Research paper

# Palynology of Neogene sediments at the Gray Fossil Site, Tennessee, USA: Floristic implications

Diana Ochoa [a,*], Michael Whitelaw [b], Yu-Sheng (Christopher) Liu [a], Michael Zavada [a]

[a] Department of Biological Sciences, East Tennessee State University, P.O. Box 70267, Johnson City, TN, 37614, USA
[b] Department of Geosciences, East Tennessee State University, P.O. Box 70357, Johnson City, TN, 37614, USA

## ARTICLE INFO

## ABSTRACT

The Gray Fossil Site, northeastern Tennessee, is formed by multiple karst sub-basins filled with lacustrine sediments. The oldest sediments found were recently dated as Paleo-Eocene by palynological means, whereas the youngest sediments are considered Mio-Pliocene based on their faunal assemblage. In this study, we examined the Mio-Pliocene lacustrine sediments from the Gray Fossil Site to determine the Late Neogene floral characteristics of a site within the southern Appalachian Mountains. The Mio-Pliocene lacustrine sinkhole fill preserves a unique fossil assemblage, which includes invertebrate, vertebrate, and floral remains. Floral remains are represented by wood, seeds, leaves, and pollen grains. Forty-seven palynological samples from six different test-pits were analyzed. All pits exhibit a low pollen yield, a result of basic pH levels, drought, and fire events that occurred during deposition. The palynofloral assemblage has a low to moderate diversity and is largely dominated by a *Quercus–Carya–Pinus* assemblage (~90% of the palynoflora). The presence of *Pterocarya* grains supports a Late Neogene age for these lacustrine sediments. Comparison with modern pollen-based floras from North America suggests that: (1) examined pits can be discriminated into two separate groups based on their palynofloral signatures, (2) the Mio-Pliocene vegetation at the site ranged between a closed to open woodland setting, depending on the intensity and frequency of drought and fire events, and (3) the fossil palynofloral assemblage is comparable to what would be expected in the modern North American Mesophytic Forest region.

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## 1. Introduction

The Gray Fossil Site (GFS), located in Gray, Tennessee (36°22′13″N, 82°29′49″W), was discovered during excavations for a road-widening project (State Road 75 S) in May 2000 (Fig. 1). The GFS was initially interpreted as a simple paleo-sinkhole deposit formed within the Cambro-Ordovician Knox Group (Wallace and Wang, 2004; Clark et al., 2005; Shunk et al., 2006). However, a recent gravity study has revealed that it was formed by the coalescence of at least eight different sinkholes (Fig. 2) (Whitelaw et al., 2008). The sinkhole fill sequence consists of up to 42 m of dark, well-laminated lacustrine silts and clays that are rich in organic matter, and contain occasional chert and dolomite gravel lenses (Clark et al., 2005). The surficial exposures of this unit preserve the GFS fauna and flora (Shunk et al., 2006). The lacustrine sequence is disconformably overlain by oxidized subaerial sands, muds and chert-rich gravels derived from the Knox Group carbonates (Clark et al., 2005; Shunk et al., 2006).

The lacustrine basin fill has preserved a unique biota which includes both animal and plant remains (Parmalee et al., 2002; Wallace and Wang, 2004; Clark et al., 2005; Schubert and Wallace, 2006; Gong et

al., 2010; Liu and Jacques, 2010). To date, the GFS has yielded the largest known assemblage of fossil tapirs (~120 individuals), and other vertebrate remains (~40 different taxa), which include rhinoceros, gomphothere, red panda, short-faced bear, camel, saber-toothed cat, alligator, fish, and turtle, among others. The floral assemblage includes seeds, leaves, wood, and pollen from *Quercus*, *Carya*, *Ulmus*, *Liquidambar*, and *Pinus*, among others. The presence of *Teleoceras* (rhinoceros) and *Plionarctos* (short-faced bear) remains constrains the age of the site to between 7.0 and 4.5 my (Parmalee et al., 2002; Wallace and Wang, 2004). This corresponds to a Late Miocene–Early Pliocene age, or the Late Hemphillian land mammal age (Woodburne, 1987; Alroy et al., 2000). It is important to note that this Neogene age constraint is only relevant to the vertebrate fossil producing surface exposures. These exposures only occur within the lacustrine sediments, which occur over 8 m of topographic relief. The age of deeper sediments recovered from the GFS-1 core, which was drilled oven one of several localized depocenters, was indicated as Paleocene-Eocene based on the palynological content (Zobaa et al., 2011), implying a more complex basin-fill history than previously thought. Hence, in order to have clear biostratigraphic control for this study we only analyzed samples from the vertebrate-bearing horizons.

Previous studies identified the GFS as a unique site due to the combined presence of faunal and floral elements with different climatic ranges and diverse biogeographic distributions (Parmalee et al., 2002;
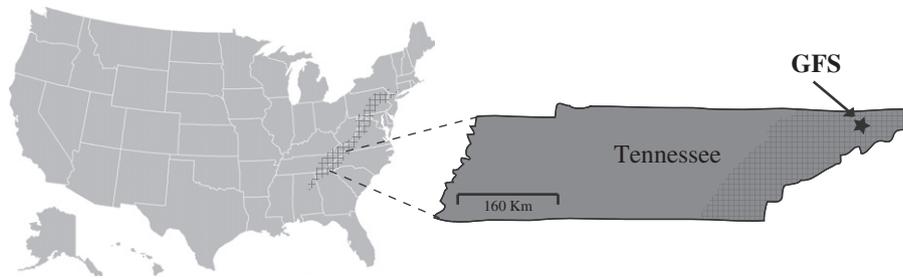
**Fig. 1.** Location map of Gray Fossil Site, northeastern Tennessee. Pattern indicates the Appalachian Mountains. Modified after Shunk et al. (2006).

Wallace and Wang, 2004; Clark et al., 2005; DeSantis and Wallace, 2008; Liu and Jacques, 2010). Warm climate taxa, such as *Alligator* (alligator) and *Tapirus* (tapir), are found with cool adapted Eurasian elements, such as *Arctomeles* (Eurasian badger) and *Pristinailurus* (red panda) (Wallace and Wang, 2004; Wallace and Schubert, 2005; Liu and Jacques, 2010). Some plant remains such as *Sinomenium* and *Vitis*, belonging to the Menispermaceae and Vitaceae families, respectively, indicate an Asian affinity (Gong et al., 2010; Liu and Jacques, 2010). Macrofloral remains and pollen preserve an association dominated by arboreal taxa (*Quercus* and *Carya*), and a herbaceous layer (Wallace and Wang, 2004). Carbon isotopic analyses on bulk rock samples, calcite crystals, and enamel from several animals confirm the dominance of C3 over C4 type plants (Shunk et al., 2006; DeSantis and Wallace, 2008; Gao et al., 2009). Based on the unique vertebrate assemblage, the large tapir population, the limited presence of equids, and the abundance of arboreal plant fossils, the Neogene sediments of the GFS have been interpreted as having been formed in a small sinkhole lake that was surrounded by a dense to moderately dense forest (Wallace and Wang, 2004; DeSantis and Wallace, 2008).

The GFS is the first Mio-Pliocene vertebrate fossil site discovered within the southern Appalachian Mountains. Study of this site is remarkably important for reconstructing local Mio-Pliocene biotic conditions and for understanding regional trends in the southern Appalachian biotic evolution. We intend to (1) reconstruct the appearance and structure of the floral community, (2) reconstruct the ecological and environmental conditions present in the southern Appalachian Mountains during GFS time, and (3) compare the GFS palynological record with modern and Mio-Pliocene pollen-based associations from eastern North America.

## 2. Methods

For this study, 47 palynological samples were collected from 6 test-pits across the GFS. These pits are designated as the Bear, Elephant,



| Pit | UTM Coordinates | | Elevation (m) | Thickness (m) | Pollen Samples |
| --- | --- | --- | --- | --- | --- |
| | Easting | Northing | | | |
| Bear | 914143.003 | 233379.483 | 502.824 | 0.864 | 16 |
| Elephant | 914088.552 | 233346.225 | 500.865 | 2.083 | 12 |
| Gator | 914156.576 | 233380.198 | 503.044 | 1.20 | 6 |
| Rhino | 914129.668 | 233355.988 | 503.417 | 0.508 | 5 |
| East | 914070.276 | 233361.875 | 499.189 | 2.00 | 4 |
| Mid | 914075.779 | 233373.305 | 498.948 | 0.70 | 4 |
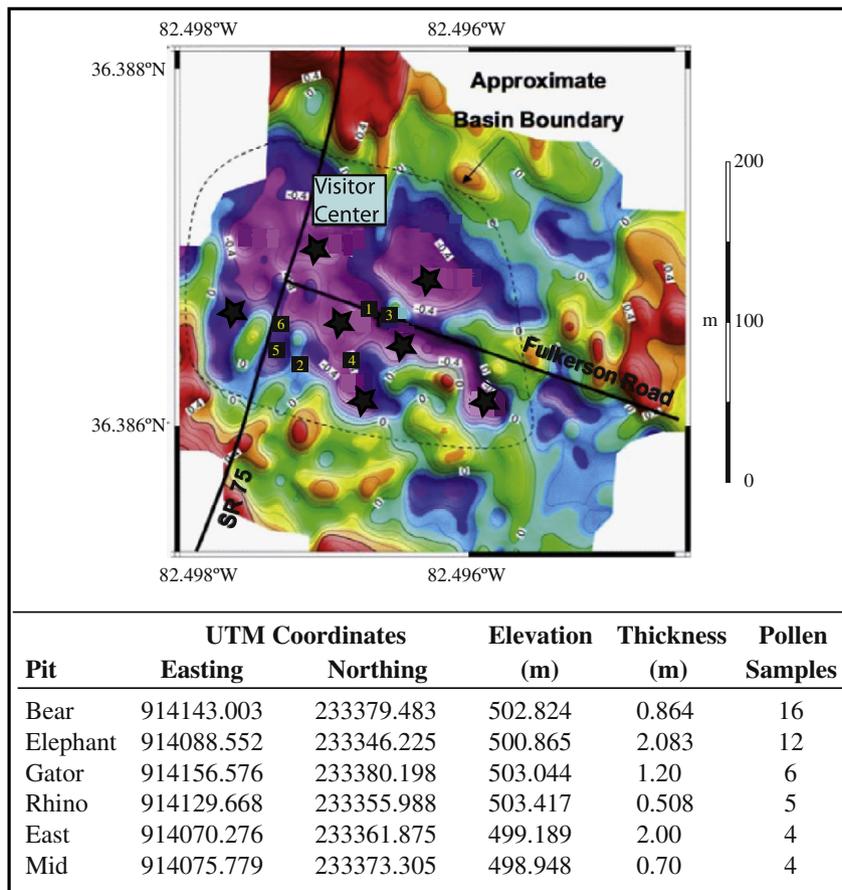
**Fig. 2.** Gravimetric map of the Gray Fossil Site. ★ = identified sinkholes, □ = test pits sampled at GFS, numbered as follows: 1 = Bear; 2 = Elephant; 3 = Gator; 4 = Rhino; 5 = Mid; 6 = East. Modified after Whitelaw et al. (2008).

Gator, Rhino, East and Mid pits (Fig. 2, Appendix A). Since samples come from the pits that are associated with vertebrate remains, we are certain of the Neogene age of these sediments. All samples were processed following standard palynological methods (Traverse, 2007) in the Paleobotany Laboratory of the East Tennessee State University Biology Department. Each sample was prepared by digestion in both 10% hydrochloric acid and 40% hydrofluoric acid, followed by sieving with 10 μm and 100 μm meshes. Prepared residues were permanently mounted in silicone oil. Each slide was examined using an Olympus BX-41 transmitted light microscope under 20×, 40× and 100× magnifications. At least 150 grains were counted per slide. Affinities were assigned following the current ICBN systematic classification of plants (McNeill et al., 2005). Stratigraphic range charts and statistical analyses were performed using the program R for Statistical Computing (R Development Core Team, 2009) by applying functions from the *Stratigraph*, *Analogue* and *Vegan* packages (Simpson, 2007; Green et al., 2010; Oksanen et al., 2010).

An estimate of pollen yield in the GFS sediments was calculated using the methodology of Traverse (2007). Traverse's method is convenient because it gives an approximation of the number of palynomorphs per gram but does not require the addition of foreign palynomorphs, such as *Lycopodium* spores or *Eucalyptus* grains, to the sample.

To describe the GFS palynoflora in terms of modern vegetation, pollen records from the six sampled GFS pits were compared with modern pollen-based floras documented in the North American Pollen Database (NAPD) (Whitmore et al., 2005, 2008). For this purpose, 525 pollen records were extracted from the database (Appendix B). The geographic distribution of these records ranges from 25°N to 43°N and from 96°W to 70°W; an area that covers the entire region described as the eastern forest domain (Bailey, 1995). In order to avoid bias due to taxonomic misidentifications, we followed the NAPD methodology and used a minimum taxonomic rank of genus level for pollen identification.

To run all statistical analysis, modern and fossil samples pollen counts were reduced to percentages based on a sum of 32 taxa, identified to either genus or family level. Taxa included in this analysis were *Abies*, *Acer*, *Ambrosia*, Anacardiaceae, Apiaceae, Aquifoliaceae, Arecaceae, *Artemisia*, Asteraceae, *Betula*, *Carya*, *Celtis*, Amaranthaceae/Chenopodiaceae, Cyperaceae, *Corylus*, Cupressaceae, Fabaceae, *Fagus*, *Liquidambar*, *Myrica*, *Nyssa*, Onagraceae, *Picea*, *Pinus*, Poaceae, *Quercus*, Ranunculaceae, *Salix*, *Taxodium*, *Tsuga*, *Taxus* and *Ulmus*. Selection of these pollen taxa was based on their importance in characterizing major vegetation regions in eastern North America (Braun, 1964; Delcourt et al., 1983; Delcourt and Delcourt, 1985; Whitmore et al., 2005).

### 2.1. Non-Metric Multidimensional Scaling

The Non-Metric Multidimensional Scaling (NMDS) method is an ordination technique widely used to analyze biological datasets (Legendre and Legendre, 1998). The NMDS was used to identify modern samples that have a floral composition similar to the GFS samples. This in turn was used to identify similar variables (e.g. temperature, precipitation, soils, etc.) that may have shaped the GFS assemblage. To improve the gradient detection ability of dissimilarity indices, a double standardization using the Wisconsin function, was performed (Oksanen et al., 2010).

### 2.2. Modern analogue assignment — dissimilarity calculations

The Modern Analogue Technique (MAT) is a quantitative analysis used to: (a) identify modern sites that are floristically or faunistically similar to fossil samples, (b) evaluate how dissimilar is the fossil assemblage from the extant communities, and (c) infer and reconstruct past climates, faunal and vegetation communities from fossil records (Overpeck et al., 1992; Guiot et al., 1993; Sawada, 2006; Simpson, 2007). To recognize analogues between the NAPD samples and the

GFS palynological record, we calculated a dissimilarity coefficient using the squared-chord distance (SCD) which has been previously recognize as the best distance metric for MAT analysis (Overpeck et al., 1992; Gavin et al., 2003; Simpson, 2007).

Samples without a modern analogue are expected to have SCDs > 0.15 (Overpeck et al., 1985; Davis, 1995; Williams et al., 2001), i.e. 0.15 is the maximum quantitative level of dissimilarity below which two samples can be regarded as analogues. Nonetheless, several studies have shown that this critical threshold depends on the ecological resolution of the vegetation and the number of taxa used to compute metric distances (Gavin et al., 2003; Wahl, 2004; Sawada, 2006). In consequence, we have implemented a Receiver Operating Characteristic (ROC, Metz, 1978) curve to assess the optimal critical limit of dissimilarity for our dataset. ROC curve maximizes the probability of identifying true positive analogs while minimizing the probability of false positive ones (Gavin et al., 2003; Wahl, 2004; Sawada, 2006; Simpson, 2007).

To apply the ROC framework, modern samples have to be *a priori* assigned to groups or types of samples (e.g. samples classified into vegetation types). In this study, the initial grouping of samples was based on the current areal forest distribution described by Dyer (2006) for eastern North America (See Fig. 6E). Although several classifications of the eastern forests have been proposed, we chose Dyer's work as an *a priori* categorization, given that (a) Dyer's regions were delimited by using only floral composition and (b) modern NAPD samples were collected during the last four decades; thus, they most likely will match the recently delimited forest zones. Modern records in the southeastern region were categorized into the Subtropical Evergreen, Southern Mixed, Mesophytic, Mississippi Alluvial Plain, Oak–Hickory, and Beech–Maple–Basswood Forest regions (See Fig. 6E). Although the Mesophytic and Southern Mixed regions include floristic subsections (i.e. Appalachian-Oak and the Oak-Pine subsection, respectively), these subsections were not used as grouping categories because their floral assemblages and geographic boundaries were not explicitly defined by Dyer (2006).

## 3. Results

### 3.1. GFS palynology

Well-preserved charcoal fragments classified as phytoclasts, according to Tyson (1995), were very common in all of the studied pits. Phytoclasts types included unstructured debris, scalariform tracheids, and dispersed angiospermous cuticles (Plate II-I and II-J). The Thermal Alteration Index (TAI) of the basin was determined by evaluating the color of *Pinus* pollen grains (Traverse, 2007). The exine walls of all examined grains exhibited colors ranging from pale yellow to yellow, and correspond to TAI values of 1 to 1+, indicating that the pollen grains and, by extension, the sediments of the GFS basin, are thermally immature.

All sediment samples collected from the GFS produced low pollen yields (Table 1). The average pollen concentration was 1387 grains/g,

**Table 1**
Pollen concentration per pit (palynomorphs/gram).

| Site name | Average pollen concentration[a] |
|---|---|
| GFS average | 1387[b] |
|   Elephant Pit | 901[b] |
|   Gator Pit | 2464[b] |
|   Rhino Pit | 905[b] |
|   East Pit | 1279[b] |
| Modern sites | |
|   Anderson Pond, TN | 812,500[c,d] |
|   Mingo Pond, TN | 1,829,300[c,d] |

[a] Palynomorphs per gram.
[b] Rounded to nearest digit.
[c] Rounded to nearest 100.
[d] Concentration value calculated for the uppermost sample of each core reported by Delcourt (1979).

with the maximum and minimum values being 2464 (Gator Pit) and 901 grains/g (Rhino Pit), respectively. These pollen concentrations are significantly lower than those reported for modern ponds in east Tennessee (Delcourt, 1979) (Table 1). It is likely that pollen preservation at the site has been affected by factors such as pH level, drought, fire activity, and sediment influx rates (Traverse, 2007). Alkaline pH values of the sinkhole lake enhanced preservation of faunal remains, but selectively degraded the pollen signal by corroding grains (Havinga, 1967; Pennington, 1996; Traverse, 2007). Poaceae, *Pinus*, and *Carya* grains are typically the most degraded grains in all sampled pits (Plates I and II). Given that degradation and corrosion appears not predominant, further analysis and comparison with other pollen assemblages should not be biased due to differential pollen preservation.

Although, the pollen yield is low, overall preservation is good. On average, unidentified pollen grains constituted ~1.14% of the total count. The maximum unidentified grain count occurred in the sample GP-4 from the Gator Pit (with 4.1% of the total counting) (Appendix A).

The 47 GFS samples collected from six pits produced a total of 10,745 palynomorphs that were classified into 87 morphotypes (Appendix A). Identification to either family or genus rank, was established for 59 (67.9%) of these pollen morphotypes. Grains with unknown affinity (28 morphotypes) represent <3% of the total palynoflora. This pollen association exhibits a low to moderate diversity and there is no significant variation of diversity within or between pits (Appendix C). The palynofloral association is largely dominated by three genera: *Quercus*, *Carya*, and *Pinus* (Plates I–II and Fig. 3). These genera comprise up to 90% of the entire count per pit (Fig. 3). Other elements present include *Abies*, *Ambrosia*, *Juglans*, *Liquidambar*, *Pterocarya*, *Ulmus*, *Tsuga* and members of the Apiaceae, Asteraceae, Malvaceae, Onagraceae, and Chenopodiaceae/Amaranthaceae families (Plates I–II and Fig. 3) (Appendix A).

### 3.2. Comparison with modern pollen-based floras

Since no significant palynofloral variation was observed between samples from the same pit (Appendix C), the counts from all samples from a pit were combined to create a total pollen spectrum for the pit (Appendix A). This produced six GFS composite samples that could be compared to modern pollen-based floras a recorded in the extensive modern dataset derived from the NAPD.

### 3.2.1. Non-Metric Multidimensional Scaling

This analysis was carried out in order to produce an ordination plot of the floral composition based on degree of similarity (Fig. 4). The stress value for this analysis was intermediate (23.2); an expected outcome since only two dimensions were used to fit the large dataset (Holland, 2008). The resulting plot shows that the distribution of modern pollen samples along Axis 1 is controlled by the latitudinal temperature gradient (Fig. 5). Samples from low latitudes and associated with warmer environments (i.e. Florida) have the most negative values; and samples from the northernmost regions, with lower mean annual temperatures, have the most positive values (Fig. 5). No evident correlation was found between Axis 1 and other factors such as precipitation, elevation, or soil type. Axis 2 relationships did not correlate with any known factors (Fig. 5). In addition, the best-differentiated modern floral groups correspond to tropical and subtropical environments currently present in Florida. The GFS samples are clearly separated from this region (Figs. 4 and 5).

The presence of fossil crocodilians is also often used as a climate indicator (Hutchison, 1982; Markwick, 1994, 1998) given documented ectothermic features of living *Alligator* relatives, such as dependency on absorption of energy and heat dissipation velocity (Colbert et al., 1946; Brisbin et al., 1982; Pough and Gans, 1982). The modern biogeographic distribution of *Alligator mississippiensis*, the only species

currently living in eastern North America, is restricted to frost-free regions (Neill, 1971; Brisbin et al., 1982; Pough and Gans, 1982; Markwick, 1994). By assuming taxonomic uniformitarianism, it is plausible to accept the current climatic tolerance range of extant *Alligator* species as a paleotemperature indicator. Since the GFS has yielded many teeth, scutes, bones, and even a complete individual of *Alligator* sp. (Schubert and Wallace, 2006), it is likely that the GFS was located in a frost-free region.

The ordination plot in Fig. 5 illustrates an evident separation of the GFS samples along Axis 1 but only a minor dispersion along Axis 2. Since distribution of the modern pollen samples along Axis 1 is controlled by temperature, this implies that temperature is also controlling the distribution of the GFS pollen assemblage. When modern samples from the NAPD, that coincide with the *A. mississippiensis* biogeographic range, are plotted on the NMDS plot, two of six GFS pits cluster within the frost-free area (Elephant and Mid pits), two towards the borderline area (Bear and East pits), and two pits (Rhino and Gator) fall outside the warmer region (Fig. 4). When NAPD samples from the Appalachian Mountains are plotted, only two pits (Rhino and Gator) share a similar temperature distribution (Fig. 4). These two pits previously plotted outside the frost-free group distribution as defined by the range of *A. mississippiensis*. The Bear and East pits plot between the frost-free and frost zones and are therefore indeterminate with respect to frost or frost-free climate preferences (Fig. 4).

Comparison to the geographically closest modern samples, defined as samples located along the Appalachian Mountains and within a 200 km radius from the site, only show a relative close relation between the Gator Pit and two modern sites (Fig. 4).These sites are the Salt Pond Mountain site in southwestern Virginia and a site located ~10 km southwest of the Tuckaleechee Caverns in eastern Tennessee. This clustering indicates great floral similarity between these sites and the Gator Pit, which may be attributed to the similar abundances of *Quercus*, *Pinus*, and *Betula*. However, none of these sites was identify as a closest modern analogue of the Gator Pit or any other GFS pit (See Dissimilarity Calculations Results and Fig. 6E).

### 3.2.2. Modern analogue assignment — dissimilarity calculations

Dissimilarity metrics calculations and ROC curves were calculate to identify NAPD closest analogues to each of the GFS pits. SCD metric has been recognized as an useful metric distance for comparisons between biomes using pollen spectra (Anderson et al., 1989; Oswald et al., 2003). In this analysis, SCD effectively differentiated modern pollen assemblages from the following forest types: Southern mixed, Mesophytic, Beech-maple-basswood, and Northern hardwood s-hemlock (See Fig. 6F, Appendix D). The oak–hickory (0.202, n = 6), Subtropical evergreen (0.198, n = 30) and Mississippi alluvial plain (0.176, n = 38) forest types could not be discriminate most likely because their sampling size (n ≤ 38).

The critical point to identify close analogues is to accurately establish a threshold value that jointly minimizes error type I and type II. Anderson et al. (1989) suggest using the 5th percentile of the distribution as critical point. In this case, the 5th percentile indicates a cutoff value of 0.214, which rather is a large cutoff given that the shape of the distribution of dissimilarities is strongly left skewed (Fig. 6A).

The ROC framework estimates 0.171 as the optimal cutoff value for the NAPD dataset. The area under the ROC curve (AUC) is a measure of the ability of the dissimilarity to differentiate between analogue (A+) and non-analogue samples (A−) (Gavin et al., 2003; Wahl, 2004; Simpson, 2007). AUC values range from 0.5 to 1, where 1 is the highest capacity to discriminate between A+ and A−. The AUC calculated in this dataset is 0.992 (Fig. 6B), indicating an excellent discrimination between the eastern North American forest types in terms of their pollen representation. The same pattern can be observed in the asymmetric density plots of the distributions of
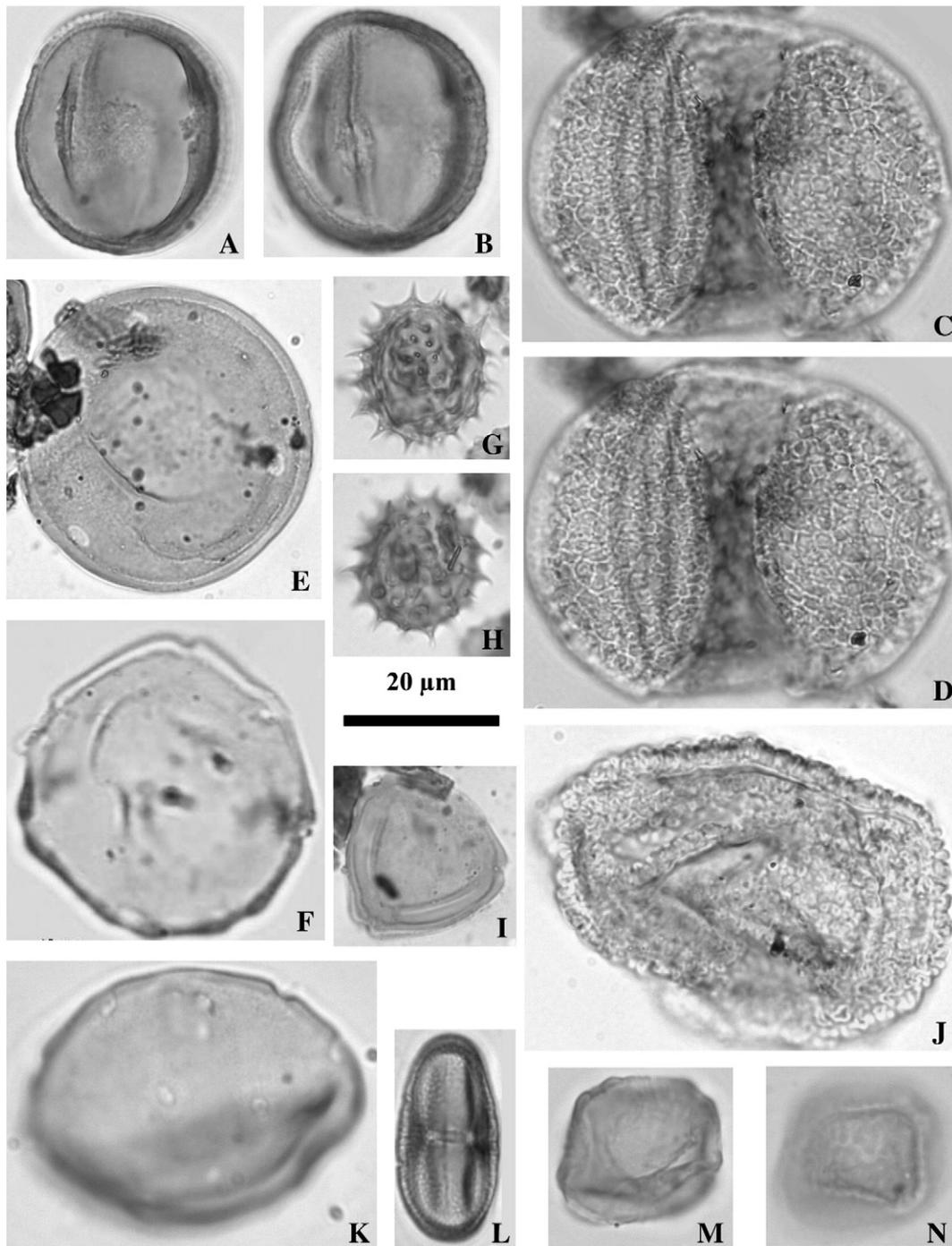
**Plate I.** Palynological association recovered from the Gray Fossil Site, plate I. Sample number is followed by the England Finder (EF) coordinate.

A–B.     *Quercus* sp., Bear Pit, sample 2b, EF: N38/2.
C–D.     *Pinus* sp., Bear Pit, sample 21, EF: L30/1.
E.       *Carya* sp., Bear Pit, sample 16b, EF: V45.
F.       *Pterocarya* sp., Mid Pit, sample 6, EF: M37/1.
G–H.     Asteraceae type, Bear Pit, sample 5 paleo 4563 unoxid, EF: L35.
I.       *Betula* sp., Gator Pit, sample 2 paleo 4565 unox, EF: O37/4.
J.       *Tsuga* sp., Gator Pit, sample 3z, EF: D37/1.
K.       *Juglans* sp., Bear Pit, sample 21b, EF: N49/1.
L.       Apiaceae type, Elephant Pit, sample 5, EF: G37.
M–N.     *Ulmus* sp., Bear Pit, sample 5-paleo 4563 oxid, EF: T37/3.

dissimilarities (Fig. 6C), where a narrow variance of A+ samples and a relatively wide variance of A− samples is shown. Likewise, the plot of the difference between the true positive fraction (TPF) and the false positive fraction (FPF) over range of dissimilarity coefficient shows the value (c = 0.171) where the maximal probability of false positive analogs is minimized (Fig. 6D).
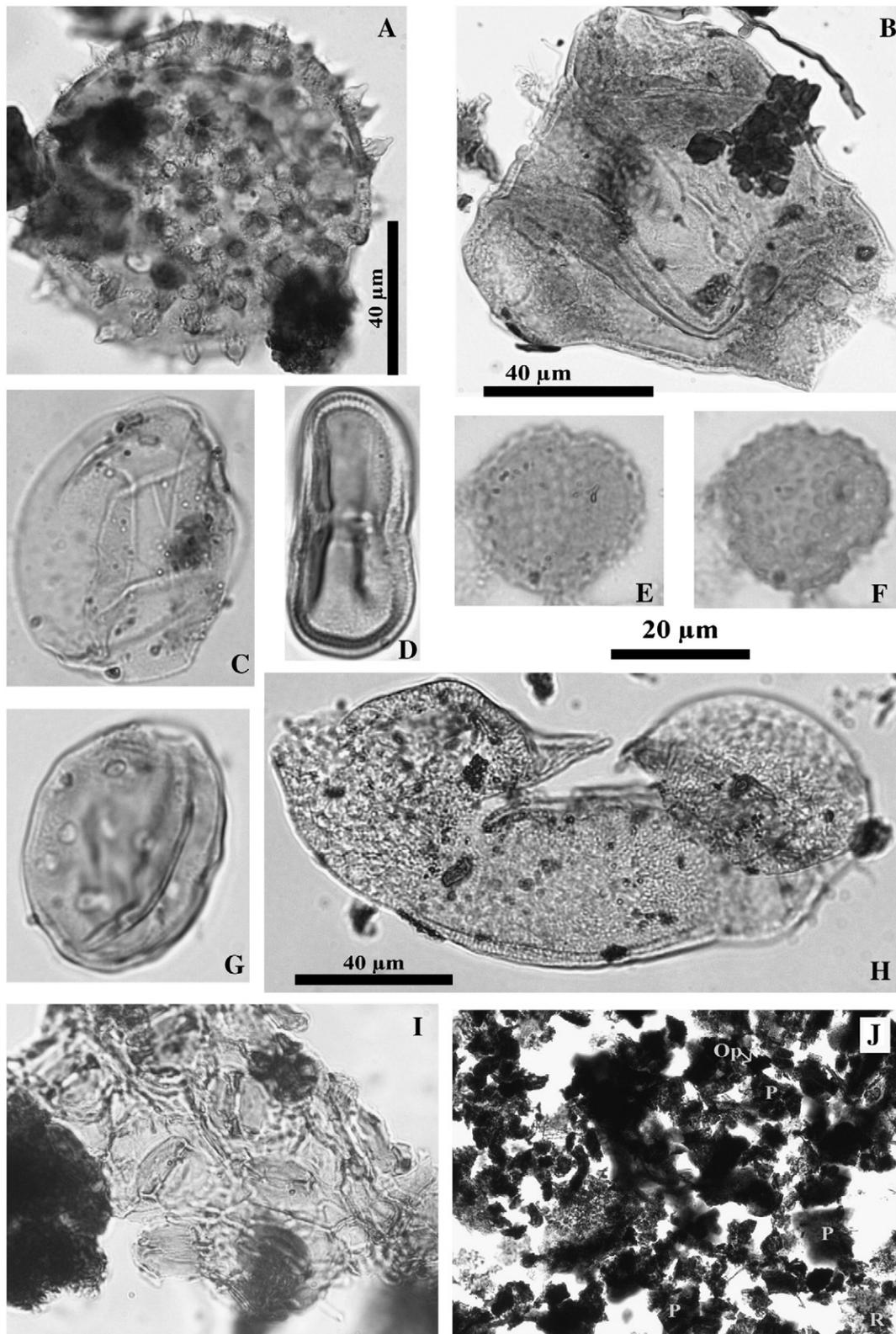
**Plate II.** Palynological association recovered from the Gray Fossil Site, plate II. Sample number is followed by the England Finder (EF) coordinate.

A.        Malvaceae type, East Pit, sample 12, EF: K31/3–4.
B.        Onagraceae type, Bear Pit, sample 2a, EF: R34.
C.        Poaceae type, Rhino Pit, sample 5b, EF: N51/3.
D.        Apiaceae type, Mid Pit, sample 6, EF: K23/1.
E–F.     *Ambrosia* sp., Elephant Pit, sample 10-paleo 4564 oxid, EF: H47.
G.        Chenopodiaceae/Amaranthaceae type, Bear Pit, sample 2a, EF: X37/1–2.
H.        *Abies* sp., East Pit, sample 3, EF: N24/1.
I.         Dispersed Cuticle, Bear Pit, sample 11a, EF: Z35.
J.         Organic Matter, Bear Pit, sample 1. Dominated by phytoclasts (P), with presence of some opaques (OP), and few resins (R).

## A) Bear Pit
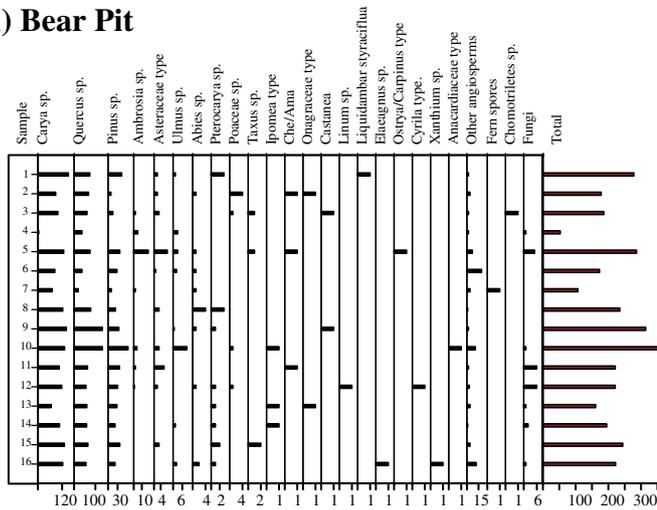


## B) Elephant Pit
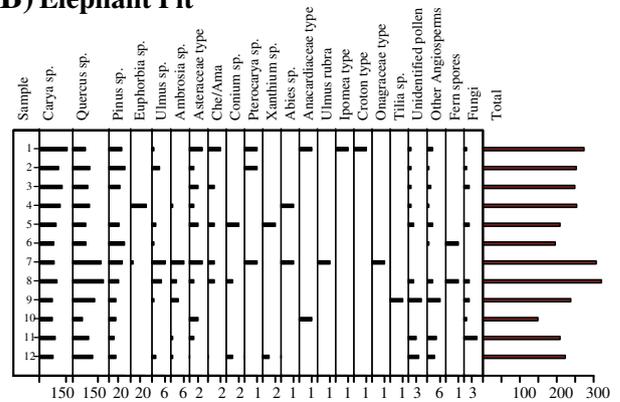


## C) Gator Pit
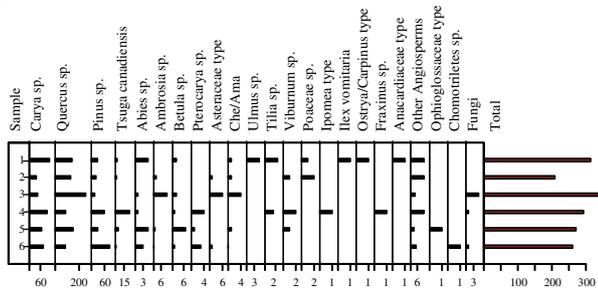


## D) Rhino Pit
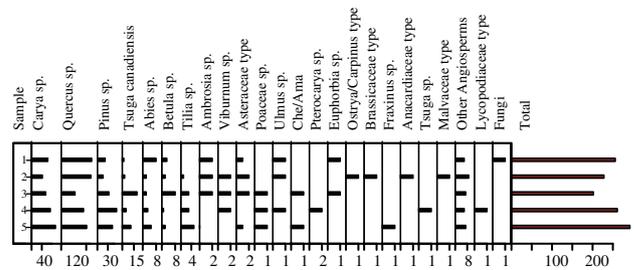


## E) East Pit
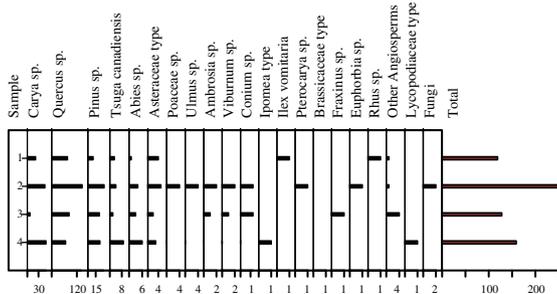


## F) Mid Pit
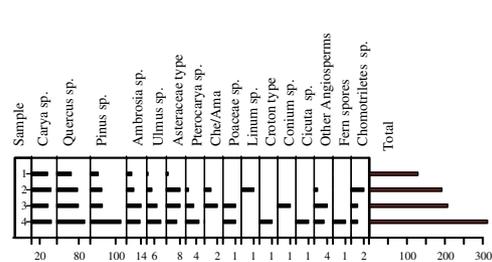


Fig. 3. Pollen diagrams of dominant taxa recovered from GFS pits. Note: Vertical axes numbers represent samples numbers and not depth values. Che/Ama = Chenopodiaceae/Amaranthaceae.

Minimum dissimilarity SCD values between each GFS pit and the NAPD samples are presented in Table 2. Using the best critical cutoff value (c = 0.171) signaled by the ROC (Fig. 6), only the Gator, Rhino and Mid pits have a modern analogue match from the NAPD dataset; Bear, Elephant and East pits do not have a positive match (Table 2). The Mid pit has two A+, whilst Gator and Rhino pits only have one each. Detected A+ are Site 4 described by Delcourt et al. (1983) and 404-Hack Pond by Craig (1969), being Site 4 a common match for all three pits (Table 2). These two NAPD sites are located within the Mesophytic forest region (Fig. 6F).

## 4. Interpretation and discussion

The GFS palynological assemblage is dominated by a *Quercus*, *Carya*, and *Pinus* association (90% of total pollen count). The remaining 10% of the flora in the Gator, Rhino and East pits is dominated by *Tsuga*, *Abies*, and *Betula*; whereas in the Bear, Elephant and Mid pits *Ambrosia*, *Ulmus*, *Abies*, Asteraceae, *Pterocarya*, Chenopodiaceae/Amaranthaceae, and Onagraceae dominate (Fig. 3).

Modern habitats dominated by the *Quercus–Carya* (oak-hickory) association commonly occur on calcareous substrata (Braun, 1964) and are usually found on xeric or low moisture soils (Braun, 1964; Abrams and Downs, 1980; Abrams, 1992; Elliott and Vose, 2010; Spira, 2011). Assuming that the *Quercus–Carya* association has not significantly changed its environmental preferences since the Late Neogene, and recognizing the fact that the GFS sediments accumulated on top of calcareous basement rocks, it is reasonable to assume that the GFS basement rocks and the calcareous substrate favored dominance of the oak-hickory association within the GFS paleoflora.

### 4.1. Disturbance factors

The tree primary disturbance factors that have affected the GFS floristic assemblage, vegetation structure, and maturity are fire activity, drought, and large animal herbivory. The occurrence of fire during deposition of the GFS sequence is indicated by (1) large amounts of charcoalified wood and charcoal debris throughout the section (Shunk et al., 2006), (2) the presence of perylene, a biochemical
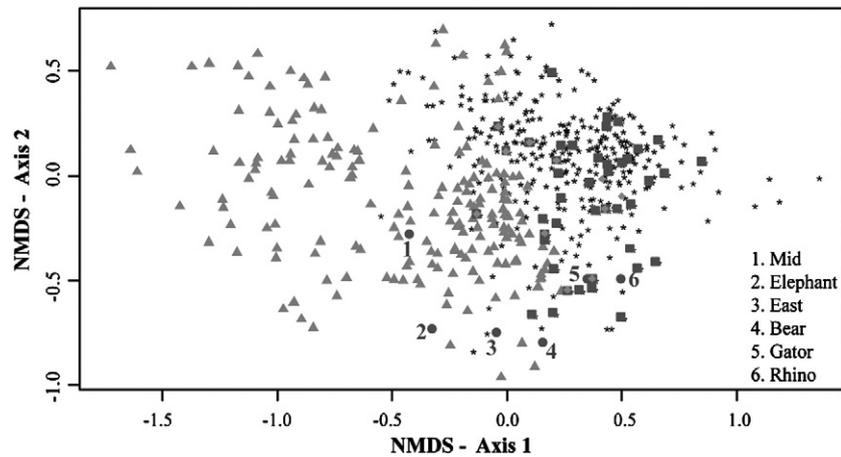
**Fig. 4.** NMDS ordination of modern pollen and GFS samples. Ordination based on degree of similarity of floral composition at genus level. ● = GFS composite samples (N = 6); ▲ = samples located within *Alligator mississippiensis* range; ■ = samples located within Appalachian Mountains; ♦ = modern samples geographically closest to the GFS. Axis 1 is controlled by minimum winter temperature (warmer temperatures to left and cooler temperatures to right).

residue that forms after fires (Jiang and Liu, 2008), and (3) the presence of macrofossils related to the canebrake *Arundinaria* (M. Zavada, in prep.), which is a fire-dependent grass (Hughes, 1966; Gagnon, 2009). Historically, fire has played an important role in maintaining extant oak–hickory communities in the southeastern United States (Wright and Bailey, 1982; Abrams, 1992; Harrod et al., 2000; Artman et al., 2005; Signell et al., 2005; Gagnon, 2009; Elliott and Vose, 2010; Spira, 2011). Mio-Pliocene fire events that occurred with the intensity indicated by the large abundance of charcoal throughout the stratigraphic section, and the presence of charcoalified logs and wood fragments suggest that tree coverage density at the GFS was comparable to a woodland (Runkle, 1985; Bond et al., 2005). Woodland is defined as a vegetation community with 25%-80% tree canopy coverage, with or without a shrub substratum and with a ground layer comprised of forbs, woody plants, vines and possibly gramineous plants (White and Madany, 1978; Taft, 1997; Anderson et al., 1999). In addition to modifying the floral assemblage, fire also tends to change the physical and chemical properties of the soil surface (Wright and Bailey, 1982; Wardle et al., 2008; Elliott and Vose, 2010) and has a substantial impact on the preservation of both palynomorphs and organic matter (Havinga, 1967; Sugita et al., 1997; Campbell, 1999). Fire activity also promotes greater runoff, increases sediment influx rates, and diminishes the relative percentage of pollen in these sediments. Thus, fire events are likely to be a significant contributor to the low pollen yields documented in the GFS samples (Table 1).

Shunk et al. (2006), based on sedimentological analysis, suggest that drought occurred during the deposition of the GFS sediments. False growth rings have been observed in well preserved GFS wood, again indicating that drought conditions at the GFS (Cook and Jacoby, 1977; Creber, 1977; Hughes et al., 1982). Two recovered wood samples preserve a 15–20 year tree ring record, but they also preserve 5–7 false growth rings in the same interval (Appendix E). A modern dendroecological study (Orwig and Abrams, 1997) has shown only 4 false growth rings in different 35-year tree-ring records from numerous angiosperm taxa growing in northern Virginia. Based on this evidence, we suggest that drought was an important influence on the frequency of fire and vegetation composition during GFS deposition (Hanson and Weltzin, 2000). Drought can also lower plant pollen production and cause water table fluctuations that may expose recently deposited plant material to oxidizing condition thus reducing pollen yields (Boyer, 1981).

Large herbivores have a significant effect on vegetation communities, particularly with regard to tree density (Huntly, 1991; Augustine and McNaughton, 1998; Maron and Crone, 2006; Vavra et al., 2007). The presence of large herbivorous taxa, such as gomphotheres, rhinoceros,

camels, peccaries, ground sloths, and tapirs at the GFS (DeSantis and Wallace, 2008), suggests that, in addition to the effect of fire, tree density would have been further reduced, perhaps to a woodland habitat. This conclusion is supported by the dominance of the oak–hickory–pine association and the abundance of herbaceous taxa that have high light requirements (e.g. *Viburnum*, *Corylus*, *Liquidambar*, *Taxodium* and species belonging to the Asteraceae, Apiaceae, Fabaceae, and Onagraceae families) (Harrod et al., 2000; Royo et al., 2010). Moreover, browsing may have enhanced fire impact by increasing the fuel load, which in turn results in more intense fires and reduced biomass (van Langevelde et al., 2003).

Analogous vegetation assemblages and disturbance events have been reported in more recent eastern North American plant communities. Plant associations dominated by oak–hickory–pine assemblages occurred in response to warmer and drier climatic conditions and fire events during the Holocene Hypsithermal Event (~8000–5000 years BP) (Delcourt, 1979; Winkler et al., 1986; Abrams, 1992). These conditions caused a shift from mesophytic forests to oak-hickory woodlands in eastern North America (Winkler et al., 1986; Abrams, 1992). Given that the GFS yields a pollen assemblage dominated by *Quercus*, *Carya*, and *Pinus*, and taking into consideration the effect of frequent fires, large herbivores, and the presence of warmer conditions during the Mio-Pliocene transition (Crowley, 1996; Zachos et al., 2001), a similar shift to oak–hickory–pine woodland is likely to have occurred at the GFS.

### 4.2. Relation of the GFS palynoflora with modern pollen-based floras

In broad terms, the GFS palynofloral assemblage is comprised of the same elements that currently occur within temperate deciduous forests of eastern North America (Braun, 1964; Delcourt et al., 1983; Delcourt and Delcourt, 1985; Walker and Oswald, 2000). *Pterocarya* is the only exotic pollen-element that is no longer present in the southern Appalachian Mountains, and it is currently confined to Asian forests (Rachele, 1976; Greller and Rachele, 1983; Sirkin and Owens, 1998). Since *Pterocarya* is known to have suffered a regional extirpation during the Middle-Late Pliocene (Greller and Rachele, 1983; Graham, 1993; Groot, 1998; Graham, 1999; Groot and Jordan, 1999), its consistent presence within sampled sediments from all pits (0.32% of the total palynoflora) confirms the Late Miocene–Early Pliocene age ascribed based on vertebrate taxa to the surficial GFS laminated sediments. The plant macrofossil record indicates that there were other exotic taxa present at the GFS (i.e. *Vitis*, *Sinomenoum*, and *Sargentodoxa*) but they are not represented in the pollen record (Gong et al., 2010; Liu and Jacques, 2010).
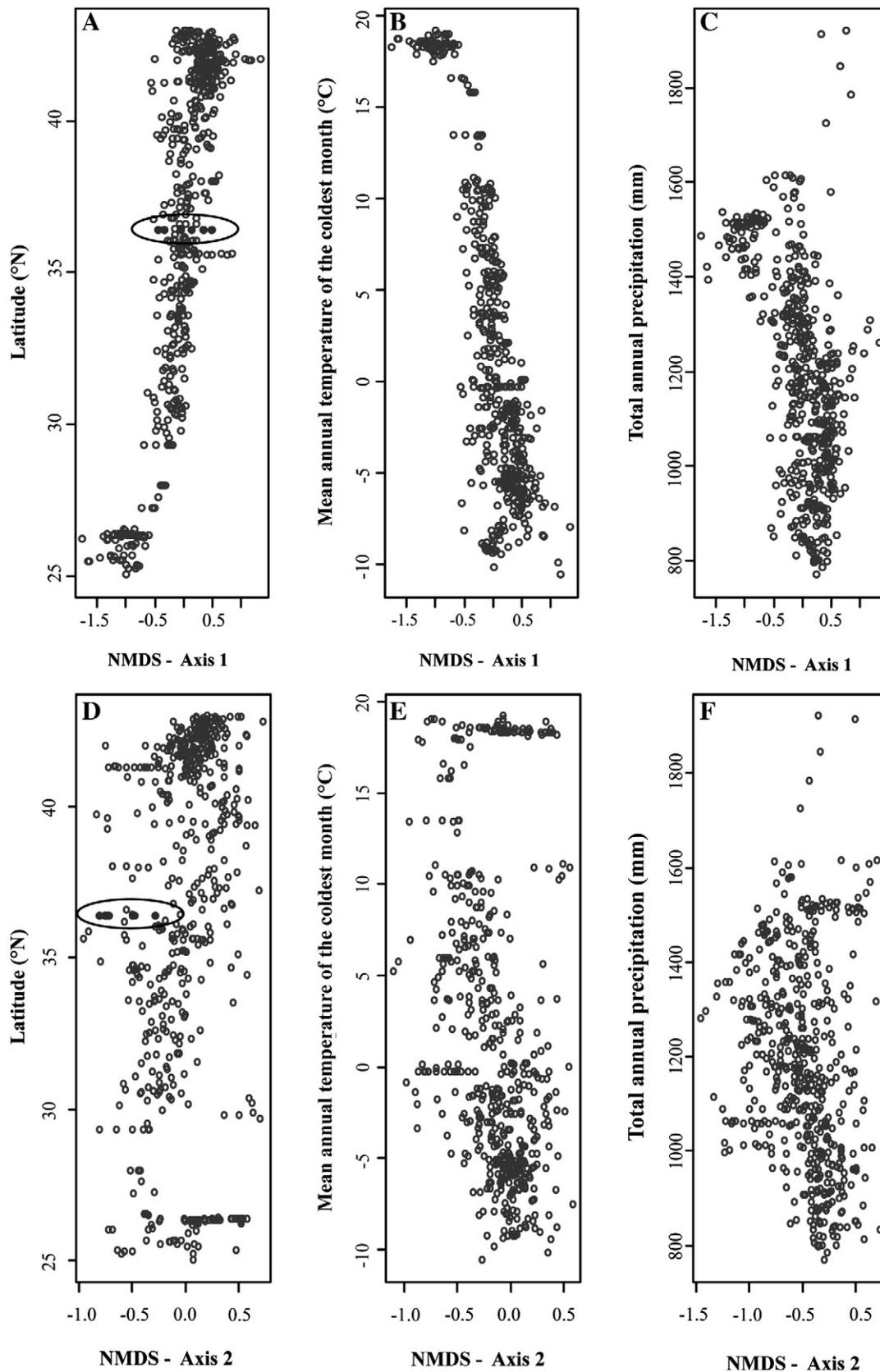
**Fig. 5.** NMDS ordination plot by axes. (A) Axis 1 vs. latitude. (B) Axis 1 vs. mean temperature of coldest month of the year. (C) Axis 1 vs. total annual precipitation. (D) Axis 2 vs. latitude. (E) Axis 2 vs. mean temperature of coldest month of the year. (F) Axis 2 vs. total annual precipitation. ● = GFS composite samples (N = 6) in frames A and D.

In terms of modern vegetation, our results indicate that only palyno-flora from three pits of the GFS, Gator, Rhino and Mid pits, share a sim-ilar composition with modern palynofloras of the Mesophytic Forest region, *sensu stricto* Dyer (2006) (Table 2, Fig. 6F). Dyer's Mesophytic Forest region is characterized by large abundances of *Quercus*, *Carya*, *Tsuga*, *Pinus*, *Acer*, *Fagus*, *Abies*, *Tilia*, and *Liriodendron*.
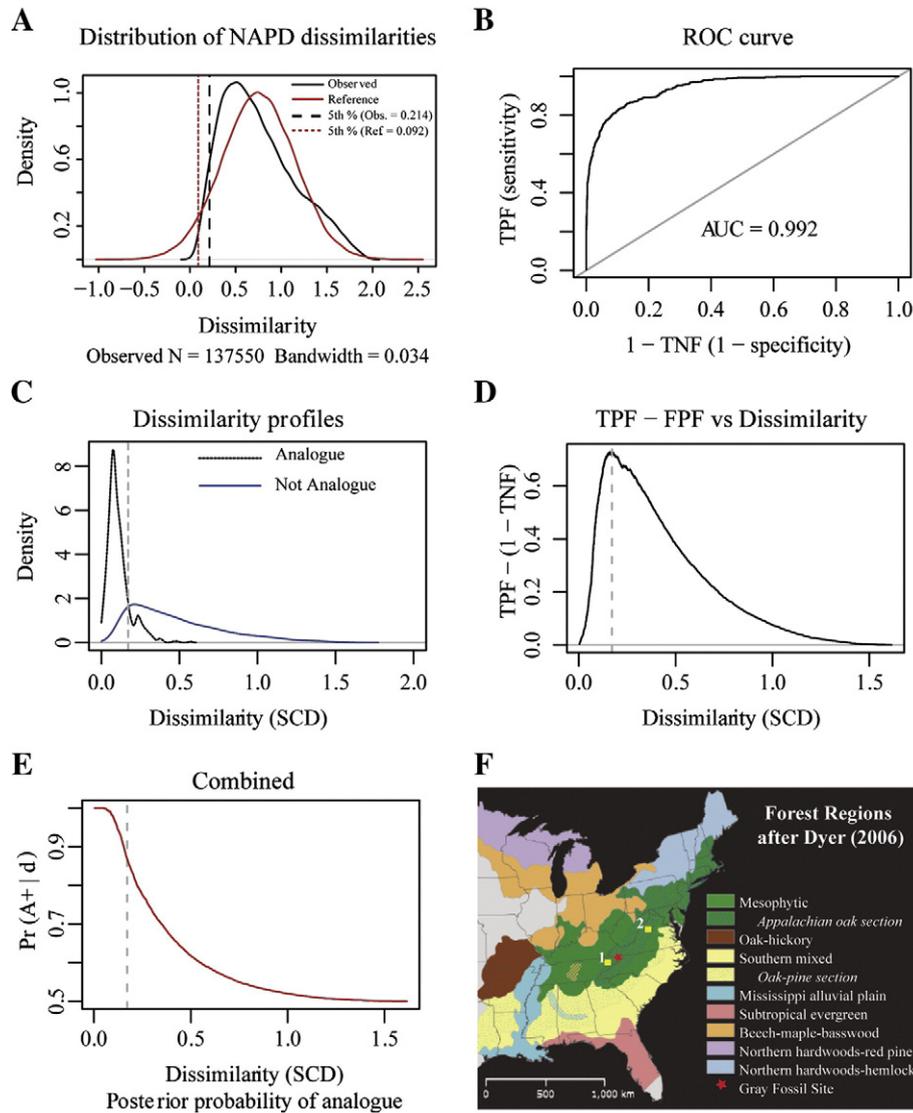
**Fig. 6.** (A) Distribution of the pair-wise dissimilarities for the NAPD samples (black line) and a reference normal distribution (red line). (B) ROC curve with AUC = 0.992 and SE = 0.00277. (C) Density plots of the distributions of the dissimilarities between analogue and non-analogue samples. (D) Difference between the true positive fraction (TPF) and the false positive fraction (FPF) as a function of dissimilarity. (E) Posterior probability of two samples being analogues as a function of the dissimilarity. (F) Location map. ★ = Gray Fossil Site, ■ = closest modern analogues at c = 0.171. Vertical dotted line showed in panels C, D and F is the optimal dissimilarity threshold (c = 0.171) based on the ROC curve.

It appears that GFS pits without a positive modern analogue may be reflecting differences in temperature, precipitation, fire frequency or the influence of GFS fauna during the deposition time. In addition, our results show that low pollen concentration values at the GFS are not preventing from finding close modern analogues (See Tables 1 and 2); however, it cannot be ruled out that low diversity values of the GFS pollen flora are result of low pollen yield caused by post-depositional diagenesis and fluctuating water levels.

### 4.3. Comparison with other Mio-Pliocene palynofloras

At least 11 Mio-Pliocene palynological studies have been published for eastern North America (Fig. 7; Appendix F). These studies include sites from Indiana (Shunk et al., 2009), Vermont (Traverse, 1955, 1994), the southeastern region (Georgia, Florida and Alabama) (Hansen et al., 2001; Rich et al., 2002; Stults et al., 2002, 2010), and New Jersey and the Delmarva Peninsula (Rachele, 1976; Greller and

**Table 2**
NAPD closest modern analogues to the GFS samples. MDV = maximum dissimilarity value for each GFS pit.

| Pit | Bear | Elephant | Gator | Rhino | Mid | East |
|---|---|---|---|---|---|---|
| MDV | 0.267 | 0.274 | 0.152 | 0.161 | 0.114 | 0.180 |
| Number of analogues (Cutoff = 0.171) | 0 | 0 | 1 | 1 | 2 | 0 |
| Closest SCD distances to NAPD samples | – | – | 0.152 | 0.161 | 0.114 0.166 | – |
| Modern NAPD analogues | None | None | Site 4[a] | Site 4[a] | Site 4[a] 404-Hack Pond[b] | None |

[a] Delcourt et al. (1983).
[b] Craig (1969).
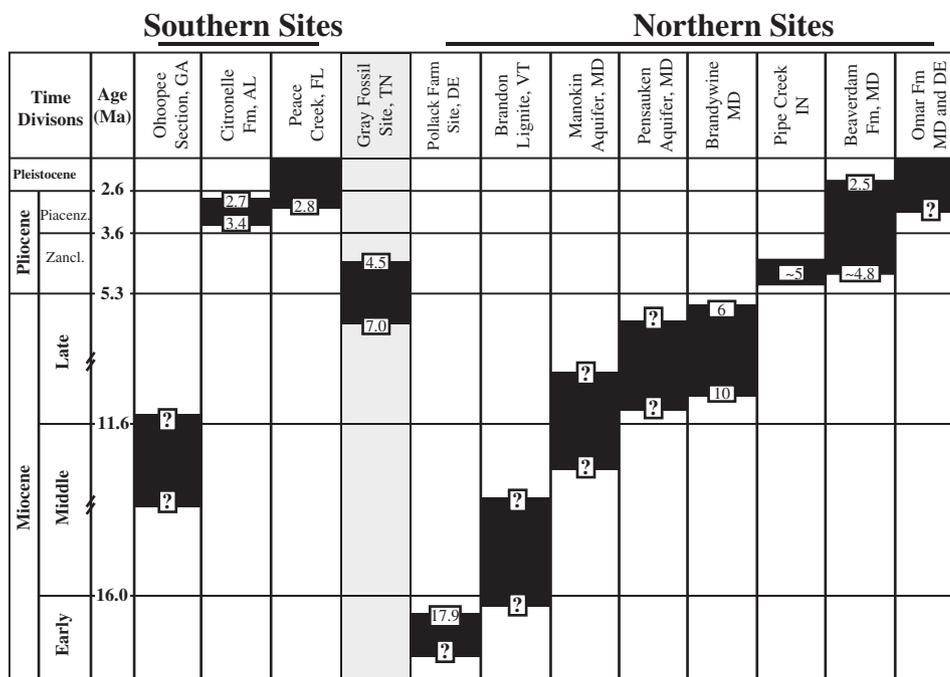
**Fig. 7.** Age model for Mio-Pliocene palynofloras from eastern North America. Age information derived from Greller and Rachele (1983), Groot (1991, 1998), Groot and Jordan (1999), Hansen et al. (2001), Rachele (1976), Rich et al. (2002), Shunk et al. (2009), Sirkin and Owens (1998), Stults et al. (2002), Traverse (1955, 1994).

Rachele, 1983; Groot, 1991, 1998; Sirkin and Owens, 1998; Groot and Jordan, 1999).

The palynofloral composition of the Mio-Pliocene sections located within the Appalachian Mountains is similar to the GFS, although some dominant and rare species vary between localities because of local conditions (Appendix F). Major compositional differences are found in pollen floras that have a marked coastal influence (i.e. Ohoopee Section, Peace Creek, and Yorktown Formation) (Appendix F). These Mio-Pliocene palynofloras are dominated by *Quercus, Carya, Pinus, Ulmus, Cyrilla, Betula, Corylus, Tsuga, Liquidambar, Nyssa,* and *Taxodium*. Elements belonging to the Amaranthaceae/Chenopodiaceae, Anacardiaceae, Apiaceae, Asteraceae, Cupressaceae, Ericaceae, and Vitaceae families are also often found. Exotic elements such as Sciadopitys, *Cyathea, Podocarpus* and *Engelhardtia* occur as well, and in some localities exotic elements with Asiatic affinities such as *Pterocarya, Eucommia, Sciadopitys, Glyptostrobus* and *Zelkova* are present. However, none of these sites report an Asiatic affinity for members of the Vitaceae (i.e. *Vitis*) (Gong et al., 2010), Menispermaceae (*Sinomenium*), or Lardizabalaceae (*Sargentodaxa*) (Liu and Jacques, 2010), which all occur at the GFS.

The similar Mio-Pliocene palynological records within the Appalachian Mountains indicate a similar composition (Appendix F). It is likely that the eastern forest domain had a continuous distribution, which ranged at least from northeastern Tennessee to the Delmarva Peninsula and represents a regional flora indicative of warmer and drier conditions. The Late Miocene pollen assemblages recovered from the Delmarva Peninsula suggest warm temperate conditions, close to those of the present day Gulf Coastal or Atlantic Coastal plains. These data also indicate that eastern North America experienced warmer conditions during the Late Miocene–Early Pliocene, and started shifting towards modern climate ranges after the Early Pliocene.

## 5. Conclusions

Based on the palynological data, we conclude that during Late Miocene- Early Pliocene time, the vegetation present at the GFS was dominated by *Quercus* (oak), *Carya* (hickory), and *Pinus* (pine). Pollen

and macrofossil evidence indicates the presence of other taxa e.g., *Acer, Abies,* Apiaceae, Asteraceae, *Betula,* Chenopodiaceae/Amaranthaceae, *Corylus,* Cupressaceae, Fagaceae, Juglandaceae, Malvaceae, *Nyssa,* Onagraceae, *Pterocarya,* Poaceae, Rosaceae *Sinomenium, Taxodium, Vitis,* and *Ulmus,* among others. Some of these elements exhibit an Asian affinity, but they are now extirpated from the region (e.g. *Pterocarya, Sinomenium* and some *Vitis* species). The presence of *Pterocarya* within the GFS assemblage confirms a Late Neogene age for the six analyzed pits at the GFS. This contrasts with previous palynological studies which indicate a Paleogene age for sediments recovered at depth from the GFS-1 core. Furthermore, the palynoflora recovered from the GFS surface pits suggests that temperature and precipitation oscillated between warm/dry and cooler/wetter conditions as that the area was frost free, promoting a vegetational shift from Mesophytic Forest to vegetation without modern analogues, when compared to present eastern North American forest.

The GFS pollen data, macrobotanical remains and other fossil evidence (e.g. charcoal and faunal association) suggest that the plant community surrounding the GFS paleolake was a successional oak–hickory–pine woodland, in which vegetation structure and habitat maturity was affected by drought, fire events and large herbivores. Similar shifts to oak–hickory–pine associations, in response to warmer and drier conditions, have previously been reported for southeastern North American plant communities during Holocene times.

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