Fossil plant stomata indicate decreasing atmospheric CO₂ prior to the Eocene-Oligocene boundary

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15 Abstract

A unique stratigraphic sequence of fossil leaves of *Eotrigonobalanus furcinervis* (extinct trees 16 of the beech family, Fagaceae) from central Germany has been used to derive an atmospheric 17 pCO₂ record with multiple data points spanning the late middle to late Eocene, two sampling 18 levels which may be earliest Oligocene, and two samples from later in the Oligocene. Using 19 the inverse relationship between the density of stomata and pCO_2 , we show that pCO_2 20 decreased continuously from the late middle to late Eocene, reaching a relatively stable low 21 22 value before the end of the Eocene. Based on the subsequent records, pCO_2 in parts of the Oligocene was similar to latest Eocene values. These results suggest that a decrease in pCO₂ 23 preceded the large shift in marine oxygen isotope records that characterizes the Eocene-24 Oliogocene transition and that when a certain threshold of pCO₂ change was crossed, the 25 cumulative effects of this and other factors resulted in rapid temperature decline, ice build up 26 on Antarctica and hence a change of climate mode. 27

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29 **1** Introduction

30 1.1 The role of pCO₂ in Cenozoic climate

The Cenozoic era is characterized by large climatic variations, including the fundamentally 1 important transition from an ice-free 'greenhouse' planet to the modern 'icehouse' planet with 2 polar glaciations. This climatic transition is generally thought to have been driven primarily 3 by changes in pCO₂ and/or the thermal isolation of Antarctica by the opening of Southern 4 Ocean gateways (DeConto and Pollard, 2003; Zachos et al., 2008; Hansen et al., 2013; Hren 5 et al., 2013; Goldner et al., 2014; Inglis et al., 2015). However, the full extent of the role of 6 pCO₂ in Cenozoic climate change remains unresolved. The most detailed Cenozoic 7 temperature and pCO₂ records are derived from marine isotope proxies (e.g. Foster et al., 8 9 2012; Pagani et al., 2011; Pearson et al., 2009; Zachos et al., 2001, 2008). Isotope records, however, may be influenced by a variety of taphonomic and diagenetic biases (see Coxall and 10 Pearson, 2007 for review; and Pagani et al., 2011), that can obscure the climatic signal, and 11 thus need independent evaluation by separate proxy records (Berling and Royer, 2011). 12

Eocene temperatures were globally much higher than today, leading to a weakened 13 equator-to-pole temperature gradient and muted seasonal cycle compared to today; the so-14 called 'Eocene equable climate problem' (Sloan and Barron, 1992; Huber and Caballero, 15 2011). Climate modelling has been able to reconstruct this climatic pattern only with 16 excessively high pCO₂ levels (~4500 ppm: Huber and Caballero, 2011), but such elevated 17 pCO₂ atmospheres do not agree with most proxy records. It has therefore been speculated that 18 Eocene climate sensitivity was elevated compared to today and/or that other forcing in 19 20 addition to high pCO₂ was involved (Caballero and Huber, 2013; Hansen et al., 2013). In 21 order to solve this enigma reliable multiple proxy records of pCO₂ are of paramount importance. 22

The fundamental climatic reorganization that occurred close to the Eocene-Oligocene 23 boundary (33.8 Ma), often referred to as the Eocene-Oligocene transition (EOT, 34-33.5 Ma), 24 had drastic consequences for biological systems. These included both terrestrial and marine 25 26 faunal and floral extinctions accompanied by evolutionary turnover (Prothero, 1994; Coxall and Pearson, 2007; Sheldon et al., 2009: Kunzmann, 2012; Kvaček et al., 2014), although 27 vegetation changes in the European terrestrial record appear to be less dramatic and more 28 gradual (Kvaček et al., 2014; Kunzmann et al., submitted). General circulation models of 29 Paleogene climate have shown that continuously declining pCO₂, amplified by Milankovitch 30 forcing and ice-albedo feedbacks, could cause significant temperature reduction. This could 31 result in a permanent continental Antarctic ice-sheet once a critical pCO₂ threshold, generally 32

considered to be < 700 ppm is crossed e.g. (DeConto and Pollard, 2003; Coxall et al., 2005; 1 Pollard and DeConto, 2005; Zachos and Kump, 2005; Pagani et al., 2011; Hansen et al., 2 2013). Modeling studies thus indicate that lowering of pCO_2 may have been the primary 3 forcer of this cooling transition (DeConto and Pollard, 2003; DeConto et al., 2008). However, 4 detailed estimates for pCO₂ for the Eocene and the Oligocene are highly variable and 5 6 sometimes contradictory or showing unexpected relationships with paleo-temperature proxy 7 records (see Pagani et al., (2005)). For example, comparing the pCO₂ record of Pearson et al., (2009: Fig. 1), which is based on measurements of Boron isotopes in planktonic foraminifera, 8 and the benthic foraminifera oxygen isotope $(d^{18}O)$ compilations of Zachos et al., (2008), it is 9 evident that in the late Eocene d¹⁸O-inferred deep ocean cooling coincided with decreasing 10 pCO₂. In contrast, there is little evidence of warming in the early Oligocene, despite a 11 surprising initial large increase in pCO₂. Overall, the pCO₂ and O isotope-based temperature 12 13 records seem to be (largely) coupled in the Eocene, but decoupled in the Oligocene. Pagani et al. on the other hand recently published compiled alkenone-based pCO₂ records and found 14 15 declining pCO₂ before and during the Antarctic glaciation (EOT and earliest Oligocene) (Pagani et al., 2011: Fig. 4), supporting the role of pCO₂ as the primary forcing agent of 16 17 Antarctic glaciation, consistent with model derived thresholds. A compounding factor of these discrepancies is that the influence of temperature on ice sheet volume is unconstrained and the 18 influence of temperature versus ice volume the $d^{18}O$ record is unresolved, with no proxy 19 identified to isolate ice sheet volume changes, complicating further the interpretation of the 20 climate proxy datasets. Independent proxy records of E-O pCO₂ are therefore desirable and 21 may support one or the other of the major prevailing scenarios outlined above, or provide 22 alternative information on Cenozoic climate change. 23

One of the four proxies that have been identified as being particularly useful for Cenozoic 24 pCO₂ reconstructions by the Intergovernmental Panel on Climate Change (initially reported in 25 the 4th IPCC report, 2007) is the terrestrial proxy based on stomatal densities of fossil plants. 26 27 Previous studies using the stomatal proxy method of pCO₂ reconstructions for the part of the Cenozoic relevant here were, however, mostly of low resolution and have been inconclusive. 28 29 Some suggested that pCO_2 was essentially stable at between 300 and 450 parts per million by volume (ppm) during the Eocene, Oligocene and Miocene (Royer, 2001; Royer et al., 2001; 30 31 Greenwood et al., 2003; Maxbauer et al., 2014) and others suggesting distinct decrease in pCO₂ across the Eocene-Oligocene boundary (Retallack, 2001). More recent studies suggest 32 33 higher and possibly rapidly decreasing pCO₂ (ranging ca. 1000-500 ppm) during the late

middle Eocene (Doria et al., 2011; Grein et al., 2011). In this issue, Liu et al. report a "late 1 Eocene" pCO₂ from a single stratigraphical level of ca. 390 ppm. However, the chronological 2 range they supply for their pCO₂ estimate (42.0-38.5 Ma) falls within the late Lutetian to 3 Bartonian in the Middle Eocene, thus recording an unusually low pCO₂ estimate for this time-4 interval characterized by high temperatures (Liu et al., 2015). Closer to the E-O boundary, 5 one study suggests that pCO₂ was significantly higher at the EOT than during the early 6 Oligocene (Roth-Nebelsick et al., 2004) and others that early Oligocene to early Miocene 7 pCO₂ was ca. 400 ppm throughout (Grein et al., 2013; Roth-Nebelsick et al., 2014). 8

9 Here we present a new stomatal proxy-based record with multiple data points spanning the
10 late middle to late Eocene, two sampling levels that according to current available evidence
11 are from the earliest Oligocene, and two samples from later in the Oligocene.

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13 1.2 The stomatal proxy method of paleo-pCO₂ reconstruction

Stomata are pores on plant leaf surfaces through which gas exchange takes place; i.e. carbon 14 15 is obtained from CO_2 and at the same time water vapour and oxygen are lost by diffusion. An inverse relationship exists between the frequency of stomata and pCO₂, as established by 16 Woodward (1987) from observations of herbarium material, showing that modern tree species 17 have responded to the anthropogenic rise in pCO_2 by reducing their stomatal frequency 18 significantly. The inverse relationship between stomatal frequency, recorded as 'stomatal 19 density' (SD = the number of stomata per mm^2) or 'stomatal index' (SI = the percentage of 20 stomata relative to epidermal cells), and pCO₂ has been repeatedly demonstrated for a wide 21 variety of plant taxa from disparate geological and ecological settings from the Palaeozoic 22 until today and is thus established as a strong proxy for paleo-pCO₂ (e.g. Beerling et al., 1998; 23 24 McElwain, 1998; Retallack, 2001; Royer et al., 2001; Kürschner et al., 2008; Steinthorsdottir et al., 2011b; 2013: Steinthorsdottir and Vajda, 2015). The increasingly close match between 25 stomatal proxy pCO₂ results and independent proxy records, actual pCO₂ measurements and 26 in some cases climate modelling (e.g. Finsinger and Wagner-Cremer, 2009; Foster et al., 27 2012; Kürschner et al., 2008; Retallack, 2001; Rundgren and Björck, 2003; Steinthorsdottir 28 and Vajda, 2015) instils growing confidence in stomatal frequency for recording past pCO_2 . 29 Strongly supporting the validity of the stomatal proxy is also the identification of the 30 mechanism by which plants control their stomatal densities based on atmospheric pCO₂. All 31 plants use the enzyme carbonic anhydrase to detect pCO₂ around their leaves (Frommer, 32

2010; Hu et al., 2010); mature leaves (early shoots) then control stomatal development of
 younger leaves through long-distance signalling (Lake et al., 2002), involving the HIC gene
 signalling pathway (Brownlee, 2001; Gray et al., 2000).

4 In order to transform stomatal frequency data derived from fossil plants into paleo pCO_2 estimates it is usually necessary to compare stomatal data from present day plants that 5 are either phylogenetically related or in other ways equivalent to the fossil plants. Nearest 6 living relatives (NLR) should be used when possible, but when these cannot be identified for 7 the fossil plants, nearest living equivalents (NLE= present day species that are of comparable 8 ecological setting and/or structural similarity to their fossil counterpart) may be used instead 9 (McElwain and Chaloner, 1995; Barclay et al., 2010; Steinthorsdottir et al., 2011a; 10 Steinthorsdottir et al., 2011b). 11

There are three stomatal paleo-pCO₂ calibration methods in use. These are (i) the 12 'stomatal ratio method' (McElwain and Chaloner, 1995; McElwain, 1998), which relies on a 13 ratio between stomatal frequencies of fossil plants and their NLE to semi-quantify pCO₂; (ii) 14 the 'transfer function method', which relies on herbarium material and/or experimental 15 16 datasets for NLR/NLE responses to calculate pCO₂ curves (e.g. Beerling and Royer, 2002); and (iii) the more recently developed taxon-independent 'mechanistic gas exchange 17 modelling' approach (e.g. Wynn, 2003; Konrad et al., 2008; Franks et al., 2014; Grein et al., 18 2013; Roth-Nebelsick et al., 2014) which all use measurements of stomatal density and pore 19 size to estimate maximum theoretical gas exchange rates, together with various 20 photosynthetic biochemical traits, and in some cases palaeoenvironmental information, to 21 estimate palaeo- CO_2 . The stomatal ratio method, which is used here, calibrates paleo- pCO_2 22 based on two so-called standardizations. The first is the 'modern' standardization that 23 assumes that the ratio between past and modern pCO_2 is 1 ($RCO_2 = 1$) and is applied to young 24 material, typically from the Quaternary. The second is the 'Carboniferous' standardization 25 that sets the ratio between past and modern pCO_2 at two times preindustrial levels of 300 ppm 26 $(RCO_2 = 2 = 600)$ (McElwain and Chaloner, 1995). Both standardizations are usually applied 27 to fossil leaf material of Cenozoic age and older to yield minimum and maximum pCO₂ 28 estimates and both standardizations will be used in this paper. 29

We have chosen not to apply the mechanistic optimization model of Konrad et al., (2008) to our study, because it has recently been shown in a modern test of the model to produce the most accurate pCO₂ estimates when used on multiple species, to derive a

consensus pCO₂ estimate from their area of overlapping pCO₂ values (Grein et al., 2013), and 1 we here study a one-species database. The optimization model produces very large and 2 species-dependent uncertainty in pCO₂ estimates when applied to individual fossil species 3 (Konrad, 2008; Roth-Nebelsick et al., 2012) and even modern species (Grein et al., 2013) for 4 which all the biochemical, environmental and anatomical parameters required to initialize the 5 model are known (Konrad, 2008; Grein et al. 2013; Roth-Nebelsick et al., 2012). We have 6 7 also not applied the mechanistic stomatal model of Franks et al. (2014) because it is shown to be highly sensitive to initial parameterization of assimilation rate resulting in +/- 500 ppm 8 error in palaeo-pCO₂ estimates (McElwain et al., 2015b). Future work on *Eotriginobalanaus* 9 furcinervis will aim to constrain likely paleo-assimilation rate for this extinct taxon by 10 applying available paleo-assimilation proxies (McElwain et al. 2015a; 2015b; Wilson et al., 11 2015) and undertaking elevated pCO₂ experiments on appropriately selected NLEs. 12

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14 2 Material and methods

15 2.1 Fossil leaf database

Eotrigonobalanus furcinervis (Rossm. 1840) Walther et Kvaček in Kvaček and Walther 1989, 16 17 an extinct evergreen Fagaceae, (Fig. 1) existed from the middle Eocene to the Oligocene-Miocene boundary and was geographically widely distributed, i.e. from central Europe to 18 19 Russia, as well as to the Mediterranean area (Mai and Walther, 2000; Velitzelos et al., 1999). It is considered as a thermophilous species that grew in evergreen broadleaved forests as well 20 21 as in mixed mesophytic forests adapted to humid and warm-temperate to subtropical climate (Mai and Walther, 2000). E. furcinervis was present in megafossil assemblages or 22 "taphocoenoses" derived from riparian forests, back swamps, peat bogs and zonal vegetation 23 and therefore the parent plant tolerated a wide range of water table conditions and soil 24 characteristics. Whereas in the Eocene it often predominated in zonal Fagaceae-Lauraceae 25 forests (Mai and Walther 2000), in the Oligocene mixed mesophytic forest it was ecologically 26 27 sub-dominant. Based on the combined fossil record of cupules, seeds and leaves, including cuticles, it is commonly accepted that the fossils represent a single long-lived but rather 28 29 variable fossil species, although minor changes in leaf anatomy have led to the distinction of two subspecies, ssp. furcinervis (mainly Eocene, rare in Oligocene) and ssp. haselbachenses 30 (only Oligocene; Kvaček and Walther 1989). The latter is distinguished by the absence of 31

pubescence (trichome clusters) on the abaxial leaf epidermis. Furthermore, a variety of leaf
morphotypes can be distinguished that have been interpreted as ecological variants (ecotypes,
see Kriegel, 2001).

Except for the material from the Kleinsaubernitz site (Fig. 2), the leaf specimens used here originate from the central German Weißelster Basin (Fig. 2), a coastal alluvial plain at the southern margin of the North German–Polish 'Tertiary' Basin (Standke, 2008). This basin is well-known for its extensive record of middle Eocene to early Miocene plant assemblages that are mainly derived from azonal vegetation, i.e. riparian and swamp forests (e.g. Mai and Walther, 2000; Kunzmann, 2012). The Knau assemblage represents the fluvial hinterland of the Weißelster lignite swamps (Mai and Walther, 2000).

The leaves used here are derived from a succession of cuticle rich taphocoenoses that 11 contain E. furcinervis ranging in age from the late middle Eocene to the end of the Oligocene 12 13 (Table 1, Fig. 3). The database analysed here consists of 233 E. furcinervis leaf cuticle fragments on as many slides, representing 151 separate individual leaf specimens (Appendix 14 15 A and Table 2). All specimens represent material used in previous taxonomic-systematic studies, they are housed in the Senckenberg Natural History Collections Dresden, Germany. 16 17 The plant fossil assemblages have been positioned on the most recent lithostratigraphy for central and East Germany (Standke, 2008; Standke et al., 2010; Fig. 3, Table 2) using 18 published information on the fossil sites (Mai and Walther, 1991; Mai and Walther, 2000; 19 Kunzmann and Walther, 2002; Hennig and Kunzmann, 2013; Ferdani, 2014) and personal 20 observations (LK). Information on dating is provided in section 2.2 below. 21

One late Oligocene locality, Kleinsaubernitz (Figs. 2, 3A), lies within the Lausitz basin, at its southern margin or even in the hinterland (Standke, 2008). Leaf specimens derive from a sediment-filled maar, volcanic in origin, preserving a parauthochtonous assemblage mainly representing zonal vegetation (Walther, 1999) in contrast to the mainly azonal vegetation from the coastal plains of the Weißelster Basin.

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28 2.2 Stratigraphy and dating

The relative stratigraphic positions for the samples from the Weißelster Basin (Figs. 2, 3A) are based on accumulating knowledge from more than 150 years of geologicalpaleontological investigations of the respective units (see Walther and Kunzmann, 2008 for summary). The samples are derived from a superposed sequence of four lignite seams and their associated strata (Table 1, Fig. 3A) the subdivisions of which can be readily recognized across different opencast mines.

It is not possible to directly correlate the plant fossil bearing horizons in the 1 Weißelster Basin to the global marine stratigraphy. Although there are a number of brackish-2 marine intercalations (Standke et al., 2010) most of these strata lack fossils suitable for 3 biostratigraphy. As is typical for lignite-bearing non-consolidated sedimentary successions 4 (i.e. gravel, sands, silts, clays) hard parts of mineralised organisms that might be used for 5 biostratigraphy in continental sequences (such as mammals and charophytes) are lacking due 6 to dissolution by humic acids originating from organic material. Non-consolidated sediments 7 do not reveal any casts or molds of these former fossils. This is also the case for any 8 9 intercalation of brackish-marine sediments in the Weißelster Basin profile. The lack of common index fossils prevents accurate stratigraphic chronology in the basin and reduces the 10 level of stratigraphic resolution compared with that typically attainable for marine deposits 11 (e.g. Roth-Nebelsick et al., 2014). Furthermore, heterogeneity in facies types (channel, 12 13 floodplain, tidal deposits, swamps) and in grain sizes of the sediments precludes the use of magnetostratigraphic methods which need longer sequences of fine-grained sediments 14 15 without facies shifts (e.g. lake sediments) to produce reliable data.

Based on a series of consecutive pollen assemblages in the Weißelster Basin strata a 16 17 regional phytostratigraphic concept was developed (Krutzsch, 1967) that can be applied to all Formations, Members and submembers, and also to all lignite seams and even individual 18 seam measures (Krutzsch, 2011). All of our investigated material is unambiguously assigned 19 to a certain unit of the regional lithostratigraphic scheme (Fig. 3A) and thus connected to a 20 respective pollen zone or subzone (Fig. 3A, Table 1). However, the pollen zonation yields 21 22 only a relative age for a given horizon within the regional palynostratigraphic framework and does not enable correlation to global stratigraphy or to the global time scale. The attempt by 23 Krutzsch (2011) to correlate the Eocene spore-pollen zones with the global timescale is used 24 25 herein (Fig. 3A) as it is the only available information to interpret our assemblages. A 'late' Eocene age (i.e. late Bartonian + Priabonian, Krutzsch 2011) for our respective assemblages 26 has been previously inferred based on floristic comparison to assemblages from the nearby 27 28 Bohemian basins (Czech Republic) some of which have absolute dates from volcanic rocks (i.e. Kučlin, Staré Sedlo, Roudníky; Kvaček et al. 2014). 29

In the younger part of the succession, marine deposits have yielded index fossils suitable for biostratigraphy. Marine strata above the Gröbers Member of the Böhlen Formation are placed into regional dinoflagellate zones D13 and D14 (Köthe, 2005; Standke et al. 2010) which are Rupelian in age. The Haselbach horizon of the Gröbers Member, including our assemblage sites Schleenhain 4 and Haselbach 2 (figs 2 and 3A), was therefore

interpreted to be basalmost Oligocene (Standke et al. 2010; Krutzsch 2011), however, the 1 only definitive information from the dinoflagellate data is that the samples must be older than 2 mid Rupelian. Lithofacies changes in the centre of the Weißelster Basin, i.e the profile in the 3 Schleenhain mine (Kunzmann and Walther, 2002) that indicate major sea level changes below 4 the sample horizon of sites Schleenhain 4 and Haselbach 2 are consistent with those that 5 occur around the Eocene - Oligocene boundary and are documented in other European 6 successions (e.g. Hooker et al., 2009). A basalmost Oligocene age for the Schleenhain 4 and 7 Haselbach 2 sites is also indicated by the first occurrence of Boehlensipollis hohlii in the 8 9 sampled horizon which places the sample in spore-pollen zone 20A/B sensu Krutzsch (2011). Boehlensipollis hohlii is regarded as a key element for the Oligocene in central and East 10 Germany (Krutzsch, 2011) and had also been treated as such in the International Geological 11 Correlation Programme (Vinken, 1988). However, it should be mentioned that Collinson 12 13 (1992) reported several occurrences of the species in the late Eocene of the UK and Frederiksen (1980) reported the species ranging from late middle Eocene to Oligocene in the 14 15 USA. Possibly the species arose in the USA and spread later via the UK into central Europe but further work is needed to securely link the occurrences of Boehlensipollis hohlii with the 16 17 marine biostratigraphy and the global time scale. In short, there are two independent pieces of evidence (lithofacies, first appearance of *Boehlensipollis hohlii*) that clearly suggest an early 18 Oligocene age for the Schleenhain 4 and Haselbach 2 samples. However, this is not 19 conclusive evidence and direct linkage to the global marine scale is currently not available. 20 The site at Kleinsaubernitz has been located on Figure 3 based on its pollen assemblage which 21 22 is zone 20G (Goth et al., 2003).

In summary, the material from the Weißelster Basin comes from a superposed 23 sequence where relative stratigraphic position is securely known (Table 1). Relative changes 24 of SD (and thus pCO₂) through the succession can be placed in context of the spore-pollen 25 zonation. However, the positions of the Eocene-Oligocene boundary and the Oligocene-26 Miocene boundary cannot be located with certainty in the Weißelster profiles. All age 27 estimates in figures 3 and 4 are based on Krutzsch's (2011) proposed correlation of the 28 regional spore-pollen zones to global sea level changes. Independent support is needed for 29 these proposals so they should be regarded as preliminary age information. 30

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32 2.3 Stomatal density quantification

Cuticles were prepared at the Senckenberg Natural History Collections Dresden as a part of 1 an existing collection. Cuticle slides were prepared using standard methods for Palaeogene 2 material. Fragments removed from leaf specimens with preparation needles were macerated 3 for 1-4 minutes in Schulze's solution. Cuticles were then neutralized with NH₄OH, washed 4 with distilled water, and upper and lower cuticles were separated using preparation needles. 5 Finally, the cuticles were stained with Safranin and affixed to slides by glycerol jelly. For this 6 study, the slides were examined microscopically by an adaptation of the methodology set out 7 by Poole and Kürschner (1999) in order to determine SD. According to this protocol, counts 8 9 from mid lamina are preferable in establishing SD, but the fragmented nature of a proportion of the fossil material did not allow establishing where individual cuticle samples were located 10 on the original leaf surface (see Fig. 1B). Individual epidermal cells were not easily 11 discernible in the majority of the *E. furcinervis* material, making SI determination impossible. 12 13 SD was obtained using a Nikon SK Light Microscope at x200 magnification with a graticule providing a counting field of 0.042 mm^2 . The graticule was centred over areas where stomata 14 15 occurred in greatest numbers (away from veins and margins where those were known, sensu Poole and Kürschner, 1999) and up to five individual counts were recorded for each slide, 16 resulting in 659 SD counts for the database of 151 leaf specimens (Table 1 and Appendix 1). 17 Data was stored in Microsoft Excel 2010 before being statistically manipulated using 18 MINITAB (version 16.1.1 for Windows). 19

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21 2.4 Choice of Nearest Living Equivalent and Paleo-pCO₂ calibration

Eotrigonobalanus furcinervis belongs to the Fagaceae, but its phylogenetic position is not 22 well defined. Based on cupule morphology, *Eotrigonobalanus* belongs to a basal clade of the 23 family, exhibiting intermediate characters between modern Trigonobalanus and Castanopsis 24 1995). However, leaf venation and leaf cuticle micromorphology place 25 (Mai, Eotrigonobalanus with Trigonobalanus and Lithocarpus, away from Castanopsis (Kvaček 26 and Walther, 1989), an affiliation recently confirmed by Denk et al., (2012). Since the 27 phylogeny of Fagaceae has changed considerably (Manos et al., 2001; Manos et al., 2008), an 28 improved systematic framework is still required to confirm the phylogenetic position of 29 Eotrigonobalanus. Because the exact relationship of Eotrigonobalanus to crown group 30 Fagaceae is unknown, a single nearest living relative (NLR) could not be obtained, hence the 31

nearest living equivalent (NLE) approach has been used for the stomatal proxy-based pCO₂
 reconstruction.

In this study, Trigonobalanus doichangensis was chosen as the NLE, due to it being a 3 basal species within the Fagaceae family and having leaf macro-morphological and leaf 4 cuticle micro-morphological similarities with E. furcinervis, including cyclocytic stomata and 5 similarly structured trichomes (Kvaček and Walther, 1989; see also Denk et al., 2012). Two 6 herbarium specimens of T. diochangensis, formerly collected in 1988, were sampled at the 7 Kew Herbarium (Royal Botanical Gardens, Kew, Richmond, Surrey, UK). Approximately 8 1cm² was cut from mid-lamina of each leaf specimen and dry mounted onto a slide. Five 9 cuticle images from each slide were taken at 200x magnification using a Leica DM2500 10 epifluorescent microscope with Leica DFC300FX camera (Leica® 312 Microsystems, 11 Wetzlar, Germany) and Syncroscopy Automontage (Syncroscopy Ltd, Cambridge, UK) 12 digital imaging software. A 0.09mm² square was superimposed on each image and stomatal 13 density was determined within this square following the protocol of Poole and Kürschner 14 (1999). SD was determined to be 546.11/mm² at pCO₂ of 351 ppm (collection year levels 15 according to NOAA ESRL data, available at www.esrl.noaa.gov). 16

Using the stomatal ratio method with *T. doichangensis* NLE for *E. furcinervis*, we calibrated paleo-pCO₂ using the equations below to derive minimum and maximum paleopCO₂ ('Modern' and 'Carboniferous' Standardization of McElwain and Chaloner, 1995), respectively:

21 Paleo-pCO_{2 min} (ppm) = ((
$$SD_{NLE} = 546.11$$
)/ SD_{fossil}) * 351 ppm

22 Paleo-pCO_{2 max} (ppm) = ((
$$SD_{NLE} = 546.11$$
)/ SD_{fossil}) * 600 ppm

23

24 3 Results

25 3.1 Stomatal density and paleo-pCO₂ estimates

SD of *E. furcinervis* range between ca. 425 and 740 stomata/mm². The lowest SD values (signifying highest pCO₂) are found in the oldest deposits, late middle to earliest late Eocene (spore-pollen zone 17), and the highest values (signifying lowest pCO₂) are found in the later late Eocene (spore-pollen zone 180), representing the most pronounced SD change during the time period covered by the dataset (Table 2, Fig. 3B), with three intermediate samples showing intermediate values (spore-pollen zones 17/18, 18u, 18uo). During this interval SD increases by >300 stomata/mm² or by ca. 75%, a very significant change indicating a sizeable decrease in pCO₂ in perhaps ca. 3.5 million years. Stomatal densities then decrease slightly again and remain around 600-650/mm² in the latest Eocene and in samples that may be earliest Oligocene as well as in the late Oligocene (spore-pollen zones 19, 20A/B, 20G, II). At the end of the Oligocene, SD decreases again to ca. 570/mm².

Paleo-pCO₂ calibrated using the stomatal densities of *E. furcinervis* will be discussed as average values and evaluated in terms of relative change, as introduced above. The largest change in palaeo-pCO₂ is the decrease from the late middle to earliest late Eocene of >250 ppm, from ca. 630 ppm to ca. 365 ppm – a decrease in pCO₂ of ca. 40% (Fig. 3B; Table 2). Concentrations of CO₂ then increase again by ca. 45 ppm to ca. 410 ppm in the latest Eocene and possibly earliest Oligocene, and further to between ca. 430-475 ppm in the late and latest Oligocene (Fig. 3B; Table 2).

14

15 4 Discussion

16 4.1 Fidelity of the Saxony stomatal pCO₂ record

The Saxony fossil leaf database is unique in that this relatively large database derives from a 17 well-constrained stratigraphic succession and consists of a single species throughout -E. 18 *furcinervis* – which is the most ideal situation when using fossil leaf material to reconstruct 19 paleo-pCO₂, since inter-species variability is eliminated and stomatal responses to pCO₂ are 20 likely to be consistent through time. The procurement of a single-species dataset from 21 multiple stratigraphic levels across several million years is not common, in particular when 22 the stratigraphy represents time intervals of significant climate and/or environmental change, 23 as is the case here. The principal challenge concerning the Saxony stomatal density record 24 was translating the stomatal signal into reliable levels of pCO₂. One of the main limitations 25 associated with the use of paleo-proxies is the preservational state of fossil material and in 26 this case the preservation of fossil leaves did not allow paleo-pCO₂ reconstruction using gas 27 exchange models for independent comparison of the results using the stomatal ratio method 28 29 because stomatal pore length could not be measured in all samples with confidence. Additionally, there is a lack of available transfer functions for potential NLEs of E. 30 furcinervis, so it was not possible either to obtain independent pCO₂ reconstructions using the 31

transfer function method. The stomatal ratio method has however been shown to closely match results produced with transfer function methods (Beerling and Royer, 2002; Royer, 2003; Barclay et al., 2010; Steinthorsdottir et al., 2011b) and is seen as a good alternative where detailed estimates of other photosynthetic parameters, which are required to initialize mechanistic models, are not readily available (McElwain, unpubl.).

6 The absence of an obvious NLE for *E. furcinervis* – an extinct species of uncertain phylogenetic affinity – further introduces potential errors in pCO₂ calibration. Although we 7 consider T. doichangensis the best available NLE, there is no guarantee that its stomatal 8 density and degree of response to pCO₂ closely mirrors that of its distant fossil relative. The 9 pCO₂ levels calibrated here appear somewhat low compared to most previously published 10 pCO₂ datasets, although broadly comparable to stomatal pCO₂ records (Fig. 4A). When 11 testing three additional potentially suitable NLE species for reconstructing pCO₂ using the 12 Saxony database; Trigonobalanus verticillata, Castanopsis cuspidata and Lithocarpus henryi, 13 the resulting paleo-pCO₂ values were extremely low – considerably lower than when using 14 the chosen NLE T. doichangensis – in many cases being lower than minimum pCO_2 levels 15 16 required to maintain sufficient plant growth and reproduction (i.e. below the ecological compensation point). This indicates that, for some reason (e.g. species-specific responses) the 17 stomatal proxy-derived pCO₂ estimates presented here based on E. furcinervis may be 18 artificially low. 19

20

4.2 Comparison with vegetation and proxy continental climate records

Paleoclimate reconstructions based on Central European megafloras reveal a sharp decline 22 in continental cold month mean temperature (Mosbrugger et al., 2005) and mean annual 23 temperature (Moraweck et al., 2015; Kvaček et al., 2014) in the late Eocene (Fig. 4B) which 24 is consistent with the timing of the pCO₂ decline that we report here (Figs. 4A,B), and with 25 global sea surface temperature trends as recorded by marine oxygen-isotopes (Fig. 4C). The 26 marine isotope curve also shows a gradual decrease of temperatures in the late Eocene, but in 27 contrast with the terrestrial records, the most pronounced and abrupt change coincides with 28 the Eocene-Oligocene boundary (Fig. 4C), suggesting that pCO₂ drawdown may have taken 29 place gradually before the slow feedback ice sheet growth was initiated and global 30 temperatures dropped suddenly in response. The possibility remains that future terrestrial 31

proxy reconstructions of pCO₂ will record a transient major drawdown of pCO₂ at the
 Eocene-Oligocene boundary. In order to resolve this, more proxy records from well constrained Early Oligocene sites must be added.

4 Furthermore, palaeo-vegetation analysis of the Weißelster and North Bohemian basins reveals that gradual restructuring of dominantly evergreen forests by immigration of 5 6 deciduous species such as Platanus neptuni, Trigonobalanopsis rhamnoides and Taxodium dubium (Kunzmann et al. submitted) took place in the late Bartonian to early Priabonian 7 interval around ca. 38 Ma (Kvaček, 2010; Teodoridis and Kvaček, in press). The temporal 8 coincidence of pCO₂ decline and major vegetation transition – from angiosperm-dominated 9 notophyllous evergreen forests to mixed mesophytic forests - suggests a potential causal role 10 of pCO₂ decline in the changing ecological composition of forests. It may have been in part 11 triggered by differential responses of evergreen and deciduous taxa to declining pCO₂ (Fig. 12 4A,B), explaining the lag between "temperatures" indicated by terrestrial vegetation and sea 13 surface temperatures recorded by marine oxygen-isotopes (Fig. 4C). The functional trait of 14 deciduousness is an adaptation to episodic cooling (Zanne et al., 2014). However, it has also 15 16 been demonstrated experimentally (McElwain et al., 2015) and on theoretical grounds (Niinemets et al., 2011) that taxa with low leaf mass per area or LMA (i.e. those that are 17 deciduous or herbaceous) and high stomatal conductance have faster photosynthetic rates than 18 evergreens at lower atmospheric pCO₂. In contrast, evergreens have higher responsiveness in 19 terms of photosynthetic rates at elevated pCO₂ (Niinemets et al., 2011). A transition from 20 21 elevated to lower CO₂ atmospheres would therefore favour the ecophysiology of deciduous or low LMA taxa over evergreen high LMA species. Further experimental investigation is now 22 required to tease apart the relative importance of 'CO₂ starvation' and increased temperature 23 seasonality on the late Bartonian to early Priabonian vegetation transition. 24

25

26 4.3 Comparison with other pCO₂ records

Previously published stomatal proxy-based pCO₂ records from the part of the Cenozoic
relevant to this paper do not always agree, but instead report highly elevated (McElwain,
1998; Doria et al., 2011; Grein et al., 2011; Smith et al., 2010), intermediate (Retallack, 2009)
or similar to modern (Royer et al., 2001) pCO₂ for the Eocene. Similarly high variability in
estimated pCO₂ levels exists for the Oligocene as well as the Miocene (Grein et al., 2013;
Kürschner et al., 2008; Roth-Nebelsick et al., 2014; Royer et al., 2001). The results reported

here are the highest stratigraphic resolution pCO₂ estimates derived from the late Eocene to 1 early Miocene basins in Saxony (see Table 2, Fig. 1 and 3). Previous studies have tended to 2 only report temporal trends on stomatal parameters (Roth-Nebelsick et al., 2004) or to lump 3 pCO₂ estimates from single Saxony localities into coarse temporal bins making cross 4 comparison difficult (Roth-Nebelsick et al., 2012). However, where individual site pCO₂ data 5 are reported (Grein et al., 2013) our estimates are in very good agreement with previous 6 studies despite differences in species and calibration approach (Table 2). For example, Grein 7 et al. (2013) report pCO₂ estimates of ~400 ppm and between ~430 to ~530 ppm respectively 8 9 for the sites Kleinsaubernitz and Witznitz (Fig. 3) using the Konrad et al. (2008) stomatal optimization model in a consensus approach on multiple species (3 - 4) including E. 10 *furcinervis* (Table 2). The optimization model produces a very large range of pCO₂ estimates 11 however (~270 to 710 ppm) when applied to *E. furcinervis* alone from stratigraphically 12 13 lumped samples from Haselbach and Profen (Table 2) (Roth-Nebelsick et al., 2012). In comparison with the study of Roth-Nebelsick et al. (2012), we report seven stratigraphically 14 15 well-resolved pCO₂ estimates spanning the same interval for which they report a single lumped average (~470 ppm) for 2 sites (Table 2). This is thus the first study therefore to 16 resolve a significant drop in paleo-pCO₂ in the late Eocene, prior to the E-O boundary from a 17 stratigraphically well constrained and relatively high-resolution record. 18

Using a rigorous generalized statistical framework, Beerling et al. (2009) revised 19 20 previously published pCO₂ estimates based on *Ginkgo* and *Metasequoia* from the early Eocene and middle Miocene upwards by 150-250 ppm. Based on this revision, average 21 stomatal proxy-based pCO₂ is 450-700 ppm in the Paleogene and 500-600 ppm in the 22 23 Neogene (Beerling et al., 2009). Interestingly, the younger set of pCO₂ estimates was fully compatible to marine proxy data and modelling results (e.g. Pagani et al., 2005; Hansen et al., 24 25 2008), whereas the older set of estimates seemed to underestimate pCO_2 compared to the other approaches, even after the upwards revision of stomatal pCO₂ values (see Fig. 4 in 26 27 Beerling et al., 2009). However, Kürschner et al. (2008) indicated that an upwards correction of 150-200 ppm – a so-called 'correction factor' – was necessary also when reconstructing 28 29 Miocene paleo-pCO₂ with two species from the Lauraceae family. Recently discrepancies between the various pCO₂ proxies have narrowed significantly, and a coherent pattern of 30 long-term Cenozoic pCO_2 has emerged, indicating pCO_2 mostly in the hundreds rather than 31 thousands of ppm, although shorter-term inter-proxy discrepancies remain (see Beerling and 32

Royer, 2011, Fig. 1). It has thus become evident that pCO₂ values reconstructed using the
 stomatal proxy do not require a correction factor.

Pearson et al. (2009) reconstructed pCO_2 for the late Eocene to early Oligocene using the 3 planktonic foraminifera boron isotope pH proxy and found that the main reduction in pCO₂ 4 took place before the main phase of EOT ice growth (ca. 33.6 Ma: DeConto et al., 2008), 5 6 followed by a sharp recovery to pre-transition levels and then a more gradual decline. Their results thus support the central role of declining pCO₂ in Antarctic ice sheet initiation and 7 8 development and agree broadly with carbon cycle modelling (e.g. Merico et al., 2008). The quantitative estimates of pCO₂ varied greatly however, according to which d¹¹B value was 9 used to derive pH, with geochemical models of the boron cycle suggesting a range of 37-39 10 % for sea water (sw) d¹¹B during this time (Simon et al., 2006). The range of pCO₂ values 11 spanned from ca. 2000-1500 ppm at the upper end and ca. 620-450 ppm at the lower end 12 (Pearson et al., 2009). Recently published alkenone-based pCO₂ records found significantly 13 declining pCO₂ before, as well as during, the Antarctic glaciation (EOT and earliest 14 Oligocene), supporting the pCO_2 pattern of Pearson et al. (2009) and the role of pCO_2 as the 15 primary forcing agent of Antarctic glaciation, consistent with model derived thresholds 16 (DeConto et al., 2008; Pagani et al. 2011; Zhang et al., 2013). The alkenone-derived dataset 17 values are overall higher – but not much higher – than those derived by stomatal densities, 18 with late Eocene values of ca. 1000 ppm, minimum value of ca. 670 at 33.57 Ma and then 19 20 gradual decline to ca. 350 ppm at the Oligocene-Miocene boundary.

In general therefore, Cenozoic stomatal proxy-based pCO₂ values, reconstructed using the 21 available methods, tend to report somewhat lower pCO₂ values than alkenone- or boron-based 22 proxies as well as those from mass balance modelling. As discussed above, isotope-based 23 proxy records depend on a range of assumptions that influence the output interpretation to a 24 large extent. In addition, it has recently been shown that the modelled pCO₂ threshold for 25 Antarctic glaciation at the EOT, routinely referred to be ca. 700 ppm (DeConto and Pollard, 26 2003), is in fact highly dependent on the type of climate model used and the configurations of 27 the model (Gasson et al., 2014), implying that the range of Cenozoic pCO_2 may be due for an 28 update. It is noteworthy that most existing stomatal proxy-based pCO₂ records report a similar 29 range of low pCO₂ values for this time interval and an internally consistent pattern is 30 emerging for the Cenozoic (see Fig. 4A). Stomatal proxy-based pCO₂ records that are 31 independently calibrated using different species/genera and families usually agree with one 32

another and show Eocene-Miocene pCO_2 in the range of 800-300 ppm (Fig. 4A). Although this discrepancy between proxies needs to be better understood before significant reevaluation of the role of pCO_2 in Cenozoic climate change is warranted, it should not be *a priori* rejected that collectively stomatal proxy records may accurately indicate lower pCO_2 levels during the Cenozoic than previously assumed.

6

7 4.4 Implications for Cenozoic climate sensitivity

The concept of Earth's climate sensitivity - usually defined as the equilibrium surface 8 temperature response to doubling of $pCO_2(2xCO_2)$ – is a key parameter for understanding the 9 mechanisms of future climate change. Recently there has been much focus on accurately and 10 11 uniformly defining this concept, but although progress has been made, discrepancies still remain. The term most in use for predicting future climate change is "equilibrium climate 12 13 sensitivity", defined as the response of global mean surface temperatures to a 2xCO₂ radiative forcing after all the fast feedbacks have occurred (changes in atmospheric temperatures, 14 clouds, water vapor, winds, snow, sea ice, etc.), but before the slow feedbacks occur (mainly 15 ice sheet, vegetation and the carbon cycle responses) and often estimated to be ca. 3°C 16 (Rohling et al., 2012; Royer et al., 2012; Hansen et al., 2013; Huber et al., 2014; the 17 Intergovernmental Panel on Climate Change report 2013 best estimate 1.5-4.5 °C). When 18 studying paleo-climate sensitivity, which has the potential to be accurately inferred from 19 high-resolution paleo-climate proxy archives, both fast and slow feedbacks must be 20 considered to define a related concept – the "Earth System sensitivity", where e.g. pCO_2 may 21 act both as forcer and as feedback, and which depends to a large degree on the initial climate 22 state (Royer et al., 2007; Hansen et al., 2013). In the Cenozoic, pCO₂ is involved in climate 23 change both as forcing and feedback, with evidence of increased climate sensitivity in warm 24 25 climates, rather than cool ones (Hansen et al., 2013).

The Eocene-Oligocene global cooling transition is represented by a large increase in deepsea benthic foraminiferal oxygen isotope values, reflecting simultaneously decrease in temperatures and increased ice sheet growth, with as of yet no proxy to accurately separate the relative effects of the two (Zachos et al., 2001; 2008). Constraining the decrease in temperature that occurred during the transition is thus a work in progress, but consensus is emerging around a ca. 2–5 °C cooling in sea surface as well as mean annual air temperature (e.g. Lear et al., 2008; Zachos et al., 2008; Liu et al., 2009; Bohaty et al., 2012; Wade et al.,

2012; Hren et al., 2013; Inglis et al., 2015; Petersen and Schrag, 2015). The EOT cooling and 1 glaciation was forced by a decrease in pCO₂ from ca. 1000 ppm to ca. 600 ppm based on 2 marine isotopes and climate modelling (e.g. DeConto et al., 2008; Pearson et al., 2009; Pagani 3 et al., 2011) or ca. 800 ppm to ca. 400 ppm based on stomatal records (e.g. Beerling and 4 Royer, 2011; this dataset) – a decrease of at least ca. 40% in pCO₂ in < 5 Ma. A simple 5 estimation of Earth System sensitivity during the EOT suggests elevated sensitivity compared 6 to today, implying an enhancing factor by fast and/or slow feedbacks, such as ice sheet 7 growth, but the radiative contribution of each is presently unknown (Lunt et al., 2010; 8 9 Goldner et al., 2012; Gasson et al., 2014; Maxbauer et al., 2014). The transition in Earth's climate mode from the Eocene greenhouse to the Oligocene icehouse was driven by changes 10 in pCO₂ (and associated feedbacks) that largely fall within the range of modern to predicted 11 future pCO_2 – albeit in opposite direction. Understanding how the Earth System responds to 12 13 radiative forcing within this range (i.e. understanding Earth System sensitivity) is of considerable interest, with the input and correlation of multiple paleo-pCO₂ proxy records 14 15 being of crucial importance.

16

17 5 Conclusions

The new terrestrial stomatal proxy-based pCO₂ record presented here, derived from fossil 18 leaves of *Eotrigonobalanus furcinervis* (extinct Fagaceae, beech tree family) from Saxony, 19 Germany, spans the late middle Eocene to latest Eocene, with two sampling levels which are 20 probably from earliest Oligocene, and two samples from later in the Oligocene. The record 21 indicates that pCO₂ decreased continuously and gradually by ca. 40% during the late Eocene, 22 from ca. 630 ppm in the late middle Eocene to ca. 365 ppm in the late Eocene and ca. 410 23 ppm near the Eocene-Oligocene boundary. Late and latest Oligocene pCO₂ was slightly 24 higher at around 430-475 ppm. Although the pCO₂ values reported here may be artificially 25 low, due to factors inherent to stomatal proxy-based calibration, they nonetheless broadly 26 agree with the pCO₂ range of previously published Eocene-Miocene stomatal proxy records, 27 indicating that Cenozoic pCO₂ may have been considerably lower than previously thought 28 based on marine proxies. The substantial late Eocene decrease in pCO₂ reported here is 29 consistent with terrestrial records of vegetation change and reconstructions of coldest month 30 mean temperatures, as well as with marine isotope records of global sea surface temperatures. 31 The substantial drop in temperatures and/or ice sheet growth that defines the Eocene-32

Oligocene boundary in the marine record is not recorded here. This may be caused by the possibility that the Saxony record does not possess the stratigraphic resolution to record such a change, or indicate that decrease in pCO₂ took place before the recorded decrease in global sea surface temperatures. The results reported here lend strong support to the theory that pCO₂ drawdown, rather than continental reorganization, was the main forcer of the Eocene-Oligocene climate change, when a 'tipping point' was reached in the latest Eocene, triggering the plunge of the Earth System into icehouse conditions.

8

9 Acknowledgements

M.S. gratefully acknowledges funding from Stockholm University postdoctoral research 10 11 fellowship SU 619-2974-12 Nat and the Bolin Centre for Climate Research. J.C.M. and A.P. acknowledge funding from Science Foundation Ireland grant SFI 08/RFP/EOB1131 and the 12 13 European Research Council grant ERC-2011-StG 279962-OXYEVOL. A.H. acknowledges funding from the Programme for Research in Third-Level Institutions (PRTLI) - Ireland, and 14 The European Regional Development Fund. MSc student Gael Giraud is acknowledged for 15 early work on the project. Sincere thanks go to Carola Kunzmann and Franziska Ferdani 16 (Dresden) for preparation of cuticle slides of *Eotrigonobalanus furcinervis*; to Zlatko Kvaček 17 (Prague) for numerous discussions on Paleogene vegetation development; to Karolin 18 Moraweck (Dresden) for discussions on paleoclimate estimation in the middle and late 19 Eocene using the Coexistence Approach and for drafting the fossil site map (Fig. 1). The 20 Royal Botanic Gardens Kew Herbarium and the National Botanic Gardens Ireland provided 21 live and herbarium specimens of Trigonobalanus doichangensis, T. verticillata, Castanopsis 22 cuspidata and Lithocarpus henryi, for analysis and selection of Nearest Living Equivalent for 23 pCO₂ calibration. Finally, Helen Coxall (Stockholm) is thanked for constructive criticism on 24 an earlier version of this manuscript and many helpful discussions. 25

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1 Tables

Table 1. Lithostratigraphic and phytostratigraphic positions of the *Eotrigonobalanus furcinervis*-containing fossil taphocoenoses in the Weißelster Basin (central Germany);
lithostratigraphy after Standke et al. 2010, spore-pollen zonation after Krutzsch (2011).

Assemblage / site	Reference for fossil flora	Formation	Member	Horizon	Epoch	Spore- pollen zone
Witznitz	Mai and Walther 1991	Cottbus	Thierbach	Witznitz	end of Oligocene	Π
Espenhain- Störmthal	Mai and Walther 1991	Cottbus	Thierbach	Witznitz	end of Oligocene	II
Haselbach 2	Mai and Walther 1978	Böhlen	Gröbers	Haselbach	earliest Oligocene?	20A/B
Schleenhain 4	Kunzmann and Walther 2012	Böhlen	Gröbers	Haselbach	earliest Oligocene?	20A/B
Schleenhain 3	Kunzmann and Walther 2002	Borna	Domsen	overlying bed of lignite seam 230	latest Eocene	19 (?)
Schleenhain 2	Ferdani 2014, Mai and Walther 2000	Borna	Bruckdorf	underlying bed of lignite seam 230 and leaf measure in lignite seam 230	late Eocene	180
Haselbach 1	Mai and Walther 2000	Borna	Bruckdorf	intercalated bed between lignite seam 23u and 23o	late Eocene	18uo
Schleenhain 1	Hennig and Kunzmann 2013	Borna	Bruckdorf	overlying bed of lignite seam 23u	late Eocene	18u
Knau	Mai and Walther 2000	Borna	uncertain	fluvial deposit	late Eocene	17/18
Profen-Süd	Fischer in Mai and Walther 2000	Profen	Wallendorf	underlying bed of lignite seam 1	late middle Eocene	17

- 1 Table 2. The Saxony *Eotrigonobalanus furcinervis* database, including spore-pollen zones
- 2 (Krutzsch 2011) and epoch inferred from them, stomatal density counts and pCO₂ calibration
- 3 results, all shown with standard deviation, average pCO_2 in bold. Comparison to previously
- 4 published stomatal proxy-based pCO₂ results from central Germany and nearby regions listed
- 5 in the far right column.

Sites		a (SD	60 M	pCO ₂	pCO ₂		Other studies
		Spore/	(stomata/mm ²)	pCO ₂ Min	Max	Average	No. of	CO_2 ppm
	Epoch	pollen zone		(ppm)	(ppm)	(ppm)	leaves	
Witznitz			569.02 +					~ 420 to ~ 530
Espenhain-	latest		108 40	351.6+	600.02 +	475 81 +		npm ¹
Störmthal	Oligocene	II	100.10	79.12	135.03	107.08	45	ppm
Kleinsaubernitz	late		623.29 ±	316.8 ±	540.71 ±	428.76 ±		$\sim 400 \text{ ppm}^1$
	Oligocene	20G	97.82	58.41	99.7	79.05	25	11
Schleenhain 4,	earliest		657.13 ±	302.5 ±	516.29 ±	409.40 ±		n/a
Haselbach 2	Oligocene (?)	20 A/B	118.98	59.31	101.23	80.27	21	
Schleenhain 3			642.88 ±	303.1 ±	517.24 ±	$410.17 \pm$		n/a
	latest Eocene	19	84.05	35.54	60.66	48.10	11	
Schleenhain 2			$740.65 \pm$	$269.56 \pm$	$460.05 \pm$	$364.80 \pm$		n/a
	late Eocene	18 o	148.90	53.01	90.48	71.74	39	
Haselbach 1	late Eocene	18 uo	$505.88 \pm$	$373.50 \pm$	$637.45 \pm$	$505.48 \pm$	2	$\sim 470 (ave.)^2$
			47.06	35.99	61.43	48.72		$\sim 270 (min)^2$
								$\sim 710 (max)^2$
Schleenhain 1			$661.18 \pm$	$296.15 \pm$	$505.43 \pm$	$400.79 \pm$		n/a
	late Eocene	18 u	90.93	44.65	76.206	60.429	4	
Knau			$495.50 \pm$	$397.33 \pm$	$678.12 \pm$	$537.73 \pm$		n/a
	late Eocene	17/18	77.80	68.7	117.25	92.98	4	
Profen-Süd			$426.14 \pm$					$\sim 470 (ave.)^2$
	late middle		83.56	$467.87 \pm$	$798.51 \pm$	633.19 ±		$\sim 270 (min)^2$
	Eocene	17		101.78	173.71	137.74	1	$\sim 710 (max)^2$

6 ¹Applying Konrad *et al.* (2008) stomatal optimization model in a multispecies consensus approach (Grein *et al.*, 2013)

7 ²Applying Konrad *et al.* (2008) stomatal optimization model to stratigraphically lumped *Eotriginobalanus furcinirvis* samples from Profen

8 and Haselbach (Roth-Nebelsick et al., 2012). n/a = Individual site CO₂ data not reported so direct comparison not possible.

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1 Figures



Figure 1. *Eotrigonobalanus furcinervis* (Rossm. 1840) Walther et Kvaček in Kvaček and
Walther 1989, A: mass occurrence of leaves in lignite, Schleenhain opencast mine, Saxony,
Germany, site Schleenhain 2, Borna Formation, Bruckdorf Member, late Eocene (Priabonian),
SPP zone 180, MMG PB SchleOE 535; B: abaxial leaf cuticle with stomata and trichome
bases, Schleenhain opencast mine, Saxony, Germany, site Schleenhain 4, Böhlen Formation,
Gröbers Member, earliest Oligocene (Rupelian), SPP zone 20A/B, slide MMG PB SchleMO
11/05 from leaf SchleMO 556/2.



Figure 2: Sites (asterisks) of *Eotrigonobalanus furcinervis*-containing fossil taphocoenoses in
central and east Germany considered in the present investigations, note: the Schleenhain and
Haselbach opencast mines revealed taphocoenoses in four and two distinct lithostratigraphic
positions respectively (see also stratigraphic chart in fig. 3). Map legend: D = Germany, CZ =
Czech Republic, PL = Poland, FR = France, NL = the Netherlands.

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Figure 3. Stratigraphic position of the assemblages with Eotrigonobalanus furcinervis, 8 regional lithostratigraphy and Krutzsch's (2011) correlation to the spore-pollen zones 9 including his proposed correlation of spore-pollen zones to global scale (see text section 2.2 10 for explanation and comments on dating uncertainty); black vertical bars next to assemblage 11 names are the temporal uncertainty (based on a combination of lithostratigraphic information 12 of the respective unit and spore-pollen zonation); bars of Schleenhain 1 and 2 are not to scale 13 because gaps in the sediment deposition of the respective units are not equivalent to the 14 15 duration of spore-pollen zones; gaps between the Eocene spore-pollen (sub-)zones illustrate gaps in the terrestrial sediment record, i.e. erosion. For horizon information see table 1. 16



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Figure 4. CO₂, vegetation and climate trends through the Cenozoic. The most significant 2 3 changes in pCO₂, forest ecosystem composition (A) and continental climate as tracked by terrestrial plants (B) take place in the late Eocene, whereas the most significant change in 4 global temperatures as tracked by marine isotopes (C) takes place at the Eocene-Oliogocene 5 boundary, indicating that the significant climate transition at the Eocene-Oligocene boundary 6 7 was preceded by a gradual decrease in pCO₂ during the late Eocene. (A): pCO₂ estimates from fossil stomata (this study pink with black error bars) in the context of existing stomatal 8 proxy estimates (in grey from Beerling and Royer, 2011) in a chronostratigraphic framework. 9 Vertical bar shows the gradual late Eocene vegetational restructuring of the dominantly 10 evergreen forests of the Weißelster and North Bohemian basins studied here (dark green to 11 light green), suggesting a potential causal role of pCO₂ decline in the changing ecological 12 forest composition (*Kunzmann and Walther, 2012; ** Kvaček et al., 2014; Kunzmann et al., 13 submitted). Note that the assigned ages for CO₂ values from this study are estimated based on 14 the biostratigraphic controls presented in Fig 3. Absolute ages were not available for any of 15 the nine fossil study sites (Table 2) although clear superposition information is available 16 throughout allowing good estimates of the temporal sequence of CO₂ estimates (see Fig 3). 17 (B): Continental temperature curve: Record of continental cold month mean temperature for 18 Central Europe during the last 45 My, redrawn from Mosbrugger et al (2005). Horizontal bars 19

represent coexistence intervals. Orange curve shows data from the Weißelster and Lausitz 1 Basins, northeast Germany; blue curve shows data from the Lower Rhine Basin, northwest 2 Germany (see Mosbrugger et al., 2005 for details). (C): Global climate (temperature) curve 3 derived from stacked records of deep-sea benthic foraminiferal oxygen-isotopes: a proxy for 4 relative changes in marine temperature in the late Eocene prior to ice build up, based on 5 updated records from Deep Sea Drilling Project and Ocean Drilling Program sites. Raw data 6 7 is smoothed by using 15-point running mean, to minimize biases introduced by uneven temporal and spatial distribution of records (data from Zachos et al., 2001; 2008; and 8 9 references therein).