1. Need approximate age

Changed as suggested.

2. Last part of sentence not clear.

Changed as suggested.

3. Last sentence needs rewriting.

Rewritten as suggested.

4. Do not see the point of the original opening sentence and references for paragraph 2.

Changed as suggested.

5. This paragraph needs rewriting to place into proper context. Right now, it does not convey a good understanding of the debate and problems.

We added a context. Hopefully, it is proper.

6. Seems awkward

Changed as suggested.

7. Why not include these in the above list??

Because these two papers reported the positive correlation between stomatal index or stomatal frequency and pCO_2.

8. Probably two good summary papers would suffice here.

Changed as suggested.

9. Not sure what this last sentence means.

We deleted some words of this sentence.

10. The last few bits can be condensed as sort of a repeat from a above.

The repeated sentence was removed.

11. Correct?

Yes, it is cited from Kouwenberg et al. (2003).

12. Things get problematic here as some of this seems to be methods. The parts on *Nageia* seemingly belong later.

The sentences should belong to the later part were deleted.

13. Split this convoluted sentence and rewrite the second bit as difficult to follow. Does this apply to *Podacarpus* or *Nageia*?

Changed as suggested.

14. Both sections? Not clear.

Yes, we are mean to "both sections".

15. Avoid Latin abbreviations in the middle of sentences.

Changed as suggested.

16. This needs slight expansion and conformity. I would list specific locations, types of material and approximate ages.

Changed as suggested.

17. As above, not quite clear what is being suggested.

We changed the whole sentence making it easily understandable.

18. Between ** This important point – species specific relationships -- needs to come earlier.

Changed as suggested.

19. Between *** I think can removed as long as clearly documneted in Tabel 1

Changed as suggested.

** Needs a brief paragraph here noting the Maoming Basin, rock units, depositional environment, and criticially, the age.**

Changed as suggested.

20. This is not crystal clear to me.

We fixed this sentence and corresponding figure 2b to make it clearly to you are readers.

21. See above. The specifics to *Nageia* should be here, but the more general procedure should be presented above.

We think it’s more proper to put the specifics to *Nageia* in front of the sentence of “Both *N. maomingensis* and *N. motleyi* are amphistomatic”.

Hello Xiaoyan:

In fixing the "Discussion", I think important and interesting to fully compare various stomatal indices in the modern analog to the known pCO_2.

For example, is use of one leaf side better than the other, or is the combination of both sides more accurate?

Discussed as suggested.

Basically, which of multiple possible methods gives the best correlation to pCO_2?

SD of the adaxial side gives the best correlation to pCO_2.

You also want to briefly discuss why some of the leaves give values offset from others.

Mentioned in the discussion part.

The pCO_2 estimates of the late Eocene in South China based on stomatal density of *Nageia* Gaertner leaves

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Abstract:

Late EoceneAtmospheric pCO_2 concentrations is haves been estimated for intervals of the Eocene based onusing various models and proxy informationies. Here we reconstructed the late Eocene (~ 40.3 XX Ma) pCO_2 based on the fossil species leaves of *Nageia maomingensis* Jin et Liu from collected from the late Eocene of Maoming Basin, Guangdong Province, China. This is the first paleoatmospheric estimates for the late Eocene of South China using stomatal data. Studies ofWe first determine relationships between atmospheric pCO_2 concentrations, stomatal density (SD) and stomatal index (SI) with using “modern” leaves of *N. motleyi* (Parl.) De Laub.., the nearest living equivalent species of to the Eocene fossils,. This work indicates that the SD inversely responds to pCO_2atmospheric CO_2 concentration, while SI has

批注 [GD1]: Need approximate age

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almost no relationships with pCO_2atmospheric CO_2 concentration. Therefore, the Eocene pCO_2 concentrations iscan be reconstructed based on both thae regression approach– and the stomatal ratio method by using the SD.is reconstructed based on the SD of the fossil leaves in comparison with *N. motleyi*. The pCO_2 result based on the regressionThe first approach gives a pCO_2 of (351.9 ± 6.6 ppmv, whereas) is more reasonable and much lower than the one based on stomatal ratio method gives a pCO_2 of 537.5 ± 56.5 (ppmv). Here, we explored the potential of *N. maomingensis* in pCO_2 reconstruction and obtained different results according to different methods, providing a new insight for the reconstruction of paleoclimate and paleoenvironment in conifers. Results suggest that the mean CO_2 concentration was 391.0 ± 41.1 ppmv or 386.5 ± 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.

批注 [GD2]: Last part of sentence not clear.

批注 [GD3]: Last sentence needs rewriting.

Keywords: pCO_2, late Eocene, *Nageia*, Maoming Basin, South China.

1 Introduction

The Eocene (55.8-33.9 Ma) generally was characterized by much warmer temperatures than present-day, although temperatures also varied significantly across this time interval (Zachos et al., 2008). Climate of the early Eocene to middle Eocene was extremely warm, particularly during ~~the early Eocene with the evidence of~~ the early Eocene Climatic Optimum (EECO; 5~~3~~1~~2~~ to ~~50~~ 53 Ma~~; Zachos et al., 2001~~), ~~also~~ and ~~known as~~ the Paleocene-Eocene Thermal Maximum (PETM~~ event~~; ~55.9~~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 ~ 55.5 to 52 Ma; and a mid-Eocene warming has been recognized, the middle Eocene Climate Optimum (MECO; ~40 Ma; Bijl et al., 2010).~~ However, ~~the~~ global climatic conditions cooled significantly by~~e occurred colder conditions during the early-middle (50 to 48 Ma) and~~ the late Eocene (40 to 36 Ma~~; Zachos et al., 2001~~). ~~The appearance of~~Indeed, small, ~~-~~ephemeral ice-sheets and Arctic sea ice likely existed during the latest Eocene ~~suggests the coldest climate of the Eocene~~ (Moran et al., 200~~4~~6; Zachos et al., 2008~~1~~).

~~Atmospheric CO_2 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).~~ M~~ost~~any authors have ~~link~~ suggested ~~the~~ that changes in temperature ~~to atmospheric CO_2 concentration~~ during the ~~entire~~ Phanerozoic were linked to atmospheric ~~(~~pCO_2~~)~~ (Petit et al., 1999; Retallack, 2001; Royer, 2006). ~~However,~~Central to these discussions are records across the Eocene, as this epoch spans the last major change from a “greenhouse” world to an “icehouse” world. The Eocene pCO_2 record remains incomplete and debated **(~~Beerling et al., 2002;~~ Kürschner et al., 2001; Royer et al., 2001; Beerling et al., 2002; Greenwood et al., 2003; Royer, 2003~~; Wing et al., 2005; Kato et al., 2011~~).

Most pCO_2 reconstructions~~estimates works~~ have focused on the Cretaceous-Tertiary

批注 [GD4]: Do not see the point of the original opening sentence and references for paragraph 2.

and Paleocene-Eocene ~~boundary~~ boundaries (65 to 50 Ma; Koch et al., 1992; Stott, 1992; Sinha and Stott, 1994; Royer et al., 2001; Beerling and Royer, 2002; Nordt et al., 2002; Royer, 2003; Fletcher et al., 2008; Roth-Nebelsick et al., 2012; 2014; Grein et al., 2013; Huang et al., 2013; Maxbauer et al.,2014~~Beerling et al., 2002; Wing et al., 2005; Kato et al., 2011~~) and the middle Eocene (Maxbauer~~Deconto~~ et al., ~~2012~~2014), while few reconstructions were conducted at the late Eocene. In addition, the pCO_2 reconstruction results have varied based on different proxies. Various methods having been used in pCO_2 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.**

The abundance of ~~Generally, s~~stomatal ~~data (stomatal density and index)~~cells can be ~~easily and accurately obtain~~measured ~~from~~ on modern leaves and well-preserved fossil l~~and~~eaves ~~modern leaves~~. Various plants show~~ing~~ ~~the~~ a negative correlation between atmospheric CO_2 concentration and stomatal density (SD), ~~or~~ stomatal index (SI), or both. As such, these parameters ~~and atmospheric CO_2 concentration~~ have been determined in fossil leaves ~~used~~ to reconstruct past pCO_2; ~~, for~~ examples include~~,~~ ~~including~~ *Ginkgo* (Retallack, 2001, 2009a; Beerling et al., 2002; Royer, 2003; ~~Retallack, 2001, 2009a;~~ Kürschner et al., 2008; 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), and *Quercus* (Kürschner et al., 1996, 2001), *Laurus* and *Ocotea* (Kürschner et al., 2008) ~~and~~

批注 **[GD5]:** This paragraph needs rewriting to place into proper context. Right now, it does not convey a good understanding of the debate and problems.

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~~multiple trees (Kürschner et al., 2008)~~. Recently, positive correlations between stomatal index or stomatal frequency and pCO_2 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 not been ~~overlooked~~ used previously in paleobotanical estimates of pCO_2 concentration.

Herein, we firstly document ~~the~~ correlations between ~~the SD and SI~~stomatal properties and atmospheric CO_2 concentrations ~~for of~~ using ~~*Nageia* using~~ leaves of the extant species *Nageia motleyi* (Parl.) De Laub. that were ~~leaves and~~ collected ~~atmospheric CO_2 concentration~~over the last two centuries. This ~~to~~ provides a training dataset for application to fossil representatives of *Nageia*. ~~Furthermore, w~~We secondly ~~estimate a new pCO_2 level for the late Eocene using the stomatal density method based~~ measure stomatal parameters on fossil *Nageia* leaves from the late Eocene of South China to estimate past CO_2 levels. The work~~,~~ provides further~~ing~~ ~~significant implications on~~insights for ~~–~~discussing Eocene ~~the~~ climate change. ~~rhythm throughout the Eocene.~~

2 Background

2.1 Stomatal proxy in pCO_2 research

Stomatal ~~proxy~~ information gathered from careful examination of leaves ~~is~~ has been widely used ~~in~~ for reconstructions of past pCO_2 concentrations (Beerling and

批注 [GD6]: Seems awkward

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批注 [GD7]: Why not include these in the above list??

Kelly, 1997; Doria et al., 2011~~REF~~). The three main parameters are stomatal density (SD), which is expressed as the total number of stomata divided by area, epidermal density (ED), which is expressed as the total number of epidermal cells per area, and the stomatal index (SI), which is ~~calculated by the calculating~~defined as the percentage of stomata among the total number of cells within an area [SI = SD×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:

$$SR = SI_{(e)} / SI_{(f)} \quad (1)$$

The stomatal ratio method is a semi-quantitative method of reconstructing pCO_2 concentrations under certain standardizations. ~~One~~ An example is the "Carboniferous standardization" (Chaloner and McElwain, 1997), ~~indicating that~~where one stomatal ratio unit ~~was~~ equals ~~to~~ two RCO_2 units ~~and~~:

$$SR = 2\ RCO_2 \quad (2)$$

and ~~Where~~ the value of RCO_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 nearest living equivalent (NLE) was collected (Berner, 1994; McElwain, 1998):

$$RCO_2 = C_{(f)} / 300 \text{ or } RCO_2 = C_{(f)} / C_{(e)} \quad (3)$$

~~Then t~~The estimated pCO_2 level ~~is~~ can then be expressed as follows:

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

批注 **[GD8]:** Probably two good summary papers would suffice here.

w~~W~~here $C_{(f)}$ is the pCO_2 ~~a~~represented by the fossil leaf, and $C_{(e)}$ is the atmospheric CO_2 of the year when the leaf of 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 RCO_2 unit:

$$SR = 1\ RCO_2 \quad (5)$$

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

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

~~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).

Kouwenberg et al. (2003) proposed some special stomatal quantification methods for ~~the~~ conifer~~s~~ leaves with stomata arranged in rows. The ~~main terms are as follows:~~

stomatal n~~n~~umber per Length (SNL) is expressed as ~~(~~the number of abaxial stomata plus the number of adaxial stomata~~)~~ divided by leaf length in millimeters. Stomatal rows (SRO) is expressed as the number of stomatal rows in both stomatal bands. Stomatal density per length (SDL) is expressed as the equation SDL = SD × SRO. T~~u~~rue stomatal density per length (TSDL) is expressed as the equation TSDL = SD × band width (in millimeters). The band width on *Nageia motleyi* leaves was measured as leaf blade width.

批注 [GD9]: Not sure what this last sentence means.

批注 [GD10]: The last few bits can be condensed as sort of a repeat from a above.

批注 [GD11]: Correct?

批注 [GD12]: Things get problematic here as some of this seems to be methods. The parts on Nageia seemingly belong later.

2.2 Review of extant and fossil *Nageia*

The genus *Nageia*~~-~~, including ~~only~~ seven living species, is a~~n~~ special group of Podocarpaceae~~-, which is~~ a large family of conifers mainly distributed in the southern hemisphere. *Nageia* has ~~with~~ broadly ovate-elliptic to oblong-lanceolate, multiveined (without a midvein), 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. *Dammaroideae* (Mill 1999, 2001).~~,~~ Both sections are ~~XX~~ mainly distributed in southeast~~ern~~ Asia and Australasia 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. 1~~a~~). Four species of the *N.* section *Nageia* ~~, 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 leaves where ~~(the~~ stomata only ~~distributed~~ occur on the abaxial side.~~) leaves, with a single exception~~ One species of this section -- *N. maxima* (De Laub.) De Laub. ~~which~~ -- is characterized by amphistomatic leaves, but where only a few stomata are found on the adaxial side (Hill and Pole, 1992; Sun, 2008). Both *N. wallichiana* (Presl) O. Kuntze and *N. motleyi*.~~,~~ of the *N.* section *Dammaroideae*~~,~~ are amphistomatic with abundant stomata distributed on both sides of the leaf. This is~~,~~ especially true for *N.* ~~*motleyi*~~ *motleyi*, which has~~ving~~ approximately

批注 **[GD13]:** Split this convoluted sentence and rewrite the second bit as difficult to follow. Does this apply to Podacarp or Nageia?

批注 **[GD14]:** Both sections? Not clear.

批注 **[GD15]:** Avoid Latin abbreviations in the middle of sentences equal stomata numbers on both surfaces (Hill and Pole, 1992; Sun, 2008).

The fossil record~~s~~ of *Nageia* can be traced back to the Cretaceous. Krassilov (1965) described *Podocarpus (Nageia) sujfunensis* Krassilov from the Lower Cretaceous of Far East Russia. Kimura et al. (1988) reported *Podocarpus* (*Nageia*) *ryosekiensis* Kimura, Ohanaet Mimoto, an ultimate leafy branch bearing a seed, from the Early Barremian in southwestern Japan. In China, a Cretaceous petrified wood, *Podocarpus* (*Nageia*) nagi Pilger, was discovered from the Dabie Mountains in central Henan, China (Yang et al., 1990). Jin et al. (2010) reported a upper Eocene *Nageia* leaf named *N. hainanensis* Jin, Qiu, Zhu et Kodrul from the Changchang Basin of Hainan Island, South China. Recently, Liu et al. (2015) found another leaf species *N. maomingensis* Jin et Liu from upper Eocene of Maoming Basin, South China.~~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 in the above studies (Krassilov, 1965; Jin et al., 2010; Liu et al., 2015) have well-preserved cuticles~~(e.g., Jin et al., 2010; Liu et al., 2015)~~, ~~the above~~ these studies are mainly concentrated on ~~the~~ morphology, systematics and phytogeography.

Here we try to reconstruct the pCO_2 concentration based on stomatal data of *Nageia maomingensis* Jin et Liu. ~~was reported described on the basised ofn four leaves with well-preserved cuticles recovered fromlate upper Eocene sedimentary rocks of South China (Liu et al., 2015).~~ Among the modern *Nageia* species mentioned

批注 **[GD16]:** This needs slight expansion and conformity. I would list specific locations, types of material and approximate ages.

批注 **[GD17]:** As above, not quite clear what is being suggested.

above, *N. motleyi* was considered as the nearest extant livingNLE (NEL) species of *N. maomingensis* (Liu et al., 2015). 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 *N. motleyi* show negative correlations with the CO_2 concentration before applying the stomatal method. Both *N. maomingensis* and *N. motleyi* are amphistomatic, indicating suggesting that both upper and lower surfaces of the leaf are needed to estimate the pCO_2 concentration sduring 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.**

批注 [GD18]: Between ** This important point – species specific relationships -- needs to come earlier

3 Material and methods

3.1 Extant leaf preparation

We examined five 12 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. 17229 (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. 18328 (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. 21151 (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. 40798 (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.

批注 [GD19]: Between *** I think can removed as long as clearly documneted in Tabel 1

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_2O_2 (1:1) and heated in the thermostatic water bath at 85 C for 8.5 hours; 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 with 1% Safranin T alcoholic solution for 5 min, sealed with Neutral Balsam and observed under the LM.

3.2 Fossil leaf preparation

** Needs a brief paragraph here noting the Maoming Basin, rock units, depositional environment, and criticially, the age.**

Maoming Basin (21°42'33.2"N, 110°53'19.4"E) is located in southwestern Guangdong, South China including Cretaceous and Tertiary strata. Tertiary strata are fluvial and lacustrine sedimentary units, divided into the Gaopengling, Laohuling, Shangcun, Huangniuling and Youganwo formations in descending order, aged from late Eocene to early Oligocene (Wang et al., 1994).

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) formations of Maoming Basin, South China (Fig. 1B, 1C in Liu et al., 2015). The age from Youganwo to Huangniuling formations is late Eocene (~ 40.3 Ma). the layersPrecise information wasregarding locations areis provided shown in Fig. 1b–c inby Liu et al., (2015). Macrofossil cuticular fragments were taken from the middle part of each fossil leaf (Fig. 2c) and treated by with Schulze's solution for approximately 1h 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

The basic stomatal parameters, SD, ED and SI, we~~a~~re counted based on analyzing ~~the~~ pictures taken with ~~the~~ a 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, 2d)~~. A total of ~~1116~~ 2816 pictures (200 × magnification of Zeiss LM) of ~~the~~ cuticles from ~~9~~ 21 leaves of *N. motleyi* were counted. Each counting field was 0.366 mm^2. We used a standard sampling protocol (Poole and Kürschner, 1999), ~~and only counted~~ counting all full stomata in the image plus stomata straddling the left and top margins, as presented in Figure 2(b), and (d).~~those stomata touching or straddling the left-hand side and top including the corner between them, provided by Fig. 2b, 2d) 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 pCO_2 reconstruction because both our fossil species and the NLE species are amphistomatic.~~

The SNL, SRO, SDL, and TSDL we~~a~~re also ~~count~~determined based on ~~the pictures taken with the light microscope (~~LM images.) ~~using the strategies stated in the background~~**A total of 2293 pictures (200 × magnification of Zeiss LM) of the cuticles from 21 leaves of *N. motleyi* were counted. Each counting field was 0.366 mm^2. None of the aforementioned counting areas overlapped and they were larger

批注 **[GD20]:** This is not crystal clear to me

批注 **[GD21]:** See above. The specifics to Nageia should be here, but the more general procedure should be presented above.

than the minimum area (0.03 mm^2) for statistics (Poole and Kürschner, 1999). In this study, the stomatal data of both surfaces are applied in pCO_2 reconstruction because both the fossil and NLE species are amphistomatic.

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~~ presented in Table 2. The SDs and ~~Sis~~SIs average ~~range from~~62.28 mm^{-2} and 3.30 %~~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 average values (70.03 mm^{-2}~~53.22–82.71~~ in SDs and ~~3.13–4.66~~3.90 % in SIs) than those from the adaxial sides. The combined SD and SI of the adaxial and abaxial surfaces average 66.14 $mm^{-2}$and 3.60 %, respectively (table 4).

~~Figure~~ Fig. 3 shows the relationships between the stomatal parameters (SD and SI) of modern *N. motleyi* and the atmospheric CO_2 concentration (SD-CO_2 relationship~~s~~ and SI-CO_2 relationship~~s~~). R^2 values in the SD~~-~~-CO_2 relationship~~s~~ from ~~both~~ the adaxial and abaxial surfaces of *N. motley* are up to ~~0.841~~ 0.4667 and ~~0.725~~0.3824 (Fig. 3a, b), suggesting that the stomatal densities of *N. motleyi* are ~~in significant~~ inverse proportion to the CO_2 concentrations. However, the figureFig. 3(c) and (d) indicate no relationships between the SIs and CO_2 concentrations for the extremely low level of the R^2 values (0.00030.2558 and 0.06080.0248). Figs. 3e and 3f based on the combined data also show that SD inversely responds to the atmospheric CO_2 concentration (R^2 =0.4421), while SI has almost no relationship with the atmospheric CO_2 concentration (R^2 =0.1177).

The mean values of SNL, SDL and TSDL are 9.81, 326.39 and 1226.93 no.·mm^{-1}, respectively (Table 5). Fig. 4 shows the relationships between SNL (SDL, TSDL) and CO_2 concentrations. The low R^2 values in the Fig. 4a and 4c indicate that SNL (R^2 = 0.0643) and TSDL (R^2 = 0.0788) have no relationship with the CO_2 concentration in this study. Fig. 4b shows that there is a weak reverse relevance between SDL and the CO_2 concentration (R^2 = 0.3154).

Compared with the SDL method, the SD-based method shows a larger R^2 value, indicating a stronger relevance between the SD and CO_2 concentrations. In this study, the pCO_2 is reconstructed based on the regression equations of SD-CO_2 relationship. Additionally,

According to the results stated above, the stomatal ratio method can be also used in estimating pCO_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 that SI is more sensitive than SD to the concentration of atmospheric CO_2

~~(Beerling, 1999) and more accurate in responding to the variation of pCO_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. 18328 are selected to reconstruct the pCO_2 concentration, because they are closest to the fitted equations in Fig. 3. This specimen was collected by Neth. Ind. For. Service from Riau on Ond. Karimon, Archipel. Ind., Malaysia, in 1934 at an altitude of 5 m and CO_2 concentration of 306.46 ppmv (Brown, 2010).~~The SD results of specimen No. 40798 are closest to the fitted equations in Fig. 3a and 3b and therefore are selected to reconstruct the pCO_2 concentration. The specimen was collected by J. Sinclair. & 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 pCO_2 concentrations based on the stomatal ratio method.~~

4.2 ~~Stomatal parameters and~~The pCO_2 estimates results

4.2.1 The regression approach

~~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~~6–8.~~ 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 mm^{-2}~~44.5 ± 2.9~~ and 1.8 %~~1.80 ±0.12~~, respectively (Table ~~5~~6). The mean SD and SI values of the abaxial surface~~values of the abaxial and abaxial surface~~ are 49.8 mm^{-2}~~48.9 ± 3.0~~ and 2.07 %~~53.22 ±2.2~~, respectively (Table ~~2, 3~~7).

Based on the regression approach, the pCO_2 was reconstructed as 351.9 ±6.6 ppmv and 365.6 ±7.6 ppmv according to the SD of adaxial and abaxial sides. The combined SD value is an average of 46.6 mm^{-2} (Table 8), giving the reconstructed pCO_2 of 358.1 ±5.0 ppmv.

4.2.2 The stomatal ratio method

~~The m~~Mean SR value~~s~~ of ~~both sides~~the adaxial side (SR=1.69 ±0.18) ~~are~~ is a little larger ~~quite similar with 1.24 ±0.13~~than that ~~in adaxial~~ of the abaxial side (SR=1.60 ± 0.11) in fossil *Nageia* leaves~~and 1.23 ±0.09 in abaxial side~~ (Tables ~~4~~ 6 and ~~5~~7). The pCO_2 ~~average~~ reconstruction results are~~of pCO_2 concentration in the late Eocene of Maoming Basin is 391.0 ±41.1~~537.5 ±56.5 ppmv (Table ~~4~~6) and ~~386.5 ± 27.8~~496.1 ±35.7 ppmv (Table ~~5~~7) ~~with a 95% confidence interval~~ based on the adaxial and abaxial cuticles, respectively. Based on the combined SD of both leaf sides, the pCO_2 result is 519.9 ±35.0 ppmv.~~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 pCO_2 level from the lower layer to~~

~~the upper ones, while the pCO_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~~ pCO_2 concentration estimates. However, Royer (2003) pointed out that it is unnecessary ~~make this~~to provide this conversion when ~~the~~ trees lived at <250 m in elevation. In this paper, the nearest living equivalent species, *Nageia motleyi*, grows at ~~51~~ 5 m in elevation with P =~~99.5~~99.9, suggesting that CO_2 concentration estimates were only underestimated by ~~0.5~~0.1%. Consequently, no correction is needed for the reconstruction result in this study. After being projected into a long-term carbon cycle model (GEOCARB III; Berner and Kothavalá, 2001), the results of this study compares well with CO_2 concentrations for corresponding age within their error ranges (Fig. 5).

5 Discussion

5.1 Stomatal parameters response to CO_2

Here, we find that SD decreases as atmospheric CO_2 concentrations increase, however, SI does not. Generally, SI is more sensitive in response to the atmospheric CO_2 concentration than SD (Beerling, 1999; Royer, 2001). However, the reverse case is not unfound. For example, Kouwenberg et al. (2003) reported that SD is better than SI in reflecting the negative relationships with CO_2 in conifer needles, accounting for the special paralleled mode of the ordinary epidermal and stomatal formation. Although *Nageia* is broad-leaved rather than needle-leaved, it also has well paralleled epidermal cells herein showing the different relationships between CO_2 and SD or SI. Compared with SD, the SDL has weaker correlation with CO_2 at a smaller R^2. The SNL and TSDL have no response to the change of CO_2. The insensitivity of SNL, SDL and TSDL might account for the characters of broad-leaved leaf shape and paralleled epidermal cells. The SNL should be applied to conifer needles with single file of stomata (Kouwenberg et al., 2003). The SDL and TSDL were considered as the most appropriate method when the stomatal rows grouped in bands in a hypo- or amphistomatal conifer needle species (Kouwenberg et al., 2003). Considering all the stomatal parameters above, SD appears to be the most sensitive to CO_2.

The SD-CO_2 correlation shows one value from leaf No. 40798 offset from the others. The SI-CO_2 correlation shows different offset values in different leaf sides. The offset values might be affected by leaf maturity and light intensity. However, it is hard to distinguish whether a fossil leaf is young or mature, or live in the sunny or shady light regimes.

The R^2 value (0.5) of SD-CO_2 based on the adaxial side is higher than from the abaxial side and the combination of both sides, indicating that the correlation of SD-CO_2 is stronger than the others parameters herein. Therefore, the SD on the adaxial side is the best in reconstructing pCO_2. The reconstruction result based on the regression approach is 351.9 ±6.6 ppmv lower than the one based on the stomatal ratio method (Table 6), and it is relatively lower than the results based on the other proxies (Fig. 6; Freeman and Hayes, 1992; Pagani et al., 2005; Maxbauer et al., 2014). However, the result based on stomatal ratio method is 537.5 ±56.5 ppmv which is closest to GEOCARB III (Fig. 5) and historical reconstruction trends (Fig. 6).

5.~~1~~ 2 Paleoclimate reconstructed history

The pCO_2 levels throughout the Cenozoic ~~was~~ were relatively lower than ~~the levels~~ through the Cretaceous (Ekart et al., 1999), but ~~it is~~ had an overall decreasing trend with some significant~~ly changes~~ increases on short-time scales (e.g. in the earliest Eocene and middle Miocene, Zachos et al., 2001, 2008; 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 pCO_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 pCO_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~~ were approximately 680 ppmv by 60 million years ago. However, Stott (1992) reconstructed pCO_2 as 450–~~-~~550 ppmv for the early Eocene based on phytoplankton. Additionally, ~~the~~ reconstructions using the stomatal ratio method based on ~~the leaves of~~ *Ginkgo*, *Metasequoia*, and Lauraceae leaves also revealed a low pCO_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 of about 800 ppmv near the Paleocene/Eocene boundary (Royer et al., 2001).

Subsequently, Smith et al. (2010) reconstructed the value of pCO_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. (~~2012~~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. Spicer et al. (2014) also reconstructed a relatively lower cool temperature than $\delta^{18}O$ records (Keating-Bitonti et al., 2011) in the middle Eocene of

Hainan Island, South China using CLAMP, indicating a not uniformly warm climate in the low latitude during the 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 pCO_2 level showed an~~ An overall decreasing trend of the pCO_2 level was presented after the ~~MECO period~~middle Eocene~~, indicating the consistance with the pCO_2 levels herein~~ (Fig. ~~5~~6; Retallack, 2009~~-~~b).

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~~6), 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 ice sheets 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~~6; Zachos et al., 2001; Roth-Nebelsick et al., 2004; Beerling and Royer, 2011). Subsequently, the CO_2 concentration decreased gradually and reached 280 ppmv until the period of the industrial revolution (Fig. ~~5~~6). Since then, however, the CO_2 concentration rebounded to present day level.

In conclusion, although various results were made by different pCO_2 reconstruction proxies at the same time, their entire decreasing tendency of pCO_2 level are remarkably consistent with each other since the Eocene (Fig. ~~5~~6). F~~urthermore,~~ig. 6 shows that during the Eocene the temperature was higher than at present. The reconstructed pCO_2 ~~concentration~~ of 351.9 ± 6.6 ppmv based on the regression approach ~~SD of fossil *Nageia* are 391.0 ± 41.1 ppmv and 386.5 ± 27.8~~is ~~ppmv,~~ ~~showing~~ shows a remarkably low pCO_2 level during the early late Eocene. The result based on the stomatal ratio method of 537.5 ± 56.5 ppmv is closely consistent with the pCO_2 changes over the geological ages (Fig. 6).

~~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 ℃ which is higher than South China (ca. 20–25 ℃; 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 1,000 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 figure 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. *Dammaroideae* (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 Conclusion

In this study, we reconstructed the late Eocene pCO_2 based on the fossil leaves of *Nageia maomingensis* Jin et Liu from the late Eocene of Maoming Basin, Guangdong Province, China. *Nageia* is a special element in conifers by its broad multi-veined leaf that lacks mid-vein. The stomatal data analysis suggests that only stomatal densities (SD) from both sides of *Nageia motleyi* leaves have significant negative correlations with the atmospheric CO_2 concentration. The SD from the adaxial side gives the best correlation to the CO_2. Based on SDs, the pCO_2 concentration is reconstructed using both the regression approach and the stomatal ratio method. The pCO_2 result based on the regression approach is 351.9 ± 6.6 ppmv, showing a relatively lower CO_2 level. The reconstructed result based on the stomatal ratio method is 537.5 ± 56.5 ppmv consistent with the variation trends based on the other proxies. Here, we explored the potential of *N. maomingensis* in pCO_2 reconstruction and obtained different results according to different methods, providing a new insight for the reconstruction of paleoclimate and paleoenvironment in conifers.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 conceentration, 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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Figure 1. Map showing the distribution of extant and fossil *Nageia* and their mean annual temperature (Modified after the map from http://www.sage.wisc.edu/atlas/maps.php?datasetid=35&includerelatedlinks=1&dataset=35).

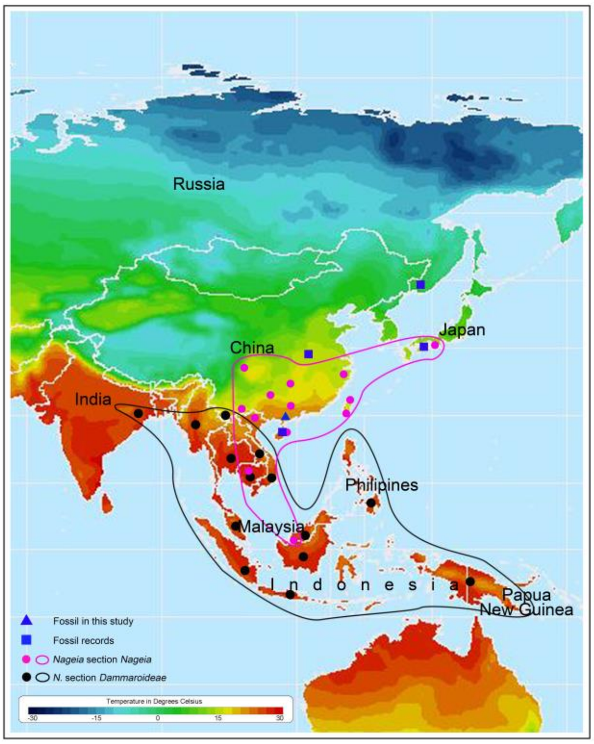

Figure 2. Sampling areas and counting rules are shown. (a) *Nageia motleyi* (Parl.) De Laub.leaf. Black squares in the middle of the leaf show the sampling areas for preparing the cuticles. (b) The abaxial side of the cuticle from *N. motleyi* leaf. Black circles show the counted stomatal complexes. (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.

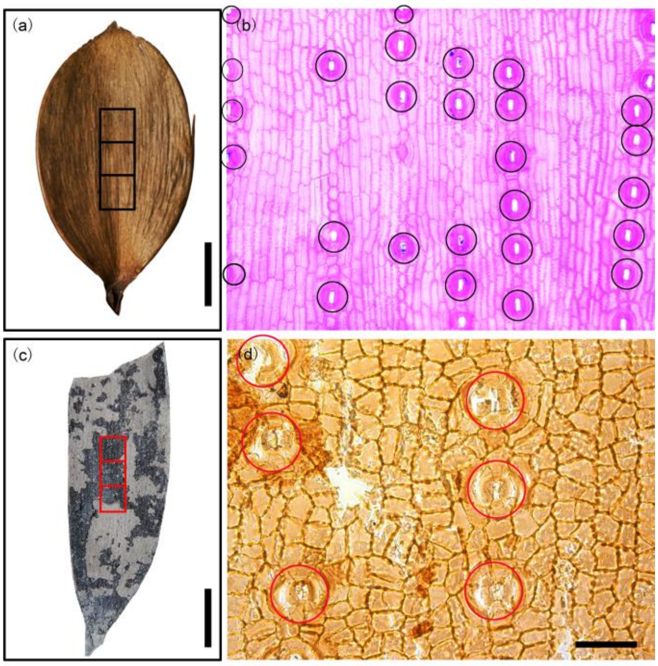

Figure 3. Correlation between SD and SI versus CO_2 concentration for modern *Nageia motleyi*. (a) Trends of SD with CO_2 concentration for the adaxial surface. (b) Trends of SD with CO_2 concentration for the abaxial surface. (c) Trends of SI with CO_2 concentration for the adaxial surface. (d) Trends of SI with CO_2 concentration for the abaxial surface. (e) Trends of SD with CO_2 concentration for the combined data of both leaf surfaces. (f) Trends of SI with CO_2 concentration for the combined data of both leaf surfaces.

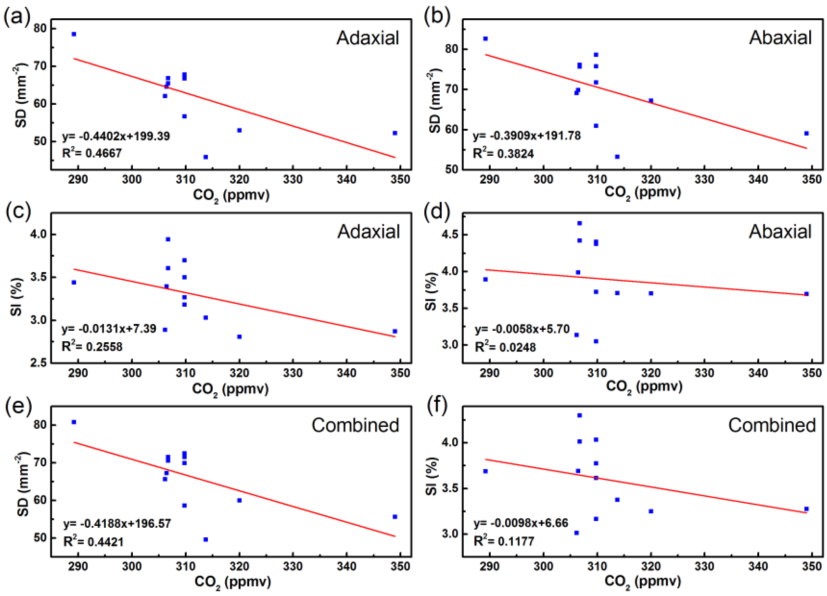

Figure 4. Correlation between SNL, SDL and TSDL versus CO_2 concentration for modern *Nageia motleyi*. (a) Trends of SNL with CO_2 concentration for the adaxial surface. (b) Trends of SDL with CO_2 concentration for the adaxial surface. (c) Trends of TSDL with CO_2 concentration for the adaxial surface.

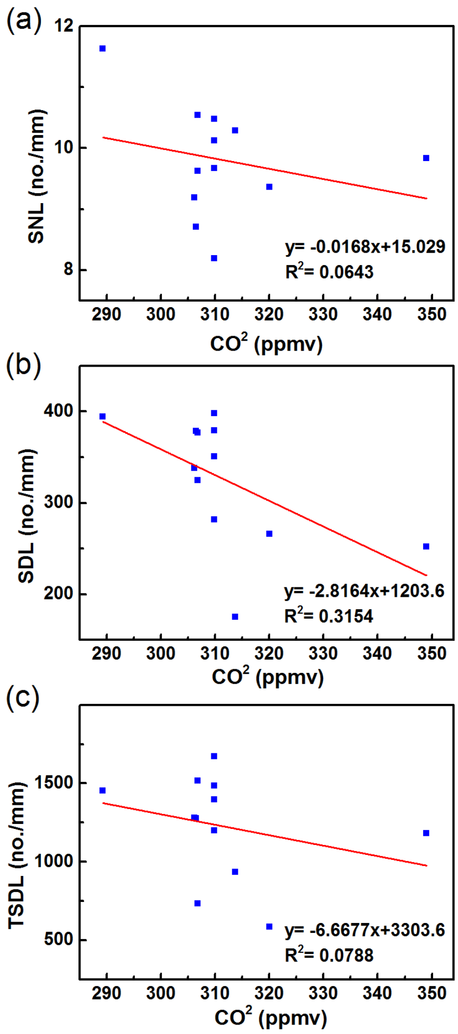

Figure ~~4~~5. The pCO_2 reconstruction results and extant CO_2 concentrations are projected onto the long-term carbon cycle model (GEOCARB III; Berner and Kothavalá 2001). The pCO_2 results based ~~on the adaxial and abaxial surfaces~~ on the regression approach and stomatal ratio method are represented by red and blue squares, respectively. ~~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).~~

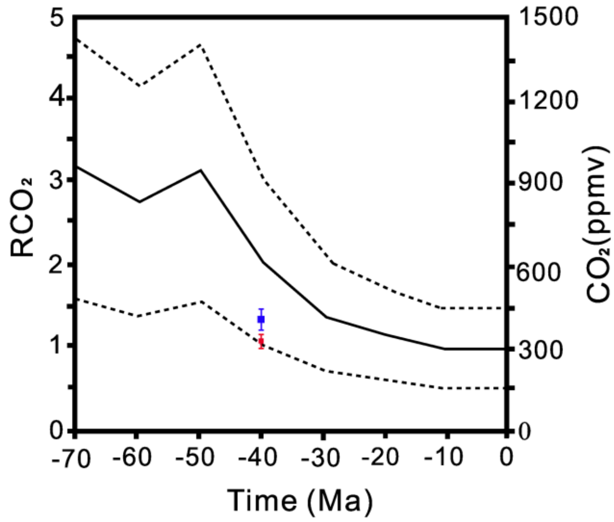

Figure ~~5~~6. Atmospheric CO_2 estimates from proxies over the past 60 million years. The horizontal dashed line indicates monthly atmospheric CO_2 concentration for March 2015 at Mauna Loa, Hawaii (401.5~~2~~ ppmv) (Pieter and Keeling, 2015). The vertical lines show the error bars. The data ~~were~~ are from the supporting data of Beerling and Royer (2011) and ~~the~~ references in Table ~~6~~9. The lower blue star shows the reconstructed result based on the regression approach. The higher one presents the result of stomatal ratio method.

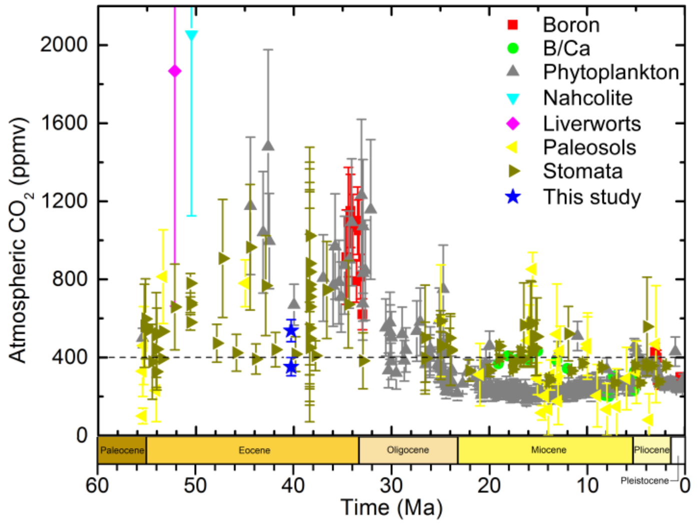

Table 1. Modern *Nageia motleyi* (Parl.) De Laub samples and atmospheric CO_2 values of their collection dates from ice core data (Brown, 2010).

Herbarium	Collection number	Collecting locality	Collectors	Number of leaf samples	Collection date	CO_2 (ppmv)
LE	No. 2649	Malaysia	Beccari, O.	1	1868	289.23
A/GH	No. bb. 17229	150 m, Riau on Ond. Karimon, Archipel. Ind.	Neth. Ind. For. Service	2	1932	306.19
A/GH	No. bb. 18328	5 m, Z. O. afd. v. Borneo Tidoengsche Landen, Archipel. Ind.	Neth. Ind. For. Service	2	1934	306.46
A/GH	No. bb. 21151	500 m, Z. O. afd. Borneo, Poeroek Tjahoe Tahoedjan, Archipel. Ind.	Neth. Ind. For. Service	2	1936	306.76
E	No. bb. 40798	51 m, Kuala Trengganu-Besut Road, Bukit Bintang Block, Gunong Tebu Forest reserve, Malaysia	Sinclair, J. and Kiah bin, Salleh	2	1955	313.73

Note: A/GH—Harvard University Herbarium, Harvard University, 22 Divinity Avenue, Cambridge, Massachusetts 02138, USA (www.huh.harvard.edu).
E—The Herbarium of Royal Botanic Garden, Edinburgh EH3 5LR, Scotland, UK (www.rbge.org.uk).
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).

Herbarium	Collection number	Collecting locality	Collectors	Number of leaf samples	Collection date	CO_2 (ppmv)
LE	No. 2649	Malaysia	Beccari, O.	1	1868	289.23
A/GH	No. bb. 17229	150 m, Riau on Ond. Karimon, Archipel. Ind.	Neth. Ind. For. Service	2	1932	306.19
A/GH	No. bb. 18328	5 m, Z. O. afd. v. Borneo Tidoengsche Landen, Archipel. Ind.	Neth. Ind. For. Service	2	1934	306.46
A/GH	No. bb. 21151	500 m, Z. O. afd. Borneo, Poeroek Tjahoe Tahoedjan, Archipel. Ind.	Neth. Ind. For. Service	2	1936	306.76
KEP	No. 30887	Kata Tinggi, Johor, Malaysia	Corner, E.J.H.	1	1936	306.76
KEP	No. 57329	Batang Padang, Perak, Malaysia	Unkonwn	2	1947	309.82
KEP	No. 57330	Batang Padang, Perak, Malaysia	Unkonwn	2	1947	309.82
KEP	No. 55897	Batang Padang, Perak, Malaysia	Unkonwn	2	1947	309.82
KEP	No. 61064	Batang Padang, Perak, Malaysia	Syed Woh	2	1947	309.82

E	No. bb. 40798	51 m, Kuala Trengganu-Besut Road, Bukit Bintang Block, Gunong Tebu Forest reserve, Malaysia	Sinclair, J. and Kiah bin, Salleh	2	1955	313.73
KEP	No. 80548	Gombak, Selangor, Malaysia	Rahim	1	1965	320.04
KEP	No. 33343	Jelebu, Negeri Sembilan, Malaysia	Yap, S.K.	2	1987	348.98

Note: A/GH—Harvard University Herbarium, Harvard University, 22 Divinity Avenue, Cambridge, Massachusetts 02138, USA (www.huh.harvard.edu).

E—The Herbarium of Royal Botanic Garden, Edinburgh EH3 5LR, Scotland, UK (www.rbge.org.uk).

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).

KEP—Kepong Herbarium, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia (http://www.frim.gov.my/).

Table 2. Summary of stomatal parameters of the adaxial surface ~~form~~ from modern *Nageia motleyi* (Parl.) De Laub.

Collection number	Collection date	CO_2 (ppmv)	SD (mm^{-2})					SI (%)				
			x	σ	s.e.	t*s.e.	n	*x*	σ	s.e.	t*s.e.	n
No.2649	1868	289.23	78.60	15.44	1.41	2.76	120	3.44	0.66	0.06	0.12	120
No.bb.17229	1932	306.19	62.14	17.20	1.78	3.50	93	2.89	0.68	0.07	0.14	93
No.bb.18328	1934	306.46	64.57	15.05	1.58	3.11	90	3.39	1.01	0.11	0.21	90
No.bb.21151	1936	306.76	65.45	11.14	1.17	2.30	90	3.94	0.74	0.08	0.15	90
No.SFN30887	1936	306.76	66.90	16.10	1.27	2.49	161	3.61	0.92	0.07	0.14	161
No.61064	1947	309.82	56.71	16.81	1.95	3.83	74	3.27	1.26	0.15	0.29	74
No.57330	1947	309.82	67.37	15.97	2.04	4.01	61	3.70	0.82	0.10	0.20	61
No.57329	1947	309.82	67.85	15.61	1.70	3.34	84	3.50	0.90	0.10	0.20	84
No.55897	1947	309.82	66.74	14.10	1.78	3.48	63	3.18	0.66	0.08	0.16	63
No.40798	1955	313.73	45.89	13.81	1.12	2.20	151	3.03	0.87	0.07	0.14	151
No.KEP80548	1965	320.04	52.94	11.25	0.85	1.67	175	2.81	0.61	0.05	0.09	175
No.FRI33343	1987	348.98	52.25	12.05	0.77	1.51	242	2.87	0.69	0.04	0.09	242
Mean	–	–	62.28	14.54	1.45	2.85	117	3.30	0.52	0.08	0.16	117

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~~ from modern *Nageia motleyi* (Parl.) De Laub.

Collection number	Collection date	CO_2 (ppmv)	SD (mm^{-2})					SI (%)				
			x	σ	s.e.	t*s.e.	n	*x*	σ	s.e.	t*s.e.	n
No.2649	1868	289.23	82.71	12.23	1.02	2.00	144	3.89	0.58	0.05	0.09	144
No.bb.17229	1932	306.19	69.16	14.23	1.48	2.90	93	3.13	0.58	0.06	0.12	93
No.bb.18328	1934	306.46	69.92	14.38	1.52	2.97	90	3.99	1.08	0.11	0.22	90
No.bb.21151	1936	306.76	75.68	15.74	1.66	3.25	90	4.66	0.88	0.09	0.18	90
No.SFN30887	1936	306.76	76.18	12.51	0.99	1.93	161	4.42	0.89	0.07	0.14	161
No.61064	1947	309.82	60.93	11.02	1.39	2.72	63	3.05	0.62	0.08	0.15	63
No.57330	1947	309.82	75.82	14.14	1.82	3.58	60	4.38	0.84	0.11	0.21	60
No.57329	1947	309.82	71.74	16.84	1.75	3.42	93	3.72	0.62	0.06	0.13	93
No.55897	1947	309.82	78.63	13.41	1.75	3.42	59	4.41	1.00	0.13	0.26	59
No.40798	1955	313.73	53.22	13.88	1.12	2.19	155	3.71	0.93	0.07	0.15	155
No.KEP80548	1965	320.04	67.22	13.97	1.07	2.09	171	3.70	0.80	0.06	0.12	171
No.FRI33343	1987	348.98	59.09	12.10	0.79	1.55	233	3.69	0.86	0.06	0.11	233
Mean	–	–	70.03	13.70	1.36	2.67	118	3.90	0.81	0.08	0.16	118

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 4. Summary of stomatal parameters of the adaxial surface of fossil *Nageia* and pCO_2 [$C_{(f)}$] estimates results.~~

Table 4. Summary of stomatal parameters of the combined data of the adaxial and abaxial surfaces from modern *Nageia motleyi* (Parl.) De Laub.

Collection number	Collection date	CO_2 (ppmv)	SD (mm^{-2})					SI (%)				
			x	σ	s.e.	t*s.e.	n	*x*	σ	s.e.	t*s.e.	n
No.2649	1868	289.23	80.84	13.74	0.85	1.66	264	3.69	0.66	0.04	0.08	264
No.bb.17229	1932	306.19	65.65	16.13	1.18	2.32	186	3.01	0.64	0.05	0.09	186
No.bb.18328	1934	306.46	67.24	14.92	1.11	2.18	180	3.69	1.08	0.08	0.16	180
No.bb.21151	1936	306.76	70.57	14.53	1.08	2.12	180	4.30	0.89	0.07	0.13	180
No.SFN30887	1936	306.76	71.54	15.12	0.84	1.65	322	4.01	0.99	0.05	0.11	322
No.61064	1947	309.82	58.65	14.54	1.24	2.43	137	3.17	1.02	0.09	0.17	137
No.57330	1947	309.82	71.56	15.61	1.42	2.78	121	4.03	0.89	0.08	0.16	121
No.57329	1947	309.82	69.90	16.33	1.23	2.41	177	3.62	0.77	0.06	0.11	177
No.55897	1947	309.82	72.49	14.95	1.35	2.65	122	3.77	1.04	0.09	0.18	122
No.40798	1955	313.73	49.60	14.31	0.82	1.60	306	3.37	0.96	0.05	0.11	306
No.KEP80548	1965	320.04	60.00	14.53	0.78	1.53	346	3.25	0.84	0.05	0.09	346
No.FRI33343	1987	348.98	55.61	12.53	0.58	1.13	475	3.28	0.88	0.04	0.08	475
Mean	–	–	66.14	14.77	1.04	2.08	235	3.60	0.89	0.06	0.12	235

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 5. Summary of stomatal parameters of the abaxial surface of fossil *Nageia* and pCO_2 [$C_{(f)}$] estimates results.

Table 5. Summary of stomatal parameters from modern *Nageia motleyi* (Parl.) De Laub (Kouwenberg et al., 2003).

Collection number	Collection date	CO_2 (ppmv)	SNL	SDL	TSDL	n
No.2649	1868	289.23	11.64	394.38	1455.10	264
No.bb.17229	1932	306.19	9.19	337.98	1280.12	186
No.bb.18328	1934	306.46	8.71	378.92	1277.63	180
No.bb.21151	1936	306.76	9.62	376.93	1517.21	180
No.SFN30887	1936	306.76	10.55	325.08	735.38	240
No.61064	1947	309.82	8.19	282.04	1200.66	133
No.57330	1947	309.82	9.67	397.83	1397.33	119
No.57329	1947	309.82	10.13	350.98	1672.50	176
No.55897	1947	309.82	10.48	379.06	1486.13	122
No.40798	1955	313.73	10.29	175.14	933.85	305
No.KEP80548	1965	320.04	9.36	266.16	585.72	263
No.FRI33343	1987	348.98	9.84	252.20	1181.51	125
Mean	–	–	9.81	326.39	1226.93	191

Table 6. The pCO_2 estimates proxies and corresponding references.

Proxies	References
Boron	Pearson et al., 2009; Seki et al., 2010
B/Ca	Tripati et al., 2009
Phytoplankton	Freeman and Hayes, 1992; Stott, 1992; Pagani et al., 1999, 2005; Henderiks and Pagani, 2008; Seki et al., 2010
Nahcolite	Lowenstein and Demicco, 2006
Liverworts	Fletcher et al., 2008
Paleosols	Cerling, 1992; Koch et al., 1992; Ekart et al., 1999; Retallack, 2009b; Royer et al., 2001
Stomata	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

Table 6. Summary of stomatal parameters of the adaxial surface of fossil *Nageia* and pCO_2 [$C_{(f)}$] estimates results.

Species	Age	SD (mm^{-2})				SI (%)				SR		pCO_2(ppmv)		$C_{(f)}$ (ppmv)	
		x	σ	s.e.	n	*x*	σ	s.e.	n	*x*	t*s.e	*x*	t*s.e	*x*	t*s.e
MMJ1-001	Late Eocene	52.5	17.1	3.1	30	2.08	0.7	0.1	30	1.35	0.19	333.6	13.9	412.1	62.0
MMJ2-003	Late Eocene	42.3	12.9	2.4	30	1.80	0.6	0.1	30	1.75	0.39	356.8	10.5	536.1	126.2
MMJ2-004	Late Eocene	39.9	13.6	2.5	30	1.66	0.6	0.1	30	1.81	0.32	362.4	11.0	554.3	101.9
MMJ3-003a	Late Eocene	43.2	17.7	3.2	30	1.67	0.7	0.1	30	1.84	0.43	354.8	14.4	564.6	135.7
Mean	Late Eocene	44.5	16.3	1.5	120	1.80	0.7	0.1	120	1.69	0.18	351.9	6.6	516.8	56.5

Note: *x*—mean; σ—standard deviation; s.e. —standard error of mean; n— numbers of photos counts (400×); t·s.e.— 95% confidence interval. pCO_2— the result based the regression approach; $C_{(f)}$— the result based on the stomatal method.

Table 7. Summary of stomatal parameters of the abaxial surface of fossil *Nageia* and pCO_2 [$C_{(f)}$] estimates results.

Species	Age	SD (mm^{-2})				SI (%)				SR		pCO_2(ppmv)		$C_{(f)}$ (ppmv)	
		x	σ	s.e.	n	*x*	σ	s.e.	n	*x*	t*s.e	*x*	t*s.e	*x*	t*s.e
MMJ1-001	Late Eocene	47.7	17.7	3.2	30	2.11	0.8	0.2	30	1.66	0.23	368.6	16.2	515.6	72.3
MMJ2-003	Late Eocene	50.9	18.3	3.3	30	2.12	0.8	0.1	30	1.57	0.23	360.9	16.6	486.0	70.7
MMJ2-004	Late Eocene	48.2	15.8	2.9	30	2.14	0.7	0.1	30	1.63	0.25	367.4	14.5	504.6	77.3
MMJ3-003a	Late Eocene	48.9	12.6	2.7	22	1.85	0.5	0.1	22	1.52	0.19	365.4	13.5	472.3	59.0
Mean	Late Eocene	48.9	16.2	1.5	112	2.07	0.7	0.1	112	1.60	0.11	365.6	7.6	496.1	35.7

Note: *x*—mean; σ—standard deviation; s.e. —standard error of mean; n— numbers of photos counts (400×); t·s.e.— 95% confidence interval. pCO_2— the result based the regression approach; $C_{(f)}$— the result based on the stomatal method.

Table 8. Summary of stomatal parameters of the combined data of the adaxial and abaxial surfaces of fossil *Nageia* and pCO_2 [$C_{(f)}$] estimates results.

Species	Age	SD (mm^{-2})				SI (%)				SR		pCO_2(ppmv)		$C_{(f)}$ (ppmv)	
		x	σ	s.e.	n	*x*	σ	s.e.	n	*x*	t*s.e	*x*	t*s.e	*x*	t*s.e
MMJ1-001	Late Eocene	50.1	17.5	2.3	60	2.09	0.8	0.1	60	1.50	0.15	349.7	10.6	471.2	47.8
MMJ2-003	Late Eocene	46.5	16.3	2.1	60	1.96	0.7	0.1	60	1.67	0.24	358.3	9.8	524.1	75.7
MMJ2-004	Late Eocene	44.0	15.8	2.0	60	1.90	0.7	0.1	60	1.73	0.17	364.3	9.5	542.9	52.6
MMJ3-003a	Late Eocene	45.6	16.1	2.2	52	1.75	0.6	0.1	52	1.73	0.28	360.5	10.4	544.6	88.3
Mean	Late Eocene	46.6	16.4	1.1	232	1.93	0.7	0.1	232	1.66	0.11	358.1	5.0	519.9	35.0

Note: *x*—mean; σ—standard deviation; s.e. —standard error of mean; n— numbers of photos counts (400×); t·s.e.— 95% confidence interval. pCO_2— the result based the regression approach; $C_{(f)}$— the result based on the stomatal method.

977 Table 9. pCO_2 estimates proxies and corresponding references.

Proxies	References
Boron	Pearson et al., 2009; Seki et al., 2010
B/Ca	Tripati et al., 2009
Phytoplankton	Freeman and Hayes, 1992; Stott, 1992; Pagani et al., 1999, 2005; Henderiks and Pagani, 2008; Seki et al., 2010
Nahcolite	Lowenstein and Demicco, 2006
Liverworts	Fletcher et al., 2008
Paleosols	Cerling, 1992; Koch et al., 1992; Ekart et al., 1999; Royer et al., 2001; Nordt et al., 2002; Retallack, 2009b; Huang et al. 2013
Stomata	Van der Burgh et al., 1993; Kürschner et al., 1996, 2001, 2008; McElwain, 1998; Royer et al., 2001, 2003; Greenwood et al., 2003; Beerling et al., 2009; Retallack, 2009a; Smith et al., 2010; Doria et al., 2011; Roth-Nebelsick et al., 2012; 2014; Grein et al., 2013; Maxbauer et al., 2014

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