little studied elsewhere in its range.

Species that provide food for indigenous people include *E. angustifolius* (Australia), *E. madopetalus* (Vietnam, Cambodia and Thailand), *E. glaber* (Indonesia), *E. cumingii* and *E. calomala* (Philippines and Sulawesi) (Phoon 2012; Li et al. 2014; Singh et al. 2015). Fruits of species such as *E. bancroftii* and *E. angustifolius* provide food sources for birds, e.g., cassowaries and native rats. Cassowaries are important fruit/seed dispersers in New Guinea (also in Australia) where *Elaeocarpus* is diverse.

Previous taxonomic studies on the genus *Elaeocarpus*

The genus *Elaeocarpus* was first established by Linnaeus (1753) with a single species, *Elaeocarpus serratus*. Since then, various local treatments of *Elaeocarpus* have been produced, e.g. for Papuasia (Schlechter, 1916; Smith, 1944; Weibel, 1968,

Weibel, 1971; Coode, 1978), Australia and New Zealand (Coode, 1984), the Pacific islands (Smith, 1953), Java (Backer and Bakhuizen van den Brink, 1963), New Caledonia (Tirel, 1982), Madagascar (Tirel, 1985), Malay Peninsula (Ridley, 1992), China (Tang and Phengklai, 2007), India, Southern India and Sri Lanka (Zmarzty, 2001; Murti, 1993). For the Malesian region, numerous papers have been published (Coode, 1978, 1981, 1996a, b, c, d, 2001a, b, c, d, e, f , 2003, 2005, 2010; Coode and Weibel, 1994), but a single regional treatment combining these available treatments is yet to be produced.

Schlechter (1916) was the first to propose an infrageneric classification of the genus, which was adopted by Smith (1944), Weibel (1968), Coode and Weibel (1994), and Coode (1978, 1981, 1984). Taxa were categorized into informal groups and sections in the infrageneric classification, based mostly on morphological characters. Twelve groups were recognized for Australia, New Zealand and Papuasia (Coode, 1978, 1981, 1984).

Crayn et al. (2006) resolved the phylogenetic relationships among the genera of Elaeocarpaceae and Tremandraceae. The latter family was found to be deeply nested in Elaeocarpaceae and is no longer recognised as a family (Coode, 2004). The phylogenetic relationships among the species of *Elaeocarpus* have been investigated (Baba, 2014; Phoon, 2015). These studies contain dense sampling of species diversity from Australasia and Western Malesia. However, few species from New Guinea were included in these studies, therefore our understanding of the relationships of species from that region is poor.

The genus *Elaeocarpus* in New Guinea

Biogeography of New Guinea and biodiversity of *Elaeocarpus* in New Guinea

New Guinea is the largest tropical island in the world and is located north of Australia. It is divided into two regions, with the western part comprising the Indonesian provinces West Papua and Papua. The eastern part of the island, with various offshore islands, forms the independent state of Papua New Guinea (PNG). The southern part of New Guinea lies on the Australian plate, and the northern part is formed from crustal fragments of different sources (Paijmans, 1976; Moore, 2003). New Guinea is part of the Malesian floristic region or Malesia. Malesia comprises Indonesia, Malaysia, the Philippines and Timor-Leste. New Guinea's flora is the richest of any island, comprising more than 13,600 species (Cámara-Leret et al. 2020; Joyce et al. 2020), and is a mixture of mostly Asian and Australian tropical rainforest lineages (Paijmans, 1976).

The genus *Elaeocarpus* was last revised for New Guinea by Coode (1978, 1981), and a number of species have been described in the four decades since (Coode, 2001a, 2003, 2005, 2010, 2014, 2019b) based largely on the extensive collections of *Elaeocarpus* made between the 1930s and 1970s. While the collections have been well studied and taxonomic concepts are well developed, only a few of the species have been included in a phylogenetic analysis therefore the relationships among them have not been investigated.

New Guinea is a centre of diversity for *Elaeocarpus* and the genus is represented there by members of eight sections – *Lobopetalum* Schltr.,

Dactylosphaera Schltr., *Elaeocarpus* L., *Blepharoceras* Schltr., *Ganitrus* Brongn. & Gris, *Monocera* Brongn. & Gris, *Oreocarpus* Schltr., *Coilopetalum* Schltr. – and an unnamed section-level group. Section *Elaeocarpus* also occurs in India and SE Asia, including Malaysia (Sabah and Sarawak), Indonesia (Kalimantan, Sumatra and Java), the Philippines and the Solomon Islands, and section *Ganitrus* occurs from India and throughout Malesia to Australia. Section *Coilopetalum* is also widespread from India to the Pacific (but not in Australia, New Caledonia or New Zealand), and section *Oreocarpus* occurs from the Philippines to Australia . The sections correspond to the nine infrageneric groupings of Coode (1978, 1981).

The arrangement of species into sections (and groups) is based on morphological study, and their relationships are still poorly understood phylogenetically. Inadequate material also has made formal description, naming and classification difficult (Coode, 1978, 1981), whilst poor representation of New Guinean samples in molecular phylogenetic analyses further limits our understanding. According to Weibel (1968), groups one to six have straight seeds, and groups seven and eight have curved seeds (as do Sect. *Acronodia* (Blume) Mast. and the *Polystachyus* group, both from western Malesia). The seeds of *E. crassus* Coode and *E. timikensis* Coode, and possibly one or two undescribed species from Western New Guinea, are unknown therefore these species remain unplaced as to group (Coode, 2019c). Furthermore, Coode (1978) has also recognised sect. '*Papuanthus*' as a synonym of sect. *Monocera*, and sect. '*Chascanthus*', as synonym of sect. *Elaeocarpus*, and has included sect. *Fissipetalum* Schltr. in Group five. This has accounted for the discrepancy in the numbers of sections and groups (M. Coode, pers. comm., 2016). Additionally, inadequate material has made formal description,

naming and classification of New Guinean *Elaeocarpus* difficult (Coode, 1978). Therefore, the list of nine sections is treated as provisional.

Coode's (1978) initial account of New Guinean *Elaeocarpus* listed 68 species, seven subspecies, and two varieties. At least seven new species were suggested, but insufficient material precluded their description. Since then, 13 additional species (and 5 subspecies, and 6 varieties) have been recorded bringing the current total estimate to 81 species: *Elaeocarpus amabilis* Kaneh. & Hatus., *E. avium* Coode*, E. bilongvinas* Coode*, E. crassus* Coode*, E. davisii* Coode*, E. gardneri* Coode*, E. johnsii* Coode*, E. myrtoides* A.C.Sm., *E. ornatus* Coode*, E. osiae* Coode*, E. rosselensis* Coode*, E. timikensis* Coode*, E. sterrophyllus* Schltr., *E. altisectus* subsp. *carrii* Coode, *E. amabilis* subsp. *piorae* Coode, *E. coloides* subsp. *ridsdalei* Coode, *E. miegei* subsp. *rosselensis* Coode, *E habbemensis* A.C.Sm. subsp. *schoddei* Coode, *E. myrtoides* subsp. *vinkii* Coode, *E. dolichostylus* Schltr. var. *dolichostylus*, *E. dolichostylus* Schltr. var. *chloranthus* (A.C.Sm.) Coode, *E. dolichostylus* Schltr. var. *hentyi* Coode, *E. polydactylus* Schltr var. *polydactylus*, *E. polydactylus* Schltr. var. *podocarpoides* Schltr., *E. polydactylus* var. *savannarum* (A.C.Sm.) Coode, *E. sericoloides* A.C.Sm. var. *sericoloides*, *E. sericoloides* var. *diffusus* Coode. In addition to the list of pubished names (Table 3.1), there may be other variants that are probably distinct and which 'need further work' (M. Coode pers. comm. 2021). New Guinean species are currently under-represented in molecular datasets. A list of taxa of *Elaeocarpus* from New Guinea is provided in Table 3.1.

Representatives of the genus are widespread throughout the island from near sea level to elevations over 3500 m. Some species are restricted to low, mid and

higher altitudes while a few are local endemics, e.g. *E. neobritannicus* (New Britain), *E. sayeri* F. Muell. (Finisterre Range). Other species have a pan-New Guinea distribution, e.g. *E. altigenus* Schltr.

The ecology of the genus in New Guinea is poorly known but where the genus is common, variation within species may be evident (Coode, 1978). Many collections come from easily accessible areas, especially in secondary forests. Further collections are required from remote and inaccessible areas where new species are expected to occur.

Methodological approach

Molecular versus morphological data

There have been discussions about the relative value of molecular versus morphological data for estimating phylogeny (Patterson et al., 1993). However, both approaches have advantages and disadvantages. For instance, morphological data can be easily accessible in fossils whereas molecular data cannot. Whilst it is possible to obtain vast numbers of molecular characters in the present genomics age, it may be difficult to obtain DNA in samples from 200-2000 years ago, due to molecules being too degraded (Wiens, 2004). For morphology-based taxonomy, species delimitation using morphological data is typically based on diagnostic characters rather than phylogenetic analysis (Wiens, 2004). In-depth knowledge of morphological characters in identifying species of study groups is critical in providing accurate species information for phylogenetic analysis.

Different types of characters are used in phylogenetic analyses to infer evolutionary relationships, e.g., phenotypic (morphology, ecology, behavior, physiology, and chemistry); genotypic or molecular (DNA sequence data, protein sequence or amino acid sequence). Phenotypic characters are usually morphological, but sometimes ecological or physiological characters are used. DNA sequence is the most widely used character source for genotypic or molecular data in systematic studies.

Due to the ease of generating DNA sequences, molecular datasets usually contain more characters than other kinds of datasets, so can potentially provide more useful characters for phylogenetic analysis (Wiens, 2004). Furthermore, molecular characters reflect gene-level changes and tend to show less homoplasy compared with morphological characters (Judd et al., 2008). Homoplasy is similarity due to independent evolution (e.g., convergence, parallelism, reversal, i.e. loss of derived features) as opposed to homology, which is similarity due to inheritance from a common ancestor. Because DNA is ‘digital’ (nucleotide characters can take only one of four discrete states: Adenine, Guanine, Cytosine and Thymine), molecular data can be easier to interpret than morphological characters, which may be potentially infinitely variable (e.g. a compound leaf may come in different forms; Judd et al., 2008).

Plant genomes and molecular markers used in Elaeocarpaceae and *Elaeocarpus* studies

Three different genomes are found in plant cells: the chloroplast (plastid), nuclear and mitochondrial genomes (Judd et al., 2008). Chloroplast (cpDNA) and mitochondrial (mtDNA) genomes exhibit uniparental inheritance (usually maternal in angiosperms) whereas the nuclear (nDNA) genome is biparental. Of the three, the nuclear genome is the largest comprising of $1.1x10^6$ – $1.1x10^{11}$ kilobase pairs (kbp): the chloroplast is 135 – 160 kbp and the mitochondrial 200 – 2500 kbp (Judd et al., 2008). Mitochondria and chloroplast genomes are both circular but differ in their structural stability. Because of its higher structural stability within both cells and species, the chloroplast genome is preferred for phylogenetic reconstruction in plants.

Molecular markers (genetic markers) are specific regions of DNA. In Elaeocarpaceae (or *Elaeocarpus*) studies, the most widely used markers for phylogenetic reconstruction include the three noncoding regions – *trnV-ndhC*, *trnH-psbA* and *trnL-trnF* from the chloroplast genome and the two nuclear regions – the internal transcribed spacer (ITS) of the nuclear ribosomal region, and the gene *Xdh* **(**Maynard, 2004; Crayn et al., 2006; Niissalo, 2011; Baba, 2014; Phoon, 2015). Other chloroplast markers have been shown to be variable within *Elaeocarpus* e.g., *petD-rpoA* and *rps16* regions in *Elaeocarpus sylvestris* var. *ellipticus* (Aoki et al., 2003). The noncoding regions of chloroplast DNA (cpDNA) evolve rapidly, thus potentially containing more information to resolve phylogenetic relationships among closely related species (Gielly and Taberlet, 1994), and have proven to be generally informative at the species level in *Elaeocarpus* (Baba, 2014; Phoon, 2015). Table 1.1 lists the primers used for each marker.

Table 1.1 Primer information for DNA regions used in Elaeocarpaceae studies.

DNA regions used in the current study are indicated by an asterix (*).

Region	Primer name	Primer sequence (5'–3')	Reference	Employed in Elaeocarpaceae/ *Elaeocarpus* studies
ITS	ITS5	GGAAGTAAAAGTCGTAAC AAGG	White et al. (1990)	Crayn et al. (2006); Niissalo, 2011; Baba, 2014; Phoon (2015) used White et al. (1990) as well as Sun et al. (1994) and Blattner (1999); Maynard, unpublished
	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)	
	ITS1	TCCGTAGGTGAACCTGCG G	White et al. (1990).	
	GNI	CGCGAGAAGTTCATTGAAC	White et al. (1990)	
petD-rpoA	cDNA-petD-u	TGAGAGAGAATGGATTATGGGAG		Aoki et al. (2003)
	cDNA-petD-1	AGGAAGGAGAGGTGGCAGTC	Sugita et al. (2006)	
	cDNA-rpoA-u	TGCTACGAAACAAATACTCCCTCA	Sugita et al. (2006)	
	cDNA-rpoA-1	ACCTCCCAAGAAAAGACGTGTATAA	Sugita et al. (2006)	
rps16	rpS16x1	GTTGCTTTYTACCACATCGTTT	Shaw et al. (2007)	Aoki et al. (2003); Baba 2014, Maynard, unpublished
	rpsR2	TCGGGATCGAACATCAATTGCAAC	Shaw et al. (2007)	

trnH-psbA spacer*	rpsF	GTGGTAGAAAGCAACGTGCGACTT	Oxelman et al. (1997)	Baba, 2014; Phoon, 2015
	trnHf_05	CGCGCATGGTGGATTCAC AATCC	Tate and Simpson (2003)	
	psbA3_f	GTTATGCATGAACGTAAT GCTC	Sang et al. (1997)	
trnL-trnF region*	Tab c	CGAAATCGGTAGACGCTA CG	Taberlet et al. (1991)	Maynard, 2004; Crayn et al., 2006; McPherson, 2008; Niissalo, 2011; Baba, 2014; Phoon, 2015
	Tab f	ATTTGAACTGGTGACACG AG	Taberlet et al. (1991)	
trnV-ndhC spacer*	trnV$^{(UAC)}$ x2	GTCTACGGTTCGARTCCGTA	Shaw et al. (2007)	Baba, 2014; Phoon, 2015
	ndhC	TATTATTAGAAATGYCCA RAAAATATCATATTC	Shaw et al. (2007)	
Xdh	X502F	TGTGATGTCGATGTATGC	Gorniak et al. (2010)	
	X1599R	G(AT)GAGAGAAA(CT)TGGAGCAAC	Gorniak et al. (2010)	

	X551F	GAAGAGCAGATTGAAGA(AT)(AT)GCC	Gorniak et al. (2010)	Phoon, 2015 followed Morton (2011)
	X1591R	AA(CT)TGGAGCAACTCCACCA	Gorniak et al. (2010)	
ycf1	Magnoliid2980F ycf1	ATTATTTGGATTGAGGAAAG	Neubig and Abbott (2010)	Proposed in this study but have not been tested
	Magnoliid3570F ycf1	ACTTATCTTCCTTGTCCCAAGC	Neubig and Abbott (2010)	

Generating molecular datasets

Sanger Sequencing: The Chain Termination method

The chain-termination method of DNA sequencing, also called Sanger sequencing (Sanger et al., 1977) has been the most commonly used method of DNA sequencing over the last 50 years. Sanger sequencing requires a high abundance of the locus to be sequenced therefore amplification of the target locus, e.g. by the Polymerase Chain Reaction (PCR), and is usually undertaken as the first step. Sanger sequencing gives high-quality sequence for relatively long stretches of DNA (up to about 900 base pairs in length) (https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/a/dna-sequencing). It is typically used to sequence individual pieces of DNA generated through PCR. Although Sanger sequencing is still commonly used, it is expensive and inefficient for larger-scale projects such as the sequencing of an entire genome or metagenome compared to new, large-scale sequencing methods that are faster and less expensive (e.g. Next generation Sequencing) (Grada and Weinbrecht 2013). The recent development and widespread adoption of target capture (a.k.a Hyb-seq, exon capture) phylogenomic methods holds much promise for more efficient generation of hundreds of markers for many samples (e.g. Baker et al., 2021).

Polymerase Chain Reaction

The polymerase chain reaction (PCR) is an effective and inexpensive technique for generating billions of copies of a molecular marker from a single or few copies, which is required for Sanger sequencing methods. In the PCR process, a thermostable enzyme (DNA polymerase) captures nucleotides, which are floating freely around it, and attaches them to

the end of the primers that have already base-paired with a longer piece of DNA (Judd et al., 2008). The strand of DNA to be copied is defined with two primers: ‘a forward primer which defines the beginning of the sequence to be amplified, and a reverse primer that defines the end’ (Bromham, 2008). PCR is both DNA-dependent and an enzyme-facilitated reaction, which works best at temperatures suitable for the specific DNA polymerase enzyme used.

PCR is achieved in three steps: denaturation, annealing and extension, which are repeated for 30 or 40 cycles using an automated thermal cycler. Test tubes that contain the reaction mix are placed in the cycler, which then heats and cools them in a rapid sequence. The reaction mix comprises the primers, the DNA of interest, the enzyme and the nucleotides (Adenine, Cytosine, Guanine, Thymine).

Denaturation – temperature of 94 °C (201.2 F) is applied to break the double stranded DNA into two single DNA strands by altering the structures without destroying them.
Annealing – temperature is reduced to 50 - 54 °C to allow the primers to pair up with the single stranded template, which is the sequence of DNA to be copied.
Extension – temperature is raised again to 72 °C creating ideal conditions for the enzyme to attach itself to the joined primer and the DNA template and commence copying.

Primers and primer sequences for PCR

Primers are small pieces of single stranded DNA, which through complementary base pairing, attach themselves at both ends of the desired segment (Judd et al., 2008). Primers are usually between 18 and 30 base pairs in length (Bromham, 2008). Primer sequences and their lengths determine the temperatures (4˚C for GC and 2˚C for AT) at which the primer will

attach to the target DNA (Bromham, 2008). Temperature varies between nucleotides due to the hydrogen bonding between them. The higher the number of hydrogen bonds, the higher the temperature or energy it requires to melt or denature them. Nucleotides G and C pair using three hydrogen bonds requiring higher temperature to denature them compared to A-T pairs, which have two hydrogen bonds. Primer design programs usually provide more accurate melting temperatures (T_m). Table 3 provides some information on various primers and primer sequences used in some Elaeocarpaceae and *Elaeocarpus* studies.

Next Generation Sequencing Methods

Next generation sequencing (NGS) refers to a set of recently developed DNA sequencing technologies. NGS methods are becoming more popular and will likely largely replace the Sanger methods in the next few years. Wilkinson et al. (2017) found that replacing Sanger with Next Generation Sequencing improves coverage and quality of reference DNA barcodes for plants. Furthermore, sequencing an entire genome or metagenome is cheaper and efficient with the NGS techniques that are faster than Sanger. Conceptually, NGS runs huge number of tiny sequencing reactions in parallel, consequently sequencing large quantities of DNA faster and more cheaply than Sanger sequencing. However, NGS although much cheaper than the Sanger sequencing methods, is still expensive for many laboratories. Furthermore, data analysis is time-consuming and requires well developed bioinformatics skills and infrastructure to manage and interpret the large volumes of sequence data generated.

Alignment of DNA sequences

The first step in phylogenetic analysis of DNA, RNA or protein sequences is to align the nucleotides (or amino acid) to identify positional homology (similarity) in the sequences (https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/a/dna-sequencing). Similarity due to homology in the sequences reflects common ancestry, as opposed to homoplasy, which is similarity due to convergent or parallel evolution. Only homology can indicate evolutionary relatedness and therefore be used for reconstructing the phylogenies.

During alignment, sequences are arranged in rows and nucleotides positioned in successive columns so identical characters are aligned (Bromham, 2008). Differences in nucleotides within a column are inferred to be due to mutations that have occurred after the split of the species from their common ancestor. Mutations may take one of two forms: substitutions, where a nucleotide is replaced by a different nucleotide (e.g. adenine for thymine); and insertion-deletion events (indels), where one or more nucleotides are inserted or deleted in the course of evolution, requiring the opening of gaps in some sequences to get the similar nucleotides to align properly. Indels are more common in noncoding sequences than coding sequences.

In order to assist in sequence alignment, homology search programs have been developed. Commonly used programs include FASTA (‘pronounced ‘fast A’, and stands for ‘FAST-All’, as the program works with any alphabet, an extension of the original ‘FAST-P’ (protein) and ‘FAST-N’ (nucleotide) alignment tools’) (Lipman and Pearson, 1985; Pearson and Lipman, 1988), BLAST (Basic Local Alignment Search Tool) (retrieved on 27 January 2021: https://blast.ncbi.nlm.nih.gov) and MAFFT (Katoh et al., 2002) among others. The

FASTA and BLAST approaches are based on string-matching or string-searching algorithms, which in ‘computer science are important classes of string algorithms that try to identify where strings (also known as patterns) are located within a larger string or text’ (https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/a/dna-sequencing). However, with the rapid increase in the number of sequences, high-speed computer programs applicable to large-scale projects are required. Hence, MAFFT was developed for multiple sequence alignment based on the fast Fourier transform (FFT), which allows rapid detection of homologous segments (Katoh et al., 2002). Multiple Alignment using Fast Fourier Transform or MAFFT is a high speed multiple sequence alignment (MSA) program, comprising of a series of algorithmic solutions for the alignment of homologous sequences. The program allows for the possibility of evolutionary events (such as mutations, insertions, deletions and rearrangements) under certain circumstances) (https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/a/dna-sequencing)..

Phylogenetic analysis

Reconstructing phylogeny

Evolutionary history and relationships of organisms can be inferred through phylogenetic analysis. Phylogenies are usually represented as branching tree diagrams (cladograms, phylograms, chronograms). Cladograms depict the branching patterns of trees only, whereas phylograms depict branch lengths as proportional to the amount of inferred evolution (character change). In chronograms, these branch lengths represent time.

The branching patterns (topologies) of trees depict branching histories of common ancestries. Three main types of relationships are recognised (monophyly, paraphyly and polyphyly). A monophyletic group consists of a common ancestor and all its descendants. A paraphyletic group also consists of the same common ancestor but lacks one or more descendent (Harrison and Langdale, 2006). Polyphyletic groups comprise the descendents of two or more ancestors and are often defined by characters that have arisen as a result of convergent evolution (homoplasy), which produces similar character states in unrelated organisms.

The three most widely used analytical approaches used to reconstruct phylogenetic relationships are maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). The analyses differ mainly in the criteria employed in the selection of the best possible trees.

Maximum parsimony

Parsimony is a non-parametric, character-based tree estimation method based on the assumption that the most likely tree is the one that requires the fewest character state changes (Hall, 2008). Parsimony assumes that organisms that evolved from a common ancestor share similar characters so the approach seeks the tree with the fewest evolutionary steps required (Judd et al., 2008). It is a simple and straightforward approach but being non-parametric it uses less information. Furthermore, under certain conditions it has been shown to be statistically inconsistent (Bromham, 2008; Hall, 2008), i.e., it does not guarantee a true tree with high probability even with infinite data. These conditions include excessive evolutionary rate variation among lineages (long branches).

The most widely used computer program for parsimony analysis is PAUP* v.4.0b10 software (Swofford, 2003). PAUP originally performed only parsimony analysis, however, the current version (version 4.0) PAUP* (* and other methods) includes distance and likelihood methods.

Maximum likelihood

Maximum likelihood (ML) is a parametric, character-based tree estimation method based on the assumption that the tree with the highest probability of character transformation is the best. ML uses an explicit character transformation model that searches for the tree with the highest compound probabilities (maximum likelihood) (Bromham, 2008; Judd et al., 2008). Tree searching in ML is similar to parsimony methods (e.g., branch swapping), although with ML, the tree is evaluated by a measure of compound probability rather than the overall length (https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/a/dna-sequencing). The tree with the highest probability of character transformation is selected as the best. The performance of ML methods depends on the selection of an appropriate evolutionary model (Hall, 2008). An appropriate model may be determined using likelihood ratio tests, such as implemented in jModel Test v. 0.11 (Posada, 2008). Because ML analysis is computationally intensive, it is slow to run with large datasets, but with its statistical consistency, ML has proven to be the best method. With large datasets, ML analysis requires ample time and a precise evolution model to run. It however, has advantage over parsimony for the reason that the estimation of the pattern of evolutionary history takes into account probabilities of character state changes from an explicit evolutionary model, which is based on and evaluated from the observed data (https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/a/dna-sequencing). A range of software packages is available to

perform ML analysis, and efficient algorithms developed recently (e.g. IQ-TREE, Nguyen et al., 2014) have made the computation of ML topologies tractable for very large datasets.

Bayesian inference

Bayesian inference (BI) involves a likelihood function but its theory and application is different from maximum likelihood analysis, although they are both parametric methods, which rely on explicit models of character evolution (Hall, 2008; Bromham, 2008). The inference of phylogeny is based on a posterior probability distribution of trees, which is the probability of a tree conditioned on the observations (Brown et al., 2010). The conditioning is accomplished using Bayes's theorem. The posterior probability distribution of trees is impossible to calculate analytically. Instead, simulation techniques such as Markov Chain Monte Carlo (or MCMC) are used to approximate the posterior probabilities of trees (Brown et al., 2010), such as in the widely used software MrBayes (Huelsenbeck and Ronquist, 2001, 2003). The BI is efficient with its computations with large data, such that the phenomenon where MCMC algorithms converge on the optimal solution given enough time.

When analyzing DNA sequences, both ML and BI methods become useful. They are both model-based methods, which use likelihood functions to choose optimal trees. In practice, BI analysis is usually much faster than ML analysis (Judd et al., 2008).

Assessing support for phylogenetic trees

To estimate the reliability of phylogenetic trees from parsimony and likelihood analyses, statistical methods are used (bootstrap and jackknife). Bootstrap is a non-parametric method and involves resampling characters with replacement, whereas jackknife resamples

without replacement. Bootstrap is the most widely used method for estimating the reliability of phylogenetic trees (Hall, 2008).

Significance and rationale of this study

The current study is important because:

I. Previous studies have provided an informal infrageneric classification based on morphology (Schlechter, 1916; Smith, 1944; Weibel, 1968; Coode, 1978, 1981, 1984; Weibel and Coode, 1994). However, natural infrageneric groups within *Elaeocarpus* have remained difficult to delimit with confidence due to the complex patterns of morphological variation among the species. Therefore, molecular data are needed to test the morphology-based classification, in order to help resolve phylogenetic relationships, and to delimit species.

II. Recent molecular studies have resolved the relationships of Australian and western Malesian species (Baba, 2014; Phoon, 2015). However, these studies have included few species from New Guinea because suitable materials were unavailable. New Guinea comprises a substantial diversity of *Elaeocarpus*, and understanding the relationships of the species from that region is critical for understanding the biogeography and evolution of the genus.

III. Within the family Elaeocarpaceae, the genus *Elaeocarpus* is characterized by the possession of fruit stones (mesocarps), which vary considerably among the species. This variation is often species specific and may be useful for species delimitation. Previous studies have compared fruit mesocarps of fossil species to those of extant species (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002, 1996a, b). The extant species (and

fossil) used in these studies are from Australia and New Zealand. The current study expands from these studies by adding species from New Guinea and Malesia, but also examines fruit morphology in a phylogenetic context to test the current morphological classification and gain insights into the evolution of fruit morphology in *Elaeocarpus*.

Overview of thesis chapters

Chapter 1 forms the general introduction of the thesis, which comprises a condensed version of my literature review. It provides contextual information on *Elaeocarpus* and Elaeocarpaceae, outlining previous work on the genus and the family. It also outlines the molecular and morphological approaches used in this study, and other similar studies. It gives a brief overview of the current study, outlining its significance, aim and objectives.

Chapter 2 assesses the taxonomic status and conservation outlook for a vulnerable species from the mountaintops of Queensland, Australia. This species has been identified as 'at risk' from climate change impacts (Costion et al. 2015) but had not been taxonomically resolved at the time. The species has formally been described with the update of the species conservation status. The chapter has been published as '*Elaeocarpus carbinensis* J.N.Gagul & Crayn (Elaeocarpaceae), a new species endemic to the Mt Carbine Tableland of northeast Queensland, Australia' (Gagul et al., 2018b).

Chapter 3 presents the results of a molecular phylogenetic analysis of *Elaeocarpus*. It provides a detailed background on the few molecular phylogenetics studies relevant to *Elaeocarpus* that have been conducted to date. These phylogenetic studies included few species from New Guinea, and with the complex morphological variation among the species of *Elaeocarpus* from New Guinea, estimation of species relationships has been difficult.

Therefore, molecular data are needed to test the morphology-based classification, in order to help resolve phylogenetic relationships, and provide a robust framework to delimit species.

The phylogeny provided in Chapter 3 comprises sequences from previous studies and the current study. The majority of the new sequences are from species native to New Guinea, with others from Japan, Cambodia, Thailand, Myanmar, and Indonesia (Sulawesi). This chapter tests the current morphology-based classification using a multi-locus molecular dataset including whole-plastome sequences for selected species. This study is the first to sequence Elaeocarpaceae plastomes, and the results confirm the superiority of multi-locus datasets for resolving relationships among species. This chapter is a collaborative effort involving several other researchers, and is being prepared as a manuscript to submit to *Australasian Systematic Botany.*

Chapter 4 discusses the taxonomic and evolutionary significance of ruminate endosperm and mesocarp lignification in *Elaeocarpus*. The research investigates important events in the development of fruits from anthesis to maturity by determining the timing of mesocarp developmental milestones, such as differentiation of the two-mesocarp layers and lignification; and the onset of endosperm rumination and its progression to maturity. Using *Elaeocarpus ruminatus*, a species endemic to Queensland, Australia, the research establishes a basis on which future studies can investigate the developmental anatomy and ontogeny of rumination and lignification across the genus *Elaeocarpus* and the relevance of this knowledge to taxonomy and evolution and the interpretation of fossil material. This chapter has been published in *Australian Systematic Botany* as ‘Fruit developmental biology and endosperm rumination in *Elaeocarpus ruminatus* (Elaeocarpaceae), and its taxonomic significance’ (Gagul et al., 2018a).

Chapter 5 investigates the evolutionary patterns of fruit morphology in *Elaeocarpus*. It provides information on different fruit types in the family, with background on fruit morphology of *Elaeocarpus*. It outlines previous studies and identifies the knowledge gaps, and sets out to expand and improve the knowledge gained from these previous studies. It also outlines the importance of this study and the contributions it will make to improve our understanding in the evolution of fruit morphology in the genus. Most importantly it uses fruit mesocarp morphology in a phylogenetic context, which is a new contribution in *Elaeocarpus* fruit studies. This chapter is a collaborative effort between myself and several other researchers, and is being prepared as a manuscript to submit to *International Journal of Plant Sciences*.

Chapter 6 comprises the general conclusions, and provides recommendations for future research in *Elaeocarpus*.

Notes on publications

Thesis chapters which have been published or prepared for journal submission have been modified to minimise repetition and to ensure a consistent style throughout the thesis. References are provided in a consolidated list at the end of the thesis rather than separately in each chapter. The terms ‘we’ and ‘our’ in the publications have been replaced with ‘I’ and ‘my’ respectively in this thesis.

Chapter 2 – *Elaeocarpus carbinensis* (Elaeocarpaceae), a new species endemic to tropical montane areas of northeast Queensland, Australia, is vulnerable due to climate change.

This chapter investigates a vulnerable species in the mountaintops of Queensland, Australia. The entity has been recognised by different informal phrase names in the past. The current study formally describes and names of the species, and investigates it's conservation outlook using Environmental Niche Modelling under a range of carbon dioxide emission scenarios. The chapter has been published as:

Gagul, J. N., Simpson, L & Crayn, D. M. (2018). *Elaeocarpus carbinensis* J.N. Gagul & Crayn (Elaeocarpaceae), a new species endemic to the Mt Carbine Tableland of northeast Queensland, Australia. *Austrobaileya*, 10(2): 247–259.

This paper was conceived by JNG and DMC. JNG conducted the study and drafted manuscript. DMC proofread and provided general guidance in morphological taxonomy. LS assisted with the environmental niche modelling. Peter Bannink assisted with species distribution maps and Nick Rockett assisted with photography.

ABSTRACT

Elaeocarpus carbinensis J.Gagul & Crayn from montane areas of the Wet Tropics bioregion of northeast Queensland, Australia is described and compared with similar species. Notes on habitat, distribution, and relationships, and a key to the allied large-fruited species is provided. The conservation outlook for the species was determined using environmental niche modelling analyses under a range of carbon dioxide emission scenarios. The results indicate that by the year 2080, suitable climate for the species will have disappeared from its current range. Thus, an IUCN Red List category of 'Vulnerable' under criteria 'restricted distribution, and plausibility and immediacy of threat' is recommended.

Keywords: Elaeocarpaceae, *Elaeocarpus*, *Elaeocarpus carbinensis*, Australia flora, Queensland flora, Wet Tropics bioregion, taxonomy, new species, environmental niche modelling, identification key

Introduction

Elaeocarpus L, the largest genus in Elaeocarpaceae, comprises more than 350 species with a mainly Indo-Pacific distribution (Coode, 2004; Phoon, 2015). New Guinea (c. 97 spp.) and Borneo (c. 70 spp.) have the highest species diversity (Coode, 2004). Australia contains 34 taxa (30 endemic), the majority of which occur along the east coast with a few extending to the Northern Territory and one species (*E. costatus* M.Taylor) on Lord Howe Island (Baba and Crayn, 2012). The genus is particularly diverse in the Wet Tropics Bioregion of northeast Queensland where 23 species are found, 16 of which are endemic to the bioregion.

The Wet Tropics is a small bioregion of c. 20,000 ha (less than 0.3% of Australia's landmass) and includes extensive tropical mountaintop habitat (c. 1000 ha, c. 5% of the bioregion, is above 1000 m elevation; Costion et al., 2015). This habitat is considered highly vulnerable to the effects of climate change (Murphy et al., 2012) because the warming signal in the tropics is amplified with elevation (Beniston et al., 1997) and the critical moisture provided by cloud cover is expected to decrease significantly with an upward shift in the elevation of cloud formation (Foster, 2001; Still et al., 1999). Impacts of climate change including range shifts and species extinctions have already been observed on tropical mountain tops (Pounds et al., 1999, 2006). A recent study predicted similar impacts on the Wet Tropics bioregion - distribution modelling of endemic montane tree species under future climate scenarios predicted 86% of species included in the study would have no suitable climate in the bioregion by 2080 (Costion et al., 2015). Among the taxa modelled in that study was a putative new species of *Elaeocarpus* (*E.* sp. Mt Misery (L.J.Webb+ 10905) Qld Herbarium).

Material of *Elaeocarpus* sp. Mt Misery (L.J.Webb+ 10905) Qld Herbarium was first collected by B. Hyland on 17 May 1973 from State Forest Reserve 143, North Mary Logging Area. The species is similar to *E. stellaris* L.S.Sm. but differs mainly in the mesocarp (equivalent to the fruit 'stone', being formed from the lignified inner mesocarp; Dettman and Clifford 2000) being smaller, with less pronounced flanges and less deeply grooved inter-flange valleys, and punctate abaxial leaf surfaces. This species is herein described as *Elaeocarpus carbinensis* J.Gagul & Crayn, and more rigorous modelling of its environmental niche undertaken to inform a conservation status recommendation.

2.2 Materials and methods

2.2.1 Specimen preparation and examination

Observations were made using the naked eye and light microscopy on dried and spirit preserved (FAA or Bang mix) material held at CNS and BRI, and on living material in the field. Dried material was rehydrated by boiling with a small amount of detergent. Measurements were made with a ruler or microscope eyepiece graticule. Information on plant growth habit and size, colour of fresh floral parts and fruit, habitat and locality were taken from the collector's notes recorded on the herbarium label and from field observations by the authors.

2.2.2 Species Distribution modelling

Environmental niche modelling (ENM) was utilised to predict the potential distribution of *E. carbinensis* under contemporary and future climates. Species distribution

models were produced in MaxEnt v. 3.3.3 (Phillips et al., 2006). The distribution models used in the previous ENM study (Costion et al., 2015) omitted several point records of this species. To ensure the species' full distribution was represented in the present analysis, Australia's Virtual Herbarium (AVH, 2016) was queried for all known synonyms, returning 13 unique locational records. All specimens used in the modeling analysis have been seen and verified by the authors (Appendix 2.1).

Climate layers were sourced from the Australian Wet Tropics Decadal Climate Change Predictions dataset sourced from the James Cook University Tropical Data Hub (Vanderwal 2011), and consisted of bioclimatic variables mapped at ~250 m resolution across the Wet Tropics bioregion. These layers had previously been created using the "climates" package in R (VanDerWal et al., 2011) using baseline climate surfaces from ANUCLIM 6.1 software with a climate baseline of 1975-2005 (Hutchinson et al., 2000). Four uncorrelated bioclimatic variables were used, previously selected from 19 bioclimatic variables using a jackknife test for importance (Costion et al., 2015): Temperature Seasonality, Maximum Temperature of Warmest Month, Mean Temperature of Wettest Quarter and Annual Precipitation. Suitable climate is defined as an area or areas providing a climate niche that the species currently occupies. This was used as a surrogate for habitat suitability following VanDerWal et al. (2009) and is referred to throughout the text as suitable habitat.

Habitat suitability was modelled with 10 replicates using the cross-validation option with linear, quadratic, product and hinge features enabled. To model habitat suitability under future climates, models were run for the years 2040, 2060 and 2080 under the intermediate (A1b), extreme (A2) and best case (B1) emission scenarios of Nakićenović et al. (2000).

2.3 Results

2.3.1 Taxonomy

Elaeocarpus carbinensis J.Gagul & Crayn sp. nov. Similar to *E. stellaris* L.S.Sm. but differs in having smaller fruits (50–55 × 35–50 mm vs 43–65 × 50–60 mm) with thinner mesocarp flanges (3–5 mm vs. 5–10 mm) that are more closely spaced (15–20 mm vs. 20–25 mm), less deeply grooved inter-flange valleys, punctate abaxial leaf surfaces, longer petals (35–40 mm vs 20–25 mm) and filaments (10–15 mm vs 6–8 mm), and petals hairy outside only (both sides in *E. stellaris*). **Typus**: Queensland, COOK DISTRICT. SFR 143, Kanawarra, Carbine LA, 16º 29' S, 145º 15' E, 1200 m, 25 Jan. 1995, *B. Gray 5938* (holotype: QRS [2 sheets]; isotype: BRI, CANB, NSW, MEL, K, L, E, MO, NYBG, P, SING, B, BO *distribuendi*).

Elaeocarpus sp. (=RFK/2907), Hyland, B.P.M. *A Revised Card Key to Rainforest Trees of North Queensland*: 139 (1982).

Elaeocarpus sp. Mt Lewis (B.P.Hyland RFK2907)**,** Thomas, M.B., & McDonald, W.J.F., Rare and threatened plants of Queensland: a checklist of geographically restricted, poorly collected and/or threatened vascular plant species Edn. 1: 24 (1987).

Elaeocarpus sp. Mt Misery (L.J.Webb+ 10905) Qld Herbarium, Guymer, G.P., Elaeocarpaceae, In R.J.F. Henderson (ed.), *Queensland Plants*: *Names and Distribution* 67 (1997).

Elaeocarpus sp. (Mt Spurgeon BH 2907RFK), Hyland, B.P.M., Whiffin, T., Christophel, D.C., Gray, B., Elick, R.W., & Ford, A.J., *Australian Tropical Rain Forest Trees and Shrubs, User Guide*: 63 (1999).

Elaeocarpus sp. (Mt Spurgeon), Cooper, W., & Cooper, W.T., *Fruits of the Australian Tropical Rainforest*: 162 (2004).

Elaeocarpus sp. 'Mount Spurgeon', Crayn, D.M., & Kupsch, K., Elaeocarpaceae in Australia. *Australian Plants* 23: 366 (2006).

Elaeocarpus sp. Mt Spurgeon (B.Hyland 2907RFK), Hyland, B.P.M., Whiffin, T., Zich, F.A., Duffy, S., Gray, B., Elick, R., Venter, S., & Christophel, D., *Australian Tropical Rainforest Plants Edition 6. Online version* (2010).

Trees to 30 m tall, buttressed, outer bark blaze yellow, white, cream or brown, speckled markedly with longitudinal stripes; stipules +/- triangular, c. 2 mm long, caducous; branchlets covered in short, white, appressed hairs < 0.5 mm long. **Leaves** simple, alternate, crowded toward the branchlet tips; petiole (15–) 20–45 (–58) mm long, +/- glabrous, usually with pulvinus at both ends, more pronounced at distal end; lamina obovate, oblanceolate or elliptic, 45–180 mm long, 19–80 mm wide, abaxial surface punctate, densely covered with small dark dots (? glands) visible (barely) to the naked eye, base cuneate, apex obtuse or slightly retuse; domatia present in secondary vein axils, 2–8 (–10) per leaf, foveolate, glabrous; margins entire or crenate; venation reticulate, +/- flush with adaxial leaf surface when fresh (slightly raised in dried material), prominent abaxially, +/- glabrous. **Inflorescences** 2–5-flowered, usually arising behind leaves, occasionally axillary, racemose but appearing +/- umbellate; peduncle 12–15 mm long, pubescent, hairs < 0.5 mm, appressed. **Flowers** white or cream; pedicels 10–18 mm long, pubescent, hairs 0.5–1 mm, spreading; calyx cream or greenish cream to brown, lobes narrowly triangular, 24–26 mm long, 5–6 mm wide at base, apex acute, densely pubescent to velvety outside, hairs 0.5–1 mm long, spreading to erect, golden-brown when dried, sericeous inside, hairs 2–3 mm long, appressed;

petals 5, free, 35–40 mm long, 10 mm wide, apex 2–3 lobed, lobes c. 5 mm long, rounded to acuminate or acute, with dense hairs on the outside, glabrous or with very few scattered hairs on the inside, indumentum extending across middle half, and along 3/4 of the length of the petal, hairs appressed, 2–3 mm long, margins entire, glabrous; ovary hairy, globular, 5-locular, c. 10 ovules per locule, sericeous, hairs c. 2 mm long, erect to appressed; style 18–22 mm long, tapering to ovary, sericeous over the lower 2/3, hairs similar to ovary, stigma not expanded; stamens numerous (c. 55), filaments very slender, 10–15 mm long, sericeous, anthers c. 8 mm long, tubular, with very short ascending hairs, longer (to c. 1 mm) along midline on back, awned, posterior tooth longer (c. 1.5 mm), backward-tilted. **Fruits** drupaceous, dark blue, or slatey to brownish grey, broadly ovoid to ellipsoid, 50–55 mm long, 35–50 mm wide, glabrous, shrinking and cracking irregularly upon drying; pedicel 15–25 mm long; outer mesocarp 1.7–2.2 mm thick, detaching cleanly from inner mesocarp (stone). **Mesocarps** ovoid–ellipsoid, 30–45 mm long, 32–40 mm wide, robust, woody; sutures 5, forming grooves on prominent longitudinal ridges (flanges), grooves becoming shallower basally; flanges 3–4 mm high, 3–5 mm thick (mesocarp appearing 5–angled in transverse section, wall c. 11 mm thick), base attenuate, apex rounded to slightly pointed; surface punctate. **Seeds** 1–3 per fruit, ellipsoid, 18–20 mm long, 8–10 mm wide; embryo straight, endosperm entire. Figs. 2.1, 2.2A.

Additional specimens examined: **Queensland, Cook:** TR 140 Cow LA, 1100 m, 26 Jan. 1975, *B. Hyland 7971* (BRI); Along the main path, c. 400 m from Mr Cooper's Camp, Mount Spurgeon National Park, 1209 m, 10 May 2010, *Y. Baba 426 et al.* (BRI, CNS); Mt Misery E of Mt Spurgeon 15.4 km NNE of Mt Carbine, site 25, centre plot 2, AMG 30980/818180, 1120 m, Nov. 1988, *L. W. Jessup GJM919* (BRI); Mt Spurgeon, 1160 m, 11 Jun. 1990, *B. Gray 5196* (QRS); ibid, *B. Gray 5197* (QRS, BRI); TR 143, Zarda LA, near

Zarda clearing, 1000 m, 27 Sep. 1973, *B. Hyland* 2907RFK (QRS); ibid, 27 Sep. 1973, *B. Hyland 2908RFK* (BRI, CANB, QRS); Mt Misery, Mt Carbine Tableland, Nov. 1972, *L. J. Webb 10905* (BRI); SFR 143, Kanawarra, Carbine LA, 1200 m, 31 Nov. 1994, *B. Gray 5825* (QRS); ibid, 21 Mar. 1991, *B. Gray 5294* (QRS, BRI); ibid, 03 Jul. 1990, *B. Hyland 25789RFK* (QRS); ibid, 1100 m, 15 Nov. 1990, *B. Hyland 14087* (QRS, BRI); SFR 143, North Mary LA, 1100 m, 17 May 1973, *B. Hyland 6731* (BRI); 32.5 km along Mt Lewis Road from Mossman - Mt Molloy Road, 1600 m, 05 Dec. 1989, *L. W. Jessup GJD3364* (BRI); Daintree National Park, NW of Black Mountain, 1260 m, 23 May 1998, PIF22897 (BRI); Cultivated Tolga, ex-Mt Lewis area beyond hut, 1150 m, 17 May 2005, *A. Ford 4312 & G. Sankowsky* (QRS)

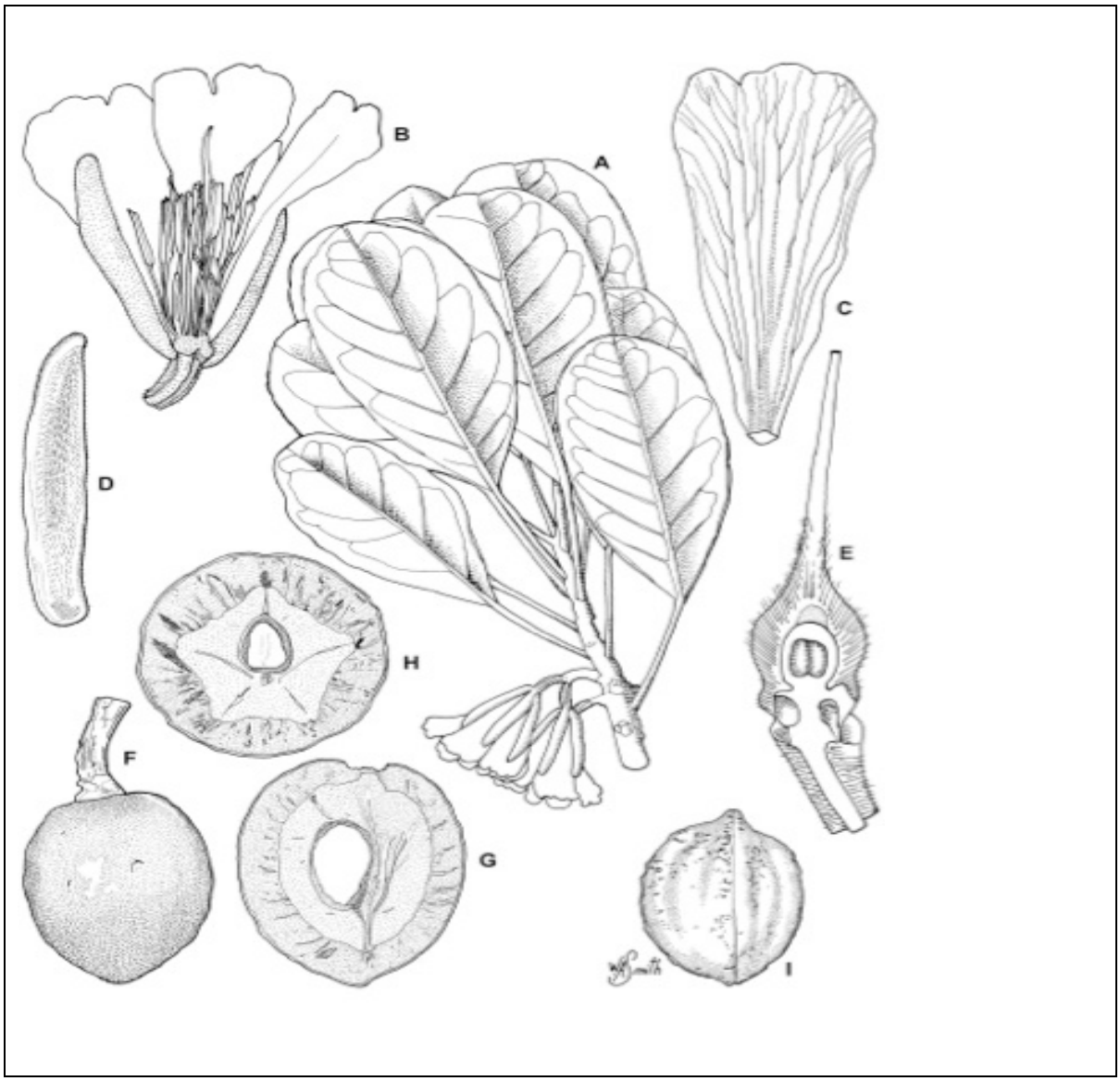


Figure 2.1 ***Elaeocarpus carbinensis.***

A. leafy twig with flowers (x0.5) B. mature flower with two petals and sepals removed (x1.5) C. petal showing inner surface (x2) D. sepal showing abaxial surface (x2) E. pistil (ovary and pedicel partly sectioned longitudinally (x3) F. fruit whole (vertical, x0.8) G. fruit (LS, x1) H. fruit (TS, x1) showing 5-angled mesocarp I. mesocarp whole, flesh removed (vertical, x1). (A – E, *Gray 5938*; F, *Hyland 14087*; G – I, *Gray 5294* [QRS]). Drawn by Will Smith.

2.3.2 Key to large-fruited *Elaeocarpus* species allied to *E. carbinensis*

1 Leaf domatia present; mesocarps with flanges……**2**

1. Leaf domatia absent; mesocarps without flanges…….....**3**

2 Mesocarp c. 30–45 mm long, c. 32–40 mm wide; flanges c. 3–5 mm thick, distance between flanges 15–20 mm, valley between flanges shallow, weakly grooved; abaxial leaf surface with small dark dots; elevational range 940–1260 m, NE QLD.....***E. carbinensis***

2. Mesocarp 41–50 mm long, 35–42 mm wide; flanges c. 5–10 mm thick, distance between flanges 20–25 mm, valley between flanges deeply grooved; abaxial leaf surface without dots; elevational range 50–500 m, NE QLD …….....***E. stellaris***

3 Fruits blackish, dark blue or dark green, 40–75 mm long, 30–50 mm wide; fibres permanently attached to mesocarp surface; anther awns present; elevational range 25–2500 m, New Guinea, Papuan Islands and the Moluccas.....***E. womersleyi***

3 Fruits dull greenish-blue to khaki, 40–55 mm long, 33–40 mm wide; fibres detaching cleanly from mesocarp surface, ornamentation punctate and pitted with irregularly scattered pits; anther awns absent; elevational range near sea level– 1200 m, NE QLD ….....***E. bancroftii***

2.3.3 Affinities

On the basis of similarities in mesocarp morphology (Rozefelds and Christophel 2002, Chapter 5) and its close molecular phylogenetic relationship with *E. stellaris* and *E. bancroftii* (Baba, 2014; Phoon, 2015; Chapter 3), *E. carbinensis* seems best placed in Group VI, Subgroup B (Coode, 1978, 1984) which comprises *E. stellaris, E. bancroftii* and *E. womersleyi*. *Elaeocarpus carbinensis* can be distinguished from these species by the following characters: domatia 2–8 per leaf (vs. 10–17 or absent); petals 5, 35–40 mm long (vs. 4, or 5 and 20–25 mm long); mesocarp flanges 5, 3–5 mm thick (vs. 5 and 5–10 mm thick, or absent) (Table 2.1). The three Australian species *E. carbinensis*, *E. stellaris* and *E. bancroftii* are distinguished from the New Guinean species *E. womersleyi* by outer mesocarp fibres, which detach cleanly from the mesocarps (persistent and permanently attached in *E. womersleyi*, Coode, 1984) (Fig. 2.2) (Table 2.1). *Elaeocarpus womersleyi* has not been included in any molecular phylogenetic study to date so its evolutionary relationships remain unclear.

A fossil mesocarp (*Elaeocarpus peteri* Rozefelds & Christophel) from late Oligocene-early Miocene (Rozefelds, 1990) deposits at Glencoe in central Queensland resembles *E. carbinensis* and *E. stellaris* in having pronounced ridges and punctate ornamentation (Rozefelds and Christophel, 1996b), but its precise relationships to extant lineages is unknown.

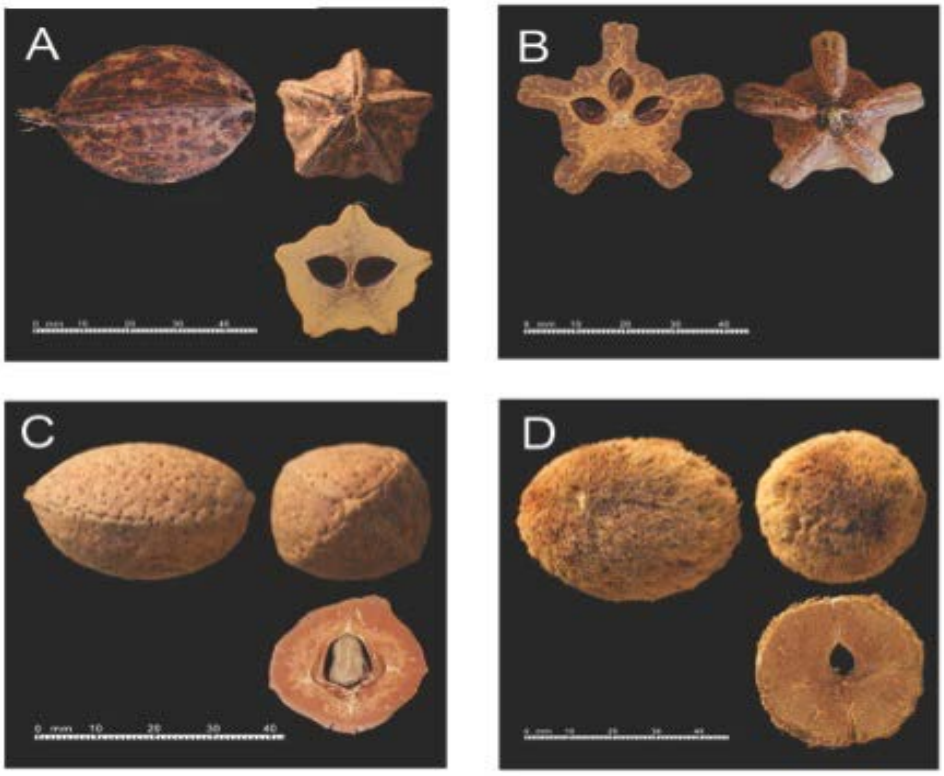


Figure 2.2 Mesocarps of *Elaeocarpus carbinensis* and similar species.

A. *E. carbinenesis* (*B. Gray 5197*, QRS), B. *E. stellaris* (*G. C. Stocker 1774*, QRS), C. *E. bancroftii* (*B. Gray 2328*, QRS), D. *E. womersleyi* (*J. Gagul 39*, CNS). Photographed by Nick Rockett.

2.3.4 Distribution and habitat

Elaeocarpus carbinensis is restricted to the Carbine Tableland west of Mossman and has been recorded on Mt Spurgeon, Mt Lewis and Mt Misery at elevations ranging from 940–1260 m. It occurs in notophyll vine forest and mixed mesophyll vine forest on soils derived from granite or a mixture of granite and basic volcanic rocks. Across the recorded localities mean annual temperature ranges between 19–20 °C, mean minimum and maximum temperatures of the coldest and warmest months range between 11–12 °C and 27–28 °C respectively, mean annual rainfall ranges between 1942–2319 mm and the mean rainfall of

the driest month ranges between 123–161 mm.

There are collections from the Windsor Tablelands (L.W. Jessup GJM1186; P.I. Forster PIF17234) of which I am unable to confirm the identity, due to unavailability of reproductive materials.

2.3.5 Predicted future distribution

Environmental Niche Modelling (ENM) under contemporary climatic conditions predicts that suitable climate for *E. carbinensis* exists across several upland regions in the northern Wet Tropics including the Windsor and Carbine Tablelands, Thornton Peak and Mt Finnigan (Fig. 2.3). Herbarium records indicate the realised distribution of the species includes the Carbine Tableland only, however. Explanations for the apparent failure of the species to fully occupy its predicted climate niche were not investigated in this study but may include a range of biotic and abiotic factors such as competitive exclusion, predation, disease, unsuitable geology/soil, or failure to recolonise after past extinction.

Environmental Niche Modelling under future climates predict a complete loss of highly and moderately suitable habitat by 2040 and of all suitable habitat by 2080 across the Wet Topics bioregion (Fig. 2.4). Although this study did not examine whether suitable habitat is predicted in other bioregions, the closest area of substantial upland rainforest outside the Wet Tropics is the Eungella region of central Queensland, located c. 250 km to the southeast. Lowland tropical savanna and cleared land separates the Wet Tropics and Eungella therefore dispersal of the large fruits of this species, which is probably achieved only by rodents (which predate the seeds) and cassowaries, to suitable habitat elsewhere (should it exist) is highly unlikely.

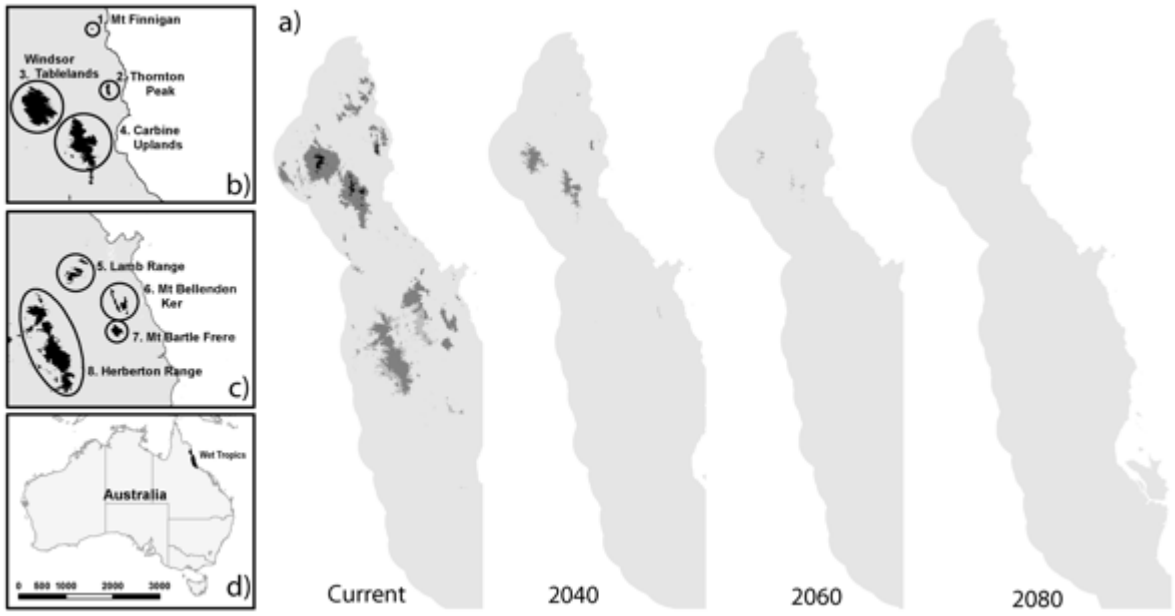


Figure 2.3 Potential distribution of *Elaeocarpus carbinensis* for contemporary and future climates under an intermediate emission scenario.

a) MAXENT species distribution models of *Elaeocarpus carbinensis* mapping habitat suitability in the Wet Tropics under current conditions and years 2040, 2060 and 2080 under an intermediate emission scenario. Highly suitable habitat is mapped in black, moderately suitable habitat in dark grey, lowly suitable habitat in light grey and unsuitable habitat in the lightest grey. Upland regions are shown for b) the northern Wet Tropics and c) the southern Wet Tropics, and the location of the Wet Tropics in Australia is shown in d).

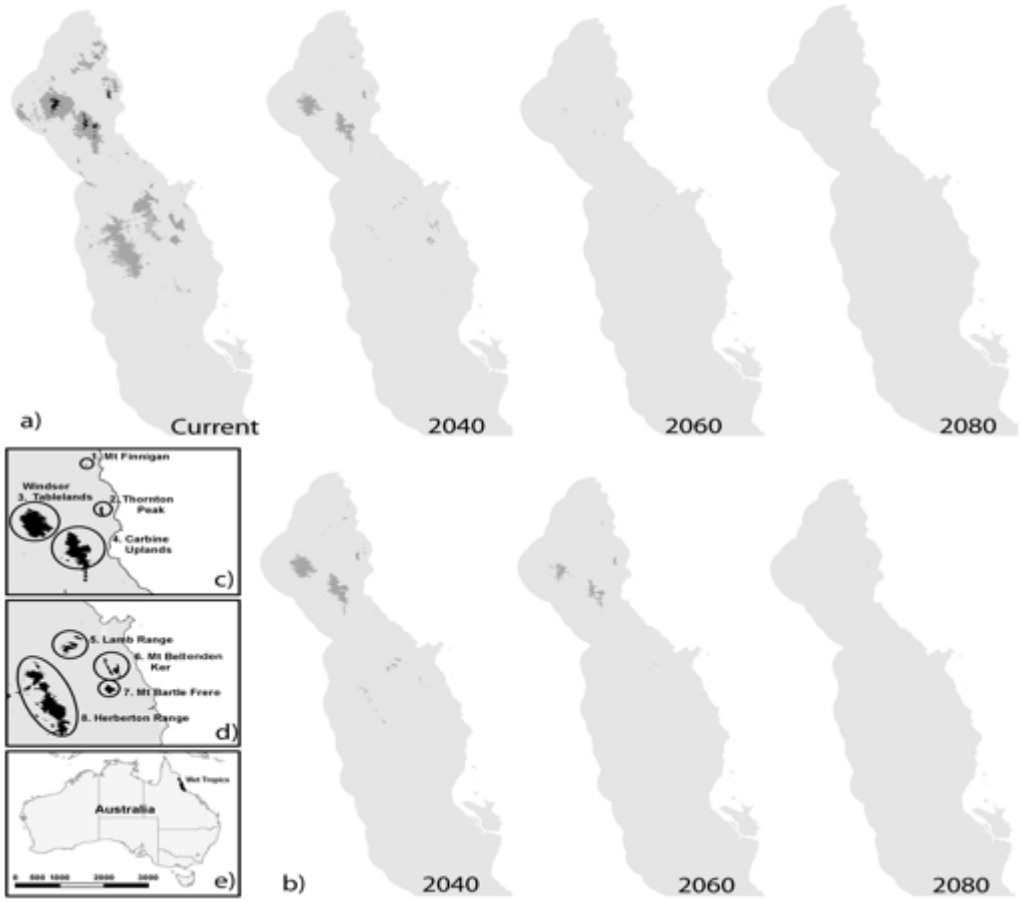


Figure 2.4 Potential distribution of *Elaeocarpus carbinensis* for contemporary and future climates under extreme and best-case emission scenarios.

a) MAXENT species distribution models of *Elaeocarpus carbinensis* mapping habitat suitability in the Wet Tropics under current conditions and years 2040, 2060 and 2080 under an extreme emission scenario. b) Habitat suitability modelled for the years 2040, 2060 and 2080 under a best-case emission scenario. Highly suitable habitat is mapped in black, moderately suitable habitat in dark grey, lowly suitable habitat in light grey and unsuitable habitat in the lightest grey. Upland regions are shown for c) the northern Wet Tropics and d) the southern Wet Tropics, and the location of the Wet Tropics in Australia is shown in e).

2.3.6 Phenology

Herbarium specimens indicate that flowering occurs in January and fruiting in March.

2.3.7 IUCN category

VU (D2). All known wild plants of *E. carbinensis* are restricted to 940–1620 m altitude, and occur within protected areas (Daintree National Park, Mt Lewis National Park and Mt Spurgeon National Park, ?Mt Windsor National Park), but complete loss of suitable habitat by 2080 is predicted by the environmental niche modeling analysis. Assessment against the IUCN red list guidelines suggests this species should be recognised as 'Vulnerable' under criteria D2 (restricted distribution, and plausibility and immediacy of threat) due to climate change (IUCN Red List Categories and Criteria, Version 13, 2017; Version 3.1, 2012).

If the model predictions are realised then the survival of the species *in situ* will depend on rapid evolutionary change and/or inherent physiological plasticity to tolerate novel climates and the novel ecological communities that differential extinction and migration will bring about. The population demographics of the species have not been studied in detail but field observations indicate all known plants are large, old trees; to date no seedlings or juveniles have been located. This suggests that generation length is likely measured in decades and that the potential for rapid evolutionary change is limited. Published studies on the physiology of the species are lacking therefore its capacity to tolerate novel climates is unknown.

Currently, the species is known from only 13 unique locational records on the Mt Carbine Tableland. Further studies are urgently required to increase knowledge of its realised

distribution, population demographics, physiology and ecology to enable a revised assessment of conservation status. In the meantime application of the precautionary principle justifies the establishment of an ex situ conservation program including both germplasm banking and cultivation of living plants.

2.3.8 Etymology

The specific epithet *carbinensis* refers to the Carbine Tableland, NE Queensland, the area to which the species is restricted.

Table 2.1 Comparison of features of *Elaeocarpus carbinensis* and similar species.

	E. carbinensis	***E. stellaris***	***E. bancroftii***	***E. womersleyi***
Distribution	940–1260 m, Mt. Spurgeon-Mt. Lewis-Mt. Misery area on Carbine Tableland.	50–500 m, Alexandra Creek-McDowall Range to Innisfail.	0–1200 m, Cooktown to Innisfail.	25–2500 m, New Guinea, Papuan Islands, Moluccas.
Habit	tree to 30 m, buttressed	tree to 25 m, may be buttressed	tree to over 30 m, buttressed, flanged or fluted	tree to 45 m, may be buttressed
Leaf margin	entire or crenate	entire or crenate	entire or sinuate	entire to slightly dentate, or sinuate
Leaf surfaces	glabrous or sparsely hairy (hairs visible with a lens), densely covered with small, barely visible, dark dots (? glands) below	glabrous above, sparsely hairy below	glabrous above, sparsely hairy below (hairs visible with a lens)	glabrous on both sides
Leaf dimensions	45–180 × 19–80 mm	80–180 × 40–90 mm	50–180 × 25–50 mm	100–150 × 40–80 mm

Petiole	(15–) 20–45 (–58) mm long; pulvinus at both ends, more pronounced at distal end.	20–55 mm long; pronounced pulvinus at both ends	10–45 mm long; pulvinus at base, apex, or both	10–30 mm long; pulvinus generally absent, sometimes weakly present at base, apex or both
Stipules	c. 2 mm, caducous	c. 2 mm, caducous	c. 1–2 mm long, deciduous	c. 1–2 mm, deciduous, sometimes caducous
Leaf domatia	present as foveoles in secondary vein axils, glabrous, 2–8 (–10) per leaf	present as foveoles in secondary vein axils, glabrous, (–5) 10–17 per leaf	absent	absent
Petals	5, white or cream, obovate, 35–40 mm long and 10 mm wide, with dense hairs on the outside, glabrous or with very few scattered hairs on the inside, divided at apex into 2–3 lobes, lobes c. 5 mm long	5, white or cream, obovate, 20–25 mm long and 10 mm wide, with dense hairs on both sides, divided at the apex into 3 lobes, lobes c. 3 mm long	4, white, obovate, c. 20–24 mm long and c. 10–18 mm wide, with sparse hairs on the outside, glabrous or with very few scattered hairs on keel on the inside, divided at apex into c. 3 (–5) lobes, lobes c. 3 mm long	4, white or cream, obovate, 30–40 mm long and 15–20 mm wide, with hairs on the inside of basal half, divided at apex into 3–5 lobes, lobe length unknown

Anther awns	present, 1–1.5 mm long	present, c. 1 mm	absent	present, c. 2 mm long
Stamens	c. 55	50–60	30–50	c. 40
Filaments	10–15 mm, with long appressed or slightly ascending hairs	6–8 mm, with long ascending or appressed hairs	5–9 mm, with long scattered ascending hairs	6–13 mm long, hairs unknown
Fruit colour	dark blue, or slatey to brownish grey	blue, shiny	dull greenish-blue to khaki	blackish, dark blue or dark green
Fruit dimensions	broad ovoid to ellipsoid, 50–55 ×35–50 mm	globose to ellipsoid, 43–65 × 50–60 mm	globose to ellipsoid, 40–55 × 33–40 mm	globose or obovoid, 40–75 × 30–50 mm
Pedicel	15–25 mm long	23–25 mm long	10–35 mm long	9–26 mm long
Mesocarp dimensions	30–45 × 32–40 mm	41–50 × 35–42 mm	30–80 × 20–70 mm	40–55 × 30–50 mm
Mesocarp fibres	detach cleanly from mesocarp	detach cleanly from mesocarp	detach cleanly from mesocarp	persistent and permanently attached to mesocarp
Mesocarp flanges	5, c. 3–5 mm thick, distance between flanges 15–20 mm, valley between flanges shallow	5, c. 5–10 mm thick, distance between flanges 20–25 mm, valley between flanges deeply grooved	absent	absent

Mesocarp ornamentation	punctate	punctate	punctate and pitted with irregularly scattered pits	fibres permanently attached
Sutures	5, prominent on flanges, becoming less grooved distally	5, prominent on flanges grooved	4 (–5), grooved, sometimes on weak ridges distally	difficult to see due to persistent fibres permanently attached to surface, but mesocarp 4–partite in TS
Locules	5	5	(2–) 4 (–5)	4 (–5)
Ovules	c. 10 per locule	4–8 per locule	9–10 per locule	6 per locule
Seeds	1–3 per fruit	1–3 per fruit	1–2 per fruit	1–2 per fruit

Where necessary, information was also taken from other sources (Coode, 1978, 1984; Dettman and Clifford, 2000; Rozefelds and Christophel, 2002; Cooper and Cooper, 2004; Phoon, 2015). Mesocarp characters for each species are illustrated in Fig. 2.2 A–D.

Chapter 3 – Molecular phylogenetics of *Elaeocarpus* (Elaeocarpaceae): a focus on New Guinean species and insights from multilocus molecular sequences

This chapter investigates the relationships of the New Guinean species of *Elaeocarpus* using multi-locus molecular sequences, and is being prepared for submission to *Australian Systematic Botany* as:

Gagul, J N., Nauheimer, L. Coode, M. J. E., & Crayn D. M. Molecular phylogenetics of *Elaeocarpus* (Elaeocarpaceae): a focus on New Guinean species and insights from multi-locus molecular sequences.

Some of the material has been presented in the preliminary report:

Gagul, J. (2016). Molecular phylogenetics of *Elaeocarpus* (Elaeocarpaceae) with a focus on New Guinean species. *Australasian Systematic Botany Society Newsletter* 167: 4–8.

and in the following presentations:

Gagul, J., Crayn, D., Gadek, P., Rozefelds, A., Thornhill, A. (2014). Systematics and evolution of the genus *Elaeocarpus* L. (Elaeocarpaceae). *Association for Tropical Biology and Conservation (ATBC) Conference.* Cairns, Australia [poster].

Gagul, J., Crayn, D., Gadek, P., Rozefelds, A., Thornhill, A. (2014). Molecular phylogenetics of *Elaeocarpus* (Elaeocarpaceae) with a focus on New Guinean species. *Research Science and Technology Conference*. Port Moresby, Papua New Guinea [talk].

Gagul, J., Rozefelds, A., Nauheimer, L., Crayn, D. (2016). Molecular phylogenetics of *Elaeocarpus* L. (Elaeocarpaceae) with a focus on New Guinean species. *Flora Malesiana 10 Conference*. Edinburgh, Scotland [talk].

Gagul, J., Crayn, D., Nauheimer, L. (2015). Molecular phylogenetics of *Elaeocarpus* L. (Elaeocarpaceae) with a focus on New Guinean species. *Australasian Systematic Botany Society Conference.* Canberra, Australia [talk].

This chapter was conceived by JNG and DMC. DMC proofread and provided general guidance in molecular phylogenetics. JNG conducted the study and wrote manuscripts. LN assisted with molecular analyses and proof reading. MJEC assisted with taxon name confirmation, proof reading and general guidance in morphological taxonomy.

ABSTRACT

The family Elaeocarpaceae comprises more than 500 species of trees and shrubs in 12 genera. Of these genera, *Elaeocarpus* is the most widespread and speciose, comprising c. 360 species distributed across Madagascar, Indo-china, Japan, Malesia, and Australasia. About 97 *Elaeocarpus* taxa occur in New Guinea. Although about 97 *Elaeocarpus* taxa occur in New Guinea, few of these have been included in previous molecular phylogenetic studies. The current study has substantially improved from the following previously neglected areas: New Guinea, New Caledonia, Japan, Cambodia, Thailand, Myanmar, and Indonesia (Sulawesi) by contributing 89 samples. I have utilised Next Generation Sequencing approach to sequence plastomes of Elaeocarpaceae, the first study to do so. Parsimony and Bayesian analyses were used to compare the performance of plastome-scale sequence datasets compared with few-marker datasets for resolving the phylogeny of *Elaeocarpus*. The results indicate that phylogenetic reconstructions based on plastome data are better resolved and better supported than few gene phylogenies. As a result of the increased sampling from New Guinea, molecular data for c. 50% of the species of *Elaeocarpus* is now available,. Of the nine currently recognised groups in New Guinea, this study has sampled representatives of six groups. Species used in the current study from New Guinea and other areas are nested within clades that have previously been identified. These clades are generally congruent with the current morphological classification.

Keywords: DNA, evolution, systematics, *Elaeocarpus*, *trnL-F, trnV-ndhC*, *trnH-psbA*, chloroplast, plastome, next generation sequencing, New Guinea.

3.1 Introduction

Elaeocarpaceae is a moderately large family of more than 500 species of trees and shrubs (Baba and Crayn, 2012; Maynard et al., 2008; Rozefelds and Christophel, 1996a). Most Elaeocarpaceae occur in rainforests, although a few, especially those formerly ascribed to Tremandraceae (namely *Platytheca* Steetz, *Tetratheca* Sm., and *Tremandra* R. Br.) occur in dry areas (Crayn et al., 2006). Elaeocarpaceae comprises 12 genera; *Aceratium* DC., *Aristotelia* L'Her., *Crinodendron* Molina, *Dubouzetia* Brongn. & Gris, *Elaeocarpus* L., *Peripentadenia* L.S Sm., *Platytheca* Steetz, *Sericolea* Schltr., *Sloanea* L., *Tetratheca* Sm., *Tremandra* R. Br. and *Vallea* L. *f*. (Coode, 2004). Of these genera, *Elaeocarpus* is the most widespread and speciose, comprising c. 360 species found in Madagascar, Indo-China, Japan, Malesia, Australasia and the Pacific (Boeira et al., 2012; Coode, 1978, 1981). Morphologically, *Elaeocarpus* is well defined. *Elaeocarpus* species are shrubs or trees up to 45 m tall, and are characterized by distinct fringed petals and drupaceous fruits. Despite considerable interest in the taxonomy of the group, the phylogenetic relationship of *Elaeocarpus* species, and particularly New Guinean species, remain unclear.

Schlechter (1916) was the first to propose an infrageneric classification of *Elaeocarpus*, which was adopted to varying degrees in the morphological taxonomies of Coode (1978, 1981, 1984), Smith (1944), Weibel (1968), and Weibel and Coode (1994). Coode (1978, 1981, 1984) proposed an infrageneric classification for Australia, New Zealand and Papuasia based primarily on seed characters, e.g., number of ovules per locule and embryo shape. Taxa were categorized into twelve morphological groupings (corresponding to sections *sensu* Coode, 1978, 1981, 1984;

Schlecter, 1916). Of the twelve morphological groupings, Groups I, II, III and IX are absent from Australia and New Zealand, and Groups X, XI, and XII are absent from New Guinea (Coode, 1978, 1981, 1984). The groups that are shared between these regions include IV, V, VI, VII, and VIII (Coode, 1978, 1981, 1984).

A few molecular phylogenetic studies have been conducted on the family **(**Baba, 2014; Crayn et al., 2006; Maynard, 2004; Niissalo, 2011; Phoon, 2015). The first substantial work on the phylogeny of Elaeocarpaceae was Crayn et al. (2006), who analysed plastid *trnL-F* and nuclear *ITS* sequences of 50 species representing the 12 genera within Elaeocarpaceae, using Parsimony and Bayesian methods. Results resolved the relationships among the genera of Elaeocarpaceae and Tremandraceae and provided strong support for the transfer of Tremandraceae into Elaeocarpaceae.

Within Elaeocarpaceae, the phylogenies of the two largest genera – *Sloanea* and *Elaeocarpus* – have been explored **(**Baba, 2014; Maynard, 2004; Niissalo, 2011; Phoon, 2015). The phylogeny of 33 species of *Sloanea* has been investigated by Niissalo (2011), utilising the same molecular markers (plastid *trnL-F* and nuclear *ITS*) and analyses methods (Parsimony and Bayesian) used by Crayn et al. (2006). His results confirmed the monophyly of *Sloanea*, and also revealed two clades within it, one representing the Old World species and the other consisting of New World species.

The phylogenetic relationships among species of *Elaeocarpus* have been investigated (Baba, 2014; Maynard, 2004; Phoon, 2015). The first molecular phylogenetic study in *Elaeocarpus* (Maynard, 2004) investigated the phylogeny of

Australian species and determined the taxonomic boundary of a putative new species (*E*. sp. 'Rocky Creek', now formalised as *E. sedentarius* Maynard & Crayn; Maynard et al., 2008). Based on analyses of nuclear ITS sequences of 32 species of *Elaeocarpus*, three *Aceratium*, one *Sericolea* and two *Peripentadenia* data using Parsimony and Likelihood analyses methods, his study resolved some relationships among the Australian *Elaeocarpus*.

Baba (2014) investigated further the phylogenetic relationships among *Elaeocarpus* species of Australia with a much-expanded dataset.
Three molecular markers – *trnL-F, trnV-ndhC* (used for the first time in molecular phylogenetics of *Elaeocarpus*) and *ITS* – were sequenced for 144 taxa (73 *Elaeocarpus* and 71 from other Elaeocarpaceae) and analysed using Parsimony and Bayesian methods. The results showed *E. holopetalus* F.Muell. to be placed outside of the main *Elaeocarpus* clade, suggesting the genus was paraphyletic. Within *Elaeocarpus* four main clades were resolved, which were generally congruent with Coode's (1978, 1981, 1984) infrageneric classification.

Phoon (2015) demonstrated that *Elaeocarpus* is monophyletic, based on a dataset of 176 taxa, 114 of which were *Elaeocarpus*. She used four molecular markers (plastid *trnL-F, trnH-psbA, trnV-ndhC* and nuclear *Xdh*), of which *trnH-psbA* and *Xdh* were used for the first time in phylogenetic analyses of Elaeocarpaceae. Her analyses showed *Elaeocarpus* to be monophyletic with *Aceratium* and *Sericolea* as the closest relatives. Within *Elaeocarpus*, Phoon (2015) identified 12 main clades including those recognised by Baba (2014), which were congruent with Coode's (1978, 1981, 1984) morphological classification. Phoon (2015) significantly increased the

understanding of the phylogenetic relationships among *Elaeocarpus* species, however she focused on species from Western Malesia (Malaysia (Peninsula Malaya, Sarawak), Singapore, Indonesia (Sumatra, Kalimantan, Java), Phillipines (Palawan); Phoon, 2015).

3.1.1 *Elaeocarpus* in New Guinea

New Guinea is one of the centres of diversity for *Elaeocarpus*, and the genus is represented there by nine sections; *Lobopetalum* Schltr., *Dactylosphaera* Schltr., *Elaeocarpus* L., *Blepharoceras* Schltr., *Ganitrus* Brongn. & Gris, *Monocera* Brongn. & Gris, *Oreocarpus* Schltr., *Coilopetalum* Schltr., and a currently unnamed section. These sections correspond to the nine infrageneric groups *sensu* Coode (1978, 1981). Section *Elaeocarpus* also occurs in India, throughout Southeast Asia (Malaysia, Indonesia - Sumatra and Java, and the Philippines), and beyond New Guinea, it extends east to the Solomon Islands., and section *Ganitrus* occurs from India throughout Malesia to Australia. Section *Coilopetalum* is also widespread from India to the Pacific (not Australia, New Caledonia or New Zealand), and section *Oreocarpus* occurs from the Philippines to Australia.. Sections *Acronodia* and *Polystachyus* are not represented in New Guinea. In New Guinea species of *Elaeocarpus* are widespread throughout the island from near sea level to elevations over 3500 m.

The most recent account of New Guinean species of *Elaeocarpus* lists 68 species, seven subspecies, and two varieties (Coode, 1978, 1981). At least seven new species were suggested but insufficient material precluded their description. Some of those species were subsequently described when sufficient material became available,

e.g., *Elaeocarpus altisectus* subsp. *carrii* Coode, *Elaeocarpus amabilis* subsp. *piorae* Coode, *Elaeocarpus coloides* subsp. *ridsdalei* Coode, *Elaeocarpus myrtoides* subsp. *vinkii* Coode, and *Elaeocarpus sericoloides* var. *diffusus* Coode (Coode, 1998; 2001a,b; 2002; 2003).

The arrangement of species into infra-generic groupings is circumscribed by morphological characteristics only. Their phylogeny is still incompletely understood.. Inadequate material has made formal description, naming and classification difficult (Coode, 1978, 1981), whilst poor representation of New Guinea samples in molecular analyses further limits our understanding of the relationships of the species with those from other regions. Although the studies of Baba (2014) and Phoon (2015) contained dense sampling of species diversity, the samples were mostly from Australia and Western Malesia, with only few (c. 12 species) from New Guinea. The New Guinean species in those studies fell within clades corresponding to the major infrageneric groups recognised by Coode (1978, 1981), but those species represent only four out of the nine groups from New Guinea. Therefore, increased sampling from New Guinea is important to develop a comprehensive understanding of the phylogenetic relationships of the species.

The current study aims to utilize phylogenetic analysis of a multilocus molecular dataset with substantially improved sampling of New Guinean species, to:

1. assess the utility of plastome-scale sequence datasets over few-marker datasets for resolving the phylogeny of *Elaeocarpus*
2. test the morphology-based classification of *Elaeocarpus*, and

3. improve the understanding of the phylogenetic relationships of *Elaeocarpus* from New Guinea.

3.2 Materials and methods

3.2.1 Sampling and plant material

The sampling strategy aimed to represent as much of the known diversity of *Elaeocarpus* and other Elaeocarpaceae from New Guinea as possible. Samples from other underrepresented areas were also included (e.g. Myanmar, Cambodia, Thailand, Indonesia (Sulawesi)).

A list of *Elaeocarpus* taxa from New Guinea was compiled using literature (Coode, 1978, 1981, 2001a, 2005, 2010) (Table 3.1), grouped according to the morphological classification of Coode (1978, 1981). This list was compared against a second list of species included in existing molecular datasets (Baba, 2014; Crayn et al., 2006; Maynard et al., 2008; Niissalo, 2011; Phoon, 2015) (Table 3.2) to identify species to sample.

Complete sampling of species is the ideal for molecular phylogenetic studies, however this was not feasible given the geographical and logistical difficulties of field work in New Guinea. Therefore an opportunistic sampling approach was adopted. Samples of dried or preserved herbarium materials were used to supplement the dataset where species could not be obtained from the field (Table 3.3). Samples were also obtained with permission from other researchers (individuals and institutions) (Table 3.4).

Fresh leaf materials were collected and desiccated in silica gel in the field. Voucher specimens (both fertile and sterile) were also collected, photographed, identified and pressed in the field. Field characters were noted *in situ*. Recordings included characters such as habit or life form of the plant, height, stem or bark colour and texture, colour of stipules if present, colours of leaves, flowers and fruits, sap and smell of bark, leaf, flower, and fruit to facilitate herbarium-based identifications. Further processing of vouchers (e.g. drying, mounting) and identification was made at the Australian Tropical Herbarium (ATH). For specimens that could not be confidently identified, high-resolution images of specimens with field notes and preliminary identification were sent to the world expert (Mark Coode) for examination. Vouchers of all samples were lodged at CNS.

A total of 89 new samples of *Elaeocarpus* and other Elaeocarpaceae were obtained for the current study - 61 samples from New Guinea, nine from Sulawesi (Indonesia), one each from Myanmar and Japan, three from Thailand, five from Cambodia and eight from Australia (Table 3). Samples whose identification was difficult to verify due to sterility of specimens included in the analysis, are labelled as *Elaeocarpus* sp. (Table 3.4).

Outgroup taxa in the molecular data comprises of members of *Aceratium*, *Crinodendron*, *Dubouzetia, Peripentadenia, Sericolea* and *Sloanea*. *Sloanea* was selected to root the phylogenetic trees on the basis of previous studies demonstrating that it was the distant relative of *Elaeocarpus* (Baba, 2014; Crayn et al., 2006; Niissalo, 2011; Phoon; 2015).

Table 3.1 Provisional checklist of *Elaeocarpus* taxa from New Guinea and its offshore islands.

Data were compiled from Coode (1978, 1981, 2001, 2005, 2010, 2019; pers. comm, 2014–2019). The great majority of taxa are endemic; those shared with other regions are indicated by an asterix (*). *Elaeocarpus coodei* Weibel is currently known from Solomon Islands only, and whether it is distinct from *E. coloides* from New Guinea is questionable. *Elaeocarpus terminalioides* is insufficiently known: it is mentioned in the notes only in Coode (1981).

Elaeocarpus altigenus Schltr.	*Elaeocarpus altisectus* Schltr. subsp. *altisectus*
Elaeocarpus altisectus Schltr. subsp. *carrii* Coode	*Elaeocarpus amabilis* Kaneh. & Hatus. subsp. *amabilis*
Elaeocarpus amabilis subsp. *piorae* Coode	*Elaeocarpus amplifolius* Schltr.
Elaeocarpus angustifolius Blume*	*Elaeocarpus arnhemicus* F. Muell.*
Elaeocarpus avium Coode	*Elaeocarpus badius* Coode*
Elaeocarpus bakaianus Coode	*Elaeocarpus bilobatus* Schltr.
Elaeocarpus bilongvinas Coode	*Elaeocarpus blepharoceras* Schltr.
Elaeocarpus branderhorstii Pulle	*Elaeocarpus buderi* Coode
Elaeocarpus coloides Schltr. subsp. *coloides*	*Elaeocarpus coloides* subsp. *ridsdalei* Coode
Elaeocarpus coodei Weibel	*Elaeocarpus crassus* Coode
Elaeocarpus crenulatus Knuth	*Elaeocarpus culminicola* Warb.*
Elaeocarpus davisii Coode	*Elaeocarpus debruynii* O.C.Schm.
Elaeocarpus dasycarpus A.C.Sm.	
Elaeocarpus densiflorus Knuth	*Elaeocarpus dolichodactylus* Schltr.*
Elaeocarpus dolichostylus Schltr. var. *dolichostylus*	*Elaeocarpus dolichostylus* Schltr. var. *chloranthus* (A.C.Sm.) Coode

Elaeocarpus dolichostylus Schltr. var. *hentyi* Coode	*Elaeocarpus elatus* A.C.Sm.
Elaeocarpus fairchildii Merr.	*Elaeocarpus filiformidentatus* R.Knuth
Elaeocarpus finisterrae Schltr.	*Elaeocarpus firmus* R.Knuth
Elaeocarpus floridanus Hemsley *	*Elaeocarpus fuscoides* R.Knuth
Elaeocarpus gardneri Coode	*Elaeocarpus habbemensis* A.C.Sm.
Elaeocarpus habbemensis A.C.Sm. subsp. *schoddei* Coode	*Elaeocarpus hartleyi* Weibel
Elaeocarpus heptadactyloides Weibel	*Elaeocarpus homalioides* Schltr.
Eleaocarpus johnsii Coode	*Elaeocarpus kaniensis* Schltr.
Elaeocarpus latescens F.Muell.	*Elaeocarpus ledermannii* Schltr.
Elaeocarpus leucanthus A.C.Sm.	*Elaeocarpus lingualis* R.Knuth
Elaeocarpus luteolus A. C. Sm.	*Elaeocarpus marafunganus* Coode
Elaeocarpus miegei Weibel subsp. *miegei**	*Elaeocarpus miegei* subsp. *rosselensis* Coode
Elaeocarpus millarii Weibel	*Elaeocarpus multisectus* Schltr.*
Elaeocarpus murukkai Coode	*Elaeocarpus myrmecophilus* A.C.Sm.
Elaeocarpus myrtoides A.C.Sm. subsp. *myrtoides*	*Elaeocarpus myrtoides* subsp. *vinkii* Coode
Elaeocarpus neobritannicus Coode	*Elaeocarpus nouhuysii* Koord.
Elaeocarpus orohensis Schltr.	*Elaeocarpus oriomensis* Weibel
Elaeocarpus ornatus Coode	*Elaeocarpus osiae* Coode
Elaeocarpus pachyanthus Schltr.	*Elaeocarpus piestocarpus* Schltr.*
Elaeocarpus poculiferus A.C.Sm.	*Elaeocarpus polyandrus* A.C.Sm.
Elaeocarpus polydactylus Schltr var. *polydactylus*	*Elaeocarpus polydactylus* Schltr. var. *podocarpoides* Schltr.
Elaeocarpus polydactylus var. *savannarum* (A.C.Sm.) Coode	*Elaeocarpus prafiensis* Weibel
Elaeocarpus ptilanthus Schltr.	*Elaeocarpus pullenii* Weibel
Elaeocarpus pycnanthus A.C.Sm.	*Elaeocarpus rosselensis* Coode
Elaeocarpus royenii Weibel	*Elaeocarpus rubescens* Weibel
Elaeocarpus sarcanthus Schltr.	*Elaeocarpus sayeri* F.Muell.
Elaeocarpus schlechterianus A.C.Sm.	*Elaeocarpus schoddei* Weibel

Elaeocarpus sepikanus Schltr.	*Elaeocarpus sericoloides* A.C.Sm. var. *sericoloides*
Elaeocarpus sericoloides var. *diffusus* Coode	*Elaeocarpus sphaericus* (Gaertn.) K. Sch. = *E. angustifolius**
Elaeocarpus sterrophyllus Schltr.	*Elaeocarpus tariensis* Weibel
Elaeocarpus terminalioides Schltr.[B]	*Elaeocarpus timikensis* Coode
Elaeocarpus trichophyllus A.C.Sm.	*Elaeocarpus undulatus* Warb.*
Elaeocarpus whartonensis A.C.Sm.	*Elaeocarpus womersleyi* Weibel

Table 3.2 Species from New Guinea used in previous molecular phylogenetic studies.

Species	Group	Collector number	Institution or Herbarium	Reference
Elaeocarpus angustifolius Blume	V (A)	D.M.Crayn 572	NSW	Baba (2014); Crayn et al. (2006); Maynard (2004); Niissalo (2011); Phoon (2015)
Elaeocarpus angustifolius Blume	V (A)	R.J.Johns 10685	K	Phoon (2015)
Elaeocarpus bakaianus Coode	V (C)	D.M.Crayn 584	NSW	Phoon (2015)
Elaeocarpus crenulatus R.Knuth	III (E)	D.M.Crayn 539	NSW	Baba (2014)
Elaeocarpus fairchildii Merr.	VI (D)	S.N.Phoon 139 et al	CNS	Phoon (2015)
Elaeocarpus multisectus Schltr.	III (A)	D.M.Crayn 561	NSW	Baba (2014); Phoon (2015)
Elaeocarpus murukkai Schltr.		D.M.Crayn 590	NSW	Maynard (2004)
Elaeocarpus myrtoides subsp. *vinkii* Coode	V (E)	D.M.Crayn 539	NSW	Phoon (2015)

Elaeocarpus nouhuysii Koord.	VI (C)	D.M.Crayn 530	NSW	Baba (2014); Phoon (2015)
Elaeocarpus nouhuysii Koord.	VI (C)	D.M.Crayn 533	NSW	Baba (2014); Phoon (2015)
Elaeocarpus polydactylus Schltr.	V (D)	D.M.Crayn 577	NSW	Baba (2014); Maynard (2004); Phoon (2015)
Elaeocarpus ptilanthus Schltr.	V (A)	D.M.Crayn 554	NSW	Baba (2014); Maynard (2004); Phoon (2015)
Elaeocarpus sarcanthus Schltr.	VIII (D)	D.M.Crayn 582	NSW	Phoon (2015)
Elaeocarpus sayeri F.Muell	VIII (C)	D.M.Crayn 557	NSW	Phoon (2015)
Elaeocarpus sphaericus K.Schum		D.M.Crayn 562	NSW	Maynard (2004)
Aceratium doggrellii C.T.White	-	M.Harrington 296	CNS	Baba (2014)
Aceratium ledermannii Schltr.	-	D.M.Crayn 534	NSW	Baba (2014); Crayn et al. (2006); Maynard (2004); Niissalo (2011); Phoon (2015)
Dubouzetia kairoi Coode	-	D.M.Crayn 578	NSW	Baba (2014); Crayn et al. (2006); Niissalo (2011); Phoon (2015)

Sericolea calophylla (Ridl.) Schltr. subsp. *grossiserrata* Coode	-	D.M.Crayn 550	NSW	Baba (2014); Crayn et al. (2006); Niissalo (2011); Phoon (2015)
Sericolea gaultheria (F.Muell.) Schltr.	-	D.M.Crayn 553	NSW	Baba (2014); Crayn et al. (2006); Maynard (2004); Niissalo (2011); Phoon (2015)
Sericolea micans var. *micans* Schltr.	-	D.M.Crayn 536	NSW	Baba (2014); Crayn et al. (2006); Phoon (2015)
Sloanea forbesii F.Muell.	-	B.J.Conn et al. 5029	NSW	Baba (2014)
Sloanea sogerensis Baker f.	-	D.M.Crayn 532	NSW	Baba (2014); Crayn et al. (2006); Niissalo (2011); Phoon (2015)

Table 3.3 Herbarium specimens used for DNA extraction.

A majority of the taxa are from New Guinea (except a few from other regions which are indicated by an asterix) which could not be sampled in the field. Only *E. crenulatus* was successfully sequenced, the rest failed to yield DNA of sufficient quality and/or quantity.

Taxon name	**Collector number**	**Herbarium or Institution Code**	**CNS DNA number**
Elaeocarpus acronodia subsp. *sandanum*	*Katik LAE70994*	BRI	G0 6920
Elaeocarpus crenulatus Knuth	DM Crayn 539	NSW	G0 7115
Elaeocarpus floridanus Hemsley	*Mair NGF1855*	BRI	G0 6240
Elaeocarpus fuscoides Knuth	*van Royen NGF15061*	BRI	G0 6918
Elaeocarpus habbemensis A.C.Sm.	Hartley 11707	BRI	G06242
Elaeocarpus lingualis Knuth	*Vandenberg NGF39988*	BRI	G0 6249
**Elaeocarpus linsmithii* Guymer	B. Hyland 1180	BRI	G0 6921
Elaeocarpus leucanthus A.C.Sm.	*Streimann LAE51751*	BRI	G0 6916
**Elaeocarpus macroceras* (Turcz.) Merr.	Bewalda 6807	BRI	G06241
Elaeocarpus marafunganus Coode	*Womersley NGF14022*	BRI	G06246
Elaeocarpus ?miegei Weibel	*Croft LAE68687*	BRI	G06248
Elaeocarpus millarii Weibel	*Katik NGF40054*	BRI	G0 7116

**Elaeocarpus nodosus* Baker f.	MacKee 14939	BRI	G0 6238
Elaeocarpus nubigenus Schltr	Robins 393	BRI	G0 6244
Elaeocarpus prafiensis Weibel	*Womersley NGF37176*	BRI	G0 6919
Elaeocarpus pullenii Weibel	*Streimann NGF44540*	BRI	G0 6916
Elaeocarpus sarcanthus Schltr.	*Womersley NGF19498*	BRI	G0 6245
Elaeocarpus sayeri var. *altigenus*	*Walker ANU739*	BRI	G0 6244
Elaeocarpus sepikanus Schltr.	*Croft LAE65413*	BRI	G0 6237
**Elaeocarpus williamsianus* Guymer	Guymer 1624	BRI	G0 6922

Table 3.4 Taxa sampled for the molecular phylogenetic study.

Indicated for each taxon are gene regions sequenced (+), not sequenced (-), and whether whole plastomes were sequenced (*). Country abbreviation: Australia (AUST), Cambodia (CAMB), Japan (JAP), Myanmar (MYAN), Papua New Guinea (PNG), Indonesia (INDO), Thailand (THAI). Abbreviations of herbaria follow Index Herbariorum: B – Herbarium, Botanischer Garten und Botanisches Museum Berlin, Zentraleinrichtung der Freien Universität Berlin, Germany; BISH – Bishop Museum, Hawaii, USA; BO – Herbarium Bogoriense, Research Centre for Biology, Cibinong, Indonesia; BRI – Queensland Herbarium, Brisbane, Australia; CEB – Herbarium Celebense, Tadulako University, Palu, Sulawesi, Indonesia; CNS – Australian Tropical Herbarium, Cairns, Australia; FU – Kyushu University Herbarium, Japan; GOET – Herbarium Göttingen, Germany; K – Royal Botanic Garden, Kew Herbarium, England; L – Naturalis Leiden Herbarium, Nederlands; MBK – The Kochi Prefectural Makino Botanical Garden, Japan; MIN – University of Minnesota Herbarium, part of the Bell Museum of Natural History, USA; NSW – National Herbarium of New South Wales, Sydney, Australia; NT – Northern Territory Herbarium, Darwin, Australia.

Taxon name	**Country**	**Collector or Accession number**	**Institution Code**	**CNS DNA number**	***trnH-psbA***	***trnL-trnF***	***trnV-ndhC***
Elaeocarpus altisectus Schltr.	PNG	SA James 594	BISH	G04574	+	+	+
Elaeocarpus angustifolius Blume	PNG	JN Gagul 18	CNS	G04561	+	-	+
Elaeocarpus angustifolius Blume	PNG	JN Gagul 20	CNS	G04562	+	-	-
Elaeocarpus angustifolius Blume	INDO	F. Brambach 576	BO, CEB, GOET, L	G04458	+	+	+

Elaeocarpus angustifolius Blume	INDO	F. Brambach 1398	CEB, GOET	G04459	+	+	+
Elaeocarpus angustifolius Blume	PNG	JN Gagul 6	CNS	G04557	+	-	+
**Elaeocarpus angustifolius* Blume	PNG	JN Gagul 45	CNS	G05190	+	+	+
Elaeocarpus arnhemicus F. Muell.	PNG	JN Gagul 1	CNS	G04467	+	+	+
Elaeocarpus blepharoceras Schltr.	PNG	JN Gagul 2	CNS	G04468	+	+	+
Elaeocarpus bokorensis Tagane	CAMB	4300	FU	G04640	+	-	-
**Elaeocarpus braceanus* Watt ex C.B.Clarke	MYAN	087274	MBK	G07118	+	+	+
**Elaeocarpus crenulatus* R.Knuth	PNG	DM Crayn 539 (herbarium material)	NSW	G07115	+	+	+
Elaeocarpus culminicola Warb.	PNG	JN Gagul 5	CNS	G04556	+	+	+
Elaeocarpus culminicola Warb.	PNG	JN.Gagul 11	CNS	G04470	+	+	+

**Elaeocarpus culminicola* Warb.	PNG	JN Gagul 31	CNS	G05175	+	+	+
**Elaeocarpus dolichostylus* var. *hentyi* Coode	PNG	JN Gagul 22	CNS	G05167	+	+	+
**Elaeocarpus dolichostylus* Schltr.	PNG	YS3G0274	MIN	G05051	+	+	+
Elaeocarpus dubius A.DC.	CAMB	4470	FU	G04641	+	-	-
Elaeocarpus firdausii Brambach, Coode, Biagioni & Culmsee	INDO	F. Brambach 1953	BO [BO 1926842], CEB, K [K000720898], L [L2055441]	G04464	+	+	+
Elaeocarpus floribundus Blume	THAI	T2974	FU	G04645	+	-	-
Elaeocarpus fuscoides R.Knuth	PNG	SA James 715	BISH	G04578	+	+	+
Elaeocarpus aff. *harunii* Coode	INDO	F. Brambach 1959	CEB, GOET, K	G04457	+	+	+
Elaeocarpus habbemensis A.C.Sm.	PNG	SA James 684	BISH	G04575	+	+	-
Elaeocarpus aff. *griffithii* (Wight) A.Gray	CAMB	3200	FU	G04638	+	-	-

**Elaeocarpus japonicus* Sieb. & Zucc.	JAP	FOS-011577	MBK	G07126	+	+	+
Elaeocarpus kaniensis Schltr.	PNG	SA James 568	BISH	G04573	+	+	+
**Elaeocarpus kaniensis* Schltr.	PNG	1599	MIN	G05064	+	+	+
**Elaeocarpus kirtonii* F.Muell. ex F.M.Bailey	AUST	M1	SYD	?	+	+	+
**Elaeocarpus kirtonii* F.Muell. ex F.M.Bailey	AUST	M1	SYD	?	+	+	+
Elaeocarpus ledermannii Schltr.	PNG	SA James 1182	BISH	G04584	+	+	+
Elaeocarpus ledermannii Schltr.	PNG	JN Gagul 3	CNS	G04554	+	+	+
Elaeocarpus macropus Warb. ex R.Knuth	INDO	F. Brambach 490	BO, CEB, GOET, K, L	G04461	+	+	+
Elaeocarpus miegei Weibel	AUST	Liddle 3246	NT	G04563	+	+	+
Elaeocarpus miegei Weibel	AUST	Liddle 3237	NT	G04564	+	-	-
Elaeocarpus cf. *multiflorus* (Turcz.) Fern.-Vill.	INDO	F. Brambach 1464	BO, CEB, GOET, K	G04460	+	+	+

Elaeocarpus multisectus Schltr.	PNG	SA James 1401	BISH	G04585	+	+	+
**Elaeocarpus murukkai* Coode	PNG	JN Gagul 27	CNS	G05172	+	+	+
Elaeocarpus musseri Coode	INDO	F. Brambach 1515	GOET	G04462	+	+	+
**Elaeocarpus nubigenus* Schltr.	PNG	JN Gagul 36	CNS	G05181	+	+	+
Elaeocarpus octopetalus Merr.	INDO	F. Brambach 555	BO, CEB, GOET	G04463	+	+	+
Elaeocarpus pachyanthus Schltr.	PNG	SA James 160	BISH	G04570	+	+	+
Elaeocarpus polydactylus Schltr.	PNG	JN Gagul 13	CNS	G04472	+	+	-
Elaeocarpus polydactylus Schltr.	PNG	JN Gagul 17	CNS	G04476	+	+	+
Elaeocarpus polydactylus Schltr.	PNG	SA James 686	BISH	G04576	+	+	+

**Elaeocarpus polydactylus* Schltr.	PNG	JN Gagul 28	CNS	G05173	+	+	+
**Elaeocarpus polydactylus* var. *nubigenus*	PNG	JN Gagul 34	CNS	G05179	+	+	+
Elaeocarpus ptilanthus Schltr.	PNG	JN Gagul 8	CNS	G04559	+	-	+
Elaeocarpus ptilanthus Schltr.	PNG	JN Gagul 9	CNS	G04560	+	-	+
Elaeocarpus ptilanthus Schltr.	PNG	JN Gagul 16	CNS	G04475	+	+	-
**Elaeocarpus ptilanthus* Schltr.	PNG	JN Gagul 21	CNS	G05166	+	+	+
Elaeocarpus pycnanthus A.C.Sm.	PNG	JN Gagul 10	CNS	G04469	-	+	+
**Elaeocarpus pycnanthus* A.C.Sm.	PNG	JN Gagul 23	CNS	G05168	+	+	+
Elaeocarpus pycnanthus A.C.Sm.	PNG	SA James 505	BISH	G04572	+	+	+
Elaeocarpus pycnanthus A.C.Sm.	PNG	SA James 746	BISH	G04581	+	+	+
**Elaeocarpus reticulatus* Sm.	AUST	M1	NSW	?	+	+	+

**Elaeocarpus reticulatus* Sm.	AUST	M2	NSW	?	+	+	+
**Elaeocarpus reticulatus* Sm.	AUST	M3	NSW	?	+	+	+
Elaeocarpus sarcanthus Schltr.	PNG	SA James 1178	BISH	G04583	+	+	+
Elaeocarpus sayeri subsp. *sayeri* F.Muell.	PNG	YUS 7434	CNS	G04565	+	+	+
**Elaeocarpus sayeri* F.Muell.	PNG	YP3A0123	MIN	G05052	+	+	+
**Elaeocarpus schlechterianus* A.C.Sm.	PNG	YP1C0058	MIN	G05056	+	+	+
**Elaeocarpus sphaericus* (Gaertn.) K.Schum.	PNG	YP3A0133	MIN	G05048	+	+	+
Elaeocarpus sphaericus (Gaertn.) K.Schum.	PNG	JN Gagul 7	CNS	G04558	-	+	-
Elaeocarpus sphaericus (Gaertn.) K.Schum.	CAMB	5718	FU	G04642	+	+	-
Elaeocarpus sphaericus (Gaertn.) K.Schum.	THAI	T3076	FU	G04646	+	+	-
Elaeocarpus sp.	PNG	2010-005	BISH	G04568	+	-	+
Elaeocarpus ledermanii	PNG	JN Gagul 4	CNS	G04555	+	+	+

Elaeocarpus sterrophyllus Schltr.	PNG	JN Gagul 14	CNS	G04473	-	+	-
Elaeocarpus tariensis Weibel	PNG	JN Gagul 12	CNS	G04471	+	+	+
Elaeocarpus tectorius (Lour.) Poir.	THAI	T3105	FU	G04647	+	-	-
Elaeocarpus teysmannii Koord. & Valeton subsp. *domatiferus* Coode	INDO	F. Brambach 744	BO, CEB, GOET, K, L	G04465	+	+	+
Elaeocarpus thorelli Pierre	CAMB	5785	FU	G04643	+	-	-
Elaeocarpus trichophyllus A.C.Sm.	PNG	YUS-A-12-13	CNS	G04566	+	-	+
Elaeocarpus whartonensis A.C.Sm.	PNG	SA James 718	BISH	G04580	+	+	-
**Elaeocarpus womersleyi* Weibel	PNG	JN Gagul 26	CNS	G05171	+	+	+
Aceratium ledermannii Schltr.	PNG	SA James 4	BISH	G04569	+	+	+
**Aceratium ledermannii* Schltr.	PNG	2147	MIN	G05066	+	+	+
Aceratium ledermannii Schltr.	PNG	JN Gagul 19	CNS	G04477	+	+	+

Aceratium muelleranum Schltr.	PNG	SA James 218	BISH	G04571	+	+	+
Aceratium oppositifolium DC.	PNG	SA James 1049	BISH	G04582	+	+	+
Sericolea brassii A.C.Sm.	PNG	SA James 687	BISH	G04577	+	+	+
Sericolea brassii A.C.Sm.	PNG	SA James 716	BISH	G04579	+	+	+
Sloanea celebica Boerl. & Koord. ex Koord.	INDO	F. Brambach 1566	CEB, GOET	G04466	+	+	+
**Sloanea nymannii* K.Schum.	PNG	JN Gagul 38	CNS	G05183	+	+	+
**Sloanea pulchra* (Schltr) A.C.Sm.	PNG	JN Gagul 46	CNS	G05191	+	+	+
Sloanea pulleniana Coode	PNG	JN Gagul 15	CNS	G04474	+	+	+
**Sloanea sogerensis* (Moore) Engl. & Krause	PNG	JN Gagul 40	CNS	G05185	+	+	+
Sloanea sogerensis Bak. f.	PNG	SA James 1417	BISH	G04586	+	+	+

3.2.2 Selection of DNA markers

For the Sanger sequencing approach, three noncoding regions from the chloroplast genome (*trnV-ndhC*, *trnH-psbA* and *trnL-trnF*) were selected to provide molecular estimates of the phylogeny. These regions have been used successfully in molecular phylogenetic studies of *Elaeocarpus* and have proven to be generally informative at the species level (Baba, 2014; Phoon, 2015). Furthermore, the noncoding regions of chloroplast DNA (cpDNA) evolve rapidly, thus potentially containing more information to resolve phylogenetic relationships among closely related species (Gielly and Taberlet, 1994). Table 3.5 lists the primers used for each marker. No sequences from GenBank were used for these markers in the current study.

For the Next Generation Sequencing (NGS) approach, sequences of up to 92 plastid genes were obtained from 27 samples (one *Aceratium*, three *Sloanea* and 23 *Elaeocarpus*, indicated by an asterix in Table 3.4). This was done to assess variation across the plastome in *Elaeocarpus*, and to determine the utility of plastome-scale data for resolving the phylogeny of *Elaeocarpus*.

Table 3.5 Primer information for DNA regions used in the current study.

Region	Primer name	Primer sequence (5'–3')	Reference	Employed in Elaeocarpaceae or *Elaeocarpus* studies
trnL-F	c	CGAAATCGGT AGACGCTACG	Taberlet et al. (1991)	Maynard (2004); Crayn et al. (2006); McPherson (2008); Niissalo (2011); Baba (2014); Phoon (2015)
	f	ATTTGAACTG GTGACACGAG	Taberlet et al. (1991)	
trnV-ndhC	trnV$^{(UAC)}$ x2	GTCTACGGTTC GARTCCGTA	Shaw et al. (2007)	Baba (2014); Phoon (2015)
	ndhC	TATTATTAGA AATGYCCARA AAATATCATA TTC		
trnH-psbA	trnH2	CGCGCATGGT GGATTCACAA TCC	Tate and Simpson (2003)	Phoon (2015)
	psbAF	GTTATGCATG AACGTAATGC TC	Sang et al. (1997)	

3.2.3 DNA extraction and isolation

Total genomic DNA was extracted from both fresh or silica-gel-dried and preserved herbarium leaf materials. Approximately 0.5 cm^2 leaf material of each species was ground using a TissueLyser (QIAGEN, Hilden, Germany). Extractions were done using commercial extraction kits (DNeasy^{TM} 96 Plant Kit; QIAGEN, Germantown, USA) following the manufacturer's protocol or using the Cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990), with modifications as described in Weising et al. (2005).

Attempts made to extract DNA from herbarium materials of species that could not be located in the field generally failed, resulting in very low amounts of DNA or no DNA. This result is consistent with the experience of previous *Elaeocarpus* researchers (D. Crayn, pers. comm., 2018) and is likely due to DNA degradation in dried preserved herbarium material. Where a low amount of DNA was obtained, multiple extractions per herbarium sheet were performed and pooled to increase DNA quantity. Using this approach DNA was successfully sequenced from only one herbarium specimen (*E. crenulatus*, D.M. Crayn 539, Tables 3.3, 3.4).

3.2.4 DNA quality control

DNA extracts of all samples were checked for concentration and quality. DNA concentration was measured using a Qubit 3.0 Fluorometer (ThermoFisher Scientific, US), and DNA quality and purity determined with NanoDrop 2000 Spectrophotometer (ThermoFisher Scientific, US) by measuring the 260/280 and 260/230 ratios. Samples that met the submission criteria (min. 100 ng total DNA and 260/280 ratio above 1.5) were then

selected and prepared further for submission to the Australian Genomic Research Facility (AGRF).

Two sequencing approaches were used in the current study: Sanger and Next Generation Sequencing (NGS).

3.2.4.1 Sanger Sequencing

The *trnL-F*, *trnV-ndhC* and *trnH-psbA* regions of the cpDNA were amplified using primers and methods described by Taberlet et al. (1991), Shaw et al. (2007), Tate and Simpson (2003) and Sang et al. (1997) (Table 3.5).

Primers 'c' and 'f' from Taberlet et al. (1991) were used to amplify the *trnL-F* region. PCR thermal cycling comprised initial denaturation of 45 s at 98°C, followed by 35 cycles of 10 s denaturation at 98°C, 30 s annealing at 65°C, 45 s extension at 72°C, concluding with a final extension of 10 min at 72°C.

Primers 'trnV$^{(UAC)}$ x2' and 'ndhC' from Shaw et al. (2007) were used to amplify the *trnV-ndhC* region. PCR thermal cycling comprised initial denaturation of 2 min at 95°C, followed by 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 55°C, 1 min extension at 72°C, and a final extension of 2 min at 72°C.

Primers 'trnH2' from Tate and Simpson (2003) and 'psbAF' from Sang et al. (1997) were used to amplify the *trnH-psbA* region. PCR thermal cycling comprised initial

denaturation of 45 s at 98°C, followed by 35 cycles of 10 s denaturation at 98°C, 30 s annealing at 64°C, 40 s extension at 72°C, and a final extension of 10 min at 72°C.

The quality of PCR products was checked using agarose gel electrophoresis on a 1% agarose gel using EZ Big Dye and Easy Ladder (Applied Biosystems, New York, USA) and run at 80 V and 350 A for 20 min. PCR products were then purified using ExoFastAP (Thermo Scientific, Waltham, Massachusetts, USA) in a 6.5 µL mixture of 5 µL PCR products and 1.5 µL of a mixture of 20 U/µL Fermentas* Exonuclease I (Exo I) and 1 U/µL of Fast AP (Thermo Scientific, Waltham, Massachusetts, USA). The final purified products were incubated at 37 °C for 15 min; 85 °C for 15 min; and hold at 10 °C, then used as DNA templates for direct sequencing.

Sequencing reactions were carried out using the amplification primers and sequencing was performed on an AB3730xl 96-capillary automated sequencer (Life Technologies, Pty Ltd, Australia) at AGRF (Brisbane, Australia).

3.2.4.2 Next Generation Sequencing (NGS)

Libraries were constructed from 100−300 ng total DNA using the TruSeq Nano DNA LT library preparation kit (Illumina, San Diego, USA) for an insert size of 350 base pairs (bp) and paired-end reads following the manufacturer's protocol. Libraries were multiplexed 96 times and DNA sequencing with 125bp paired-end reads was carried out for high throughput sequencing on an Illumina HiSeq 2500 platform at the AGRF (Melbourne, Australia). The resulting sequence reads included 92 plastid genes (Table 3.6).

The plastome raw reads were mapped, annotated and aligned using MAFFT v.7.222 (Katoh et al., 2002, 2013) and edited manually using Geneious R10 software (Kearse et al., 2012). Due to unavailability of published *Elaeocarpus* plastome reference sequences in GenBank, sequences of *E. murukkai* (J. Gagul 27, CNS G05172, Table 3.4) were assembled and annotated, and used as a reference for assembly of sequences of the other samples in the study. Raw plastome sequence data from five samples representing two species (2 x *E. kirtonii* and 3 x *E. reticulatus*) were obtained from Dr Maurizio Rossetto (Royal Botanic Gardens, Sydney) and assembled and annotated together with the plastome data from the current study. Assemblies were carried out with the highest quality threshold and a minimum coverage of ten reads. The quality of the assemblies was checked and edited manually where required.

The annotated, aligned and edited sequence data of regions *trnL-F*, *trnV-ndhC* and *trnH-psbA* were then retrieved from the whole plastome data for each of the 27 samples. Those sequences were then combined with the *trnL-F*, *trnV-ndhC* and *trnH-psbA* obtained by Sanger sequencing. Sequences from each sample were assembled, edited manually using Geneious R10 and aligned using MAFFT.

3.2.4.3 Datasets

Four sequence datasets were compiled for analysis: (1) 27 samples, three plastid spacers (NGS), 2,016 bp, (2) 27 samples, 92 plastid genes (NGS), 70,912 bp, (3) 231 samples, three plastid spacers, 2,923 bp, and (4) 231 samples, combined 92 plastid genes (NGS) for 27 taxa and three plastid spacers (Sanger) for 204 samples, 73,835 bp.

Datasets one and two (three plastid spacers versus 92 plastid genes for the same 27 samples) were analysed and the results compared to assess the utility of few-marker sequence datasets versus plastome-scale sequence datasets for phylogenetic reconstruction in *Elaeocarpus*. Datasets three and four were analysed to estimate the phylogeny of *Elaeocarpus* using the largest taxon sample available. The difference between datasets three and four was the inclusion in dataset four of 92 plastid genes for 27 of the 231 samples.

3.2.4.4 Phylogenetic analysis

Phylogenetic relationships were inferred using maximum likelihood (ML) and Bayesian inference (BI) methods. Best-fit nucleotide substitution models were determined for all four datasets using ModelFinder (Kalyaanamoorthy et al., 2017) and the Bayesian information criteria (BIC) (Schwarz, 1978), and implemented in IQ-TREE v1.6.1 (Nguyen et al., 2014).

Maximum likelihood analyses were performed in IQ-TREE v1.6.1 applying the best-fit model (HKY+F for dataset 1; TVM+F+1 for dataset 2; TN+F+I+G4 for dataset 3, and K3PU+F+1+G4 for dataset 4). Branch support was estimated using 1000 non-parametic bootstrap trees.

Bayesian analyses were executed in MrBayes v2.2.4 (Huelsenbeck and Ronquist, 2001) in Geneious Prime v11 applying the best-fit model from the model finder analyses that were available in MrBayes (HKY for dataset 1; GTR+I for dataset 2; GTR+I+G4 for dataset 3, and HKY+I+G4 for dataset 4). Four independent Markov Chain Monte Carlo (MCMC) runs with heated chain temperature of 0.2 and subsampling frequency of 1,000 were carried

out for 2,000,000 generations sampling trees every 500,000 generations. A maximum clade credibility tree was calculated from the runs with posterior probability values (PP) plotted, with PP values greater than or equal to 0.95 considered strong support. Trees were viewed and exported using Figtree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

3.2.4.5 Clade support and credibility values

Clade support was assessed by bootstrap and posterior probability analyses. Bootstrap support of 50% or less was considered to be no support, 51–75% weak support, 76–90% moderate support, and 91–100% strong support. Posterior probability values greater than or equal to 0.95 were considered strong support, 0.85–0.94 moderate support, 0.75–0.84 weak support and values less than 0.65 no support.

Table 3.6 Plastid genes generated from 27 Elaeocarpaceae samples.

The samples used are indicated by an asterix in Table 3.4.

accD	*clpP-p3*	*ndhI*
atpA	*matK*	*ndhJ*
atpB	*ndhA-p1*	*ndhK*
atpE	*ndhA-p2*	*petA*
atpF-p1	*ndhB-p1*	*petB-p2*
atpF-p2	*ndhB-p2*	*petD-p2*
atpH	*ndhC*	*petG*
atpI	*ndhD*	*petL*
ccsA	*ndhE*	*petN*
cemA	*ndhF*	*psaA*
clpP-p1	*ndhG*	*psaB*
clpP-p2	*ndhH*	*psaC*

psaI
psaJ
psbA
psbB
psbC
psbD
psbE
psbF
psbH
psbI
psbJ
psbK
psbL
psbM
psbN
psbT
psbZ
rbcL
rpl14
rpl16-p2
rpl2-p1
rpl2-p2
rpl20
rpl22
rpl23
rpl33
rpl36
rpoA
rpoB
rpoC1-p1
rpoC1-p2
rpoC2
rps11
rps12-p1
rps12-p2
rps12-p3
rps14
rps15
rps16-p1
rps16-p2
rps18
rps19
rps2
rps3
rps4
rps7
rps8
trnH-psbA
trnL-trnF
trnV-ndhC
ycf1
ycf2
ycf3-p1
ycf3-p2
ycf3-p3
ycf4

3.3 Results

3.3.1 Elaeocarpaceae data

The current molecular dataset comprises sequences from 231 Elaeocarpaceae samples, the largest data currently available for Elaeocarpaceae. Sequences of the three plastid spacers (*trnH-psbA*, *trnL-F* and *trnV-ndhC*) were obtained from 62 samples (Table 3.4) using Sanger sequencing, and an additional 27 samples using NGS. Sequences of these spacers from an additional 142 samples were available from previous studies (Baba, 2014; Phoon, 2015). Whole plastome sequences were obtained from 27 samples (23 *Elaeocarpus*, one *Aceratium* and three *Sloanea*). Of the 23 *Elaeocarpus* samples, five (2 x *E. kirtonii* and 3 x *E. reticulatus*) are from Australia, one (*E. japonicus*) from Japan, one (*E. braceanus*) from Myanmar, and 19 (*E. angustifolius, E. crenulatus, E. dolichostylus, E. dolichostylus var. hentyi*, *E. kaniensis, E. murukkai, E. nubigenus*, *E. polydactylus, E. polydactylus* var. *nubigenus*,, *E. ptilanthus, E. pycnanthus, E. sayeri, E. schlechterianus, E. sphaericus, E. sterrophyllus* and *E. womersleyi*) from New Guinea. These samples are indicated by an asterix in Table 3.4.

3.3.2 Phylogenetic relationships

Phylogenies reconstructed using datasets 1 and 2 (three plastid spacers versus 92 plastid genes for the same 27 samples) were compared to assess the utility of plastome-scale sequence datasets versus few-marker sequence datasets for reconstructing phylogenetic relationships in *Elaeocarpus*. Those reconstructed using datasets 3 and 4 were compared to estimate the phylogeny of *Elaeocarpus* using the

largest taxon sample available. The difference between datasets 3 and 4 was the inclusion in dataset 4 of 92 plastid genes for 27 of the 231 samples.

3.3.2.1 Phylogenies of 27 samples: plastid-three spacers (dataset one) versus 92 genes (dataset two).

Dataset 1 and dataset 2 comprised the same sample set (27 samples). The difference between these datasets is dataset 1 comprised sequences of three plastid spacers (aligned length 2,016 bp), and dataset 2 comprised sequences of 92 plastid genes (aligned length 70,912 bp). Each dataset was analysed with both ML and Bayesian analyses to compare the differences in the resolution of species relationships in the phylogenies.

The phylogenetic reconstruction based on dataset 1 (three plastid spacers) is presented as Fig. 3.1 and that based on dataset 2 (92 plastid genes) is presented as Fig. 3.2. The phylogenies constructed from these two datasets were similar in many aspects of the topology and backbone support, although there were several differences in the resolution of some nodes, and the arrangement of lineages at the deeper nodes within *Elaeocarpus* (Figs 3.1, 3.2). Analysis of the 92-gene dataset (dataset 2) produced a generally better resolved and supported estimate of phylogenetic relationships (Fig. 3.2) compared to the three-spacer dataset (dataset 1; Fig. 3.1).

In both analyses there is maximum support for the monophyly of *Elaeocarpus* and for its sister relationship with *Aceratium* (PP 1, Figs 3.1, 3.2). The clade comprising *Sloanea* also received maximum support in both phylogenies except the grouping of *S. pulchra* and *S. sogerensis* in the 92-gene phylogeny received

maximum support (PP 1, Fig. 3.2) whereas this relationship in the three-spacer phylogeny was unsupported (PP 0.63, Fig. 3.1).

Within the *Elaeocarpus* clade, three main clades (corresponding to Groups V, VII and VIII) were identified in both phylogenies, and all were strongly supported (Figs 3.1, 3.2).

The Group V (*Ganitrus*) clade comprises two main sub clades in the 92-gene phylogeny, both receiving maximum support (PP 1) (Fig. 3.2). One clade comprises *E. angustifolius*, *E. sphaericus*, *E. ptilanthus* and *E. kaniensis*, and the other comprises *E. polydactylus*, *E. polydactylus* var. *nubigenus, E. nubigenus*, *E. murukkai, E. dolichostylus* and *E. dolichostylus* var. *hentyi*. Only the first clade was resolved (PP 1) in the three-spacer phylogeny (Fig. 3.1). *Elaeocarpus braceanus* was placed sister to the Group V clade with maximum support in both analyses.

The Group VII (*Oreocarpus*) clade comprises *E. reticulatus* and *E. kirtonii* from Australia, and *E. sterrophyllus* from New Guinea, and receives maximum support in both analyses (PP 1, Figs 3.1, 3.2). Internal phylogenetic structure was not resolved in the three-spacer analysis except for the two samples of *E. kirtonii*, which were clustered together as a strongly supported group (Fig. 3.1, PP=0.95; Fig. 3.2, PP=1.00), whereas the 92-gene analysis resolved this clade fully with maximum support for all nodes. Notably the sample of *E. sterrophyllus* grouped with one of the samples of *E. reticulatus*, rendering the latter species paraphyletic on these data.

The Group VIII (*Coilopetalum*) clade comprises the New Guinean species *E. sayeri*, *E. crenulatus* and *E. pycnanthus*, plus *E. japonicus* from Japan, and received

strong support in both analyses (PP 0.96, Fig. 3.1; PP 1.00, Fig. 3.2). The relationship between *E. sayeri* and *E. crenulatus* was resolved with maximum support by both analyses (PP 1, Figs 3.1, 3.2). The 92-gene analysis further resolved the relationships of *E. pycnanthus* and *E. japonicus* with maximum support: the latter was placed sister to the rest, with *E. pycnanthus* sister to the *E. sayeri* - *E. crenulatus* clade.

The relationships among the three major clades (Groups V, VII and VIII), *E. womersleyi,* and *E. schlechterianus* differ between the two analyses. In the three spacer analysis, *E. schlechterianus* was placed sister to the clade comprising *E. braceanus* and Group V, and *E. womersleyi* was placed in a polytomy with that clade plus Group VII. In the 92 plastid gene analysis, *E. womersleyi,* and *E. schlechterianus* are placed with maximum support as successive sisters to a clade comprising Groups VII and VIII in sister relationship.

Overall, phylogenies based on the three plastid spacers and 92 plastid genes for the same sample set (27 samples) were well-resolved and supported, and largely congruent with each other (Figs 3.1, 3.2). Where differences in resolution were evident, the phylogeny based on dataset 2 (92 plastid genes) was more fully resolved and more strongly supported. For instance, the clade comprising *E. crenulatus, E. sayeri, E. japonicus* and *E. pycnanthus* received strong support, except the support values differed only slightly, i.e., PP 0.96 in the three-spacer gene phylogeny (dataset 1, Fig. 3.1), and PP 1 in the 92 genes phylogeny (dataset 2, Fig. 3.2). Thus, there was better resolution in the 92 gene phylogeny than in the three spacer phylogeny.

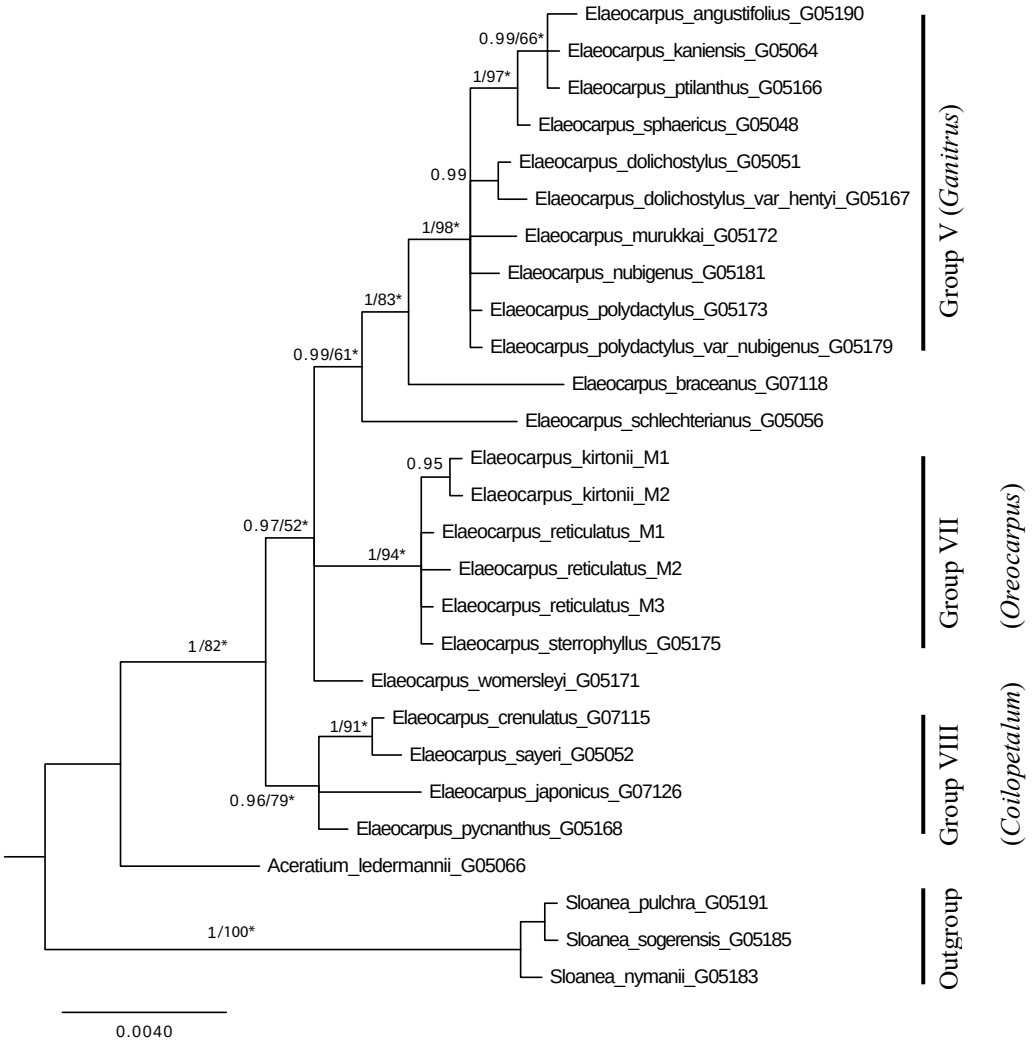


Figure 3.1 Phylogenetic relationships based on analysis of sequences of 3 plastid spacers (Dataset 1).

Support values are displayed adjacent to nodes, with posterior probability (PP) values to the left of bootstrap (BS) values with an asterix. Support values PP<0.65 and BS<50% are not shown.

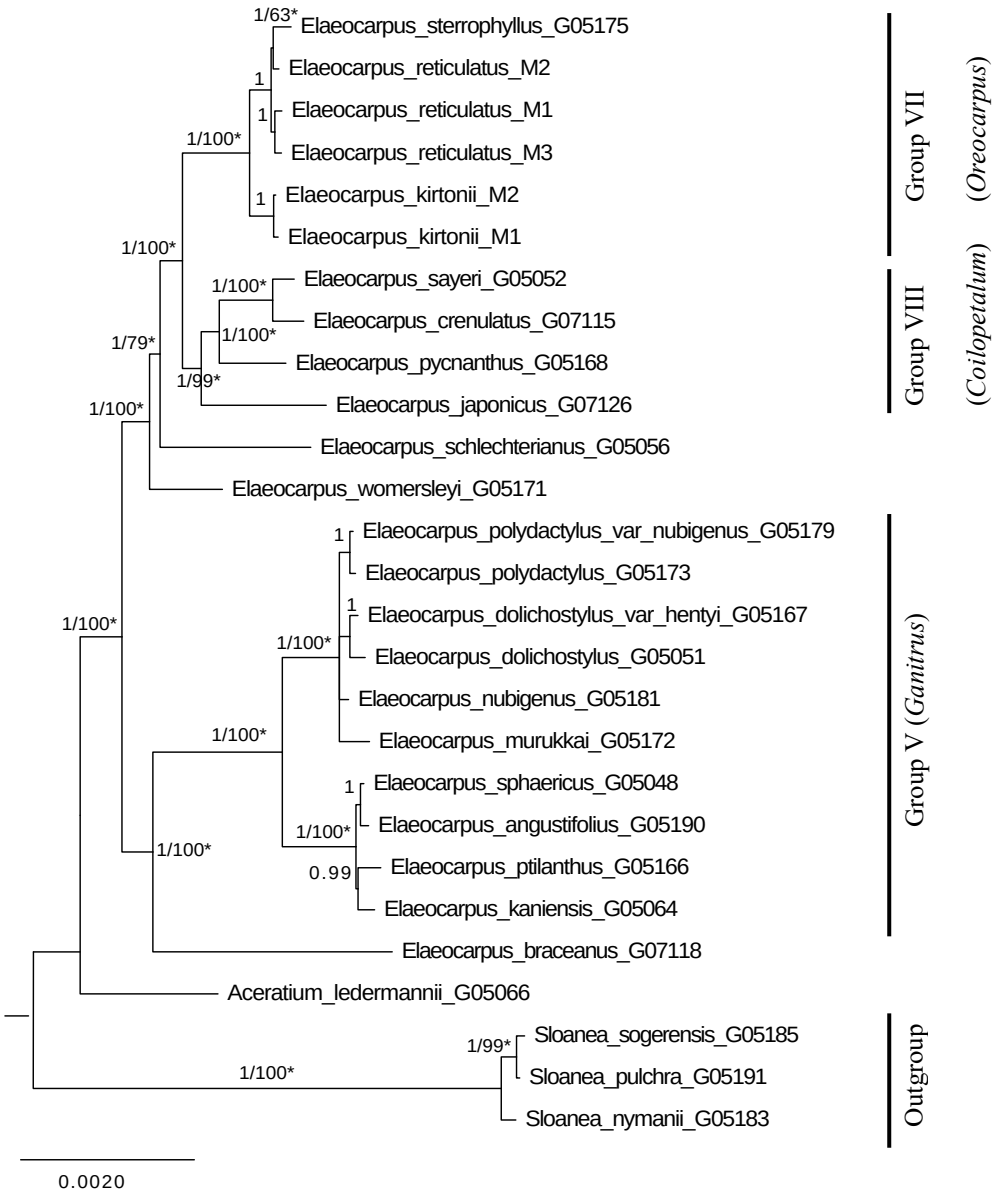


Figure 3.2 Phylogenetic relationships based on analysis of sequences of 92 plastid genes (Dataset 2).

Support values are displayed adjacent to nodes, with posterior probability values to the left of bootstrap values with an asterix. Support values PP<0.65 and BS<50% are not shown.

3.3.2.2 Phylogenies of 231 samples: three plastid spacers (dataset 3), versus three plastid spacers plus 92 genes for 27 of the 231 samples (dataset 4).

Dataset 3 and dataset 4 comprised the same sample set (231 samples). The sample set comprised of both *Elaeocarpus* and other Elaeocarpaceae. Other Elaeocarpaceae samples included *Aceratium* (9 samples, 3 spp.), *Crinodendron* (2 spp.), *Dubouzetia* (3 spp.), *Peripentadenia* (2 spp.), *Sericolea* (4 samples, 3 spp.), *Sloanea* (6 samples, 5 spp.). The current study contributed 89 samples from Elaeocarpaceae to those generated in previous studies: *Aceratium* (5 samples, 3 spp.), *Eleaocarpus* (77 samples, c. 45 spp.), *Sericolea* (2 spp.) and *Sloanea* (6 samples, 5 spp.) (Table 3.4).

The difference between dataset 3 and dataset 4 is that dataset 3 comprises sequences of three spacers only (231 samples, aligned length 2,923 bp), and dataset 4 comprises sequences of an additional 92 plastid genes for 27 of the 231 samples (aligned length 73,835 bp). Each dataset was analysed with both ML and Bayesian analyses to compare the differences in the resolution of and support for relationships.

The phylogenetic reconstruction based on the three-spacers dataset (dataset 3) is presented in Fig. 3.3 and compared with dataset 4 (92 genes and three spacers for 27 samples, three spacers for 204 samples) phylogeny for the same sample set (Fig. 3.4). Different topologies, relationships and resolutions were evident in the phylogenetic reconstructions (Figs 3.3, 3.4), although the species relationships in both were generally congruent with the current classification. Support for most nodes in both phylogenies are weak or unsupported (Figs 3.3, 3.4). Furthermore, for those

clades that are well-supported, the relationships between these clades are mostly unsupported or weakly supported, thus they remain largely unresolved.

The levels of resolution at the main nodes and among species were also different in the phylogenies (Figs 3.3, 3.4). For instance, the lineage comprising species in the *Obovatus* group received weak support (PP 0.7) in the analysis of dataset 3 (Fig. 3.3), but moderate support (PP 0.82) in the analysis of dataset 4. In both phylogenies, the Australian sample of *Elaeocarpus arnhemicus* (CNS1852) is not resolved with the *Obovatus* clade (Figs 3.3, 3.4), whereas this relationship has been recovered in previous studies (Baba, 2014; Phoon, 2015). Furthermore, in both analyses the New Guinean sample of *E. arnhemicus* (G04467) is grouped with *E. coorangooloo* (CNS1939) of Australia with strong support (PP 0.98, Fig. 3.3; PP 0.99, Fig. 3.4), suggesting *E. coorangooloo* is more closely related to *E. arnhemicus* of New Guinea rather than that of Australia. The phylogenies show unsupported or weak with some moderately supported clades at the deeper nodes, whilst the shallow nodes however, received mostly moderate with some highly supported clades (Fig. 3.3).

The monophyly of *Elaeocarpus* was supported in both phylogenies, except *E. holopetalus* was placed outside the main *Elaeocarpus* clade (PP 0.95, Fig. 3.3; PP 0.97, Fig. 3.4).

Within the *Elaeocarpus* clade, four main clades (corresponding to Groups IV, V, VII and the *Obovatus* clade) were identified in both phylogenies, but with different levels

of resolution (Figs 3.3, 3.4). Apart from those clades, other groups have been marked on the phylogenies to illustrate the affinities of the New Guinean species (Figs 3.3, 3.4).

Overall, the results showed substantial differences in the resolution and support for relationships, and where differences occurred, generally the results of dataset 4 were most informative. For instance, the lineage comprising Group VIII species from New Guinea (*E. ledermannii, E. sayeri, E. crenulatus, E. fuscoides, E. trichophyllus* and *E. whartoensis*) received maximum support (PP 1) in the analysis of dataset 4 (Fig. 3.4), whereas for dataset 3 that lineage received moderate support (PP 0.75). Analysis of dataset 4 produced generally better resolved phylogeny than that of dataset three.

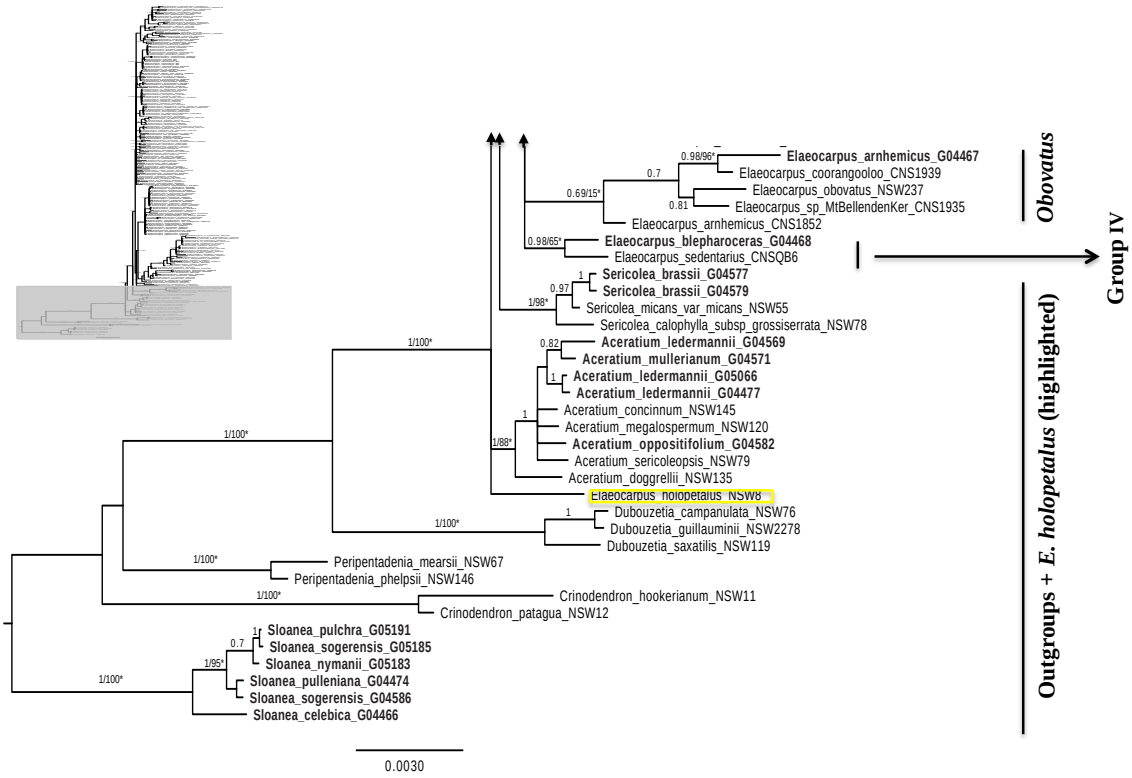


Figure 3.3 Phylogenetic relationships based on analysis of dataset 3 comprising sequences of three plastid spacers for 231 samples.

Support values are displayed adjacent to nodes, with posterior probability values to the left of bootstrap values with an asterix. Support values PP<0.65 and BS<50% are not shown. Samples added from the current study are indicated in bold text.

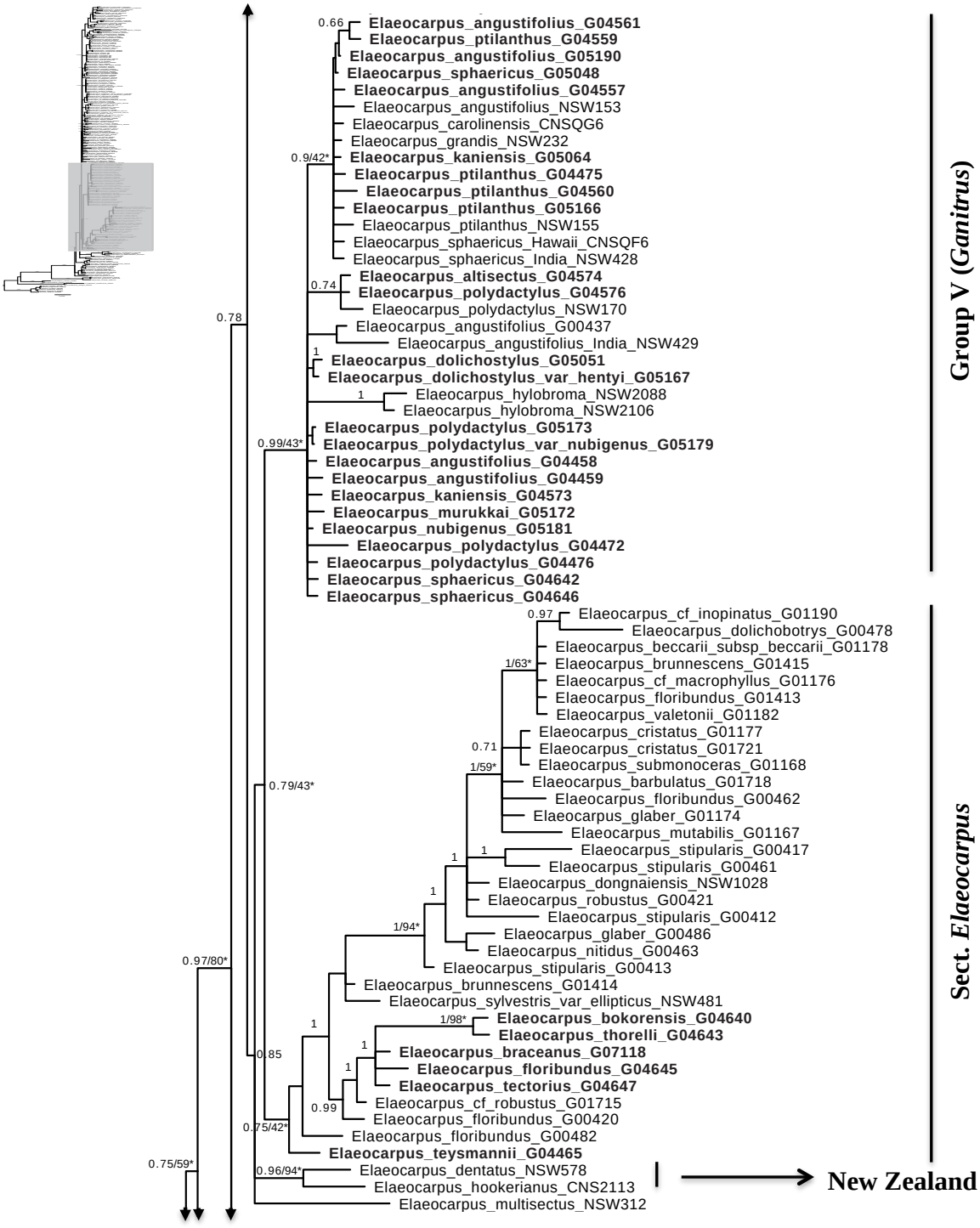


Figure 3.3. (continued).

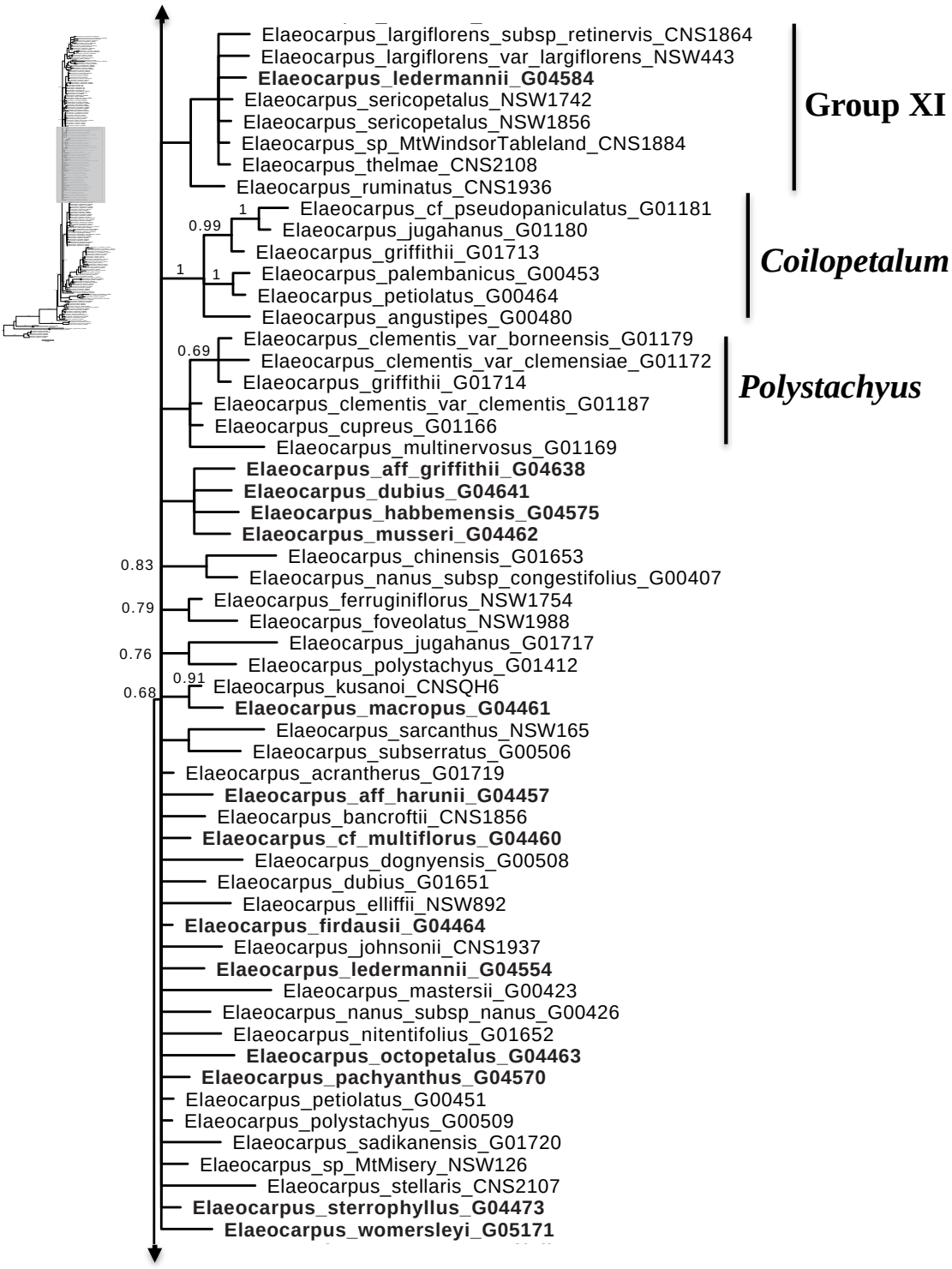


Figure 3.3. (continued).

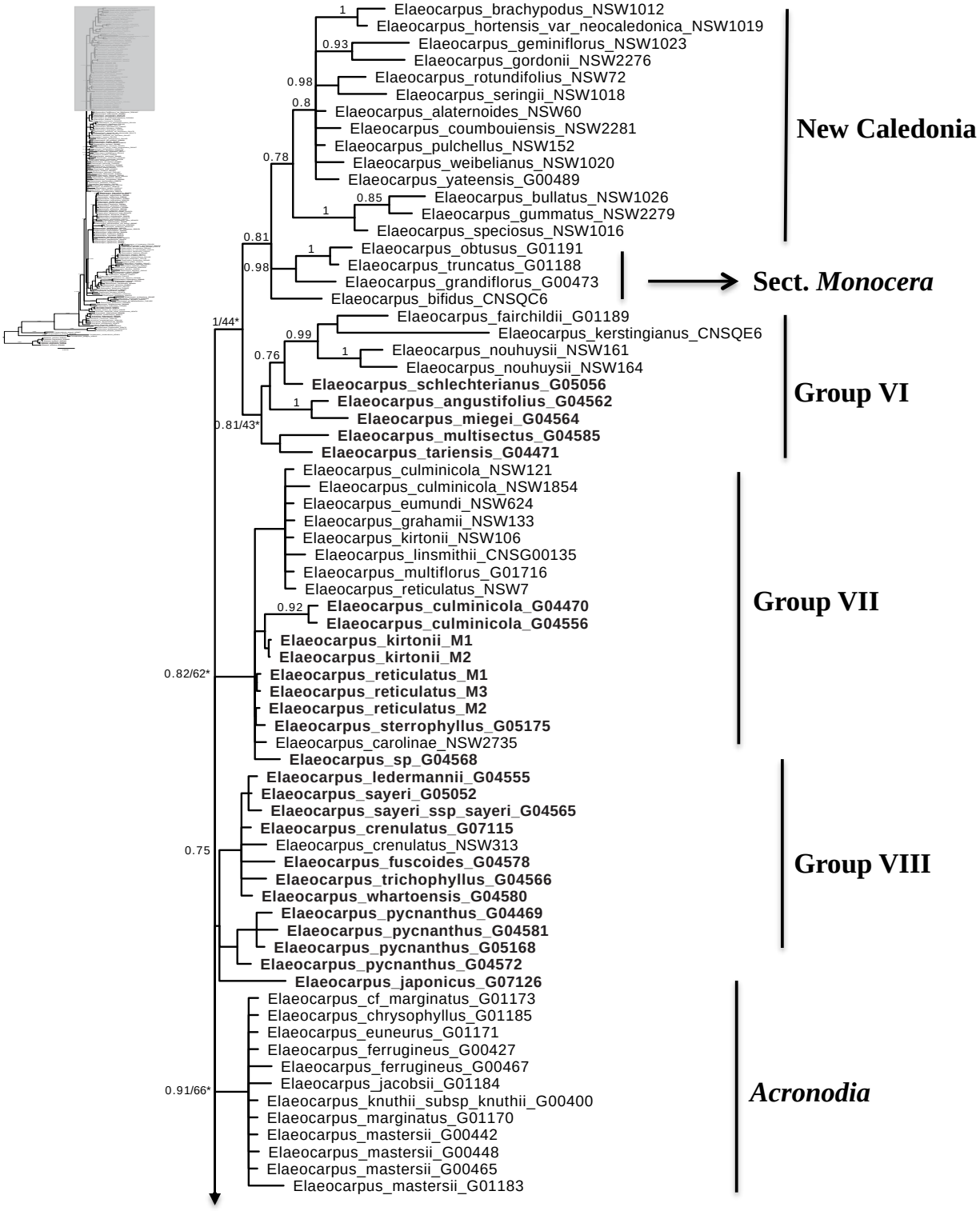


Figure 3.3. (continued).

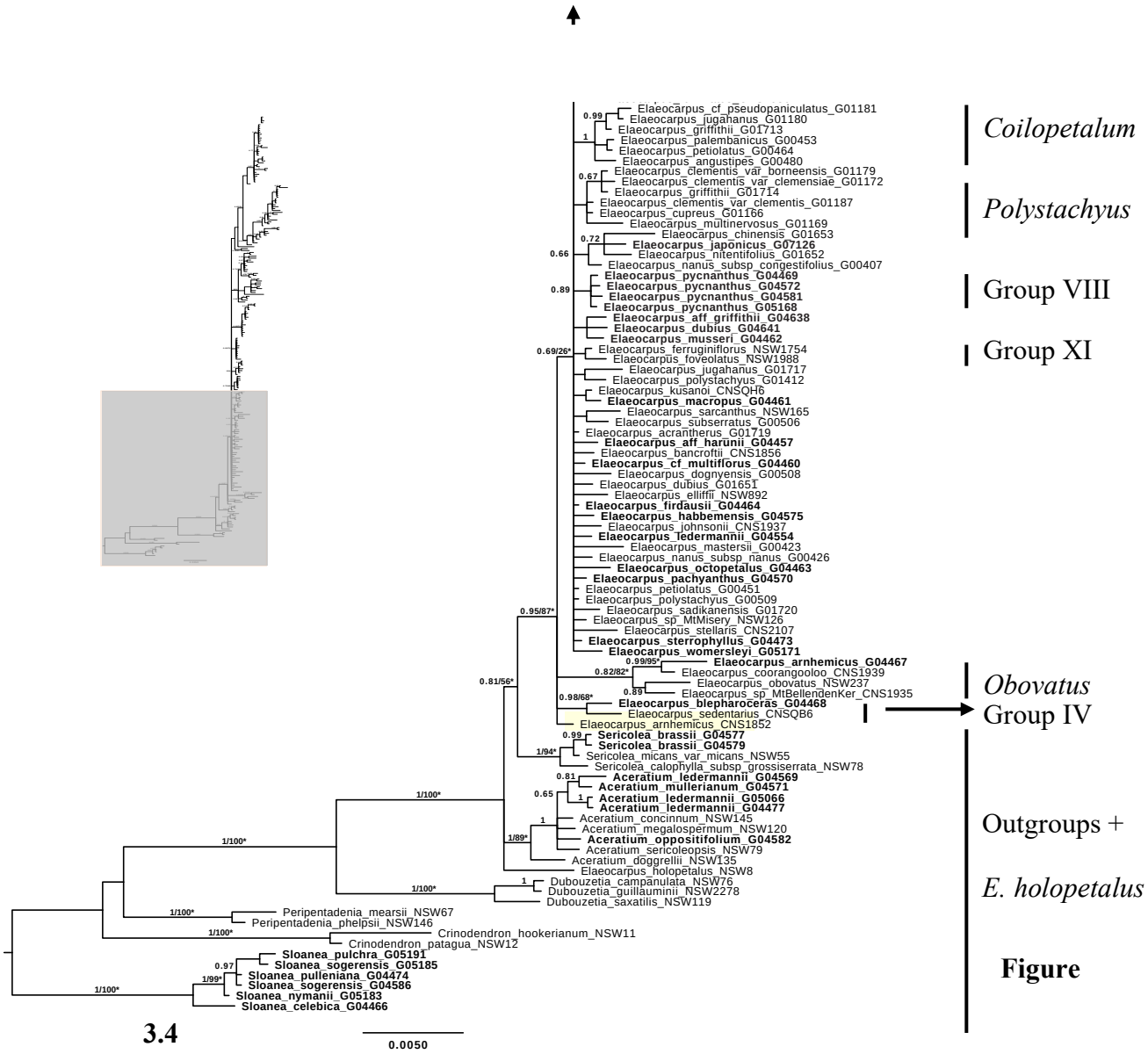


Figure 3.4

Phylogenetic relationships based on analysis of dataset 4, a combined alignment of three plastid spacers for 231 taxa and an additional 92 plastid genes for 27 of those taxa.

Support values are displayed adjacent to nodes, with posterior probability values to the left of bootstrap values with an asterix. Support values PP<0.65 and BS<50% are not shown. Samples added from the current study are in bold text.

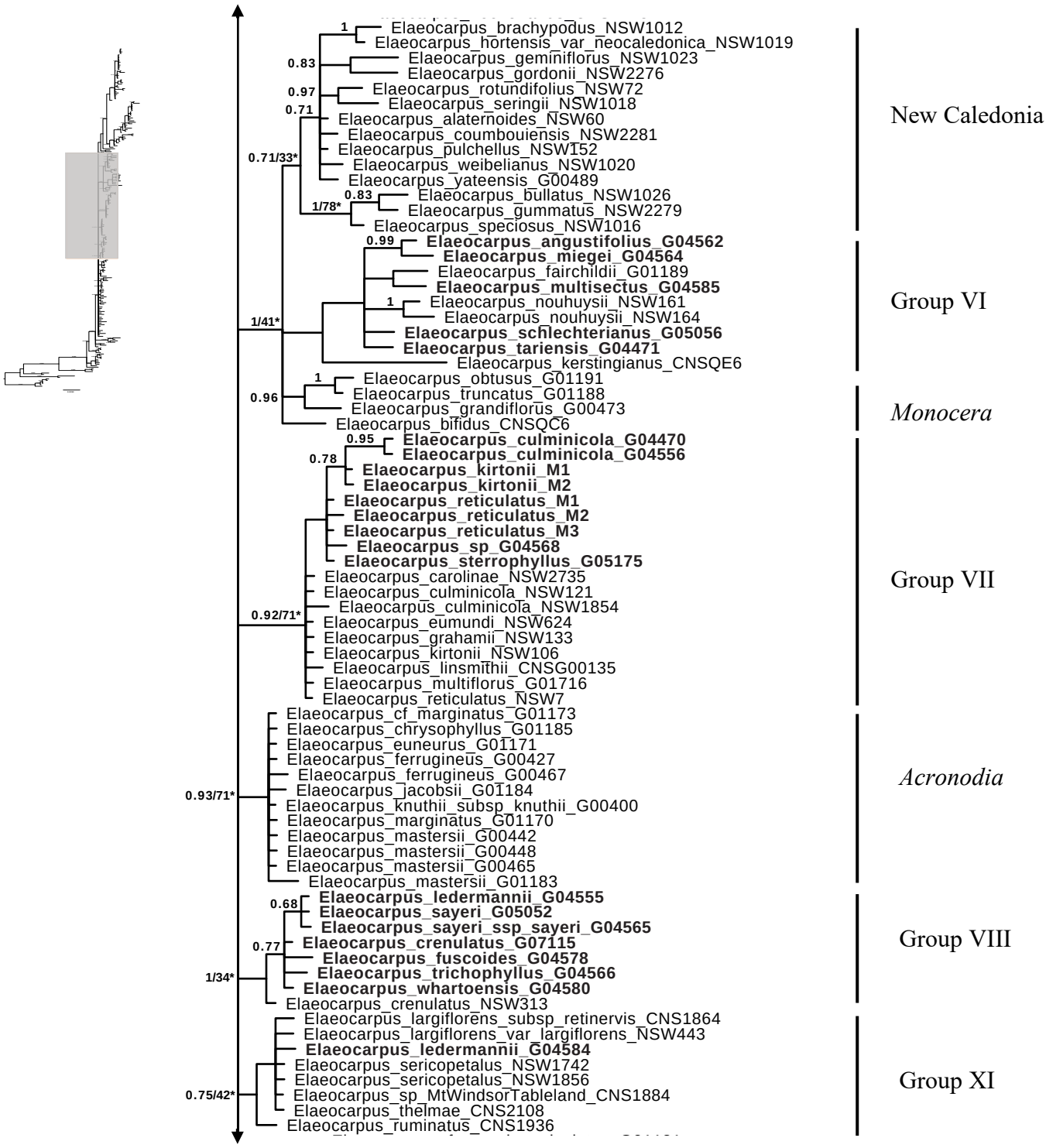


Figure 3.4. (continued).


Elaeocarpus_angustifolius_G04561
Elaeocarpus_angustifolius_G05190
Elaeocarpus_angustifolius_NSW153
Elaeocarpus_ptilanthus_G04559
Elaeocarpus_angustifolius_G04557
Elaeocarpus_carolinensis_CNSQG6
Elaeocarpus_grandis_NSW232
Elaeocarpus_kaniensis_G05064
Elaeocarpus_ptilanthus_G04475
Elaeocarpus_ptilanthus_G04560
Elaeocarpus_ptilanthus_G05166
Elaeocarpus_ptilanthus_NSW155
Elaeocarpus_sphaericus_G05048
Elaeocarpus_sphaericus_Hawaii_CNSQF6
Elaeocarpus_sphaericus_India_NSW428
Elaeocarpus_angustifolius_G00437
Elaeocarpus_angustifolius_India_NSW429
Elaeocarpus_angustifolius_G04458
Elaeocarpus_angustifolius_G04459
Elaeocarpus_sphaericus_G04642
Elaeocarpus_sphaericus_G04646
Elaeocarpus_altisectus_G04574
Elaeocarpus_polydactylus_G04576
Elaeocarpus_polydactylus_NSW170
Elaeocarpus_polydactylus_G04472
Elaeocarpus_dolichostylus_G05051
Elaeocarpus_dolichostylus_var_hentyi_G05167
Elaeocarpus_hylobroma_NSW2088
Elaeocarpus_hylobroma_NSW2106
Elaeocarpus_kaniensis_G04573
Elaeocarpus_murukkai_G05172
Elaeocarpus_nubigenus_G05181
Elaeocarpus_polydactylus_G04476
Elaeocarpus_polydactylus_G05173
Elaeocarpus_polydactylus_var_nubigenus_G05179
Elaeocarpus_teysmannii_G04465
Elaeocarpus_cf_inopinatus_G01190
Elaeocarpus_dolichobotrys_G00478
Elaeocarpus_beccarii_subsp_beccarii_G01178
Elaeocarpus_brunnescens_G01415
Elaeocarpus_cf_macrophyllus_G01176
Elaeocarpus_floribundus_G01413
Elaeocarpus_valetonii_G01182
Elaeocarpus_cristatus_G01177
Elaeocarpus_cristatus_G01721
Elaeocarpus_submonoceras_G01168
Elaeocarpus_barbulatus_G01718
Elaeocarpus_floribundus_G00462
Elaeocarpus_glaber_G01174
Elaeocarpus_mutabilis_G01167
Elaeocarpus_stipularis_G00417
Elaeocarpus_stipularis_G00461
Elaeocarpus_dongnaiensis_NSW1028
Elaeocarpus_robustus_G00421
Elaeocarpus_stipularis_G00412
Elaeocarpus_glaber_G00486
Elaeocarpus_nitidus_G00463
Elaeocarpus_stipularis_G00413
Elaeocarpus_bokorensis_G04640
Elaeocarpus_thorelli_G04643
Elaeocarpus_braceanus_G07118
Elaeocarpus_floribundus_G04645
Elaeocarpus_tectorius_G04647
Elaeocarpus_cf_robustus_G01715
Elaeocarpus_floribundus_G00420
Elaeocarpus_brunnescens_G01414
Elaeocarpus_sylvestris_var_ellipticus_NSW481
Elaeocarpus_floribundus_G00482
Elaeocarpus_multisectus_NSW312
Elaeocarpus_dentatus_NSW578
Elaeocarpus_hookerianus_CNS2113
0.86
0.88
0.77
1/47*
1
0.85/22*
0.95
1
1
0.98
1
0.98
1/93*
1/98*
0.99
0.99
1/27*
1
0.92/45*
0.98/95*
Group V
Section
Elaeocarpus
New Zealand

Figure 3.4. (continued).

3.4 Discussion

My study provides insights into the utility of plastome-scale sequence datasets versus few-marker datasets for phylogenetic reconstruction in *Elaeocarpus*, and it estimates the phylogeny of *Elaeocarpus* using the largest taxon sample available. The phylogeny provides a basis to assess the current morphology-based classification in order to understand the phylogenetic relationships of New Guinean species with those of other regions. This study has also expanded the molecular data of species from other regions such as Indonesia (Sulawesi), Japan, Myanmar, Thailand, Cambodia and Australia for a wider geographical coverage. My analysis shows the samples used in the current study are nested within the clades identified previously, and are congruent with the current morphological classification.

3.4.1 Assessing the utility of plastome-scale sequence datasets over few-marker datasets for resolving the phylogeny of *Elaeocarpus*

Within Elaeocarpaceae, the intergenic spacers *trnL-F*, *trnV-ndhC* and *trnV-ndhC* have been widely used (Crayn et al., 2006; Baba, 2014; Phoon, 2015). Of these spacers *trnL-F* and *trnV-ndhC* were found to be less variable at the species level than *trnH-psbA*. In previous studies, some samples repeatedly failed to amplify for *trnL-F* and *trnV-ndhC* when using the Sanger approach (Y. Baba and S.N. Phoon pers. comm, 2018). In the current study, many samples also failed to amplify for *trnL-F* and *trnV-ndhC* when using the Sanger approach. Therefore, the current study has utilised an approach never before used on Elaeocarpaceae, i.e., the genome skimming approach using NGS technology. My analysis showed that plastome-scale data,

including the intergenic spacers, could be retrieved with a high success rate using the genome skimming approach. Therefore, I recommend that future molecular phylogenetic studies in Elaeocarpaceae pursue a genome skimming approach.

The current study is the first to generate plastome-scale sequence datasets for Elaeocarpaceae to compare their utility with that of previous approaches which used a few selected plastid markers for phylogenetic reconstruction in the group. The results showed that the plastome-scale data substantially improved resolution and support for relationships, compared to that of the three-spacer dataset (Figs 3.1, 3.2). Based on these results, and the observation that standard PCR failed to amplify many samples for the selected intergenic spacers, it is recommended that future phylogenetic studies of Elaeocarpaceae should avoid a few gene, Sanger approach. Clearly, plastomics has value, but recent studies have demonstrated value in nuclear marker-based phylogenomics using target capture approaches and universal bait sets such as Angiosperms353 (Johnson et al., 2019) for reconstructing phylogenies in species rich tropical groups (e.g. *Nepenthes*; Murphy et al., 2020; Nauheimer et al., 2021) either alone or in combination with existing Sanger data (e.g. Urticaceae; Wells et al., 2021). While no studies have yet explored the utility of target capture approaches in *Elaeocarpus*, preliminary data representing a single exemplar of each genus of Elaeocarpaceae (D. Crayn, pers. comm., 2021) suggests the Angiosperms353 bait set will generate datasets of unprecendented power for resolving relationships in the family and in species rich genera such as *Elaeocarpus, Sloanea* and *Tetratheca.*

3.4.2 Testing the morphology-based classification of *Elaeocarpus* using molecular data: New Guinean samples and their phylogenetic relationships

The following discussion is based on the results of the analysis of the large 231 sample datasets (3 and 4), particularly dataset 4 (three plastid spacers for 231 taxa and an additional 92 plastid genes for 27 of those taxa), which is currently the largest dataset available for *Elaeocarpus*. In addition to the amount of sequence data, this study has substantially expanded the geographical sampling over previous studies, particularly for New Guinea, a region with the greatest *Elaeocarpus* diversity (c. 97 spp.), distributed in nine groups. Six of these nine groups, namely Groups III, IV, V, VI, VII and VIII, plus the *Obovatus* group (Baba, 2014) were sampled in the current phylogenetic assessment. Most species were nested within clades corresponding to the morphological groups in which they were placed by Coode (1978, 1981). Groups III, VI and VIII did not form clades in the present analysis; the relationships of the New Guinean species of these groups are further discussed. Apart from *Elaeocarpus*, there are samples of species from other genera in the current molecular data: *Aceratium*, *Dubouzetia*, *Sericolea*, *Sloanea*, *Crinodendron* and *Peripentadenia*. Of these genera, only *Aceratium*, *Dubouzetia*, *Sericolea* and *Sloanea* are represented in New Guinea, with *Sericolea* being endemic there (c. 11 spp.) (Coode, 1981). The genera *Crinodendron* and *Peripentadenia* are absent from New Guinea.

3.4.2.1 Group three (III) - (sect. *Elaeocarpus* L.)

Species of section *Elaeocarpus* are found throughout Malesia. In New Guinea there are currently eight taxa recognised, which are distributed in two subgroups (subgroup A and subgroup B). Subgroup A comprises three species (*E. homalioides* Schltr., *E. multisectus* Schltr., and *E. royenii* Weibel). Subgroup B comprises five

described species (*E. leucanthus* A. C. Sm., *E. millarii* Weibel, *E. oriomensis* Weibel, *E. prafiensis* Weibel, *E. pullenii* Weibel), and two undescribed species (*E*. sp. ?nov.2 and *E*. sp. ?nov.3 *sensu* Coode, 1981). The two undescribed species are now regarded to be one species based on studies of three herbarium specimens (*Henty* NGF16877*, Foreman & Galore* NGF45798 and *Streimann & Kairo* LAE51537), but additional fertile material particularly with flowers, is required for a formal description (M. Coode, pers. comm., 2016). Within this group, there is uncertainty as to whether Subgroup B is sufficiently distinct to recognise formally. The group as a whole can be distinguished by its 3(-5)-locular ovaries, two ovules per locule and typically much-divided petals (M. Coode, pers. comm., 2018).

In terms of molecular representation from this group, two samples of *E. multisectus* (NSW312, G04585) were included in the present analysis. Attempts were made to extract DNA from herbarium material of *E. leucanthus, E. millarii*, *E. prafiensis* and *E. pullenii* but the available specimens failed to yield sequenceable DNA.

The molecular analysis placed the two samples of *E. multisectus* in different positions on both phylogenies (Figs 3.3, 3.4). Sample NSW312 is placed without support as sister to sect. *Elaeocarpus* (Figs 3.3, 3.4), whereas sample G04585 is placed within the maximally supported sect. *Monocera* (Group VI) clade. However, the species from sect. *Monocera* clade include *E. tariensis, E. miegei, E. nouhuysii, E. schlechterianus* and *E. fairchildii* from New Guinea, and *E. kerstingianus* from the Caroline Islands, which are not related to *E. multisectus* morphologically. Therefore, the position of the sample of *E. multisectus* (G04585) in sect. *Monocera* of Group VI

rather than in sect. *Elaeocarpus* of Group III calls into question the identity of the sample. Although *E. multisectus* and its relatives are not found in western Malesia, there are floral similarities with species found in that areas, e.g., flowers that open widely at anthesis (Phoon, 2015). Therefore, the former position is not to be disputed and the latter is possibly a misidentified specimen. Future phylogenetic studies should focus on obtaining molecular data from the Solomon Islands and western New Guinea to more comprehensively test the monophyly of this group.

Elaeocarpus multisectus Schltr. (syn. *E. salomonensis* Knuth, *E. solomonensis* A.C.Sm.) is a tree species scattered throughout Papuasia, growing in primary forests. In New Guinea it occurs from near sea level to c. 670 m elevation. It has been recorded from Vogelkop and Mimika (including Adi Island in Fakfak) to the west, and Madang, Morobe, Southern and Western Highlands, Gulf, West New Britain and Western Provinces to the east including Bougainville. In the Solomon Islands it has been recorded at 1200 m altitude in Guadalcanal (Coode, 1978,1981).

3.4.2.2 Group four (IV) - (sect. *Blepharoceras* Schltr.)

Within Group IV, a single species (*Elaeocarpus blepharoceras* Schltr. syn. *E. tafaensis* A.C.Sm.) is currently known from mainland New Guinea (Coode, 1978). Morphologically, *E. blepharoceras* is very similar to the Australian *E. sedentarius* Maynard & Crayn in having a pale green to glaucous abaxial leaf surface, fruits that are triangular in transverse section and dense radial fibres in the outer mesocarp (Coode 1978, 1981, 1984; Maynard et al., 2008; Phoon, 2015). This character combination is restricted to these two species only within *Elaeocarpus*. However, *E.*

sedentarius is currently unassigned to a group, despite its morphological similarities with *E. blepharoceras*, which is placed in Group IV.

Elaeocarpus blepharoceras has been sequenced for the first time in the current study (J.Gagul 002, CNS; G04468) (Table 3.4). The molecular analysis shows *E. blepharoceras* and *E. sedentarius* form a robust clade with strong support (PP 0.98) suggesting they are closely related (Figs 3.3, 3.4). This has confirmed a relationship previously inferred from morphological studies (Coode, 1978, 1984; Maynard et al., 2008). Therefore, *E. sedentarius* should be placed in Group IV based on the current phylogenetic analysis.

Furthermore, both these species have radial fibres in the outer mesocarp that are permanently attached to the inner mesocarp (stones). This character is not restricted to these species, but also occurs in *E. johnsonii* F.Muell. ex C.T.White from Australia and *E. womersleyi* Weibel from New Guinea (Coode, 1978, 1984; Maynard et al., 2008).

With respect to morphological taxonomy, both *E. johnsonii* and *E. blepharoceras* are placed in Group IV. Molecular evidence does not support this however, and places *E. johnsonii* (CNS1937) on an unresolved lineage within a moderately supported clade on both phylogenies (PP 0.69, Figs 3.3, 3.4). Previous molecular analyses have not resolved the relationships of *E. johnsonii*. Baba (2014) found *E. johnsonii* and *E. ruminatus* formed a small clade, whereas Phoon (2015) found it to be on a separate lineage sister to the Group VII clade. The relationships of

this species require further investigation, with additional samples and nuclear markers.

3.4.2.3 Group five (V) - (sect. *Ganitrus* Brongn. & Gris)

The Group V of *Elaeocarpus* is a widespread and complex group, taxonomically. In Papuasia (a botanical region consisting of the mainland New Guinea, the Bismarck Archipelago and the Solomon Islands (as defined by Warburg 1890, and used by Womersley 1978)) it is more complex and speciose (Coode, 2010). This group currently comprises *E. altisectus* Schltr., *E. angustifolius* Blume, *E. avium* Coode, *E. bakaianus* Coode, *E. buderi* Coode, *E. dasycarpus* A.C.Sm., *E. densiflorus* Knuth, *E. dolichostylus* Schltr., *E. kaniensis* Schltr., *E. murukkai* Coode, *E. ornatus* Coode, *E. orohensis* Schltr, *E. osiae* Coode, *E. polydactylus* Schltr and *E. ptilanthus* Schltr. (Coode, 1978, 1981, 2005, 2010).

Representatives of the *Ganitrus* from other regions have been recorded, i.e., *E. trichopetalus* Merr. & Quisumb. (Sulawesi and Philippines), *E. ramiflorus* Merr. (Philippines), and *E. cyanocarpus* Maingay ex Mast. (Indonesia, Malaysia, Philippines) (Coode, 2010). These species share the following morphological character states: spherical mesocarps (fruit stones), bastionate mesocarp ornamentation, straight embryos and entire endosperm.

The current study provides new sequence data for *E. angustifolius* (G04561, G05190, G04557), *E. dolichostylus* (G05051), *E. dolichostylus* var. *hentyi* (G05167), *E. kaniensis* (G05051), *E. murukkai* (G05172), *E. nubigenus* (G05181), *E. polydactylus* (G04476, G05173, G05179), *E. polydactylus* var. *nubigenus* (G05179),

E. ptilanthus (G04559, G04560, G04475, G05166) and *E. sphaericus* (G05048, G04642) from New Guinea; *E. sphaericus* (G04642) from Cambodia; *E. angustifolius* (G04458, G04459) from Sulawesi; and *E. sphaericus* (G04646) from Thailand (Table 4). Molecular analyses show these taxa are nested within the clades identified in previous phylogenetic studies (Baba, 2014; Phoon 2015) and are congruent with Coode's (1978, 1981, 1984) morphological taxonomy, but with different levels of resolution (Figs 3.3, 3.4). *Elaeocarpus sphaericus* and *E. angustifolius* from Cambodia and Indonesia (Sulawesi) are placed together on a separate lineage within the main *Ganitrus* clade.

The monophyly of the *Ganitrus* clade is strongly supported (PP 1, Figs 3.1, 3.2, 3.4; PP 0.99, Fig. 3.3). Also placed within the clade is *E. hylobroma* (NSW2088, NSW2106), a recently described species (Baba and Crayn, 2012) from Australia. *Elaeocarpus hylobroma* has morphological similarities to *E. tariensis* of Group VI from New Guinea and *E. carolinae* of Group VII from Australia. Therefore, until its phylogenetic relationships are more fully resolved, *E. hylobroma* is tentatively placed in Group V but unassigned with respect to subgroup.

The fossil species *Elaeocarpus spackmaniorum* Rozefelds from Guildford in Victoria (*NMVP53926, NMVP53982*) shows close morphological affinities with the *Ganitrus* group, in having five-partite, spherical mesocarps with bastionate ornamentaion (Rozefelds and Christophel, 2002; Dettman and Clifford, 2000).

Only a few species of section *Ganitrus* extend beyond New Guinea, e.g. *E. grandis*, *E. dolichostylus* and *E. angustifolius*. *Elaeocarpus angustifolius* (syn. *E.*

sphaericus sensu K.Schum., *E. drymophilus* Domin, *E. cyanocarpus* Maingay ex Mast.), commonly known as the blue or silver quandong, or blue 'fig', is a widespread tropical species. It is morphologically complex and highly variable across its range, which extends from India through Malesia (including New Guinea), continental Asia and to Australia, Fiji and other Pacific Islands. In Australia it is distributed in the Northern Territory. Plants in Queensland and New South Wales have been assigned to this species (e.g. Coode, 1984) but are now regarded as *E. grandis* (CHAH 2021).

The *E. dolichostylus* complex includes *E. dolichostylus* Schltr. subsp. *dolichostylus* (syn. *E. ulapensis* Knuth), *E. dolichostylus* var. *chloranthus* A.C.Sm. (syn. *E. chloranthus* A.C.Sm.), and *E. dolichostylus* var. *hentyi* (syn. *E. dolichostylus* subsp. *collinus* Coode) (Coode, 2010). In eastern New Guinea (PNG), members of this group have been collected from East Sepik and Sandaun (formerly West Sepik), Madang (Karkar Is.), Morobe, Southern Highlands and Western Highlands, Oro (formerly Northern), and New Ireland Provinces, with possible occurrences in New Britain. In western New Guinea, they have been recorded from all districts except Fakfak. Apart from New Guinea, *E. dolichostylus* also occurs in Indonesia (Sulawesi and Maluku (Coode, 1978, 1981). Members of the *E. dolichostylus* complex are mostly trees that grow to c. 25 m tall occurring in both primary and secondary forests including swampy areas, from sea level to c. 750 m elevation (Coode, 1978, 1981).

Further analyses including additional samples of *E. dolichostylus* species from Indonesia (Sulawesi and Maluku) and New Guinea are required to resolve

relationships within this group, and to better understand its geographical variability and delimitation.

3.4.2.4 Group six (VI) - (sect. *Monocera* Brongn. & Gris)

Group VI is large, possibly polyphyletic, and currently comprises 20 taxa distributed in five subgroups in New Guinea (Coode, 1978, 1981).

Within Group VI, Subgroup A is monotypic, comprising *E. polyandrus* A.C.Sm., found mostly in the Solomon Islands with some specimens resembling this taxon collected from Bougainville, Papua New Guinea (Coode, 1978, 1981). During the current study this species could not be located and collected, and DNA extraction from herbarium material failed to yield useable DNA (Table 5). Fresh or silica dried leaf samples of this species may be required for DNA analysis in the future.

Subgroup B is monotypic also, comprising *E. womersleyi* Weibel, distributed in New Guinea, Papuan Islands and the Moluccas. Similarities in mesocarp morphology suggest *E. womersleyi* is related to large fruited species from Australia with thick robust inner mesocarps namely *E. bancroftii* F.Muell., *E. stellaris* L.S.Sm. from Group VI, Subgroup B, and *E. carbinensis* J.N.Gagul & Crayn (currently unassigned to a group) (Gagul et al., 2018; Rozefelds and Christophel 2002; Coode, 1978, 1981, 1984). Its mesocarp morphology suggests it is best placed in Group VI, Subgroup B. The three Australian species are distinguished from the New Guinea *E. womersleyi* by the outer mesocarp fibres, which detach cleanly from the inner mesocarp when the outer mesocarp and exocarp rot away (attached, at least for some time, in *E. womersleyi*, Coode, 1978, 1981, 1984). This subgroup seems to extend as

far west as Sulawesi. A specimen from Sulawesi (*Kjellberg 1611*) bears a strong resemblance to *E. stellaris*, particularly in the fruit, although the fruit of *E. stellaris* is consistently larger (> 50 mm versus c. 40 mm diam.) and lacks the 'cogwheel' at the base (M. Coode, pers. comm., 2018). The sculpturing on *Kjellberg 1611* looks much more profound than in *E. stellaris*, and whether it has more fertile locules (or just 4?), is yet to be confirmed (D. Crayn, pers. comm., 2018). Additionally, the leaves of *E. stellaris* have secondary veins that make a sharper angle with the midrib, and have longer petioles (mostly > 30 mm) than *Kjellberg 1611* (10–20 mm) (D. Crayn, pers. comm., 2018). Based upon these characters it is reasonable to hypothesise a relationship to *E. stellaris*, *E. carbinensis* J.N.Gagul & Crayn, *E. bancroftii* and *E. womersleyii* (see Chapter 2, Fig. 2.2 and Table 2.1 for details of the morphology of these four species) but additional morphological and molecular data of this taxon are required to test this. A fossil mesocarp (*Elaeocarpus peteri* Rozefelds & Christophel) from late Oligocene-early Miocene deposits at Glencoe in central Queensland resembles *E. carbinensis* and *E. stellaris* in having pronounced ridges and punctate ornamentation (Rozefelds and Christophel, 1996), but its precise relationship to extant lineages is unknown.

Subgroup C in New Guinea currently comprises seven species: *E. amplifolius* Schltr., *E. badius* Coode, *E. finisterrae* Schltr., *E. nouhuysii* Koord., *E. piestocarpus* Schltr., *E. schlechterianus* A.C.Sm., and *E. undulatus* Warb.

Subgroup D comprises six taxa: *E. coloides* Schltr., *E. coodei* Weibel, *E. fairchildii* Merr., *E. rubescens* Weibel, *E. tariensis* Weibel, and *Elaeocarpus* sp.?nov. 4. *Elaeocarpus fairchildii* is morphologically very similar to *E. coloides*, but differs in

the leaf and petiole measurements (Coode, 1978, 1981). It also has an unpleasant flower smell (which also occurs in *E. coloides*). Additional material is needed to determine if there are significant morphological differences between the species. Meanwhile, the undescribed *Elaeocarpus* sp.?nov. 4 in Coode 1978 (p. 222: *Hartley* 12526), is probably *E. filiformidentatus* R.Knuth, and does not belong in this subgroup (M. Coode, pers. comm., 2018).

Subgroup E comprises five taxa: *E. debruynii* O.C.Schm., *E. hartleyi* Weibel, *E. miegei* Weibel, *E. neobriticanus* Coode, and *Elaeocarpus* sp.?nov. 5. The status of *Elaeocarpus* sp.?nov. 5 is currently unclear due to the availability of numerous morphologically similar specimens (*NGF25016, NGF25668, NGF28089, LAE52295, Takeuchi 6967*). It also bears a close similarity to (but is distinct from) *E. miegei*, a species found in New Guinea and Australia. Good specimens with flowers of the undescribed taxon are needed for formal description (M. Coode, pers. comm., 2015). The phylogenetic relationships of the majority of species in this group from New Guinea are unknown. Representatives from subgroups B, C, D and E (except subgroup A) were included in the present study: *E. womersleyi* (G05171), *E. fairchildii* (G01189; Phoon, 2015), *E. tariensis* (G04471), *E. nouhuysii* (NSW161), *E. schlechterianus* (G05056) from New Guinea, *E. kerstingianus* (CNSQE6) from the Caroline Islands, and *E. miegei* (G04564) from Australia.

The species above comprise a lineage in both phylogenies, with moderate support in the 92-gene phylogeny (PP 0.81, Fig. 3.4) and weak support in the 3-spacer phylogeny (PP 0.52, Fig. 3.3). However, among those species are two non-group VI species, i.e. *E. multisectus* (G04585) of Group III and *E. angustifolius* (G04562) of

Group V, which are possibly misidentified (or contaminated) samples (Figs 3, 4). *Elaeocarpus kerstingianus* (CNSQE6) of the Caroline Islands is also nested within this clade, and is morphologically similar to Group VI species. The relationships of *E. womersleyi* are unresolved.

3.4.2.5 Group seven (VII) - (sect. *Oreocarpus* Schltr.)

Group VII currently comprises two species from New Guinea, *E. culminicola* Warb. and *E. sterrophyllus* Schltr. (Coode, 2019c). Within this group only *E. culminicola* extends beyond New Guinea, to NE Australia, Sulawesi and the Moluccas, Luzon (Philippines), and the Bismarck Archipelago (Phoon, 2015; Coode, 2019c). Evolutionary divergence analysis suggests that *E. culminicola* originated in Australia about 1.49 Mya (Phoon, 2015). *Elaeocarpus culminicola* is an understory tree species occurring in forests, from sea level to 2750 m, mostly between 1000 – 2000 m elevation (Coode, 1978, 1981, 1984). In Australia the group contains six species: *E. grahamii* F. Muell., *E. carolinae* Hyland & Coode, *E. kirtonii* F.M.Bailey, *E. reticulatus* Sm., *E. eumundi* F.M.Bailey and *E. linsmithii* Guymer (Coode, 1984).

Elaeocarpus culminicola was initially recognised as the only species from New Guinea in Group VII (Coode, 1978, 1981). *Elaeocarpus sterrophyllus,* a morphologically similar species was inadequately described due to lack of sufficient materials – a single collection in the Cycloop Mountains, Jayapura in western New Guinea – and unplaced in a group. (Coode, 1981, 1987). *Elaeocarpus sterrophyllus* is now known to occur in eastern New Guinea based on *Womersley NGF24507*, *Womersley NGF43926*, *Katik LAE62008, Streimann & Kairo NGF47513*, and *Streimann & Kairo NGF39044* (Coode, 2019c). In light of the additional materials

and new data, *E. sterrophyllus* is now formally recognised as distinct and is closely related to *E. culminicola* (Coode, 2019c). It is morphologically similar to *E. culminicola*, differing in its height (to 5 m versus to 30 m in *E. culminicola*), petal length (mostly 2.7–3 cm versus up to 0.9–1.9 cm in *E. culminicola*), and style length (c. 2.5 cm versus up to 1.5 cm in *E. culminicola*) (Coode, 2019c). Specimens collected from PNG in the current study confirm the occurrence of *E. sterrophyllus* in eastern New Guinea (J. Gagul 14, CNS; G04473) (Table 3.4). However, the specimens of *E. sterrophyllus* recorded from PNG seem to be restricted to Wau Subdistrict, Morobe Province. Further morphological and molecular samplings from Western New Guinea will provide further insights into this entity.

The current study has contributed ten samples of Group VII taxa: 2 x *E. sterrophyllus* (G04473, G05171), 2 x *E. culminicola* (G04470, G04556), 3 x *E. reticulatus* (M1, M2, M3), 2 x *E. kirtonii* (M1, M2), and *E.* sp. (G04568). The phylogenetic analysis shows that these species form a clade that is strongly supported in the 92 genes phylogeny (PP 0.92, Fig. 3.4), but with moderate support in the 3-spacer phylogeny (PP 0.82, Fig. 3.3). However, one sample of *E. sterrophyllus* (G04473) should be further investigated, because it does not group with the other sample of this species.

Overall, the current phylogenetic study supports the placement of *E. sterrophyllus* within Group VI (Coode, 2019c). In future molecular analysis, more samplings of *E. culminicola* and *E. sterrophyllus* from New Guinea and their varieties are recommended for better resolution and delimitation.

3.4.2.6 Group eight (VIII) - (sect. *Coilopetalum* Schltr.)

This is a large and widespread group (c. 18 spp.), with its members distributed in four subgroups (Coode, 1978, 1981). Subgroup A is endemic to New Guinea and comprises two species: *E. fuscoides* Knuth and *E. trichophyllus* A.C.Sm. Subgroup B comprises two species: *E. poculiferus* A.C.Sm. and *E. pycnanthus* A.C.Sm. Subgroup C comprises six species: *E. altigenus* Schltr., *E. filiformidentatus* Knuth, *E. habbemensis* A.C.Sm., *E. luteolus* A.C.Sm., *E. sayeri* F.Muell. and *E. whartonensis* A.C.Sm., all endemic to New Guinea (Coode, 1978, 1981). Subgroup D is diverse and geographically widespread (Coode, 1978, 1981) comprising nine taxa: *E. branderhorstii* Pulle, *E. elatus* A.C.Sm., *E. floridanus* Hemsley, *E. ledermannii* Schltr., *E. lingualis* Knuth, *E. pachyanthus* Schltr, *E. sarcanthus* Schltr., *E. sepikanus* Schltr. and *Elaeocarpus* sp.?nov. 6. *Elaeocarpus* sp.?nov. 6 is represented by a single collection from New Ireland, PNG and additional fertile material is needed for formal description and naming (Coode, 1978, 1981). *Elaeocarpus branderhorstii* includes the following synonyms: *E. microdontus* Schltr., *E. subinteger* Schltr., *E. lancipetalus* Merr. *Elaeocarpus ledermannii* includes the following synonyms: *E. confertifolius* Knuth, *E. brevirostris* A.C.Sm., *E. idenburgensis* A.C.Sm. and *E. fluviatilis* A.C.Sm.

A total of 17 samples comprising 10 species from Group VIII were used in the current study: Subgroup A (*E. crenulatus* (G07115, NSW313), *E. fuscoides* (G04578), *E. trichophyllus* (G04566). Subgroup B (*E. pycnanthus* (G04469, G04572, G04581, G05168); Subgroup C (*E. habbemensis* (G04575), *E. sayeri* (G05052), *E. sayeri* ssb. *sayeri* (G04565), *E. whartonensis* (G04580); and Subgroup D (*E. ledermannii*, G04555, G04584, G04554), *E. pachyanthus* (G04570), *E. sarcanthus*

(NSW165). Attempts were made to extract useable DNA from herbarium materials of *E. lingualis* and *E. sepikanus* but these attempts were not successful (Table 3.3).

The 92-genes analysis strongly supports the monophyly of these species (PP 1, Fig. 3.4). The 3-spacer phylogeny also supported the placement, except it only received moderate support (PP 0.75, Fig. 3.3). On the 92-gene phylogeny the four samples of *E. pycnanthus* were all placed in a lineage outside the clade, while that of 3-spacer was unresolved. Overall, the current molecular study is congruent with Coode's (1978, 1981) morphological study. Additional molecular sampling may improve the resolution and placement of *E. pycnanthus*.

Species from the current study which may have possibly been misidentified as Group VIII species due to sterile specimens include: *E. habbemensis* (G04575), *E. sarcanthus* (NSW165) and *E. ledermannii* (G04584, G04554). *Elaeocarpus ledermannii* (G04584) is however, placed in the clade that comprises Group XI species from Australia with ruminate endosperm, with moderate support (PP 0.75, Fig. 3.4), while *E. ledermannii* (G04554), *E. habbemensis* (G04575) and *E. sarcanthus* (NSW165) are unresolved, although *E. sarcanthus* forms a small lineage with *E. subserratus* (G00506) with a moderate support (PP 0.76, Fig. 3.4).

3.4.2.7 The *Obovatus* group

The *Obovatus* group has recently been proposed as a new group for New Guinean *Elaeocarpus* (Baba, 2014). Support for this is seen in the inclusion of *E. arnhemicus*, a species distributed in New Guinea, northeastern Australia, and Java in Indonesia (Phoon, 2015). This confirms Coode's (1978, 1981, 1984) suspicion that *E.*

arnhemicus is close to *E. obovatus*, which was the basis of his removal of *E. arnhemicus* from Group V (Coode, 2010).

Elaeocarpus arnhemicus is restricted to dry scrubland or woodland areas, from sea level to 200 m elevation (Coode, 1978, 1981, 1984, 2010). In New Guinea, *E. arnhemicus* is mostly distributed in the southern region, especially in Central, Western and Milne Bay Provinces, although specimens have been reported from forests in New Britain, and from Lake Wanum in the Markham Valley of Morobe Province (Coode, 1978, 1981). *Elaeocarpus arnhemicus* was initially placed in Group V, Subgroup D (Coode, 1978, 1981, 1984). However, new data from additional material of *E. arnhemicus* (and *E. sericoloides*) have been removed from the initial position (sect. *Fissipetalum*, Group V) and placed with *E. obovatus* G.Don of Australia, based on floral similarities (Coode, 2010). Another species from Australia that resembles *E. arnhemicus* and *E. obovatus* is *E. coorangooloo* J.F.Bailey & C.T.White (Coode 1984). *Elaeocarpus coorangooloo* was initially placed in Group VI, Subgroup E with *E. miegei* Weibel and *E. hartleyi* Weibel from New Guinea based on its glabrous ovary, awnless anthers and petals with narrow divisions (Coode, 1984).

Previous molecular phylogenetic studies have suggested that the phylogenetic relationships of *E. arnhemicus* are with the Australian species *E. obovatus* (*E. obovatus* subsp. *umbratilis*; Baba et al., 2020) and *E. coorangooloo* (Baba, 2014; Phoon, 2015; Baba et al., 2020). However, these studies included only an Australian sample of *E. arnhemicus* (CNS1852) (Baba, 2014; Phoon, 2015). The current study adds *E. arnhemicus* from New Guinea (J. Gagul 1, G04467; Table 3.4) to the

molecular dataset, and phylogenetic analysis shows the placement of *E. arnhemicus* to be congruent with the previous molecular studies (Figs 3.3, 3.4). This supports the view that *E. coorangooloo* should be placed in the *Obovatus* group, rather than Coode's (1984) placement in Group VI, but this needs to be tested with additional molecular sampling of other New Guinean species from Group VI (E).

The two *E. arnhemicus* samples (G04467, CNS1852) from New Guinea and Australia do not group together: *E. arnhemicus* from New Guinea is sister to *E. coorangooloo* whereas *E.arnhemicus* from Australia is sister to the rest of the species from the *Obovatus* group. Whether this species is non-monophyletic should be investigated using additional molecular samplings from both regions. The holotype of *Elaeocarpus reedyi* F.Muell. (MEL68068, *https://plants.jstor.org/stable/viewer/10.5555/al.ap.specimen.mel68068*), a synonym of *E. arnhemicus*, was collected from Yule Island, New Guinea, the same locality as the specimen of *E. arnhemicus* from the current study. Should future studies confirm the New Guinean specimens of *E. arnhemicus* as a lineage distinct from the Australian material, the name *Elaeocarpus reedyi* F. Muell. is potentially available.

3.4.2.8 Non-New Guinean samples used in the current study, and their phylogenetic relationships

Sequences of 25 previously unsampled species from other regions were included: five samples from Cambodia (*E. thorelli*, G04643; *E. sphaericus*, G04642; *E*. aff. *griffithii*, G04638; *E. dubius*, G04641; *E. bokorensis*, G04640); nine from Sulawesi (*E. angustifolius*, G04458 and G04459; *E. firdausii*, G04464; *E.* aff. *harunii*, G04457; *E. macropus*, G04461; *E.* cf. *multiflorus*, G04460; *E. musseri*, G04462; *E.*

octopetalus, G04463; *E. teysmannii*, G04465); one from Japan (*E. japonicus*, G07126); three from Thailand (*E. floribundus*, G04645; *E. tectorius*, G04647; *E. sphaericus*, G04646), one from Myanmar (*E. braceanus*, G07118) and six samples from Australia (*E. miegei*, G04564; *E. reticulatus* (M1, M2, M3), *E. kirtonii* (M1, M2) (Table 3.4).

Australian samples

Elaeocarpus miegei Weibel has been included in the molecular analyses for the first time. This species mostly occurs in rainforests up to elevations of 1450 m (rarely to 2600 m), and is part of Group VI, Subgroup E (VIE) (Coode, 1978, 1981, 1984). This subgroup also comprises four other taxa (*E. debruynii* O.C.Schm., *E. hartleyi* Weibel, *E. neobriticanus* Coode, and *Elaeocarpus* sp. nov.5) from New Guinea (Coode, 1978, 1981). In Australia *E. miegei* occurs only on the Tiwi Islands, Northern Territory. It is also known from Maluku (Kepulaun Kai and Kepulaun Aru), Indonesia, and from the Solomon Islands. Unfortunately, no samples from Indonesia or the Solomon Islands were available for this study..

Molecular analysis shows *E. miegei* (G04564) is nested within Group VI species, with moderate support on the 92-gene phylogeny (PP 0.81, Fig. 3.4), and weak support in the 3-spacer phylogeny (PP 0.52, Fig. 3.3). However, on both phylogenies *E. miegei* forms a small strongly supported lineage (PP 1, Fig. 3.4; PP 0.99, Fig. 3.3) with one sample of *E. angustifolius* (G04562), within the clade that comprises the majority of the Group VI species. This unexpected placement suggests this sample of *E. angustifolius* should be re-evaluated as a possible mis-identification. Furthermore, two other species (*E. hartleyi* and *E. debruynii*) within Group VI from New Guinea, which together with *E. miegei* should form the core Subgroup (VIE;

Coode, 1984), are currently unavailable. Meanwhile *E. kirtonii* and *E. reticulatus* from Australia are nested within the Group VII clade with moderate support in the 3-spacer phylogeny (PP 0.82) (Fig. 3.3), and strong support in the 92 gene phylogeny (PP 0.92) (Fig. 3.4), which confirms their placement with the rest of the Group VII species (including *E.* sp., G04568 from New Guinea).

Southeast Asian samples

The Cambodian sample of *E. sphaericus* (G04642) is placed with *E. sphaericus* (G04646) from Thailand and *E. angustifolius* (G04458 and G04459) from Sulawesi in the main *Ganitrus* (Group V) clade, with moderate support (PP 0.77) (Fig. 3.4). *Elaeocarpus thorelli* (G04643) is placed with *E. bokorensis* (G04640) in a small strongly supported lineage (PP 1, Figs 3.3, 3.4), well resolved with the clade that comprises *E. floribundus* (G04645) of sect. *Elaeocarpus*, and *E. tectorius* (G04647) from Thailand, *E. braceanus* (G07118) from Myanmar and *E. teysmannii* (G04465) from Indonesia (Sulawesi), and *E.* cf. *robustus* (Fig. 3.4) with maximum support (PP 1). Other Cambodian species (*E.* aff. *griffithii*, G04638 and *E. dubius*, G04641) are placed with *E. musseri* (G04462), without support (PP 0.53, Fig. 3.4) in an unresolved clade (PP 0.69). *Elaeocarpus macropus* (G04461) is placed with *E. kusanoi* (CNSQH6) in a strongly supported lineage (PP 0.91, Fig. 3.3), while the relationships of *E. firdausii* (G04464), *E.* cf. *multiflorus* (G04460), *E.* aff. *harunii* (G04457) and *E. octopetalu*s (G04463) from Sulawesi are unresolved.

Elaeocarpus japonicus (G07126) from Japan, also a new sequence added from the current study is placed with *E. nitentifolius* and *E. chinensis* in a moderately

supported clade (PP 0.72, Fig. 3.4), sister to a lineage comprising *E. nanus* subsp. *congestifolius* of the *Acronodia* group with a support of PP 0.66 (Fig. 3.4).

3.4.3 Improving the understanding of phylogenetic relationships of *Elaeocarpus* from New Guinea

In order to improve our understanding of phylogenetic relationships of *Elaeocarpus* from New Guinea, we require samples of species from there that are currently unavailable in the molecular dataset. This includes samples of species from Group I, Group II and Group IX. Future studies should focus on obtaining samples of species from these groups to build a comprehensive and robust phylogenetic reconstruction for New Guinean *Elaeocarpus*, and to determine their phylogenetic relationships.

3.4.3.1 Group one (I) - sect. *Lobopetalum* Schltr.

Group I is monotypic and currently comprises a single species (*E. bilobatus* Schltr.), restricted to New Guinea mainland (Coode, 1978, 1981). Morphologically *E. bilobatus* is likely to be placed next to or nested within sect. *Elaeocarpus*, based on the shared character two ovules per loculus. The inadequately known *E. bilongvinas* Coode (which is missing from Coode, 1978) may possibly also belong here. It differs from *E. bilobatus* principally in having a three locular ovary (Coode, 1981).

3.4.3.2 Group two (II) - sect. *Dactylosphaera* Schltr.

Group II currently comprises species *E. dolichodactylus* Schltr., *E. heptadactyloides* Weibel, *E. marafunganus* Coode and *E. myrmecophilus* A.C.Sm. *Elaeocarpus dolichodactylus* is widespread in New Guinea and possibly extends west

to Maluku. *Elaeocarpus heptadactyloides* and *E. myrmecophilus* occur in Western New Guinea, and *E. marafunganus* in PNG. Morphologically, the group seems coherent and might be nested within sect. *Elaeocarpus*, sharing three locular ovaries and two ovulate locules, but differing in the possession of thickened apices of the petal divisions. However, with the lack of molecular data, the monophyly of this group is difficult to determine.

3.4.3.3 Group nine (IX) - no current published section name

This group does not correspond to any named section, and comprises the species *E. schoddei* Weibel (Coode, 1978, 1981), *E. amabilis* Kaneh. & Hatus and *E. myrtoides* A.C.Sm. (Coode, 2005). Future studies are recommended to document both morphological and molecular data for a better understanding of these species and their relationships. Due to the unavailability of fruiting specimens of *E. schoddei*, it remains unclear whether the seed is straight or curved (M. Coode, pers. comm., 2018). Fruit morphology, particularly seed characters are important in species (and sectional) level classification, thus infrageneric taxonomic placement can only be achieved when additional material and data become available.

3.5 Conclusions

Reconstructing the phylogenetic relationships of species in *Elaeocarpus* is key to understanding the history and evolution of the genus, particularly in species-rich tropical regions such as New Guinea. New Guinea harbours a substantial fraction of the species diversity of *Elaeocarpus*, however few species of *Elaeocarpus* from there have been included in previous molecular analyses. In this study I substantially

expanded the molecular dataset of *Elaeocarpus* with respect to taxa from New Guinea and other under-sampled regions. I found that generally, the newly sampled species fell within clades that have previously been identified, and those clades are broadly congruent with the current morphological classification.

The current study has significantly expanded chloroplast data of *Elaeocarpus* with representatives of seven out of the nine currently accepted infrageneric groups in New Guinea. Samples of species from groups III, IV, V, VI, VII, VIII and the *Obovatus* group have been included in the molecular analyses, while groups I, II and IX have no current representation.

The current phylogenetic framework is built on the results of previous studies to address questions on evolutionary history and species complexes. A sample of c. 50% of the known species diversity was used. The phylogenetic analyses were done on a much-expanded dataset using Maximum Likelihood and Bayesian Inference. Furthermore, high throughput sequencing approach has been utilized to sequence whole plastomes of 27 Elaeocarpaceae samples, all novel data contributed from this study.

Results of these analyses show the phylogeny is generally congruent with previous studies, although with differences in the level of resolution. The current study places *E. holopetalus* on a distinct lineage outside the main *Elaeocarpus* clade, suggesting the genus *Elaeocarpus* is paraphyletic. This position is consistent with previous studies. *Aceratium* and *Sericolea* are resolved as the closest relatives to the *Elaeocarpus*.

Previous studies identified 12 major clades within *Elaeocarpus*, nine of which broadly agreed with the morphological classification. A majority of the newly sequenced species from New Guinea and the other regions are nested within the clades identified previously, and relationships of most are congruent with the current morphological groupings. The topologies are only partly resolved, but with inclusion of nuclear DNA markers and extensive sampling representing the known diversity, a much better resolution in the relationships at the species level may potentially be achieved.

Continuation of molecular sampling from New Guinea and follow up studies should focus on sampling species of groups that are currently unavailable in the molecular data. Most samples in the current data were collected especially where access was easy, with few from remote difficult accessible localities. Alternatively, exploring different techniques to extract DNA from dried preserved herbarium materials (e.g. the cetyl trimethylammonium bromide (CTAB) protocol, or adopting a target capture sequencing approach which is more amenable to the use of degraded material than Sanger sequencing – see Hart et al., 2016), the difficult to sample species from New Guinea can be extracted to expand the New Guinea data in the molecular dataset. Furthermore, formal morphological descriptions of the currently recognised putative new species from New Guinea will improve species delimitation within the groups.

The main aim of the current study was to utilize phylogenetic analysis of a multilocus molecular dataset with substantially improved sampling of New Guinea

species. The study has addressed the New Guinea sampling gap together with increasing taxa representation from other under-sampled areas for a comprehensive phylogeny. It has achieved its aim by:

- increasing sampling from New Guinea, but also from Indonesia (Sulawesi), Cambodia, Thailand, Japan and Myanmar, which provides improved understanding of the relationships of *Elaeocarpus*, especially in the evolution of *Elaeocarpus* in particular context to the New Guinean species and their relationships,
- resolving the relationships using a phylogenetic framework established from previous studies,
- testing the morphology-based classification against molecular data especially for the New Guinean taxa,
- enhancing understanding of the evolution of *Elaeocarpus* in the Malesian region including a strong representation of New Guinean species,
- showing significant improvement in multi-locus analysis compared to few selected genes.

3.6 Recommendation for future studies

The following recommendations for future Elaeocarpaceae phylogenetic studies are made:

- Utilise a genome skimming or target capture sequencing approach,
- utilise nuclear markers to improve species level resolution in the relationships,
- obtain molecular samples of species from Group I, Group II and Group IX from New Guinea, which are currently unavailable, but also species from other groups that are not represented in the molecular dataset,

- obtain both morphological and molecular samplings of the undescribed putative species of New Guinea recognised by Coode (1978, 1981) for description and delimitation,
- obtain additional molecular samplings of species with fibrous mesocarps, particularly *E. johnsonii, E. sedentarius, E. blepharoceras* and *E. womersleyi* to test current morphological placement. Molecular samples of *E. womersleyi* from New Guinea, Papuan Islands and the Moluccas are required for better resolution. *Elaeocarpus johnsonii* is currently placed with *E. blepharoceras* (Group IV) from New Guinea in the morphological classification but molecular data refutes this, and does not place them together. Utilising nuclear DNA may help improve and illuminate the relationship of *E. johnsonii* to other species,
- obtain additional samples of species from the *Obovatus* group particularly, *E. arnhemicus* and *E. sericoloides* from New Guinea, to confirm the placement of *E. coorangooloo* in the *Obovatus* group, rather than Group VI
- obtain additional molecular samples of *E. culminicola* and *E. sterrophyllus* and their varieties from New Guinea,
- obtain additional molecular samplings of *E. hylobroma*, *E. tariensis* and *E. carolinae* to confirm the tentative placement of *E. hylobroma* in Group V currently, despite it's morphological similarity to *E. tariensis* from Group VI (New Guinea) and *E. carolinae* of Group VII (Australia)

Chapter 4 – Fruit developmental biology and endosperm rumination in *Elaeocarpus ruminatus* F.Muell. (Elaeocarpaceae), and its taxonomic significance.

This chapter investigates the development of mesocarp formation and endosperm rumination in fruits and seeds of *Elaeocarpus* and has been published as:

Gagul, J. N., Tng, D. Y.P. & Crayn, D. M. (2018). Fruit developmental biology and endosperm rumination in *Elaeocarpus ruminatus* F. Muell. (Elaeocarpaceae), and its taxonomic significance. *Australian Systematic Botany*, 31: 409–419.

The paper was conceived by JNG, who conducted the study and wrote the manuscript. The idea for project was conceived by JNG and DMC; the later assisted with proof reading and general guidance in taxonomy. DYPT assisted with anatomical analysis

and general guidance in anatomy. Bruce Wannan and Chris Quinn assisted with anatomical and cell interpretation. Wendy Cooper assisted with fruit sampling. Nick Rockett assisted with photography.

ABSTRACT

The genus *Elaeocarpus* is the largest genus in the family Elaeocarpaceae, comprising more than 350 species of trees and shrubs with a mainly Indo-Pacific distribution. About 28 species in the genus, including nine species from Australia, are known to possess ruminate endosperm. To provide a basis for understanding fruit development and endosperm rumination in the genus and therefore its taxonomic and evolutionary significance, I studied the fruit anatomy of *Elaeocarpus ruminatus* F.Muell at different developmental phases (petal-fall to maturity). I found lignin in pericarp and ovary wall tissues in the earliest stages of development. In contrast, endosperm rumination occurs only after fruits have fully expanded, and becomes more pronounced as fruits ripen. Its phylogenetic distribution suggests that ruminate endosperm is a derived, albeit homoplasious character in *Elaeocarpus*. Comparative studies on related species will be instructive in determining the utility of ruminate endosperm for informing infra-generic taxonomy of the genus, and gaining insight into its adaptive significance.

Additional keywords: fruit morphology, plant anatomy, seeds

4.1 Introduction

Fruit and seed characteristics feature heavily in plant classification and description. For example, in some plant groups, such as the Annonaceae, the presence of rumination in endosperm tissue (ingrowths of the seed coat) has been used as a taxonomic marker in infrageneric classifications (van Setten and Koek-Noorman, 1992). Ruminate endosperm is widespread in the Angiospermae, having been recorded in the seeds of at least 58 families representing most of the major clades (Bayer and Appel, 1996; Werker, 1997; van Balgooy et al., 2015). However, the taxonomic relevance of this condition has not been investigated in detail for most of the plant families in which it occurs.

One group in which ruminate endosperm is poorly understood is the Elaeocarpaceae, a moderately large family of Gondwanan origin comprising more than 500 species of trees and shrubs (Crayn et al., 2006). The largest genus, *Elaeocarpus* L., comprises more than 350 species of trees and shrubs typically found in tropical and subtropical mesic forests of the Old World tropics (excluding mainland Africa) (Coode, 2004). Based on published and anecdotal reports, at least 28 species of *Elaeocarpus* possess ruminate endosperm (Coode, 1984; Phoon, 2015). In the most comprehensive treatment of the subject so far, Corner (1976) described seeds of five *Elaeocarpus* species (*E. edulis* Teysm. et Binn., *E. ganitrus* Roxb., *E. petiolatus* (Jack) Wall., *E. robustus* Roxb., and *E. serratus* L.), one of which (*E. edulis*) is considered a synonym of *Aceratium oppositifolium* DC. Of the four *Elaeocarpus* species, *E. petiolatus* has a ruminate endosperm, for which he examined only

immature fruits and made a brief observation of the condition. To date, the nature and development of ruminate endosperm in the genus has not been examined.

Ruminate endosperm is a homoplasious trait within *Elaeocarpus*, having arisen independently at least twice (Phoon, 2015; Chapter 4 this thesis). This feature accords well with some subclades within the genus, but our understanding of its evolutionary patterns is limited. More fundamentally, the developmental anatomy and ontogeny of *Elaeocarpus* fruits and seeds is little studied. The current study, although based on a single species may seem disproportionate given the taxa within *Elaeocarpus* with ruminate endosperm. However, it sets the basis upon which future studies can explore and investigate the developmental anatomy of rumination and its significance in *Elaeocarpus* taxonomy and evolution.

Fruits of *Elaeocarpus* develop from flowers with a superior 2–5(–8) locular, hairy or glabrous ovary, with 2–12 anatropous ovules per locule (Dettmann and Clifford, 2000) arranged in two rows (Coode, 1984). Not all ovules develop into seeds, some are aborted or compressed during fruit development. In *Elaeocarpus*, only one ovule per locule develops into a seed whereas in *Sloanea* two to several ovules per locule normally develop.

In *Elaeocarpus*, fruits are small (< 1 cm diameter) to large (4–7.5 x 3–5 cm) drupes, variously blue in most species with a few (e.g. *E. holopetalus* F.Muell.*, E. johnsonii* F.Muell.*, E. ruminatus*, *E. grandiflorus* Sm.) exhibiting brown, black or red fruits at maturity. Iridescent blue fruits are characteristic of some *Elaeocarpus* species (e.g. *E. angustifolius* Blume), a colour often mistaken for blue pigmentation, but which actually is due to epidermal microstructure, which affects light interference (Lee, 1991).

Anatomically, fruits are generally two-layered. The outer layer (beneath the exocarp) is typically fleshy and sometimes fibrous, whereas the inner layer, commonly referred to as the fruit stone, is woody with an often conspicuously sculptured surface. The fruit stones of some *Elaeocarpus* species have ornamental, cultural or religious uses (e.g. *E. angustifolius*, *E. ganitrus*: Phoon, 2012; Li et al., 2014; Singh et al., 2015).

As has been found in Anacardiaceae (Wannan and Quinn, 1990), the two main layers of *Elaeocarpus* fruit comprise the outer and inner mesocarp (Dettman and Clifford, 2000). Developmentally, the inner mesocarp becomes woody through the deposition of lignin in cell walls, resulting in a durable casing that protects the seed and may function as the unit of endozoochorous dispersal via vertebrate vectors (Rossetto et al., 2008; 2009; Baba and Crayn, 2012; Corlett, 2017). In *Elaeocarpus*, the durability of these structures is evidenced by a number of fossilised mesocarps (11–17 fossil taxa) having been recorded from Tertiary deposits in Australia and New Zealand (Dettman and Clifford, 2000; Rozefelds and Christophel, 2002), India (Bera et al., 2004) and China (Xiaoyan pers. comm. 2016).

Dettmann and Clifford (2000) have described fruits and seeds of two species of *Elaeocarpus* (*E. angustifolius*, *E. reticulatus* Sm.), in which they compared the lignified inner mesocarps of these species with fossil *Elaeocarpus* fruits. However, little attention has been accorded to date to the ontogeny of lignification in *Elaeocarpus* mesocarps. The present study aimed to fill this knowledge gap by undertaking a comprehensive developmental study, from petal-fall to fully ripened fruit, of endosperm rumination and mesocarp development in *E. ruminatus*. For this species I sought to determine:

1. the timing of mesocarp developmental milestones such as differentiation of the two mesocarp layers and lignification;
2. the onset of endosperm rumination and its progression to maturity.

I discuss the taxonomic and evolutionary significance of ruminate endosperm and lignification in *Elaeocarpus*.

4.2 Materials and methods

4.2.1 Study species and sampling

Thirty four taxa of *Elaeocarpus* are found in Australia, nine of which possess ruminate endosperm (Coode, 1984; Phoon, 2015, Chapter 4). For this study, I selected *E. ruminatus* (locally known as brown or grey quandong) for investigation. The species is endemic to Queensland, Australia and is distributed from northeast Queensland and southwards to coastal central Queensland, where it is relatively common. It grows as a tree to 40 (–50) m tall in a wide variety of rain forests ranging in elevation from 200–1160 m a.s.l. (Cooper and Cooper, 2004). Flowering and fruiting occur between November and July (Coode, 1984). Young fruits are hairy but hairs start to disappear (4–6 weeks after petal-fall) becoming completely glabrous at maturity (late in development). Fruits are globular to slightly oval in shape, c. 13 mm long, 10–13 mm wide and are brownish-green or dull blue when ripe. Upon drying, the fruit surface cracks irregularly. With respect to taxonomy, *E. ruminatus* is placed in group XI, subgroup A (*sensu* Coode, 1984), a monotypic group. Phylogenetically *E. ruminatus* is sister to a clade comprised of eight other Australian taxa (*E. elliffii* B.Hyland & Coode*, E. ferruginiflorus* C.T.White*, E. foveolatus* F.Muell.*, E.*

largiflorens C.T.White subsp. *largiflorens, E. largiflorens* C.T.White subsp. *retinervis* B.Hyland & Coode, *E. sericopetalus* F.Muell*., E. thelmae* B.Hyland & Coode, *E.* sp. Mt. Windsor), corresponding to group XI subgroup B (XIB)(Coode 1984; Phoon 2015). *Elaeocarpus ruminatus* differs from members of XIB in having fruit 2–locular (3–locular in XIB), floral disks glabrous (hairy in XIB), anthers awned (not awned in XIB) and mesocarp ornamentation rugose (smooth and granulose in XIB) (Coode, 1984) (Fig. 4.1). Rozefelds and Christophel (1996a; 2002) placed *E. ruminatus* with group VII species (*E. carolinae* Hyland & Coode, *E. culminicola* Warb., *E. eumundi* F.M.Bailey and *E. reticulatus*) based on their echinate ornamentation.

Flowering and fruiting phenology of a mature individual of *E. ruminatus* growing in upland rainforest on the Atherton Tableland (17º 24' 44.0" S, 145º 42' 07.0" E, 707 m elevation), Queensland, was monitored from petal-fall over a period of 22 weeks (Nov. 2014 – Apr. 2015). During this period, fruits were sampled every two weeks to fruit maturity (total 12 samplings). Fruits were observed to develop at different rates on different branches, and so sampling was limited to one branch. Each sampling comprised five (5) fruits collected and preserved in 70% ethanol.

4.2.2 Specimen preparation

I prepared longitudinal and transverse sections from a minimum of two samples of *E. ruminatus* fruits from each collection interval using a GLS1 portable microtome (Gärtner et al., 2014). Sections were stained with Toluidine blue, which stains lignified tissues green/blue. The stained sections were then mounted on glass slides with glycerine jelly and examined at 6.7x and 10x magnifications using a

stereomicroscope (Nikon SMZ 745T, Nikon Corporation, Minato-ku, Tokyo, Japan) and at 40x and 100x with a light microscope (Nikon ECLIPSE Ci-L, Nikon Corporation, Minato-ku, Tokyo, Japan). Representative sections were photographed using a digital camera (Nikon DS-Fi2, Nikon Corporation, Minato-ku, Tokyo, Japan).

4.2.3 Morphological and anatomical observations

Observations and measurements of morphological and anatomical features were made on representative transverse (TS) and longitudinal sections (LS) of fruits and seeds from different developmental stages. Descriptions use standard botanical terminology (Corner, 1976; Coode, 1984; Rozefelds, 1990; Wannan and Quinn, 1990; von Teichman and van Wyk, 1993; Dettmann and Clifford, 2000; Phoon, 2015) and for mesocarp structure I followed the interpretations of Dettman and Clifford (2000).

For ease of description and interpretation, I categorised the stages of fruit development of the study species into early and late. The early stage was defined as the start of the fruit set (1–10 weeks), where fruit growth has been initiated after flowers were pollinated and fertilized, which coincided with the first sampling. The late stage (11–18 weeks) was defined as from the first appearance of brown coloration on the fruit indicating maturity. The early stage also corresponds to a rapid fruit growth phase, while the late stage represented a phase of maturation (Fig. 4.2).

4. 3 Results

4.3.1 Morphological and anatomical observations at different fruit developmental stages

Flowers of *E. ruminatus* were observed to open at different times, meaning fertilisation and fruit development probably occurs somewhat asynchronously both within and among inflorescences. Nevertheless, a consistent pattern observed in this study was an initial rapid increase in fruit size (from c. 2 to c. 13 mm) over the first 10 weeks followed by a more gradual maturation period (from c. 13 to c. 15 mm) from week 10 to 22 (Fig. 4.2).

Throughout fruit development, there is a clear demarcation between the main tissue layers (exocarp, mesocarp and endocarp) of the pericarp (Figs 4.3–4.7). The exocarp, the thin outermost layer of the pericarp, remains attached to the mesocarp throughout fruit development. The mesocarp differentiates into an outer fibrous and gritty-textured fleshy layer, and an inner woody layer that becomes heavily lignified. The outer mesocarp and exocarp detach together from the inner mesocarp in mature fruits. The endocarp comprises an undifferentiated tissue layer surrounding the seed. I outline the different phases and discuss my morphological and anatomical observations individually.

Figure 4.1 Inner mesocarp surface detail of representatives of *Elaeocarpus* from Group XI.

A. The study species *Elaeocarpus ruminatus* (Gray 3669, QRS) exhibits rugose surface ornamentation. The related species show granulose ornamentation: B. *E. sericopetalus* (Gray 3030, QRS); and C. *E. elliffii* (Irvine 1541, QRS). Photographed by Nick Rockett.

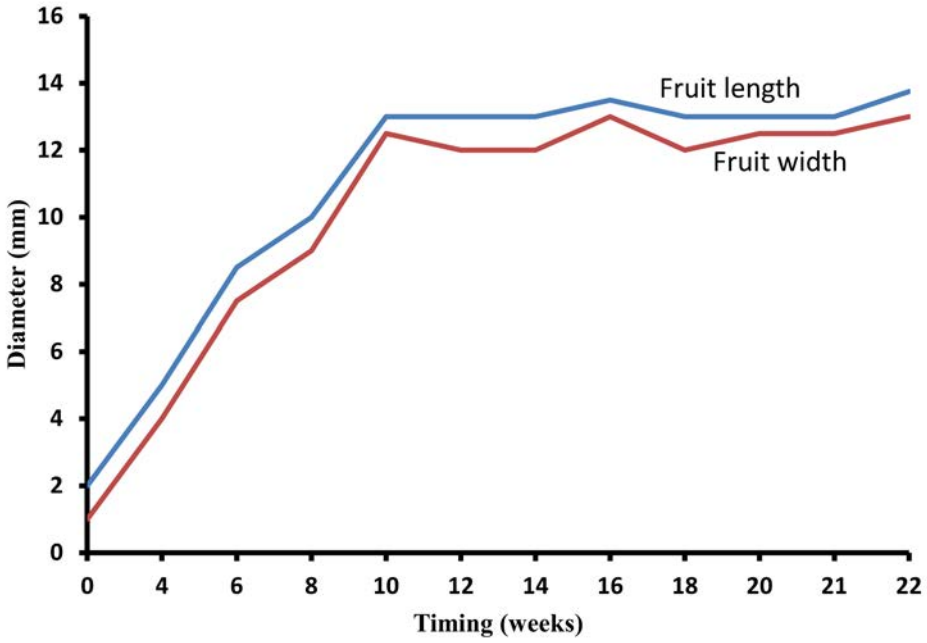


Figure 4.2 Development of *Elaeocarpus ruminatus* fruits.

Fruits show an initial rapid increase in size until ~10 weeks after petal fall, followed by a maturation period where no further significant size changes occur.

Table 4.1 Growth and development of fruit and seed in *Elaeocarpus ruminatus*.

Outer and inner mesocarp, and locule measurements were made on fruit cross-sections. Range measurements are included for fruits observed (n = 3).

Days after petal fall	Length (mm)	Width (mm)	Outer mesocarp wall (mm)	Inner mesocarp wall (mm)	Locule (mm)
Early phase (1–10 weeks)					
0	2	1	0.2–0.5	0.2	1.5
28	5	4–5	0.5–1	0.5	2
42	8–9	7–8	2–2.5	0.5–1	3
56	10	9	2–2.5	0.5	5
70	13	12–13	2–3	0.5–1.5	7
Late phase (11–22 weeks)					
84	13	12	2.5–3	1	7
98	13	12	1–2	0.5–1	7
112	13–14	13	2–2.5	0.5–1	8
126	13	12	2–2.5	0.5–1	8
140	13	12–13	2–2.5	1	7.5
154	13–14	13	c.2	0.5–1	8

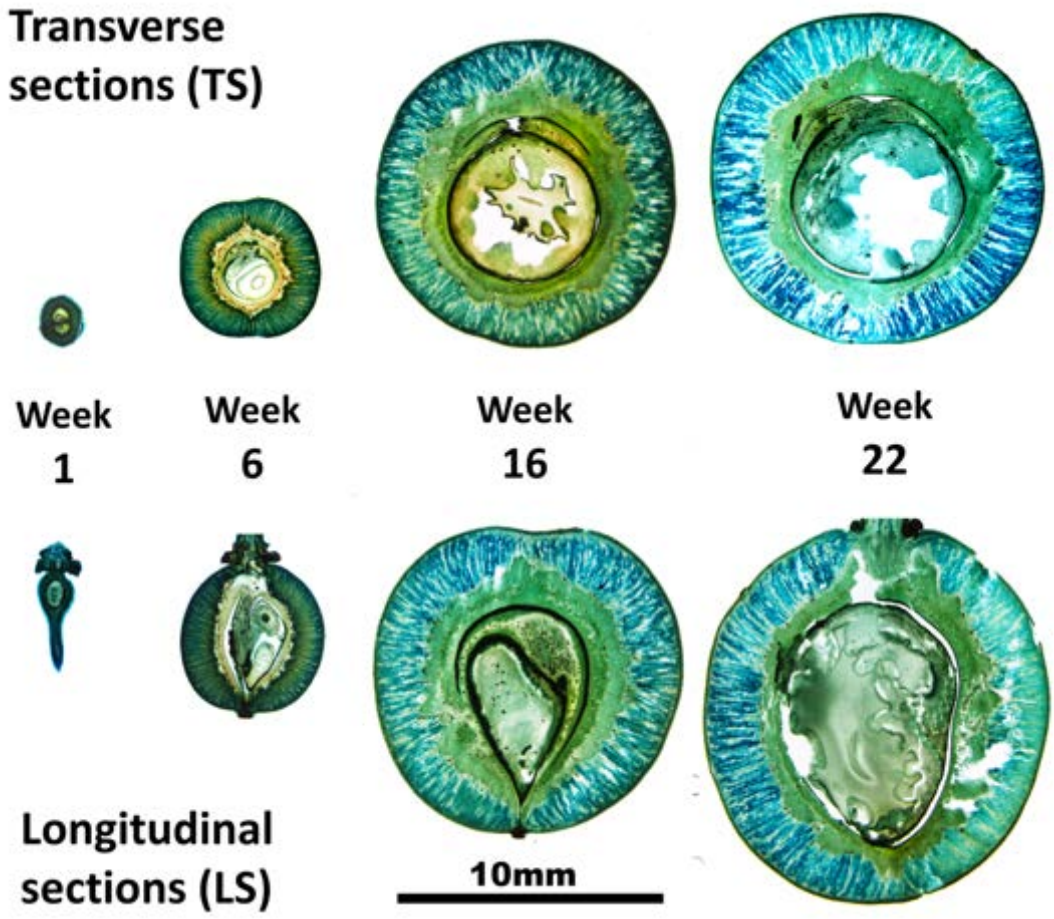


Figure 4.3 Developmental anatomy of *Elaeocarpus ruminatus* fruits.

Transverse (top) and longitudinal (bottom) sections of developing fruits at Weeks 1, 6, 16 and 22 post-petal fall. Sections from Weeks 1 and 6 represent samplings of early fruit developmental stages (Weeks 1–10), whereas those from Weeks 16 and 22 are from the late developmental stage (Weeks 11–22; See Materials and Methods). Structures are interpreted and labeled in Figs 4.4–4.7.

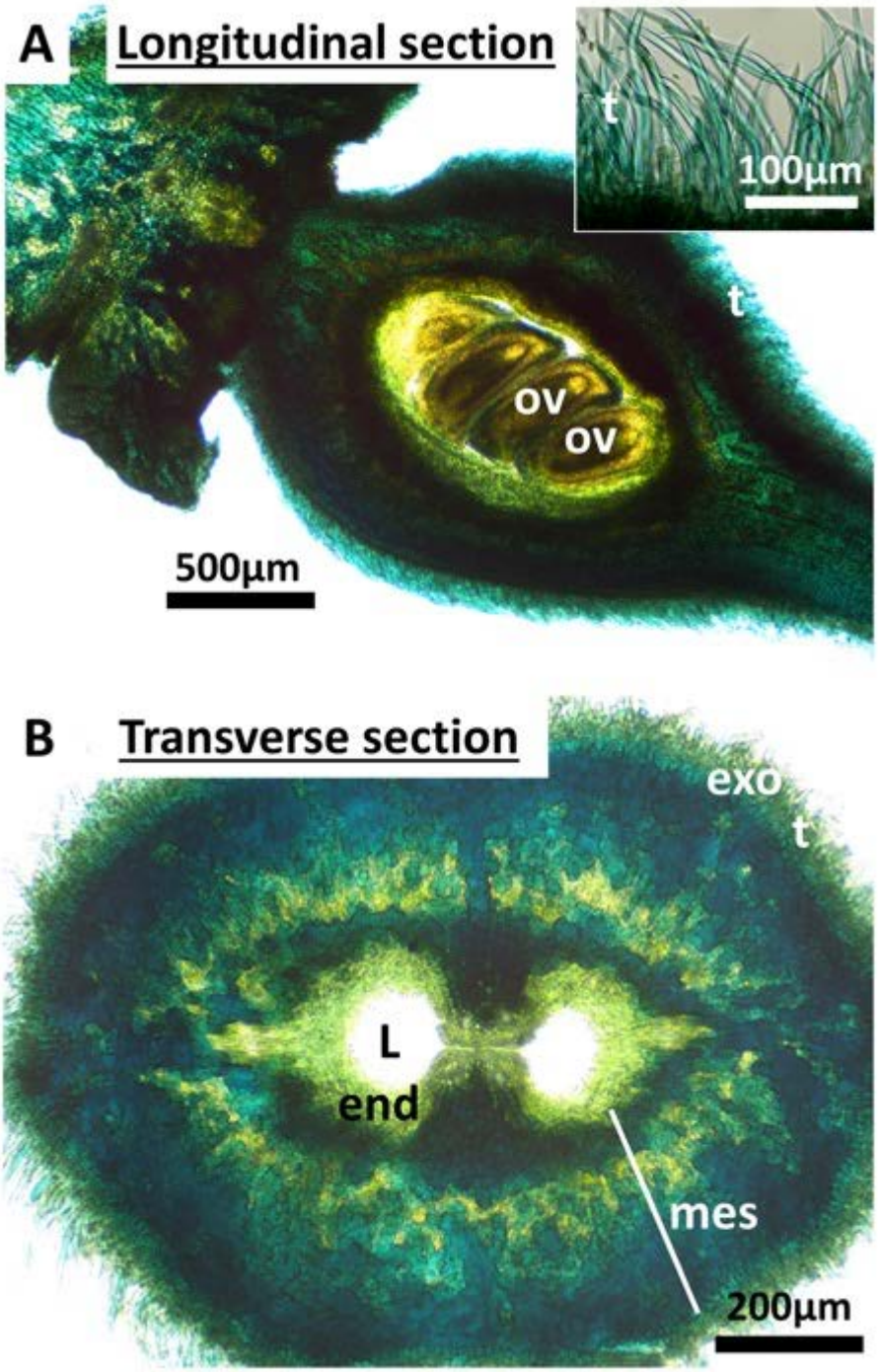


Figure 4.4 Developmental anatomy of early stage *Elaeocarpus ruminatus* fruits.

A. A longitudinal section of early stage fruits. B. A tranverse section of early stage fruits of *Elaeocarpus ruminatus*. Inset in A is a closeup of the trichomes on the exocarp. end, endocarp; exo, exocarp; mes, mesocarp; L, locule; ov, ovule; t, trichomes.

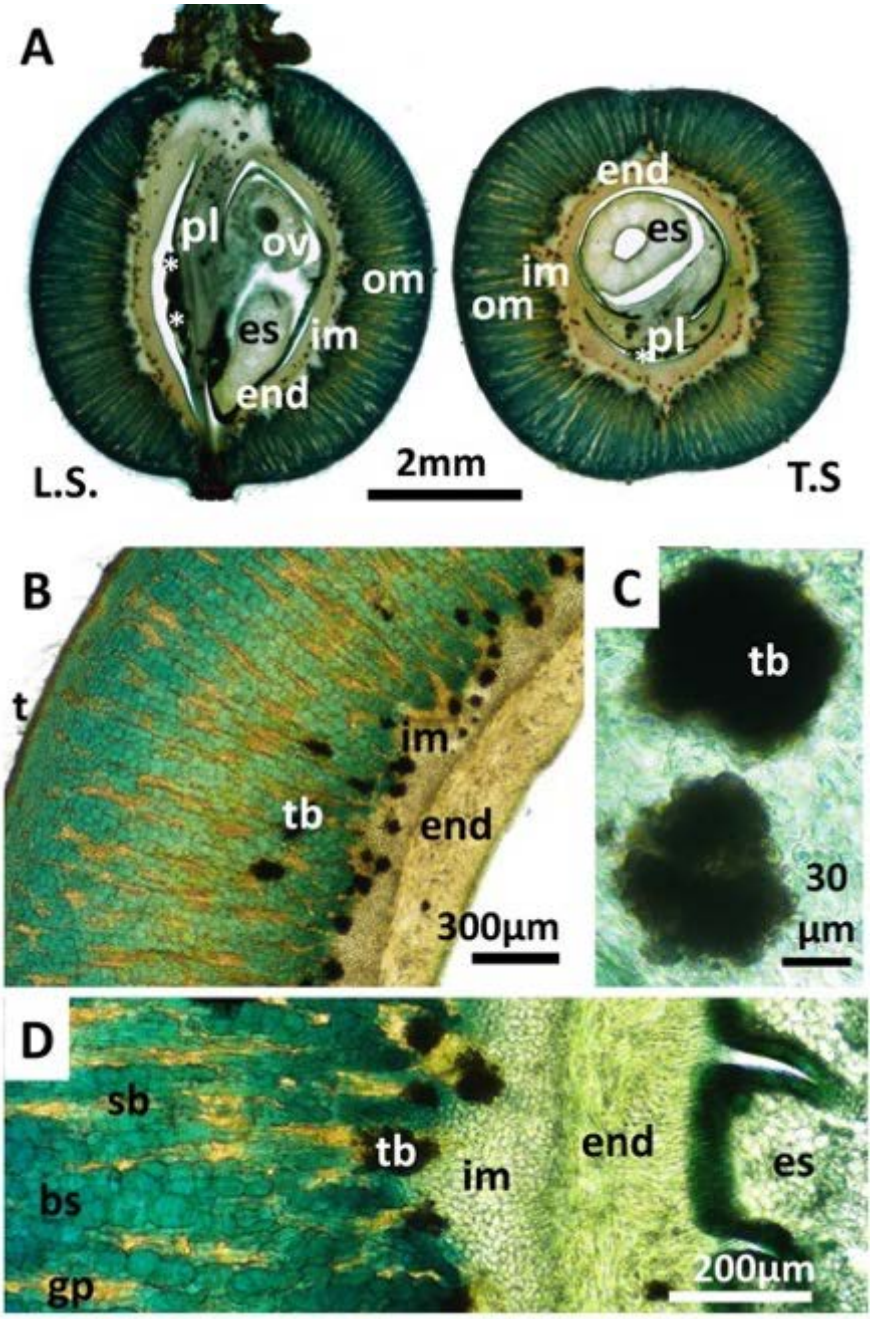


Figure 4.5 Developmental anatomy of early stage *Elaeocarpus ruminatus* fruits.

A. Longitudinal and transverse sections of *Elaeocarpus ruminatus* fruits at an early development stage (Week 4), showing well defined mesocarp layers. Aborted ovules (*) in locules are prominent as the main fertilised ovule develops. B. The layers of the pericarp are well demarcated: C. frequent, dark tanniferous bodies scattered throughout the fruit. D. A few shallow trenches are sometimes observable on the outline of the endosperm (es). bs, brachysclereids; end, endocarp; exo, exocarp; es, endosperm; im, inner mesocarp; om, outer mesocarp; sb, sclerenchyma bundles; t, trichomes; tb, tanniferous bodies.

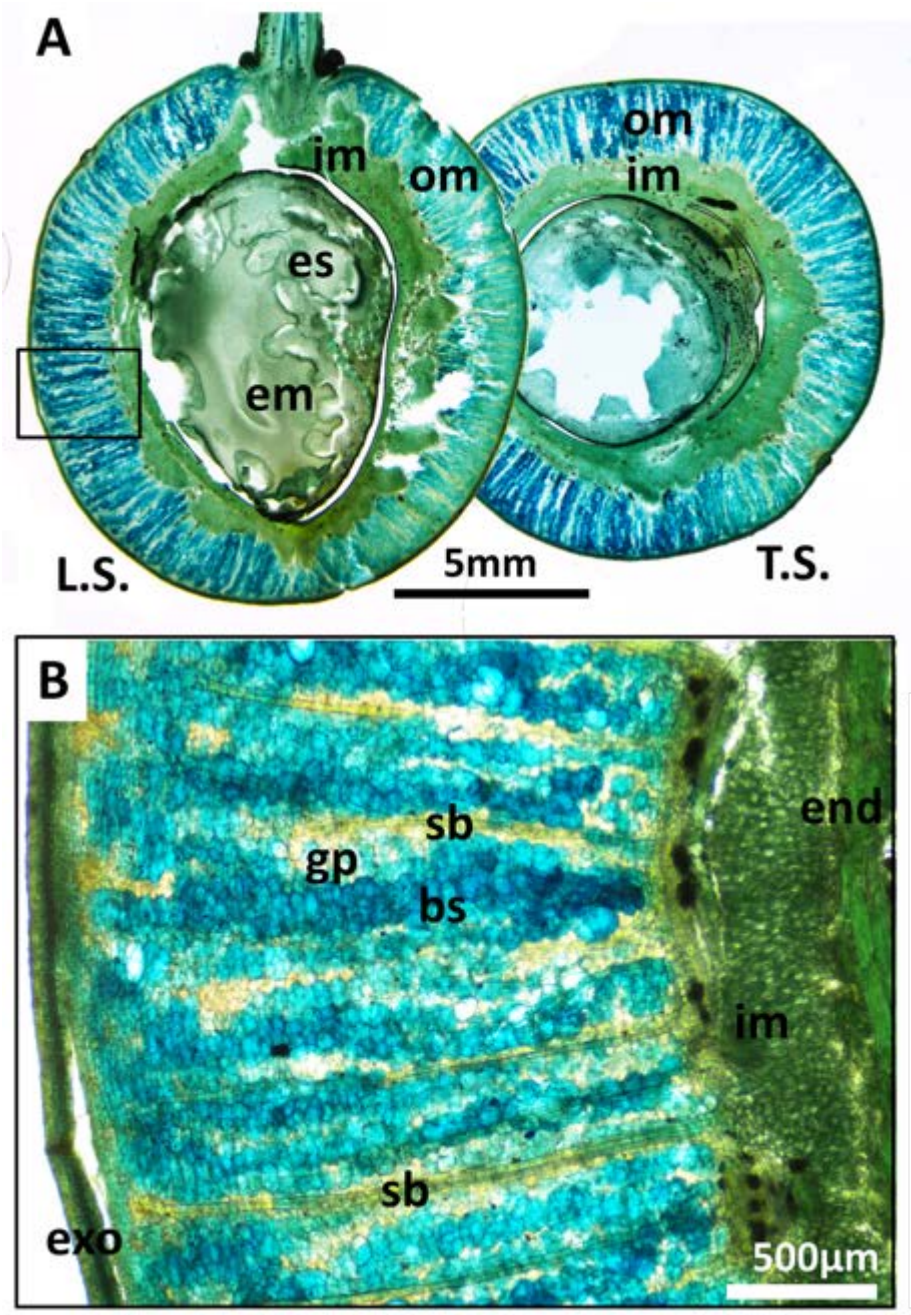


Figure 4.6 Developmental anatomy of late stage *Elaeocarpus ruminatus* fruits.

A. Longitudinal and transverse sections of *Elaeocarpus ruminatus* fruits at late developmental stage (Week 22), showing well defined mesocarp layers and extensive endosperm ruminations. B. Magnification of a section of the pericarp, showing the distribution of the tissue and cell types. bs, brachysclereids; em, embryo; end, endocarp; es, endosperm; exo, exocarp; gp, ground parenchyma; im, inner mesocarp; om, outer mesocarp; sb, sclerenchyma bundles.

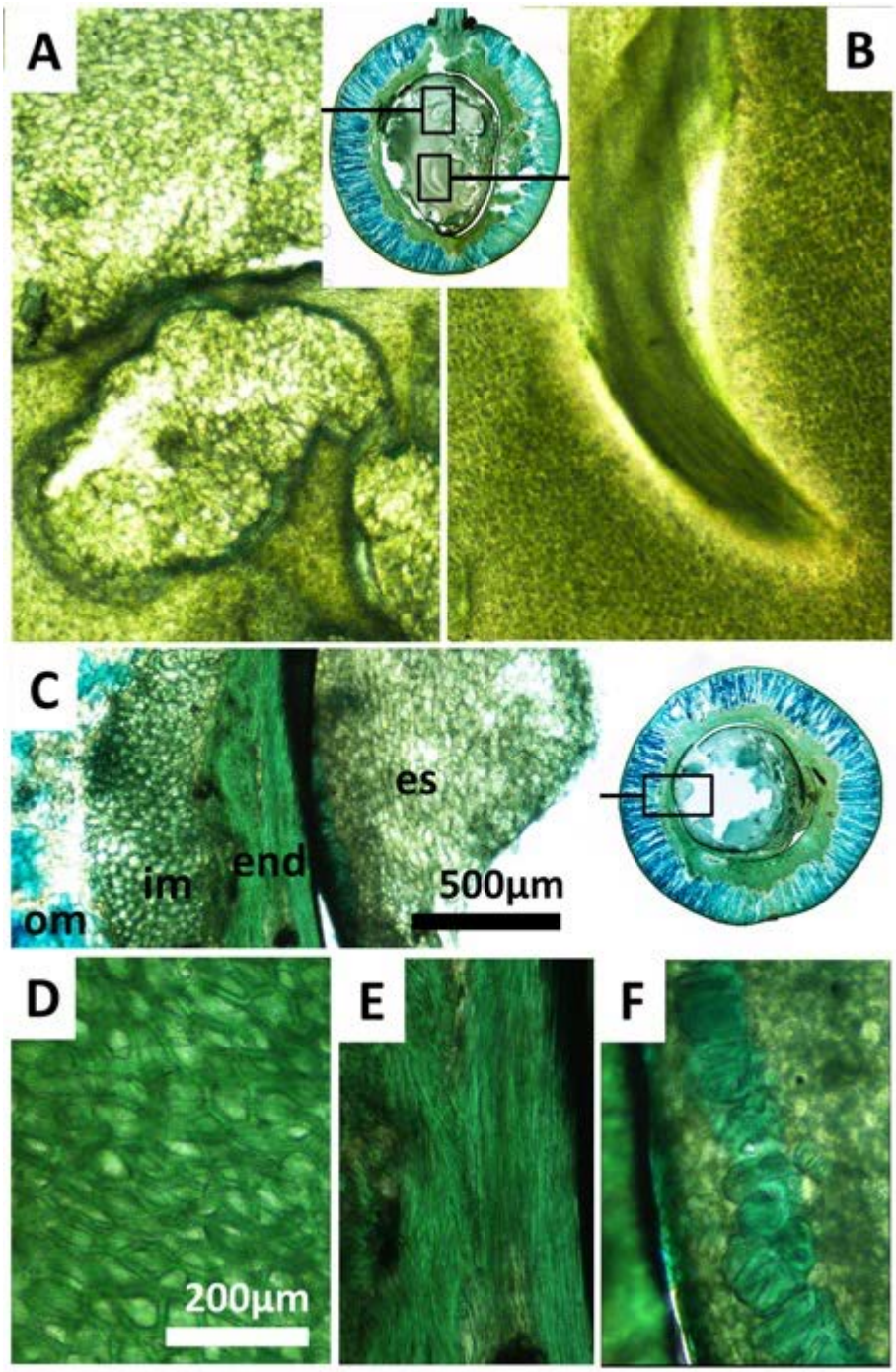


Figure 4.7 Developmental anatomy of late stage *Elaeocarpus ruminatus* fruits.

Longitudinal sections of late-stage (Week 22) fruits showing: A. details of endosperm rumination; and B. curved embryo. C. A fruit cross-section shows the clear demarcation of the tissue layers. D. The mesocarp consisting of lignified sclereids. E. The endocarp consisting of compressed fibres. F. The endosperm of ground tissue with rumination outlines often lined with bands of thick-walled macrosclereids. end, endocarp; es, endosperm; im, inner mesocarp; om, outer mesocarp.

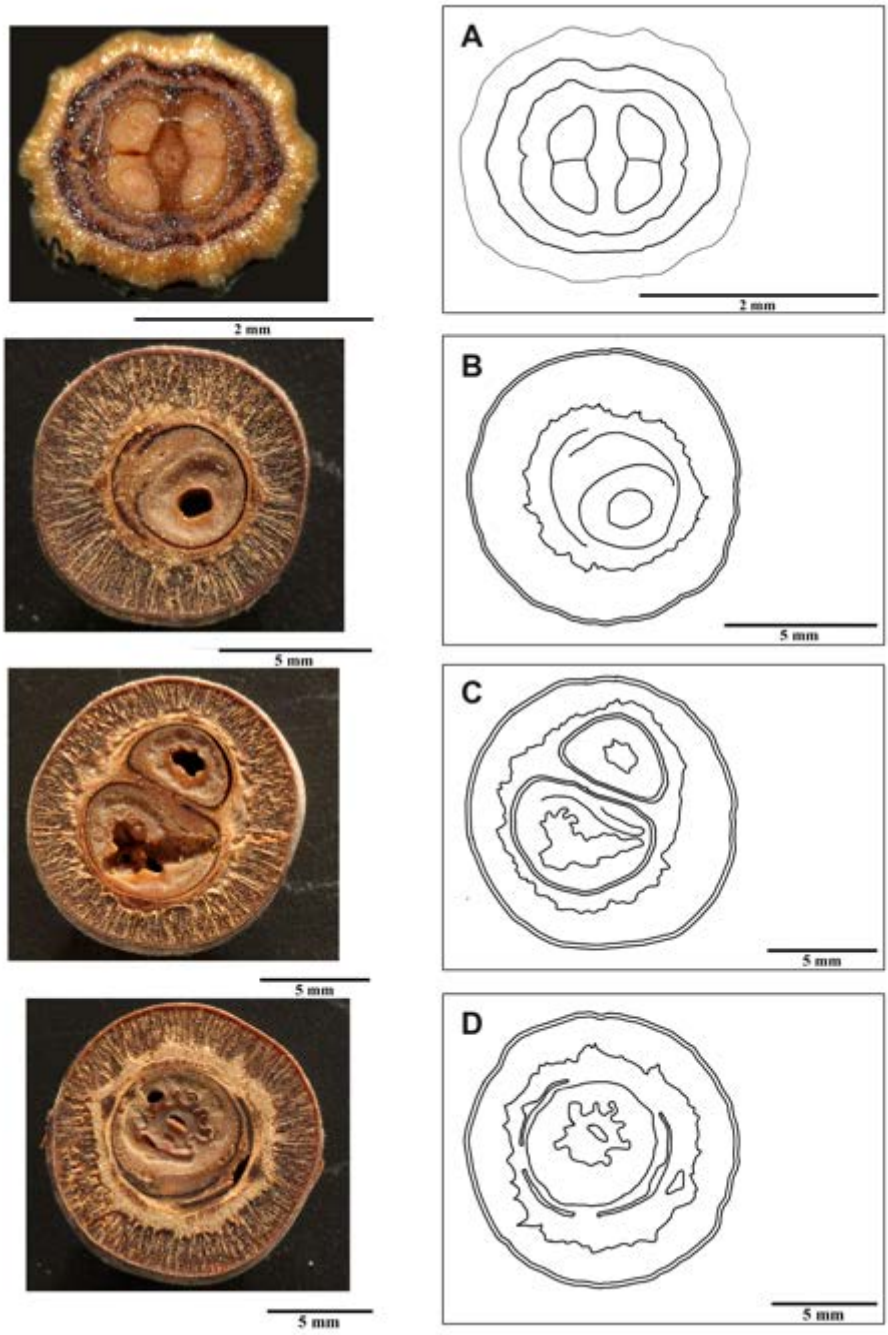


Figure 4.8 Representative transverse sections of *Elaeocarpus ruminatus* fruits and corresponding line drawings.

A. Two-locular, hairy ovary at Week 1. B. Pericarp at Week 6 (no evidence of endosperm rumination). C, D. Pericarps showing endosperm rumination at Weeks 10 and 14 respectively. D. Other ovules are aborted, with only one developing into a mature seed.

4.3.2 Early stage (Weeks 1–10)

At the earliest developmental stage (week 1) the fertilized gynoecium in longitudinal section bears the typical elongated shape of a pistil, 3–5 mm long (Figs 4.3, 4.4A), with a bulbous ovary at the base extending into a pointed style. The entire young fruit is covered with a dense layer of unicellular trichomes 0.20–0.32 mm long (Fig. 4.4A, inset). The ovary is 2–locular (Figs 4.4B, 4.8) with each locule bearing up to eight anatropous ovules, arranged in two rows (Fig. 4.4A). In some slightly more developed fruits (e.g. week 4), the placenta was obvious (Fig. 4.5A), and contained tanniferous inclusions (Figs 4.5B–D).

Fruit growth is rapid at this stage (Table 4.2, Fig. 4.2). The young fertilized ovaries (fruits) are variable in size, ranging between c. 2 × c. 1 mm, and 5–9 × 4–7 mm. Usually one ovule per fruit develops into a seed and the rest are aborted. In TS of fruits, the curvature of the seed may in certain sectional planes give the false impression that two seeds are present (Fig. 4.8C).

By 4–10 weeks after petal fall, the fruits are globular (Figs 4.3, 4.5A) and still green, but the dense layer of trichomes on the exocarp has thinned out (Fig. 4.5B). Beneath the trichomes, the exocarp is characterised by at least two thin layers of small and tightly packed non-lignified epidermal cells (Fig. 4.5B), which are hard to distinguish in young fruits.

The layers of the mesocarp at the very earliest stage are not well differentiated. However, by the fourth to sixth week of development (Fig. 4.5) the mesocarp is clearly segregated into two distinct layers. The outer fibrous layer

comprises brachysclereids interspersed with ground parenchyma and sclerenchyma bundles, and the inner layer comprises a continuous group of white/clear non-lignified cells (Figs 4.5B–D) many of which become lignified later in development (Figs 4.6B, 4.7C–D). The thickness of the outer mesocarp wall ranges between 0.2–2.5 mm and that of the inner mesocarp between 0.2–1.0 mm. Both outer and inner mesocarp tissues contain black tanniferous cells (Figs 4.5B–D).

Between weeks 1–6, the endocarp comprises a layer of elongated and irregularly shaped cells forming a non-staining tissue layer (Fig. 4.5D). Between weeks 7–9 however, the endocarp layer stains green (see Late Stage). The endosperm also appears to be lined with dark-staining macrosclereids (see Late Stage).

By weeks 8–10, the endocarp becomes differentiated into three layers, the middle layer containing elongate irregularly arranged lignified cells; while the cell layers above and beneath were distinctively darker, more compact and harder to differentiate. The inner surface of the endocarp comprises a layer of non lignified inner epidermal cells.

4.3.3 Late stage (Weeks 11–22)

Fruit size varies little (c. 13–14 × c. 12–13 mm; Fig. 4.2) throughout the late stage of fruit development, indicating cessation of fruit expansion. From 18 weeks, maturation, ripening and senescence of the fruits occur, indicated by the gradual loss of green colour and development of brownish-green or dull blue colour on the outer surface of the exocarp. Upon drying, the exocarp cracks irregularly on the surface. Anatomically, the exocarp is comprised of an epidermal layer and two hypodermal layers with tightly packed cells (Fig. 4.6B). Trichomes are absent at this stage.

By around 10 weeks endosperm rumination is evident, and by 22 weeks the degree of rumination is at its most pronounced (Figs 4.3, 4.7A). The curved outline of the embryo is also evident (Fig. 4.7B). Coincident with the cessation of fruit expansion, the seeds apparently cease expanding (Table 4.2) but continue to develop anatomically. Seed maturity is achieved by week 22 at which point seeds measure c. 9 × 6 mm.

Within the ovules and endosperm, black tanniferous inclusions are abundant and rumination is extensive. The placenta is clearly distinct. There are colourless non-lignified tissues in the seeds (Figs 4.7A–B).

With respect to the mesocarp, the sclerenchyma bundles are well-developed and extend radially from the inner mesocarp to the exocarp (Fig. 4.6B), forming a reticulate fibrous network throughout the outer mesocarp.

With the outer mesocarp removed, the inner mesocarp surface appears rugose. Two external sutures (mesosutural) are evident, which form indistinct ridges c. 1.0 mm high that extend down to the slightly pointed ends of the mesocarp. When fully developed the inner lignified mesocarp (stone) is ovoid-ellipsoid in shape and varies in size from c. 10–12 × c. 8–10 mm (Fig. 4.1).

The thickness of the outer mesocarp wall is variable, and ranges between 2–3 mm, and between 0.5–1 mm in inner mesocarp, in both intermediate and late stages of fruit development (Table 4.2). This indicates that internal/anatomical growth and development is more gradual and uniform indicating fruit expansion ceases at the end of the intermediate phase (12 weeks) of development.

Anatomically, the outer mesocarp tissues are comprised mostly of heavily lignified stone cells or sclereids, with inclusions of parenchyma ground tissue interspersed among them (Fig. 4.6B). The cells are thick-walled and larger than inner mesocarp cells. Both outer and inner mesocarp cells appear isodiametric, and there are also scattered dark tanniferous inclusion bodies.

At 22 weeks, the inner mesocarp layer is also clearly lignified (Fig. 4.7C), consisting of an obvious c. 1 mm thick band of isodiametrical sclereids with large lumens and pitted cell walls (Fig. 4.7D). The endocarp is comprised of elongate, tangentially arranged but somewhat disorganised and compressed fibres (Fig. 4.7E), which in earlier developmental stages were not as apparent. In the fully developed endosperm, the outlines of the ruminations are often lined with dark-staining macrosclereids (Fig. 4.7F).

4.4 Discussion

4.4.1 Fruit morphology

Within Elaeocarpaceae, fruits of *Elaeocarpus* and the closely related genus *Aceratium* DC. are drupes (Coode, 2004, Rozefelds and Christophel, 1996a). Members of both have fruits with outer mesocarps that are persistent and permanently attached to the surface of the inner woody mesocarps. In his description of *Aceratium oppositifolium* (as *E. edulis*), Corner (1976) described fibrovascular strands within the mesocarp, a character which I also noticed in *E. ruminatus*. However, the mesocarps of *A. oppositfolium* are succulent (Corner ,1976). In contrast, the inner woody mesocarps in *Elaeocarpus* fruits range from poorly lignified (*E. sedentarius* Maynard

& Crayn, *E. blepharoceras* Schltr. and *E. johnsonii*) to strongly lignified (*E. bancroftii* F.Muell. & F.M.Bailey*, E. womersleyi* Weibel*, E. stellaris* L.S.Sm. and *E. carbinensis* J.Gagul & Crayn (Gagul et al., 2018b)).

The mesocarp of *Elaeocarpus* fruits differentiates into two distinct layers. The outer layer is succulent or fibrous with a gritty texture, and in the majority of *Elaeocarpus* species, this layer detaches cleanly from the inner mesocarp (stone). However, in some species (e.g. *E. blepharoceras, E. johnsonii, E. sedentarius*, *E. womersleyii*) the fibres remain attached, a feature that has taxonomic implications pending further investigation.

In *E. ruminatus* both inner and outer mesocarp tissues contain black tanniferous cells (Figs 4.5B–C). While the taxonomical significance of these inclusions requires further study, similar tanniferous bodies have been reported in the mesocarps of mature fruits of *E. sphaericus* (Gaertn.) K.Schum. (=*E. angustifolius*) and *E. tectorius* (Lour.) Poir (Shah et al., 2010; Muthuswamy and Senthamarai, 2014), species that are not closely related to *E. ruminatus*.

4.4.2 Timing and development of lignification and rumination in *Elaeocarpus ruminatus*

Deposition of lignin commences in the earliest phases of fruit development, before the seed develops. In addition to non-staining ground tissue, a large proportion of the tissues of the pericarp and ovary wall stained blue, signifying the presence of lignin at the very earliest fruit developmental stages, i.e. c. 1 week post-anthesis. The lignified cells at this stage appear to be mostly brachysclereids (stone cells); fibres are absent. As the fruit develops, lignification commences and continues, resulting in an

increase in size and thickness of the lignified layer of the inner mesocarp and the differentiation of fibre cells.

The development of endosperm ruminations, on the other hand, appears to be decoupled from lignification of the mesocarp, and begins to develop c. 10 weeks after petal fall when fruit expansion is complete. Throughout the late developmental stage, endosperm rumination becomes increasingly pronounced, reaching its maximum extent by about week 22 (Fig. 4.3).

4.4.3 Ruminate endosperm and *Elaeocarpus* systematics

Ruminate endosperm has been reported in 28 taxonomically diverse taxa in *Elaeocarpus* (Phoon, 2015) (Table 4.1), and with reference to my study. *Elaeocarpus ruminatus* is closely related to eight other Australian taxa that have curved embryos and ruminate endosperm (Chapter 3: Fig. 3.3, 3.4; Table 3.1), seven of which are placed in group XIB (*E. elliffii, E. ferruginiflorus, E. foveolatus, E. largiflorens* subsp. *largiflorens, E. largiflorens* subsp. *retinervis, E. sericopetalus, E. thelmae*) and *E.* sp. Mt. Windsor (as yet unassigned to a group). As yet, detailed molecular phylogenetic study has been unable to fully resolve the relationships of *E. ruminatus* (XIA) and group XIB species (Chapter 3), but furture investigations into mesocarp features of these species may shed additional insights of the relationship between these groups and also with the unassigned *E.* sp. Mt. Windsor.

The bulk of the other 19 species known to exhibit ruminate endosperm belong to the *Acronodia*, *Coilopetalum*, and *Polystachyus* groups, and are primarily from the Indomalayan region. Further investigation and thorough re-examination of fresh or liquid-preserved seeds material from these species, plus a wider range of *Elaeocarpus*

species including those whose seed condition is unknown, across the geographical range of the genus is recommended to gain further insights into the taxonomic distribution of ruminate endosperm in the genus. So far, the occurrence of this condition in other Elaeocarpaceae genera is unknown.

Table 4.2 Occurrence of ruminate endosperm within *Elaeocarpus*.

Data derived with modification from Phoon (2015).

Taxon	Region	Infrageneric group and subgroup	Reference
Elaeocarpus chrysophyllus Merr.	Western Malesia	Acronodia	Coode (1996b)
Elaeocarpus clementis var. *borneensis* (Ridl.) Coode	Western Malesia	Polystachyus	Coode (1996c)
Elaeocarpus clementis var. *clemensiae* (R.Knuth) Coode	Western Malesia	Polystachyus	Coode (1996c)
Elaeocarpus clementis Merr. var. *clementis*	Western Malesia	Polystachyus	Coode (1996c)
Elaeocarpus cupreus Merr.	Western Malesia	Polystachyus	Coode (1996c)
Elaeocarpus elliffii B.Hyland & Coode	Australia	XI B	Coode (1984)
Elaeocarpus euneurus Stapf ex Ridl.	Western Malesia	Acronodia	Coode (1996b)
Elaeocarpus ferrugineus (Jack) Steud.	Western Malesia	Acronodia	Coode (1996b)
Elaeocarpus ferruginiflorus C.T.White	Australia	XI B	Coode (1984)

Elaeocarpus foveolatus F.Muell.	Australia	XI B	Coode (1984)
Elaeocarpus jacobsii Coode	Western Malesia	Acronodia	Coode (1996b)
Elaeocarpus knuthii Merr. subsp. *knuthii*	Western Malesia	Acronodia	Coode (1996b)
Elaeocarpus kusanoi Koidz.[A]	Caroline Island, central Pacific	Coilopetalum	Coode (unpublished)
Elaeocarpus largiflorens C.T.White subsp. *largiflorens*	Australia	XI B	Coode (1984)
Elaeocarpus largiflorens subsp. *retinervis* B.Hyland & Coode	Australia	XI B	Coode (1984)
Elaeocarpus marginatus Stapf ex Weibel	Western Malesia	Acronodia	Coode (1996b)
Elaeocarpus mastersii King	Western Malesia	Acronodia	Coode (1996b)
Elaeocarpus multiflorus (Turcz.) Fern.-Vill.[B]	Indonesia	Coilopetalum	Coode (2001d)
Elaeocarpus multinervosus R.Knuth	Western Malesia	Polystachyus	Coode (1996c)
Elaeocarpus nanus subsp. *congestifolius* (R.Knuth) Coode	Western Malesia	Acronodia	Coode (1996b)
Elaeocarpus nanus Corner subsp. *nanus*	Western Malesia	Acronodia	Coode (1996b)
Elaeocarpus nitentifolius Merr. & Chun	Western Malesia	Acronodia	Tang & Phengklai (2007); Weibel (1968)

Elaeocarpus petiolatus (Jack) Wall.	Malesia, Pacific Islands.	Coilopetalum	Coode (1998)
Elaeocarpus polystachyus Wall. ex Müll. Berol.	Western Malesia	Polystachyus	Coode (1996c)
Elaeocarpus ruminatus F. Muell.	Australia	XI A	This paper; Coode (1984)
Elaeocarpus sericopetalus F. Muell.	Australia	XI B	Coode (1984)
Elaeocarpus thelmae B. Hyland & Coode	Australia	XI B	Coode (1984)
Elaeocarpus sp. Mt Windsor Tableland (L.W.Jessup & GJM 1378) Qld Herbarium	Australia	Unassigned	NA; information from voucher (M. Godwin C 3030 (CNS), B. Hyland) 5541 (CNS

[A]Embryo and endosperm uncertain.

[B]Endosperm uncertain.

Within Elaeocarpaceae, all except *Sericolea* and some *Elaeocarpus* have straight embryos (Coode, 2004). The seeds with straight embryos have broad cotyledons. Non-straight (curved) embryos are of two types in Elaeocarpaceae: 1) weakly curved embryos with wide cotyledons (e.g., *E. holopetalus* F. Muell., probably *E. costatus* M.R.F.Taylor, and *Sericolea*), and 2) strongly curved embryos with narrow cotyledons, which occur in many species of *Elaeocarpus*.

Phylogenetically, the curved embryo has a single origin within *Elaeocarpus*, whereas ruminate endosperm has evolved at least twice (Chapter 5: Figs 5.13, 5.14).

Interestingly, seed rumination has evolved only within lineages having strongly curved seeds.

Although the great majority of *Elaeocarpus* species with ruminate endosperm also have curved embryos, this does not appear to be a strict association. Some species with curved embryos lack ruminate endosperm e.g. *Elaeocarpus culminicola*, *Elaeocarpus floridanus* Hemsley, *Elaeocarpus habbemensis* A.C.Sm., and *Elaeocarpus sarcanthus* Schltr. (Coode, 1978, 1995; Chapter 4). Apart from *Elaeocarpus*, two species of *Sericolea* (*S. calophylla* subsp. *grossiserrata* Coode; *S. micans* Schltr. var. *micans*), within Elaeocarpaceae have curved embryos and entire endosperm (Coode, 1978, 2004; Phoon, 2015).

As a morphological character, ruminate endosperm appears to be a derived, homoplasious character within *Elaeocarpus* (Chapter 5). However, the character may still be useful for infrageneric classification or for use in field identification.

The functional significance and evolutionary advantages of possessing ruminate endosperm are not well understood, due to insufficient knowledge of seed biology and physiology of taxa with ruminate seeds (Bayer and Appel, 1996). I speculate that the increased surface area of the endosperm tissue may confer some as yet unknown benefit to the developing embryo, such as the storage and facilitation of water, and protection from predators. Endosperm tissue ingrowths may contain secondary compounds and oils that make the seeds less attractive to predators (Goebel, 1933; Periasamy, 1990), but to date no studies in *Elaeocarpus* that test this claim have been published.

4.5 Conclusions

The development of ruminations in seed endosperm and the lignification of tissues in drupes may have taxonomic value for some families of angiosperms, particularly in species-rich tropical regions. However, the development of these characteristics within maturing fruit of *Elaeocarpus* has never been studied in detail. Examining developing fruits of a widespread tree species of *Elaeocarpus* in northeastern Australia, I found that lignification of the inner mesocarp layer occurs in the early developmental stages, while endosperm rumination develops as fruits near maturity. Although homoplasious at genus level, endosperm rumination may be a taxonomically useful character at the infrageneric levels, and follow up studies should focus on comparisons between closely-related species.

Chapter 5 – Evolutionary patterns of fruit mesocarp and seed morphology in *Elaeocarpus* (Elaeocarpaceae) and the implications with a focus on the New Guinea radiation.

This chapter investigates the evolutionary patterns of fruit morphology in *Elaeocarpus* and is being prepared for submission to International Journal of Plant Sciences as:

Gagul, J. N., Rozefelds, A., Nauheimer, L. & Crayn, D. M. Evolutionary patterns of fruit mesocarp morphology in *Elaeocarpus* (Elaeocarpaceae).

Some of the material was also presented at the Australasian Systematic Botany Society conference:

Gagul, J. N., Rozefelds, A. & Crayn, D. M. (2016). Fruit mesocarp morphology of *Elaeocarpus* (Elaeocarpaceae): a phylogenetic survey. *Australasian Systematic Botany Conference*. Alice Springs, Australia [poster].

The research in this chapter was conceived by JNG and DMC. JNG conducted the study and wrote the chapter. DMC provided general guidance in molecular phylogenetics. AR assisted with mesocarp morphology interpretation of extant and

fossil species, proof reading and general guidance in paleobotany. LN provided guidance and assisted in the ancestral character state reconstruction of mesocarp and seed characters. They all proofread chapter drafts.

ABSTRACT

Elaeocarpus is the largest genus in the family Elaeocarpaceae with about 360 species. The majority of the species exhibit drupaceous fruits that contain woody fruit stones. However, the evolution of fruit morphology and its value in taxonomy and paleobotanical relevance is poorly known. In the present study, I examined the fruit and seed morphology of 54 extant and 22 fossil species of *Elaeocarpus*, focusing on five characters: mesocarp shape, mesocarp surface ornamentation, embryo shape, endosperm ornamentation and locule number. To infer the evolution of these characters, an ancestral character state reconstruction analysis was performed using a molecular phylogeny of the genus. The results show that the common ancestor of the genus *Elaeocarpus* most likely had ovoid-ellipsoid mesocarps with fibrous ornamentation, entire (non-ruminate) endosperm, and straight embryos. This study is currently the first attempt at placing mesocarp anatomy of *Elaeocarpus* in an evolutionary context. Due to the limitations of sampling for both morphological assessment and phylogenetic reconstruction, the evolution of these traits is only partially understood. Improved estimate of the phylogenetic relationships and a more comprehensive database of mesocarp for the species included in the phylogenetic trees will be required to provide improved knowledge.

Keywords: *Elaeocarpus*, morphology, mesocarp, phylogenetics

5.1 Introduction

The Elaeocarpaceae is a family of trees and shrubs comprising more than 550 species in 12 genera (Coode, 2004), occurring mostly in South America, Australasia, and Southeast Asia, with outliers in Madagascar (Crayn et al., 2006). Within Elaeocarpaceae, fruits are either dehiscent (*Crinodendron*, *Dubouzetia, Peripentadenia*, *Platytheca, Sloanea, Tetratheca*, *Tremandra* and *Vallea*) or indehiscent (berries: *Aristotelia, Sericolea*; or drupes: *Aceratium*, *Elaeocarpus*) (Coode, 2004). In those taxa with drupaceous fruits, the seed coats (which are usually papery or membranous) have no protective function, and the inner layer of the fruit is hardened to protect the seeds (Dettmann and Clifford, 2000). These structures are commonly referred to as fruit stones. Berries in Elaeocarpaceae on the other hand have an outer skin and inner fleshy mass, containing seeds that have a hardened seed coat (e.g. *Sericolea, Aristotelia*).

The term 'endocarp' has been used to describe the woody fruit stones in *Elaeocarpus* by most researchers working on extant and fossil material (Coode 1978, 1984; Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002; Liu et al., in press). However, Dettmann and Clifford (2001) have argued that the term mesocarp is preferable because the woody layer of the fruit is derived from the inner mesocarp. This woody inner mesocarp encases the woody endocarps surrounding each seed. Following the terminology of Dettmann and Clifford (2001) and also to be consistent with recent studies (Gagul et al., 2018a), I use the term mesocarp in this study to refer to the fruit stones of *Elaeocarpus*.

Taxonomic studies on *Elaeocarpus* have been based on a range of vegetative (leaves) and reproductive (embryo, floral, fruit and seed) characters, but only some have included mesocarp morphology (Schlechter, 1916; Smith, 1944; Weibel, 1968; Coode 1978, 1981, 1984, 2005, 2010). In his revision of *Elaeocarpus* of Australia and New Zealand, and Papuasia, Coode (1978, 1981,1984) noted that *Elaeocarpus* mesocarps can be morphologically diverse, but did not study them in detail, focusing mostly on other reproductive and vegetative features. A few studies have documented the morphological diversity in the fruit mesocarps and have studied them in detail (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002). Phoon (2015) examined the evolution of embryo and endosperm characters of seeds of *Elaeocarpus* fruits in a phylogenetic context, concluding that ruminate endosperm and curved embryos are homoplasious traits that have risen independently at least twice and three times respectively in *Elaeocarpus*. Her study provides a good evaluation of the utility of seed morphology to test current infrageneric classifications of the genus. However, her study was limited by the very sparse available data on the occurrence of endosperm rumination and embryo shape across the genus. Further, she did not investigate the evolution of fruit mesocarps.

The woody mesocarps of *Elaeocarpus* fruits are morphologically highly distinctive and vary in size, shape and ornamentation (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002, Gagul et al., 2018). Most *Elaeocarpus* species have deeply ornamented (sculptured) fruit mesocarps, although a few have mesocarps that are poorly lignified consist of persistent fibres within a soft matrix (*E. blepharoceras* Schltr., *E. johnsonii* F.Muell.; *E. sedentarius* Maynard & Crayn). *Elaeocarpus*

mesocarps fossilize readily and can sometimes be conspicuous in fossil deposits, and therefore comparisons with extant species are possible (Dettmann and Clifford, 2000; Rozefelds and Christophel, 1996a, b, 2002; Liu et al., in press). For instance, fossil *Elaeocarpus* fruits from Australia, New Zealand, India and China have been compared with extant species in their respective floras, and these studies suggest that fruit morphology may provide characters that may be useful in defining species groups (clades) within the genus (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002; Bera et al., 2004; Liu et al., in press). Additionally, these fossils can potentially be used to calibrate molecular evolutionary clocks, enabling the derivation of minimum dates for the divergence times of clades on phylogenetic trees (Crayn et al., 2006; Phoon, 2015).

Rozefelds (1990) recognised that significant variation occurred in mesocarp morphology, which allowed him to match fossil species to groups of extant species (Rozefelds, 1992; Rozefelds and Christophel, 1996a, b). Similarly, Dettmann and Clifford (2000) noted that fruit mesocarps displayed high morphological variation in Australian species and recognised a number of distinct morphotypes. They have used two extant species (*E. angustifolius* with straight and *E. reticulatus* Sm. with curved embryos respectively: see Weibel 1968) as a basis for comparison with their 16 fossil species, which they grouped into five mesocarp types based on surface ornamentation (Dettmann and Clifford, 2000). Rozefelds and Christophel (2002) accepted some of Dettmann and Clifford's groupings such as the punctate and smooth but recognised the fossulate type as bastionate. In total they recognised seven distinct ornamentation types in *Elaeocarpus* – *baculate*, *bastionate*, *echinate*, *granulose*, *punctate*, *smooth* and *verrucate* – and two mesocarp shapes – spherical and ovoid-ellipsoid) (Appendix

4.1). In a recent study by Liu and colleagues (in press), six new fossil species from China were described and matched to the extant species using mesocarp morphology. They included an additional ornamentation type (*rugose*), bringing the total to eight. Their study reported the first reliable fossils of *Elaeocarpus* mesocarps from East Asia, indicating the genus had already colonized there by the late Oligocene. The scope of those studies has concentrated on fruit specimens of *Elaeocarpus* from Australia, New Zealand and East Asia particularly China. The current study revisited the groupings proposed by Dettmann and Clifford (2000), Rozefelds and Christophel (2002) and Liu et al. (2020), and tested those categories against new data from the additional species I examined from New Guinea, New Caledonia and western Malesia, with additional seed characters pertaining to the embryo, endosperm and locules.

5.2 Materials and methods

5.2.1 Taxon sampling

I surveyed 54 extant and 22 fossil species (16 from Australia and New Zealand plus 6 from China) of *Elaeocarpus* fruit mesocarps (stones) (Table 5.1). For fossil species, information on ornamentation, mesocarp shape, locule numbers, age range and geographical localities was obtained from the literature. For extant species, five mesocarps per species were sampled from specimens from the Queensland Herbarium (BRI), Australian National Herbarium (CANB) and Australian Tropical Herbarium (CNS) and those used previously by Rozefelds (1990). For the following species, field collections were undertaken to source fruits not available on accessible herbarium specimens, or to supplement existing collections: *E. angustifolius* (field

collection - J. Gagul 18), *E. blepharoceras* (J. Gagul 22), *E. culminicola* (J. Gagul 31), *E. dolichostylus* var. *hentyi* (J. Gagul 22), *E. polydactylus* (J. Gagul 12), *E. ptilanthus* (J. Gagul 8), *E. womersleyi* (J. Gagul 26) (Appendix 5.1). The identities of the examined specimens were checked against current literature and in some cases confirmation was sought from experts (e.g. Mark Coode, Edinburgh, UK).

Only mature fruits were sampled as determined by the coloration of outer mesocarps in the fresh state described on herbarium sheet labels (blue in most species, except a few e.g., *E. ruminatus*, brownish-green or dull blue).

5.2.2 Specimen preparation and measurement

5.2.2.1 Extant taxa

For extant species, both preserved or dried and fresh materials were used. Mature fruits from herbarium specimens and spirit collections were rehydrated by soaking in water for 1 – 5 days to soften the outer mesocarps. To avoid using immature fruits, field labels on the specimens were consulted for record of blue coloration, which indicates maturity of the fruits in most *Elaeocarpus* species (although not all fruits turn blue at maturity, e.g. *E. ruminatus* turns brown). The outer mesocarp and exocarp of the rehydrated fruits were removed using scalpel blades and forceps and cleaned thoroughly with a toothbrush to expose the mesocarp surface. In a few species, however, radial mesocarp fibres are permanently attached to the mesocarp making it difficult to expose the surface. In these cases, whole fruits were transversely sectioned to view the internal anatomy.

Fruits were sectioned transversely using an array of fine-toothed saws (fret wood cutting saw). Large fruits were clamped to the sides of a table or wooden cutting board to hold them in place during sectioning, while small fruits were sectioned using scalpel blades. Transverse sections (TS) were polished using a series of progressively finer sandpaper grades - 80, 160, 240, and 400 grit. The sectioned surfaces were washed thoroughly between each stage of polishing to remove any grit.

5.2.2.2 Fossil species

Information on fossil species was mostly obtained from the literature (Dettmann and Clifford, 2000; Rozefelds and Christophel, 1996a, b; Rozefelds, 1992, 1990; Liu et al., in press), and observations of fossil materials from the Queensland Museum. Data for fossil material included mesocarp shape, mesocarp ornamentation, locule number, age-range, and locality.

Both qualitative data (e.g. mesocarp shape, ornamentation type, embryo and endosperm shapes) and quantitative data (e.g. mesocarp dimensions, and locule number) were scored into a data matrix (Appendix 5.1). For the quantitative data, measurements were made using the graduated scale on an Olympus S30 or S40 stereoscope, vernier caliper or a hand-held ruler. Up to five mesocarps per specimen were sampled and length and width measurements were made for each mesocarp.

5.2.3 Characters and definitions for mesocarps

Rozefelds and Christophel (2002) identified seven distinct ornamentation types in *Elaeocarpus*, namely *baculate, bastionate, echinate, granulose, punctate, smooth* and *verrucate*. Liu et al. (in press) recorded *rugose* ornamentation in their

study, bringing the ornamentation types to eight. Descriptions of those eight characters, with additional information from the current study and a list of species possessing the character types are provided in Appendix 5.1. Within those characters, there are also variations. For instance, within the punctate ornamentation, there is 'punctate and pitted', and 'punctate with longitudinal ridges appearing stellate in TS'. Throughout the chapter, I use punctate for 'punctate and pitted' as they both refer to the same surface description. The current study has added an additional character 'fibrous' (Figs 5.1, 5.4, 5.7, 5.9). The study also identified mesocarps that do not match Rozefelds and Christophel's scheme, and categorised them as 'miscellaneous' types. Thus, nine distinct mesocarp morphologies were defined and selected for analysis (Appendices 5.1, 5.3).

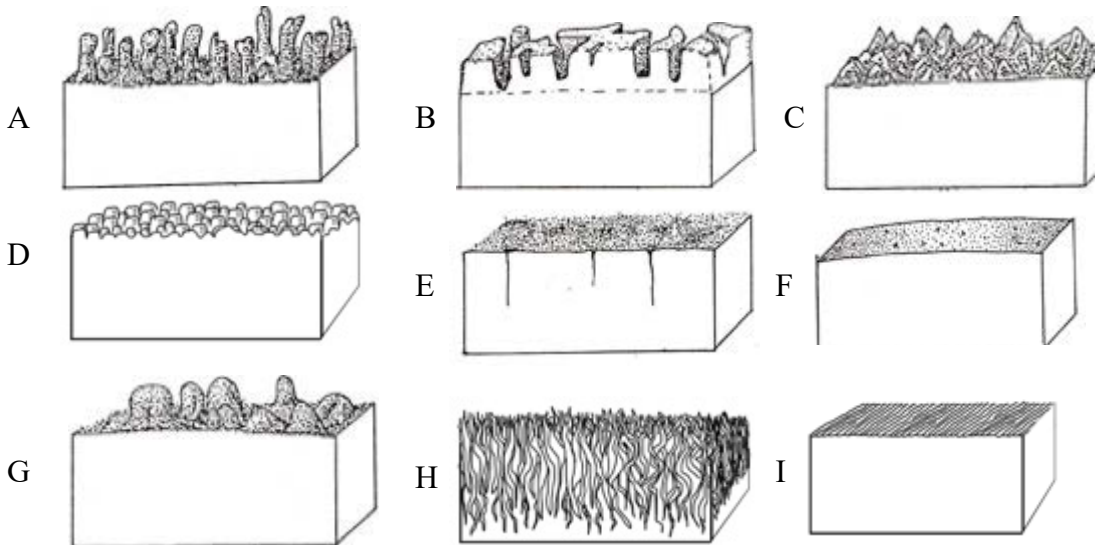


Figure 5.1 Patterns of surface sculpturing of *Elaeocarpus* mesocarps.

A. baculate, B. bastionate, C. echinate, D. granulose, E. smooth or almost smooth, F. punctate, G. verrucate, H. fibrous, I. rugose. Line drawings A, B, C, E, F and G

are reproduced with permission from Rozefelds (1990) and Rozefelds (1996), and D, H and I were drawn by the author.

5.2.4 Characters and definitions for seeds

Coode (1984) studied embryo and endosperm characters in seeds of *Elaeocarpus* in detail and observed that the endosperm was either entire or ruminate (Fig. 5.2 A, B), and the embryo was either straight or curved (Fig. 5.2 B, C).

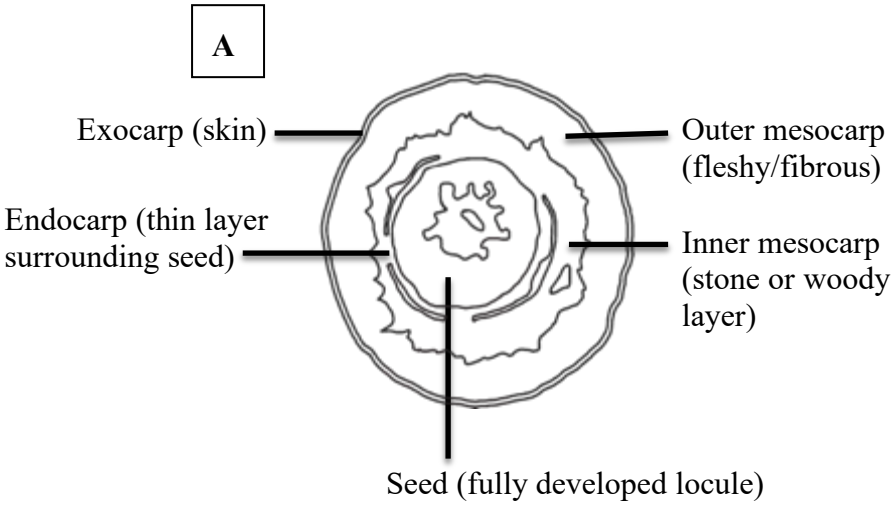
In the current study, seeds are fully developed ovules, and number of seeds per fruit is determined using sectioned fruits.

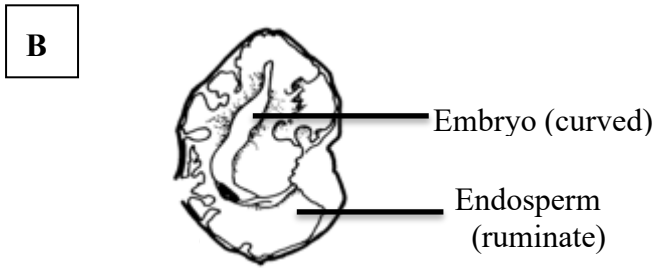
Variation in fruit morphology was scored in terms of the following five characters: shape and surface ornamentation of stone for mesocarp, and embryo shape, endosperm ornamentation and locule numbers for seeds. The geographical location of surveyed specimens was also recorded.

1. Mesocarp shape – two broad state categories were observed: ovoid-ellipsoid (0) and spherical (1).
2. Mesocarp surface ornamentation or sculpturing – nine broad categories were observed: baculate - surface bearing rod-like projections with obtuse to rounded apex (0), bastionate - surface appearing brain-like, sometimes with prominent tunnels underneath (1), echinate - surface bearing sharp, raised processes (2), fibrous - surface obscured by attached radial mesocarp fibres (3), granulose - surface bearing small, subcircular to circular grain like structures (4), punctate - surface irregularly pitted (5), punctate with stellate ridges (6), rugose - surface appearing wrinkly (7), smooth or almost smooth (8) and verrucate - surface with

irregular rounded to globular structures (9). Specimens with obscure surface characters that I could not confidently assign to a category were considered ‘miscellaneous’. (Fig. 5.1)

3. Embryo shape – three broad character states: straight (0), curved (1), straight with hooked tip (2) (Fig. 5.2)
4. Endosperm ornamentation – two states: entire (0), ruminate (1) (Fig. 5.2)





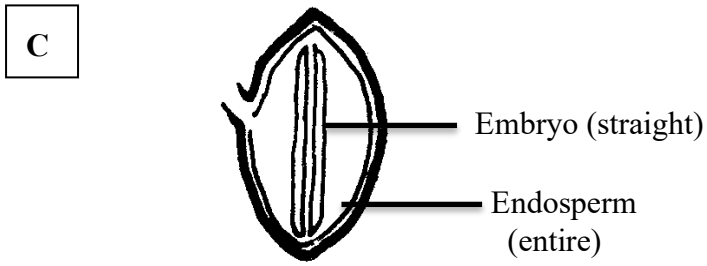


Figure 5.2 Sections of *Elaeocarpus* fruit and seed.

A. Transverse section of a whole fruit, B. Transverse section of a curved embryo with ruminate endosperm, C. Transverse section of a straight embryo with entire

endosperm (Coode, 1984; Phoon, 2015, Gagul et al., 2018a). Illustrations have been modified from Gagul et al. (2018) (A), and Phoon (2015) (B, C).

Table 5.1 Source of fruit samples of *Elaeocarpus* species.

Abbreviations of herbaria follow Index Herbariorum: BRI – Queensland Herbarium, Brisbane, Australia; CANB – Australian National Herbarium, Canberra, Australia; CNS – Australian Tropical Herbarium, Cairns, Australia; K – Royal Botanic Garden, Kew, United Kingdom; MBK – The Kochi Prefectural Makino Botanical Garden, Japan; NSW – National Herbarium of New South Wales, Australia. All samples of the extant species have been seen by the author except otherwise stated. Information of fossil species has been taken from the literature. They are indicated by an asterix (*). AMF – Australian Museum; GST – Geological Survey of Tasmania; NMVP – Museum of Victoria; QMP – Queensland Museum; SYS – The Museum of Biology, Sun Yat-sen University, Guangzhou, China.

Species name	**Collector and collector/museum number**	**Institution or herbarium/museum code**
E. alaternoides Brongn. & Gris	P. Morat 6288	BRI
E. altigenus Schltr.	Walker ANU 739	CANB
**E. angularis* (F.Muell.) Selling	NMVP 53565, NMVP 6017	NMVP
E. angustifolius Blume	J. Gagul 18	CNS
E. arnhemicus F.Muell.	B. Hyland 11243	BRI
E. bancroftii F.Muell.	B. Gray 2328	BRI
**E. bivalve* (F.Muell.) Dettmann &	MMF 36220	Unknown

Clifford		
E. blepharoceras Schltr.	J. Gagul 2	CNS
E. braceanus Watt ex C.B.Clarke	K. Fujikawa 94360	MBK
**E. brachyclinis* (F.Muell.) Selling	NMVP 6060	NMVP
E. carbinensis J.Gagul & Crayn	B. Gray 5197	CNS
E. carolinae B.Hyland & Coode	B. Hyland 3171 RFK	BRI
**E. cerebriformis* Rozefelds & Christophel	UAY001	Unknown
**E. clarkei* (F.Muell.) Selling	AMF 9281	AMF
E. coorangooloo J.F.Bailey & C.T.White	B. Hyland 12637	BRI
**E. couchmanii* (F.Muell.) Dettmann & Clifford	NMVP 53920	NMVP
E. culminicola Warb.	J. Gagul 31	CNS
**E. cunningii* Rozefelds	QMF 16768	QMF
E. dentatus (J.R.Forst. & G.Forst.) Vahl	Specimen not vouchered	BRI
E. dolichostylus Schltr.	YS3G0274	CNS
E. dolichostylus var. *hentyi*	J. Gagul 22	CNS
E. elliffii B.Hyland & Coode	A. K. Irvine 1478	BRI
E. eumundi F.M.Bailey	B. Gray 3278	BRI
E. ferruginiflorus C.T.White	B. Hyland 13460	BRI
E. foveolatus F.Muell.	B. Hyland 13654	BRI
E. fuscoides R.Knuth	P. van Royen NGF15061	CANB

E. glaber Blume	A.J.G.H. Kostermans 56	BRI
E. grahamii F.Muell.	B. Hyland 13428	BRI
E. grandis F.Muell.	B. Gray 2749	BRI
E. griffithii (Wight) A.Gray	F. H. Endert 2028	BRI
E. habbemensis A.C.Sm.	T. G. Hartley 11707	CANB
E. holopetalus F.Muell.	Constable 6978 (QRS 001692)	BRI
E. hylobroma Y.Baba & Crayn	L. Brass 221	BRI
E. johnsonii F.Muell.	T. S. Risley 428	BRI
**E. johnstonii* (F.Muell.) Dettmann & Clifford	Fig. 60-a. Mueller in Johnston, 1882.	Unknown
E. kirtonii F.Muell. ex F.M.Bailey	A. K. Irvine 1414	BRI
E. largiflorens C.T.White subsp. *largiflorens*	B. Hyland 13745	BRI
E. ledermannii Schltr.	P. J. Darbyshire 8255	CANB
E. linsmithii Guymer	B. Hyland 13606	BRI
**E. lynchii* (F.Muell.) Selling	NMVP 6033, NMVP 6034	NMVP
**E. mackayi* (F.Muell.) Kirchheimer	NMVP 53562	NMVP
E. miegei Weibel	A. Gillinson NGF25754	CANB
**E. muelleri* Ettingsh.	Pl.14, Fig. 4 in Ettingsh., 1886.	Unknown
E. multiflorus (Turcz.) Fern.-Vill.	Kuswata 287	BRI
E. multisectus Schltr.	J.S. Womersley NGF24978	BRI

**E. nanningensis* Liu & Jin	NN-334	SYS
E. nubigenus Schltr.	R. Pullen 5405	CANB
**E. occulatus* Rozefelds & Christophel	AMF 11111	AMF
**E. peteri* Rozefelds & Christophel	QMF 18088	QMF
**E. pleioclinis* (F.Muell.) Dettmann & Clifford	NMVP 53747	NMVP
E. polydactylus Schltr.	J. Gagul 17	CNS
E. polystachyus Wall. ex Mull.Berol	Kia 32414	BRI
**E. presikkimensis* Liu & Jin	GP-001	SYS
**E. prerugosus* Liu & Jin	ZL-012	SYS
**E. prelacunosus* Liu & Jin	ZL-006	SYS
**E. preserratus* Liu & Jin	ZL-018	SYS
**E. preprunifolioides* Liu & Jin	ZL-153	SYS
E. ptilanthus Schltr.	J. Gagul 8	CNS
E. pycnanthus A.C.Sm.	P. van Royen NGF18259	CANB
E. reticulatus Sm.	Specimen not vouchered	Specimen not vouchered
E. robustus Roxb.	Ngadiman 34726	BRI
E. rotundifolius Brongn. & Gris	G. McPherson 4852	BRI
**E. rozefeldsii* Dettmann & Clifford	QMF 50123	QMF
E. ruminatus F.Muell.	Drew 76	BRI

E. sarcanthus Schltr.	J. Womersleyi NGF19498	CANB
E. sedentarius Maynard & Crayn	D.J. Maynard DJM 02 (Fig. 3 in Maynard et al., 2008)	NSW
E. sericopetalus F.Muell.	B. Gray 3030	BRI
E. seringii Montrouz.	G. McPherson 5556	BRI
**E. spackmaniorum* Rozefelds	QMF15440	QMP
E. stellaris L.S.Sm.	G. C. Stocker 1774	BRI
E. sterrophyllus Schltr.	J. Gagul 14	CNS
E. tariensis Weibel	J.S. Womersley NGF43624	BRI
E. thelmae B.Hyland & Coode	B. Hyland 13508	BRI
**E. trachyclinis* (F.Muell.) Selling	NMV 53758	NMV
E. trichophyllus A.C.Sm.	T. G. Hartley 13253	BRI
E. weibelianus Tirel	G. McPherson 1728	BRI
E. womersleyi Weibel	J. Gagul 26	CNS

5.2.5 Data analysis

To infer the character evolution of mesocarp and seed morphology in *Elaeocarpus*, I reconstructed the phylogeny of the genus and performed an ancestral character state reconstruction (ASR) based on this phylogeny. The molecular dataset from Chapter 3 was used, but optimized for the ASR (see below).

5.2.5.1 Molecular dataset

The molecular dataset consisted of three chloroplast markers: *trnV-ndhC*, *trnH-psbA* and *trnL-trnF*. These markers were selected to provide molecular estimates of the phylogeny, as they have been used successfully in molecular phylogenetic studies of *Elaeocarpus* and have proven to be generally informative at the species level. The dataset used in Chapter 3 was optimized for the ancestral state reconstruction by removing all non-*Elaeocarpus* samples except for three *Sericolea* samples that were used for outgroup rooting. In addition, *E. holopetalus* was removed, as it was not placed with the remaining *Elaeocarpus* samples in the broader analysis undertaken in Chapter 3. The alignment was further optimized by removing all but one sample per taxon as well as by removing identical sequences. The final dataset comprised 54 samples.

5.2.5.2 Alignment and phylogenetic reconstruction

Sequences were aligned using MAFFT v7.450 (Katoh and Standley, 2013) and the alignment was subsequently corrected manually. Phylogenetic trees were reconstructed using MrBayes v3.2.6 (Ronquist and Huelsenbeck, 2003) with the GTR+G model and four gamma categories, two runs of four heated chains each with

two million generations each and a sample frequency of 1000. An allcompat consensus tree was generated after a burn-in of 25%. Clade support for the consensus tree was estimated using posterior probability of clades across the sampled states.

5.2.5.3 Character state scoring and matrix

A character state matrix was generated for each of the five mesocarp and seed morphology characters investigated: mesocarp shape, surface ornamentation, embryo shape, endosperm rumination and locule number. A total of 76 species (54 extant and 22 fossil species) were examined in this study. However, only the extant taxa that were included in the molecular phylogenetic analysis (Chapter 3) were scored for ancestral state reconstruction.

Those species which had obscure surface characters or which had mesocarps that were too small to confidently interpret were deemed ‘miscellaneous’ and scored in the matrix as missing data. Those specimens are illustrated in Fig. 5.10, and tend to be the smaller fruits, on which the surface characters are more difficult to observe and interpret. Character state coding is provided in a matrix table (Appendix 5.4).

5.2.5.4 Ancestral character reconstructions

Ancestral character states were reconstructed for each of the five morphological characters. The ASR was performed in the software RASP 4.0 (Yu et al., 2020) using the Bayesian Binary MCMC (BBM) method (Ronquist and Huelsenbeck, 2003) based on the MrBayes consensus tree. The Markov Chain Monte Carlo analyses were performed with 10 heated chains of 50,000 cycles sampling

every 100 generations and discarding the first 100 sampled stated. The model for state evolution was set to fixed frequencies and equal among site variation. Only nodes with posterior probability of at least 0.9 in the consensus tree were taken into account for the reconstruction of ancestral states to avoid misinterpretation of unsupported nodes. Because not all tips had character states assigned, nodes that have no descendant with states were not taken into account for reconstruction.

5.3 Results

In this study, I investigated the fruit morphology of 76 species including 54 extant species from New Guinea, Australia, New Zealand, New Caledonia and Malesia (Appendix 5.1), 16 fossil species from Australia, representing 11 morphological groups (Groups I, IV, V, VI, VII, VIII, XI, *Coilopetalum, Obovatus, Oreocarpus, Polystachyus*), and 6 fossil species from China (Appendix 5.2). Using my observations, I tested the ability of the mesocarp classification groupings of Rozefelds and Christophel (2002) to characterise the variation in fruit morphology across the genus. I found that the most of the species studied could be placed into the existing categories. However, 15 samples from my survey did not fit in any category mostly due to obscurity in the surface characters in smaller fruits, a majority of which are from New Guinea (Fig. 5.10).

I compiled the following data based on the surface characters. The numbers in brackets represent the number of species with the surface type: bastionate (15), baculate (1), echinate (9), fibrous (3), granulose (6), punctate (9), rugose (7), smooth (7) and verrucate (2) ornamentation (Figs 5.3 – 5.10, Appendices 5.1, 5.3). Species that are described as having fibrous mesocarps include *E. blepharoceras, E. sedentarius* and *E. johnsonii*. These species have fibres that are persistent and permanently attached to the inner mesocarps. A morphologically unrelated species (*E. womersleyi*) also has fibres on the mesocarps, but the fibres are not persistent and are only attached to the surface of the outer mesocarp. Those fibres eventually rot away and expose a punctate mesocarp surface (Figs 5.4, 5.8, Appendix 5.3). *Elaeocarpus johnsonii* has mesocarps that are obovoid in shape (with a truncate base and acute apex) (Fig. 5.4, Appendix 5.3) in contrast with the irregularly spherical mesocarps in

E. blepharoceras, the spherical mesocarps in *E. womersleyi*, and the spherical to 3–4 lobed mesocarps in *E. sedentarius*.

5.3.1 Mesocarp morphology observation and description

Mesocarp morphology is highly variable within the genus and ranges from soft and fibrous to thick and woody. In the woody mesocarps, the inner portion is lignified and becomes robust in the developmental process, which renders them amenable to fossilisation. Dettmann and Clifford (2000), Rozefelds and Christophel (2002) and Liu et al. (2020) have documented mesocarp morphologies of both extant and fossil species. Those studies, however, focused mainly on the species of *Elaeocarpus* from Australia, New Zealand and China, and did not include species with fibrous mesocarps. Although species with fibrous mesocarps may not fossilize readily compared to woody stones, they are important in classification. In the current study, the mesocarp data from Dettmann and Clifford (2000), and Rozefelds and Christophel (2002) was revisited and their trait classification tested against new observations.

Elaeocarpus fruits have lignified inner mesocarps (stones) surrounded by either succulent (composed of parenchyma cells) or fibrous outer mesocarps and an external thin epicarp (skin). A majority of the species have thick, strongly lignified mesocarp walls, but a few have poorly lignified mesocarps with soft and thin walls, such as those with fibrous mesocarps (Fig. 5.4).

Figs 5.3–5.10. Variation in mesocarp surface ornamentation in *Elaeocarpus*, arranged according to the infrageneric grouping.

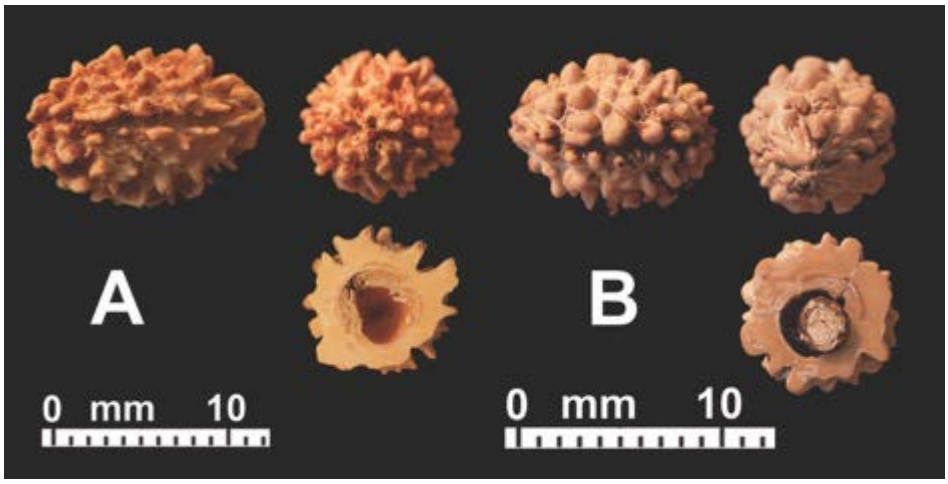


Figure 5.3 Selected mesocarps of *Elaeocarpus* species from the *Obovatus* group.

Mesocarps exhibit baculate (A), and verrucate (B) ornamentation. A. *Elaeocarpus arnhemicus*, B. *E. coorangooloo.* Seeds of A were lost during sectioning.

Figure 5.4 Whole fruits of *Elaeocarpus* species from Group IV showing soft and poorly lignified inner mesocarps and fibrous outer mesocarps.

The fibres are permanently attached to the surface of the inner mesocarps. A. *Elaeocarpus sedentarius* (spherical to 3–4 lobed), B. *E. blepharoceras* (irregularly spherical), C. *E. johnsonii* (obovoid with truncate base, acute apex). Image A was reproduced from Maynard et al. (2008) with permission.

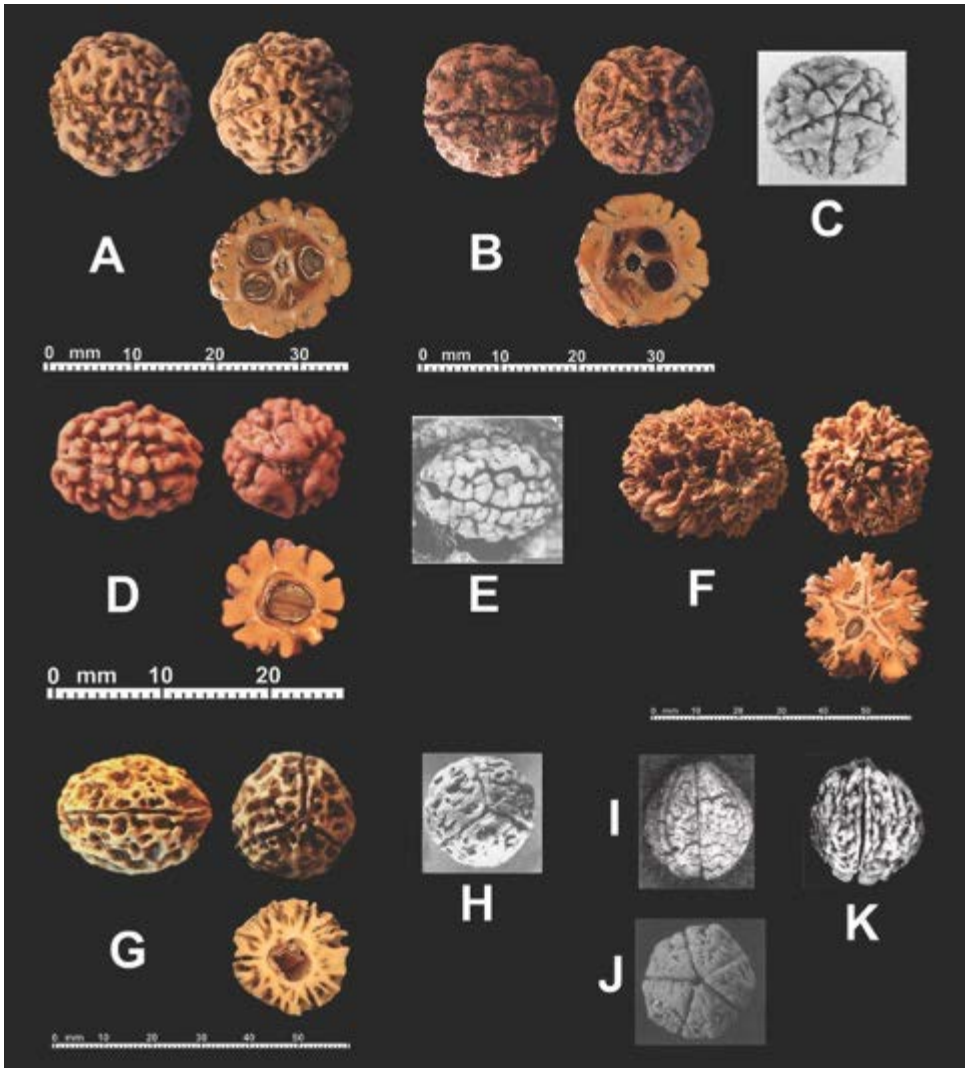


Figure 5.5 Selected mesocarps of *Elaeocarpus* species from the *Ganitrus* group. These mesocarps show showing bastionate ornamentation. Fossil species are indicated by an asterix (*). A. *Elaeocarpus angustifolius*, B. *E. dolichostyllus*, C. **E. spackmaniorum*, D. *E. hylobroma*, E. **E. cerebriformis*, F. *E. ptilanthus*, G. *E. braceanus*, H. **E. mackayi*, I. **E. trachyclinis*, J. **E. couchmanii*, K. **E. occultus*. *Elaeocarpus ptilanthus* has extreme bastionate structure, with tunnels or cavities underneath. Images of fossil species were reproduced with permission from Dettmann and Clifford (2000) and Rozefelds and Christophel (1996a, 2002). Seeds of B were removed.

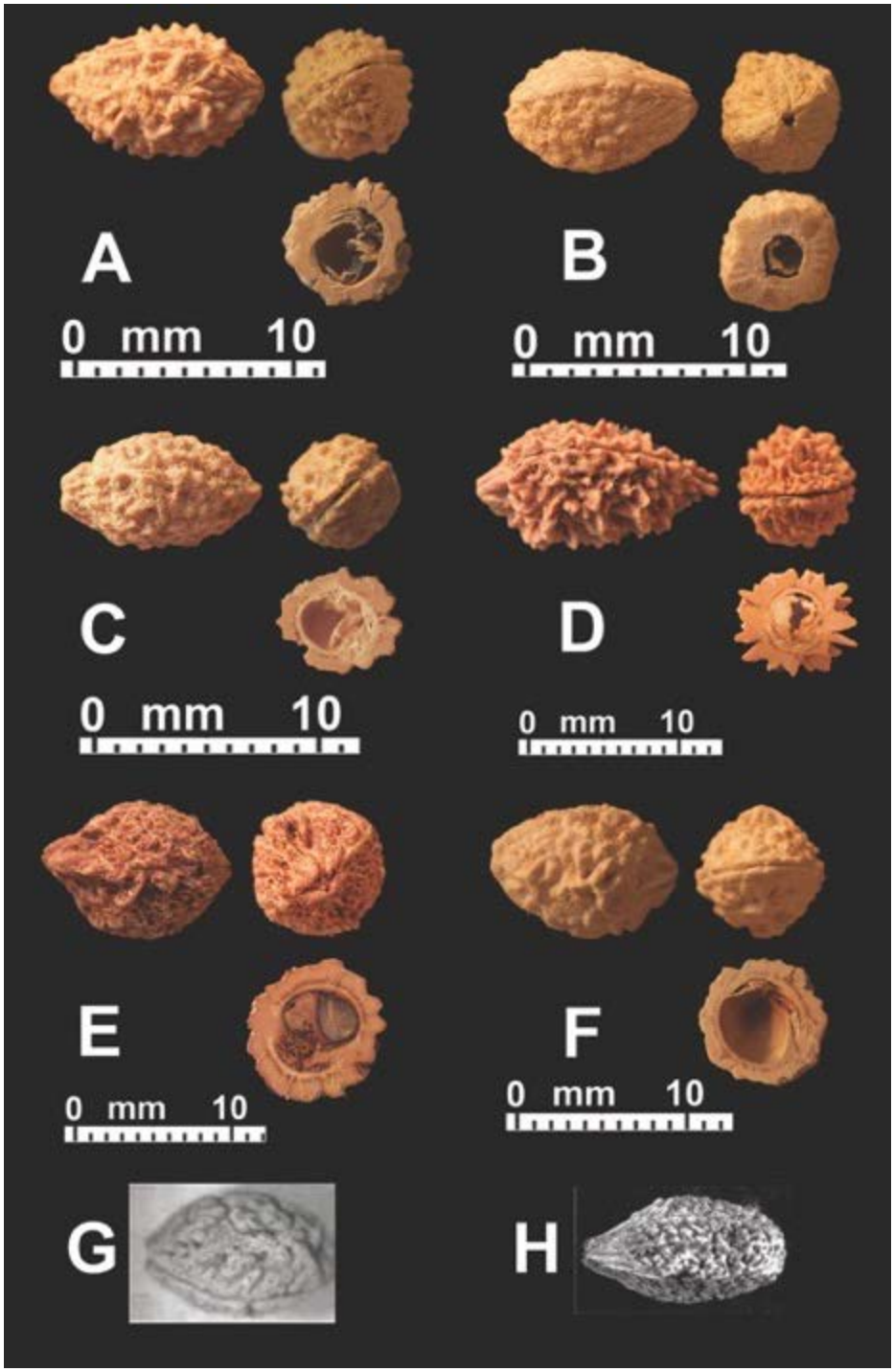


Figure 5.6 Selected mesocarps of *Elaeocarpus* species from Group VII.

These mesocarps exhibit echinate ornamentation. Fossil species are indicated by an asterix (*). A. *Elaeocarpus grahamii*, B. *E. carolinae*, C. *E. reticulatus*, D. *E. eumundii* (strongly echinate), E. *E. culminicola*, F. *E. kirtonii*, G. **E. cunningii*, H. **E. lynchii*. Images G and H were reproduced with permission from Dettmann and Clifford (2000). Seeds of A, B, C and F were removed.

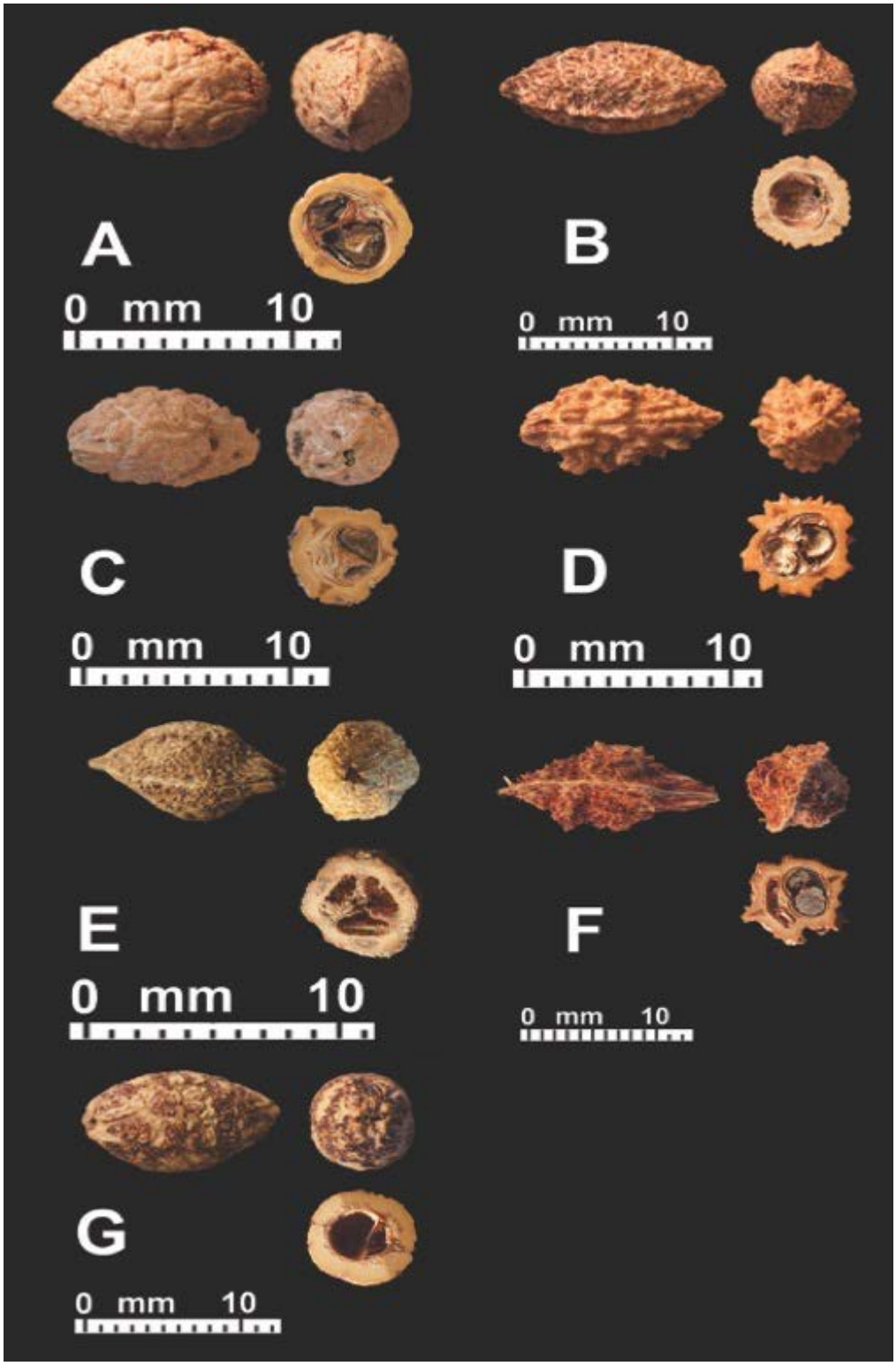


Figure 5.7 Selected mesocarps of New Guinean *Elaeocarpus* species from Group VIII.

These mesocarps exhibit rugose (A, C, D, F) or granulose ornamentation (B, E, G). A. *Elaeocarpus altigenus*, B. *E. fuscoides*, C. *E. habbemensis*, D. *E. ledermannii,* E. *E. pycnanthus*, F. *E. sarcanthus*, G. *E. trichophyllus*. Seeds of B and G were removed.

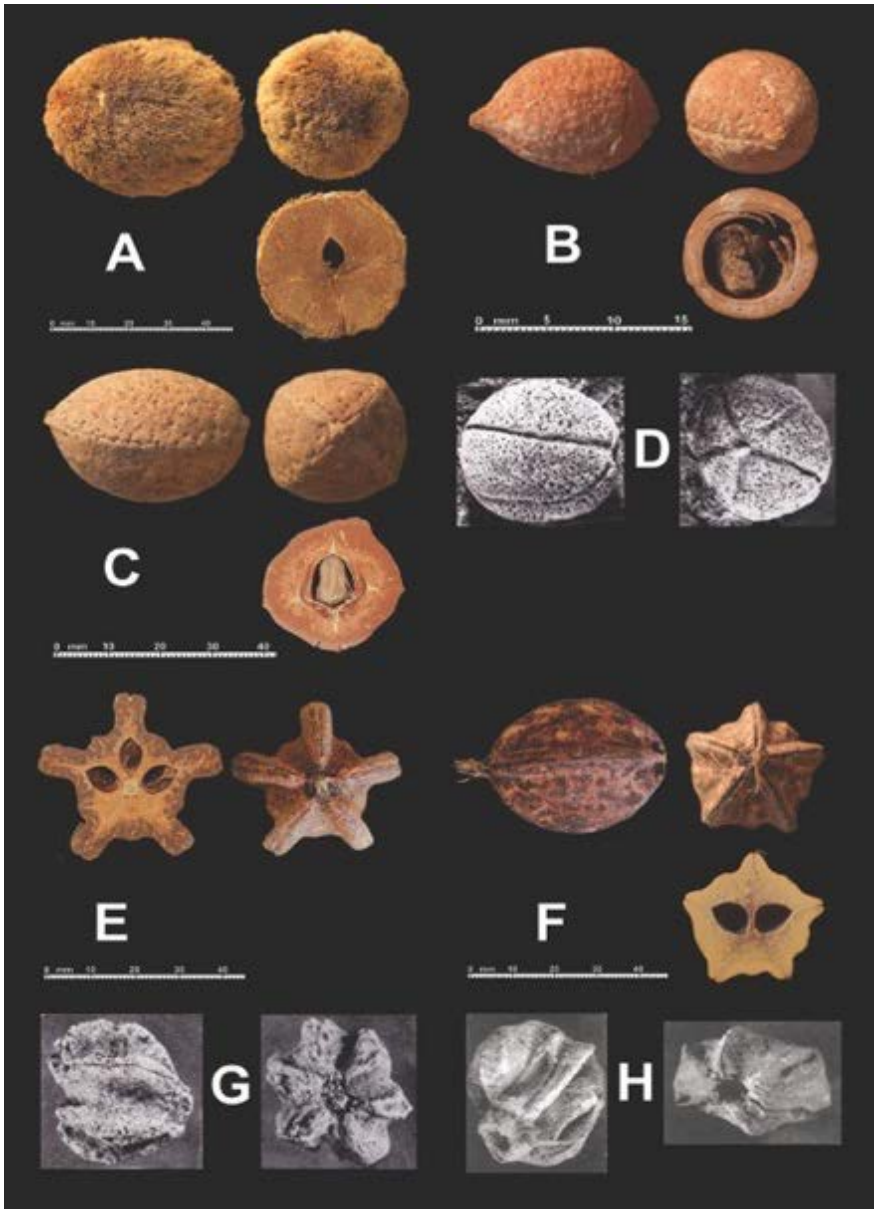


Figure 5.8 Mesocarps of *Elaeocarpus* species from Group VI.

These mesocarps exhibit surfaces that are punctate (A, B, C, D), or punctate with longitudinal ridges and appearing stellate in transverse section (E, F, G, H). A. *Elaeocarpus womersleyi* (the mesocarp surface of *E. womersleyi* appears punctate when fibres are removed), B. *E. linsmithii*, C. *E. bancroftii*, D. **E. clarkii*, E. *E. stellaris* (more prominent ridges), F. *E. carbinensis* (less prominent ridges), G. **E. peteri*, H. **E. rozefeldsii*. Images A, B, C and F were photographed by Nick Rockett; D, G and H were reproduced from Rozefelds (1990) with permission. Fossil species are indicated by an asterix (*). Seeds of A and F were removed.

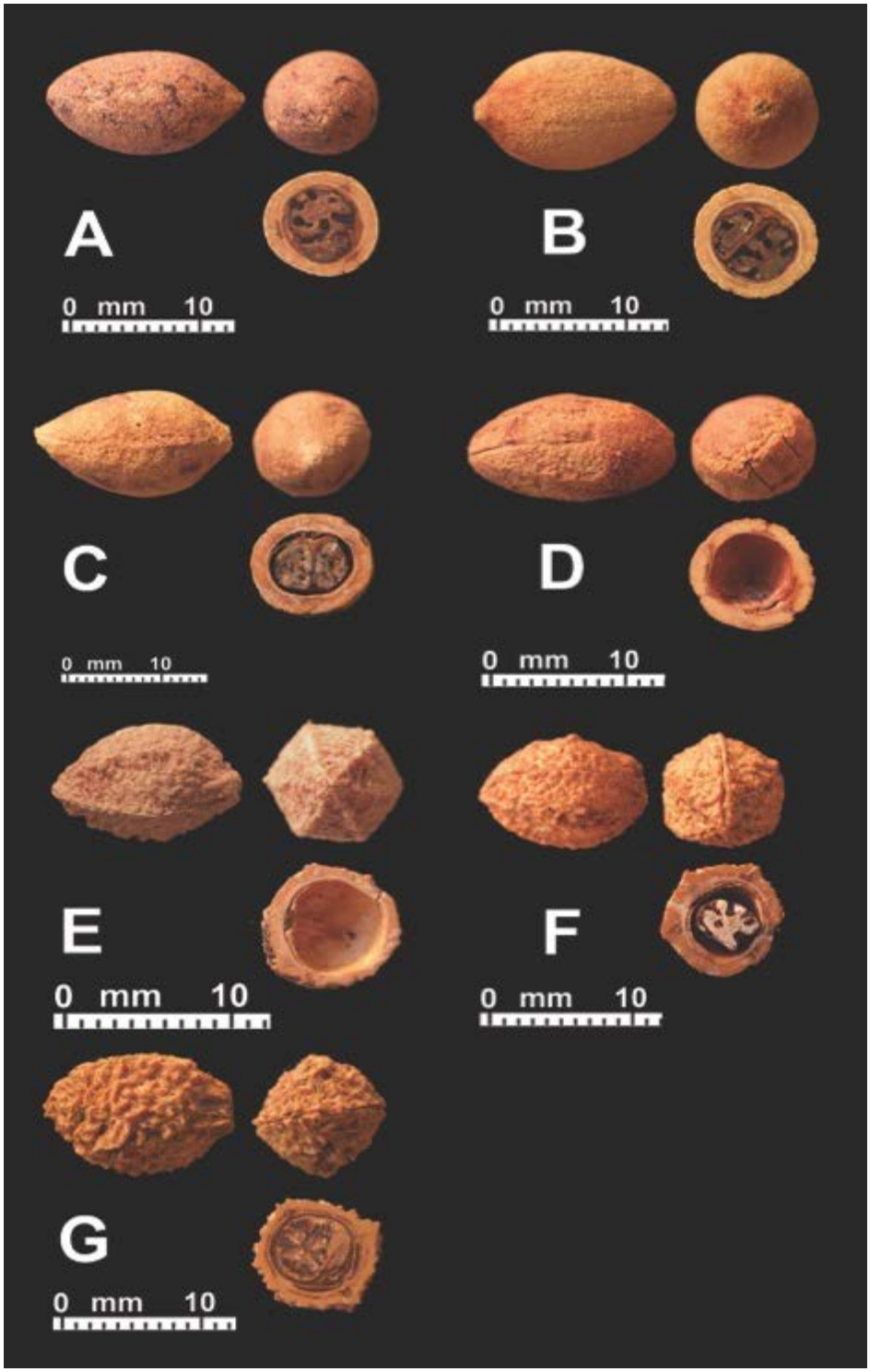


Figure 5.9 Selected mesocarps of *Elaeocarpus* species from Group XI.

These mesocarps exhibit smooth or almost smooth surface ornamentation (A, B, C, D), granulose (E, F), rugose (G). A. *Elaeocarpus foveolatus*, B. *E. ferruginiflorus*, C. *E. thelmae,* D. *E. largiflorens* subsp. *largiflorens*, E. *E. elliffii,* F. *E. sericopetalus,* G. *E. ruminatus*. Seeds of D and E were removed.

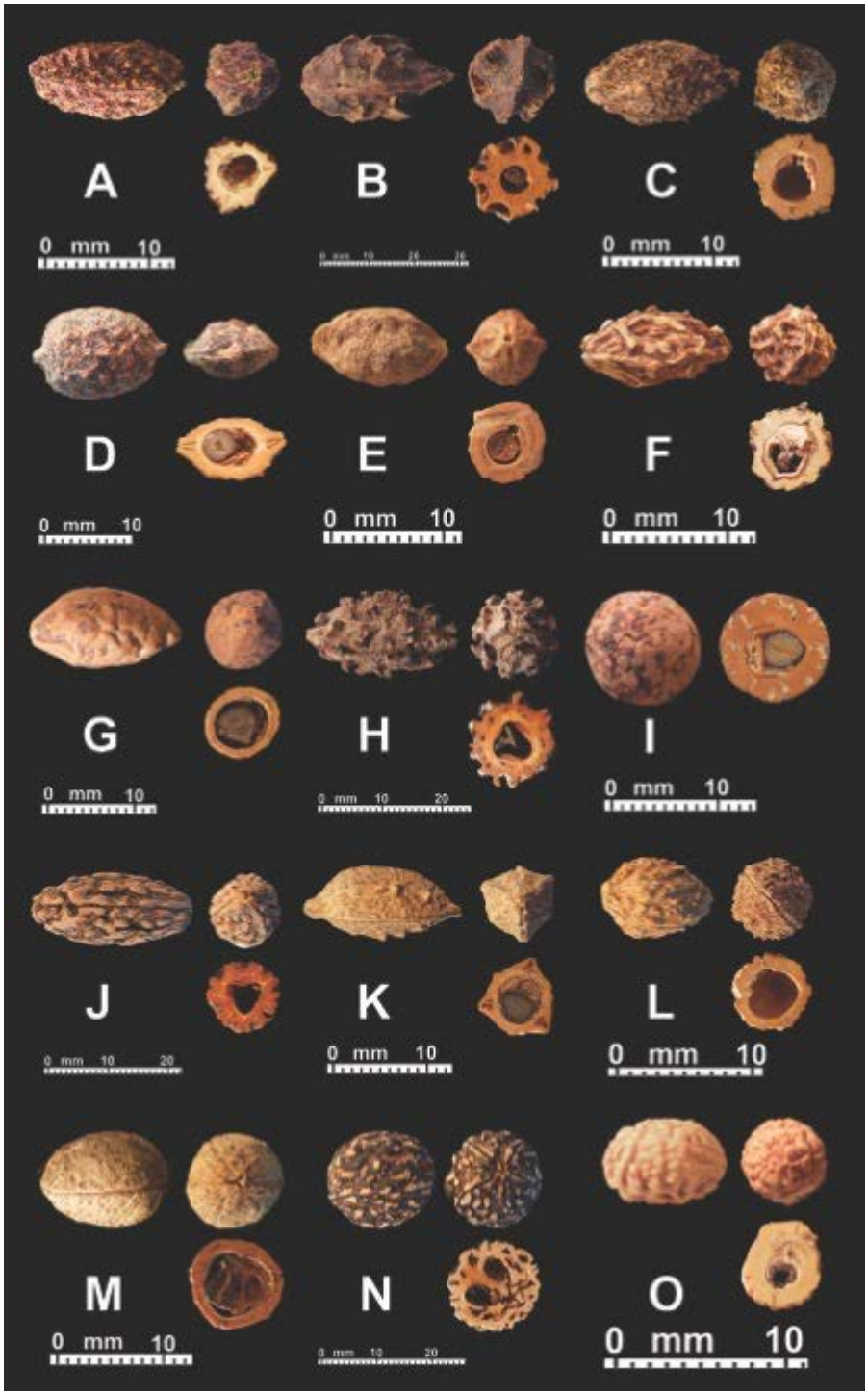


Figure 5.10 Miscellaneous mesocarps of *Elaeocarpus* species.

These mesocarps do not match the current surface ornamentation grouping by Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002, A. *Elaeocarpus alaternoides*, B. *E. weibelianus*, C. *E. seringii*, D. *E. rotundifolius,* E. *E. dentatus*, F. *E. miegei*, G. *E. tariensis*, H. *E. multisectus*, I. *E. nubigenus*, J. *E. glaber*, K. *E. multiflorus*, L. *E. griffithii*, M. *E. polystachyus*, N. *E. robustus*, O. *E. holopetalus*. Seeds of C, J and L were removed.

5.3.2 Phylogeny

The phylogeny reconstructed by Bayesian analysis of 231 samples is shown in Appendix 5.6. Relatively few nodes receive strong support (posterior probability values >50%). Some key clades resolved by this analysis, which are consistent with those resolved by the more inclusive analysis undertaken in Chapter 3 are as follows:

Group IV (PP 1.0): *E. blepharoceras* and *E. sedentarius*;

Obovatus group (PP 1.0): *E. arnhemicus, E. coorangooloo* and *E. obovatus*;

Group V (*Ganitrus* group, PP 1.0): *E. hylobroma*, *E. altisectus*, *E. murukkai*, *E. polydactylus*, *E. nubigenus*, *E. dolichostylus*, *E. dolichostylus* var. *hentyi*, *E. carolinensis*, *E. grandis*, *E. sphaericus*, *E. ptilanthus*, *E. angustifolius*, *E. kaniensis*;

Group VII (PP 1.0): *E.* sp. G04568, *E. culminicola*, *E. sterrophyllus*, *E. reticulatus*, *E. grahamii*, *E. kirtonii*, *E. carolinae*, *E. linsmithii*, *E. eumundi*, *E. multiflorus*.

5.3.3 Reconstructing ancestral fruit and seed morphological states

The ancestral character state reconstruction provided estimates for the likelihood of character states at ancestral nodes. The resulting probabilities are shown as pie charts at nodes on the figures and given as percentages in the table (Appendix 5.5). The state with the highest probability at each node is referred to as most likely ancestral state for the clade it defines. Only nodes with high node support as well as nodes with at least one descendant that had character states scored are depicted.

Mesocarp shape: A majority of the species studied have ovoid-ellipsoid mesocarps, whereas others are spherical, ovoid-obovoid, ellipsoid or ovoid in shape.

The ancestral state reconstruction suggests that ovoid-ellipsoid is the ancestral trait (marginal probabilities 66.37% ovoid-ellipsoid, 29.50% spherical) and that spherical mesocarps evolved later (Fig. 5.11). The ovoid-ellipsoid trait is conserved in species of most groups across the phylogeny. Species with spherical mesocarps are found in Group IV (sect. *Blepharoceras – E. blepharoceras* and *E. sedentarius*), and the *Ganitrus* group *(E. nubigenus, E. dolichostyllus, E. grandis, E. ptilanthus* and *E. angustifolius)* (Fig. 5.11). The mesocarps within Group IV vary somewhat from 'true' spherical, e.g. spherical to 3- or 4-lobed in *E. sedentarius* and irregularly spherical in *E. blepharoceras.* The species with 'true' spherical mesocarps are mostly distributed in the *Ganitrus* group.

***Mesocarp surface ornamentation*:** The mesocarp ornamentation varies within *Elaeocarpus*, with the majority of species having echinate and bastionate ornamentation. A minority of species exhibit variously baculate, granulose, punctate, rugose, smooth, verrucate and fibrous mesocarp ornamentation (Appendix 5.1). The ancestral state reconstruction shows that the most likely ancestral state for the genus *Elaeocarpus* is fibrous (64.5% marginal probability) (Appendix 5.5). This state is conserved in *E. blepharoceras* and *E. sedentarius* of Group IV (Fig. 5.12). Verrucate mesocarp ornamentation evolved in the ancestor of *E. coorangooloo* in the *Obovatus* group (Fig. 5.12) and is also known in the fossil species *E. johnstonii*. Other species belonging to the *Obovatus* group have baculate mesocarp ornamentation, as exemplified by *E. arnhemicus*. Fruit of *E. obovatus* subsp. *umbratilis* was unavailable for scoring. Echinate ornamentation is largely conserved in most of the species in Group VII (*E. culminicola, E. grahamii, E. kirtonii, E. carolinae, E. eumundii*). For the other ornamentation types, bastionate ornamentation is found in all sampled

members of the *Ganitrus* clade (*E. hylobroma, E. polydactylus, E. dolichostyllus, E. grandis, E. ptilanthus, E. angustifolius*) while granulose, rugose and smooth are conserved in Group XI from Australia (*E. ferruginiflorus, E. foveolatus, E. ruminatus, E. largiflorens, E. thelmae*) and Group VIII from New Guinea (*E. habbemensis, E. pycnanthus, E. fuscoides, E. tricophyllus, E. sayeri, E. ledermannii*) (Fig. 5.12).

Species with punctate ornamentation may have mesocarps bearing pronounced longitudinal ridges (*E. stellaris, E. carbinensis*), or not (*E. bancroftii, E. linsmithii*). Phylogenetic analysis places *E. linsmithii* with Group VII species, but the position of *E. bancroftii* is unresolved. Likewise, the positions of *E. stellaris* and *E. carbinenesis* are unresolved. Therefore, the evolution of punctate ornamentation cannot be determined with the present dataset.

The mesocarps of species with bastionate ornamentation are mostly spherical in shape, and most of them belong to the *Ganitrus* group. Ancestral state reconstruction also places species with these traits (bastionate and spherical) together in the *Ganitrus* clade on the phylogeny (Fig. 5.12). However, this may not be a strict association, because some species in sect. *Elaeocarpus* also have spherical mesocarps with bastionate ornamentation, e.g., *E. braceanus* (Appendix 5.1).

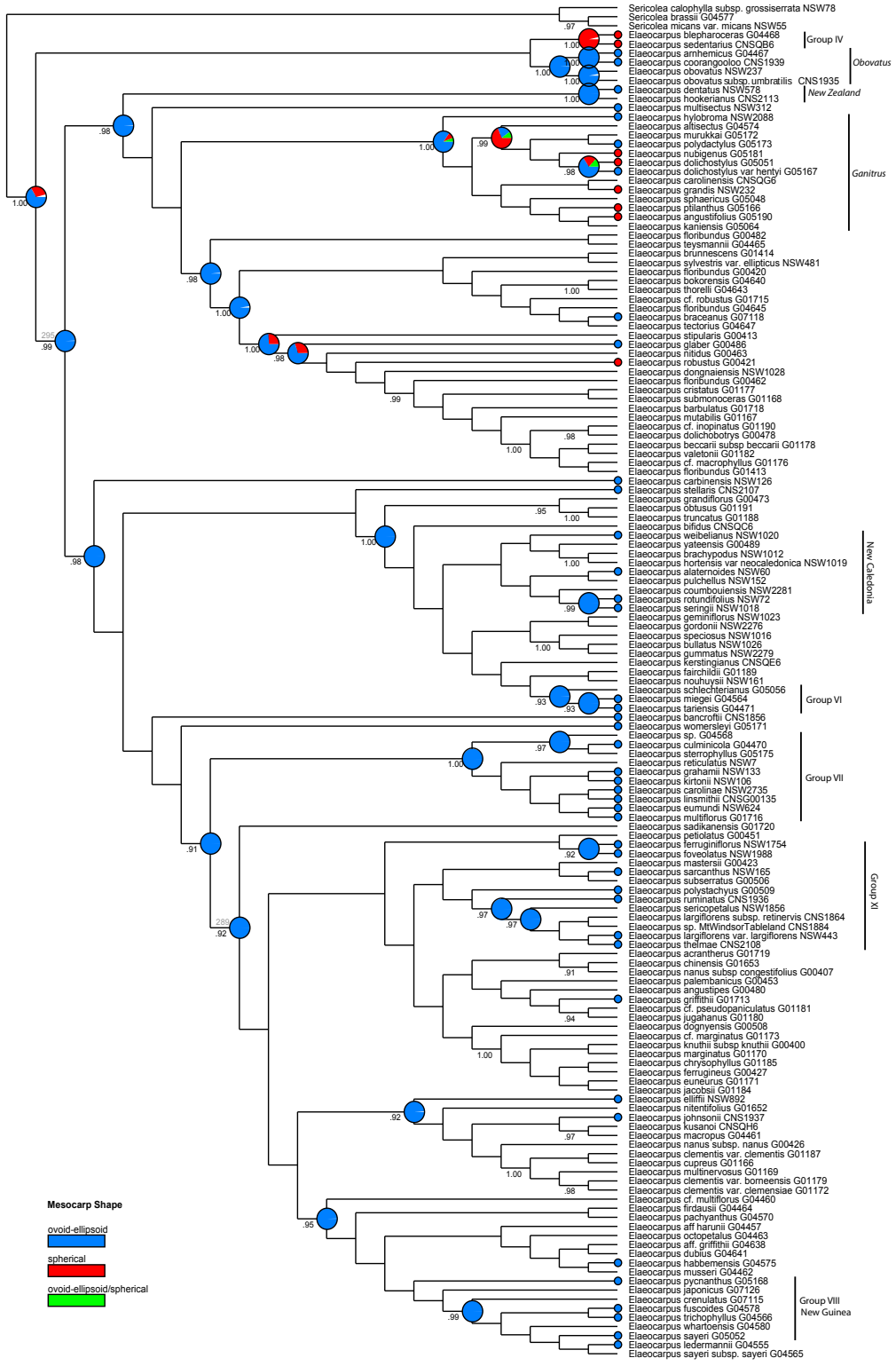


Figure 5.11 Ancestral state reconstruction of mesocarp shape.

Pie charts represent the marginal probabilities of states reconstructed at that node. Numbers below branches are posterior probabilities for well-supported branches (>0.90).

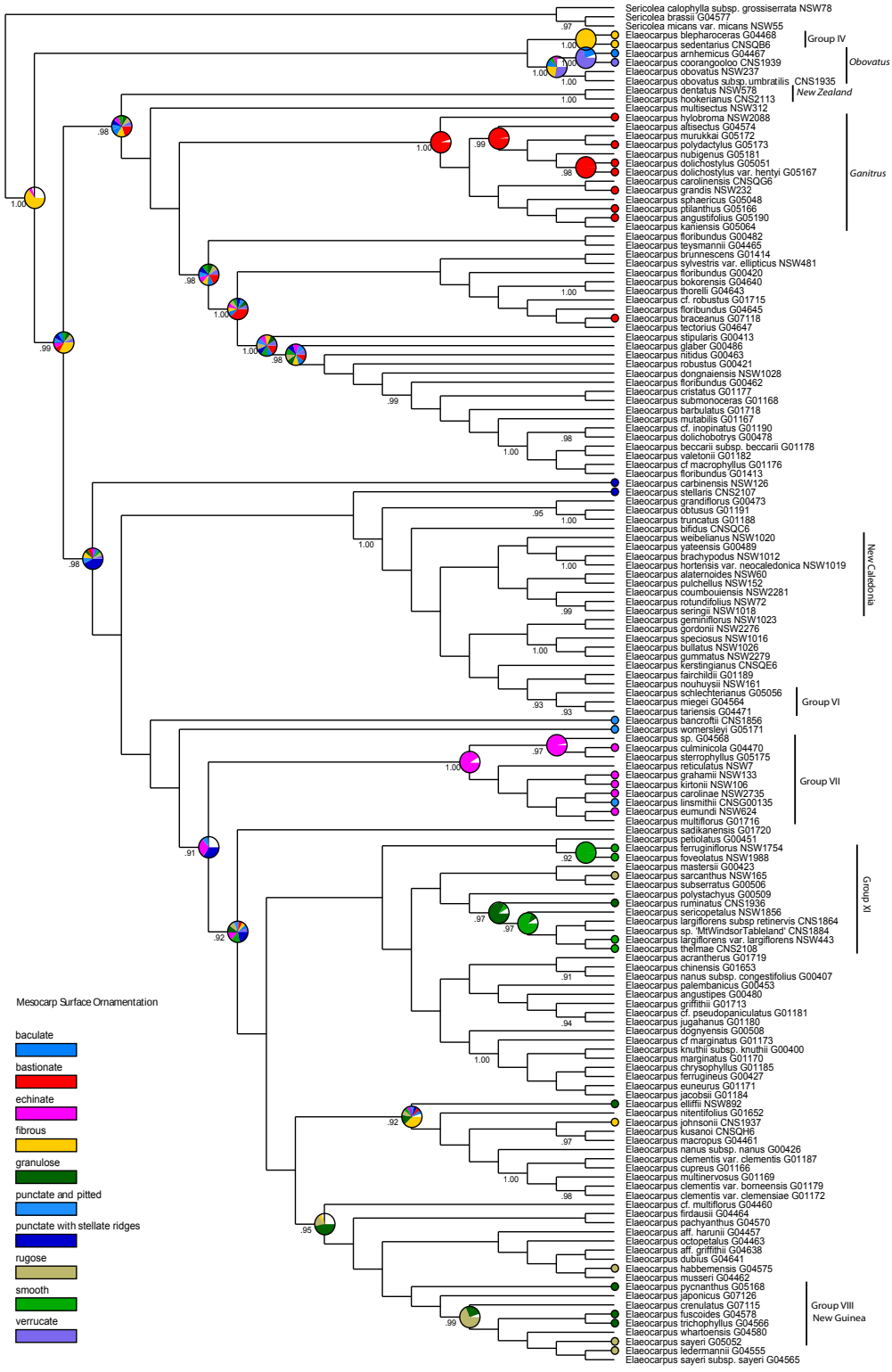


Figure 5.12 Ancestral state reconstruction of surface ornamentation.

Pie charts at nodes represent the marginal probabilities of states reconstructed at that node. Numbers below branches are posterior probabilities for well-supported branches (>0.90).

***Embryo shape*:** The straight embryo is reconstructed as the ancestral condition in *Elaeocarpus* (99.84%). The species with straight embryos are distributed in the following groups: New Caledonia, Group VI, Group V (*Ganitrus*), sect. *Elaeocarpus,* the *Obovatus* and Group IV (sect. *Blepharoceras*). However, in most cases the relationships of the species are mostly unresolved in the clades corresponding to those groups. Exceptions include the New Caledonia clade (*E. weibelianus, E. alaternoides, E. rotundifolius* and *E. seringii*), the *Ganitrus* clade (*E. polydactylus, E. nubigenus, E. dolichostylus, E. dolichostylus* var. *hentyi, E. grandis, E. ptilanthus, E. angustifolius*), the *Obovatus* clade (*E. arnhemicus, E. coorangooloo*) and the Group IV clade (*E. blepharoceras, E. sedentarius*). The relationships of species *E. bancroftii, E. carbinensis, E. stellaris*, and *E. womersleyi* with straight embryos are unresolved (Fig. 5.13).

Curved embryos have evolved at least once in species from Group VII (*E. culminicola, E. grahamii, E. kirtonii, E. carolinae, E. linsmithii, E. eumundii*) and *E. multiflorus* from Indonesia) (Fig. 5.13), Group VIII (*E. fuscoides, E. tricophyllus*, *E. sayeri* and *E. ledermannii*), and Group XI (*E. ferruginiflorus* and *E. foveolatus*). The relationships of *E. ruminatus*, *E. largiflorens* var. *largiflorens*, *E. thelmae*, *E. griffithii* and *E. polystachyus* are unresolved in those clades (Fig. 5.13).

Endosperm ornamentation: the endosperm is either entire or ruminate. The ancestral state reconstruction (Fig. 5.14) shows that entire endosperm is the ancestral state in *Elaeocarpus.* Ruminate endosperm is recorded only is species belonging to Group XI, plus *E. multiflorus* and *E. elliffii.*

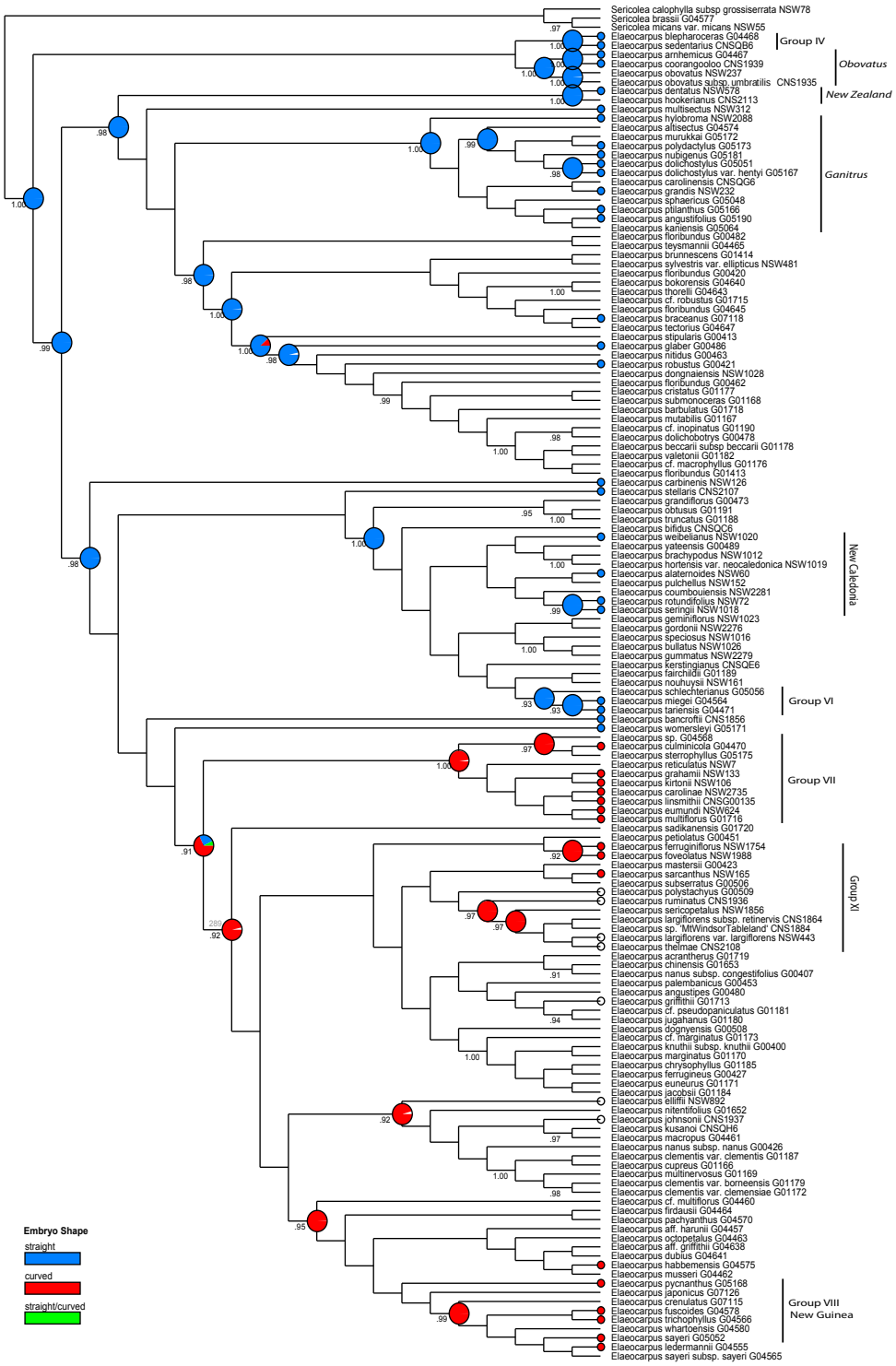


Figure 5.13 Ancestral state reconstruction of embryo shape.

Pie charts at nodes represent the marginal probabilities of states reconstructed at that node. Numbers below branches are posterior probabilities for well-supported branches (>0.90).

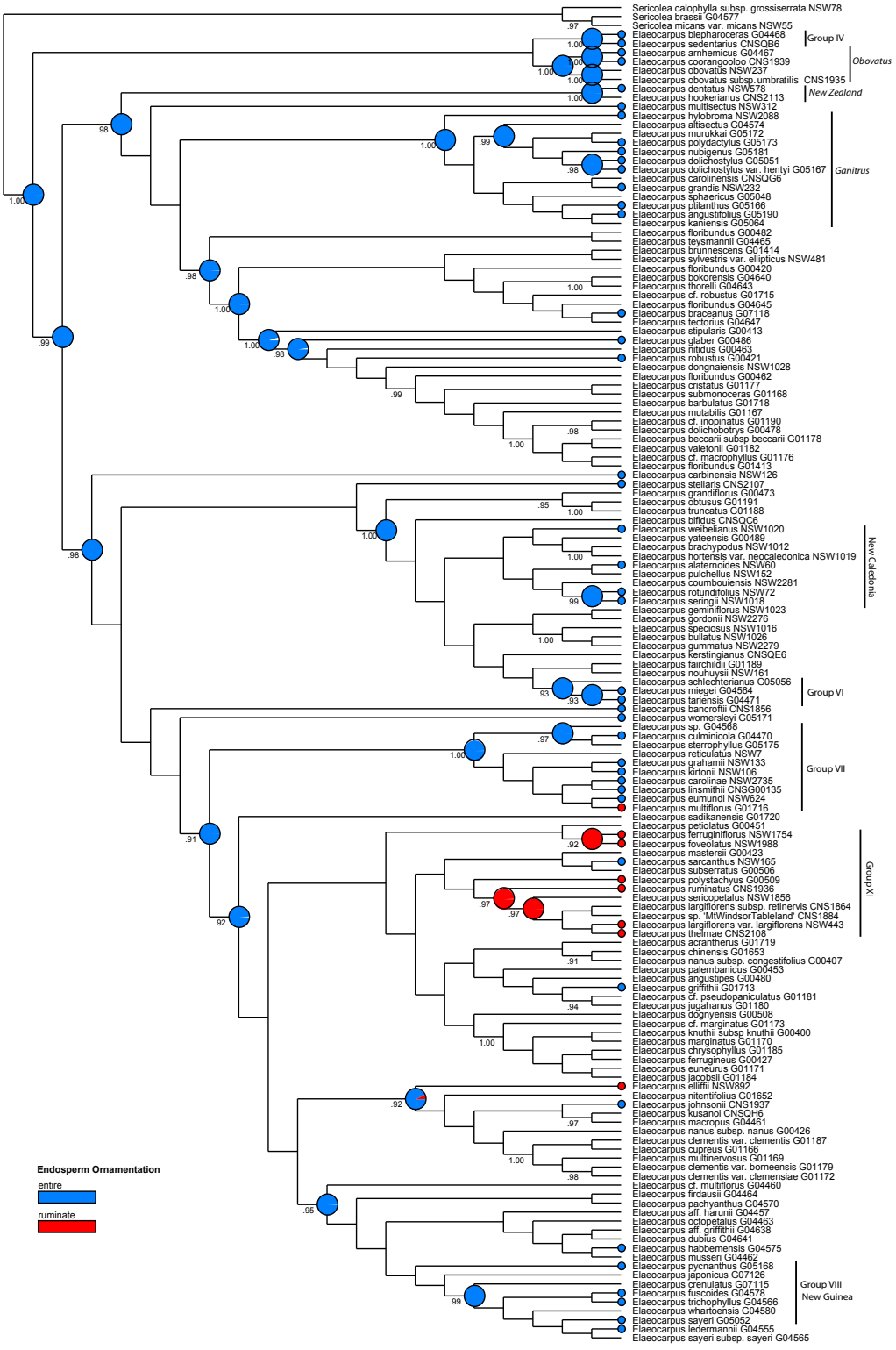


Figure 5.14 Ancestral state reconstruction of endosperm ornamentation.

Pie charts at nodes represent the marginal probabilities of states reconstructed at that node. Numbers below branches are posterior probabilities for well-supported branches (>0.90).

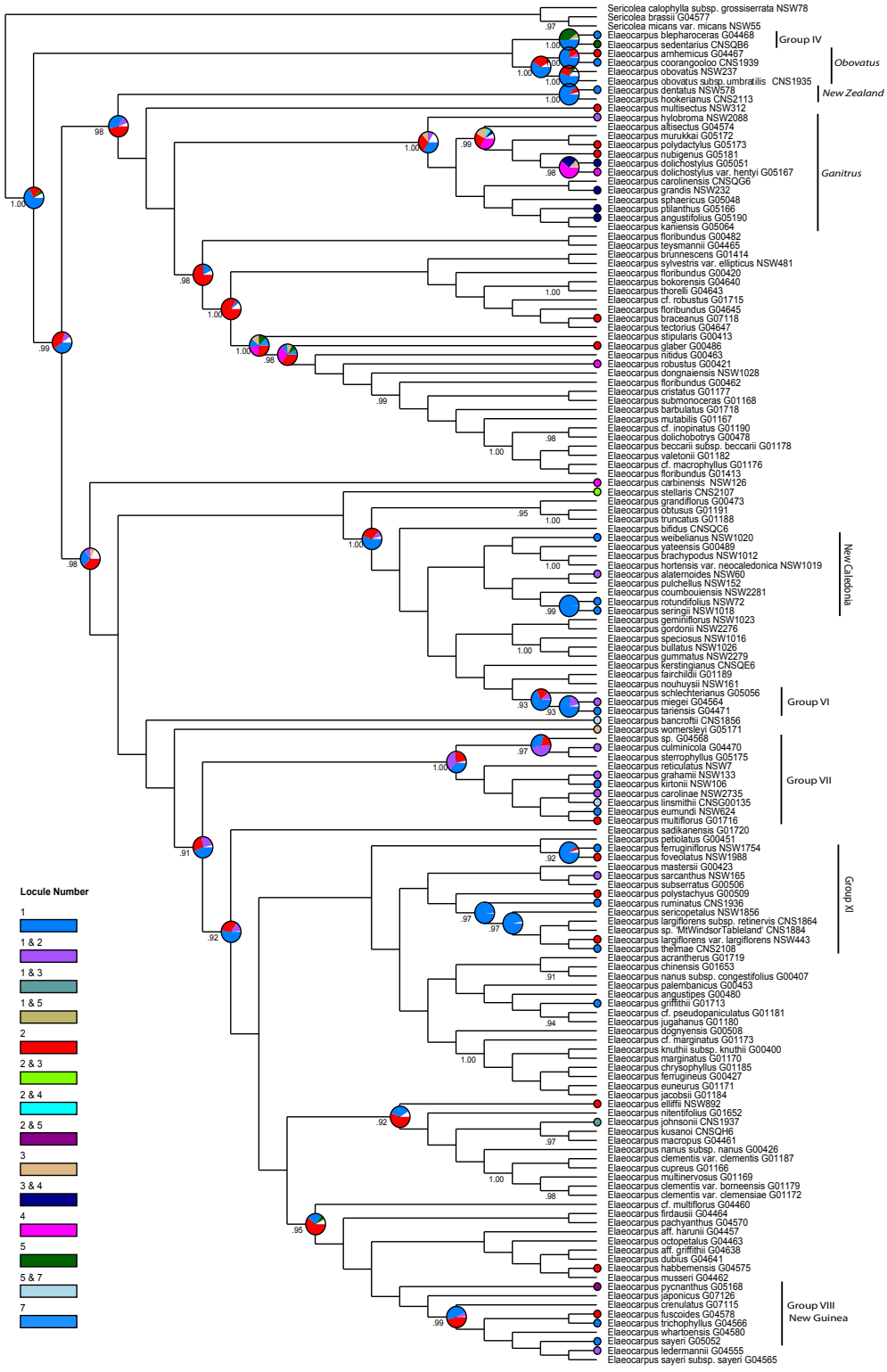


Figure 5.15 Ancestral state reconstruction of locule number.

Pie charts at nodes represent the marginal probabilities of states reconstructed at that node. Numbers below branches are posterior probabilities for well-supported branches (>0.90).

5.4 Discussion

Fruit and seed characters were surveyed and ancestral states reconstructed using a molecular phylogeny in an attempt to test the utility of mesocarp and seed morphological traits used in previous fruit morphological analyses of *Elaeocarpus* species. Previous studies have compared species from Australia, New Zealand and China to fossil species based on mesocarp shape, mesocarp ornamentation and locule number (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002; Liu et al., in press). Another study traced the evolution of embryo shape and endosperm characters of seeds in species of *Elaeocarpus* (Phoon, 2015). The current study expands on those studies by including a much greater number of species from New Guinea, one of the centres of diversity for the genus, and additional species from New Caledonia, western Malesia and Australia (Table 5.1; Appendix 5.1), and including mesocarp characters in the ancestral state reconstruction analyses. Mesocarp surface ornamentation is shown to be highly variable among *Elaeocarpus* species. Previous studies have documented eight distinct mesocarp surface ornamentation types and the current study added one new state (i.e., fibrous) (Appendix 5.1). The current study attempted to interpret the evolution of these character states, including reconstructing the ancestral condition, but was limited by relatively few clades being robustly resolved, and incomplete sampling of fruit characters for species included in the phylogenetic analysis. While mesocarp surface ornamentation remains a good character to delimit species within *Elaeocarpus*, further work is required to understand its evolution and determine the utility of the states as indicators of relationship. Each ornamentation state is discussed below based on the observations and ancestral state reconstruction analysis completed in this study.

Baculate ornamentation – previous studies have recorded baculate ornamentation in *E. arnhemicus* (Appendices 5.1, 5.3, Fig. 5.3) and *E. obovatus* subsp. *umbratilis* Y.Baba & Crayn (as *Elaeocarpus* sp. Mt Bellenden Ker; the species was not scored in this study) (Rozefelds and Christophel, 2002; Baba et al., 2020). No additional occurrences were recorded in the current study. *Elaeocarpus arnhemicus* is a subcanopy tree that grows to 10 – 12 m tall that is restricted to dry scrubland or woodland areas, from sea level to 200 m elevation (Coode, 1978, 1984; Baba et al., 2020). It occurs in New Guinea, the northeastern part of Australia, and Java in Indonesia (Phoon, 2015; Baba et al., 2020) and flowers from mid-March to mid-August (Baba et al., 2020). *Elaeocarpus obovatus* subsp. *umbratilis* is also a subcanopy tree of 10 – 20 m that grows in wet, upland rainforest, and flowers from late October to early December (Baba et al., 2020). Molecular phylogenetic, population genetic and morphometric studies place *E. arnhemicus* and *E. obovatus* subsp. *umbratilis* in the *Obovatus* group, which also comprises *E. obovatus* subsp. *obovatus* and *E. coorangooloo* from Australia (Baba, 2014; Baba et al., 2020; Phoon, 2015). Of these species, *E. coorangooloo* differs in having verrucate surface ornamentation (Figs 5.1, 5.3; Appendices 5.1, 5.3; Rozefelds and Christophel, 2002) (rugose ornamentation in Baba et al., 2020). Samples of additional species from the *Obovatus* group, particularly *E. sericolioides* and *E. arnhemicus* from New Guinea and Indonesia may further illuminate the evolution of mesocarp ornamentation in this group and inform taxonomic delimitation. With respect to seed characters, the species from the *Obovatus* group all have straight embryos and entire endosperm (Coode, 1978, 1984; Baba et al., 2020).

Bastionate ornamentation – bastionate ornamentation is restricted to the *Ganitrus* group, and found in species that are widespread in South East Asia and the Pacific including Australia and New Guinea. Fossils, however, suggest an Australian origin (Phoon, 2015). The bastionate processes vary within the species. For example, in *E. ptilanthus* they are pronounced and have fibres that are interlocked between them and are sometimes difficult to remove, and the surface is generally rough. Species such as *E. angustifolius* and *E. grandis* have a brain-like sculpture and the surface is generally smooth. The species with bastionate ornamentation have straight embryos and entire endosperm. Species with spherical fruits with bastionate ornamentation mostly have three or more locules (e.g., *E. grandis, E. williamsianus, E. dolichostylus, E. ptilanthus*, and the fossil species *E. spackmaniorum* and *E. mackayii*) (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002). Within bastionate and spherical shaped fruits with more than 3 locules, there is variation. For instance, the fossil species *E. oculatus* has bastionate surface ornamentation with mesosutural ridges, and is 5-locular (Rozefelds and Christophel, 2002). Additionally, there are species that have bastionate ornamentation with linear ridges on the surface (e.g., fossil species *E. couchmanii* with 8 locules) (Dettmann and Clifford, 2000). Species with ovoid-ellipsoid fruits with bastionate ornamentation have three or fewer locules (e.g. *E. hylobroma, E. nubigenus*; and fossil species *E. cerebriformis*) (Rozefelds and Christophel, 1996c).

The ancestral state reconstruction analysis shows most species with bastionate ornamentation form a clade which corresponds to Group V or the *Ganitrus* clade, and which confirms previous fruit morphological analysis based on mesocarp morphology (Rozefelds and Christophel, 2002; Dettmann and Clifford, 2000; Coode, 1978, 1984).

The clade comprises samples of species *E. hylobroma, E. polydactylus, E. dolichostylus, E. dolichostylus* var. *hentyi, E. grandis, E. ptilanthus* and *E. angustifolius* (Fig. 5.12). One other species shows bastionate ornamentation: *E. braceanus*. This species is part of a larger clade that includes the *Ganitrus* clade, which supports a single origin of bastionate ornamentation. However, ornamentation type is unknown in all other (c. 30) members of this clade.

Echinate ornamentation – echinate ornamentation is restricted to species belonging to the Group VII from Australia and New Guinea and sect. *Oreocarpus* from western Malesia, but fossils suggest an Australian origin (Phoon, 2015). For instance, *E. culminicola* is estimated to have originated in Australia and diverged about 1.49 Mya in the Pleistocene (Phoon, 2015). *Elaeocarpus culminicola* is an understory tree species occurring in forests, from sea level to 2750 m elevation, mostly between 1000 and 2000 m elevation (Coode, 1978, 1984). It is scattered throughout New Guinea, and northeastern Australia, also extending to Indonesia (Sulawesi) and the Philippines (Phoon, 2015). Based on seed characters, the species from Group VII and sect. *Oreocarpus* have curved embryos and entire endosperm (Coode, 1978, 1981, 1984). The ancestral state reconstruction shows that species from Group VII (*E. culminicola, E. grahamii, E. kirtonii, E. carolinae* and *E. eumundii*) form a clade that also includes *E. linsmithii* from Group VI. This confirms previous studies, which suggested that *E. linsmithii* belongs in Group VII rather than Group VI. The relationships of other Group VII species in that clade - *E. sterrophyllus*, *E. reticulatus* and *E. multiflorus* - are unresolved. The current observations and examinations also reveal that even within echinate type mesocarps, there are variations. For instance, *E. fuscoides* and *E. pycnanthus* from New Guinea have

mesocarps with weakly echinate surface, and *E. undulatus* (not included in the current analysis) has echinate surfaces but the mesocarps are flattened with wings. Echinate mesocarps are found in only one clade, which supports a single origin of this state and suggests it is a useful diagnostic marker for this clade.

Fibrous ornamentation – this character state has been documented in taxonomic studies (Coode, 1978, 1981, 1984; Maynard et al., 2008), but not in fruit mesocarp studies (Dettmann and Clifford, 2000; Rozefelds and Christophel, 2002; Liu et al., in press). Fibrous mesocarp is added (together with rugose) from the current study to the seven known mesocarp types, bringing the total to nine. The current study found the fibrous mesocarps to be of two types: fibrous and soft with fibres extending to the inner mesocarp (e.g. *E. johnsonii, E. sedentarius, E. blepharoceras*), and fibrous and robust with fibres extending to the outer mesocarp (*E. womersleyi*). The terms 'soft' and 'robust' refer to the lignified inner mesocarp or fruit stone (herein described as mesocarp). Thus, fibrous mesocarps with soft woody inner mesocarps are poorly lignified and have a thin mesocarp wall compared to the heavily lignified mesocarps that are robust and have a thick mesocarp wall (*E. womersleyi*, Fig. 5.12). Currently, fibrous mesocarps are known only in *E. johnsonii, E. sedentarius* and *E. blepharoceras* (with poorly lignified mesocarps), and *E. womersleyi* (with heavily lignified mesocarp).

Molecular phylogenetic analysis shows *E. sedentarius* and *E. blepharoceras* form a robust clade (see also Chapter 3), but *E. johnsonii* (with the same character) is phylogenetically distant from them (Fig. 5.12). In the current taxonomy, both *E. johnsonii* and *E. blepharoceras* are placed in Group IV (Coode, 1978, 1984) and *E.*

sedentarius is unplaced (Maynard et al., 2008). *Elaeocarpus sedentarius* shares with *E. blepharoceras* (Group IV or sect. *Blepharoceras*) morphological features such as pale green to glaucous abaxial leaf surfaces, fruits that are triangular in transverse section, and dense radial fibres within the outer mesocarps (Coode 1978, 1984; Maynard et al., 2008; Phoon, 2015). These similarities would support a placement of *E. sedentarius* in Group IV. However, Group IV is not monophyletic: *E. johnsonii* is placed distantly to *E. sedentarius* and *E. blepharoceras*. Apart from the persistent fibres and poorly lignified inner mesocarps, these species also share straight embryos and entire endosperm seed characters. The species differ, however, in locule number and fruit shape. *Elaeocarpus blepharoceras* has 2 locules in an irregularly spherical shaped fruit, *E. sedentarius* 1 or 2 locules in a spherical to 3- or 4-lobed fruit, and *E. johnsonii* 3(or 4) locules in an obovoid fruit with a truncate base and acute apex (Fig. 4.8) (Appendix 5.1). *Elaeocarpus womersleyi*, which has persistent mesocarp fibres (but differs in its inner mesocarp which is strongly lignified and thick) is currently placed in Group VI, subgroup B (VI B), rather than in Group IV with species that have fibrous mesocarps. These results suggest group IV should be revised.

Although the fibrous mesocarp was reconstructed as ancestral for all *Elaeocarpus*, this result has to be taken with caution. The high number of character states and large proportion of missing information makes the estimate on the basal node sensitive to the influence of the basal nodes, which include taxa with fibrous ornamentation. More data are required to confidently estimate the ancestral state in this case. It is worth noting that the genus *Aceratium*, which is consistently resolved as one of the closest relatives to *Elaeocarpus* (Chapter 3), has fibrous outer mesocarps and poorly developed inner mesocarps, which is consistent with the reconstruction.

Granulose ornamentation – this character is found in some species from Group XI from Australia (see Fig. 5.12). Other species from Group XI, e.g. *E. ferruginiflorus*, *E. foveolatus*, *E. largiflorens* var. *largiflorens* and *E. thelmae*, have smooth or almost smooth mesocarps. Granulose mesocarps have also been recorded in species from New Guinea (*E. pycnanthus*, *E. fuscoides* and *E. trichophyllus* of Group VIII), but not from New Caledonia or western Malesia. Based on the ancestral state reconstruction, the New Guinean species are grouped together, and those from Australia are in a separate clade. The relationships of the species within each clade are generally poorly resolved (Fig. 5.12). Granulose ornamentation has likely evolved multiple times and is therefore of limited use as a taxonomic marker.

Punctate ornamentation – mesocarps with punctate ornamentation may also have prominent longitudinal ridges (*E. stellaris*, *E. carbinensis*), or lack the ridges (*E. bancroftii* and *E. linsmithii*) (Gagul et al., 2018; this study). Currently punctate mesocarps with longitudinal ridging are known only from Australia, although there has been a recent report of an undescribed taxon with that mesocarp type from Sulawesi (Coode pers. comm, 2018). There are no records of species from New Guinea with such ornamentation type, although fruits of *E. womersleyi* may sometimes appear punctate if the persistent fibres rot away (Coode, 1978). The relationships of these species are unresolved (Chapter 3). With respect to morphological taxonomy these species are currently placed in Group VI (Coode, 1978, 1981, 1984). Group VI, subgroup B comprises the large fruited species *E. bancroftii* (with mesocarps measuring 30–80 x 20–70 mm), *E. carbinenesis* (30–45 x 32–40 mm) and *E. stellaris* (41–50 x 35–43 mm) (Gagul et al., 2018b; Chapter 2,

Table 2.1). Mesocarps of fossil species have been matched to extant species from Group VI (B), e.g., *E. clarkei* (F.Muell.) Selling*, E. peteri* Rozefelds & Christophel (=*E. peteri*) (Rozefelds and Christophel, 2002).

Smooth ornamentation – mesocarps with smooth or almost smooth ornamentation have been recorded in the following species: *E. ferruginiflor*us, *E. foveolatus*, *E. largiflorens* and *E. thelmae* belonging to Group XI from Australia. Ancestral state reconstruction indicates the trait may have evolved independently in two lineages: *E. ferruginiflor*us + *E. foveolatus*, and *E. largiflorens* + *E. thelmae*. However, sparse sampling of mesocarp traits in related species renders the evolution of this trait obscure.

Verrucate ornamentation – this character is uncommon and is recorded so far only in *E. coorangooloo* of Australia (the *Obovatus* group), and *E. johnstonii*, a fossil species also from Australia. The current study did not record any additional species with verrucate ornamentation from New Guinea, New Caledonia or western Malesia. *Elaeocarpus coorangooloo* and *E. arnhemicus* are sisters (Fig. 5.12) and while they differ in mesocarp ornamentation (*E. arnhemicus* has baculate ornamentation) they share other characters such as ovoid-ellipsoid mesocarp, straight embryo and entire endosperm. (Figs 5.11, 5.13, 5.14).

5.4.1 Embryo and endosperm in seeds of *Elaeocarpus*

Embryo and endosperm characters are informative seed traits for phylogenetic analysis (Phoon, 2015). The embryo is straight, curved, or straight with a hooked tip in *Elaeocarpus*. Seeds with curved embryos are found in species belonging to Group VII, Group VIII and Group XI, while species with straight embryos are found in the

following groups: New Caledonia, *Ganitrus* group, sect. *Elaeocarpus*, *Obovatus* group and Group IV. Only *Elaeocarpus holopetalus* has embryos that are straight with a hooked tip (Coode, 1984). *Elaeocarpus holopetalus* has several other character states that are unique in *Elaeocarpus* such as undivided petal tips (versus divided), and dark purple or black fruits (versus variously blue or green, or rarely red or brown). This condition might be an intermediate state between straight and curved seeds (Weibel, 1968; Coode, 1984; Phoon, 2015). Fruit developmental biology studies in future may illuminate our understanding, and to determine at which stage of development does this condition occur, and if it has any taxonomic significance. Additionally, in molecular phylogenetic studies conducted to date *E. holopetalus* is not consistently resolved in a clade with the rest of *Elaeocarpus* (Baba, 2014; Phoon, 2015; Chapter 3). Based on the ancestral state reconstruction, straight embryo is the ancestral trait and curved embryo has a single origin basal to a derived clade comprising approximately half of all sampled species including those belonging to Group VII, Group VIII and Group IX (Fig. 5.13).

Endosperm is either entire or ruminate in seeds of *Elaeocarpus*. The ancestral state reconstruction indicates that entire endosperm is the ancestral condition and is found in most of the sampled species across the phylogeny, particularly in the *Ganitrus*, New Caledonia, sect. *Elaeocarpus*, the *Obovatus*, and Groups IV, VI, VII and VIII (Fig. 5.14). *Elaeocarpus multiflorus* is placed within a strongly supported clade (Group VII) that is characterised by entire endosperm. In this species the endosperm is only slightly ruminate, which may represent an early stage of evolutionary development of the ruminate condition.

5.4.2 Groupings based on the mesocarp and seed character analysis

Group IV (sect. *Blepharoceras*) – Group IV species have irregularly spherical or spherical to 3- or 4-lobed fibrous mesocarps, and seeds with straight embryos and entire endosperm. Currently, the two species with these characters are grouped together (*E. sedentarius* and *E. blepharoceras*) on all phylogenies with strong support (Figs 5.11 – 5.14). *Elaeocarpus johnsonii* also has fibrous mesocarps, straight embryos and entire endosperm, but it does not group with the aforementioned Group IV species. This species differs in its mesocarp shape, being obovoid with truncate base and acute apex (generally spherical in outline in *E. sedentarius* and *E. blepharoceras*).

Group V (the *Ganitrus* group) – species in this group have spherical mesocarps that have bastionate ornamentation, straight embryos and entire endosperm. These species are distributed in South East Asia and the Pacific including Australia and New Guinea, but records from fossil species with bastionate ornamentation suggest an Australian origin. These species are grouped together on the phylogenies with strong internal node support (PP 1.0, Figs 5.12 – 5.14).

Group VI – species from this group have ovoid-ellipsoid mesocarps with verrucate, echinate or smooth surface ornamentation, and seeds with straight embryo and entire endosperm. Two species (*E. tariensis* and *E. miegei*) were included in the current study and they group together with moderate support (PP 0.93, Figs 5.11, 5.13, 5.14). *Elaeocarpus tariensis* is restricted to New Guinea while *E. miegei* extends beyond New Guinea to Australia (Tiwi Islands, Northern Territory), Indonesia (Key Island and Aru Island), and the Solomon Islands (M. Coode, pers. comm. 2018).

Group VII – Group VII species have ovoid-ellipsoid mesocarps with echinate ornamentation, curved embryos and entire endosperm. These species are mostly from Australia, New Guinea and New Caledonia. The results of this study support the placement of *E. linsmithii* in this group as originally proposed by Coode (1984) albeit conjecturally because seed characters of *E. linsmithii* were unknown. The current study has investigated fruit specimens of *E. linsmithii* (ELS-01) (CNS), from Mt. Lewis, Queensland (1261 m elevation, S 16'31. 167'; E 145' 16.69') and the embryo of all three samples are curved and the endosperm entire. In contrast, previous studies of mesocarp morphology (Rozefelds and Christophel, 2002) suggested a placement in Group VI, Subgroup D with *E. bancroftii* instead based primarily on its ovoid-ellipsoid shape, punctate mesocarp surface ornamentation, and 2–5 locules. But that study did not take seed characters into account. The two species differ in mesocarp sizes, with *E. linsmithii* having smaller mesocarps (14–15 x 9–10 mm vs 30–80 x 20–70 mm in *E. bancroftii*), and embryo types, with *E. linsmithii* having curved embryos and *E. bancroftii* having straight embryos. Furthermore, *E. linsmithii* is an upland species found between 1200–1600 m elevation, whereas *E. bancroftii* is found at 0–1200 m elevation.

Group VIII – Group VIII species surveyed in the current study include *E. habbemensis, E. pycnanthus, E. fuscoides, E. trichophyllus* and *E. ledermannii*. Those species have ovoid-ellipsoid mesocarps with granulose or rugose surface ornamentation. Seeds of species from this group have curved embryos and entire endosperm. Ancestral state reconstruction places them together with a well-supported internal node (PP 0.99, Figs 5.11 – 5.14).

Group XI – Group XI species have ovoid-ellipsoid mesocarps with smooth, granulose or rugose surface ornamentation, and curved embryo with ruminate endosperm. The ancestral state reconstruction places *E. ferruginiflorus* and *E. foveolatus* together (PP 0.92, Figs 5.13, 5.14), and *E. largiflorens* var. *largiflorens* and *E. thelmae* together mostly unresolved, based on the endosperm and embryo characters.

The *Obovatus* group – the *Obovatus* group comprises species with ovoid-ellipsoid mesocarps, baculate or verrucate surface ornamentation, straight embryos and entire endosperm. So far only *E. arnhemicus* and *E. obovatus* subsp. *umbratilis* (not sampled) have been recorded to have baculate ornamentation. These species are related to *E. coorangooloo* with verrucate ornamentation. Due to the number of different ornamentation types in this group and the incomplete sampling, the ancestral state analysis was unable to reconstruct the evolution of this character (Appendix 5.5).

Section *Elaeocarpus* – section *Elaeocarpus* species have spherical, ellipsoid or ovoid-ellipsoid mesocarps with bastionate ornamentation (sometimes with longitudinal ridges), straight embryos and entire endosperm. The current study has documented only *E. braceanus, E. glaber* and *E. robustus*, none of which are resolved in the current analysis.

5.4.3 Informative and non-informative or variable characters

Both external and internal features are useful to describe fruit morphology. External morphology of mesocarps (e.g., size, shape, sutures and surface ornamentation) of larger fruits is easily distinguishable, providing informative data, but on smaller mesocarps some features, particularly sutures and ornamentation on

the surface may be reduced and difficult to interpret. Sutures usually reflect the number of locules when they are conspicuous, but in smaller mesocarps, it is evident internally (in transverse section). It is useful to confirm with sectioned mesocarps if unsure of the locule numbers.

For internal morphology documentation and description of seeds, embryos, endosperms and fibre arrangement (especially persistent fibres that are permanently attached) are only possible through sectioning. Seeds must not be confused with locules. Locules can be fertile (seed bearing) or infertile. Remnants of locules seen internally are evident on the external segments, but not necessarily in all instances. For instance, a fruit with four segments externally may have more or less than four locules.

In *Elaeocarpus*, fruits develop from a multi-locular ovary at anthesis (except *E. ruminatus* with a 2-locular ovary with 7–8 ovules in each; Coode, 1984). However, not all develop into fully matured seeds, some are aborted or compressed during development.

Defining embryo shape and endosperm morphology are also only possible with sectioned mesocarps, but again it depends on the nature of mesocarp, whether it is fresh or dry. In my observations I found that sections of dried mesocarps could sometimes lead to misleading interpretations of ruminate endosperms and curved embryos. Therefore, fresh fruits are recommended to be sectioned if they are available to confirm embryo and endosperm morphology.

5.4.4 Notes on fossils, and the implications of the ancestral state reconstructions for interpreting them

To date, mesocarp characters (shape, size, surface ornamentation) and locule number have been used to compare fossil species to extant species in order to infer the relationships of fossil species. However, seed characters such as embryo and endosperm are rarely preserved in fossilised materials therefore these characters, while useful for defining clades within *Elaeocarpus*, are of limited use for determining the relationships of fossils.

Other studies have confirmed fossil species from Australia bear resemblance of extant species in the *Ganitrus* group, e.g., *E. spackmaniorum* and *E. mackayi* (Rozefelds, 1990; Rozefelds and Christophel, 1996a, b, 2002; Dettmann and Clifford, 2000; Liu et al., 2020). The species in the *Ganitrus* group have 5-locular fruits. An additional species (*E. occulatus* Rozefelds & Christophel), also with a spherical, 5-locular mesocarp, 'mesosutural ridges' and deep foramina has been described (Rozefelds and Christophel (2002), which does not resemble an extant species from Australia. However, an extant species (*E. weibelianus*; *G. McPherson 1728*, BRI) from New Caledonia with mesosutural ridges has been documented in the current study. The prominent ridging is aligned along the sutures, with baculate – bastionate processes between the ridges. This species has a 2-locular mesocarp with straight seeds and belongs to Group IV of the infrageneric classification (Tirel, 1983). A transverse section (TS) or CT scan of the internal anatomy of *E. occulatus* would perhaps be informative (DeVore et al., 2006). Meanwhile, other fossil species such as *E. couchmanii* (F.Muell.) Dettmann & Clifford and *E. lynchii* (F.Muell.) Selling do not match any existing groups from Australia, although their characters may suggest

certain groups. For instance, *E. couchmanii* has a spherical mesocarp with a bastionate surface ornamentation and 8-locular, which is characteristic of the *Ganitrus* group. Whether it should be considered part of the *Ganitrus* should internal anatomy be studied. *Elaeocarpus lynchii* has an ovoid-ellipsoid mesocarp, with an echinate ornamentation and is 5-locular, a combination of characters which does not resemble any known extant species from Australia. The current fossil records clearly demonstrate that *Elaeocarpus* was diverse by Miocene in Australia (Rozefelds and Christophel, 2002). Liu et al., (2020) have utilized CT technology to study the internal anatomy of fossil mesocarps resulting in description of six new species. These species have mostly been compared to extant species from China, and broadly grouped into bastionate, echinate, punctate, rugose and smooth. They have noticed that smaller fruits with weak ornamentation are difficult to group, and that ornamentation can vary within a fruit. Their study provides evidence for earliest occurrences of *Elaeocarpus* in the Northern Hemisphere.

Fossil fruit data can be used to calibrate phylogenetic analyses and provide minimum dates for the divergence times of clades. Four fossil species have been used (e.g. Crayn et al., 2006) to calibrate molecular evolutionary rates to estimate ages of lineage divergences, and to investigate historical biogeography: (i) *E. spackmaniorum* Rozefelds (25 Mya) resembling *E. angustifolius*, (ii) *E. lynchii* (F.Muell.) Selling (14 Mya) resembling *E. grahamii*, (iii) *E. rozefeldsii* Dettmann & Clifford (28 Mya) resembling *E. stellaris* and *E. carbinensis*, and (iv) *E. mackayi* (F.Muell.) Kirchheimer (20 Mya) resembling species of the *Ganitrus* and sect. *Elaeocarpus*. Apart from that study, fruit morphology has rarely been discussed in a phylogenetic context, although a rich fossil record of *Elaeocarpus* fruit stones exists. The current

study aimed to resolve the phylogenetic affinities of fossil mesocarps of *Elaeocarpus,* and while the estimated molecular phylogeny and the database of mesocarp morphologies of extant species represent a significant advance over previous studies, they are insufficient at present to confidently place the fossils in a phylogenetic context. This study has however provided a solid foundation for future studies. Those studies should: 1) investigate the use of phylogenomic datasets generated using genome skimming (shown to be successful in Chapter 2) and target capture (e.g. Baker et al., 2021) approaches for resolving species relationships, and 2) more comprehensively sample mesocarp morphology using the structured approached developed in the present study with a focus on taxa for which suitable material was unavailable to me during this study.

5.5 Conclusion

This study is the first attempt at placing mesocarp anatomy and morphology of *Elaeocarpus* in an evolutionary context, based on a sample of c. 15% of the total species diversity with a focus on species from western Malesia, New Guinea, Australia and New Caledonia. I have found that the most informative characters are the mesocarp surface sculpturing, the embryo shape and the endosperm ornamentation. Mesocarp surface ornamentation is useful in delimiting species with larger fruits, however in smaller fruits, surface characters are often poorly developed and hence difficult to interpret. A majority of the smaller mesocarps showed insufficient development of mesocarp morphology to be unambiguously scored against the current character states, and additional states to accommodate them could not be confidently defined. Interpreting the mesocarp morphology of the small fruited species requires a more comprehensive knowledge of mesocarp ancestral states across

the phylogeny than has been possible to achieve in this study. Both an improved estimate of the phylogenetic relationships (which will require denser species sampling and the use of more informative molecular markers) and a more comprehensive database of mesocarp for the species included in the phylogenetic trees will be required to provide this improved knowledge.

Although not homologous at group (and sectional) level, mesocarp surface ornamentation is a taxonomically useful character at the species level. For seed characters both embryo shape and endosperm ornamentation are useful in taxonomic and phylogenetic classification. Locule number may assist in placing fossils in a phylogenetic context, but this will require a better estimate of the phylogeny and a more complete database of fruit character states than was possible here.

The current study provides a basis and framework for future studies which should focus on lineages within *Elaeocarpus* that have not been sampled adequately in either molecular and fruit morphological analyses to date. Furthermore, the use of non-destructive CT scanning technology can provide detailed information on the nature of the internal anatomy of the fruit, such as the distribution of fibres and vasculature, in both modern and fossil material, which may assist with interpretation of fossils.

Chapter 6 – Conclusions and Future Directions

6.1 Overview of the thesis

This dissertation comprises several, related research topics, which are each addressed by separate data chapters. As each data chapter contains separate conclusions, this final chapter provides an overall summary of the key findings of the thesis. The last section outlines potential future research areas.

The broad aim of the thesis was to investigate the systematics and evolution of *Elaeocarpus* with a focus on the New Guinean taxa, using a combined molecular and morphological approach. While pondering the scope of this task, a study appeared that documented a climate risk to an undescribed *Elaeocarpus* species found in the Wet Tropics of Australia. This prompted me to undertake an investigation of the taxonomy and conservation outlook of this species (Chapter 2).

The molecular analysis increased the sampling of *Elaeocarpus* from under represented regions compared with previous studies (Chapter 3). One such region is New Guinea, which contains substantial *Elaeocarpus* diversity.

The morphological analysis involved fruit morphology, and comprised two distinct studies: 1) an anatomical study of fruit development from anthesis to fruit maturity (Chapter 4), and 2) a test of the concordance between the existing *Elaeocarpus* classification and fruit mesocarp morphology, using a molecular phylogeny (Chapter 5).

The respective researches conducted have each achieved their aims in the following ways:

1. Describing and formally naming a new species of conservation concern from the Wet Tropics of Queensland, Australia.
2. Producing a phylogenetic reconstruction of the genus *Elaeocarpus*, which includes substantially expanded representation of species from New Guinea and from other under-sampled regions.
3. Testing the current infrageneric classification using molecular data, with a focus on New Guinean species.
4. Increasing the molecular sampling of *Elaeocarpus* to c. 50% of the known diversity from Australia, New Zealand, New Caledonia, western Malesia and the Pacific region including New Guinea.
5. Providing the first plastome-scale data from species of Elaeocarpaceae.
6. Undertaking the first developmental study of endosperm rumination in seeds and lignification in the mesocarp of *Elaeocarpus*.

7. Reconstructing the evolution of fruit charaters (and determining ancestral states) and testing the congruence with current *Elaeocarpus* morphological classification using mesocarp morphology.

6.2 A new species endemic to Wet Tropics and its conservation outlook

This study (Chapter 2) aimed to determine the taxonomic and conservation status of an undescribed species in the Wet Tropics of Queensland, Australia. A recent study indicated that this entity, among others, is at risk from climate change, so the current study investigated the taxonomic status and conservation outlook for the species, to inform conservationists and policy makers. This study indicated the entity is distinct from related species and it has been formally described and named as *Elaeocarpus carbinensis* J.N.Gagul and Crayn. Environment Niche Modelling based on possible future climates, predicts a complete loss of highly and moderately suitable habitat by 2040, and of all suitable habitat by 2080 across the Wet Topics. Assessment against the IUCN red list guidelines suggests this species should be recognised as 'Vulnerable'. The results of this study informed subsequent Chapters of this thesis.

6.3 Molecular phylogenetics of *Elaeocarpus*

The main aim of this part of the study (Chapter 3) was to utilize phylogenetic analysis of a multilocus molecular dataset with substantially improved sampling of *Elaeocarpus* from New Guinea. The study has addressed the New Guinean sampling

gap together with increasing taxonomic representation from other under-sampled areas. It has achieved its aim by:

1. Increasing sampling from New Guinea, but also from Sulawesi (Indonesia), Cambodia, Thailand, Japan and Myanmar, which provides improved understanding of the relationships and evolution of *Elaeocarpus*.
2. Testing the morphology-based classification against molecular data, especially for the New Guinean taxa.
3. Demonstrating the value of plastome scale datasets in improving phylogenetic estimation in *Elaeocarpus* over datasets comprising a few selected genes.

Reconstructing the phylogenetic relationships of species in *Elaeocarpus* is key to understanding the history and evolution of the genus, particularly in species-rich tropical regions such as New Guinea. The current study has significantly expanded chloroplast data of *Elaeocarpus* with representatives of seven out of the nine currently known groups in New Guinea. Samples of species from groups III, IV, V, VI, VII, VIII and the *Obovatus* group have been included in the molecular analyses, while groups I, II and IX have no current representation, due to unavailability of samples. The current phylogenetic framework is built on the results of previous studies, bringing the sample of the known *Elaeocarpus* diversity to c.50 %. The phylogenetic analyses were done on a much-expanded dataset using Maximum Likelihood and Bayesian Inference approaches. High throughput sequencing has been utilized to sequence whole plastomes of 27 Elaeocarpaceae samples, all of which are novel data contributed by this study.

Results of these analyses show the phylogeny is largely congruent with previous studies, although with differences in the level of resolution. The current study places *E. holopetalus* on a distinct lineage outside the main *Elaeocarpus* clade, suggesting that the genus *Elaeocarpus* may be paraphyletic. This position is consistent with previous studies. The closest relatives of *Elaeocarpus* appear to be *Aceratium* and *Sericolea*. A majority of the newly sequenced species from New Guinea and the other regions are nested within the clades identified previously, and relationships of most are congruent with the current morphological groupings.

Our understanding of the phylogenetic relationships of the species from New Guinea has improved, but substantial work remains to be done to comprehensively understand *Elaeocarpus* evolution. Many more representatives from the main lineages represented in New Guinea are needed to rigorously test the current morphological groupings, and to provide a better resolution in the relationships, at both species and sectional (or groups) level. Furthermore, the topologies remain weakly supported at many nodes. In future studies, the inclusion of nuclear DNA markers and more extensive sampling of the known diversity may improve resolution and support leading to further insights.

6.4 Fruit developmental biology and endosperm rumination

The aim of this study (Chapter 4) was to investigate two key processes in fruit development in *Elaeocarpus* i.e., endosperm rumination in seeds and lignification in fruit mesocarps. Ruminate endosperm and fruit stone features are heavily utilised in *Elaeocarpus* classification, particularly of fossil species. In examining developing fruits of a widespread species of *Elaeocarpus* in northeastern Australia – *E. ruminatus*

- I found that mesocarp lignification occurs in early developmental stages, while endosperm rumination becomes more apparent near maturity. The development of ruminations in seed endosperm and lignification of tissues in fruit mesocarps have taxonomic value for Elaeocarpaceae. Endosperm rumination is a taxonomically useful character at the infrageneric level.

6.5 Evolutionary patterns of fruit mesocarp and seed morphology

The aim of this study (Chapter 5) was to determine evolutionary patterns of fruit morphology in the genus in order to: 1) test the fruit morphology-based classification of *Elaeocarpus*, and 2) improve our understanding of evolutionary patterns of fruits within *Elaeocarpus*, particularly for placing fossil taxa in an evolutionary framework.

Based on the mesocarp and seed character examination:

1. Group IV species have irregularly spherical or spherical to 3-4 lobed mesocarps, that are fibrous and have straight embryo with entire endosperm.
2. Group V (*Ganitrus* group) species have spherical mesocarps that have bastionate ornamentation, straight embryo and entire endosperm. These species are distributed in South East Asia and the Pacific including Australia and New Guinea. Based to fossil records, species with bastionate ornamentation may have originated from Australia.
3. Group VI species have ovoid-ellipsoid mesocarps that have verrucate, echinate or smooth surface ornamentation, and seed characters with straight embryo and entire endosperm.

4. Group VII species have ovoid-ellipsoid mesocarps with echinate ornamentation, curved embryo and entire endosperm. These species are mostly from Australia, New Guinea and New Caledonia.
5. Group VIII species have ovoid-ellipsoid mesocarps with echinate, verrucate, smooth, rugose or finely pitted rugose surface ornamentation. The seeds of species from this group are curved with either entire or slightly ruminate endosperm.
6. Group XI species have ovoid-ellipsoid mesocarps that have either smooth or granulose surface ornamentation, and curved embryo with ruminate endosperm. Within this group, those with smooth and granulose ornamentation group together respectively.
7. The *Obovatus* group comprises species with ovoid-ellipsoid mesocarps, baculate or verrucate surface ornamentation, straight embryo and entire endosperm. So far, only *E. arnhemicus* and *E. obovatus* subsp. *umbratilis* have been recorded to have baculate ornamentation. Those species are related to *E. coorangooloo*, which has verrucate ornamentation.
8. Section *Elaeocarpus* species have either spherical, ellipsoid or ovoid-ellipsoid mesocarps with bastionate ornamentation, sometimes bastionate with linear ridging. The embryo is straight and endosperm is entire in the seeds of the species belonging to sect. *Elaeocarpus*.
9. The New Caledonian species have ovoid-ellipsoid mesocarps with either bastionate, echinate or verrucate surface ornamentation, and seeds with straight embryo or entire endosperm.

Based on ancestral character states reconstruction, few nodes received strong support (posterior probability values >50%). Key clades resolved by this analysis are consistent with those resolved by the more inclusive analysis undertaken in Chapter 3. These clades include Group IV, the *Obovatus*, Group V (*Ganitrus*) and Group VII.

6.6 Recommendations for future research

During the current research, I identified various potential future research directions for *Elaeocarpus* (detailed below). As morphological taxonomy studies are extensively done on *Elaeocarpus* to establish the infrageneric classification, future studies should continue to focus on molecular phylogenetics to include species that are currently not represented in the molecular dataset. They should also focus on fruit anatomy and development, which are less explored in *Elaeocarpus*. The genus is large and speciose, so establishing a comprehensive and robust phylogenetic relationship of the known diversity across *Elaeocarpus* is challenging and will require ongoing work. The current phylogenetic framework contains c. 50 % *Elaeocarpus* diversity covering New Guinea, Australia and New Zealand, New Caledonia and the western Malesia. The anatomical and developmental study conducted for this thesis, provides a framework for future studies in *Elaeocarpus*. The research has also investigated fruit mesocarp morphology to improve our understanding in the evolution of genus.

Recommendations for future investigation are listed under the respective studies carried out for the thesis:

6.6.1 Molecular phylogenetics of *Elaeocarpus*

For molecular phylogenetics, future studies should:

- utilise genome-scale sequencing approaches (e.g. genome skimming or target capture).
- utilise nuclear markers to improve species level resolution in the relationships.
- obtain molecular samples of species from Group I, Group II and Group IX from New Guinea (which are currently unavailable) for a comprehensive phylogenetic inclusion of New Guinean representatives with *Elaeocarpus* from other regions.
- continue to obtain molecular samples of species from other regions that are not represented in the molecular dataset for a wider geographical inclusion.
- obtain both morphological and molecular samplings of the putative new species from New Guinea identified by Coode (1978, 1981) for description and delimitation within the groups.
- obtain additional molecular samplings of species with fibrous mesocarps, particularly *E. johnsonii, E. sedentarius, E. blepharoceras* and *E. womersleyi* to test the current morphological placement. Molecular samples of *E. womersleyi* from Maluku (Indonesia), New Guinea, and Papuan Islands (PNG) are required for better resolution. *Elaeocarpus johnsonii* is currently placed with *E. blepharoceras* (Group IV) from New Guinea in the morphological classification but molecular data refutes this, and does not place them together. Utilising nuclear DNA may help improve and clarify the relationship of *E. johnsonii* to other species.
- obtain additional samples of species from the *Obovatus* group particularly, *E. arnhemicus* and *E. sericoloides* from New Guinea, to confirm *E. coorangooloo*'s placement in the *Obovatus* group, rather than in Group VI.

- obtain additional molecular samples of *E. culminicola* and *E. sterrophyllus* and their varieties from New Guinea for better resolution and delimitation.
- obtain additional molecular samplings of *E. hylobroma*, *E. tariensis* and *E. carolinae* to confirm the placement of *E. hylobroma*. *Elaeocarpus hylobroma* is tentatively placed in Group V currently, but it is morphologically similar to *E. tariensis* from Group VI (New Guinea) and *E. carolinae* of Group VII (Australia).

6.6.2 Fruit developmental biology

The study on developmental anatomy and ontogeny of *Elaeocarpus* fruits and seeds has been limited. Future study should focus on examining the fruits with fibrous mesocarps. As a morphological character, it is uncertain whether fruits with persistent fibrous mesocarps are derived through evolution within *Elaeocarpus*. However, the character may still be useful for infrageneric classification or for use in field identification. A thorough investigation of taxa from other genera within Elaeocarpaceae, plus a wider range of *Elaeocarpus* species including those whose mesocarp condition is unknown, is recommended to gain further insights into the taxonomic distribution of taxa with fibrous mesocarps in the genus. The functional significance and evolutionary advantages of possessing fibrous mesocarps are not well understood, due to insufficient knowledge of fruit biology and physiology of taxa with fibrous mesocarps.

6.6.3 Fruit mesocarp and seed morphology of extant and fossil materials

Future studies should focus on lineages within *Elaeocarpus* that have not been sampled adequately in either molecular and fruit morphological analyses to date. This

includes sampling more species from New Guinea, New Caledonia and western Malesia because of the morphological diversity that exists in the mesocarps. Additionally, more species should be scored for ancestral character state reconstruction analyses to infer character evolution of mesocarp and seed morphology in the genus, because the evolution of fruit morphology is poorly known and therefore its value in taxonomy and paleobotany is currently limited. The use of non-destructive CT scanning technology is recommended in future studies to provide detailed information on internal anatomy of the fruits in both modern and fossil material, which may assist with interpretation of fossils. A more comprehensive knowledge of mesocarp ancestral states across the phylogeny is required in future studies to interpret the mesocarp morphology of the small-fruited species. The current mesocarp surface ornamentation categories are useful in delimiting large fruited species with more define characters. However, in smaller fruits, surface characters are often poorly developed and hence difficult to interpret. As noticed in this study, a majority of the smaller mesocarps showed insufficient development of mesocarp morphology to be unambiguously scored against the current character states, and additional states to accommodate them could not be confidently defined. Both an improved estimate of the phylogenetic relationships (which will require denser species sampling and the use of more informative molecular markers) and a more comprehensive database of mesocarp for the species included in the phylogenetic trees will be required to provide this improved knowledge.

REFERENCES

Ali, S., Yu, Y., Pfosser, M., & Wetschnig, W. (2012). Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies). *Annals of Botany,* 109: 95–107.

Aoki, K., Suzuki, T. & Murakami, N. (2003). Intraspecific sequence variation of chloroplast DNA among the component species of evergreen broad-leaved forests in Japan. *Journal of Plant Research*, 116: 337–344.

AVH (2016). *Australia's Virtual Herbarium*. Council of Heads of Australasian Herbaria, http://avh.chah.org.au, accessed 10 June 2016.

Baba, Y. (2014). Evolution, systematics and taxonomy of *Elaeocarpus* (Elaeocarpaceae) in Australasia. Ph.D. thesis. James Cook University, Cairns.

Baba, Y. & Crayn, D. (2012). *Elaeocarpus hylobroma* (Elaeocarpaceae): a new species endemic to mountain tops in north-east Queensland, Australia. *Kew Bulletin*, 67: 743–750.

Baba, Y., Rossetto M. & Crayn, D. M. (2020). Identifying evolutionary lineages in the *Elaeocarpus obovatus* complex: population genetics and morphometric analyses support a new subspecies, *Elaeocarpus obovatus* subsp. *umbratilis*, from northern Queensland, Australia. *Australian Systematic Botany,* 33: 346–379.

Backer, C. A. & Bakhuizen van de Brink, R. C. (1963). Elaeocarpaceae. *Flora of Java: (Spermatophytes only)*. (pp. 396–400). P. Noordhoff, Groningen.

Balick, M. J. (2009). Ethnobotany of Pohnpei. Kings Times Printing Press, Ltd, China.

Baker, W. J., Coode, M. J. E., Dransfield, S., Harley, M. M., Hoffmann, P. & Johns, R. J. (1998). Patterns of distribution of Malesian vascular plants. In: R. Hall & J. D. Holloway (eds) *Biogeography and Geological Evolution of SE Asia.* (pp. 243–258). Backhuys Publishers, Leiden.

Baker, W. J., Bailey, P., Barber, V., Barker, A., Bellot, S., Bishop, D., Botigué, L. R., Brewer, G., Carruthers, T., Clarkson, J. J., Cook, J., Cowan, R. S., Dodsworth, S., Epitawalage, N., Françoso, E., Gallego, B., Johnson, M. G., Kim, J. T., Leempoel, K., Maurin, O., McGinnie, C., Pokorny, L., Roy, S., Stone, M., Toledo, E., Wickett, N. J., Zuntini, A. R., Eiserhardt, W. L., Kersey, P. J., Leitch, I. J., & Forest, F. (2021). A comprehensive phylogenomic platform for exploring the Angiosperm Tree of Life. *Systematic Biology*, syab035, https://doi.org/10.1093/sysbio/syab035.

Bayer, C., Appel, O. (1996) Occurrence and taxonomic significance of ruminate endosperm. *Botanical Review,* 62: 301–310.

Beccari, O (1877-1890) Malesia; raccolta di osservazioni botaniche intorno alle piante dell'arcipelago Indo-Malese e Papuano pubblicata da Odoardo Beccari, destinata principalmente a descrivere ed illustrare le piante da esso raccolte in quelle regioni durante i viaggi eseguiti dall'anno 1865 all'anno 1878 ... Volumes 1-3 (Pubblicazione del R. Instituto di studi superiori pratici e di perfezionamento: Firenze/Genova).

Beniston, M., Diaz, H. F & Bradley, R. S. (1997). Climatic change at high elevation sites: an overview. *Climate Change,* 36: 233–251.

Bera, S., De, A., De, B. (2004) First record of *Elaeocarpus* Linn. fruits from the upper Siwalik sediments (Kimin formation) of Arunachal Pradesh. *Journal of the Geological Society of India,* 64: 350–352

Blackburn, D. T. & Sluiter, I. R. K (1994). The Oligo–Miocene coal floras of southeastern Australia. In 'History of the Australian vegetation. Cretaceous to recent'. (Ed. R. S. Hill) pp. 328–367. Cambridge University Press.

Boeira, A. S. P., Vincentini, A. & Ribeiro, J. E. L. D. S. (2012). Three new species of *Sloanea* L. (Elaeocarpaceae) from the Central Amazon, Brazil. *Brazilian Journal of Botany*, 35 (1): 119–123.

Blattner, F. R. (1999). Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *Biotechnology*, 27: 1180–1185.

Brambach, F., Coode, M., Biagioni, S. & Culmsee, H. (2016). *Elaeocarpus firdausii* (Elaeocarpaceae), a new species from tropical mountain forests of Sulawesi. *PhytoKeys*, 62: 1–14.

Bromham, L. (2008). Reading the story in DNA. New York: Oxford University Press.

Bromham, L., Duchêne, S., Hua, X., Ritchie, A. M., Duchêne, D. A., and Ho, S. Y. (2018). Bayesian molecular dating: opening up the black box. *Biological Reviews,* 93: 1165–1191.

Brown, J. M., Hedtke, S. M., Lemmon, A. R. & Lemmon, E. M. (2010). When trees grow too long: investigating the causes of highly inaccurate Bayesian branch-length estimates. *Systematic Biology*, 59: 145–161.

Burrows, C. J. (1995). A macrofossil flora from sediments in a lagoon marginal to Lake Coleridge, Canterbury, New Zealand. *New Zealand Journal of Botany*, 33:

519–522.

Burrows, C. J. (1997). An interglacial macrofossil flora from Schulz Creek, north Westland, New Zealand. *New Zealand Journal of Botany*, 35: 187–194.

Cámara-Leret, R., Frodin, D. G., Adema, F., Anderson, C., Appelhans, M. S., Argent, G., Arias Guerrero, S., Ashton, P., Baker, W. J., Barfod, A. S., Barrington, D., Borosova, R., Bramley, G. L. C., Briggs, M., Buerki, S., Cahen, D., Callmander, M. W., Cheek, M., Chen, C.-W., Conn, B. J., Coode, M. J. E., Darbyshire, I., Dawson, S., Dransfield, J., Drinkell, C., Duyfjes, B., Ebihara, A., Ezedin, Z., Fu, L.-F., Gideon, O., Girmansyah, D., Govaerts, R., Fortune-Hopkins, H., Hassemer, G., Hay, A., Heatubun, C. D., Hind, D. J. N., Hoch, P., Homot, P., Hovenkamp, P., Hughes, M., Jebb, M., Jennings, L., Jimbo, T., Kessler, M., Kiew, R., Knapp, S., Lamei, P., Lehnert, M., Lewis, G. P., Linder, H. P., Lindsay, S., Low, Y. W., Lucas, E., Mancera, J. P., Monro, A. K., Moore, A., Middleton, D. J., Nagamasu, H., Newman, M. F., Nic Lughadha, E., Melo, P. H. A., Ohlsen, D. J., Pannell, C. M., Parris, B., Pearce, L., Penneys, D. S., Perrie, L. R., Petoe, P., Poulsen, A. D., Prance, G. T., Quakenbush, J. P., Raes, N., Rodda, M., Rogers, Z. S., Schuiteman, A., Schwartsburd, P., Scotland, R. W., Simmons, M. P., Simpson, D. A., Stevens, P., Sundue, M., Testo, W., Trias-Blasi, A., Turner, I., Utteridge, T., Walsingham, L., Webber, B. L., Wei, R., Weiblen, G. D., Weigend, M., Weston, P., De Wilde, W., Wilkie, P., Wilmot-Dear, C. M., Wilson, H. P., Wood, J. R. I., Zhang, L.-B. & Van Welzen, P. C. (2020). New Guinea has the world's richest island flora. *Nature*, 584: 579–583.

CHAH - Council of Heads of Australian Herbaria (2020). Australian Plant Census. http://www.chah.gov.au/apc/index.html. Date Accessed 24 July 2020.

CHAH - Council of Heads of Australian Herbaria (2021). Australian Plant Census. http://www.chah.gov.au/apc/index.html. Date Accessed 14 November 2021.

Chapman, F. (1935). Plant remains of lower Oligocene age from near Blanche Point, Aldinga, South Australia. *Transactions of the Royal Society of South Australia*, 69: 237–240.

Christophel, D.C & Greenwood. (1987). A megafossil flora from the Eocene of Golden Grove, South Australia. *Transactions of the Royal Society of South Australia*, 111(3): 155–162

Christophel, D. C., Harris, W. K, & Syber, A. K. (1987). The Eocene flora of the Anglesea locality, Victoria. *Alcheringa*, 11: 303–323.

Collinson, M. E., Manchester, S. R., Wilde, V & Hayes, P. (2010). Fruit and seed floras from exceptionally preserved biotas in the European Paleogene. *Bulletin of Geosciences*, 85: 155–162.

Coode, M. J. E. (1978). Elaeocarpaceae in Papuasia. *Brunonia*, 1: 131–298.

– (1981). Elaeocarpaceae. In E. E. Henty (Ed.), *Handbooks of the Flora of Papua New Guinea* (Vol. 2). Melbourne: Melbourne University Press.

– (1984). *Elaeocarpus* in Australia and New Zealand. *Kew Bulletin*, 509–520.

– (1987). *Crinodendron*, *Dubouzetia* and *Peripentadenia,* closely related in Elaeocarpaceae. *Kew Bulletin,* 42: 777–814.

– (1996a). *Elaeocarpus* for Flora Malesiana: notes, new taxa and combinations in sect. *Elaeocarpus*: 2. *Kew Bulletin*, 51: 83–101.

– (1996b). *Elaeocarpus* for Flora Malesiana: notes, new taxa and combinations in the *Acronodia* group. *Kew Bulletin*, 267–300.

– (1996c). *Elaeocarpus* for Flora Malesiana: the '*Polystachyus* group': 2. *Kew Bulletin*, 51: 649–666.

– (1996d). Elaeocarpaceae: In M.J.E Coode, J. Dransfield, L.L. Forman, D. W. Kirkup & I.M. Said (eds) *A checklist of the flowering plants & gymnosperms of Brunei Darussalam*. 90–95. Ministry of Industry and Primary Resources, Brunei Darrussalam.

– (2001a). *Elaeocarpus* in New Guinea – new taxa in the *Debruynii* subgroup of the *Monocera* group. Contribution to the Flora of Mt Jaya, V. *Kew Bulletin,* 56: 449–460.

– (2001b). *Elaeocarpus* for Flora Malesiana: the *Fissipetalum* group in central Malesia. *Kew Bulletin,* 461–463.

– (2001c). *Elaeocarpus* for Flora Malesiana: the *E. stipularis* complex, *E. nitidus* group and *E. barbulatus*. *Kew Bulletin,* 56: 513–565.

– (2001d). *Elaeocarpus* for Flora Malesiana: the *Coilopetalum* group in Sulawesi & Maluku. *Kew Bulletin,* 56: 837–874.

– (2001e). *Elaeocarpus* for Flora Malesiana: the *Coilopetalum* group in the Lesser Sunda Islands. *Kew Bulletin,* 56: 875–883.

– (2001f). *Elaeocarpus* for Flora Malesiana: the *Verticellatae* subgroup of the *Monocera* group and a new Philippine species. *Kew Bulletin,* 56: 885–901.

– (2002). *Elaeocarpus* in New Guinea: *E. sericoloides* (*Fissipetalum* group), *E. royenii* & *E. multisectus* (sect. *Elaeocarpus*). Contributions to the Flora of Mt Jaya, IX. *Kew Bulletin*, 925–935.

– (2003). *Elaeocarpus* for Flora Malesiana: two new taxa in the *Coloides* subgroup of the *Monocera* group from New Guinea. *Kew Bulletin*, 453–458.

– (2004). Elaeocarpaceae. In: K. Kubitzki, K. U. Kramer, P. S. Green, J. G. Rohwer & V. Bittrich (eds). The families and genera of vascular plants, VI. Flowering plants Dicotyledons: *Celastrales, Oxalidales, Rosales, Cornales, Ericales*. (pp. 135–144) Springer-Verlag.

– (2005). *Elaeocarpus* for Flora Malesiana: *E. crenulatus, E. myrtoides* and *E. amabilis* from New Guinea, reconsidered. *Kew Bulletin*, 305–311.

– (2010). *Elaeocarpus* for Flora Malesiana: new taxa and understanding in the *Ganitrus* group. *Kew Bulletin*, 65 (3): 355–399.

– (2014). *Elaeocarpus* for Flora Malesiana: the *Monocera* group in western Malesia. *Kew Bulletin*, 69: article 9487.

– (2019a). *Elaeocarpus* for Flora Malesiana: 5 new taxa. *Kew Bulletin* 74: article 36.

– (2019b). *Elaeocarpus* for Flora Malesiana: the *Dactylosphaera* group (Sulawesi to New Guinea). *Kew Bulletin*, 74: article 37.

– (2019c). *Elaeocarpus* for Flora Malesiana: the *Oreocarpus* group in Malesia. *Kew Bulletin*, 74: article 35.

Coode, M. J. E. & Weibel, R. (1994). *Elaeocarpus* for Flora Malesiana: notes, new taxa and combinations in sect. *Elaeocarpus*: 1. *Kew Bulletin*, 49: 235–259.

Cooper, W. & Cooper, W. T. (2004) Fruits of the Australian Tropical Rainforest. Nokomis Editions, Melbourne, Australia.

Corlett, R. T. (2017) Frugivory and seed dispersal by vertebrates in tropical and subtropical Asia: An update. *Global Ecology and Conservation,* 11: 1–22.

Corner, E. J. H. (1976). The Seeds of Dicotyledons. Cambridge University Press, Cambridge, UK.

Costion, C. M., Simpson, L., Pert, P., & Crayn, D. M. (2015). Will tropical mountaintop plant species survive climate change? Identifying key knowledge gaps using species distribution modelling in Australia. *Biological Conservation,* 191: 322–330.

Crayn, D. M, Rossetto, M & Maynard, D. J. (2006). Molecular phylogeny and dating reveals an Oligo-Miocene radiation of dry-adapted shrubs (Tremandraceae) from rainforest tree progenitors (Elaeocarpaceae) in Australia. *American Journal of Botany,* 93(9): 1328–1342.

Dettmann, M. E., & Clifford, H. T. (2002). The fossil record of *Elaeocarpus* L. fruits. *Memoirs of the Queensland Museum*, 46: 461–497.

Dardick, C.D., Callahan, A. M., Chiozzotto, R., Schaffer, R. J., Piagnani, M. C. & Scorza, R. (2010). Stone formation in peach fruit exhibits spatial coordination of the lignin and flavonoid pathways and similarity to Arabidopsis dehiscence. *BMC Biology,* 8: article 13.

Dardick, C.D., Callahan, A. M. (2014). Evolution of the fruit endocarp: molecular mechanisms underlying adaptations in seed protection and dispersal strategies. *Frontiers in Plant Science,* 5: article 284.

DeVore, M. L, Kenrick, P, Pigg, K.B. & Ketcham, R. A. (2006). Utility of high resolution x-ray computed tomography (HRXCT) for paleobotanical studies: an example using London Clay fruits and seeds. *American Journal of Botany,* 93 (12): 1848–1851.

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus,* 12: 13–15.

Erdei, B. & Rakosi, L. (2009) The Middle Eocene flora of Csordakút (N Hungary). *Geologica Carpathica,* 60: 43–57.

Ettingshausen, C. v. (1883). Beiträge zur Kenntniss der Tertiärflora Australiens. *Denkschriften Kaiserlichen Akademie Wissenschaften Wien Part 1,* 47: 101–148.

Ettingshausen, C. v. (1886). Beiträge zur Kenntniss der Tertiärflora Australiens. *Denkschriften Kaiserlichen Akademie Wissenschaften Wien Part 2,* 53: 81–184.

Felsenstein, J. (2004). *Inferring Phylogenies*. Sinauer Associates, Sunderland, Mass.

Foster, P. (2001). The potential negative impacts of global climate change on tropical montane cloud forests. *Earth-Science Reviews*, 55: 73–106.

Gagul, J. (2009). Plant diversity and plant lore of a community in the Pawaiia area of the Simbu Province, Papua New Guinea. *Science in New Guinea*, 29: 79–92.

Gagul, J. (2016). Molecular phylogenetics of *Elaeocarpus* (Elaeocarpaceae) with a focus on New Guinea species. *Australasian Systematic Botany Society Newsletter*, 167: 4–8.

Gagul, J. (2016). Fruit mesocarp morphology of *Elaeocarpus* (Elaeocarpaceae): a phylogenetic survey. *Australasian Systematic Botany Society Conference.* Alice Springs, Australia. [poster].

Gagul, J.N., Tng, Y. P. D & Crayn, D.M. (2018a). Fruit developmental biology and endosperm rumination in *Elaeocarpus ruminatus* (Elaeocarpaceae), and its taxonomic significance. *Australian Systematic Botany*, 31: 409–419.

Gagul, J.N., Simpson, L. & Crayn, D.M. (2018b). *Elaeocarpus carbinensis* J.N.Gagul & Crayn (Elaeocarpaceae), a new species endemic to the Mt Carbine Tableland

of northeast Queensland, Australia. *Austrobaileya*, 10(2): 247–259.

Gärtner, H., Lucchinetti, S. & Schweingruber, F. H. (2014) New perspectives for wood anatomical analysis in dendrosciences: the GSL1-microtome. *Dendrochronologia,* 32: 47–51

Gielly L., Taberlet P. 1994. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus rbcL sequences. *Molecular Biology and Evolution,* 11: 769–777.

Goebel, K. (1933) Organographic der Pflanzen. Ed. 3. Vol. 3. Fischer, Jena, Germany.

Górniak, M., Paun, O., & Chase, M. W. (2010). Phylogenetic relationships within Orchidaceae based on a low-copy nuclear coding gene, Xdh: Congruence with organellar and nuclear ribosomal DNA results. *Molecular Phylogenetics and Evolution*, 56(2): 784–795.

Grada, A and Weinbrecht, K. (2013). Next-generation sequencing: methodology and application. *The Journal of Investigative Dermatology*, 133 (8): e11.

Hably L. (2007) New European records and first evidence of epidermal structures of *Sloanea* L. from the Italian Oligocene – revised type and original material of Principi 1916. *Rivista Italiana di Paleontologia e Stratigrafia,* 113: 449–457

Hall, B. G. (2008). Phylogenetic trees made easy: A How-To-Manual. Sinauer Associates, Inc. USA.

Hart, M. L., Forrest, L. L., Nicholls, J. A., & Kidner, C. A. (2016). Retrieval of hundreds of nuclear loci from herbarium specimens. *Taxon,* 65(5): 1081–1092.

Harrison, J.C., & Langdale, J.A. (2006). A step by step guide to phylogeny reconstruction. *Plant Journal,* 45(4): 561-572.

Heath, T. A., Huelsenbeck, J. P. & Stadler, T. (2014). The fossilized birth-death process: A coherent model of fossil calibration for divergence time estimates. *Proceedings of the National Academy of Sciences* 111(29): E2957-E2966.

Hill, R. S & Macphail, M. K. (1983). Reconstruction of the Oligocene vegetation at Pioneer, northeast Tasmania. *Alcheringa*, 7(4): 281–299.

Horrocks, M., Bulmerc, S. & Gardnerd, R. O. (2008). Plant microfossils in prehistoric archaeological deposits from Yuku rock shelter, Western Highlands, Papua New Guinea. *Journal of Archaeological Science*, 35 (2): 290–301.

Horrocks, M. & Wozniak, J. A. (2008). Plant microfossil analysis reveals disturbed forest and a mixed-crop, dryland production system at Te Niu, Easter Island. *Journal of Archaeological Science*, 35: 126–142.

Hu, L., Pan, H., Zhou, Y., & Zhang, M. (2011). Methods to improve lignin's reactivity as a Phenol substitute and as replacement for other Phenolic compounds: A brief review. *BioResources*, 6(3): 3515–3525.

Huang, T. C. (1972). Pollen Flora of Taiwan. National Univiversity Press, Taipei.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17: 754–755.

Hutchinson, M. F., Houlder, D. J., Nix, H. A. & McMahon, J. P. (2000). ANUCLIM User Guide, Version 5.1. Centre for Resource and Environmental Studies, Australian National University: Canberra.

Ikuse, M. (1956). Pollen Grains of Japan. Hirokawa Pub. Co., Tokyo (in Japanese).

IUCN. (2012). IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK.

IUCN Standards and Petitions Subcommittee (2017). Guidelines for Using the IUCN Red List Categories and Criteria. Version 13. Prepared by the Standards and Petitions Subcommittee. http://www.iucnredlist.org/documents/RedListGuidelines.pdf.

Johnson, M. G., Pokorny, L., Dodsworth, S., Botigué, L. R., Cowan, R. S., Devault, A., Eiserhardt, W. L., Epitawalage, N., Forest, F., Kim, J. T., Leebens-Mack, J. H., Leitch, I. J., Maurin, O., Soltis, D. E., Soltis, P. S., Wong, G. K., Baker, W. J., Wickett, N. J. (2019). A universal probe set for targeted sequencing of 353 nuclear genes from any flowering plant designed using k-medoids clustering. *Systematic Biology,* 68(4): 594-606.

Joyce, E. M., Thiele, K. R., Slik, F. J. W. & Crayn, D. M. (2020). Checklist of the vascular flora of the Sunda-Sahul Convergence Zone. *Biodiversity Data Journal,* 8: e51094.

Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F., & Donoghue, M. J. (2007). Plant Systematics: A Phylogenetic Appoach. 3rd edition. Massachusetts: Sinauer Associates Inc.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods,* 14(6): 587–589.

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30(14): 3059–3066.

Katoh, K. & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics,* 28: 1647–1649.

Kershaw, A. P., & Sluiter, I. R. (1982). Late Cenozoic pollen spectra from the Atherton Tableland, north-eastern Australia. *Australian Journal of Botany*, 30(3): 279–295.

Kershaw, A. P., Martin, H. A., & McEwen-Mason, J. R. C. (1994). The Neogene: a period of transition. In R. S. Hill (Ed.), *History of the Australian Vegetation: Cretaceous to Recent* (pp. 299–327). Cambridge: Cambridge University Press.

Krauss, B. H. (1993). Plants in Hawaiian Culture. University of Hawaii Press, USA.

Kumar, G, Karthik, L and Rao, K. V. B (2014). A Review on Medicinal Properties of *Elaeocarpus ganitrus* Roxb. ex G.Don. (Elaeocarpaceae). *Research Journal of Pharmacy and Technology* 7(10): 1184–1186

Kvaček, Z., Hably, L. & Manchester, S. R. (2001) *Sloanea* (Elaeocarpaceae) fruits and foliage from the Early Oligocene of Hungary and Slovenia. *Palaeontographica Abteilung B Palaeophytologie*, 259: 113–124.

Kvaček, Z. (2002). Late Eocene landscape, ecosystems and climate in northern Bohemia with particular reference to the locality of Kučlin near Bilina. *Bulletin*

of the Czech Geological Survey, 77: 217–236.

Lee, D.W. (1991). Ultrastructural basis and function of iridescent blue colour of fruits in *Elaeocarpus*. *Nature*, 349: 260–262.

Lombardo, A. V., Osorio, S., Borsani, J., Lauxmann, A. M., Bustamante, A. C., Budde, O. C., Andreo, S. C., Lara, V. M., Fernie, R. A. & Drincovich, F. M. (2011). Metabolic profiling during peach fruit development and ripening reveals the metabolic networks that underpin each developmental stage. *Plant Physiology*, 157(4): 1696 – 1710.

Li, F., Li, J., Liu, B., Zhuo, J., Long, C. (2014) Seeds used for Bodhi beads in China. *Journal of Ethnobiology and Ethnomedicine,* 10: 15.

Linnaeus, C. (1753). Species plantarum: exhibentes plantas rite cognitas, ad genera relates, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas, 1: 515. Impensis Laurentii Salvii, Holmiae.

Lipman, D. J & Pearson, W. R. (1985). Rapid and sensitive protein similarity searches.*Science,* 227(4693): 1435–1441.

Liu, X.-Y., Manchester, S. R., Rozefelds, A. C., Quan, C., & Jin, J.-H. (in press) First fossil fruits of Elaeocarpus (Elaeocarpaceae) in East Asia: Implications for phytogeography and paleoecology. *Journal of Systematics and Evolution*. doi:https://doi.org/10.1111/jse.12684

Luly, J., Sluiter, I. R & Kershaw, A. P. (1980). Pollen studies of Tertiary brown coals: preliminary analyses of lithotypes within the Latrobe Valley, Victoria. *Monash Publications in Geography*, 23: 1–78.

Mabberly, D. J. (2006). The plant Book: a portable dictionary of higher plants. Cambridge University Press, UK.

Macphail, M. K., Alley, N. F., Truswell, E. M., & Sluiter, I. R. H. (1994). Early Tertiary vegetation: evidence from spores and pollen. In R. S. Hill (Ed.), *History of the Australian Vegetation: Cretaceous to Recent* (pp. 189–261). Cambridge: Cambridge University Press.

Mahomoodally M. F., Sookhy V. (2018). Ethnobotany and Pharmacological Uses of *Elaeocarpus floribundus* Blume (Elaeocarpaceae). In: Ozturk M., Hakeem K. (eds) Plant and Human Health, Volume 1. Springer, Cham.

Manchester, S. R. & Kvaček, Z. (2009) Fruits of *Sloanea* (Elaeocarpaceae) in the Paleogene of North America and Greenland. *International Journal of Plant Sciences,* 170: 941–950.

Maynard, D. (2004). A molecular phylogeny for Australian *Elaeocarpus* (Elaeocarpaceae) and the affinities of a putative new taxon. Honours thesis, University of New South Wales, Australia.

Maynard, D., Crayn, D., Rossetto, M., Kooyman, R. & Coode, M. J. E. (2008). *Elaeocarpus sedentarius* sp. nov. (Elaeocarpaceae) – morphometric analysis of a new, rare species from eastern Australia. *Australian Systematic Botany*, 21(3): 192–200.

Martin, H. A. (1998). Late Cretaceous-Cainozoic palynology of the Poonarunna No. 1 well, central Australia. *Transactions of the Royal Society of South Australia,* 122(3-4): 89–138.

McPherson, H. (2008). Phylogenetics and evolutionary dynamics of *Tetratheca* (Elaeocarpaceae). The University of New England.

McPherson, H., Porter, C., Rymer, P.D., Crayn, D.M., & Rossetto, M. (2008). Isolation and characterization of polymorphic microsatellite loci from *Tetratheca ericifolia* (Elaeocarpaceae). *Molecular Ecology Resources*, 8: 867–869.

Moore, C. (2003). New Guinea: Crossing Boundaries and History. University of Hawaii Press, Honolulu.

Murphy, B., Forest, F., Barraclough, T., Rosindell, J., Bellot, S., Cowan, R., Golos, M., Jebb, M., Cheek, M. (2020) A phylogenomic analysis of *Nepenthes* (Nepenthaceae). *Molecular Phylogenetics and Evolution,* 144: article 106668.

Murphy, H., Liedloff, A., Williams, R. J., Williams, K. J. & Dunlop, M. (2012) Queensland's biodiversity under climate change: terrestrial ecosystems. CSIRO Climate Adaptation Flagship Working Paper No. 12C. http://www.csiro.au/resources/CAF-working-papers.html

Murti, S. K. (1993). Botanical Survey of India. In: B. D. Sharma & M. Sanjappa (eds). Flora of India, Vol. 3 (pp. 528–563). Government of India, Calcutta.

Muthuswamy, R., Senthamarai, R. (2014). Pharmacognostical studies on the fruit of *Elaeocarpus oblongus* Gaertn. *Pharmocognosy Journal,* 6(3): 72–78.

Nakićenović, N., Alcamo, J., Davis, G., De Vries, B., Fenhann, J., Gaffin, S., Gregory, K., Grübler, A., Jung, T. Y., Kram, T., Lebre La Rovere, E., Michaelis, L., Mori, S., Morita, T., Pepper, W., Pitcher, H., Price, L., Riahi, K., Roehrl, A., Rogner, H.-H., Sankovski, A., Schlesinger, M., Shukla, P., Smith, S., Swart, R., Van Rooijen, S., Victor, N. & Dadi, Z. (2000). Emissions Scenarios - Special Report of Working Group III of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Nauheimer, L., Weigner, N., Joyce, E., Crayn, D., Clarke, C., & Nargar, K. (2021). Hybphaser: A workflow for the detection and phasing of hybrids in target capture data sets. *Applications in Plant Sciences,* 9(7): e11441.

Neubig, M. K. & Abbott, R. J. (2010). Primer development for the plastid region *ycf1* in Annonaceae and other magnoliids. *American Journal of Botany*, 97(6): 52–55.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2014). IQ-TREE: A fast and effective stochastic algorithm for estimating Maximum-Likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274.

Niissalo, M. A. (2011). Biogeography and Phylogeny of *Sloanea.* M.Sc. Thesis. University of Edinburgh, Edinburgh.

O'Dowd, D. J., Brew, C. R., Christophel, D. C., & Norton, R. A. (1991). Mite-plant associations from the Eocene of southern Australia. *Science,* 252: 99–101.

Oxelman, B., Lidén, M. & Berglund, D. (1997). Chloroplast *rps*16 intron phylogeny of the tribe *Sileneae* (*Caryophyllaceae*). *Plant Systematics and Evolution* 206: 393–410.

Patterson, C., Williams, D. M., & Humphries, C. J. (1993). Congruence between molecular and morphological phylogenies. *Annual Review of Ecology and Systematics,* 24: 153–188.

Paijmans, K. (1976). New Guinea Vegetation. Australian National University Press, Canberra.

Pearson, W. R & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proceedings of the National Academy of Sciences of the United States of America,* 85(8): 2444–2448.

Pennington, T. D., & Wise, R. (2017). The genus *Sloanea* (Elaeocarpaceae) in America. Milbourne Port, England: Dh Books.

Periasamy, K. (1990). The ruminate seed. *Indian Review of Life Sciences,* 10: 141–168.

Phillips, S. J., Anderson, R. P. & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling,* 190: 231–259

Phoon, S. N. (2012). *Elaeocarpus angustifolius*. *Flora of Peninsular Malaysia Online Newsletter,* 122/26.

Phoon, S. N. (2015). Systematics and Biogeography of *Elaeocarpus* (Elaeocarpaceae). Ph.D. thesis. James Cook University, Cairns.

Pole, M. (1993). Early Miocene flora of the Manuherikia Group, New Zealand. 5. Smilacaceae, Polygonaceae, Elaeocarpaceae, *Journal of the Royal Society of New Zealand*, 23 (4): 289–302.

Posada, D. (2008). jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, 25: 1253–1256.

Pounds, J. A., Fogden, M. P. L. & Campbell, J. H. (1999). Biological response to climate change on a tropical mountain. *Nature,* 398: 611–615.

Pounds, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogen, M. P. L., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., Puschendorf, R.,

Ron, S. R., Sanchez-Azofeifa, G. A., Still, C. J. & Young, B. E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature,* 439: 161–167.

Prakash, U. & Dayal, R. (1964). Fossil woods resembling *Elaeocarpus* and *Leea* from the Deccan Intertrappean beds of Mahurzaei near Nagpur. *Palaeobotanist* 12: 121–127.

Premathilake, R., & Nilsson, S. (2001). Pollen morphology of endemic species of the Pollen morphology of endemic species of the Horton Plains National Park, Sri Lanka. *Grana*, 40: 256–279.

Ramasubbu, R. & Irudhyaraj, D. F. (2016). Reproductive biology of *Elaeocarpus blascoi* Weibel, an endemic and endangered tree species of Palni Hills, Western Ghats, India. *Current Science*, 110(2): 234–240.

Rao, R. R & Hajra, P. K. (1986). Methods of Research in Ethnobotany, Pawan Kumar for Scientific Publishers, India.

Ridley, H. N. (1922). Polypetalae. The flora of Malay Peninsula. 1: 309–321. L. Reeve & Co., Ltd., London.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19(12): 1573–1574.

Rossetto, M., Kooyman, R., Sherwin, W. & Jones, R. (2008) Dispersal limitations, rather than bottlenecks or habitat specificity, can restrict the distribution of rare and endemic rainforest trees. *American Journal of Botany,* 95: 321–329.

Rossetto, M., Crayn, D., Ford, A., Mellick, R. & Sommerville, K. (2009) The influence of environment and life-history traits on the distribution of genes and

individuals: a comparative study of 11 rainforest trees. *Molecular Ecology,* 18: 1422–1438.

Rozefelds, A. C. (1990). A taxonomic study of extant and fossil fruits of the genus *Elaeocarpus* (Elaeocarpaceae) in Australia and New Zealand. Honours thesis, Department of Botany, University of Adelaide, Australia.

Rozefelds, A. C., & Christophel, D. C. (1996a). *Elaeocarpus* (Elaeocarpaceae) endocarps from the Early to Middle Miocene Yallourn formation of Eastern Australia. *Muelleria*, 9: 229–237.

– (1996b). *Elaeocarpus* (Elaeocarpaceae) endocarps from the Oligo-Miocene of eastern Australia. *Papers and Proceedings of the Royal Society of Tasmania* 130: 4–48.

– (2002). Cenozoic *Elaeocarpus* (Elaeocarpaceae) fruits from Australia. *Alcheringa*, 26 (2): 261–274.

Sachse, M. (2005) A remarkable fossiliferous mass flow deposit in the Eocene Eckfeld Maar (Germany) – sedimentological, taphonomical, and palaeoecological considerations. *Facies,* 51: 173–184

Sanderson, M.J. (2004) r8s, version 1.70 user's manual. Section of evolution and ecology. Davis, CA: University of California.

Sang, T., Crawford, D. J & Stuessy, T. F. (1997). Chloroplast DNA phylogeny, reticulate evolution and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany*, 84: 1120–1136.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain terminating inhibitors. *Proceedings of the National Academy of Sciences, U.S.A.,* 74: 5463.

Sauquet, H. (2013). A practical guide to molecular dating. *Comptes Rendus Palevol*, 12: 355–367.

Schlechter, R. (1916). Die Elaeocarpaceen Papuasiens. In: C. Lauterbach (ed) Beitrage zur Flora von Papuasien V. *Botanische Jahrbucher* 54: 92–155.

Schwarz, G. (1978). Estimating the dimension of a model. *The Annals of Statistics*, 6(2): 461–464.

Shah, G., Shiri, R., Kumar, R., Singh, H. & Nagpal, N. (2010) Pharmacognostical standardization of fruits of *Elaeocarpus sphaericus* (Gaertn). K. Schum. *Pharmacognosy Journal,* 2: 565–571.

Shaw, A. J., Lickey, E. B., Schilling, E. E., & Small, R. L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany,* 94: 275–288.

Shubharani, R., Roopa, P & Sivaram, V. (2013). Pollen morphology of selected bee forage plants. *Global Journal of Bio-Science and Biotechnology*, 2 (1): 82–90.

Singh, B., Ishar, M. P. S., Sharma, A., Arora, R. & Arora, S. (2015) Phytochemical and biological aspects of Rudraksha, the stony endocarp of *Elaeocarpus ganitrus* (Roxb.): a review. *Israel Journal of Plant Sciences,* 62: 265–276.

Smith A. C. (1944). Studies of Papuasian plants, VI. *Journal of the Arnold Arboretum* 25: 104–121 & 222–298.

Smith A. C. (1953). Studies of Pacific Island plants, XV – the genus *Elaeocarpus* in the New Hebrides, Fiji, Samoa, and Tonga. *Contributions from the United States National Herbarium* 30(5): 523–575.

Still, C. J., Foster, P. N. & Schneider, S. H. (1999). Simulating the effects of climate change on tropical montane cloud forests. *Nature,* 398: 608–610.

Sugita, M., Miyata, Y., Maruyama, K., Sugiura, C., Arikawa, T., & Higuchi, M. (2006). Extensive RNA editing in transcripts from the PsbB operon and RpoA gene of plastids from the enigmatic moss *Takakia lepidozioides*. *Bioscience, Biotechnology, and Biochemistry*, 70: 2268–2274.

Sun, Y., Skinner, D. Z., Liang, G. H., & Hulbert, S. H. (1994). Phylogenetic analysis of Sorghum and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics*, 89: 26–32.

Swofford, D. L. (2003). PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991). Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Molecular Biology*, 17: 1105–1109.

Tang, Y. & Phengklai, C. (2007). *Elaeocarpus*. In: Wu, Z. Y., Raven, P. H. & Hong, D. Y. (eds.), Flora of China. 12: 223–235. Science Press, Beijing, & Missouri Botanical Garden Press, St. Louis.

Tani, E., Polidoros, A. & Tsaftaris, A. (2007). Characterization and expression analysis of FRUITFULL-and SHATTERPROOF-like genes from peach (*Prunus persica*) and their role in split-pit formation. *Tree Physiology,* 27: 649–659.

Tate, J. & Simpson, B. B. (2003). Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany*, 28: 723–737.

Tirel, C. (1982). Éléocarpacées, Monimiacées, Amborellacées, Athérospermatacées, Triméniacées, Chloranthacées. Flore de la Nouvelle-Calédonie et Dépendances. *Muséum National d'Histoire Naturelle, Paris*, 11: 82.

Tirel, C. (1985). Elaeocarpacées. *Fl. Madagascar*. 125: 1–53.

Tissot, C., Chikhi, H. & Nayar, T. S. (1994). Pollen of wet evergreen forests of the Western Ghats, India. Publications du department d'ecologie, no. 35. Institut Francais de Pondichery.

Truswell, E.M., Kershaw, A.P. & Sluiter, I.R. (1987) The Australian-Southeast Asian connection: evidence from the palaeobotanical record. In: Whitmore, T.C. (ed.), Biogeographical Evolution of the Malay Archipelago. (pp. 32–49). Clarendon Press: Oxford.

Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T. W., McNeill, J., Monro, A. M., Prado, J., Price, M. J. & Smith, G. F. (2018). *International code of nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017.* (Vol. 159). Glashütten: Koeltz Botanical Books.

Umar, R., Khalid, N., Sukari, M., Rahmani, M., Abdul, A. & Hamidi, D. (2013). Phenolic contents, antioxidant and cytotoxic activities of *Elaeocarpus floribundus* Blume. *Pakistan Journal of Pharmaceutical Sciences,* 26: 245–250.

Van Balgooy, M. M. J., Low, Y. W. & Wong, K. M. (2015) Spot characters for the identification of Malesian seed plants: a guide. Natural History Publications (Borneo), Kota Kinabalu, Malaysia.

Van Setten, A. K. & Koek-Noorman, J. (1992) Fruits and seeds of the Annonaceae:

Morphology and its significance for classification and identification. *Bibliotheca Botanica,* 142: 1–101.

Vanderwal, J., Shoo, L. P., Johnson, C. N. & Williams, S. E. (2009). Abundance and the environmental niche: environmental suitability estimated from niche models predicts the upper limit of local abundance. *American Naturalist,* 174: 282–291.

Vanderwal, J. J., Beaumont, L., Zimmermann, N. E., Lorch, P. & Blodget, D. (2011). *Climates*. v.0: 1-1.4: Methods for working with weather and climate. Software package www.rforge.net/climates/.

Von Teichman, I. & Van Wyk, A. E. (1993) Ontogeny and structure of the drupe of *Ozoroa paniculosa* (Anacardiaceae). *Botanical Journal of Linnean Society,* 111: 253–263.

Wannan, B. S., Quinn, C. J. (1990) Pericarp structure and generic affinities in Anacardiaceae. *Botanical Journal of Linnean Society,* 102: 225–252.

Warburg, O. (1890). Beiträge zur Kenntniss der papuanischen Flora [Contributions to the knowledge of Papuan flora]. In Engler (ed.) Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie XIII: 230–450.

Werker E (1997) Seed anatomy. In Encyclopaedia of Plant Anatomy, Band 10, Teil 3. Gebrüder Borntraeger, Berlin, Germany.

Weibel, R. W. (1968). Morphologie de l'embryon et de la graine des *Elaeocarpus*. *Candollea*, 23: 101–108.

Weibel, R. W. (1971). Espéces nouvelles du genre *Elaeocarpus* provenant de la Nouvelle-Guinée et de ses dépendances, des îles Salomons et de Célébes. *Candollea*, 26: 359–382.

Weising, K., Nybom, H., Wolff, K. & Kahl, G. (2005). DNA fingerprinting in plants: principles, methods, and applications. CRC Press, Boca Raton, USA

Welch, J. J. & Bromham, L. (2005). Molecular dating when rates vary. *Trends in Ecology and Evolution*, 20 (6): 320–327.

Wells, T., Maurin, O., Dodsworth, S., Friis, I., Cowan, R., Epitawalage, N., Brewer, G., Forest, F., Baker, W. & Monro, A. K. (2021). Combination of sanger and target-enrichment markers supports revised generic delimitation in the problematic 'urera clade' of the nettle family (Urticaceae). *Molecular Phylogenetics and Evolution, 158*, 107008.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis (Ed.), PCR Protocols: A Guide to Methods and Applications. (pp. 315–322). Academic Press.

Wiens, J. J. (2004). Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution,* 58(1): 193–197.

Wilkinson, J. M., Szabo, C., Ford, S. C., Yarom, Y., Croxford, E. A, Camp, A & Gooding, P. (2017). Replacing Sanger with Next Generation Sequencing to improve coverage and quality of reference DNA barcodes for plants. *Scientific Reports,* 7: 46040.

Womersley, JS (ed.) (1978) 'Handbooks of the flora of Papua New Guinea.' Vol. 1 (Melbourne University Press: Carlton) 278 pp.

Yu, Y., Blair, C. & He, X. (2020). RASP 4: ancestral state reconstruction tool for multiple genes and characters. *Molecular Biology and Evolution,* 37: 604–606.

Zmarzty, S. (2001). Revision of *Elaeocarpus* (Elaeocarpaceae) section *Elaeocarpus* in Southern India and Sri Lanka. *Kew Bulletin,* 56: 405–447.

Zwickl, D.J., & Hillis, D.M. (2002). Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology*, 51: 588–598

APPENDICES

Appendix

1.1 Glossary of terms used in this thesis.

The following abbreviations, signs and symbols are used throughout the thesis, categorized under each heading.

Taxonomy and classification

aff.	affinity = close to
c.	circa = about
distribuendi	to be distributed
et al.	et alia = and others
ibid.	ibidem = in the same book or reference
sp.	species (singular)
spp.	species (plural)
ssp. or subsp.	subspecies
sp. nov.	species nova = new species
var.	variety

Author and collector name abbreviations

Author name abbreviations follow the classical Draft Index of Author Abbreviations (Mabberly, 2006) protocol. Last names of authors and collectors are used throughout where acceptations are made for initials of first names where there is a possibility of confusion with other authors and collectors.

Conservation

ANUCLIM	Software package used for spatial modeling of environmental and natural resources
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
IUCN	International Union for Conservation of Nature
ENM	Environmental Niche Modeling
MAXENT	Maximum entropy modelling of species' geographic distributions
VU (D2)	'Vulnerable' under criteria D2 (restricted distribution, and plausibility and immediacy of threat) due to climate change

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Molecular phylogenetics

AGRF	Australian Genome Research Facility
cpDNA	chloroplast DNA
ITS	Internal Transcribed Spacer
mtDNA	mitochondrial DNA
nDNA	nuclear DNA
kbp	kilo base pairs
bp	base pairs
trnH-psbA, trnL-F, trnV-ndhC	intergenic spacers of cpDNA
Xdh	Xanthine Dehydrogenase
BI	Bayesian Inference
MAFFT	Multiple sequence alignment program
MESQUITE	Software for evolutionary biology, designed to analyse comparative data about organisms
ML	Maximum Likelihood
MP	Maximum Parsimony
PAUP	Phylogenetic analysis using PAUP
CTAB	Cetyl trimethylammonium bromide
DNA	Deoxyribonucleic acid
NGS	Next Generation Sequencing (next gen sequencing)
PCR	Polymerase Chain Reaction

WPS	Whole Plastome Sequencing

Nucleotide and amino acid character codes

A	ADENINE
C	CYTOSINE
G	GUANINE
T	THYMINE

Fruit morphology and anatomy (as used in this study)

bs	brachysclereids
gp	ground parenchyma
end	endocarp
es	endosperm
exo	exocarp
IN	Inner Mesocarp
L	Locule
LS	Longitudinal Section
mes	mesocarp
OM	Outer Mesocarp
ov	ovule
pl	placenta
sb	sclerenchyma bundles
t	trichomes
TS	Transverse Section
tb	anniferous bodies.

Other abbreviations, signs and symbols

+/-	plus minus: more or less
&	and
°C	degree Celsius
%	percentage
<	greater than

>	less than
=	equal to
*****	asterix
µm	micrometer
asl	above sea level
AVH	Australasian Virtual Herbarium
cm	centimeter
e.g.	exempli gratia = for example
FAA	Formaldehyde Alcohol Acetic Acid
GIS	Geographical Information Systems
i.e.	id est = that is
JCU	James Cook University
m	meter
mm	millimeter
NGF	New Guinea Forces
pers. comm	personal communication
s	seconds
SEM	Scanning Electron Microscope
SFR	State Forest Reserve
UPNG	University of Papua New Guinea
v.	version

Appendix 2.1 Specimens used for environmental niche modelling of *Elaeocarpus carbinensis*.

Specimens collected from the same location are excluded.

Catalog number	Previous name	Collector and Number
CNS 134375.1	*Elaeocarpus* sp. Mt Spurgeon (B.Hyland 2907RFK)	Y. Baba 426, C. Kilgour & K. Schulte
QRS 127312.1	*Elaeocarpus* sp. Mt Spurgeon (B.Hyland 2907RFK)	A. Ford 4312 & G. Sankowsky
QRS 93786.1	*Elaeocarpus* sp. Mt Spurgeon (B.Hyland 2907RFK)	B. Gray 5196
QRS 105520.1	*Elaeocarpus* sp. Mt Spurgeon (B.Hyland 2907RFK)	B. Gray 5938
BRI AQ0233078	*Elaeocarpus* sp. Mt Misery (L.J.Webb+ 10905)	B. Hyland 6731
BRI AQ0419732	*Elaeocarpus* sp. Mt Misery (L.J.Webb+ 10905)	B. Hyland 7971
BRI AQ0020524	*Elaeocarpus* sp. (Mt Misery L.J.Webb+ 10905)	L.J. Webb 10905
CBG 9102543.1	*Elaeocarpus* sp. nov. (Mt Lewis 1)	I.R. Telford 11342
QRS 94486.1	*Elaeocarpus* sp. Mt Spurgeon (B.Hyland 2907RFK)	B. Hyland 14087
QRS 93638.1	*Elaeocarpus* sp. Mt Spurgeon (B.Hyland 2907RFK)	B. Hyland 25789RFK
BRI AQ0849050	*Elaeocarpus* sp. (Mt Misery L.J.Webb+ 10905)	B. Hyland 2907RFK
BRI AQ0485068	*Elaeocarpus* sp. Mt Misery (L.J.Webb+ 10905)	L.W. Jessup GJD3364
BRI AQ0486550	*Elaeocarpus* sp. Mt Misery (L.J.Webb+ 10905)	L.W. Jessup GJM919

Source: Herbarium specimens seen and verified.

Appendix 5.1 Mesocarp morphologies of extant *Elaeocarpus* species based on the literature and observations from the current study. Photographs of the mesocarps of these species are provided in Appendix 5.3. The term 'miscellaneous' is used here to refer to mesocarp surface sculpturing that does not fit the paleobotany scheme recognised by others (Dettmann and Clifford, 2000; Rozefelds and Christophel, 1990a, 1996a, b, 2002; Liu et al., in press) (Fig. 5.14). Only mesocarp shape, surface sculpturing, embryo shape, endosperm ornamentation characters and locule numbers have been used in the ancestral state reconstruction analysis. Refer to Appendix 5.4 for the character coding.

Species names	Group	Mesocarp shape	Mesocarp surface sculpture	Embryo shape	Endosperm ornamentation	Locule number	Voucher/ collection number	Herbarium/ Institution code	Reference
Elaeocarpus alaternoides Brongn. & Gris	*Dicera*	Ovoid-ellipsoid	Miscellaneous	Straight	Entire	2–3	P. Morat 6288	BRI	This study; Phoon (2015); Tirel (1983)
Elaeocarpus altigenus Schltr. (= *E. sayeri* var. *altigenus*)	VIIIC	Ovoid-ellipsoid	Rugose	Curved	Entire	2 (?4)	ANU 739	CANB	This study; Coode (1978, 1981)
Elaeocarpus angustifolius Blume (=*E. sphaericus*)	VA	Spherical	Bastionate	Straight	Entire	3–5	J. Gagul 18	CNS	This study; Coode (1978, 1981, 1984, 2010); Dettmann &

									Clifford (2000); Rozefelds & Christophel (2002); Phoon (2015)
Elaeocarpus arnhemicus F.Muell.	VD	Ovoid-ellipsoid	Baculate	Straight	Entire	3 (4 external sutures)	B. Hyland 11243	BRI	Coode (1978, 1981, 1984); Rozefelds and Christophel (2002)
Elaeocarpus bancroftii F.Muell.	VIB	Ovoid-ellipsoid	Punctate and pitted	Straight	Entire	2–5	B. Gray 2328	BRI	This study; Phoon (2015); Coode (1984); Dettmann and Clifford (2000); Rozefelds and Christophel (1996b); Rozefelds (1990, Hons thesis)
Elaeocarpus blepharoceras Schltr.	IV	Spherical (irregularly spherical with skin)	Fibrous (to inner mesocarp)	Straight	Entire	2	J. Gagul 2	CNS	This study; Maynard et al. (2008); Coode (1978, 1981)
Elaeocarpus braceanus Watt ex C.B.Clarke	*Elaeocarpus*	Ovoid-ellipsoid (more ellipsoidal)	Bastionate	Straight	Entire	?3	K. Fujikawa 94360	MBK	This study

Elaeocarpus carbinensis J.Gagul and Crayn	Unassigned	Ovoid-ellipsoid	Punctate with longitudinal ridging, mesocarp appearing stellate in TS (less prominent stellate ridges)	Straight	Entire	5–7	B. Gray 5197	CNS	This study; Gagul et al. (2018); Phoon (2015); Dettmann and Clifford (2000); Rozefelds and Christophel (1996b)
Elaeocarpus carolinae B.Hyland and Coode	VII	Ovoid-ellipsoid	Echinate	Curved	Entire	2–3	B. Hyland 3171 RFK	BRI	This study; Coode (1984); Phoon (2015); Rozefelds (1990a); Dettmann and Clifford (2000)
Elaeocarpus coorangooloo J.F.Bailey and C.T.White	VIE	Ovoid-ellipsoid	Verrucate	Straight	Entire	2	B. Hyland 12637	BRI	This study; Phoon (2015); Coode (1984); Rozefelds (1990, Hons. thesis)
Elaeocarpus culminicola Warb.	VII	Ovoid-ellipsoid	Echinate	Curved	Entire	2–3	J. Gagul 31	CNS	Coode (1978, 1981,1984, Coode, in review)

Elaeocarpus dentatus (J.R. and G.Forst.) Vahl	VD	Ovoid-ellipsoid	Miscellaneous. Foramina on ridges	Straight	Entire	2	Voucher unknown	BRI	This study; Ian Geary (2016 pers. comm.); Phoon (2015); Coode (1984)
Elaeocarpus dolichostylus Schltr.	VA	Spherical	Bastionate	Straight	Entire	3–5	YS3G027 4	CNS	This study; Coode (1978, 1981)
Elaeocarpus dolichostylus var. *hentyi*	VA	Ovoid-ellipsoid	Bastionate	Straight	Entire	5	J. Gagul 22 (image of mesocarp unavailabl e)	CNS	This study; Coode (1978, 1981).
Elaeocarpus elliffii B.Hyland and Coode	XIB	Ovoid-ellipsoid	Granulose	Curved	Ruminate	3 (6 external segment s)	A. K. Irvine 1478	BRI	This study; Phoon (2015); Coode (1998); Rozefelds (1990, Hons. thesis)
Elaeocarpus eumundi F.M.Bailey	VII	Ovoid-ellipsoid	Echinate	Curved	Entire	2	B. Gray 3278	BRI	This study; Coode (1984); Phoon (2015); Rozefelds (1990a); Dettmann and Clifford (2000)

Elaeocarpus ferruginiflorus C.T.White	XIB	Ovoid-ellipsoid	Smooth	Curved	Ruminate	2	B. Hyland 13460	BRI	Coode (1984)
Elaeocarpus foveolatus F.Muell.	XIB	Ovoid-ellipsoid	Smooth	Curved	Ruminate	3	B. Hyland 13654	BRI	Coode (1984)
Elaeocarpus fuscoides R.Knuth	VIIIA	Ovoid-ellipsoid	Granulose	Curved	?Entire	3 (4 external sutures)	P. van Royen NGF15061	CANB	This study; Coode (1978, 1981)
Elaeocarpus glaber Blume	*Elaeocarpus*	Ovoid-ellipsoid	Miscellaneous. More strong processes towards base, sutures longitudinally aligned	Straight	Entire	3	A.J.G.H. Kosterm ans 56	BRI	This study; Phoon (2015); Coode (in prep.)
Elaeocarpus grahamii F.Muell.	VII	Ovoid-ellipsoid	Echinate	Curved	Entire	2–3	B. Hyland 13428	BRI	This study; Phoon (2015); Coode (1998); Rozefelds (1990, Hons. thesis)
Elaeocarpus grandis F.Muell.	VA	Spherical	Bastionate	Straight	Entire	3–5	B. Gray 2749	BRI	This study; Coode (1984); Phoon (2015); Dettmann and Clifford (2000);

									Rozefelds and Christophel (2002)
Elaeocarpus griffithii (Wight) A.Gray	*Coilopetalum*	Ovoid-ellipsoid	Miscellaneous	Curved	Entire	2	F.H. Endert 2028	BRI	This study; Phoon (2015); Coode (1998)
Elaeocarpus habbemensis A.C.Sm.	VIIIC	Ovoid-ellipsoid	Rugose	Curved	Entire	3	T. G. Hartley 11707	CANB	This study; Coode (1978, 1981)
Elaeocarpus holopetalus F.Muell.	X	Ovoid-ellipsoid	Miscellaneous	Straight with hooked tip	Entire	2	Constable 6978 (QRS 001692)	BRI	This study, Phoon (2015); Coode (1984)
Elaeocarpus hylobroma Y.Baba and Crayn	V (tentative)	Ovoid-ellipsoid	Bastionate	Straight	Entire	(2-)3	L. Brass 221	BRI	This study; Baba & Crayn (2012; Phoon (2015); Dettmann and Clifford (2000); Rozefelds and Christophel (1996a)
Elaeocarpus johnsonii F.Muell.	IV	Ovoid-ellipsoid (obovoid with skin)	Fibrous	Straight	Entire	3(-4)	T. S. Risley 428	BRI	Coode (1984)

Elaeocarpus kirtonii F. Muell. ex F.M.Bailey	VII	Ovoid-ellipsoid	Echinate	Curved	Entire	2	A. K. Irvine 1414	BRI	This study; Phoon (2015); Coode (1984); Rozefelds (1990, Hons. thesis)
Elaeocarpus largiflorens C.T.White subsp. *largiflorens*	XIB	Ovoid-ellipsoid	Smooth	Curved	Ruminate	3	B. Hyland 13745	BRI	Coode (1984)
Elaeocarpus ledermannii Schltr.	VIIID	Ovoid-ellipsoid	Rugose	Curved	Entire	2–3	P. J. Darbyshire 8255	CANB	This study; Coode (1978, 1981)
Elaeocarpus linsmithii Guymer	VII	Ovoid-ellipsoid	Punctate (inner mesocarp wall not thick)	Curved	Entire	2–5	B. Hyland 13606	BRI	This study; Phoon (2015); Coode (1984); Rozefelds and Christophel (1996b); Rozefelds (1990, Hons thesis)
Elaeocarpus miegei Weibel	VIE	Ovoid-ellipsoid	Miscellaneous	Straight	Entire	2–3	A. Gillison NGF25754	CANB	Coode (1978, 1981, 1984)

Elaeocarpus multiflorus (Turcz.) Fern.-Vill.	*Coilopetalum*	Ovoid-ellipsoid	Miscellaneous	Curved	Ruminate	3	Kuswata 287	BRI	This study; Phoon (2015); Coode (2001d)
Elaeocarpus multisectus Schltr.	IIIA	Ovoid-ellipsoid	Miscellaneous. Suture line depressed but well sculptured, with widely scattered processes	Straight	Entire	3	J.S. Womersley NGF24978	BRI	This study; Coode (1978,1981); Phoon (2015)
Elaeocarpus nubigenus Schltr.	VD	Spherical	Miscellaneous	Straight	Entire	3	R. Pullen 5405	CANB	Coode (1978, 1981)
Elaeocarpus polydactylus Schltr.	VD	Ovoid-ellipsoid	Bastionate	Straight	Entire	3 (?4)	J. Gagul 17	CNS	This study; Phoon (2015); Coode (1978, 1981)
Elaeocarpus polystachyus Wall. ex Mull. Berol	*Polystachyus*	Ovoid-ellipsoid	Miscellaneous. Shallow furrows on ridges	Curved	Ruminate	3	185709	BRI	This study; Phoon (2015); Coode (1996c)
Elaeocarpus ptilanthus Schltr.	VA	Spherical	Bastionate (extreme sculpturing with tunnels underneath)	Straight	Entire	3–5	J. Gagul 8	CNS	This study; Coode (1978, 1981)

Elaeocarpus pycnanthus A.C.Sm.	VIIIB	Ovoid-ellipsoid	Granulose	Curved	?Entire	1–3	P. van Royen NGF18259	CANB	This study; Coode (1978, 1981)
Elaeocarpus reticulatus Sm.	VII	Ovoid-ellipsoid	Echinate (apex round, not sharp or pointy)	Curved	Entire	2–3	Voucher unknown	Voucher unknown	This study; Coode (1984); Phoon (2015); Rozefelds (1990a); Dettmann and Clifford (2000)
Elaeocarpus robustus Roxb.	*Elaeocarpus*	Spherical	Miscellaneous. Linear ridging	Straight	Entire	5	Ngadiman 34726	BRI	This study; Phoon (2015); Coode (1996a)
Elaeocarpus rotundifolius Brongn. & Gris	I	Ovoid-ellipsoid (flat)	Miscellaneous. Resembles *E. undulatus*	Straight	Entire	2	G. McPherson 4852	BRI	This study; Phoon (2015); Tirel (1983)
Elaeocarpus ruminatus F.Muell.	XIA	Ovoid-ellipsoid	Granulose	Curved	Ruminate	2	Drew 76	BRI	This study; Gagul et al. (2018); Coode (1984); Phoon (2015); Rozefelds (1990a); Dettmann and Clifford (2000)

Elaeocarpus sarcanthus Schltr.	VIIID	Ovoid-ellipsoid	Rugose	Curved	Entire	2–3	J. Womersleyi NGF19498	CANB	This study; Coode (1978, 1981); Phoon (2015)
Elaeocarpus sayeri F.Muell.	VIIIC	Ovoid-ellipsoid	Rugose	Curved	Entire	2	D.M.Crayn 557	NSW	This study; Phoon (2015)
Elaeocarpus sedentarius Maynard & Crayn	Unassigned	Spherical (spherical to 3–4 lobed with skin)	Fibrous	Straight	Entire	1–2			This study; Maynard et al. (2008)
Elaeocarpus sericopetalus F.Muell.	XIB	Ovoid-ellipsoid	Granulose	Curved	Ruminate	3 (6 external segments)	B. Gray 3030	BRI	This study; Phoon (2015); Coode (1998); Rozefelds (1990, Hons. thesis)
Elaeocarpus seringii Montrouz.	I	Ovoid-ellipsoid	Miscellaneous. Surface obscure with persistent fibres, non-descriptive	Straight	Entire	2	G. McPherson 5556	BRI	This study; Phoon (2015); Tirel (1983)
Elaeocarpus stellaris L.S.Sm.	VIB	Ovoid-ellipsoid	Punctate with longitudinal ridging, mesocarp appearing	Straight	Entire	5–7	G. C. Stocker 1774	BRI	This study; Phoon (2015); Coode (1984); Dettmann and Clifford (2000);

			stellate in TS (more prominent stellate ridges)						Rozefelds and Christophel (1996b)
Elaeocarpus sterrophyllus Schltr.	VII	Ovoid-ellipsoid	Echinate	Curved	Entire	2	J. Gagul 14	J. Gagul 14 (image unavailable)	Coode (2019)
Elaeocarpus tariensis Weibel	VID	Ovoid-ellipsoid	Miscellaneous. Shallow furrows on ridges	Straight	Entire	2	J.S. Womersley NGF43624	BRI	This study; Coode (1978, 1981)
Elaeocarpus thelmae B.Hyland & Coode	XIB	Ovoid-ellipsoid	Smooth	Curved	Ruminate	2	B. Hyland 13508	BRI	Coode (1984)
Elaeocarpus trichophyllus A.C.Sm.	VIIIA	Ovoid-ellipsoid	Granulose	Curved	?Entire	2	T. G. Hartley 13253	BRI	Coode (1978, 1981)
Elaeocarpus weibelianus Tirel	IV	Ovoid-ellipsoid	Miscellaneous. Prominent ridging aligned along sutures, with processes between the ridges	Straight	Entire	2	G. McPherson 1728	BRI	This study; Phoon (2015); Tirel (1983)
Elaeocarpus womersleyi Weibel	VIB	Ovoid-ellipsoid	Fibrous (to outer mesocarp); ?pun	Straight	Entire	4	J. Gagul 26	CNS	This study; Coode (1978, 1981)

		(obovoid with skin)	ctate when fibres rot away						

Appendix 5.2 Mesocarp morphologies of fossil *Elaeocarpus* species based on the literature and observations from the current study.

Fossil materials are from Australia and South China. Images of the fossil species are provided in Rozefelds, 1990a; Rozefelds and Christophel, 1996a, b, 2002; Dettman and Clifford, 2000; Liu et al., in press.

Fossil species	Age range	Locality	Mesocarp shape	Surface ornamentation	Locule number	References
Elaeocarpus angularis (F. Muell.) Selling	Early-Middle Miocene	Reform Co. Shaft, Smythe's Creek, Haddon, Victoria, Australia	Ovoid	Near smooth	3	Dettmann and Clifford (2000)
Elaeocarpus bivavle (F. Muell.) Dettmann & Clifford	Late Middle – early Late Miocene	Black Lead, Gulgong, NSW, Australia	Ovoid	Near smooth	2	Dettmann and Clifford (2000)
Elaeocarpus brachyclinis (F. Muell.) Selling	Early-Middle to Late Miocene	Reform Co. Shaft, Smythe's Creek, Haddon, Victoria, Australia	Ovoid-ellipsoid	Punctate	2–5	Rozefelds and Christophel (1996b); Dettmann and Clifford (2000)

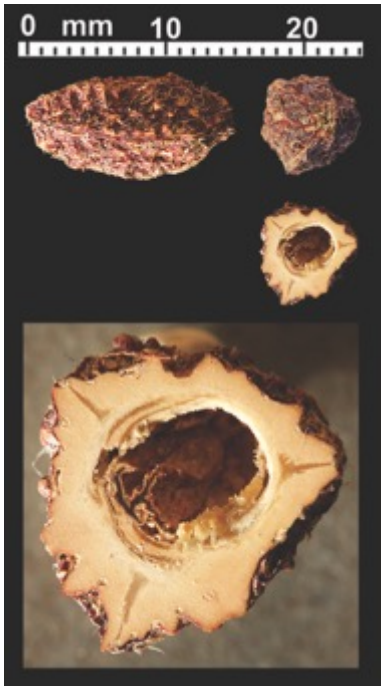
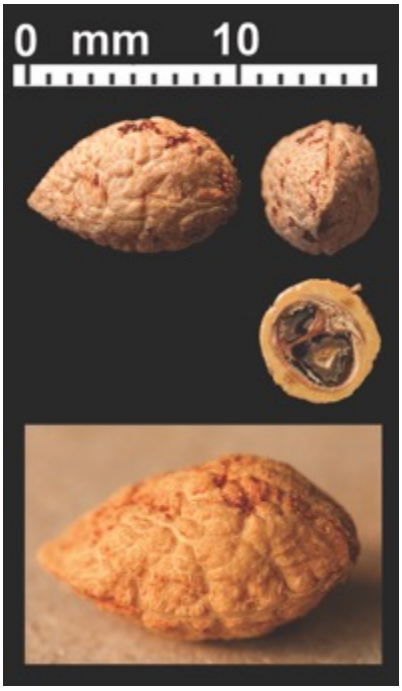
Elaeocarpus cerebriformis Rozefelds & Christophel	Late Early-Late Miocene	Yallourn Formation in the Yallourn Coal Mine, Latrobe Valley, Victoria, Australia	Ovoid-ellipsoid	Bastionate	(2-)3	Rozefelds and Christophel (1996a); Dettmann and Clifford (2000)
Elaeocarpus clarkei (F. Muell.) Selling	Oligocene-Miocene	Haddon, Victoria and Gulgong, NSW, Australia	Ovoid-ellipsoid	Punctate	2–5	Rozefelds and Christophel (1996b); Dettmann and Clifford (2000)
Elaeocarpus couchmanii (F. Muell.) Dettmann & Clifford	Early-Late Miocene	Crucible Shaft or Reefton Shaft Smythe's Creek, Victoria, Australia	Spherical	Bastionate	8	Dettmann and Clifford (2000)
Elaeocarpus cunningii Rozefelds 1990	Late Oligocene-Early Miocene	3 km north of Glencoe Homestead, near Capella, Queensland, Australia	Ovoid-ellipsoid	Echinate	2–3	Rozefelds (1990); Dettmann and Clifford (2000)
Elaeocarpus johnstonii (F. Muell.) Dettmann & Clifford	Oligocene	Beaconsfield (Brandy Creek), Tasmania, Australia	Ellipsoidal	Irregular verrucae	5	Dettmann and Clifford (2000)
Elaeocarpus lynchii (F. Muell.) Selling	Early to Late Miocene	Crucible Shaft, Nintingbool, Haddon, Victoria, Australia	Ovoid-ellipsoid	Echinate	5	Dettmann and Clifford (2000)

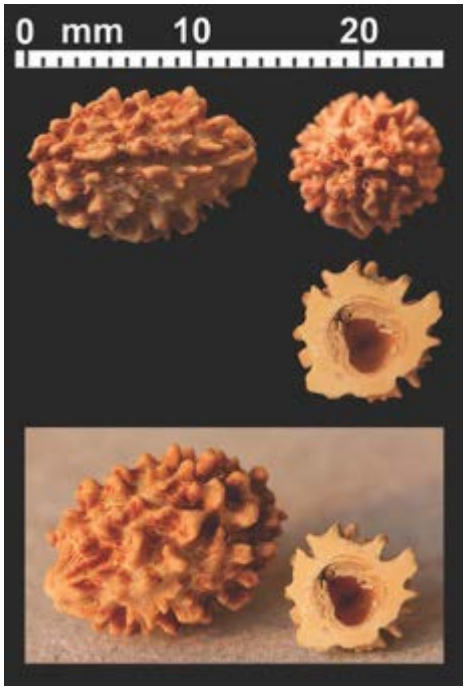
Elaeocarpus mackayi (F. Muell.) Kirchheimer	Early Oligocene-Miocene	Reform Co. Shaft, Smythe's Creek, Haddon, Victoria, Australia	Spherical	Bastionate	3–5	Dettmann and Clifford (2000); Rozefelds and Christophel (2002)
Elaeocarpus muellari Ettingsh	Early Miocene	Newstead near Elsmore, NSW, Australia	Spherical	Bastionate	5	Rozefelds and Christophel (2002)
Elaeocarpus occulatus Rozefelds & Christophel	Unknown	Deep Leads, Haddon, Victoria, Australia	Spherical	Bastionate with mesosutural ridges	5	Rozefelds and Christophel (2002)
Elaeocarpus nanningensis sp. nov.	Oligocene	Nanning Basin, Guangxi, South China	Ellipsoid	Slightly rugose with punctate; sutures confluent with mesocarp wall	3	Liu et al. (in press)
Elaeocarpus prelacunosus sp. nov.	Miocene	Nankang Basin, Guangxi, South China	Ovoid	Generally bastionate with spines; sutures distinct with longitudinal ridges	3	Liu et al. (in press)
Elaeocarpus preprunifolioides sp. nov.	Miocene	Nankang Basin, Guangxi, South China	Ellipsoid	Rugose to echinate with small spines and sutures distinct sutures distinct with spines and fluted apex	3	Liu et al. (in press)
Elaeocarpus prerugosus sp. nov.	Miocene	Nankang Basin, Guangxi, South China	Flatten ellipsoid	Generally bastionate, rarely pointed and	2	Liu et al. (in press)

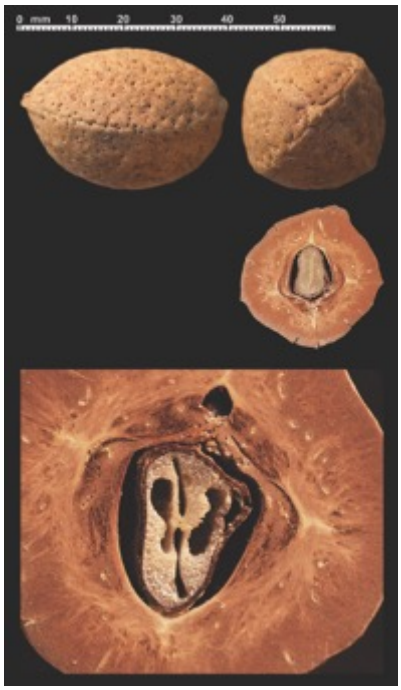
				echinate; sutures with bastionate ridges		
Elaeocarpus preserratus sp. nov.	Miocene	Nankang Basin, Guangxi, South China	Ovoid	Punctate with some fine grooves; sutures confluent with mesocarp wall	3	Liu et al. (in press)
Elaeocarpus presikkimensis sp. nov.	Miocene	Guiping Basin, Guangxi, South China	Ellipsoid	Smooth and regularly punctate with a few shallow grooves; sutures largely confluent with mesocarp wall	3	Liu et al. (in press)
Elaeocarpus peteri Rozefelds & Christophel (=*E. peterii*)	Late Oligocene-Early Miocene	Glencoe, mid eastern Queensland	Ovoid-ellipsoid	Punctate with prominent stellate ridges	5–7	Rozefelds and Christophel (1996b); Dettmann and Clifford (2000)
Elaeocarpus pleioclinis (F. Muell.) Dettmann & Clifford	Early-Middle Miocene	Crucible Co Shaft, Nintingbool, Haddon, Victoria	Ovoid-ellipsoid	Smooth or pitted	8	Dettmann and Clifford (2000)
Elaeocarpus rozefelds Dettmann & Clifford	Early-Late Oligocene	South Blackwater Coal Pty Ltd Hole R8736, Near Blackwater, Queensland	Ovoid-ellipsoid	Punctate with prominent stellate ridges	5–7	Dettmann and Clifford (2000)

Elaeocarpus spackmaniorum Rozefelds	Oligocene to Early Miocene	3 km north of Glencoe Homestead, near Capella, Queensland	Spherical	Bastionate	3–5	Dettmann and Clifford (2000); Rozefelds and Christophel (2002)
Elaeocarpus trachyclinis (F. Muell.) Selling	Oligocene-Miocene	Reform Co. Shaft, Smythe's Creek, Haddon, Victoria	Spherical	Bastionate	5	Dettmann and Clifford (2000)

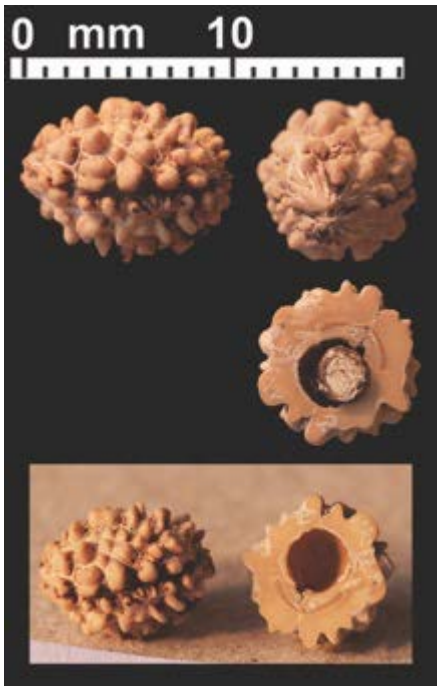
Appendix 5.3 Fruit mesocarps of extant *Elaeocarpus* species documented in the current study. All mesocarps reported here have been seen by the author. Mesocarp images of *E. dolichostylus* var. *hentyi, E. grandis* and *E. sterrophyllus* are unavailable.

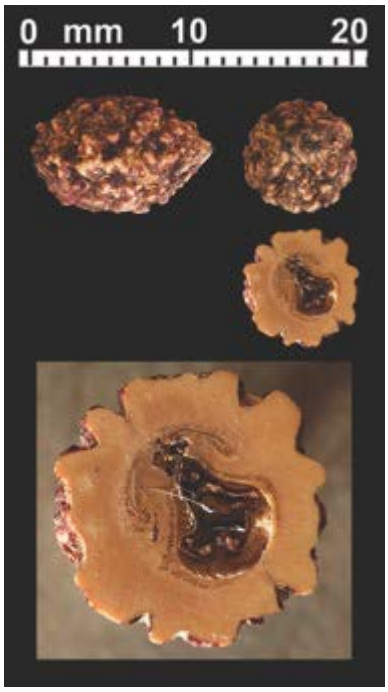
Elaeocarpus alaternoides Brongn. & Gris  Specimen examined: *P. Morat 6288* (BRI)	*Elaeocarpus altigenus* Schltr.  Specimen examined: *Walker ANU 739* (CANB)

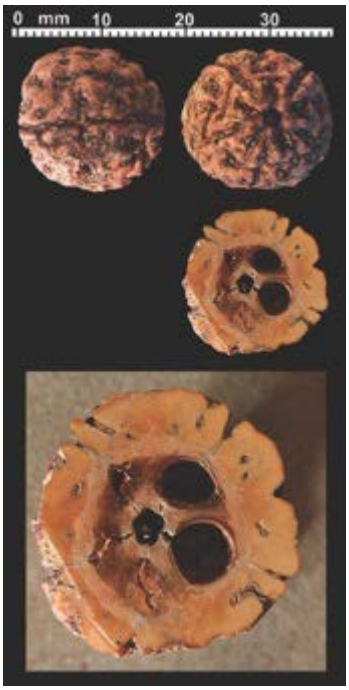
Elaeocarpus angustifolius Blume  Specimen examined: *J. Gagul 18* (CNS)	*Elaeocarpus arnhemicus* F.Muell.  Specimen examined: *B. Hyland 11243* (BRI)

Elaeocarpus bancroftii F.Muell.	*Elaeocarpus blepharoceras* Schltr.
	
Specimen examined: *B. Gray 2328* (BRI)	Specimen examined: *J. Gagul 2* (CNS). Whole fruit is displayed, as the mesocarp is fibrous and not distinctly layered.

Elaeocarpus braceanus Watt ex C.B.Clarke	*Elaeocarpus carbinensis* Gagul & Crayn
	
	Specimen examined: *B. Gray 5197* (CNS)

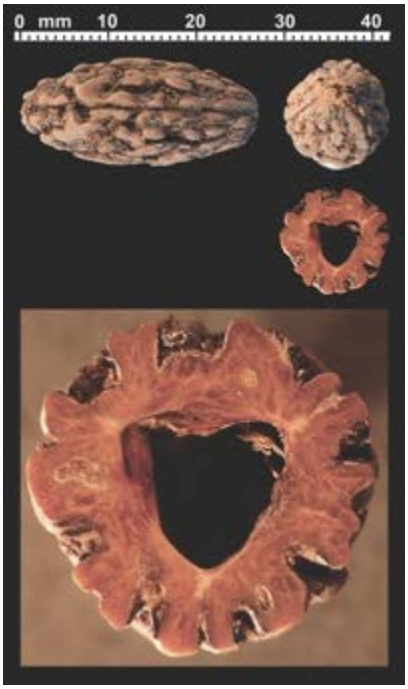
Elaeocarpus carolinae B.Hyland & Coode  Specimen examined: *B. Hyland 3171 RFK* (BRI)	*Elaeocarpus coorangooloo* J.F.Bailey & C.T.White  Specimen examined: *B. Hyland 12637* (BRI)

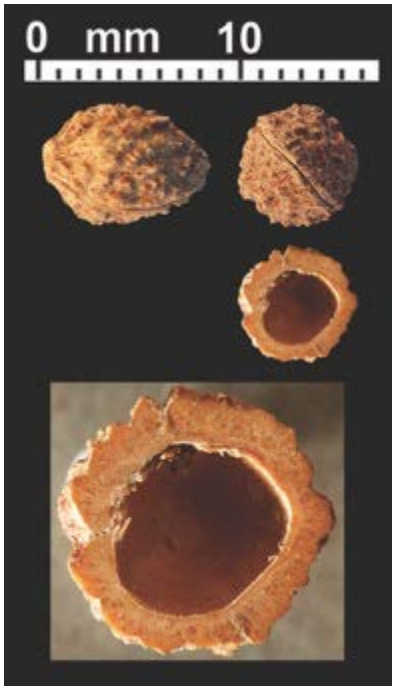
Elaeocarpus culminicola Warb. 	*Elaeocarpus culminicola* Warb.  *J. Gagul 31* (CNS)

Elaeocarpus dentatus (J.R. & G.Forst.) Vahl  Fruit specimen only available from BRI Specimen not vouchered	*Elaeocarpus dolichostylus* Schltr.  Specimen examined: *YS3G0274* (CNS)

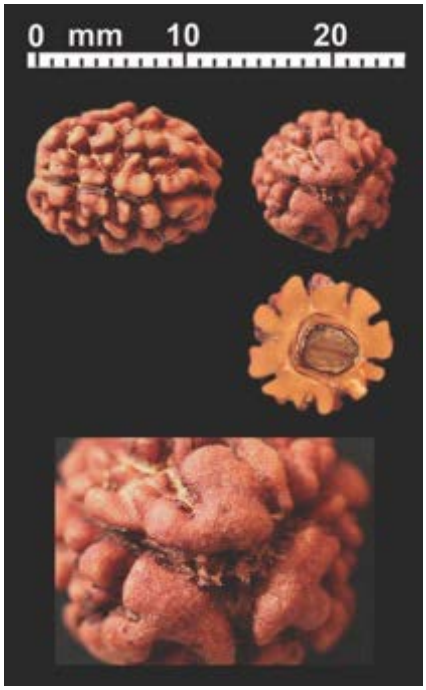
Elaeocarpus elliffii B.Hyland & Coode	*Elaeocarpus eumundii* F.M.Bailey
	 Specimen examined: *B. Gray 3278* (BRI)

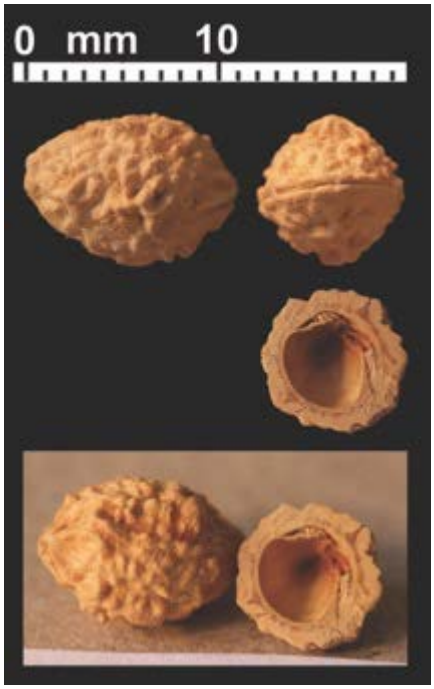
Elaeocarpus ferruginiflorus C.T.White  Specimen examined: *B. Hyland 13460* (BRI)	*Elaeocarpus foveolatus* F.Muell.  Specimen examined: *B. Hyland 13654* (BRI)

Elaeocarpus fuscoides R.Knuth  Specimen examined: *P. van Royen NGF15061* (CANB)	*Elaeocarpus glaber* Blume  Specimen examined: *A.J.G.H. Kostermans 56* (BRI)

Elaeocarpus grahamii F.Muell.  Specimen examined: *B. Hyland 13428* (BRI)	*Elaeocarpus griffithii* (Wight) A.Gray  Specimen examined: *F. H. Endert 2028* (BRI)

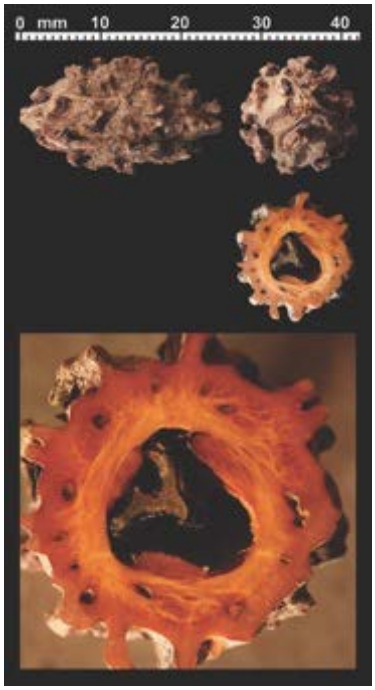
Elaeocarpus habbemensis A.C.Sm.  Specimen examined: *T. G. Hartley 11707* (CANB)	*Elaeocarpus holopetalus* F.Muell.  Specimen examined: *Constable 6978* (QRS 001692, BRI)

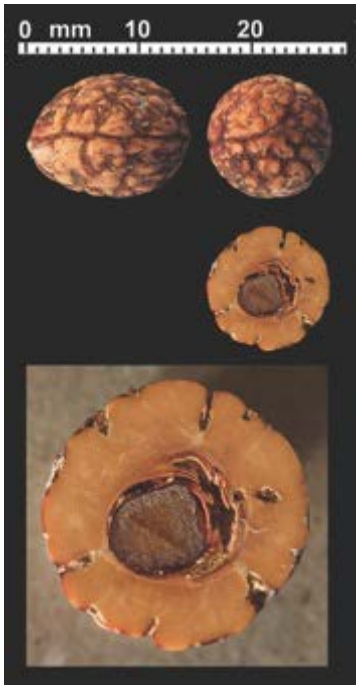
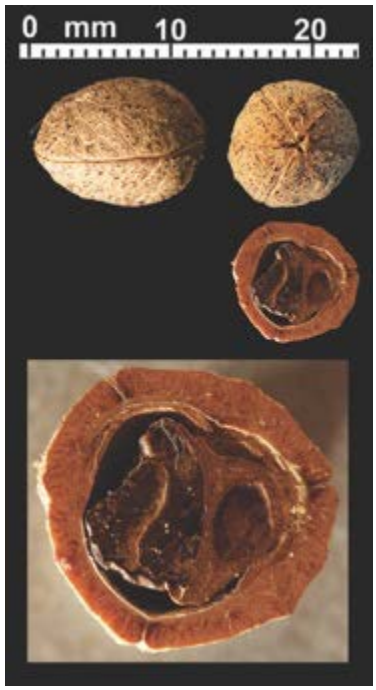
Elaeocarpus hylobroma Y.Baba & Crayn	*Elaeocarpus johnsonii* F.Muell.
	
Specimen examined: *L. Brass 221* (BRI)	

Elaeocarpus kirtonii F.Muell. ex F.M.Bailey  	*Elaeocarpus largiflorens* C.T. White subsp. *largiflorens*  Specimen examined: *B. Hyland 13745* (BRI)

Elaeocarpus ledermannii Schltr.  Specimen examined: *P. J. Darbyshire 8255* (CANB)	*Elaeocarpus linsmithii* Guymer  Specimen examined: *B. Hyland 13606* (BRI)

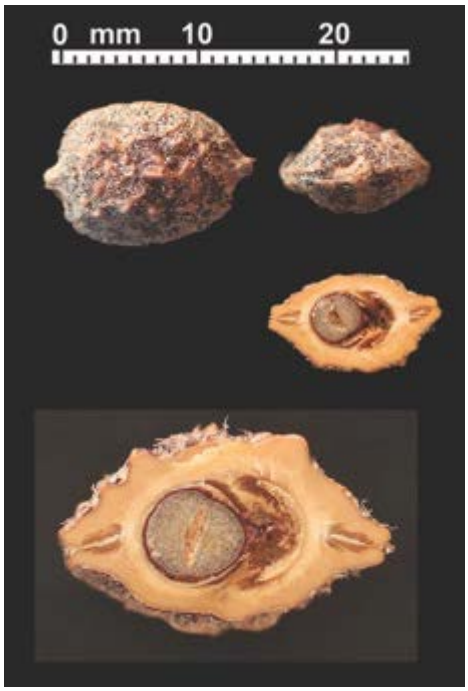
Elaeocarpus miegei Weibel  Specimen examined: *A. Gillison NGF25754* (CANB)	*Elaeocarpus multiflorus* (Turcz.) Fern.-Vill.  Specimen examined: *Kuswata 287* (BRI)

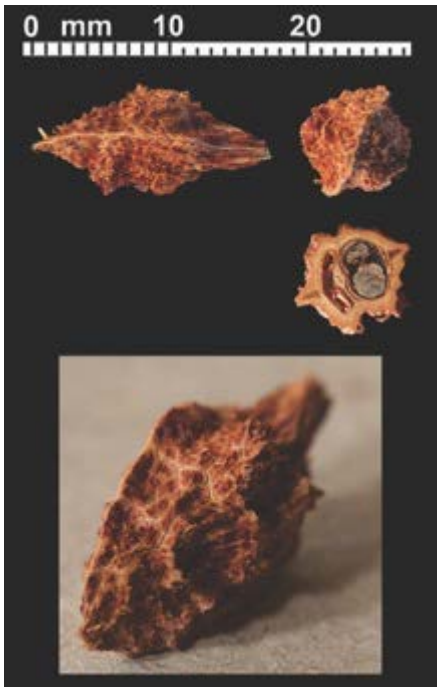
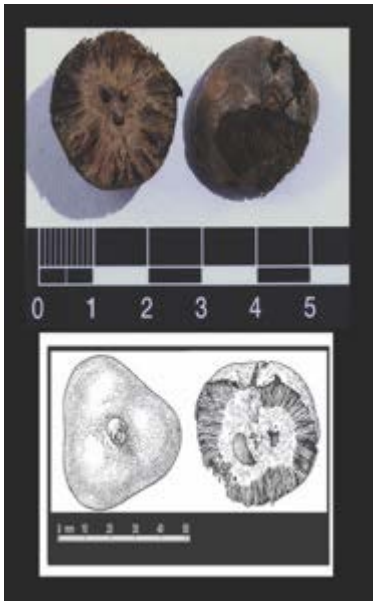
Elaeocarpus multisectus Schltr.  Specimen examined: *J. S. Womersley NGF24978* (BRI)	*Elaeocarpus nubigenus* Schltr.  Specimen examined: *R. Pullen 5405* (CANB)

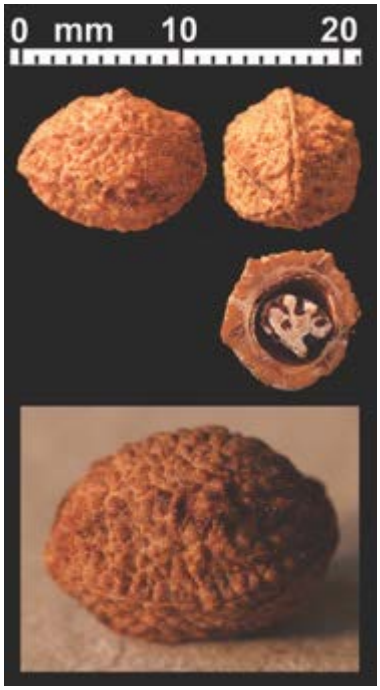
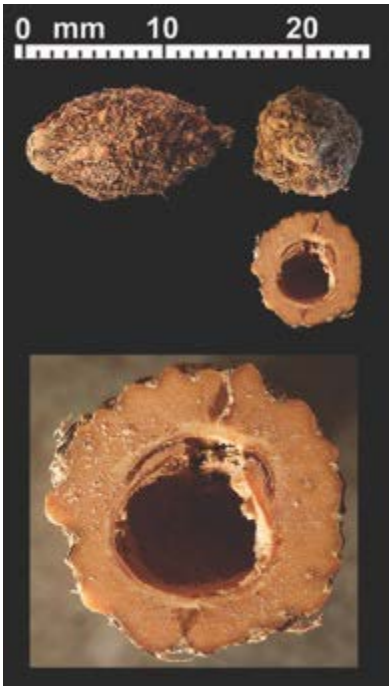
Elaeocarpus polydactylus Schltr.  Specimen examined: *J. Gagul 17* (CNS)	*Elaeocarpus polystachyus* Wall. ex Mull.Berol  Specimen examined: *Kia 32414* (BRI)

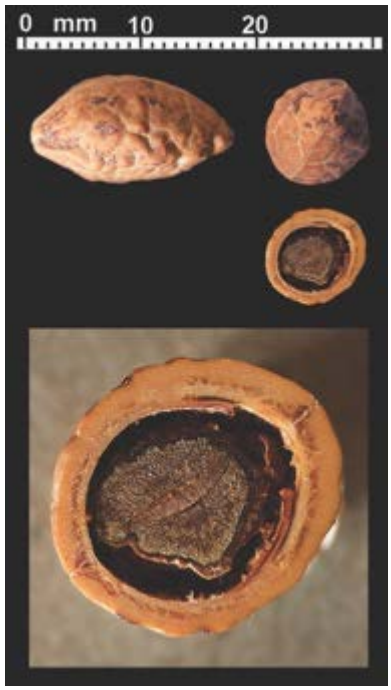
Elaeocarpus ptilanthus Schltr.	*Elaeocarpus pycnanthus* A.C.Sm.
	
Specimen examined: *J. Gagul 8* (CNS)	

Elaeocarpus reticulatus Sm. 	*Elaeocarpus robustus* Roxb.  Specimen examined: *Ngadiman 34726* (BRI)

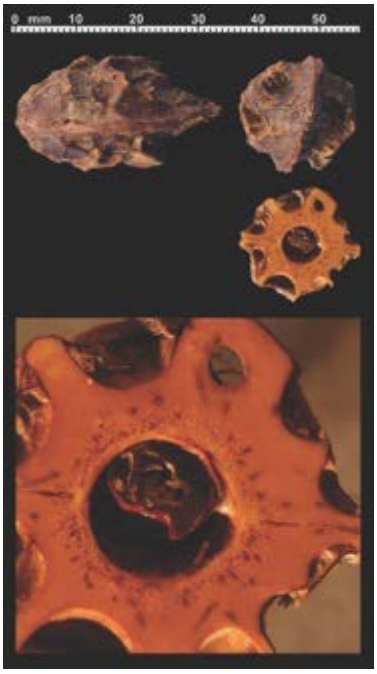
Elaeocarpus rotundifolius Brongn. & Gris  Specimen examined: *G. McPherson 4852* (BRI)	*Elaeocarpus ruminatus* F.Muell.  Specimen examined: *Drew 76* (BRI)

Elaeocarpus sarcanthus Schltr.	*Elaeocarpus sedentarius* Maynard & Crayn
 Specimen examined: *J. Womersleyi NGF19498* (CANB)	 Specimen examined: Top (*WGS 84*, BRI). Bottom (*D. J. Maynard DJM02*, Fig. 3 in Maynard et al., 2008). Reproduced with permission from CSIRO publication.

Elaeocarpus sericopetalus F.Muell.  Specimen examined: *B. Gray 3030* (BRI)	*Elaeocarpus seringii* Montrouz.  Specimen examined: *G. McPherson 5556* (BRI)

Elaeocarpus stellaris L.S.Sm.	*Elaeocarpus tariensis* Weibel
	
Specimen examined: *G. C. Stocker 1774* (BRI)	Specimen examined: *J. S. Womersley NGF43624* (BRI)

Elaeocarpus thelmae B.Hyland & Coode  Specimen examined: *B. Hyland 13508* (BRI)	*Elaeocarpus trichophyllus* A.C.Sm.  Specimen examined: *T. G. Hartley 13253* (BRI)

Elaeocarpus weibelianus Tirel	*Elaeocarpus womersleyi* Weibel
 Specimen examined: *G. McPherson 1728* (BRI)	 Specimen examined: *J. Gagul 26* (CNS)

Appendix 5.4 Morphological character states scored for ancestral state reconstruction. Coding of mesocarp and seed characters for mapping onto the molecular phylogenetic trees was as follows: *mesocarp shape* – ovoid-ellipsoid = 0, spherical = 1; *mesocarp surface ornamentation or sculpturing* – baculate = 0, bastionate = 1, echinate = 2, fibrous = 3, granulose = 4, punctate = 5, punctate with stellate ridges = 6, rugose = 7, smooth or almost smooth = 8, verrucate = 9; *embryo shape* – straight = 0, curved = 1, straight with hooked tip = 2; *endosperm ornamentation* – entire = 0, ruminate = 1. Fossil species are indicated by an asterix (*); for these species embryo and endosperm characters were not observable and were scored as not applicable (NA). The term 'miscellaneous' (MIS) is used here to refer to mesocarp surface sculpturing that does not fit the paleobotany scheme recognised by others (Dettmann and Clifford, 2000; Rozefelds and Christophel, 1990a, 1996a, b, 2002; Liu et al., in press).

Species	Mesocarp shape	Mesocarp surface ornamentation	Embryo shape	Endosperm ornamentation
Elaeocarpus alaternoides Brongn. & Gris	0	MIS	0	0
Elaeocarpus altigenus Schltr. (= *E. sayeri* var. *altigenus*)	0	7	1	0
Elaeocarpus angustifolius Blume (=*E. sphaericus*)	1	1	0	0
**Eleaocarpus angularis* (F.Muell.) Selling	0	8	NA	NA
Elaeocarpus arnhemicus F.Muell.	0	0	0	0
Elaeocarpus bancroftii F.Muell.	0	5	0	0
**Elaeocarpus bivalve* (F.Muell.) Dettmann & Clifford	0	8	NA	NA

Elaeocarpus blepharoceras Schltr.	1	3	0	0
Elaeocarpus braceanus Watt ex C.B.Clarke	0	1	0	0
**Elaeocarpus brachyclinis* (F.Muell.) Selling	0	5	NA	NA
Elaeocarpus carbinenesis J. Gagul & Crayn (=*Elaeocarpus* sp. Mt Misery)	0	6	0	0
Elaeocarpus carolinae B.Hyland & Coode	0	2	1	0
**Elaeocarpus cerebriformis* Rozefelds & Christophel	0	1	NA	NA
**Elaeocarpus clarkei* (F.Muell.) Selling	0	5	NA	NA
Elaeocarpus coorangooloo J.F.Bailey & C.T.White	0	9	0	0
**Elaeocarpus couchmanii* (F.Muell.) Dettmann & Clifford	1	1	NA	NA
Elaeocarpus culminicola Warb.	0	2	1	0
**Elaeocarpus cunningii* Rozefelds	0	2	NA	NA
Elaeocarpus dentatus (J.R. & G.Forst.) Vahl	0	MIS	0	0
Elaeocarpus dolichostylus Schltr.	1	1	0	0
Elaeocarpus dolichostylus var. *hentyi* Coode	0	1	0	0
Elaeocarpus elliffii B.Hyland & Coode	0	4	1	1
Elaeocarpus eumundi F.M.Bailey	0	2	1	0
Elaeocarpus ferruginiflorus C.T.White	0	8	1	1
Elaeocarpus foveolatus F.Muell.	0	8	1	1
Elaeocarpus fuscoides R.Knuth	0	4	1	0
Elaeocarpus glaber Blume	0	MIS	0	0
Elaeocarpus grahamii F.Muell.	0	2	1	0
Elaeocarpus grandis F.Muell.	1	1	0	0
Elaeocarpus griffithii (Wight) A.Gray	0	MIS	1	0
Elaeocarpus habbemensis A.C.Sm.	0	7	1	0
Elaeocarpus holopetalus F.Muell.	0	MIS	2	0
Elaeocarpus hylobroma Y.Baba & Crayn	0	1	0	0

Elaeocarpus johnsonii F.Muell.	0	3	0	0
**Elaeocarpus johnstonii* (F.Muell.) Dettmann & Clifford	0	9	NA	NA
Elaeocarpus kirtonii F.Muell. ex F.M.Bailey	0	2	1	0
Elaeocarpus largiflorens C.T.White subsp. *largiflorens*	0	8	1	1
Elaeocarpus ledermannii Schltr.	0	7	1	0
Elaeocarpus linsmithii Guymer	0	5	1	0
**Elaeocarpus lynchii* (F.Muell.) Selling	0	2	NA	NA
**Elaeocarpus mackayi* F.Muell	1	1	NA	NA
Elaeocarpus miegei Weibel	0	MIS	0	0
**Elaeocarpus muelleri* Ettingsh.	1	1	NA	NA
Elaeocarpus multiflorus (Turcz.) Fern.-Vill.	0	MIS	1	1
Elaeocarpus multisectus Schltr.	0	MIS	0	0
**Elaeocarpus nanningensis* sp.nov	0	7&5	NA	NA
Elaeocarpus nubigenus Schltr.	1	MIS	0	0
**Elaeocarpus occulatus* Rozefelds & Christophel	1	1	NA	NA
**Elaeocarpus peterii* (=*petersii*) Rozefelds & Christophel	0	6	NA	NA
**Elaeocarpus pleioclinis* (F.Muell.) Dettmann & Clifford	0	8	NA	NA
Elaeocarpus polydactylus Schltr.	0	1	0	0
Elaeocarpus polystachyus Wall. ex Mull. Berol	0	MIS	1	1
**Elaeocarpus prelacunosus* sp.nov	0	1	NA	NA
**Elaeocarpus preprunifolioides* sp.nov	0	7&2	NA	NA
**Elaeocarpus prerugosus* sp.nov	0	1&2	NA	NA
**Elaeocarpus preserratus* sp.nov	0	5	NA	NA
**Elaeocarpus presikkimensis* sp.nov	0	8&5	NA	NA

Elaeocarpus ptilanthus Schltr.	1	1	0	0
Elaeocarpus pycnanthus A.C.Sm.	0	4	1	0
Elaeocarpus reticulatus Sm.	0	2	1	0
Elaeocarpus robustus Roxb.	1	MIS	0	0
Elaeocarpus rotundifolius Brongn. & Gris	0	MIS	0	0
**Elaeocarpus rozefelds* Dettmann & Clifford	0	6	NA	NA
Elaeocarpus ruminatus F.Muell.	0	4	1	1
Elaeocarpus sarcanthus Schltr.	0	7	1	0
Elaeocarpus sayeri F.Muell.	0	7	1	0
Elaeocarpus sedentarius Maynard & Crayn	1	3	0	0
Elaeocarpus sericopetalus F.Muell.	0	4	1	1
Elaeocarpus seringii Montrouz.	0	MIS	0	0
**Elaeocarpus spackmaniorum* Rozefelds	1	1	NA	NA
Elaeocarpus stellaris L.S.Sm.	0	6	0	0
Elaeocarpus sterrophyllus Schltr.	0	2	1	0
Elaeocarpus tariensis Weibel	0	MIS	0	0
Elaeocarpus thelmae B.Hyland & Coode	0	8	1	1
**Elaeocarpus trachyclinis* (F.Muell.) Selling	1	1	NA	NA
Elaeocarpus trichophyllus A.C.Sm.	0	4	1	0
Elaeocarpus weibelianus Tirel	0	MIS	0	0
Elaeocarpus womersleyi Weibel	0	5	0	0

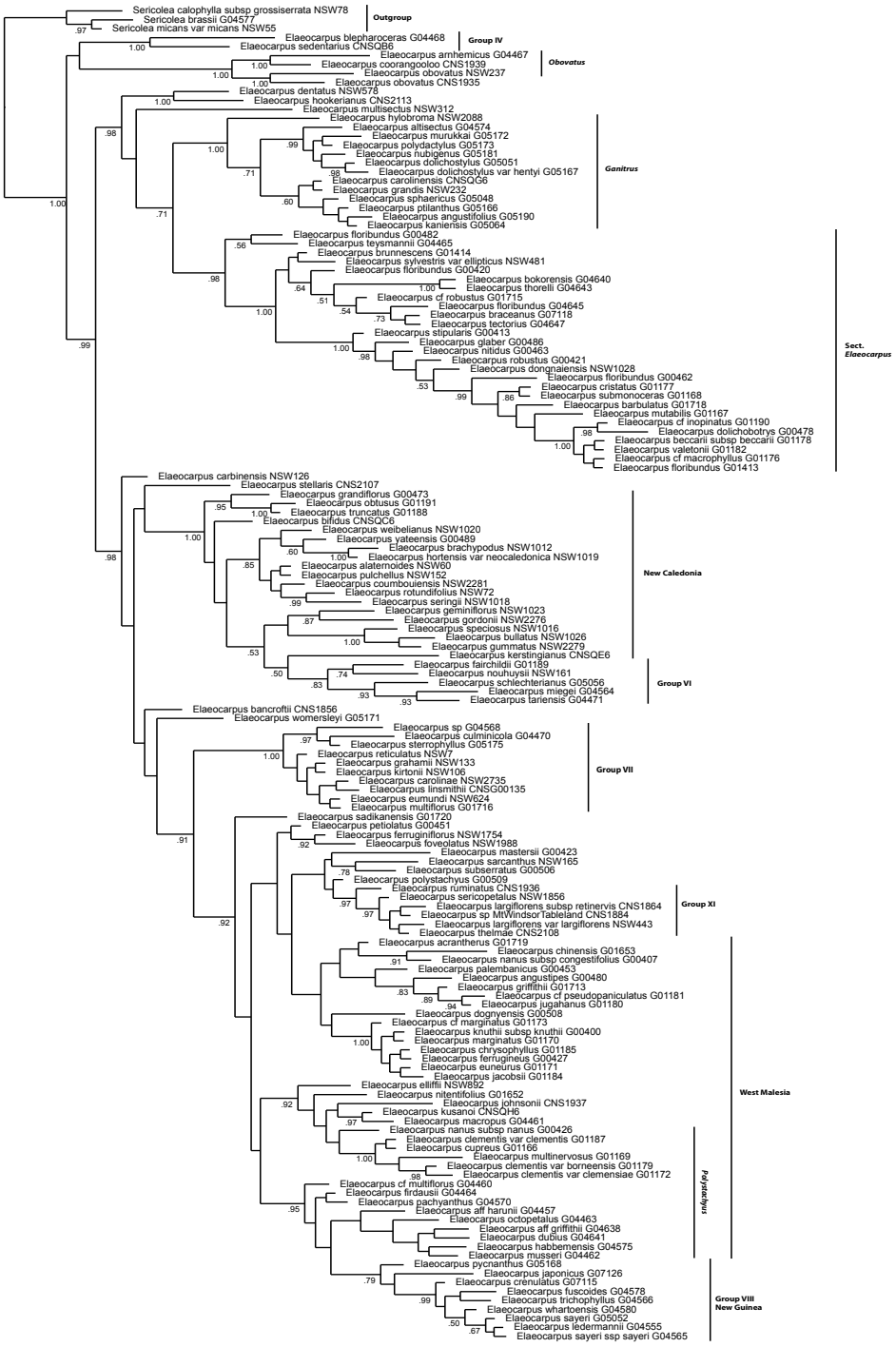
Appendix 5.5 Percentage of each trait represented in the different character states based on the ancestral state reconstruction. Letters represent the different traits, which correspond to different colours on the phylogenies (Figs 5.3 – 5.7).

Node ID	Endosperm Ornamentation	Embryo Shape	Surface Ornamentation	Mesocarp Shape	Locule No.
296	A 99.86	A 99.84	D 64.57 C 5.90 J 4.49 A 3.72 I 3.57 B 3.54 F 3.43 G 3.36 E 3.35 H 3.33	A 66.37 B29.50 AB 4.13	A 66.78 B 19.98 E 5.61 AB 2.61 C 1.46 D 1.10
295	A 99.94	A 99.89	D 32.53 B 8.51 C 8.08 F 8.02 G 8.00 A 7.52 I 6.88 E 6.79 H 6.65 J 6.64	A 99.25	A 40.39 B 39.37 AB 10.97 C 2.73 E 1.85
294	A 99.96	A 99.73	G 42.53 F 9.42 D 8.83 E 6.11 B 5.82 C 5.62 A 5.60 I 5.35 H 5.32 J 5.00	A 99.92	B 37.74 A 27.03 AB 9.65 C 8.06 E 3.85 BC 2.88 AC 2.06 D 1.52 BE 1.38
290	A 99.81	B 67.88 A 24.95 AB 7.17	G 34.08 C 29.70 F 9.29 B 4.49 E 4.34 H 3.80 I 3.66 D 3.48 A 3.41 J 3.16	A 99.92	A 44.12 B 28.45 AB 24.02
289	A 98.77	B 96.45 A 2.78	G 22.36 I 14.44 C 12.04 E 10.75 H 8.89 F 7.82 B 6.20 D 5.99 A 5.77 J 5.38	A 99.90	A 52.16 B 32.44 AB 13.96

286	A 99.39	B 99.43	E 47.89 H 16.55 D 5.16 I 4.84 G 4.74 C 4.16 B 4.16 A 4.15 F 4.15 J 3.85	A 99.49	B 60.60 A 24.31 E 6.81 D 2.49 C 2.36 F 2.25
281	A 99.83	B 99.73	H 68.00 E 26.73 EH 1.89	A 99.83	B 46.21 A 46.05 AB 6.23
269	A 92.75 B 6.82	B 95.59 A 4.07	D 37.43 E 15.62 H 7.74 I 6.19 C 5.89 F 5.48 G 5.46 B 5.43 A 5.43 J 4.98	A 98.82 B 1.10	B 55.79 A 35.14 C 4.27 AB 1.43
240	B 99.22	B 99.84	E 84.09 I 7.16 D 1.90 H 1.37	A 99.92	A 98.71
239	B 99.75	B 99.86	I 80.96 E 10.77 H 1.40 D 1.07	A 99.84	A 96.88 B 1.59
232	B 99.51	B 99.89	I 99.77	A 99.96	A 89.22 B 5.54 AB 4.66
231	A 99.53	B 97.71 AB 1.29 A 1.01	C 91.03 F 3.14 G 1.31	A 99.93	A 38.85 AB 34.21 B 23.63
224	A 99.85	B 99.72	C 96.89	A 99.92	AB 45.65 A 34.21 B 19.93
221	A 99.90	A 99.87		A 99.85	A 54.50 B 32.99 AB 8.13 C 1.49
215	A 99.76	A 99.65		A 99.67	A 67.10 B 21.30 AB 10.72
214	A 99.95	A 99.95		A 99.91	A 78.51 AB 18.16 B 3.17
205	A 99.97	A 99.98		A 99.93	A 99.53

198	A 99.93	A 99.92	B 20.21 D 13.34 A 10.86 F 8.22 G 8.10 C 8.09 I 7.86 E 7.83 H 7.80 J 7.41	A 99.20	B 46.67 A 36.67 AB 10.99 C 2.44
195	A 99.35	A 99.48	B 17.50 A 9.80 D 9.33 C 9.10 F 9.10 G 9.10 I 9.10 E 9.08 H 9.08 J 8.57	A 98.67 B 1.26	B 74.93 A 17.80 C 2.39 D 1.53 E 1.12 F 1.03
194	A 98.65 B 1.27	A 98.88 B 1.03	B 37.74 A 7.84 D 6.85 C 6.81 H 6.81 I 6.81 G 6.81 F 6.81 E 6.81 J 6.44	A 97.60 B 2.34	B 84.43 A 5.19 C 2.61 D 2.49 E 2.41 F 2.36
193	A 95.49 B 4.46	A 88.68 B 11.27	B 15.58 A 9.51 I 9.42 G 9.42 H 9.42 F 9.42 C 9.42 D 9.42 E 9.42 J 8.79	A 74.15 B 25.79	B 28.02 D 19.83 A 13.39 C 13.25 E 12.98 F 12.29
192	A 97.93 B 2.00	A 96.52 B 3.41	B 10.68 A 10.00 D 9.98 E 9.98 H 9.98 I 9.98 G 9.98 C 9.98 F 9.98 J 9.30	A 69.69 B 30.11	B 36.67 D 28.46 A 8.90 C 8.85 E 8.49 F 8.23
169	A 99.95	A 99.93	B 95.54 A 1.32	A 84.68 B 9.52 AB 5.79	A 33.62 B 30.36 C 10.60 AB 9.38 D 4.66 AC 3.27 BC 2.96 AD 1.44 BD 1.30
162	A 99.93	A 99.93	B 98.25	B 68.98 A 21.05 AB 9.97	D 32.63 B 25.86 C 21.16 BD 6.66 CD 5.45 BC 4.32 A 1.37 BCD 1.11

159	A 99.96	A 99.98	B 99.78	A 65.63 B 21.41 AB 12.96	D 60.18 CD 27.61 C 10.51
157	A 99.75	A 99.92		A 99.91	A 87.82 B 8.56 AB 1.91
155	A 99.83	A 99.87	J 28.78 D 22.26 A 11.00 I 7.16 C 6.01 B 4.97 F 4.90 G 4.85 E 4.83 H 4.82	A 99.28	A 58.74 B 33.56 AB 3.46 E 1.54
154	A 98.74 B 1.20	A 98.75 B 1.22		A 97.15 B 2.79	A 55.01 B 25.47 E 5.82 C 4.62 D 4.53 F 4.06
153	A 99.96	A 99.96	J 68.83 A 25.44 I 1.05	A 99.94	A 76.67 B 16.61 AB 5.55
152	A 99.96	A 99.95	D 99.67	B 96.46 AB 2.90	A 48.41 E 41.43 AE 7.16 B 1.05



Appendix 5.6 Phylogeny of *Elaeocarpus* estimated by Bayesian analysis, rooted using *Sericolea* as outgroup. Numbers below the branches are posterior probabilities (shown only for branches that receive > 0.50 support).

Publications and presentations from Ph.D. candidature

List of publications

Papers published from this thesis are listed below. Reprints of published chapters (chapters 2, 4 and parts of 3) are attached.

Gagul, J. N., Simpson, L & Crayn, D. M. (2018). *Elaeocarpus carbinensis* J.N. Gagul & Crayn (Elaeocarpaceae), a new species endemic to the Mt Carbine Tableland of northeast Queensland, Australia. *Austrobaileya*, **10(2)**: 247–259.

Gagul, J. N., Tng, D. Y. P. & Crayn, D. M. (2018). Fruit developmental biology and endosperm rumination in *Elaeocarpus ruminatus* F. Muell. (Elaeocarpaceae), and its taxonomic significance. *Australian Systematic Botany*, **31**: 409–419.

Gagul, J. N. (2016). Molecular phylogenetics of *Elaeocarpus* (Elaeocarpaceae) with a focus on New Guinea species. *Australasian Systematic Botany Society Newsletter*, **167**: 4–8.

List of presentations

Gagul, J. N., Crayn, D. M., Gadek, P., Rozefelds, A. & Thornhill, A. (2014). Molecular phylogenetics of *Elaeocarpus* (Elaeocarpaceae) with a focus on New

Guinea species. *Research Science and Technology Conference*. Port Moresby, Papua New Guinea [oral].

Gagul, J. N., Crayn, D. M., Gadek, P., Rozefelds, A. & Thornhill, A. (2014). Systematics and evolution of the genus *Elaeocarpus* L. (Elaeocarpaceae). *Association for Tropical Biology and Conservation Conference*. Cairns, Australia [poster].

Gagul, J. N., Crayn, D. M. & Nauheimer, L. (2015). Molecular phylogenetics of *Elaeocarpus* L. (Elaeocarpaceae) with a focus on New Guinea species. *Australasian Systematic Botany Society Conference.* Canberra, Australia [oral].

Gagul, J. N., Rozefelds, A., Nauheimer, L. & Crayn, D. M. (2016). Molecular phylogenetics of *Elaeocarpus* L. (Elaeocarpaceae) with a focus on New Guinea species. *Flora Malesiana 10 Conference*. Edinburgh, Scotland [oral].

Gagul, J. N., Rozefelds, A. & Crayn, D. M. (2016). Fruit mesocarp morphology of *Elaeocarpus* (Elaeocarpaceae): a phylogenetic survey. *Australasian Systematic Botany Society Conference.* Alice Springs, Australia [poster].