Moderate levels of Eocene $p$CO$_2$ indicated by Southern Hemisphere fossil plant stomata
Margret Steinthorsdottir, Vivi Vajda, Mike Pole and Guy Holdgate

1. GEOLOGICAL SETTING AND CHRONOLOGY
Fossil plant material analysed for this study was derived from ten localities in Australia and New Zealand, spanning the Eocene. The sites have been the subject of numerous palaeobotanical studies and are relatively well-dated, within the constraints of Cenozoic Southern Hemisphere chronostratigraphy where chemo- and magnetostratigraphy is partly lacking, and where “mammalian zones” (widely used in Northern Hemisphere Cenozoic stratigraphy), are missing. None of the sites have been radiometrically dated, with chronology based on correlation of the associated microfloras to the basin palynostratigraphic scheme (Holdgate et al. 2017). Paleobotanical and palynological analyses from these sites show that Australia and New Zealand were mostly covered by tropical-subtropical rainforest vegetation throughout the Eocene, although with significant changes in vegetation composition and structure, both geographically and temporally (Christophel and Greenwood, 1988; 1989; Carpenter et al., 1994; Christophel, 1994; Macphail et al., 1994; McLoughlin and Hill, 1996; Vadala and Greenwood, 2001; Hill, 2004, Holdgate et al., 2017). Below, we briefly list the Eocene geological setting of the relevant parts of Australia and New Zealand, and describe each locality as well as the associated chronology in more detail.

1.1. Australia
During the early Eocene southern Australia lay between palaeolatitudes 55ºS to about 65ºS. Marine conditions existed along the southern margin and thick clastic sediments were deposited in several coastal basins, many of which extend from the present onshore to offshore. Carbonate deposition was rare to absent. Bass Strait between southern Australia and Tasmania was a swampy alluvial plain surrounding large freshwater to saline lakes (MacPhail, 2007). During the middle-late Eocene Australia began to separate from the Antarctic and drifted north so that the southern basins lay around palaeolatitudes of ~55ºS to ~62ºS. Carbonate shelf sedimentation commenced from the west whereas in the east, thick lignite sequences accumulated in the Noarlunga and Willunga Embayments of the St. Vincent Basin (Fairburn, 1998; 2000), at Anglesea in the Torquay Sub-basin, Bass Basin and in the Gippsland Basin (Holdgate and Sluiter, 1991; Holdgate et al., 2000). Peripheral and offshore to SW Tasmania coast lay the Sorrell Basin. Updip outcrops to this basin occur in Macquarie Harbour around Regatta Point.

The Cenozoic climatic change data derives mostly from palynology in exploration wells and cores drilled within the Gippsland, Bass, Otway and Murray Basins. Here, relatively continuous clastic deposition preserves abundant macro- and microfossils of the Eocene vegetation. The microfloras mostly represent coastal plain communities whereas the macrofossil assemblages come from thick brown coal measures and associated clastic sediments in Victoria, South Australia and Tasmania. The sediments are dated mainly from the Esso-BHP zonation developed for the Gippsland Basin (Partridge, 1971; Stover and Partridge, 1973). These palynological zonation schemes are widely used to date and correlate Cenozoic sediments across the southern Australia region. Most of the margins appear to have had an everwet climate, and although there was no central arid zone as today, there are indications that the central region was drier and may have had a distinct ‘monsoonal’ or ‘dry rainforest’-type vegetation (Greenwood, 1991; 1996; Carpenter et al., 2011). Tropical vegetation extended to high latitudes including dominant conifer such as Nothofagus subgenus Brassospora, and some survivors from the end-Cretaceous extinction, e.g. Ginkgo, extinct cycads and unidentified broad-leaved angiosperms and conifers (McGowran and Hill, 2015). Even the tropical mangrove palm...
*Nypa* is present at SW Tasmania’s Regatta Point (Pole and Macphail, 1996). In the later Eocene, a
cooling is indicated by the disappearance of the paratropical biome and *Nothofagus* increased in
dominance (Pocknall, 1989; Holdgate et al., 2009; Raine et al., 2009; Holdgate et al., 2017),
temporarily interrupted by a warm period during the Mid Eocene Climatic Optimum (at ~40 Ma). This
MECO event can be discerned in the floras at Maslin Bay, Anglesea, Nerriga and Golden Grove
locations of southern Australia (Scriven et al., 1995; McGowran and Hill, 2015).

For the Gippsland Basin and in general for the southern Eocene coal basins in Australia, coal
seam palynology records show late middle Eocene (T2) coals formed under megathermic conditions
characterized by high-gymnosperm contents, late Eocene (T1) coals formed under mesothermic
conditions characterized by reduced-gymnosperm contents and earliest indications of palaeoclimate
cooling. Earliest Oligocene T0 coal record (33.9-31.5 Ma) contains high gymnosperm palynology
profile, very similar to the T2 coals. The earliest indication of cooler climes only begins after this coal
formed as indicated by low-gymnosperm high-*Nothofagus* (southern beech) pollen proportions
(Holdgate et al., 2017). This suggests in Gippsland the earliest evidence for major glacial cooling (by
inference the Oi1 event) occurs immediately above the T0 coal seam where early to late Oligocene
Morwell Formation sands, clays and coals contain low counts of gymnosperms (<10%) but high
average proportions of *Nothofagus* (50%). This is the main definitive indicator that climate had cooled
between the Eocene and Oligocene. This agrees with the current ocean drilling position of the earliest
(Oi1) glacial event shortly above the Eocene–Oligocene boundary (Pocknall, 1989; Holdgate et al.,
2009; Raine et al., 2009; Holdgate et al., 2017).

1.1.1. Anglesea Power Station
The Anglesea Power Station coal mine locality is situated c. 3 km north-west of the coastal town of
Anglesea in Victoria. The sediments belonging to the Eastern View Formation of the Torquay Basin,
comprise a rich and abundant macroflora preserved in fluvio-lacustrine clay and clay-sand lenses in
the overburden from the Anglesea open-cut coal mine (Christophel et al., 1987; Greenwood et al.,
2003a; Hill et al., 2018). The flora correlates near the lower boundary of the Gippsland Basin’s middle
*Nothofagidites asperus* zone and thus the boundary between the middle and late Eocene
(Bartonian/Priabonian), ~38 Ma +/-1 Ma (Christophel et al., 1987; Greenwood et al., 2003a) (Fig.
DR1). The macroflora at Anglesea is dominated by fossil angiosperms, which are very well preserved
and diverse, including several species of Lauraceae (Christophel et al., 1987), Proteaceae (Christophel,
1984; Hill and Christophel, 1988; Carpenter et al., 2016) and Myrtaceae (Christophel and Lys, 1986).
Christophel et al. (1987) regarded the Anglesea vegetation as being closest to extant Complex
Mesophyll Vine Forest and considered that its composition and structure was similar to that of the
vegetation at modern Noah Creek in north-eastern Queensland.

1.1.2. Golden Grove
Located in South Australia, the Golden Grove locality is found in a sandstone quarry near Adelaide
(Christophel et al., 1992), where fossil leaves are preserved in clay lenses. The sediments belong to the
North Maslin Sands in the St. Vincent Basin of South Australia, correlated with the Lower
*Nothofagidites asperus* Zone A of the Gipsland Basin, ~45–39 Ma (Partridge, 1999; Greenwood et al.,
2003a), in the middle Eocene, mostly Lutetian (Fig. DR1).

1.1.3. Nelly Creek
The Nelly Creek locality is situated north of Adelaide in the interior of South Australia, ca. 1 km south
of Lake Eyre South’s southern shore (Christophel et al., 1992). The macroflora is preserved in clays
belonging to the Eyre Formation. Both sites include diverse rain forest taxa, including Lauraceae.
These two floras, together with the Anglesea flora, express a physiognomic signature consistent with
that of the litter found in Webb's Complex Notophyll Vine Forest or Complex Mesophyll Vine Forest
(Christophel and Greenwood, 1988; Christophel, 1995). It is considered broadly contemporaneous with Golden Grove (Fig. DR1).

1.1.4. Dean’s Marsh
The Dean's Marsh locality, Eastern View Formation (Douglas and Ferguson, 1988; Christophel, 1995), ~80 km northwest of the Anglesea Power Station, contains a rich macroflora in fluvio-lacustrine sediments (Greenwood et al., 2003a). The flora displays plant physiognomic characteristics that are very similar to those of the Anglesea locality, although no species are held in common (Christophel, 1995). Dean's Marsh locality is correlated to the basal Eastern View Formation, belonging to the *Malvacipollis diversus* Zone C of Partridge (1998) and thus inferred to belong in the early Eocene (Ypresian) (Fig. DR1) (Douglas and Ferguson, 1988) at ~53–52 Ma (Greenwood et al., 2003a). The deposition of the Dean’s Marsh flora may thus have coincided with the early Eocene cooling interval also detected in North America (Greenwood et al., 2003a).

1.1.5. Hasties
The Hasties deposits are located in northeastern Tasmania, Australia, with well-preserved fossil plant remains found in a carbonaceous to slightly lignitic sedimentary unit (Pole, 1992). The Hasties assemblage is characterised by a high diversity of conifers, with 14 species in over seven genera (Pole, 1992). There were also a variety of angiosperms including Lauraceae and Nothofagus. The sediments have been assigned to the Lower *Nothofagidites asperus* Zone within the Gippsland Basin (Macphail in Bigwood and Hill 1985), which is middle to late Eocene (Fig. DR1), broadly contemporaneous or slightly younger than the Golden Grove and Nelly Creek localities centring around ~40 Ma +/-1-2 Ma (Bartonian-Lutetian) (Tarran et al., 2016).

1.1.6. Regatta Point
The carbonaceous mud and sands at Regatta Point locality outcrop around the edges of the Macquarie Harbour on the western side of Tasmania and belong to Macquarie Harbour Formation. Based on sedimentary features and the presence or absence of certain pollen and dinoflagellates, it was interpreted that the depositional environment was a tidal estuary with freshwater swamps (Pole, 1998; Pole, 2007), with all of the here studied fossil leaves deriving from the freshwater community. The fossil-plant assemblage of mostly dispersed cuticle fragments studied here record evidence of mesothermal rainforest and mangrove vegetation (Pole, 2007). M.K. Macphail (in Bigwood and Hill, 1985) dated a sample from approximately the same stratigraphic horizon as belonging to the early Eocene *Malvacipollis diversus* Zone of Stover and Partridge (1973). Furthermore, A.D. Partridge (pers. comm. 1993 to M.K. Macphail) considered that the sample was late early Eocene, within the range of ~55–48.5 Ma (Ypresian) (Pole and Macphail, 1996) and this age is accepted for the Regatta Point fossil plant material studied here (Fig. DR1).

1.2. New Zealand
During the Eocene New Zealand was located at higher latitudes compared to present, although at lower latitudes relative to Australia. Over the course of the Eocene, the localities studied here moved north from about 56–58 °S to about 49–52 °S (based on Matthews et al. 2016, using GPlates software). New Zealand’s vegetation over the Eocene was generally forested (Pocknall 1989, 1990) but distinct changes over the period are apparent in the palynological record (Raine 1984, 2004). Earliest Eocene samples are dominated by conifer pollen, but later in the early Eocene, probably in response to increased warmth, they become dominated by *Myricipites harrisii*, a taxon usually regarded as Casuarinaceae. In the middle Eocene, Nothofagus, the southern beech, became prominent, in response to cooler conditions (Pocknall, 1989; Holdgate et al., 2009; Raine et al., 2009; Holdgate et al., 2017).
1.2.1. Otaio River
The coal-bearing sediments of the Taratu (or Broken River) Formation at Otaio Gorge, South Canterbury, New Zealand have yielded palynofloras that initially indicated a “Mangaorapan to Heretaungan” age (early–middle Eocene) (Pocknall, 1984). This was later broadened to a “Mangaorapan to Porangan (or possibly Bortonian)” age (Pocknall, 1990). The coal sequence however is overlain by the marine Kauru Formation, with molluscs that Marwick (1960) regarded as ‘perhaps lower mid Eocene’, which were in turn about 30 m below more clearly Bortonian marine fossils. More recent work (E. Crouch in Maxwell, 2003; Fordyce et al. 2009) has identified dinoflagellates suggesting a Waipawan or Mangaorapan age for the Kauru Formation. Considering Pocknall’s (1984, 1990) age ranges, this would constrain the age of the carbonaceous sequence to the Mangaorapan (~51–52 Ma, Raine et al., 2015), and likely early within it. However, Pocknall (1990, p. 62) noted that “all samples are dominated by Casuarinaceae”, which indicates they date to the MH1 Zone of Raine (1984). Morgans et al. (2004) dated the base of the MH1 Zone as “middle Waipawan”. Thus an older, mid-late Waipawan age is also possible (earliest Eocene, ~52–54 Ma). However, Pancost et al. (2013) obtained a PM3b Zone (of Morgans et al., 2004) palynological sample from the Otaio River, earlier in the Waipawan. If the coal-bearing sediments are PM3b Zone, then the age is 54–56 Ma (Morgans et al. 2004. Raine et al. 2015). If the coal-bearing sediments extend into the MH1 Zone, then with an upper constraint of marine somewhere in the Waipawan or Mangaorapan, the Otaio River samples may date from ~51–56 Ma. In either case, they correlate with the early Eocene Ypresian zone of the international stratigraphy system (Fig. DR1).

1.2.2. Gannon’s Bridge
The Gannon’s Bridge locality, near Reefton in Victoria Forest Park on the northwest of the South Island of New Zealand, contains carbonaceous mud, with dispersed well-preserved leaf cuticle fragments. The bed lies at the foot of cliffs that form the north bank of the Waitahu River, downstream of Gannon’s Bridge, north of Reefton. The macrofossil assemblage is dominated by the conifer Libocedrus and a variety of Myrtaceae, Lauraceae, Proteaceae are also present (Pole, unpublished). Palynological data (Pole, unpublished) indicate Zone MH3 of Raine (1984). The area was mapped as Kaiatan by Suggate (1957) and correlates with the Brunner Coal Measures. The New Zealand Kaiatan chronostratigraphic zone spans the boundary between the middle Eocene Bartonian and the late Eocene Priabonian zones in the international chronostratigraphy, at ~39–37 Ma (Fig. DR1).

1.2.3. & 1.2.4. Huntly Mine and Rotowaro
Leaf cuticle is well-preserved in these coal-bearing sediments, belonging to the Waikato Coal Measures, at the adjacent Rotowaro and Huntly Mine localities on New Zealand’s north island. Although the precise location of these samples with respect to the major coal seams is still uncertain, based on Edbrooke et al. (1994), the Huntly material is regarded as Runangan (34.6–36.7 Ma, Raine et al. 2015), coincident with the latest Bartonian of the middle Eocene in the international chronostratigraphy, but the Rotowaro material may be as old as Kaiatan (36.7–39.1 Ma, Raine et al. 2015), in the late Eocene Priabonian. Due to the uncertainties connected to this stratigraphy, we plot the Huntly Material at 34.5–37 Ma and the Rotowaro material as 36.5–39 Ma (Fig. DR1).

2. PALEO-pCO2 RECONSTRUCTION: METHODS AND RESULTS

2.1. The Lauraceae cuticle database
The Southern Hemisphere plant dataset utilized in this study derives from two sources. One – the “Christophel database” – consists of 46 leaf cuticle microscope slides amassed during previously published and unpublished taxonomic work by David Christophel. This database is hosted at the
Melbourne Museum, Melbourne, Victoria, Australia (Dataset DR1). This historical leaf material was collected in the 1970’s and 80’s at the Anglesea open cut coalmine and at Deans Marsh in Victoria, as well as the Golden Grove and Nelly Creek localities, in South Australia, Australia (Fig.1). The other database – the “Pole database” consists of 46 cuticle microscope slides accumulated by Mike Pole. The leaf material was collected at the Rotowaro, Huntly mine, Gannon’s Bridge and Otaio River localities in New Zealand, as well as the Hasties and Regatta Point localities in Tasmania, Australia (Fig.1). This database is hosted at the School of Environment, University of Auckland, New Zealand (Dataset DR1). Cuticles belonging to the family Lauraceae is present at all localities, with the majority of specimens not identifiable to a lower taxonomic level, due to generalised cuticle morphology. Lauraceae cuticle is characterized by stomata, with paracytic subsidiary cells overarching guard cells, with cuticular scales between and simple trichome bases (Hill, 1986). This morphology is common in the warmer Australasian rainforests today, and is not known to be produced by any other plant family. Although advances have been made in characterising the cuticle of extant genera of Lauraceae (Christophel and Rowett, 1996; Christophel et. al, 1996) the epidermal cuticular surface morphology of the Lauraceae specimens studied here in most cases prevents assigning specimens to lower taxonomic rank (Fig. DR2). This is further compounded by differences in preservation, thickness and degree of staining of the cuticle specimens, which can affect the overall visual impression. However, the Anglesea dataset also includes several specimens belonging to the lauracean genus Cinamommum, previously classified based on leaf macro- and micro-morphology by D. Christophel (Fig. DR2).

Leaves and leaf fragments were separated from the sediment, macerated with 10% aqueous chromium trioxide to provide cleared abaxial cuticle specimens, which were stained in safranin and mounted on microscope glass slides in glycerin jelly, following conventional paleobotanical procedures. In general, epidermal cells are small (ca. 15–20 μm along the long axis and ca. 8–12 μm along the short axis), quadratic to rectangular-polygonal in shape, with straight, usually thick, non-undulate cell walls. Simple hair bases, or trichome-attachment scars, are usually present, sometimes abundantly – mostly located on veins, less commonly on areoles. Stomata are easily identified and dense in inter-vein areas (Fig. DR2 A-C). Stomatal pore length is normally ca. 8.5–10 μm. Overall, the shape of stomatal complexes is rectangular to circular, most often appearing visually lighter than surrounding epidermal cells, probably indicating that the stomatal cuticle is comparatively thin (and thus absorbed less stain). Veins consist of rows of several epidermal cells (ca. 7–8 × 20–25 μm in size), which are longer and slimmer than areolar epidermal cells. Trichome detachment scars are small, circular in cross-section, with thickened cuticle around the detachment zone and surrounded by ca. 20 μm long epidermal cells in the equivalent of the stomatal actinocytic arrangement.

Many of the specimens from the Christophel database derive from entire-leaf specimens, a collection of which is also hosted at the Melbourne museum, and were thus identified based both on leaf macro- and micro-morphology. This allowed including cuticles belonging to genus Cinamomnum from Anglesea Power Station (Fig. DR2 C), to compare stomatal densities between the highly generalised Lauraceae dataset, and a smaller single-genus dataset. The Pole cuticle database consists mostly of dispersed cuticle specimens and specimens were identified based on micromorphology.

2.2. Image analysis
The leaf cuticle specimens were all photographed at 200 × magnification to best capture abaxial epidermal morphology, taking 7 photographs evenly distributed across each cuticle specimen surface (sensu Poole and Kürschner, 1999), using light microscopy with mounted cameras. The Christophel database was photographed at the University of Melbourne, using a Zeiss Photomicroscope 2 with a Leica DFC320 camera attachment and associated FireCam V3.4.1. software. The Pole database was photographed at Stockholm University, using a Leica microscope with a mounted Leica DF310 FX camera and the associated LAS v3.8 software. Images were annotated with grids, delimiting areas of 300 × 300 μm, or 200 × 200 μm in the case of exceedingly small (and thus many) cells, and all the
stomata as well as the epidermal cells within each grid counted, using the free software ImageJ (1.46
h; http://imagej.nih.gov/ij).

2.3. Stomatal proxy palaeo-pCO2 reconstruction
The stomatal proxy uses the inverse relationship between densities of stomata on the leaf surface
and the concentration of CO2 in the atmosphere, to reconstruct past pCO2 using the stomatal densities of
fossil plants (Woodward, 1987). The inverse relationship is the physiological expression of plants’
response of minimizing loss of water through transpiration when CO2 is abundantly available in the
atmosphere. The stomatal proxy has been applied to a wide variety of plant taxa from disparate
geological, palaeo-ecological and palaeo-climatological settings to reconstruct palaeo-pCO2 from the
Palaeozoic until today, and is now considered a strong proxy (McElwain and Steinhorsdottir, 2017).
Stomatal frequency of plant leaves is quantified most reliably as the stomatal index (SI), since this is
affected less than SD by additional environmental and physiological parameters (such as light, leaf
expansion, etc.: Salisbury, 1927). Stomata and epidermal cells within each annotated grids were
counted and SI calculated as SI (%) = [SD / (SD + ED)] × 100, where SD is the number
of stomata/mm² and ED is the number of epidermal cells/mm². Five–seven images were counted for each
leaf cuticle specimen and the average obtained for SI. The mean values were confirmed by cumulative
mean statistical analysis, showing statistically stable values obtained by 4–5 counts per specimen and
5–6 cuticle specimens from each of the localities.

In some cases a species-specific relationship in the SI response to pCO2 may influence pCO2
reconstruction using fossil plants of a different species (Kelly and Beerling, 1995; Kürschner et al.,
1997; Haworth et al., 2010), but taxonomic groups generally show conservatism in the SI-pCO2
relationship within genera, and even at the family or order level, clustering together with similar SI
values, as well as displaying similar response directions and magnitudes to changes in pCO2
(McElwain et al., 2002; Barclay et al., 2010; Haworth et al., 2013; Steinhorsdottir et al., 2011;
Steinhorsdottir et al., 2016b). This conservatism is particularly useful when reconstructing pCO2 from
the pre-quaternary fossil record, which does not typically contain many fossil plants that are
conspecific with modern plants.

Eocene pCO2 was calibrated using four nearest living equivalent (NLE) species for the fossil
Lauraceae: Litsea glutinosa, L. fuscata, L. stocksii and Neolitsea dealbata in the stomatal ratio
method, as well as a Laurus nobilis transfer function. In addition, we calibrated pCO2 based on fossil
Cinnamomum specimens from Anglesea with NLE Cinnamomum camphora (see Table 1).

Three methods of stomatal proxy palaeo-pCO2 reconstructions are currently in use: 1) the
empirical stomatal ratio method, which utilizes the ratio between the SI of fossil plants and the SI of
extent nearest living relatives or equivalents (NLR or NLE), grown in known pCO2 (natural or
experimental), to estimate palaeo-pCO2 (McElwain and Chaloner, 1995); 2) the also empirical transfer
function method, which uses herbarium and/or experimental datasets of NLR/NLE responses to
variations in pCO2 to construct regression curves on which fossil SI can be plotted to infer palaeo-pCO2
(e.g. Kürschner et al., 2008; Barclay and Wing, 2016); and 3) mechanistic gas exchange modelling,
which is taxon-independent, relying on morphological measurement data, but also requires input of
additional parameters, such as leaf δ13C (Konrad et al., 2008; Franks et al., 2014; Konrad et al., 2017).
Here the historical databases are without leaf δ13C data, thus we utilized the stomatal ratio and transfer
function methods to reconstruct Eocene pCO2. Below we explain the methods used in more detail, as
well as provide the specific equations used, with average pCO2 results listed in Table 1 and individual
calculations for each specimen in Dataset DR1.

2.3.1. The stomatal ratio method
The stomatal ratio method (McElwain and Chaloner, 1995; McElwain, 1998), employs the ratio
between the stomatal density or index of a fossil plant and the stomatal density or index of the plant’s
nearest living relative (NLR) or nearest living taxonomical/morphological/ecological equivalent (NLE), in relation to the ratio between the known \( pCO_2 \) (modern) and palaeo-\( pCO_2 \). The so-called modern \( pCO_2 \) was historically set at 300 ppm (defined as preindustrial \( pCO_2 \)), but post-industrial \( pCO_2 \): SI couples are now mostly used. The ratio between \( SI_{modern}/SI_{fossil} \) to \( pCO_2_{fossil}/pCO_2_{modern} \) is assumed to be 1:1 (McElwain, 1998) and the stomatal ratio calibration is expressed by the equation:

\[
pCO_2_{palaeo} = \frac{SI_{NLE}}{SI_{fossil}} \times pCO_2_{modern}. \tag{1}
\]

Here, three subtropical–tropical rainforest tree species, which have previously been used in the stomatal ratio method for mid Eocene palaeo-\( pCO_2 \) calibrations (McElwain, 1998), were utilized as NLEs for the Australian and New Zealand Lauraceae database. These are \( Litsea glutinosa \) (synonym \( L. sebifera \)), \( L. fuscata \) and \( L. stocksii \). We used the stomatal ratio equation of McElwain (1998), with modern standardisation:

\[
pCO_2_{palaeo} = \frac{SI_{NLE}}{SI_{fossil}} \times 360 \tag{2}
\]

With SI_{NLE} being 19% for \( L. glutinosa \), 18.3% for \( L. fuscata \) and 14.4% for \( L. stocksii \) at 360 ppm \( pCO_2 \) (see McElwain, 1998).

We additionally used the Australian tropical rainforest species \( Neolitsea dealbata \), closely related to \( Litsea \), and possibly the best NLE to the Southern Hemisphere fossil Lauraceae studied here, with the published SI of ~17% at ~300 ppm (18.18% at 295.5 ppm and 15.88% at 309 ppm, see Greenwood et al., 2003b):

\[
pCO_2_{palaeo} = 17/SI_{fossil} \times 300 \tag{3}
\]

As mentioned above, SI values are relatively stable within genera and even higher taxonomic groups, but plant responses to \( pCO_2 \) are sometimes species/genus specific (Royer, 2001). This may introduce errors into calibrations using broader taxonomic groups, such as the Lauraceae. Here, we tested the \( pCO_2 \) values obtained with Lauraceae against specimens from Anglesea power station that have previously been assigned to genus \( Cinnamomum \) in the Christophel database. We use the published SI of sub-tropical species \( Cinnamomum camphora \), at 12% average (full range 10–14%) at 390 ppm (Qing et al., 2010).

\[
pCO_2_{palaeo} = 12/SI_{fossil} \times 390 \tag{4}
\]

2.3.2. Transfer functions

To test the results obtained with the stomatal ratio method, we further employed the transfer function of Kürschner et al. (2008) constructed for Miocene extinct Lauraceae, based on the extant species \( Laurus nobilis \) (laurel, or the common bay leaf), calibrated from a set of historical herbarium leaves, with an added correction factor of 150 ppm, since the leaves systematically underestimated \( pCO_2 \) (Kürschner et al., 2008):

\[
pCO_2_{palaeo} = 10^{3.173 - [0.5499 \times \log (SI_{fossil})]} + 150 \tag{5}
\]

The evergreen Mediterranean species \( L. nobilis \) was originally assigned as the specific NLE for the extinct Neogene European species \( Laurus abchasica \) (Kürschner et al., 2008; Kürschner and Kvacek, 2009) and may thus not be the most fitting NLE for the Southern Hemisphere Lauraceae studied here. However, we chose to include this calibration for easier comparison of previously published \( pCO_2 \).
values using this transfer function (Kürschner et al., 2008; Steinthorsdottir et al., 2016a, Steinthorsdottir et al., 2016b; Steinthorsdottir et al., 2018), in principal considering it as a standardised Lauraceae (Fig. DR2 D).

Finally, we calibrated \( pCO_2 \) using the published transfer function based on \( N. dealbata \) (Greenwood et al., 2003b), expressed by the equation:

\[
pCO_2_{\text{palaeo}} = -7.6871 \times SI_{\text{fossil}} + 419.48
\]  

(6)

However, the \( pCO_2 \) values produced by this transfer function using the Southern Hemisphere Lauraceae SI were very low, to an unrealistic degree (< 350 ppm) considering the warm Eocene climate, and significantly lower than the \( pCO_2 \) values obtained using all the other calibrations. We therefore concluded that this transfer function is not applicable to the fossil leaves studied here and omitted the results from further analyses. The unexpectedly low \( pCO_2 \) produced may be due to \( N. dealbata \)'s species-specific response to \( pCO_2 \) not being applicable to the more generalised Lauraceae fossil cuticle dataset studied here, or it could be the result of specific habitat and environmental influences \( N. dealbata \) is subjected to in the wild. Greenwood et al. (2003b) suggest that SI in this species may be additionally influence by irradiance, growing in hilly rainforest environments with frequent thick cloud cover. Quality and amount of irradiance have been shown to influence SI (Lake et al., 2002), and this may help explain why the transfer function was not applicable here.

While errors are difficult to quantify for stomatal proxy-based \( pCO_2 \) estimates, one way forward to reduce uncertainties and discrepancies in the Eocene record would be to (re-) calibrate \( pCO_2 \) using all currently available methods of paleo-\( pCO_2 \) reconstruction, as well as identify sections with multiple common taxa (e.g. Lauraceae, \textit{Ginkgo} and \textit{Metasequoia}) and quantify the potential differences in \( pCO_2 \) obtained between methods and/or taxa.

References


Stover, L., and Partridge, A., 1973, Tertiary and Late Cretaceous spores and pollen from the


---

**Figure DR1.** Chronological correlation of the Australian and New Zealand localities. Formal divisions of the Eocene follow Cohen et al. (2013) and informal subseries (early, middle, late) follow Luterbacher et al., (2005) and Head et al. (2017). New Zealand stages follow Morgans et al. (2004), and the Australian Gippsland Basin spore-pollen zones (defined by Partridge, 2006, and Stover and Partridge, 1973), follow the correlation of Holdgate and Gallagher (2003, fig. 10.18).
Figure DR 2. Micromorphology of fossil Lauraceae specimens and macromorphology of a nearest living equivalent. A: epidermal surface of Lauraceae leaf specimen nr. 997. The image shows typical morphology of epidermal cells and stomata in the leaf cuticle database, without trichome scars. Scale bar is 100 µm. B: Epidermal surface of leaf specimen Box 1.8., displaying abundant trichome scars. The turquoise rectangle illustrates how stomata (marked by dots) and epidermal cells were counted within engraved grids, to determine stomatal indices. Scale bar is 100 µm. C: epidermal surface of *Cinnamomum* sp. from Anglesea, with genus-characteristic oil glands (dark dots). Specimen nr. 218. Scale bar is 100 µm. D. Macromorphology of a typical Lauraceae leaf and one of the nearest living equivalents used for palaeo-pCO$_2$ calibration in this study – *Laurus nobilis*. Length ~10 cm including petiole. Image from Wikimedia.

Appendix DR2: full data set

2019329_AppendixDR2.xlsx