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# Atmospheric paleo-$CO_2$ estimates based on *Taxodium distichum* (Cupressaceae) fossils from the Miocene and Pliocene of Eastern North America

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ABSTRACT

Neogene atmospheric paleo-$CO_2$ estimates based on fossils of the extant cupressaceous conifer species *Taxodium distichum* from the Brandywine Formation of Maryland and the Citronelle Formation of southern Alabama are presented. These are important as the first such estimates from eastern North American paleofloras, and provide new evidence from a time for which the role of $CO_2$ in climate change is controversial. Comparisons of the stomatal density (SD) of the fossil leaf cuticles to a calibration curve constructed from modern leaves of the same species collected over the last century of anthropogenic $CO_2$ increase produces Miocene and Pliocene atmospheric paleo-$CO_2$ mean estimates of 360 and 351 ppmv, respectively. Although the temporal resolution of the fossil sites is low, these results are in agreement with multiple independent proxies that indicate near modern $CO_2$ levels during this interval, and demonstrate the utility of *T. distichum* leaves as instruments for stomatal frequency analysis.

## 1. Introduction

The use of fossil plant cuticle micromorphology as an atmospheric paleo-$CO_2$ proxy has become an important tool for understanding the pattern and causes of climate change throughout Phanerozoic history (Royer et al., 2001). Most of the original research demonstrating a stomatal response to changing atmospheric $CO_2$ in living plants (Woodward, 1987), as well as most fossil studies, involves $C_3$ angiosperms; however, some gymnosperms have also been shown to be useful and exhibit certain advantages. For example, *Ginkgo* and *Metasequoia* fossils used to estimate Paleocene – Miocene paleo-$CO_2$ appear identical to extant species (Royer et al., 2001), and these and many other extant gymnosperm species probably evolved during times of elevated $CO_2$ (Haworth et al., 2010).

In this paper, we demonstrate that megafossil remains from the Miocene Brandywine Formation of Maryland and the Pliocene Citronelle Formation of southern Alabama – including leafy shoots, isolated leaves, pollen cones, isolated seed cone scales, and seeds – are assignable to the extant cupressaceous conifer species *Taxodium distichum* (Bald Cypress) (Fig. 1). Multiple organs support this determination, but the unique arrangement of stomatal bands on the leaves is the most compelling feature that allies these fossils with *T. distichum* as opposed to the other extant *Taxodium* species and the similar extinct form *T. dubium*. Based on this finding, we provide the first estimates of atmospheric paleo-$CO_2$ based on comparisons of the fossil leaf cuticles to a stomatal response curve derived from freshly collected and historical herbarium specimens of this species. This is noteworthy, as the late Neogene was an important time of climate change with major biotic responses including the $C_3$ to $C_4$ plant transition (Cerling et al., 1997). Furthermore, the Citronelle Formation flora existed during, or immediately following, the Pliocene warm interval, which has become a major research focus due to its relevance as a model for future climate change responses (Dowsett and Caballero, 2010; Stults et al., 2010).

### 1.1. Brief review of fossil and extant Taxodium

Fossils of *Taxodium* are first recognized from the Late Cretaceous in Europe and North America, and become widespread in these regions during the Paleogene and Neogene (Knobloch and Mai, 1986; Aulenback and LePage, 1998). Historically, the European species have been assigned to several extinct species; however, in an extensive review, Kunzmann et al. (2009) have determined that all of the Neogene records should be considered as variants of the single extinct species *T. dubium*. Most of the Neogene records from North America are also attributed to this species (e.g., Huggins, 1985). Since the Neogene, the distribution of *Taxodium* has greatly contracted. Only three extant species are recognized: *T. distichum* and *T. ascendans* (sometimes recognized as a variety of *T. distichum*) from the lower Atlantic and Gulf Coastal Plain extending north to southern Illinois along the Mississippi Valley; and *T. mucronatum* from southernmost Texas, Mexico and Guatemala (Little, 1971).

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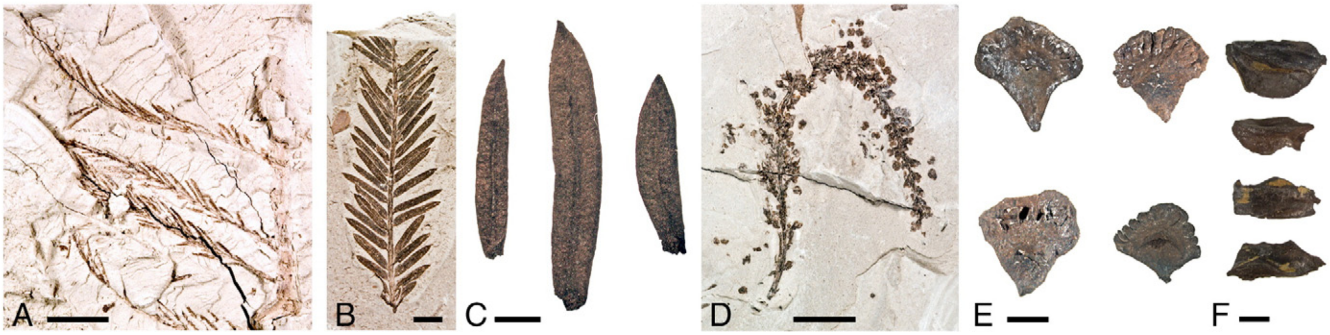

**Fig. 1.** Representative *Taxodium distichum* fossils from the Citronelle Formation (A–D) and Brandywine Formation (E–F). A) Leafy shoots attached to stem. Scale bar = 10 mm. B) Isolated deciduous shoot. Scale bar = 5 mm. C) Isolated leaves. Scale bar = 1 mm. D) Complete male shoot system with attached pollen cones. Scale bar = 10 mm. E) Isolated ovulate cone scales. Scale bar = 2 mm. F) Isolated seeds. Scale bar = 1 mm.

*T. distichum* prefers subhumid to humid climates with abundant precipitation and an extended growing season with mean annual temperatures of 12–25 °C, and mean annual precipitation of approximately 1000 mm (Little, 1971; Thompson et al., 1999). It grows principally below 30 m above sea level, which is relevant to its utility in $CO_2$ proxy studies as potentially confounding altitudinal effects on stomatal numbers are mitigated (Woodward and Bazzaz, 1988). It is a long-lived tree (often greater than 1000 years), and can form extensive, pure stands in wet soils and periodically flooded sites (Conner et al., 1986; Conner, 1988; Farjon, 2005). It is noteworthy in the context of this study that the fossil localities occur within the current range of *T. distichum*.

*T. distichum* is deciduous with dimorphic shoots. The long shoots are indeterminate, while its short shoots are determinate and shed seasonally (Watson, in Flora of North America, 1993+). Leaves are narrow with tapered apices (approximately 1.5 mm wide, 5–19 mm long), linear to lanceolate, and flattened. Leaves are spirally arranged on the shoot and twisted at the base so that the entire shoot occupies one plane (Godfrey, 1988; Vidakovic, 1991; Watson, Flora of North America, 1993+). Abundant stomata are randomly oriented in two broad bands on the abaxial leaf surface, one on each side of the midvein. Fewer stomata are present in the narrower bands of the adaxial surface (Plate 2A). Many workers have described the stomata as occurring mostly in individual rows within the bands, although additional stomata do occur in groups between rows (Bertrand, 1874; Mahlert, 1885; Coulter, 1889; Florin, 1931; Cross, 1940; Farjon, 2005). Our observations of abundant extant and fossil material indicate that consistent rows of stomata are exceedingly difficult to recognize, as should be expected based on Cross's (1940) report of substantial intercalary growth during leaf ontogeny. When dealing practically with fossils of *T. distichum* from both localities described here, it is even more difficult to recognize rows (Fig. 2B–C). This has important implications for the counting strategy and stomatal counts employed in this study as detailed below.

### 1.2. Conifers in stomatal paleo-$CO_2$ proxy research: The case for *Taxodium* stomatal density

In many angiosperm paleo-$CO_2$ based studies, stomatal index (SI = percentage of stomatal density relative to stomatal density plus epidermal cell density) is often utilized in preference to stomatal density (SD = total no. stomata/area), as it is usually less affected by variables like water availability and leaf position potentially affecting leaf expansion and cell size (Salisbury, 1927; Ticha, 1982; Poole et al., 1996; Royer, 2001). SI has also been shown to be a robust proxy for change in atmospheric $CO_2$ concentration in the extant gymnosperms *Gingko* and *Metasequoia* (e.g., Royer, 2003; Haworth et al., 2010). In more recent conifer based studies, true stomatal density per length (TSDL) has been the preferred metric in species with stomatal rows or bands (e.g., Kouwenberg et al., 2003), but was not successfully employed in this study due mainly to sudden increases in the bandwidths in samples from the most recent years (years 2002 and younger). The suggestion that the number of stomatal rows could be used as a proxy for bandwidth (Kouwenberg et al., 2003) was not taken due to the difficulty in accurately discerning stomatal rows, especially in the fossil specimens of *T. distichum* (Fig. 2B-C).

In this study, SD alone is used for several additional reasons. Conifer leaf development itself mitigates cell size issues, as stomatal maturation generally occurs later than in angiosperms, and there is typically a fixed ratio of stomatal/epidermal cell complexes (Esau, 1977; Croxdale, 2000; Kouwenberg et al., 2003). Epidermal cell size variation due to water availability and differential light exposure that can confound SD-based studies is also a relatively minor consideration for *T. distichum* due to its occurrence in swamp habitats with very moist to submerged soils, and the relatively open crown of this species (Thompson et al., 1999). Most importantly, SD results with several conifer species have shown significant responses to atmospheric $CO_2$ change (Kouwenberg et al., 2003; Lammertsma et al., 2011), and a response is demonstrated here based on historical herbarium specimen records (Fig. 3). Finally, SD had to be used in this study, as it is not possible to consistently delimit the boundaries of the non-guard cell epidermal cells on the fossil *Taxodium* leaves from both localities due to preservational factors and the delicate nature of the cuticles.

## 2. Materials and methods

### 2.1. Geological setting and fossil preparation

The Brandywine Formation was considered Pleistocene (Clark, 1915) until regional palynological investigations indicated a Late Miocene (~11.2 to 6.5 Ma) age (Pazzaglia et al., 1997). The discovery of fossil plants – including the *T. distichum* fossils used in this study – from a temporary exposure approximately 20 km southeast of Washington D.C. also support this older age determination due to the presence of abundant taxa now restricted to Asia (McCartan et al., 1990). The Brandywine Formation was part of a braided stream system typical of those that still characterize the Salisbury Embayment (Ward & Powars, 1989), and the flora accumulated in a fluvial upland environment, probably within a sub-channel intermittently reconnected to a main channel during flooding events (McCartan et al., 1990). Forty-nine plant taxa were originally identified from the Brandywine flora, including a reference to *Taxodium* c.f. "*T. distichum*." Based on a qualitative consideration of the plant assemblage, climate during the time of deposition was warm temperate with periods of extended rainfall (McCartan et al., 1990).

The Citronelle Formation extends along the northeastern Gulf of Mexico Coastal Plain from the Florida panhandle to eastern Texas. The age of the formation has been a source of considerable controversy, but more recent studies convincingly reaffirm the original Pliocene designation of Matson (1916), and indicate that deposition occurred sometime between 3.4 and 2.7 Ma (Otvos, 1997, 1998). The Citronelle