VARIATION IN STOMATAL NUMBERS OF *GLOSSOPTERIS* LEAVES FROM THE LOWER PERMIAN OF PARANÁ BASIN, BRAZIL

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ABSTRACT – The stomatal density and index in compressed leaves of *Glossopteris communis* from two different roof shales from the Lower Permian in Paraná Basin, Brazil (Western Gondwana) have been investigated to test the possible relationship with modeled global changes in atmospheric CO₂ during the Phanerozoic. The obtained parameters show that the genus *Glossopteris* from the Cool Temperate biome can be used as CO₂-proxy, despite the impossibility of being compared with living relatives or equivalents. When confronted with already published data for the Tropical Summer Wet biome, the present results confirm the detection of low levels of atmospheric CO₂ during the Early Permian, as predicted by the modeled curve. Nevertheless, the lower stomatal numbers detected at the climax of the coal interval (Faxinal Coalfield, Sakmarian) when compared to the higher ones obtained in leaves from a younger interval (Figueira Coalfield, Artinskian) could be attributed to temporarily high levels of atmospheric CO₂. Therefore, the occurrence of an extensive peat generating event at the southern part of the basin and subsequent greenhouse gases emissions from this environment may have been enough to reverse regionally and temporarily the reduction trend in atmospheric CO₂. Additionally, the Faxinal flora is preserved in a tonstein layer, which is a record of volcanic activity that could also cause a rise in atmospheric CO₂. During the Artinskian, the scarce generation of peat mires, as revealed by the occurrence of thin and discontinuous coal layers, and the lack of volcanism evidence would be insufficient to affect the general low CO₂ trend.

Key words: paleo-CO₂ proxy, pteridosperms, fossil cuticles, Rio Bonito Formation, peat-forming floras, Gondwana.

RESUMO – Frequências estomáticas foram calculadas em cutículas de *Glossopteris communis*, procedentes de dois afloramentos do Permiano Inferior na bacia do Paraná, com o objetivo de relacioná-las com variações na concentração atmosférica de CO₂ modeladas para o Fanerozoico. Os resultados indicam que as glossopterídeas do bioma Temperado Frio podem ser utilizadas como equivalentes climáticos para inferência de níveis de CO₂ paleoatmosférico, apesar da impossibilidade de estabelecer um equivalente ecológico atual. Quando confrontados com dados obtidos para o bioma Tropical de Verão Úmido, os resultados aqui apresentados confirmam a detecção de baixos níveis de CO₂ na atmosfera durante o período, de acordo com o modelamento da curva. Porém, as frequências estomáticas mais baixas detectadas no clímax do intervalo formador de carvão (jazida de Faxinal, Sakmariano), quando comparadas às frequências mais altas obtidas nas folhas de intervalo mais jovem (jazida de Figueira, Artinskiano), poderiam ser atribuídas a níveis temporariamente altos de CO₂ na atmosfera. A ocorrência de extenso evento gerador de turfa na parte sul da bacia, com a consequente emissão de gases-estufa deste ambiente, poderia ter sido suficiente para reverter a tendência de queda de CO₂ atmosférico em âmbito regional ainda que de forma temporária. Além disso, a flora de Faxinal está preservada em uma camada de *tonstein*, registro de atividade vulcânica que poderia ter afetado os níveis de CO₂. No Artinskiano, a formação de turfeiras em menor escala, evidenciada por camadas de carvão descontínuas e delgadas, sem registro de vulcanismo, teria sido insuficiente para afetar a tendência de baixo CO₂.

Palavras-chave: equivalente paleoclimático para CO₂, pteridospermas, cutículas fósseis, Formação Rio Bonito, floras formadoras de turfa, Gondwana.

INTRODUCTION

The applicability of fossil plant leaves as biosensors of Earth's paleoatmospheric CO₂ fluctuations is being increasingly ratified through the comparisons between the

stomatal numbers (stomatal density and index) of fossil, living and herbarium material (McElwain, 1998; Kouwenberg *et al.*, 2003; Wagner *et al.*, 2005) since Woodward (1987) observed an inverse correlation between stomatal frequency in extant plants and the concentration of atmospheric CO₂. Even

though earlier studies were focused on historical and recent pre-historical approaches (Beerling & Chaloner, 1992, 1993; Kürschner *et al.*, 1996), this method has encouragingly been applied to cuticular analyses of leaves from different geological intervals, from the Early Devonian through the Holocene (Van der Burgh *et al.*, 1993; McElwain & Chaloner, 1995, 1996; Kürschner *et al.*, 1998; McElwain *et al.*, 1999; Beerling, 2002). In general, the estimated results based on stomatal parameters help to calibrate the curve of Phanerozoic levels of paleoatmospheric CO₂ calculated by the GEOCARB model (Berner, 1991, 1994; Berner & Kothavala, 2001).

Different authors pointed out that environmental factors such as temperature and humidity can influence stomatal density by altering the size and/or spacing of the epidermal cells (Salisbury, 1927; Tichá, 1982; Roth-Nebelsick, 2005). However, atmospheric CO₂ is the main factor that influences the development of stomata from epidermal cell initials, thus directly affecting the number of stomatal pores on the leaf surface. Calculation of the stomatal index, which is a ratio of the number of stomatal cells to the total number of cells (epidermal and stomatal) on the leaf surface, separates the direct effects of atmospheric CO₂ from the indirect effects of all other environmental parameters on stomatal numbers (McElwain, 2003). Therefore the stomatal index is a much more stable parameter and recent data ratify it as being amongst the most robust proxies for paleoatmospheric CO₂ (Royer *et al.*, 2001a; Beerling *et al.*, 2009).

When dealing with Mesozoic and Cenozoic floras, results from fossils have been compared with close living relatives (McElwain *et al.*, 1999; Chen *et al.*, 2001; Royer *et al.*, 2001b), called nearest living relative (NLR) species (Mosbrugger, 1999). The method is based on the premise that climatic tolerances of the fossils would be similar to those of their NLR (Taylor *et al.*, 2009).

In an attempt to overcome the difficulties of identifying taxonomic affinities between extinct and extant forms in large time spans (*i.e.* Paleozoic to Recent), comparisons have been made between fossil and nearest living equivalent (NLE) species. These are, as far as possible, species from the present day of comparable ecological setting and/or structural similarity to their fossil counterparts (McElwain & Chaloner, 1995). However, it is not always possible to find living matches to extinct plant species.

Cleal *et al.* (1999) presented an alternative approach to this problem focusing on the foliage of a single species, *viz.* *Neuropteris ovata* Hoffmann, a Paleozoic pteridosperm (Medullosales) from the Equatorial swamp forest, to test whether its stomatal frequencies work as CO₂-proxies at different stratigraphic levels during the Upper Carboniferous. They observed a marked drop in stomatal density (SD) and stomatal index (SI) in the very Early Stephanian that could reflect a rise in paleoatmospheric CO₂ due to a drastic contraction of tropical forests.

The results obtained by Retallack (2001) for atmospheric CO₂ through SI values in herbarium and fossil leaves of *Ginkgo* since late Triassic and Paleozoic pteridosperms (*Lepidopteris*, *Tatarina* and *Rachyphyllum*) as CO₂ proxies, agreed partially with the geochemical models proposed for comparison by the author. A transfer function, in which

stomatal parameters of extant species calibrated against CO₂ are replaced by those of fossil species (Roth-Nebelsick, 2005), available for *Ginkgo* was applied to the stomatal measurements in the pteridosperms in order to calculate the corresponding atmospheric CO₂ levels.

Vördding & Kerp (2008) analyzed cuticles of the Upper Permian pteridosperm species *Peltaspermum martinsii* (Germar) Poort & Kerp from different coeval outcrops in the Southern Alps to test its applicability as a paleo-CO₂ proxy. The authors also employed a transfer function previously determined for *Ginkgo* leaves in order to establish comparisons with the atmospheric CO₂ models. Their estimates for the paleoatmospheric CO₂ levels resulted much higher than those of the geochemical models. Stomatal parameters were considered by the authors as speculative due to the use of a transfer function which has been established for a distinct taxon.

Vördding *et al.* (2009) tested stomatal parameters of a pteridosperm, *Odontopteris brardii* (Brongniart) Sternberg, from two geologically and ecologically different localities in Europe (Germany and France) in the late Stephanian. Similar values found in all specimens suggested that habitat-specific factors did not significantly affect SI. The results inferred from the SI values, despite being a little lower than the ones from the paleoatmospheric CO₂ models used for comparison, still corroborate the hypothesis of low levels of the gas during the late Paleozoic.

However, the determination of species which could be modern analogues to the extinct group of pteridosperms, abundant in the fossil record and often with well preserved cuticles, still remains an unresolved matter.

The *Glossopteris* Flora

In the Gondwanan realm, the Phylum Pteridospermophyta (Taylor *et al.*, 2009) was mainly represented by the Glossopteridales, which dominated the flora during the Permian (Gastaldo *et al.*, 1996). The plants of this group were prominent elements of temperate ecosystems, primarily those of wetlands, and a swampy habitat has been inferred by their dominance in taphofloras which have been recovered mainly from strata associated with Permian Gondwana peat and coals (Anderson & Anderson, 1985; Pigg, 1990; Guerra-Sommer *et al.*, 1991; Pigg & McLoughlin, 1997; Banerjee, 2005). Nevertheless, fossil remains are known from fluvial and lacustrine deposits as well (Archangelsky *et al.*, 1996; Srivastava, 1997; Prevec *et al.*, 2009, 2010; Holz *et al.*, 2010), suggesting that this group of plants could also occur at wetter areas of seasonally dry environments.

Over 200 morphospecies of the genus *Glossopteris* have been described from the Permian rocks of the Gondwanaland countries, *viz.* Africa, Antarctica, Australia, India and South America (Stewart & Rothwell, 1993), many of them are based exclusively on the macromorphology of the impression leaves. Epidermal features of leaf adpressions have been used to distinguish a number of *Glossopteris* species in material recovered mainly from roof shale levels (Pant & Gupta, 1968, 1971; Pant & Singh, 1971; Guerra-Sommer, 1992).

In Brazilian Gondwana, abundant and well preserved

Glossopteris compressed leaves have been described from muddy and silty sand layers interbedded with coal seams in coalfields in the northeastern and southern areas of the coal bearing Rio Bonito Formation from the Lower Permian of the Paraná Basin. *Glossopteris* leaves are also known from older glaciocontinental facies of the Itararé Group (Asselian to Sakmarian) and younger geologic strata of Irati, Teresina and Rio do Rasto formations (Late Artinskian to Wuchiapingian), however they are in the form of impressions only (Guerra-Sommer & Cazzulo-Klepzig, 2000).

The present study is based on the compression leaves of *Glossopteris communis* Feistmantel, collected from the roof shale sediments of the Rio Bonito Formation exposed in Figueira Coalfield, in the northeastern, and Faxinal Coalfield, in the southern Paraná Basin. Besides their macromorphology, the similar epidermal features among the *Glossopteris* leaves allowed us to consider them as a single biological entity. The

use of a single taxon from a specific habitat (peat swamps in the present study) is an important requirement when comparing the effects of altered atmospheric CO₂ regimes on parameters like stomatal frequency (Cleal *et al.*, 1999).

The aim of this study is (i) to test whether cuticles of the extinct Gondwanan taxon *Glossopteris communis* are suitable as proxies for CO₂ concentrations in the paleoatmosphere, and (ii) to find lines of evidence to support the inferences from the obtained stomatal patterns.

GEOLOGICAL AND PALEBOTANICAL SYNTHESIS

The Paraná Basin is a large (1.4.10⁶ km²) intracratonic sag basin covering part of southern Brazil, Paraguay, Uruguay and Argentina (Figure 1). Basin floor subsidence, in addition to Paleozoic sea-level changes, created six second-order

Figure 1. Schematic geological map showing the main supersequences of the Paraná Basin (modified from Santos *et al.*, 2006). Coalfields are represented according to Horbach *et al.* (1986). Faxinal Coalfield is indicated by arrow 1 and Figueira Coalfield by arrow 2.

sequences deposited from the Ordovician to Late Cretaceous, separated by regional unconformities (Milani, 1997). The coal-bearing Rio Bonito Formation, which is the subject of this study, occurs at the base of the second order Carboniferous-Early Triassic sequence (Gondwana I Supersequence). Extensive peat swamps developed during the deposition of Rio Bonito Formation in Paraná Basin at a paleolatitude of approximately 50°S, included in the Cool Temperate biome (Rees *et al.*, 2002).

The study area comprises the Faxinal Coalfield, in the southeastern outcrop belt of the Rio Bonito Formation in southern Brazil, Rio Grande do Sul State, and the Figueira Coalfield, located in the eastern area of the Brazilian portion of the basin in Paraná State (Figure 1).

The Faxinal Coalfield, mined by the Companhia de Pesquisas e Lavras Minerais - COPELMI, is located near the town of Arroio dos Ratos, about 120 km west of Porto Alegre, Rio Grande do Sul State. The Faxinal coal succession includes five coal seams, named I, IM, M, MS and S from the base to the top of the sequence. The present study focuses on a fossiliferous argillite approximately 7 cm thick, interbedded with the upper coal seam (S). Petrographic studies in this argillite reveal a volcanic ash-fall origin for this sediment, thus identifying it as a tonstein (Guerra-Sommer *et al.*, 2008a). Ion microprobe (SHRIMP II) dating of zircons from the tonstein by Guerra-Sommer *et al.* (2008b) yielded an age of 290.6 ± 1.5 Ma (Sakmarian).

According to Simas (2008), the original thickness of the unconsolidated ash has been inferred at about 28 cm. This evidence suggests the existence of an active and widespread explosive volcanic event in the Lower Permian of Western Gondwana. Volcanic activities in the surrounding areas have been assumed as ignition sources for wildfires, which produced large amounts of charcoal at the basal level of the tonstein layer (Jasper *et al.*, 2009).

The compression taphoflora preserved in the Faxinal tonstein is predominantly gymnospermous (Guerra-Sommer, 1992), consisting of glossopterid fragments that constitute about 70% of the entire assemblage. Taxa identified include *Glossopteris brasiliensis* Guerra-Sommer, *G. papillosa* Guerra-Sommer, *G. similis-intermittens* Guerra-Sommer, *Plumsteadia semnes* Rigby and *Platycardia* sp. Cordaitalean taxon *Rufloia gondwanensis* Guerra-Sommer and *Sphenopteris* cf. *ischanovens* are subdominant elements (Guerra-Sommer, 1988).

Palynological analyses of the Faxinal coals indicate the dominance of bisaccate (striate and non-striate) and monosaccate pollen grains related to glossopterids, conifers and cordaitaleans (Cazzulo-Klepzig *et al.*, 2007). These pollen grains are followed by a low proportion of spores of lycopsids, filicopsids and scarce sphenopsids which emphasizes the minor contribution of pteridophytic plants to the peat-coal forming vegetation (Cazzulo-Klepzig *et al.*, 2009).

The stratigraphic analyses conducted by Schneider *et al.* (1974) in the states of Santa Catarina and Paraná defined three members for the Rio Bonito Formation: Triunfo (base), Paraguaçu (intermediate) and Siderópolis (top). According

to those authors, in the State of Paraná, the thickness of the Rio Bonito Formation decreases northwards, mainly because of the thinning and wedging out of the Triunfo Member, which disappears at the south of the State of São Paulo.

Soares & Cava (1982) concluded that the most extensive peat formation event, located in the northern area of the Paraná State, was associated to deltaic plain environments correlative to the upper part of the Triunfo Member and at the base of Paraguaçu Member. Morrone & Daemon (1985) and Della Fávera *et al.* (1994) agreed with these inferences.

According to Zacharias & Assine (2005), different facies associations from the Rio Bonito Formation in the northeast of the Paraná State result from sedimentation on an irregular depositional surface. Peat swamps, of restricted occurrence, developed in paleoenvironments related to delta plains in the central portion of the estuary and were included in the basal part of the Paraguaçu Member.

The coal seam in Figueira Coalfield occurs on average at 40 m depth and is subdivided into a lower seam (0.4 m thick) and an upper seam (0.2 m thick) separated by a silty-sandstone layer of approximately 0.4 m thickness (Levandowski & Kalkreuth, 2009).

Palynological data are not available for Figueira Coalfield. The only results concerning microfloristic analyses were registered by Ricardi-Branco *et al.* (2002), who identified megaspores of the genera *Lagenosporites*, *Sublagenicula* and *Setosisporites*, of botanical affinity with arborescent lycophytes from siltstones immediately overlying the coal bed.

The Figueira paleoflora, recovered from siltstone layers interbedded with coal seams in different outcrops, was categorized by Ricardi-Branco & Rösler (2004) under the following plant communities: swamp community [*Brasilodendron* cf. *B. pedroanum* (Carruthers) Chaloner, Leistikov & Hill and *Paracalamites australis* Rigby]; flood plain community [*Sphenophyllum brasiliensis* Rösler, *Annularia occidentalis* Rösler, *Asterotheca derbyi* Rösler, *Pecopteris cambuyensis* (Read) Rösler, *Sphenopteris lobifolia* Morris and *Glossopteris communis* Feistmantel] and elevated terrain community (*Paranocladus dusenii* Florin and *Paranospermum cambuiense* Ricardi-Branco). Using coal petrography techniques Sbaraini (2006) inferred that the peat-forming flora was composed mainly by arborescent plants, developed in transitional paleoenvironment controlled by fluvial and tidal processes.

Based on sequence stratigraphy, Holz *et al.* (2010) proposed a chronostratigraphic chart (Figure 2) for the Paraná Basin in which the Rio Bonito succession is included in two third order sequences. In relation to previous studies, this chart presents a new stratigraphic model for peat generation at northeastern and southern regions of the basin. The Siderópolis Member and its coal seams in Santa Catarina and Paraná States are linked to the Late Paleozoic Third-Order Sequence (LPTS) number 4 (Artinskian); in Rio Grande do Sul State, the Rio Bonito Formation is not formally subdivided into different lithostratigraphic members and the coal seams are included in the underlying LPTS-3 (Sakmarian). This new stratigraphic model is adopted in the present study.

Figure 2. Simplified chronostratigraphic chart of the Late Carboniferous/Permian succession of the eastern border of the Paraná Basin indicating the approximate position of the Faxinal and Figueira Coalfields (modified from Holz *et al.*, 2010). Stratigraphic position of the Faxinal Coalfield is based on radiometric age determination according to Guerra-Sommer *et al.* (2008b). Stratigraphic position of the Figueira Coalfield is based on sequence stratigraphy according to Holz *et al.* (2010).

MATERIAL AND METHODS

Material

Numerous leaves of *Glossopteris* occur haphazardly in different fossiliferous layers of the slabs. Most of the compressions are fragments of the detached leaves and some of them correspond to leaves organically attached to short shoots in whorls (Guerra-Sommer, 1988). Selected leaves are oblong to lanceolate in shape, with entire margins, midrib extends from the base (when present) to the apex and veins are a little arched.

The re-examination of the material described by Fittipaldi & Rösler (1985) and Guerra-Sommer (1992) indicates that assemblages from Figueira and Faxinal Coalfields show an overall similarity in regard to the epidermal micromorphology, particularly the stomatal complex and the clear delimitation

of costal and intercostal zones on one of the leaf faces. However, the stomatal pattern of the *Glossopteris communis* leaves described by Fittipaldi & Rösler (1985) for the Paraná State remained insufficiently known because their stomatal description was based on the sparse ones from the adaxial leaf surface and no stomatal counts were carried out.

Pant & Gupta (1968) described small cuticular pieces of *Glossopteris communis* derived from macerated material showing polygonal to irregular-shaped cells with straight walls and gave an emended diagnosis of this species. However, only non-stomatiferous zones have been observed.

Methods

Cuticles were mechanically pulled out from the surface of the *Glossopteris* leaves preserved as large fragments on the rock matrix (Figure 3) with the help of dissecting needles and

Figure 3. Rock slabs bearing compression specimens of *Glossopteris* leaves. **A**, tonstein slab from the Faxinal Coalfield stored in the paleobotanical collection of DPE-IG/UFRGS under the number PB4835; **B**, siltstone slab from the Figueira Coalfield housed in the collection of GSA-IGc/USP under the number GP/3E-6990. Scale bars = 2 cm.

scalpel and then macerated in hydrofluoric acid for about 48 hours (Kerp, 1990). The dissolution of the rock allowed the cuticles to be released into the liquid and then to be filtered and gently washed in distilled water until neutralization.

Cuticles from Figueira Coalfield were sampled from a total of 11 leaf fragments. They were bleached with dilute Schulze solution for approximately 48 to 60 hours, using Fittipaldi & Rösler's (1982) maceration process. After being washed with distilled water, the cuticles were treated with 10% KOH for five minutes and then washed again.

The dilute Schulze solution was also employed in the preparation of the material from Faxinal Coalfield but it did not work. Then an alternative method derived by Gray (1965) was employed to make use of saturated dry Schulze solution (70% HNO_3 and KClO_3 7:1).

Cuticles from Faxinal Coalfield were sampled from a total of 59 leaf fragments. They were macerated with the dry Schulze solution for two hours, then gently washed and put in 5% KOH solution for about five minutes and washed again with distilled water as per Kerp's procedure (1990).

All cuticles were dehydrated in pure glycerin (Krings & Kerp, 1997) and mounted in glycerin jelly slides with the help of a stereoscopic microscope Leica S8 APO using incident and transmitted light under magnifications between 20x and 160x.

Slides were examined using a Zeiss Axioplan 2 microscope with Plan-Neofluar objectives and H/DIC II differential interference contrast filter (Kerp & Krings, 1999). Pictures were taken using a Sony/Ziess Cyber-shot DSC-S75 camera having 3.3 Megapixels at 400x magnification and the images were analyzed with the Zeiss Axio Vision 4.8.1 software.

Epidermal cells and stomata were counted and measured in the intercostal, stomatiferous zones. The stomatal density (SD) was determined by manually marking the number of stomata on the computer screen and dividing the result by the area value of the observed field to obtain the number per square millimeter (Salisbury, 1927).

The stomatal index (SI) was calculated using the formula $SI = 100S/(E+S)$ introduced by Salisbury (1927), where S represents the number of stomata and E the number of epidermal cells per unit area (cf. Kerp, 1990). This formula expresses the stomatal numbers independently of the size of the epidermal cells in order to compensate for the effects of leaf expansion (Poole & Kürschner, 1999). All cells were counted manually by marking them on the computer screen with the help of a software.

Data analysis was performed using the MULTIV 2.63 for Windows statistical software (Pillar, 2006). ANOVA with permutation tests (Pillar & Orlóci, 1996) was employed to evaluate the effects of the factor 'site' (Faxinal vs. Figueira) on the stomatal results. Euclidean distances were used as dissimilarity index, and the sum of squares between groups (Qb statistics) was used as test criterion (Legendre & Legendre, 1998).

The fossils from Figueira Coalfield are housed at the collection of the Departamento de Geologia Sedimentar e Ambiental, Instituto de Geociências, Universidade de São Paulo (GSA-IGc/USP) under the code GP3E. The fossils from Faxinal Coalfield and all the slides prepared for this study are housed under the code PB at the collection of the Paleobotanical Section of the Departamento de Paleontologia e Estratigrafia, Instituto de Geociências, Universidade Federal do Rio Grande do Sul (DPE-IG/UFRGS), Porto Alegre, Brazil.

RESULTS

The material from Faxinal Coalfield yielded excellent slides and, from a total of 59 leaf fragments sampled, 43 cuticle fragments have been analyzed.

The cuticles from Figueira Coalfield were sampled from a total of 11 leaf fragments. However, only 5 fragments yielded well preserved cuticles to be useful for measurements and photographs.

Epidermal cells were clearly visible in the Faxinal material (Figure 4), while in the Figueira cuticles the cell walls were not always preserved. Therefore, cell counts could not be obtained for the most part of Figueira material.

The adaxial leaf surface of *Glossopteris communis* is highly cutinized, mostly devoid of stomata and is composed of elongate, subrectangular cells. Papillae are absent from this surface. The abaxial epidermis is less cutinized, with elongated polygonal or subrectangular cells bearing a single papilla in the costal zones, while the cells of the intercostal zones consist mostly of subsidiary cells which are polygonal, sometimes subtriangular, subquadrangular, oval or irregularly shaped. Stomata of abaxial surface are haplocheilic and occur randomly oriented and sunken, with 4 to 8 (generally 5-7) subsidiary cells arranged in irregular rings, each bearing a prominent hollow papilla arching over the stomatal pore in the center. Subsidiary cells are sometimes shared between adjacent stomata (Figure 4).

The results of stomatal counts are given in table 1. Calculations for both SD and SI for the two stratigraphical levels indicate an increase of approximately 20% in stomatal numbers from the older (Sakmarian) to the younger (Artinskian) strata. Mean densities increased from 234.73 mm⁻² in the Faxinal Coalfield to 284.14 mm⁻² in the Figueira Coalfield, and indices increased respectively from 15.7 (mean) to 18.9. The trend in stomatal density observed in the Sakmarian to the Artinskian coalfields in the Rio Bonito Formation is represented in Figure 5.

SI calculation could be carried out on only one cuticle fragment from Figueira Coalfield and therefore statistical analysis could only be applied to the SD data.

The difference found for SD between the two sites is statistically significant, being P between groups = 0.0018 (Table 2). The test applied is sufficiently robust and did not require data transformation, despite the sampling difference between the two sites in the data here presented (Pillar & Orlóci, 1996).

Table 1. Calculation results of epidermal cell density (ED), stomatal density (SD) and stomatal index (SI). **A**, results for the Faxinal Coalfield (Sakmarian); **B**, results for the Figueira Coalfield (Artinskian). **Abbreviations:** **NL**, number of leaves sampled; **NC**, number of cuticle fragments analyzed; **NE**, number of epidermal cells counted; **NS**, number of stomata counted; **n.a.**, not available.

(A) Faxinal Coalfield (Sakmarian)	ED	SD	SI
Mean	1269.74	234.73	15.7
Minimum	977.68	160.78	12.6
Maximum	1779.03	328.06	18.75
Standard deviation	203.80	36.26	1.51
Coefficient of variation (%)	16.05	15.45	9.66

NL=59; NC=43; NE=5355; NS=992

(B) Figueira Coalfield (Artinskian)	ED	SD	SI
Mean	979.14	284.14	18.9
Minimum	n. a.	204.04	n. a.
Maximum	n. a.	481.26	n. a.
Standard deviation	n. a.	74.81	n. a.
Coefficient of variation (%)	n. a.	26.33	n. a.

NL=5; NC=17; NE=90; NS=355

Figure 4. Transmitted light microscopy of the stomatal pattern in *Glossopteris communis* under 400x magnification. Stomatal apparatus is composed of 4-8 subsidiary cells, generally 5-7, each bearing a hollow papilla which arches over the stomatal pore in the center. * = note the subsidiary cell shared between two adjacent stomatal apparatus. Slide of the Faxinal Coalfield material stored in the DPE-IG/UFRGS collection under the number PB4801-37-021. Scale bar = 100 μ m.

Figure 5. Trends in stomatal density and index in *Glossopteris communis* observed in the Sakmarian (Faxinal Coalfield) to Artinskian (Figueira Coalfield) of the Paraná Basin, Brazil (after Cleal *et al.*, 1999). The Faxinal sample is shown against the absolute dating by Guerra-Sommer *et al.* (2008b). The Figueira sample is plotted according to data by Holz *et al.* (2010). The bar for each sample represents the total range in stomatal numbers observed, joined by the mean values through a dashed line. Stomatal index of Figueira samples is based on only one count.

Table 2. Results of ANOVA data analysis with permutation tests to evaluate the effects of the factor 'site' (Faxinal vs. Figueira) on the stomatal densities. * = *P* probabilities generated for sum squares (Qb), except for interactions, where $F=Qb/Qw$ was employed as testing criterion.

Source	Sum squares (Q)	$P(Qb_{NULL} \geq Qb) *$
site		
Between groups	29742	0.0018

DISCUSSION

There are no published records that could be directly compared to the present results. Earlier studies by Pant & Gupta (1968) for cuticular analyses in different species of *Glossopteris* from the Raniganj Coalfield, India (Wordian), do not include stomatal counts for *G. communis*, as did not the description by Fittipaldi & Rösler (1985). Based exclusively on micromorphological features Guerra-Sommer (1992) described a new species for the Faxinal Coalfield, *G. papillosa*, which could be *G. communis*. Given that the specific epithet *papillosa* had already been employed to designate a *Glossopteris* species from India by Srivastava (1969), the designation by Guerra-Sommer (1992) is invalid.

Taking into account that *Glossopteridales* is an extinct group of plants with no living relatives or equivalents, this paper does not apply the transfer functions available for Ginkgo to SI values of *Glossopteris communis*. As a consequence, the data here obtained cannot be plotted on the curve modeled by Berner & Kothavala GEOCARB III (2001) and Berner GEOCARBSULF (2006) for atmospheric CO_2 variation in the Phanerozoic.

Thus, this study adopted the alternative approach given by Cleal *et al.* (1999) of comparing data of a single species from different fossil assemblages in similar paleoenvironments, but within distinct stratigraphic levels, to test the capability of detection of CO_2 atmospheric fluctuations by *Glossopteris communis*.

According to Royer (2006), low levels of atmospheric CO_2 are estimated for the Namurian-Kazanian interval. Different authors (Crowley & Berner, 2001; Berner & Kothavala, 2001; Royer *et al.*, 2004) associated this event to an extensive Phanerozoic glaciation, suggesting strong coupling between CO_2 and temperature. Isbell *et al.* (2003), instead of a continuous record of ice, argued for two shorter phases of glaciation. The coal interval from the Lower Permian of Paraná Basin can be associated with the termination of the second ice phase and was developed during a cool phase, from 290 until 267 Ma (Royer, 2006).

The stomatal numbers obtained in *Glossopteris communis* can be confronted to those of the Early Permian conifer *Lebachia frondosa* (Renault) Florin. This species, among other taxa, was used by McElwain & Chaloner (1995) to support the hypothesis that stomata parameters in fossil leaves would have potential value for detecting changes in the atmospheric CO_2 concentration through geological time. The difference range between the stomatal numbers here calculated and of *L. frondosa* is narrow. Whereas mean SDs in *G. communis* vary between 234.73 and 284.14, in *L. frondosa* it corresponds to 309. The mean SI values obtained for such a distinct species as a conifer (14.6) from the Tropical Summer Wet biome and glossopterid (15.7 to 18.9) from the Cool Temperate biome (*sensu* Rees *et al.*, 2002) also display uniformity (*cf.* Beerling & Royer, 2002). A consistency is evidenced through the inferences of global low levels of atmospheric CO_2 during the Lower Permian as predicted by the GEOCARB model (Berner, 1991, 1994; Berner & Kothavala, 2001). On strictly northern hemisphere reconstructions published so far, this could be the first cross-check with southern hemisphere stomatal data as expected by Beerling & Royer (2002).

Glossopteris communis in the Sakmarian has lower stomatal numbers than in the Artinskian, implying relatively higher levels of atmospheric CO_2 during the former interval. Many factors could have influenced this trend. Considering the absolute age of 290.6 ± 1.5 Ma (Sakmarian) for the Faxinal

Coalfield (Guerra-Sommer *et al.*, 2008b) and the relative age of the Figueira Coalfield (Artinskian) according to the chronostratigraphic chart by Holz *et al.* (2010) for the Permian of Paraná Basin, a probable time span of about 8 Ma should have occurred between peat formation in the southern and the northeastern part of the basin. Additionally, the multiple coal seams (five or more), continuous and above one meter thickness registered at the Sakmarian southern coalfields are indicative of extensive peat accumulating swamps, generated in a barrier-lagoon depositional system, and found behind the barrier (Alves & Ade, 1996; Holz *et al.*, 2010). On the other hand, only one thin (about 0.6 m thick) and discontinuous coal bed is found in the northeastern part of the basin at the Artinskian, formed in restricted peat swamp areas developed in saturated ambits of delta plain systems (Zacharias & Assine, 2005). During the generation of peat mires in these northern areas, wetland forests were disappearing in the Brazilian Paraná Basin as indicated by palynostratigraphic data (Holz *et al.*, 2010). An overall change in flora composition is marked by the transition of *Vittatina costabilis* Zone to the *Luekisorites virkkiae* palynozone right after the coal deposition of Figueira Coalfield attributed to climate change and tectonic activity (Holz *et al.*, 2010).

A relation between the low stomatal numbers of *Glossopteris communis* in the Sakmarian (Faxinal Coalfield) of the southern Paraná Basin and the development of extensive mires can be inferred here. This important and extensive event of peat generation may have been sufficient to reverse regionally and temporarily the reduction trend in atmospheric CO₂ according to the global curves established by the GEOCARB model (Bernier & Kothavala, 2001). Studies in present-day peatlands point out that this type of environment plays a significant role in the emission of greenhouse gases, especially under warming and flooding conditions (Billings *et al.*, 1982; Aerts & Ludwig, 1997; McKenzie *et al.*, 1998). On the other hand, the younger peat mires developed in the northern areas of the basin were very sparse and therefore insufficient to affect the general low trend in atmospheric CO₂.

It must also be observed that the integration of the results presented in this study with previously published data for the Faxinal Coalfield (Guerra-Sommer *et al.*, 2008a; Jasper *et al.*, 2009) allows us to infer that volcanic events could be identified as one of the potential sources of greenhouse gases.

According to Guerra-Sommer *et al.* (2008b), the volcanism detected in the southern Brazilian coal interval through the presence of tonstein layers was interpreted as the same which produced the Choiyoy Group in western Argentina (López-Gamundi, 2006). Nevertheless, unpublished data (J.O.S. Santos, pers. comm.) leads to the hypothesis that in the Brazilian region the explosive event occurred in still unidentified nearby areas.

CONCLUSIONS

Finer-scale patterns of atmospheric CO₂ variations were detected for an 8 Ma interval in the Lower Permian of the

Paraná Basin using stomatal parameters in *Glossopteris communis* leaves from two assemblages of different age intervals (Sakmarian and Artinskian).

The lower stomatal numbers have been observed at the climax of the peat deposition interval (Sakmarian) during a phase of globally estimated low paleoatmospheric CO₂, and could be related to a temporary increase in CO₂ levels due to extensive areas of peat accumulation and/or under intense ash fall due to volcanic activities.

The results show that the Permian taxon *Glossopteris* can be used as CO₂-proxy. Although the evidence does not give direct results about the past atmosphere which could be compared to present-day levels, it does reveal fluctuations within the Sakmarian-Artinskian interval during the development of peat-forming systems in Brazilian sequences.

In order to corroborate the present results, future lines of investigation will include cuticular analyses of fossil plants from Permian coal-bearing strata from South Africa and India.

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