The \( pCO_2 \) estimates of the late Eocene in South China based on stomatal density of \textit{Nageia} Gaertner leaves

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Abstract

late Eocene $p$CO$_2$ concentration is estimated based on the species of *Nageia maominggensis* Jin et Liu from the late Eocene of Maoming Basin, Guangdong Province. This is the first paleoatmospheric estimates for the late Eocene of South China using stomatal data. Studies of stomatal density (SD) and stomatal index (SI) with *N. motleyi* (Parl.) De Laub., the nearest living equivalent species of the fossil, indicate that the SD inversely responds to atmospheric CO$_2$ concentration, while SI has almost no relationships with atmospheric CO$_2$ concentration. Therefore, the $p$CO$_2$ concentration is reconstructed based on the SD of the fossil leaves in comparison with *N. motleyi*. Results suggest that the mean CO$_2$ concentration was $391.0 \pm 41.1$ ppmv or $386.5 \pm 27.8$ ppmv during the late Eocene, which is significantly higher than the CO$_2$ concentrations documented from 1968 to 1955 but similar to the values for current atmosphere indicating that the Carbon Dioxide levels during that the late Eocene at that time may have been similar to today.

1 Introduction

The Eocene (55.8–33.9 Ma) was characterized by much warmer temperatures than present-day, although temperatures also varied significantly across this time interval. Climate of the early Eocene to middle Eocene was extremely warm, particularly during the early Eocene Climatic Optimum (EECO; 52 to 50 Ma; Zachos et al., 2001), also known as the Paleocene–Eocene Thermal Maximum (PETM event; $\sim$ 55.8 Ma; Wing et al., 2005; Kato et al., 2011). A series of sudden and extreme global warming events (hyperthermals; Deconto et al., 2012) have been described $\sim$ 55.5 to 52 Ma; and a mid-Eocene warming has been recognized, the middle Eocene Climate Optimum (MECO; $\sim$ 40 Ma; Bijl et al., 2010). However, the global climate occurred colder conditions during the early-middle (50 to 48 Ma) and the late Eocene (40 to 36 Ma; Zachos et al.,
The appearance of small-ephemeral ice-sheets during the latest Eocene suggests the coldest climate of the Eocene (Zachos et al., 2001).

Atmospheric CO₂ concentrations have been well correlated with global surface temperature change (Mann et al., 1998; Crowley, 2000; Barnett et al., 2001; Harries et al., 2001; Levitus et al., 2001; Mitchell et al., 2001). Most authors link the changes in temperature to atmospheric CO₂ concentration during the entire Phanerzoic (pCO₂) (Petit et al., 1999; Retallack, 2001; Royer, 2006). However, the Eocene pCO₂ record remains incomplete and debated (Beerling et al., 2002; Kürschner et al., 2001; Royer et al., 2001; Greenwood et al., 2003; Royer, 2003; Wing et al., 2005; Kato et al., 2011).

Most pCO₂ estimates works have focused on the Paleocene–Eocene boundary (Beerling et al., 2002; Wing et al., 2005; Kato et al., 2011) and the middle Eocene (Deconto et al., 2012). In addition, the pCO₂ reconstruction results have varied based on different proxies. Various methods having been used in pCO₂ reconstruction mainly include the computer modeling methods: GEOCARB-I, GEOCARB-II, GEOCARB-III, GEOCARB-SULF and the proxies ice cores, paleosol carbonate, phytoplankton, nahcolite, Boron, and stomata parameters.

Generally, stomatal data (stomatal density and index) can be easily and accurately obtained from well-preserved fossil and modern leaves. Various plants showing the negative correlation between stomatal density (SD) or stomatal index (SI) and atmospheric CO₂ concentration have been used to reconstruct pCO₂, including Ginkgo (Beerling et al., 2002; Royer, 2003; Retallack, 2001, 2009a; Smith et al., 2010), Metasequoia (Royer, 2003; Doria et al., 2011), Taxodium (Stults et al., 2011), Betula (Kürschner et al., 2001; Sun et al., 2012), Neolitsea (Greenwood et al., 2003), Quercus (Kürschner et al., 1996, 2001) and multiple trees (Kürschner et al., 2008). Recently, positive correlations between stomatal index or stomatal frequency and pCO₂ have been reported based on fossil Typha and Quercus (Bai et al., 2015; Hu et al., 2015). However, the tropical and subtropical moist broadleaf forest conifer tree Nageia has been overlooked in paleobotanical estimates of pCO₂ concentration.

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Herein, we firstly document the correlation between the SD and SI of extant *Nageia motleyi* (Parl.) De Laub. leaves and atmospheric CO$_2$ concentration to provide a training dataset for application to fossil representatives. Furthermore, we estimate a new pCO$_2$ level for the late Eocene using the stomatal density method based on fossil *Nageia* from the late Eocene of South China, providing significant implications on discussing the climate change rhythm throughout the Eocene.

2 Background

2.1 Stomatal proxy in pCO$_2$ research

Stomatal proxy is widely used in reconstructions of pCO$_2$ concentration. The main parameters are stomatal density (SD) which is expressed as the total number of stomata divided by area, epidermal density (ED) expressed as the total number of epidermal cells per area and stomatal index (SI) calculated by the calculating the percentage of stomata among the total number of cells within an area \([SI = SD \times 100/(SD + ED)]\). Woodward (1987) considered that both SD and SI had inverse relationships with atmospheric CO$_2$ during the development of the leaves. Subsequently, McElwain (1998) created the stomatal ratio (SR) method to reconstruct pCO$_2$. SR is a ratio of the stomatal density or index of a fossil \([SD(f)\) or \(SI(f)\)] to that of corresponding nearest living equivalent \([SD(e)\) or \(SI(e)\)], expressed as follows:

\[
SR = \frac{SI(e)}{SI(f)}
\]  

The stomatal ratio method is a semiquantitative method of reconstructing pCO$_2$ concentrations under certain standardizations. One is the “carboniferous standardization” (Chaloner and McElwain, 1997) indicating that one stomatal ratio unit was equal to two RCO$_2$ units and:

\[
SR = 2RCO_2
\]
Where the value of $R_{CO_2}$ is the $pCO_2$ level divided by the pre-industrial atmospheric level (PIL) of 300 ppm (McElwain, 1998) or that of the year when the NLE was collected (Berner, 1994; McElwain, 1998):

$$R_{CO_2} = C_{(f)}/300 \text{ or } R_{CO_2} = C_{(f)}/C_{(e)} \tag{3}$$

Then the estimated $pCO_2$ level is expressed as follows:

$$C_{(f)} = 0.5 \times C_{(e)} \times SD_{(e)}/SD_{(f)} \text{ or } C_{(f)} = 0.5 \times C_{(e)} \times SI_{(e)}/SI_{(f)} \tag{4}$$

Where $C_{(f)}$ is the $pCO_2$ and $C_{(e)}$ is the atmospheric $CO_2$ of the year when the NLE species was collected (McElwain and Chaloner, 1995, 1996; McElwain 1998). The equation adapts to the $pCO_2$ concentration prior to Cenozoic era.

Another standardization, the “Recent standardization” (McElwain, 1998), is expressed as one stomatal ratio unit being equal to one $R_{CO_2}$ unit:

$$SR = 1R_{CO_2} \tag{5}$$

According to the equations stated above, the $pCO_2$ concentration can be expressed as:

$$C_{(f)} = C_{(e)} \times SD_{(e)}/SD_{(f)} \text{ or } C_{(f)} = C_{(e)} \times SI_{(e)}/SI_{(f)} \tag{6}$$

Where $C_{(f)}$, $C_{(e)}$, $SD_{(e)}$, $SD_{(f)}$, $SI_{(e)}$ and $SI_{(f)}$ are stated above. This standardization is usually used for reconstruction based on Cenozoic fossils (Chaloner and McElwain, 1997; McElwain, 1998; Beerling and Royer, 2002).

### 2.2 Review of extant and fossil Nageia

The genus *Nageia* including only seven living species, is a special group of Podocarpaceae which is a large family of conifers mainly distributed in the Southern
Hemisphere with broadly ovate-elliptic to oblong-lanceolate, multiveined (without a mid-vein), spirally arranged or in decussate, and opposite or subopposite leaves (Cheng et al., 1978; Fu et al., 1999). Generally, Nageia is divided into Nageia Sect. Nageia and Nageia Sect. Dammarioideae (Mill, 1999, 2001), mainly distributed in eastern Asia from north latitude 30° to nearly the equator and coastal mountain areas and island areas of the western Pacific Ocean, including South China, South Japan, Malaya and Indonesia, New Guinea, and other Pacific islands (Fu, 1992; Fig. 1a). Four species, ie., Nageia nagi (Thunberg) O. Kuntze, N. fleuryi (Hickel) De Laub., N. formosensis (Dummer) C. N. Page, and N. nankoensis (Hayata) R. R. Mill, of the N. section Nageia, have hypostomatic (the stomata only distributed on the abaxial side) leaves, with a single exception of N. maxima (De Laub.). De Laub. which is characterized by amphistomatic leaves but only a few stomata on the adaxial side (Hill and Pole, 1992; Sun, 2008). Both N. wallichiana (Presl) O. Kuntze and N. motleyi, of the N. section Dammarioideae, are amphistomatic with abundant stomata distributed on both sides of the leaf, especially N. motleyi having approximately equal stomata on both surfaces (Hill and Pole, 1992; Sun, 2008).

The fossil records of Nageia can be traced back to the Cretaceous with the evidence from Far East Russia, Japan and Henan, China (Krassilov, 1965; Matsuo, 1977; Kimura et al., 1988; Yang, 1990) and be extended into the Eocene of Hainan Island and Guangdong (Maoming), South China (Jin et al., 2010; Liu et al., 2015; Fig. 1a). Although some of the Nageia fossil materials described above have well-preserved cuticles (eg. Jin et al., 2010; Liu et al., 2015), the above studies are mainly concentrated on the morphology, systematics and phytogeography.

Nageia maomingensis Jin et Liu was reported based on four leaves with well-preserved cuticles recovered from late Eocene of South China (Liu et al., 2015). Among the modern Nageia species mentioned above, N. motleyi was considered as the nearest extant living (NEL) species of N. maomingensis (Liu et al., 2015). Both N. maomingensis and N. motleyi are amphistomatic indicating that both upper and lower surfaces of the leaf are needed to estimate the $pCO_2$ concentration during the late Eocene.
However, because of the species-specific inverse relationship between atmospheric CO$_2$ partial pressure and SD (Woodward and Bazzaz, 1988), it is necessary to explore whether the SD and SI of the *N. motleyi* show the negative correlations with the CO$_2$ concentration before applying the stomatal method.

3 Material and methods

3.1 Extant leaf preparation

We examined five specimens of extant *Nageia motleyi* from different herbaria (Table 1): (1) the specimen numbered 2649 (the herbarium of the V. L. Komarov Botanical Institute of the Russian Academy of Sciences) was collected by Beccari O. from Malaysia in 1868, (2) the specimen numbered bb. 17 229 (Harvard University Herbarium) was collected by Neth. Ind. For. from Riau on Ond. Karimon, Archipel. Ind. in the latitude of 150 m in 1932, (3) the specimen numbered bb. 18 328 (Harvard University Herbarium) was collected from Z. O. afd. v. Borneo Tidoengsche Landen, Archipel. Ind., Malaysia in the latitude of 5 m in 1934, (4) the specimen numbered bb. 21 151 (Harvard University Herbaria) was collected from Z. O. afd. Borneo, Poeroek Tjahoe Tahoedjan, Archipel. Ind. in the latitude of 500 m in 1936, (5) the specimen numbered bb. 40 798 (Herbarium of Royal Botanic Garden, Edinburgh) was collected by Sinclair, J. and Kiah bin, Salleh from Gunong Tebu Forest reserve, Malaysia in the latitude of 51 m in 1955 (Table 1). We removed one or two leaves from each specimen, and took three fragments (0.25 mm$^2$) from every leaf (Fig. 2a) and numbered them for analysis.

The numbered fragments were boiled for 5–10 min in water. Subsequently, after being macerated in a mixed solution of 10 % acetic acid and 10 % H$_2$O$_2$ (1 : 1) and heated in the thermostatic water bath at 85°C for 8.5 h; the reaction was stopped when the specimens fragments turned white and semitransparent; The cuticles were then rinsed with distilled water until the pH of the water became neutral. After that the cuticles were treated in Schulze’s solution (one part of potassium chlorate saturated solution
and three part of concentrated nitric acid) for 30 min, rinsed in water, and then treated with 8 % KOH (up to 30 min) and the abaxial and adaxial cuticles were separated with a hair mounted on needle. Finally, the cuticles were stained by 1 % Safranin T alcoholic solution for 5 min, sealed with Neutral Balsam and observed under the LM.

### 3.2 Fossil leaf preparation

Four fossil leaves of *Nageia maomingensis* were recovered from the late Eocene of the Youganwo Formation (MMJ1-001) and the Huangniuling Formation (MMJ2-003, MMJ2-004 and MMJ3-003) of Maoming Basin, South China (Fig. 1b and c). Macrofossil cuticular fragments were taken from the middle part of each fossil leaf (Fig. 2c) and treated by Schulze’s solution for approximately 1 h and 5–10 % KOH for 30 min (Ye, 1981). The cuticles were observed and photographed under a Carl Zeiss Axio Scope A1 light microscope (LM). All fossil specimens and cuticle slides are housed in the Museum of Biology of Sun Yat-sen University, Guangzhou, China.

### 3.3 Stomatal counting strategy and calculation methods

SD, ED and SI are counted based on analyzing the pictures taken with the light microscope (LM) using the standard sampling protocol, only counting those stomata touching or straddling the left-hand side and top including the corner between them, provided by Poole and Kürschner (1999; Fig. 2b and d). A total of 1116 pictures (200× magnification of Zeiss LM) of the cuticles from 9 leaves of *N. motleyi* were counted. Each counting field was 0.366 mm$^2$. In *Nageia maomingensis*, 112 views (400× magnification) of the abaxial side and 150 views (400× magnification) of the adaxial side of cuticles were counted with an area of 0.092 mm$^2$. None of the counting areas above overlapped and they were larger than the minimum area (0.03 mm$^2$) for statistics (Poole and Kürschner, 1999). In this study, the stomatic data of both surfaces are applied in $p$CO$_2$ reconstruction because both our fossil species and the NLE species are amphisomatic.
4 Results

4.1 Correlations between the CO$_2$ concentrations and stomatal parameters of *Nageia motleyi*

The SD and SI data of the adaxial sides of *N. motleyi* leaves are shown in Table 2. The SDs and SIs range from 45.89 to 78.6 (mm$^{-2}$) and from 2.89 to 3.94 (%), respectively. However, the SDs and SIs data of the abaxial sides, summarized in Table 3, give significantly higher values (53.22–82.71 in SDs and 3.13–4.66 in SIs) than those from the adaxial sides. Figure 3 shows the relationships between the stomatal parameters (SD and SI) of modern *N. motleyi* and the atmospheric CO$_2$ concentration (SD-CO$_2$ relationships and SI-CO$_2$ relationships). R$^2$ values in the SD-CO$_2$ relationships from both the adaxial and abaxial surfaces of *N. motley* are up to 0.841 and 0.725 (Fig. 3a and b), suggesting that the stomatal densities of *N. motleyi* are in significant inverse proportion to the CO$_2$ concentrations. However, the Fig. 3c and d indicate no relationships between the SIs and CO$_2$ concentrations for the extremely low level of the R$^2$ values (0.003 and 0.0608).

According to the results stated above, the stomatal ratio method can be used in estimating $p$CO$_2$ concentration of the late Eocene based on the stomatal densities (SDs) of the fossil species *N. maomingensis* and the extant species *N. motleyi*. Beerling (1999) and Royer (2001) considered both the SD and SI vary with economical and biological factors such as irradiance, temperature, and water supply, but the SI is more sensitive than the SD to the concentration of atmospheric CO$_2$ (Beerling, 1999) and more accurate in responding to the variation of $p$CO$_2$ concentration (Royer, 2001). However, the study of Kouwenberg et al. (2003) indicated that the SD better reflects the negative relationships with atmospheric CO$_2$ concentration.

The SD results of specimen No. 40 798 are closest to the fitted equations in Fig. 3a and b and therefore are selected to reconstruct the $p$CO$_2$ concentration. The specimen was collected by J. Sinclair and Salleh Kiah Bin from Gunong Tebu Forest Reserve,
Malaysia, in 1955 at an altitude of 51 m and a CO$_2$ concentration of 313.73 ppmv during that time (Brown, 2010). Therefore, the SD from the adaxial and abaxial surfaces of *N. maomingensis* and its NLE species *N. motleyi* are used to recover $p$CO$_2$ concentrations based on the stomatal ratio method.

**4.2 Stomatal parameters and $p$CO$_2$ estimates results**

After being projected into a long-term carbon cycle model (GEOCARB III; Berner and Kothavalá, 2001), the results of this study compares well with the CO$_2$ concentrations for corresponding age within their error ranges (Fig. 4). The summary of stomatal parameters of the extant and fossil *Nageia* and reconstruction results are provided in Tables 2 and 3, respectively. SD and SI values were calculated for all samples of the extant and fossil *Nageia*. The mean SD and SI values of the adaxial surface are 44.5 ± 2.9 and 1.80 ± 0.12, respectively (Table 5). The mean SD values of the abaxial and abaxial surface are 48.9 ± 3.0 and 53.22 ± 2.2, respectively (Table 2 and 3). The mean SR values of both sides are quite similar with 1.24 ± 0.13 in adaxial side and 1.23 ± 0.09 in abaxial side (Tables 4 and 5). The average reconstruction results of $p$CO$_2$ concentration in the late Eocene of Maoming Basin is 391.0 ± 41.1 ppmv (Table 4) and 386.5 ± 27.8 ppmv (Table 5) with a 95% confidence interval based on the adaxial and abaxial cuticles, respectively. Clearly the two estimates are rather similar with a difference of 5 ppmv in mean value, which is clearly less than their own standard error, indicating that the reconstructions based on both sides are consistent in this fossil species. Table 4 shows gradually increasing $p$CO$_2$ level from the lower layer to the upper ones, while the $p$CO$_2$ estimated results based on the abaxial side are random with the highest result in lowest layer (Table 5).

The partial pressure of CO$_2$ decreases with elevation (Gale, 1972). Jones (1992) proposed that the relationship between elevation and partial pressure in the lower atmosphere can be expressed as $P = -10.6E + 100$, where $E$ is elevation in kilometers and $P$ is the percentage of partial pressure relative to sea level. Various studies corroborate that SI and SD of many plants have positive correlations with altitude (Körner
and Cochrane, 1985; Woodward, 1986; Woodward and Bazzaz, 1988; Beerling et al., 1992; Rundgren and Beerling, 1999) while they are negatively related to the partial pressure of CO$_2$ (Woodward and Bazzaz, 1988). Therefore, it is essential to take elevation calibration into account during the $p$CO$_2$ concentration estimates. However, Royer (2003) pointed out that it is unnecessary make this conversion when the trees lived at $< 250$ m in elevation. In this paper, the nearest living equivalent species, *Nageia motleyi*, grows at 51 m in elevation with $P = 99.5$, suggesting that CO$_2$ concentration estimates were only underestimated by 0.5%. Consequently, no correction is needed for the reconstruction result in this study.

5 Discussion

5.1 Paleoclimate reconstructed history

The $p$CO$_2$ throughout the Cenozoic was relatively lower than the levels through the Cretaceous (Ekart et al., 1999), but it is had an overall decreasing trend with some significantly changes on short-time scales (e.g. in the earliest Eocene and middle Miocene (Zachos et al., 2001; Wing et al., 2005; Lowenstein and Demicco, 2006; Fletcher et al., 2008; Zachos et al., 2008; Bijl et al., 2010; Kato et al., 2011). There is a wide range in $p$CO$_2$ estimates for the Paleogene, reflecting both problems in the various proxies. Both the fractionation of carbon isotopes by phytoplankton (Freeman and Hayes, 1992) and analysis of paleosol (fossil soil) carbonates (Ekart et al., 1999) demonstrate that carbon dioxide levels were less than 1000 ppmv before the Cretaceous–Tertiary boundary and have been decreasing since the Paleocene.

Based on the measurements of palaeosol carbon isotopes, Cerling (1991) reported that $p$CO$_2$ levels for the Eocene and Miocene through to the present was lower than 700 ppmv. Fletcher et al. (2008) also showed that an atmospheric CO$_2$ levels of approximately 680 ppmv by 60 million years ago. However, Stott (1992) reconstructed $p$CO$_2$ as 450–550 ppmv for the early Eocene based on phytoplankton. Additionally, the re-
constructions using the stomatal ratio method based on the leaves of *Ginkgo*, *Metasequoia*, and Lauraceae also revealed a low $p$CO$_2$ level between 300 and 500 ppmv during the early Eocene (Kürschner et al., 2001; Royer et al., 2001; Greenwood et al., 2003; Royer, 2003) except a single high estimate about 800 ppmv near the Paleocene/Eocene boundary (Royer et al., 2001).

Subsequently, Smith et al. (2010) reconstructed the value of $p$CO$_2$ ranging from 580 ± 40 to 780 ± 50 ppmv using the stomatal ratio method (recent standardization) based on both SI and SD. A climatic optimum occurred in the middle Eocene (MECO): the reconstructed CO$_2$ concentrations are mainly between 700 and 1000 ppmv during the late middle Eocene climate transition (42–38 Ma) using stomatal indices of fossil *Metasequoia* needles, but concentrations declined to 450 ppmv toward the top of the investigated section (Doria et al., 2011). Jacques et al. (2014) used CLAMP to calibrate climate change in Antarctica during the early-middle Eocene, suggesting a seasonal alternation of high- and low-pressure systems over Antarctica during the early-middle Eocene. The two results of our study agree well with the estimates of approximately the same period based on phytoplankton (Freeman and Hayes, 1992; Ekart et al., 1999; Pagani et al., 2005) and other stomatal studies (McElwain, 1998) (Fig. 5). The $p$CO$_2$ level showed an overall decreasing trend after the MECO period, indicating the consistence with the $p$CO$_2$ levels herein (Fig. 5; Retallack, 2009b).

The ice-sheets started to appear in the Antarctic during the late Eocene (Zachos et al., 2001), then the temperature suffered an apparent further decrease from the late Eocene to the early Oligocene (Roth-Nebelsick et al., 2004), which resulted in the Antarctic being almost fully covered by ice-sheets. Subsequently, the climate variation was comparatively stable with a little wobbling in temperature during the Oligocene period (Fig. 5), while a small and ephemeral late Oligocene Warming was present in the latest part of the Oligocene, resulting in reducing the ice sheets in Antarctic to a minimum and forming a brief period of glaciation at that time (Zachos et al., 2001). During the middle Miocene, a quick rise in temperature was shown, which was followed by a small glaciation (Fig. 5; Zachos et al., 2001; Roth-Nebelsick et al., 2004;
Beerling and Royer, 2011). Subsequently, the CO₂ concentration decreased gradually and reached 280 ppmv until the period of the industrial revolution (Fig. 5). Since then, however, the CO₂ concentration rebounded.

In conclusion, although various results were made by different pCO₂ reconstruction proxies at the same time, their entire decreasing tendency of pCO₂ level are remarkably consistent with each other since the Eocene (Fig. 5). Furthermore, during the Eocene the temperature was higher than at present. The reconstructed pCO₂ concentration based on the SD of fossil Nageia are 391.0 ± 41.1 ppmv and 386.5 ± 27.8 ppmv, showing a remarkably low pCO₂ level during the early late Eocene.

5.2 Implications from Nageia motleyi ecology

Nageia motleyi is restrictedly distributed in the southern half of Malay Peninsula, adjacent Sumatra, and southern Borneo (Fig. 1a) with the mean annual temperature of ca. 25–30°C which is higher than South China (ca. 20–25°C; Fig. 1a). This species is generally scattered in the canopy of primary and secondary rainforests on massive substrates and situations from well-drained, even arid, slopes to waterlogged peat swamps at elevations of 15–500 (~ 1000) m (Eckenwalder, 2009) and in Borneo surviving where there is deep peat in a mixed ramin-peat swamp, ridges, and hill sides in bindang-dipterocarp forest, and 1000 m on podsolic sandy loam (Coomes and Bellingham, 2011). All the living ecological characteristics of N. motleyi provide a significant implication that the temperature during the late Eocene might have been similar to that in the area where N. motleyi grows today.

Palynological assemblages from the late Eocene of Maoming Basin of Guangdong (Aleksandrova et al., 2012) suggest that the Youganwo Formation was humid, and the Huangniuling Formation had an increase of average annual temperatures and humidity during this period. Additionally, according to the winged-fruits Shorea maomingensis Feng, Kodrul et Jin (Dipterocarpaceae) recovered from the late Eocene of the Huangniuling Formation of the Maoming Basin and the living conditions of modern Shorea,
Feng et al. (2013) point out the occurrence of seasonally dry climate at that time and a temperature higher than today.

In this article, we reconstructed the $pCO_2$ of the late Eocene as 391.0 ± 41.1 ppmv and 386.5 ± 27.8 ppmv, which are distinctly higher than the CO$_2$ level of 289.23–313.73 ppmv from extant leaves collected from 1968 to 1955 (Table 1), but similar to the extant CO$_2$ concentration of 387.35–401.52 ppmv from 2009 to 2015 (Brown, 2010; Pieter and Keeling, 2015). Compares with the reconstruction results in Fig. 5, our estimates show comparatively low $pCO_2$ concentration during the late Eocene. Combined with the low $pCO_2$ and the living conditions of $N$. sect. Demmaroideae (adapted to warm areas of East Asia) (Fig. 1), we conclude that the other factors may have played a role in the global climate changing process. Owing to the totally decreasing trend of the global climate change from the late Eocene reconstructed based on the proxies of stomata, paleosols, phytoplankton and B/Ca (Fig. 5), the plants of $N$. Sect. Demmaroideae migrated toward south and ultimately disappeared from South China (Fig. 1).

6 Conclusions

The stomatal data analysis suggests only the stomatal densities from both sides of $Nageia motleyi$ leaves have significant negative correlations with the atmospheric CO$_2$ concentration, suggesting that we can estimate the $pCO_2$ of the Eocene in South China based on the stomatal densities of the Eocene fossil leaves of $N. maomingensis$ and their nearest living equivalent species $N. motleyi$. Based on the stomatal ratio method, $pCO_2$ concentration of the late Eocene of Maoming Basin, Guangdong Province, is reconstructed as 391.0 ± 41.1 ppmv (based on the adaxial side of leaf cuticles) and 386.5 ± 27.8 ppmv (based on the abaxial side of leaf cuticles), showing low $pCO_2$ levels during the globally warm epoch of the Eocene, which is significantly higher than the historical CO$_2$ concentrations from 1868 to 1955 (around the industrial atmospheric level, 300 ppmv) and similar to the concentration of today.

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**Table 1.** Modern *Nageia motleyi* (Parl.) De Laub samples and atmospheric CO$_2$ values of their collection dates from ice core data (Brown, 2010).

<table>
<thead>
<tr>
<th>Herbarium</th>
<th>Collection number</th>
<th>Collecting locality</th>
<th>Collectors</th>
<th>Number of leaf samples</th>
<th>Collection date</th>
<th>CO$_2$ (ppmv)</th>
</tr>
</thead>
<tbody>
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<td>No. 2649</td>
<td>Malaysia</td>
<td>Beccari, O</td>
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<td>289.23</td>
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<tr>
<td>A/GH</td>
<td>No. bb. 17229</td>
<td>150 m, Riau on Ond, Karimon, Archipel. Ind.</td>
<td>Neth. Ind. For. Service</td>
<td>2</td>
<td>1932</td>
<td>306.19</td>
</tr>
<tr>
<td>E</td>
<td>No. bb. 40798</td>
<td>51 m, Kuala Trengganu-Besut Road, Bukit Bintang Block, Gunong Tebu Forest reserve, Malaysia</td>
<td>Sinclair, J. and Kiah bin, Salleh</td>
<td>2</td>
<td>1955</td>
<td>313.73</td>
</tr>
</tbody>
</table>

Note: A/GH – Harvard University Herbarium, Harvard University, 22 Divinity Avenue, Cambridge, Massachusetts 02138, USA (www.huh.harvard.edu).
LE – The Herbarium of the V.L. Komarov Botanical Institute of the Russian Academy of Sciences, Prof. Popov Street 2, Saint Petersburg 197376, Russia (www.binran.ru).
Table 2. Summary of stomatal parameters of the adaxial surface form modern *Nageia motleyi* (Parl.) De Laub.

<table>
<thead>
<tr>
<th>Collection number</th>
<th>Collection date</th>
<th>CO₂ (ppmv)</th>
<th>SD (mm⁻²)</th>
<th>SI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.2649</td>
<td>1868</td>
<td>289.23</td>
<td>78.60</td>
<td>15.44</td>
</tr>
<tr>
<td>No.bb.17 229</td>
<td>1932</td>
<td>306.19</td>
<td>62.14</td>
<td>17.20</td>
</tr>
<tr>
<td>No.bb.18 328</td>
<td>1934</td>
<td>306.46</td>
<td>64.57</td>
<td>15.05</td>
</tr>
<tr>
<td>No.bb.21 151</td>
<td>1936</td>
<td>306.76</td>
<td>65.45</td>
<td>11.14</td>
</tr>
<tr>
<td>No.40 798</td>
<td>1955</td>
<td>313.73</td>
<td>45.89</td>
<td>13.81</td>
</tr>
</tbody>
</table>

Note: *x* – mean; *σ* – standard deviation; s.e. – standard error of mean; *n* – numbers of photos counts (40×); *t*s.e. – 95 % confidence interval.
Table 3. Summary of stomatal parameters of the abaxial surface form modern *Nageia motleyi* (Parl.) De Laub.

<table>
<thead>
<tr>
<th>Collection number</th>
<th>Collection date (year)</th>
<th>CO₂ (ppmv)</th>
<th>SD (mm⁻²)</th>
<th>SI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.2649</td>
<td>1868</td>
<td>289.23</td>
<td>82.71</td>
<td>3.89</td>
</tr>
<tr>
<td>No.bb.17 229</td>
<td>1932</td>
<td>306.19</td>
<td>69.16</td>
<td>3.13</td>
</tr>
<tr>
<td>No.bb.18 328</td>
<td>1934</td>
<td>306.46</td>
<td>69.92</td>
<td>3.99</td>
</tr>
<tr>
<td>No.bb.21 151</td>
<td>1936</td>
<td>306.76</td>
<td>75.68</td>
<td>4.66</td>
</tr>
<tr>
<td>No.40 798</td>
<td>1955</td>
<td>313.73</td>
<td>53.22</td>
<td>3.71</td>
</tr>
</tbody>
</table>

Note: $x$ – mean; $\sigma$ – standard deviation; s.e. – standard error of mean; $n$ – numbers of photos counts (40×); $t^*\text{s.e.}$ – 95% confidence interval.
Table 4. Summary of stomatal parameters of the adaxial surface of fossil *Nageia* and \( pCO_2 \) \([C(f)]\) estimates results.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>SD (mm(^{-2}))</th>
<th>SI (%)</th>
<th>SR</th>
<th>( C_{(f)} ) (ppmv)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( x )</td>
<td>( \sigma )</td>
<td>s.e.</td>
<td>( n )</td>
</tr>
<tr>
<td>MMJ1-001</td>
<td>late Eocene</td>
<td>52.5</td>
<td>17.1</td>
<td>3.1</td>
<td>30</td>
</tr>
<tr>
<td>MMJ2-003</td>
<td>late Eocene</td>
<td>42.3</td>
<td>12.9</td>
<td>2.4</td>
<td>30</td>
</tr>
<tr>
<td>MMJ2-004</td>
<td>late Eocene</td>
<td>39.9</td>
<td>13.6</td>
<td>2.5</td>
<td>30</td>
</tr>
<tr>
<td>MMJ3-003a</td>
<td>late Eocene</td>
<td>43.2</td>
<td>17.7</td>
<td>3.2</td>
<td>30</td>
</tr>
<tr>
<td>Mean</td>
<td>late Eocene</td>
<td>44.5</td>
<td>16.3</td>
<td>1.5</td>
<td>120</td>
</tr>
</tbody>
</table>

Note: \( x \) – mean; \( \sigma \) – standard deviation; s.e. – standard error of mean; \( n \) – numbers of photos counts (400×); t*s.e. – 95 % confidence interval.
Table 5. Summary of stomatal parameters of the abaxial surface of fossil *Nageia* and \( p\text{CO}_2 \) \( C_{(f)} \) estimates results.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>SD (mm(^{-2}))</th>
<th>SI (%)</th>
<th>SR</th>
<th>( C_{(f)} ) (ppmv)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( x )</td>
<td>( \sigma )</td>
<td>s.e.</td>
<td>n</td>
</tr>
<tr>
<td>MMJ1-001</td>
<td>late Eocene</td>
<td>47.7</td>
<td>17.7</td>
<td>3.2</td>
<td>30</td>
</tr>
<tr>
<td>MMJ2-003</td>
<td>late Eocene</td>
<td>50.9</td>
<td>18.3</td>
<td>3.3</td>
<td>30</td>
</tr>
<tr>
<td>MMJ2-004</td>
<td>late Eocene</td>
<td>48.2</td>
<td>15.8</td>
<td>2.9</td>
<td>30</td>
</tr>
<tr>
<td>MMJ3-003a</td>
<td>late Eocene</td>
<td>48.9</td>
<td>12.6</td>
<td>2.7</td>
<td>22</td>
</tr>
<tr>
<td>Mean</td>
<td>late Eocene</td>
<td>48.9</td>
<td>16.2</td>
<td>1.5</td>
<td>112</td>
</tr>
</tbody>
</table>

Note: \( x \) – mean; \( \sigma \) – standard deviation; s.e. – standard error of mean; \( n \) – numbers of photos counts (400x); t*s.e. – 95% confidence interval.
Table 6. The \( p\text{CO}_2 \) estimates proxies and corresponding references.

<table>
<thead>
<tr>
<th>Proxies</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boron</td>
<td>Pearson et al. (2009); Seki et al. (2010)</td>
</tr>
<tr>
<td>B/Ca</td>
<td>Tripati et al. (2009)</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>Freeman and Hayes (1992); Stott (1992); Pagani et al. (1999, 2005); Henderiks and Pagani (2008); Seki et al. (2010)</td>
</tr>
<tr>
<td>Nahcolite</td>
<td>Lowenstein and Demicco (2006)</td>
</tr>
<tr>
<td>Liverworts</td>
<td>Fletcher et al. (2008)</td>
</tr>
<tr>
<td>Paleosols</td>
<td>Cerling (1992); Koch et al. (1992); Ekart et al. (1999); Retallack (2009b); Royer et al. (2001)</td>
</tr>
<tr>
<td>Stomata</td>
<td>Van der Burgh et al. (1993); Kürschner et al. (1996); McElwain (1998); Kürschner et al. (2001); Greenwood et al. (2003); Royer (2003); Kürschner et al. (2008); Beerling et al. (2009); Royer et al. (2001); Retallack (2009a); Smith et al. (2010); Doria et al. (2011)</td>
</tr>
</tbody>
</table>
Figure 1. Map showing the distribution of extant and fossil *Nageia* and their mean annual temperature (Modified after the map from http://nelson.wisc.edu/sage/data-and-models/atlas/maps.php?datasetid=35&includerelatedlinks=1&dataset=35).
Figure 2. Sampling areas and counting rules are shown. (a) Nageia motleyi (Parl.) De Laub. leaf. Black squares in the middle of the leaf show the sampling areas for preparing the cuticles. (b) The abaxial side of the cuticle from N. motleyi leaf. Black circles show the counted stomatal complexes. (c) N. maomingensis Jin et Liu. Red squares in the middle of the leaf indicate the sampling areas. (d) The abaxial side of the fossil cuticle. Red circles show the counted stomatal complexes. Scale bars: (a) and (c) = 1 cm; (b) and (d) = 50 µm.
Figure 3. Correlation between SD and SI vs. CO₂ concentration for modern *Nageia motleyi*. (a) Trends of SD with CO₂ concentration for the adaxial surface. (b) Trends of SD with CO₂ concentration for the abaxial surface. (c) Trends of SI with CO₂ concentration for the adaxial surface. (d) Trends of SI with CO₂ concentration for the abaxial surface.
Figure 4. The $p$CO$_2$ reconstruction results and extant CO$_2$ concentrations are projected onto the long-term carbon cycle model (GEOCARB III; Berner and Kothavalá, 2001). The $p$CO$_2$ results based on the adaxial and abaxial surfaces are represented by blue and red, respectively. The CO$_2$ concentrations from 1968 to 1955 are showed by light red and those from 2009 to 2015 are in light blue (they are not the CO$_2$ concentrations of Holocene).
Figure 5. Atmospheric CO$_2$ estimates from proxies over the past 60 million years. The horizontal dashed line indicates monthly atmospheric CO$_2$ concentration for March 2015 at Mauna Loa, Hawaii (401.52 ppmv) (Pieter and Keeling, 2015). The vertical lines show the error bars. The data were from the supporting data of Beerling and Royer (2011) and the references in Table 6.