Early Jurassic (late Pliensbachian) CO2 concentrations based on stomatal analysis of fossil conifer leaves from eastern Australia

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**Abstract**

The stomatal index (a measure of stomatal density) of an extinct Australian Early Jurassic araucariacean conifer species, Allocladus helgei Jansson, is used to reconstruct the atmospheric carbon dioxide concentration (pCO2) in the Early Jurassic. The fossil leaves are preserved in a single bed, palynologically dated to late Pliensbachian (~185-183 Mya). Atmospheric pCO2 is estimated from the ratios between the stomatal index of A. helgei and the stomatal indices of three modern analogs (nearest living equivalent plants). CO2 concentration in the range of ~750-975 ppm was calibrated from the fossil material, with a best-estimated mean of ~900 ppm. The new average pCO2 determined for the late Pliensbachian is thus similar to, although ~10% lower, than previously inferred minimum concentrations of ~1000, based on data from the Northern Hemisphere, but may help constrain pCO2 during this period. Our results are the first pCO2 estimates produced using Jurassic leaves from the Southern Hemisphere and show that i) paleo-atmospheric pCO2 estimates are consistent at a global scale, though more investigations of Southern Hemisphere material are required, and ii) the stomatal proxy method can now be used without the context of relative change in pCO2 when applying the correct methodology.

1. Introduction

Stomata are pores on plant leaf surfaces, through which gas exchange takes place, i.e. carbon is acquired for photosynthesis from carbon dioxide (CO2) and water vapor and oxygen are lost by diffusion. The inverse relationship between stomatal density (often recorded as the percentage of stomata relative to stomata plus epidermal cells on the leaf surface and referred to as stomatal index or SI) and pCO2 has been repeatedly demonstrated for a wide range of plant taxa from different geological and ecological settings from the Paleozoic until present and this relationship has thus been established as a strong proxy for paleo-pCO2 (e.g. Woodward, 1987; Beerling and Chaloner, 1993; McElwain and Chaloner, 1995; Kürschner et al., 1996; Beerling et al., 1998; McElwain et al., 1998; Retallack, 2001; Royer, 2001; Rundgren and Björk, 2003; Roth-Nebelsick, 2005; McElwain and Haworth, 2009; Barclay et al., 2010; Steinthorsdottir et al., 2011, 2013). Stomatal density can also be used to test atmospheric models based on other proxy data (McElwain et al., 2002; Garcia-Amorena et al., 2006; Haworth et al., 2013). Although the plant gene HIC signaling pathway, which encodes the plant gene HIC signaling pathway, which encodes the protein HIC and is responsible for the regulation of stomatal development, has been mapped (Gray et al., 2000; Lake et al., 2002) and a large majority of (in particular gymnosperm) woody plants has been shown to react to elevated pCO2 (Royer, 2001; Haworth et al., 2013), some plant taxa do not respond by lowering their stomatal densities with elevated pCO2 (e.g. Haworth et al., 2011b). To obtain optimal results, we propose that only proven responders among modern analog taxa should be used in paleo-pCO2 reconstructions. Stomatal proxy-based paleo-pCO2 records have been obtained almost exclusively from the Northern Hemisphere and, so far, no studies have been carried out on Southern Hemisphere Jurassic leaf fossils. To our knowledge, the only previously published stomatal proxy-based pCO2 estimates from the Mesozoic of the Southern Hemisphere are for the Triassic and the Cretaceous (Retallack, 2002; Passalia, 2009). This is particularly striking since the Southern Hemisphere flora arguably hosts the most nearest living equivalents (NLEs) to Mesozoic counterparts. Consequently, it would be ideal to test the applicability of using fossil leaves and extant taxa from the same families within the same hemisphere to reconstruct pCO2. Several relict conifers possess a high “ceiling of response”, a concept that refers to the diminution of stomatal response to pCO2 after a certain level is reached (Kouwenberg et al., 2003; Haworth et al., 2010, 2011a). This level is usually fairly low for angiosperm trees that have evolved and adapted to the Cenozoic “icehouse” conditions and pCO2 of ~300 ppm (Beerling and Chaloner, 1993; Kouwenberg et al., 2003). Conversely, the araucariacean conifers used as modern analogs here, clearly demonstrate that they retain the stomatal responses developed in the Mesozoic greenhouse world, in which pCO2 reached >600 ppm (Berner, 2006). This conserved response makes these relict
conifers ideal to employ as nearest living equivalents or modern analogs when reconstructing pCO2 for the Mesozoic, in addition to other plant groups with reconstructing Mesozoic records, such as Ginkgoales (Beering et al., 1998; Chen et al., 2001; Kouwenberg et al., 2003; Royer et al., 2003; Haworth et al., 2011a; Steinhorsdottir et al., 2011).

For this study, we selected exceptionally well-preserved leaves of the araucariacean conifer *Allocladus helgei* (Jansson in Jansson et al., 2008a) from the Early Jurassic (Pliensbachian) of eastern Australia, and used the stomatal proxy method to reconstruct paleo-pCO2. The study aims to constrain pCO2 for the Early Jurassic based on Southern Hemisphere fossil plants, and to test the reliability of the stomatal method in the reconstructions of deep time pCO2 using proven relict Southern Hemisphere conifer responders.

2. Geological setting

The broad intracratonic Clarence–Moreton Basin, located largely onshore in northeastern New South Wales and southern Queensland (Fig. 1), was developed at a time of widespread subsidence in eastern Australia and incorporates entirely non-marine sediments of latest Triassic to Early Cretaceous age. The Clarence–Moreton sedimentary succession is divided into three subgroups, in turn divided into several formations. The fossil material in this study derived from strata is laterally equivalent to the Gatton Sandstone within the Marburg Subgroup comprising of sandstones, mudstones and shale deposited in a braided alluvial environment (Jansson et al., 2008b). The succession exposed at Inverleigh quarry represents the lower part of the Marburg Subgroup and is represented by – 15 m of organic-rich mudstones, siltstones and shale intercalated with massive to planar and cross-laminated sandstone units deposited in a floodbasin setting. The – 10 cm thick siltstone bed hosting the plant material of this study occurs about 4.1 m above the quarry floor (see fig. 3 of Jansson et al., 2008a; fig. 4 of Jansson et al., 2008b; McLoughlin et al., in press).

Based on palynostratigraphy, the sediments exposed in the quarry were initially dated as encompassing the Pliensbachian — early Toarcian (Jansson et al., 2008a,b) but subsequent palynological studies have revealed that the succession is constrained to the Pliensbachian. The age determination was based on comparisons with several Australian Jurassic palynological zonation schemes (see Helby et al., 1987 and references therein), and the Pliensbachian age was based mainly on the large portion of *Classopolis* pollen in combination with the scarce representation of the gymnosperm pollen *Callialasporites* spp. Representatives of the latter genus reached much higher relative abundance within lower Toarcian palynological assemblages (Helby et al., 1987).

3. Material and methods

3.1. Fossil leaf database

Ten exceptionally well-preserved leaves and leaf fragments of *A. helgei* were obtained by macerating siltstones collected from Inverleigh quarry in 2006 (Jansson et al., 2008a). *A. helgei* has a unique combination of cuticular and macromorphological characters that place it within Araucariaceae (Jansson et al., 2008a). The leaves are spirally arranged on the shoots (Fig. 2A), generally – 8–9 mm long and ~ 5 mm wide, triangular to narrowly ovate, uni-veined with a short acuminate tip and denticulate leaf margins (Fig. 2B). Stomata occur on one leaf surface only, interpreted to be the adaxial (upper) surface (Jansson et al., 2008a). Stomata are arranged in two rows, separated and flanked by zones of rectangular epidermal cells (Fig. 2C–D). Stomatal aperture orientation relative to the long axis of leaves is variable. Each stoma is circular to oval, surrounded by a narrow band of 4–6 subsidiary cells in a cyclocytic arrangement sensu Prabhakar (2004). On *A. helgei* leaf cuticle surfaces, most stomata occur as circular open holes, since guard cells are generally not preserved (Jansson et al., 2008a). The leaves studied here include whole leaves and leaf fragments, easily identifiable to species based on macromorphology (Fig. 2A–B). The cuticle morphology is well-preserved and identical in all specimens (Fig. 2C–D). Based on previous analyses of pollen, plant assemblages and sediments from the Inverleigh quarry, the *A. helgei*-dominated flora grew on waterlogged floodplains in a warm humid paleoclimate (Jansson et al., 2008a,b). Illustrated macrofossils (prefix LO) are registered in the fossil collections of the Geology Department at Lund University.

3.2. Laboratory methods

The siltstone samples were carefully treated with dilute hydrofluoric acid (35%), and subsequently sieved through a 63 μm mesh before *A. helgei* leaves and leaf fragments were handpicked under stereomicroscope and dried. No further treatment was necessary in order to study the leaf surfaces. The fossil leaves were dry-mounted, adaxial side (stomatal surface) up, on glass slides and studied using an epifluorescence microscope (Olympus BX51FT-5). The surface of each leaf was photographed at several sites at × 200 magnification, using a mounted microscope camera (INFINITY2-1C) and associated software (Analyze 6.0). The images were evenly distributed across the mid-leaf areas, away from edges, midrib and veins to minimize variation and provide optimal results, according to the methods of Poole and Kürschner (1999). The images were annotated using the software Imaged (1.39u, NIH, USA) by engraving 0.1 mm² grids on each image (~ 316 × 316 μm); all stomata and epidermal cells (including subsidiary cells) within these grids were then counted and recorded. Data was entered into Microsoft Excel spreadsheets. Following the methodology of Poole and Kürschner (1999), eight images were counted for each leaf fragment and the mean was obtained for stomatal indices. The means were confirmed by cumulative mean statistical analysis.

3.3. Stomatal analysis and pCO2 calibrations

For calibration of late Pliensbachian pCO2 based on mid-leaf stomatal index, we use the NLE approach, incorporating the stomatal ratio (SR) method of McElwain and Chaloner (McElwain and Chaloner, 1995, 1996), which compares the ratio between stomatal indices of fossil plants and their nearest living relatives or equivalents (NLR or NLE) in
relation to the ratio between known pCO2 and paleo-pCO2. The SR method was established using a range of NLE and modern SI values for current pCO2 matched against equivalent values for ancient pCO2 inferred from Berner's (1994) GEOCARB II model (McElwain et al., 1998). The pCO2 data derived using the SR method showed a close fit to independently estimated pCO2 values and also to pCO2 records constructed using experimental data (Beierling et al., 1998). Based on analyses of best fit to the GEOCARB pCO2 curve, a “Carboniferous standardization” and a “Modern standardization” were established for calibrating paleo-pCO2 (Chaloner and McElwain, 1997; McElwain et al., 1998). The Carboniferous standardization is used for calibrations involving Mesozoic and Paleozoic fossil plants: the ratio of past pCO2 (RCO2) to the pre-industrial pCO2 of ~300 ppm was inferred to be ~2:1 (2RCO2) (Chaloner and McElwain, 1997). Stomatal indices, reflecting changes in pCO2, also display this ratio of approximately 2:1 for the Paleozoic and Mesozoic relative to pre-industrial levels (McElwain and Chaloner, 1996; McElwain et al., 1998). The concentration of paleo-CO2 is thus calibrated based on this ratio:

$$pCO_2^{\text{paleo}} = \frac{SI_{\text{NLE}}}{SI_{\text{fossil}}} \times 600.$$ 

An alternative approach, the transfer function method, is often used to reconstruct paleo-pCO2. Using this method, inverse regression analyses are applied to quantify paleo-pCO2 from SI response datasets derived from herbarium and/or CO2 fumigation experiments. This method relies on the assumption that the NLE should behave in the same way as its fossil counterpart, but provides a more “quantitative” approach to reconstructing paleo-pCO2. However, there are several caveats to consider. Firstly, it is only possible to infer equivalent responses between fossil and modern plants when working with the same species, or at least closely related species, which are very seldom available. Even when working with the same species, transfer functions may give widely differing results for separate localities across the same time interval, whereas the more simplified NLE approach gives similar results between localities (see Steinthorsdottir et al., 2013). The SI of fossil plants commonly falls outside the “response slope” for modern plants and falls instead on the flat part of the curve. Therefore, even when using this quantitative approach, the response curve typically needs to be extended beyond the dataset, bringing its own set of uncertainties. In addition, the transfer function method often produces values that are unrealistic (commonly too low) and inconsistent with other proxies and other transfer function based results. The NLE approach appears to give more consistent results with both other NLE stomatal proxy results, with alternative proxy data, and with modeling estimates (see Steinthorsdottir et al., 2011). Therefore, we employ the simpler, more “qualitative” NLE approach.

NLRs (same species or genus) are generally not available further back in time than the Neogene, so a NLE for the Pliensbachian leaves studied here must be established before pCO2 calibrations can be performed. Following the protocol of Steinthorsdottir et al. (2011), we limit the choice of NLEs to proven responders of pCO2, assuming that these will more accurately reflect the physiological responses of the fossil equivalent plants. Araucariacean conifers are probably the most appropriate NLEs for A. helgei (Jansson et al., 2008a). Based on the elevated pCO2 experiments of Haworth et al. (2010, 2011a), two araucariacean species were selected: Wollemia nobilis and Agathis australis, together with one cupressacean conifer bearing broadly similar scale-leaves to A. helgei: Athrotaxis cupressoides. The NLE’s stomatal indices under control or ambient conditions (pCO2 ~ 380 ppm) were 12.3%, 14.4% and 11.2% respectively. The two araucariacean NLEs were

![Fig. 2. Morphology of Allocladus helgei. A and B are respectively a photo and SEM image showing the leaf macro-morphology of A. helgei (from Jansson et al., 2008a). B and C show adaxial A. helgei leaf cuticle micro-morphology, including stomata and epidermal cell shape and orientation, under epifluorescence at ×100 and ×200 magnification respectively (photo: M. Steinthorsdottir).]
selected based on their close phylogenetic relationship and analogous cuticle morphology to the fossil conifer studied here, whereas the cupressaceous NLE was chosen based on its similar leaf macro-morphology to \textit{A. helgei}. Perhaps other araucariaceous conifer species would be better NLEs to \textit{A. helgei}, such as \textit{Araucaria heterophylla}, closely matching in both morphology and ecology, but we consider it essential that NLEs are proven CO\textsubscript{2} responders and, therefore, chose to focus on taxa that have been used in elevated CO\textsubscript{2} experiments. See Section 3.4 below for a brief description of each NLE.

The chosen NLEs are Southern Hemisphere scale- and broad-leaved conifers that possess high ceilings of response to pCO\textsubscript{2} changes (response begins at \textasciitilde 500 ppm; Haworth et al., 2010, 2011a). The high response ceilings indicate adaptation to higher CO\textsubscript{2} for these conifers than for modern angiosperm trees (Beerling and Chaloner, 1993; Kouwenberg et al., 2003), probably illustrating the more coniferous preferences, and the proven ability to respond to CO\textsubscript{2}. Here three NLEs were distributed during the Mesozoic (Kershaw and Wagstaff, 2001; Vajda and Wigforss-Lange, 2006, 2009; Panti et al., 2012). Three NLEs were selected for \textit{A. helgei}, based on taxonomy, ecological preference and stomatal adaptations and gas exchange relationships. Using the mean SI values derived from the use of multiple similar NLEs is a superior approach to selecting just one NLE, since species-specific variability in SI may be minimized and a more accurate SI signal of paleo-pCO\textsubscript{2} may emerge. Each chosen NLE, its morphology, ecological preference and taxonomic affinity, is listed briefly below.

### 3.4. Nearest living equivalents

The fossil conifer species studied here, \textit{A. helgei}, has previously been placed within the Araucariaceae, based on macro- and micro-morphology (Jansson et al., 2008b). The relative abundance of Araucariaceae fossils in the geological record has provided an extensive understanding of the family's past distribution and evolution (Stockley, 1982, 1994; Pole, 1995; Chambers et al., 1998; Kunzmann, 2007; Pole, 2008; Pole and Vajda, 2009). Araucariaceans are today predominantly found in the Southern Hemisphere, but were globally distributed during the Mesozoic (Kershaw and Wastaff, 2001; Vajda and Wigforss-Lange, 2006, 2009; Panti et al., 2012). Three NLEs were selected for \textit{A. helgei}, based on taxonomy, morphology, ecological preferences, and the proven ability to respond to CO\textsubscript{2}. Of the three species, two belong to Araucariaceae: \textit{W. nobilis} and \textit{A. australis}, while the third: \textit{A. cupressoides}, belongs to Cupressaceae.

#### 3.4.1. \textit{W. nobilis}

\textit{W. nobilis} (Wollemi pine) was discovered in 1994 in a national park not far from Sydney, Australia (Jones et al., 1995; McLoughlin and Vajda, 2005). Phylogenetic analyses indicate that Wollemia is a sister group to \textit{Agathis} (Gilmore and Hill, 1997; Stefanovic et al., 1998; Liu et al., 2009). \textit{W. nobilis} is an evergreen tall tree, reaching a maximum height of \textasciitilde 40 m. The leaves are variable in shape, but mainly long and narrow, \textasciitilde 3–9 cm long and 2–6 mm broad, and spirally inserted (although typically basally twisted to form plagiotropic juvenile leaf arrangements or orthotrophic four-ranked adult leaf arrangements), with stomata occurring in rows (Jones et al., 1995). Stomata are arranged in uni-seriate discontinuous rows of \textasciitilde 5–6 stomata, with their long axis predominantly parallel to the long axis of the leaf (in adult foliage), separated within each row by squarish epidermal cells. Stomatal rows are separated by regions of elongate epidermal cells. The morphology of the stomatal complexes sees each stomatal opening surrounded by 4–6 subsidiary cells, and with the epidermal cells immediately surrounding these modified in shape to form a second cycle around the subsidiary cells (Chambers et al., 1998).

#### 3.4.2. \textit{A. australis}

\textit{Agathis} ranges from southeast Asia, through northeastern Australia to northern New Zealand, New Caledonia and Fiji (Pole, 2008). New Caledonia is a center of diversity for the genus with five species. \textit{A. australis} (New Zealand kauri) is a tall tree (up to 50 m), with a straight cylindrical trunk (1–4 m thick) and an extensive crown; the lowermost retained branches on mature trees are commonly at least 15 m from the ground (Sando, 1936). Leaves are thick, elliptical to lanceolate, about 3–7 cm long, 1 cm broad, and multi-veined. Stomata occur in rows; the stomatal openings are circular to rectangular, surrounded by four subsidiary cells (Haworth et al., 2011a). Macrofossils confidently attributed to \textit{Agathis} have only been recorded from Cenozoic strata (Carpenter and Pole, 1995; Hill et al., 2008; Pole, 2008). Macrofossils ascribed to this genus from older strata (e.g., White, 1981; Daniel, 1989; Cantrill, 1992) lack definite characters for this genus and likely belong to the large diversity of Mesozoic representatives of the family that has been lost to extinction.

#### 3.4.3. \textit{A. cupressoides}

\textit{A. cupressoides} (pencil pine) is a Southern Hemisphere evergreen cupressaceous conifer endemic to Tasmania. The conifer's leaf morphology is scale-like, rhombic, \textasciitilde 4–5 mm long and 1–2 mm wide. Stomata are irregularly arranged across the abaxial leaf surface, most abundant distally (Haworth et al., 2010). Stomatal complexes are oval, with the stomatal pore circular to rectangular and surrounded by 5–7 subsidiary cells. Epidermal cells are elongate and irregularly rectangular. \textit{A. cupressoides} is endemic to Tasmania, Australia, where it grows at 700–1300 m altitude, with an ecological preference for cool, moist conditions (Cullen and Kirkpatrick, 1988; van der Ham et al., 2001; Haworth et al., 2010). We include \textit{A. cupressoides} here due to the superficial similarity of leaf macro-morphology and preference for wet environments, but we consider it as the least appropriate of the three selected NLE species.

### Table 1

<table>
<thead>
<tr>
<th>Leaf nr.</th>
<th>Mean SI</th>
<th>pCO\textsubscript{2} NLE \textit{A. cupressoides}</th>
<th>pCO\textsubscript{2} NLE \textit{W. nobilis}</th>
<th>pCO\textsubscript{2} NLE \textit{A. australis}</th>
<th>Mean pCO\textsubscript{2} per leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.50\textsuperscript{+1;−0.90}</td>
<td>707.4</td>
<td>776.8</td>
<td>909.5</td>
<td>797.9</td>
</tr>
<tr>
<td>2</td>
<td>8.93\textsuperscript{+1;−0.50}</td>
<td>752.5</td>
<td>826.4</td>
<td>967.5</td>
<td>848.8</td>
</tr>
<tr>
<td>3</td>
<td>8.38\textsuperscript{+1;−0.42}</td>
<td>801.9</td>
<td>880.7</td>
<td>1031.0</td>
<td>904.5</td>
</tr>
<tr>
<td>4</td>
<td>9.33\textsuperscript{+1;−1.05}</td>
<td>720.3</td>
<td>791.0</td>
<td>936.0</td>
<td>812.4</td>
</tr>
<tr>
<td>5</td>
<td>8.7\textsuperscript{+1;−0.00}</td>
<td>771.5</td>
<td>847.3</td>
<td>992.0</td>
<td>870.3</td>
</tr>
<tr>
<td>6</td>
<td>8.96\textsuperscript{+1;−0.53}</td>
<td>750.0</td>
<td>823.7</td>
<td>964.3</td>
<td>846.0</td>
</tr>
<tr>
<td>7</td>
<td>8.80\textsuperscript{+1;−0.30}</td>
<td>763.6</td>
<td>838.6</td>
<td>981.8</td>
<td>861.4</td>
</tr>
<tr>
<td>8</td>
<td>8.62\textsuperscript{+1;−0.00}</td>
<td>779.6</td>
<td>856.1</td>
<td>1002.3</td>
<td>879.4</td>
</tr>
<tr>
<td>9</td>
<td>8.06\textsuperscript{+1;−1.06}</td>
<td>833.7</td>
<td>915.6</td>
<td>1072.0</td>
<td>940.4</td>
</tr>
<tr>
<td>10</td>
<td>9.52\textsuperscript{+1;−0.67}</td>
<td>705.9</td>
<td>777.2</td>
<td>907.6</td>
<td>756.2</td>
</tr>
<tr>
<td>Mean</td>
<td>8.88\textsuperscript{+1;−0.46}</td>
<td>758.6\textsuperscript{+1;−41.0}</td>
<td>833.2\textsuperscript{+1;−52.7}</td>
<td>975.6\textsuperscript{+1;−45.4}</td>
<td>853.7\textsuperscript{+1;−46.1}</td>
</tr>
</tbody>
</table>
The mean stomatal index based on ten leaves of A. helgei is 8.88% + / − standard deviation of 0.48% (Table 1). Pliensbachian pCO2 calibrated using this mean SI, using the stomatal proxy method with Carboniferous standardization and the three chosen NLEs, was found to have a mean value of 855.7 ppm (Fig. 3 and Table 1). The lowest pCO2 value, using the NLE A. cupressoides is 758.6 ppm, whereas W. nobilis and A. australis yield pCO2 of 833.2 ppm and 975.4 ppm respectively (Fig. 3). We speculate that since W. nobilis and A. australis may be more closely related to A. helgei than A. cupressoides, the former are more appropriate NLEs to A. helgei. Focussing on the pCO2 values obtained using only the araucarian conifers raises the calibrated Pliensbachian pCO2 to a mean of 904.3 ppm (≈900 ppm, see Fig. 3).

5. Discussion and conclusions

The ability to predict the effects of currently unfolding climate change requires detailed knowledge about the link between atmospheric CO2 and climate through geological time. Although it may be impossible to reconstruct pCO2 based on stomatal density at high resolution since the rise of vascular plants, throughout the last 400 Ma of the Phanerozoic, every opportunity to add to our knowledge about past pCO2 should be seized. We suggest that the stomatal proxy method, using ancient conifers with appropriate NLEs, is sufficiently well-established to reconstruct pCO2 at any point in the geological timescale, including intervals of relatively stable pCO2, without the background of relative change.

5.1. Comparison with previous Early Jurassic pCO2 estimates

While major events, pre- and postdating the Early Jurassic, such as the mass extinction events outlined below, have been studied intensely using multiple-proxy data and carbon cycle modeling, the intervening “non-event” time periods, i.e. the Sinemurian and Pliensbachian, have not received the same attention. During the Early Jurassic, greenhouse conditions prevailed with generally very high atmospheric pCO2 inferred (Cerling, 1991; Yapp and Poths, 1996; Ekart et al., 1999; McElwain et al., 1999; Retallack, 2001; Beerling and Royer, 2002; Berner, 2006; Retallack, 2009). However, this has commonly been based on “background” values close to stage boundaries characterized by significant global change (see Fig. 4).

Previous stomatal-based pCO2 estimates from the Jurassic have been carried out on Northern Hemisphere floras from intervals immediately preceding and/or following major extinctions or turnovers in the geological record. Results from stomatal-proxy based pCO2 estimates across the Triassic-Jurassic (Tr–J) boundary interval in Germany, Greenland and the UK, based on conifer, ginkgoalean, bennettitalean and seed-fern leaves, reveal pCO2 at ~2000–2500 ppm during the boundary interval, whereas Late Triassic (Rhaetian) and Early Jurassic (Hettangian) levels were significantly lower, at ~1000–1500 ppm (McElwain et al., 1999; Bonis et al., 2010a; Steinthorsdottir et al., 2011). The Tr–J boundary is characterized by global extinctions of species both in the marine and terrestrial realms followed by ecosystem collapse defined by significant carbon cycle perturbations traced in the carbon stable isotope record (Raup and Sepkoski, 1982; Palfy et al., 2001; Hesselbo et al., 2002; Kiessling et al., 2007; Lucas and Tanner, 2007; McElwain et al., 2007, 2009; Aikikuni et al., 2010). Signals of elevated temperatures, increased runoff and marine anoxia (indicated by black shale formation) are evident at this time (Bonis et al., 2010b; Richoz et al., 2010; Steinthorsdottir et al., 2012). A narrowing consensus on the causal mechanism of this disruption of the ecosystem centers on global warming, driven by the relatively rapid increase of pCO2 resulting from eruptions within the Central Atlantic Magmatic Province (CAMP), is probably aggravated by the enhanced release of methane and/or volcanic pollutants into the atmosphere (McElwain et al., 1999; Hesselbo et al., 2002; McElwain et al., 2007; van de Schootbrugge et al., 2009; Ruhl et al., 2011; Lindstrøm et al., 2012; Vajda et al., 2013). Although past studies have concentrated on Tr–J boundary events, they also establish “background” conditions for the ensuing Early Jurassic pCO2 at ~1000–1500 ppm.

A subsequent mostly marine mass extinction took place in the earliest Toarcian (183 Ma; Hallam, 1987; Palfy and Smith, 2000; Gomez and Goy, 2011; Cúneo et al., 2013), during the so-called Toarcian Ocean Anoxic Event (T-OAE). Severe environmental perturbations occurred at this time resulting in extinctions of biota and preservation of organic-rich sediments in the marine realm (Aberhan and Baumiller, 2003; McElwain et al., 2005; Dera et al., 2010; Gill et al., 2011). Further, the T-OAE is characterized in the carbon isotope record by a distinct negative excursion and the formation of black shale, indicating anoxia (Jiménez et al., 1996; Hesselbo et al., 2000; Beerling et al., 2002). This event is most likely related to CO2 outgassing by the Karoo–Ferrar eruptions (Hesselbo et al., 2000; Palfy and Smith, 2000; Beerling and Brentnall, 2007). The concentration of atmospheric CO2 during
the T-OAE appears to have doubled, from ~1000 ppm to ~2000 ppm (Beerling and Royer, 2002; McElwain et al., 2005; Berner, 2006; Retallack, 2009). Early Jurassic background pCO2 is thus confirmed at around 1000 ppm.

Our calibrated pCO2 values of ~900 ppm, based on Pliensbachian araucariacean conifer leaf fossils from southeastern Australia are consistent with high pCO2 values relative to the present, but slightly less than the Early Jurassic pCO2 estimates of approximately 1000 ppm as outlined above, based on different plant groups for the Northern Hemisphere. The Pliensbachian pCO2 constructed here falls close to the median range (and well within the error envelope) of pCO2 reconstructed using the GEOCARB II and III models (Berner, 1994; Berner and Kothavala, 2001; Fig. 4). Based on the pCO2 reconstructed here using the stomatal method coupled with previous calculations of Early Jurassic “background pCO2 values” as detailed above, we suggest that Early Jurassic pCO2 (Hettangian to Pliensbachian) was approximately 900 ppm (Fig. 4).

5.2. The importance of multiple locality, global pCO2 reconstructions

Although CO2 is the primary driver of climate change on geological timescales (Royer et al., 2004), and has been shown to be well-mixed in the global atmosphere (Keeling et al., 1989), some climate change episodes have been attributed to internal dynamics, i.e. changes in heat distribution via insolation, ocean currents or wind systems, typically because paleo-pCO2 records commonly show little change and/or contradictory results. Examples in the geological record include the Eocene–Oligocene boundary event (~34 Mya) and the Younger Dryas climatic change (~12 800 cal years BP), when oxygen isotopes and other proxies indicate large changes in temperatures, but changes in pCO2 records are small or enigmatic (Monnin et al., 2001; Zachos et al., 2001; Broecker, 2006; Coxall and Pearson, 2007). However, the results of more recent paleo-pCO2 studies are changing this view in unexpected ways. For instance, it seems that both the above-mentioned cooling events may have been preceded by fairly abrupt but transient rises in pCO2 (Pagani et al., 2011; Steinthorsdottir et al., 2013), which may then have forced a chain of feedbacks to unfold, leading eventually to colder climates and associated CO2 drawdown. Recent records show dynamic behavior of pCO2 across these climate change transitions, and demonstrate that there is clearly still much to learn regarding the coupling of pCO2 and climate on short and long timescales. It is, therefore, important to obtain pCO2 data preferably from multiple localities and both hemispheres, when reconstructing paleoclimates, in order to discriminate conditions that were global from those that were of regional or local extent. Identical stomatal indices from multiple sources on separate continents will strongly indicate a global climatic signature and help explain mechanisms of climate change.

The results presented here are the first pCO2 estimates to derive from Southern Hemisphere Jurassic plant material, but a few previously published Southern Hemisphere stomatal proxy-based estimates do exist. Passalida (2009) employed late Aptian (mid-Cretaceous) conifer and Ginkgo leaf cuticles from Argentina, revealing that mid-Cretaceous pCO2 estimates range from 700 to 1400 ppm, which is also consistent with Northern Hemisphere results for Aptian pCO2 levels. Retallack (2002) collated, mostly from previously published taxonomical studies, stomatal indices from several genera and multiple localities in both hemispheres and calibrated atmospheric pCO2 for the Triassic based on this data. Several Southern Hemisphere datasets were included based on Lepidopteris leaves from Early Triassic of Antarctica and Australia (McLoughlin et al., 1997), Middle Triassic of Australia (Townrow, 1966) and Late Triassic of Australia (Townrow, 1965), Argentina (Baldoni, 1972) and South Africa (Townrow, 1956, 1960; Anderson and Anderson, 1989). Results from these datasets confirmed the Northern Hemisphere results of overall highly elevated Triassic pCO2.

5.3. The potential of Australian fossil conifers in paleo-pCO2 reconstructions

With time, the relatively new stomatal proxy method is becoming increasingly well-established in paleo-pCO2 reconstructions, after most caveats have been addressed and early criticism rebuked (McElwain and Haworth, 2009; Haworth et al., 2013). In particular, the method is considered to provide the most accurate results when using reliable NLEs such as araucariacean conifers (Haworth et al., 2011a), and it now seems possible to accurately determine long-term pCO2 patterns using fossil leaves, even in the absence of supporting proxies.

The stomatal ratio method is continually being refined and strengthened by e.g. establishing specific criteria for the NLE plants that are used in calibrations and by accumulating independent supporting evidence of the stomata-based pCO2 values. We suggest that the best practice in Mesozoic pCO2 reconstructions should include not only selecting NLEs that are the closest equivalents in terms of phylogeny and ecology (as per Chaloner and McElwain (1997)), but also applying multiple species of proven CO2 responders with closely matching stomatal indices. This should maximize the probability that the NLEs are expressing real physiological responses to pCO2, which evolved in the Mesozoic and thus reflect the same responses in fossil Mesozoic plants.

The relative abundance of Araucariaceae in the fossil record has assisted paleobotanists to unravel much of this family’s past evolution and geographical distribution. Even though Araucariaceae primarily flourishes in the Southern Hemisphere today, it is evident that this distribution is a remnant of its past geographically widespread range, which includes most parts of the Northern Hemisphere back to at least the Jurassic (Stockey, 1982, 1994; Vajda, 2001; Kunzmann, 2007). While this family’s taxonomy has been documented in many studies, analyses of stomata as a tool for interpreting climatic signals through pCO2 estimates have not been performed on Southern Hemisphere araucariacean fossil conifers. We contend that the Southern Hemisphere Araucariaceae leaf assemblages constitute a true geological treasure, especially as the close modern relatives to these middle and late Mesozoic taxa presently flourishing in this region. The Australian record is particularly significant in this regard, with key macrofossil assemblages characterized by organic preservation represented through the Early Jurassic (Cattamarra Coal Measures: McLoughlin and Pott, 2009), Middle Jurassic (Walloon Coal Measures: Gould, 1980), Late Jurassic (Talbragar Fossil Fish Beds: White, 1981), Early Cretaceous (Otway and Strzelecki groups: Douglas, 1969; McLoughlin et al., 2002; Nagalingum et al., 2005), mid-Cretaceous (Otway Group and Winton Formation: Cantrill, 1991, 1992; Pole, 2000) and Late Cretaceous (Waarre Formation: Douglas, 1965).

5.4. Concluding remarks

Based on the stomatal method, using fossil leaves from the Australian araucariacean conifer A. helgei, we reconstructed pCO2 for the late Pliensbachian at ~900 ppm. This atmospheric CO2 concentration is slightly lower but broadly consistent with the results from stomatal-based pCO2 reconstructions for adjacent geological time periods based on equivalent methodologies in the Northern Hemisphere, and also with the lower ranges of GEOCARB II and GEOCARB III (Berner, 1994; Berner and Kothavala, 2001) modeled pCO2 (Fig. 4).

Constraining the uncertainty associated with predictions of the likely impacts of future climate change is a topical field of scientific research. Records of biotic turnover coupled with well-constrained atmospheric models provide important insights into how past ecosystems responded to climate change. Amassing an extensive database of past pCO2 interpretations will provide improved reconstructions of the scale and rate of atmospheric pCO2 changes and permit improved predictions of biotic responses to future climate change. Australian conifers are emerging as an ideal group to be utilized in stomatal-based pCO2 reconstructions, due to their long evolutionary lineages, and proven retained responses to high levels of pCO2. With the recent advances
in stomatal proxy research, it is now highly feasible to utilize fossil conifers with the appropriate NLEs to reconstruct pCO$_2$, enabling a more extensive range of fossil leaf material to be employed in paleo-pCO$_2$ reconstructions.

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