

ABSTRACT

LOCKLEAR, JARED RICKY. Fossil *Taxodium* Seeds and Palynological Assessment of a Pleistocene Age Locality from Southeastern North Carolina. (Under the direction of James Earl Mickle).

A recently discovered locality in an open aggregate mine pit owned by the Martin Marietta Corporation near Wilmington, North Carolina (34° 22. 368' N, 77 ° 50.356' W), has produced abundant macrofossil remains that show a wide range of diversity. The exact environmental setting is uncertain, but appears to be channel-fill or lacustrine deposit, based on the matrix. The material is preserved in loose clay and was recovered from the spoil heap. Palynological analysis and comparisons to other southeastern sites suggests a Pleistocene age and also shows a wide range in diversity. Pollen types found include, but are not limited to, *Pinus*, *Taxodiaceous* types, *Picea*, *Alnus*, *Liquidambar*, *Betula*, *Quercus*, *Ilex*, Monolete Fern types, *Ambrosia spp.*, and other *Compositae*. Fungal, diatom, and protozoan palynomorphs are also present, which presents evidence of a wetland or riverine setting. The locality lacks *Isoetes* spores, considered to be an indicator of an interglacial period. Macrofossil specimens were recovered by maceration and include abundant well preserved seeds, fruit, flowers, conifer and angiosperm wood, cones, and insect fossils. Plant fossils include cf. *Fagus*, *Pinus*, *Quercus*, and *Vitus*. A dominant seed type in the fossil flora is *Taxodium*, based on key characteristics such as overall size, raised marginal features, and lack of wings. Comparisons with other species in the genus and statistical analysis suggest that this may be a new fossil species of *Taxodium*. Little is known of Pleistocene macrofossil floras of the southeastern United States and interglacial palynological assessments are absent for North Carolina. The study of this locality will add to our knowledge of this time period in this region.

Fossil *Taxodium* Seeds and Palynological Assessment of a Pleistocene Age Locality from
Southeastern North Carolina

by
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DEDICATION

I dedicate this body of work to my two nephews: Kaden Xenophen Oxendine and Brendon Emmanuel Locklear. Both were born or had a major influence on my life while I was in the process of completing this research.

I also dedicate this body of work to my great grandmothers: Lizzie Bell Locklear, Irene Deese, and Maggie Jane Oxendine. All showed me great love, and will be forever in my heart.

BIOGRAPHY

Jared Locklear was born in Lumberton, North Carolina. He is the oldest child of Ricky and Jeri Locklear, with two younger siblings, Emmanuel Locklear and Brittany Locklear. Jared completed his baccalaureate degree with honors in Biology with two concentrations in Botany and Environmental Science at the University of North Carolina at Pembroke, Pembroke, North Carolina in May of 2008. Jared enrolled in the Master of Science program in Plant Biology at North Carolina State University, Raleigh, North Carolina, in August 2008. He is currently under the supervision of the Paleobotanist Dr. James E. Mickle, and he plans to enroll and continue his education for the fulfillment of the Ph.D in Plant Biology.

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TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	ix
INTRODUCTION	1
Overview of the <i>Taxodium</i> genus and Current Research	1
Importance of the Research	2
MATERIALS AND METHODS	4
Geology and Description of the Marietta Locality	4
Materials and Techniques for Data Collection	5
Identification of Taxa	7
PALYNOLGICAL ASSESSMENT.....	10
Background Information.....	10
Paleofloristics of the Martin Marietta Locality.....	12
Dating the Locality.....	14
TAXODIUM SEEDS OF THE MID-LATE PLEISTOCENE.....	16
Descriptions of Extant Species.....	16
Descriptions of Extinct Species.....	21
Current Knowledge of <i>Taxodium</i> Seeds.....	25
Statistical Analysis and Comparison.....	28
Description and Systematics of <i>Taxodium macrosperma</i> Locklear and Mickle.....	31
Discussion.....	33
REFERENCES.....	88

LIST OF TABLES

PALYNOLOGICAL ASSESSMENT

Table 1.1	Palynological assessment results and relative abundance.....	41
-----------	--	----

TAXODIUM SEEDS OF THE MID-LATE PLEISTOCENE

Table 2.1	Summary of important morphological features of <i>Taxodium</i> species.....	42
Table 2.2	Anatomy of <i>Taxodium</i> seed testa layers.....	43
Table 2.3	Data Summary of Discriminant Statistics using the Candisc and Discrim procedures.....	44
Table 2.4	Class level information of the four <i>Taxodium</i> species.....	44
Table 2.5	Table showing the results of the four multivariate tests run by SAS in the Discrim and Candisc procedures.....	45
Table 2.6	Canonical Correlations, Eigenvalues, and Likelihood Ratios of the discriminating variables and the groupings (class levels).....	46
Table 2.7	Cross-validation Summary using Quadratic Discriminant Function.....	47
Table 2.8	Error Counts for the Cross-validation Summary.....	47
Table 2.9	Data Summary of Discriminant Statistics using the Candisc and Discrim procedures.....	48
Table 2.10	Class level information of the three <i>Taxodium</i> species.....	48
Table 2.11	Table showing the results of the four multivariate tests run by SAS in the Discrim and Candisc procedures.....	49
Table 2.12	Canonical Correlations, Eigenvalues and Likelihood Ratios of the discriminating variables and the groupings.....	50
Table 2.13	Cross-Validation Summary using Quadratic Discriminant Function.....	51
Table 2.14	Error Counts for the Cross-validation Summary.....	51

Table 2.15	<i>Taxodium</i> evolutionary history with time table, along with notes about origins and discovery of species.....	52
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LIST OF FIGURES

MATERIALS AND METHODS

Figure 1.1	Aerial photo of the Martin Marietta Aggregate Mine.....	53
Figure 1.2	Spoil heaps of the Martin Marietta Locality.....	54
Figure 1.3	Photo of the Martin Marietta Aggregate Mine.....	55
Figure 1.4	Map of North Carolina and site location.....	56

PALYNOLOGICAL ASSESSMENT

Figure 2.1	Atmospheric CO ₂ and glacial cycle of the Late Pleistocene (Ruen 2005).....	57
Figure 2.2	Generalized Pollen Diagram for Bladen County, NC (Frey 1953).	58
Figure 2.3	Sample pollen found at the Martin Marietta Locality.....	59

TAXODIUM SEEDS OF THE MID-LATE PLEISTOCENE

Figure 3.1	<i>Taxodium distichum</i> distribution map. (Little 2011).....	60
Figure 3.2	<i>Taxodium ascendens</i> distribution map (Flora of North America Editorial Committee, eds. 1993+).....	61
Figure 3.3	<i>Taxodium mucronatum</i> distribution map (Little 2011).....	62
Figure 3.4	<i>Taxodium distichum</i> seeds.....	63
Figure 3.5	<i>Taxodium ascendens</i> seeds.....	64
Figure 3.6	<i>Taxodium mucronatum</i> seeds.....	65
Figure 3.7	Fossil <i>Taxodium</i> seeds (<i>Taxodium macrosperma</i>), not cleaned with any chemicals.....	66
Figure 3.8	Fossil <i>Taxodium</i> seeds (<i>Taxodium macrosperma</i>), cleaned with H ₂ O ₂ and then 10% HCl.....	67
Figure 3.9	Longitudinal cross section of fossil <i>Taxodium</i> seeds (<i>Taxodium macrosperma</i>) at 10x.....	68

Figure 3.10	Longitudinal cross section of fossil <i>Taxodium</i> seeds (<i>Taxodium macrosperma</i>) at 5x.....	69
Figure 3.11	<i>Taxodium macrosperma</i> whole seed cross section (25x, 15kv).....	70
Figure 3.12	<i>Taxodium macrosperma</i> whole seed cross section (25x, 15kv).....	71
Figure 3.13	<i>Taxodium macrosperma</i> seed wall cross section (180x, 15kv).....	72
Figure 3.14	<i>Taxodium macrosperma</i> seed cross section (230x, 15kv).....	73
Figure 3.15	<i>Taxodium macrosperma</i> seed cross section (750x, 15kv).....	74
Figure 3.16	Graph of the Seed Length and Seed Width Ratios (SLSW_Ratio) of the three extant <i>Taxodium</i> and the fossil <i>Taxodium</i> , <i>Taxodium macrosperma</i>	75
Figure 3.17	Graph of the Seed Length and Seed Width Ratio (SLSW_Ratio) compared to Seed Length (Length) of the three extant <i>Taxodium</i> species and the fossil <i>Taxodium</i> , <i>Taxodium macrosperma</i>	76
Figure 3.18	Graph of the Seed Length and Seed Width Ratio (SLSW_Ratio) compared to Seed Width (Width) of the three extant <i>Taxodium</i> species and the fossil <i>Taxodium</i> , <i>Taxodium macrosperma</i>	77
Figure 3.19	Graph of the Seed Length (Length) and the Seed Width (Width) of the three extant <i>Taxodium</i> species and the fossil <i>Taxodium</i> , <i>Taxodium macrosperma</i>	78
Figure 3.20	3D Scatter Plot of the Seed Length and Seed Width Ratios (SLSW_Ratio) by Seed Length (Length) and Seed Width (Width) of the three extant <i>Taxodium</i> species and the fossil <i>Taxodium</i> , <i>Taxodium macrosperma</i>	79
Figure 3.21	Fossil <i>Taxodium</i> seeds (<i>Taxodium macrosperma</i>) Holotype.....	80
Figure 3.22	<i>Taxodium macrosperma</i> exotesta (200x, 15kv).....	81
Figure 3.23	<i>Taxodium macrosperma</i> exotesta (800x, 15kv).....	82

Figure 3.24	<i>Taxodium macrosperma</i> seed cross section (25x, 15kv) with endotesta curling and clearly visible.....	83
Figure 3.25	Broken <i>Taxodium macrosperma</i> seed (35x, 15kv) with degrading embryo/nucellus, and testa layering clearly visible...	84
Figure 3.26	Micropylar end of <i>Taxodium macrosperma</i> seed longitudinal section (35x, 15kv).....	85
Figure 3.27	<i>Taxodium distichum</i> seed anatomy (USDA Plants 2011).....	86
Figure 3.28	Proposed evolutionary history of <i>Taxodium</i>	87

INTRODUCTION

Overview of the *Taxodium* Genus and Current Research

The genus *Taxodium* was widespread throughout the Tertiary. Today it is now restricted to a few natural environments, and as a cultivar (Aulenback and LePage 1998). *Taxodium* is a genus of the family Cupressaceae, and taxonomic synonymous with Taxodiaceae. The genus *Taxodium* consists of three extant species that are now restricted to the southeastern United States and Mexico (Weakley 2010). The three species are: *Taxodium distichum* (Linnaeus) L.C. Richard, commonly called Bald-cypress, *Taxodium ascendens* Brongniart, commonly called Pond-cypress, and *Taxodium mucronatum* Tenore, commonly called Mexican cypress (Weakley 2010). The three taxa are deciduous or evergreen trees in warmer climates, and they are morphologically similar with few distinguishing characteristics (Farjon 2005). They are typically treated as either three distinct species or one species with three varieties, because they display close similarity morphologically. Much debate over the proper classification of the species, including arguments over proper nomenclature, has been ongoing since the early 19th century (Tsumura *et al.* 1999).

Fossil *Taxodium* species indicate that the genus was widespread throughout North America, Europe, and East Asia during the Cretaceous until the Pleistocene (Tsumura *et al.* 1999). The earliest reported occurrence of the genus was described by Aulenback and LePage as *Taxodium wallissii* Aulenback et LePage sp. nov. found during the late Cretaceous (1998). *Taxodium dubium* (Sternberg) Heer was ubiquitous throughout the early Tertiary (Chaney 1950). After the last glacial maximum during the Pleistocene, and the succession of the oceans, the three extant taxa settled into their current habitats (Tsumura *et al.* 1999).

Research over the past 20 years on extant *Taxodium* species is focused on genetic diversity and differentiation within the genus. Dr. Yoshihiko Tsumura's lab at the Bio-resources Technology Division, Forestry and Forest Products Research Institute, Kukizaki, Ibaraki, Japan has analyzed the genetic differences between species in the genus (Tsumura *et al.* 1999). Lickey and Walker (2002) of Appalachian State University, North Carolina analyzed the population genetics of *Taxodium*, building upon Tsumura's initial genetic analysis. The latest paleobotanical research on species of *Taxodium* includes Aulenback and LePage's (1998) analysis of *Taxodium wallissii*, and Kunzmann *et al.*'s (2009) analysis of *Taxodium* during the Paleogene and Neogene periods. Berry's (1907, 1909a, 1909b) floristic analyses of Pleistocene and Holocene localities throughout the southeastern United States have yielded very little to no statistical analysis or morphological comparisons of discovered *Taxodium* specimens, but offered general descriptions and details of found specimens and site localities.

Importance of Research

Current Pleistocene records of North Carolina are palynological based with no quantitative macrofossil evaluations performed (Whitehead 1964; Whitehead 1981; Frey 1951; Frey 1955; Hussey 1993; Delcourt and Delcourt 1991; Delcourt and Delcourt 1985). The analysis and review of the Martin Marietta locality *Taxodium* species will be one of the first during the Pleistocene in North Carolina, and for the southeastern coastal plain in general. Because this topic is of large debate, this research will help to provide a potential solution to our vague understanding of *Taxodium* after the Miocene. There is much speculation and rather few studies of *Taxodium* during this span of time; however this study will provide information about *Taxodium* especially during the Pleistocene.

The Pleistocene was a period of climatic instability, with seven glacial cycles occurring throughout the last 600,000 years (Delcourt and Delcourt 1991). However, the focus of palynological research has been on the last glacial maximum, the Wisconsin, into the current Holocene Epoch (Bryant and Holloway 1985). Evidence will be proposed that argues that the Martin Marietta locality sediments were deposited during an interglacial period which is older than the current Holocene. This would date the Martin Marietta sediments up to 120,000 years in age, probably Sangamon interglacial, and will also be one of the first studies during this time period for eastern North Carolina (Bryant and Holloway 1985; Stahle 2005). Analysis and evaluation of this locality will add to our knowledge about interglacial periods in North Carolina where much is still unknown about species migration patterns, refugia locations, and overall paleoclimatic conditions (Delcourt and Delcourt 1991). Understanding past interglacial-glacial transitions will allow for us to gain a better understanding of present flora and fauna distributions, affects of climatic changes, and how to prepare for future climatic changes and their effects on flora-fauna ecology.

MATERIAL AND METHODS

Geology and Description of the Martin Marietta Locality

The Martin Marietta locality can be found in New Hanover County, of the outer coastal plain of North Carolina, a few miles north of Wilmington at the Martin Marietta Quarry. Aerial photo of the site can be seen in Figure 1.1. Map of North Carolina and site location can be found in Figure 1.4. The geology of the Martin Marietta locality is uncertain. The Martin Marietta quarry is located on the Castle Hayne formation, an Eocene limestone based formation which is famous for its aquifer system. Studies of the Castle Hayne formation and of this particular quarry have been conducted but none so of Pleistocene age (Upchurch 1973; Baum, Harris and Zullo 1978; Ward and Blackwelder 1980; Baum, Harris, and Dres 1985; Zullo and Harris 1986; Harris, Zullo, and Otte 1986). However, it is mentioned that younger strata vary in thickness across the state depending on the locality (Baum, Harris, and Zullo 1978). Fossil plant material was collected from the spoil heaps. Photos of the spoil heaps, and the mine can be found in Figures 1.2 and 1.3. Integrity of sediment layers has not been compromised at this particular mine, with heap deposits being uniform. The Pleistocene sediments are treated as waste or overburden. The sediment layers in which fossil samples occur are clay based. The site appears to be a channel basin or historic lacustrine deposit based on the surrounding topography and local sediments. It can be concluded that these sediments (clay based) are Pleistocene in age, rather than Holocene (sand based) or Eocene (limestone).

Though not directly associated with the Castle Hayne Formation, a recent study of Pleistocene sedimentary formations was conducted along the Intracoastal Waterway near Myrtle Beach, South Carolina (Stahle *et al.* 1990). The oldest formation, Canepatch,

included plant fossil material which dated around 125,000 to 135,000 years B.P. (Stahle *et al.* 1990). The study found buried at the site abundant cypress (*Taxodium*) fossil remains in peaty clay deposits. Stahle *et al.* (1990) report that *Taxodium* remains (trunks and stumps) have been discovered along the Atlantic Coastal Plain in peaty clay deposits. They argue that the magnitude and timing of Pleistocene sea level changes along the coast and the significance of *Taxodium* deposits all of the relative same age, probably were buried around the same time from a large change in sea level across the coastal plain some 125,000 years B.P. (Stahle *et al.* 1990).

Materials and Techniques for Data Collection

A. Pre-Laboratory Collections

Lateral sampling was conducted by Drs. James E. Mickle and Vince Schnieder. Fossil collections were from the spoil heaps of Martin Marietta Mine. Samples were stored in large plastic containers and individual sediment samples were wrapped in paper for protection. Extant seeds were collected from the North Carolina State University Herbarium, and *T. mucronatum* seeds were donated from the University of Naples Botanical Gardens, Naples, Italy.

B. Macrofossil Macerations

Macrofossil isolation was conducted using a modified maceration technique. Fossil material was imbedded in loose clay-based sediment. This technique was developed by trial and error based on the nature of the fossil preservation and sediment composition. Initially, samples were placed in tap water with soaps high in sodium bicarbonate (NaHCO₃). This process released the plant material from the matrix, but was slow and time consuming. A modified approach used hydrogen peroxide (H₂O₂) increments starting with 15% concentrations. H₂O₂

concentrations of 15%, and 10% had a negative effect on the macrofossils and in some cases destroyed samples completely. Using a series of lower concentrations, it was shown that a concentration of 3% H₂O₂ yielded little or no damaging results over a period of a few days. It was determined that samples placed in 3% H₂O₂ over a period of two or more days would be adequate for fossil isolation in the clay-based sediment. Loose sediment and fossil samples were then sifted (3um-2000um series) for isolation. Fossils were allowed to air dry on paper towels or mesh which had no visible negative effects (water can damage specimens).

C. Palynological Preparations

After consulting with Dr. Martin Farley of the University of North Carolina at Pembroke, Department of Geology and Alfred Traverse's (2007) book *Paleopalynology* 2nd Edition published by Springer, we developed a standard technique for the palynological preparations for loose clay sediments (Madhumi 2002; Traverse 2007). Dr. Martin Farley provided expertise on protocol procedures and aided in developing a specific protocol for the Pleistocene material. A sediment sample of 20-25 grams was taken from a core of collected material and pulverized with mortar and pestle until the sediment was granular. The pulverized sediment was transferred to 100 mL Tripour beakers for the removal of carbonates, by covering the sediment with 10% hydrochloric acid (HCl). The process of washing the sediment with HCl was repeated until no reaction occurred to ensure the cleaning of the samples from sediment. Sediment was washed with water for neutralization, and then covered in 49% hydrofluoric acid (HF) for 24 hours or more until chemical reaction was complete. Additional HF washes were conducted over a period of two weeks, at least twice per week, to ensure removal of excessive amounts of organics and siliceous material.

After neutralization with water washes, the sediment residue was placed in sterile water (dH₂O). Phenol (C₆H₅OH) was added to the palynological sample to prevent fungal or bacterial growth in the medium. Acetolysis was considered but found to be unnecessary.

D. Slide Preparations

After palynological preparations were completed and neutralization had occurred, sample residues were then mixed with glycerin jelly and mounted on standard microscope slides with coverslips (No. 1.5 22x40mm, No. 1 22x30mm, and No 1.5 22x22mm). To dry the mounting medium, slides were kept upside down on toothpicks on a warming table at 40°C for 2 days and then the coverslip was ringed with clear nail polish creating a tight seal, and preventing degradation of the slides (Madhumi 2002). For this locality, 20+ slides were prepared. Excess palynological samples not mounted were stored in a glass 100mL flask with a few drops of phenol to prevent microbial growth. To prevent contamination of the samples, all preparatory material and tools were washed in bleach and work place conditions sterilized accordingly. Stored samples were also placed in air tight containers to prevent contamination during slide preparations.

Identification of Taxa

A. Macrofossil Cross Sections

Fossil specimens were imbedded in synthetic plastic and cut using a Buehler IsoMet low speed saw. Cut sections were approximately 0.025 of an inch. Cross sections were labeled based on specimen number and cross section number. This technique was used to reconstruct, section by section, the internal anatomy of the *Taxodium* seeds. Comparisons with modern *Taxodium* species were conducted based on described characteristics in literature descriptions of the species.

B. Palynoflora's and Modern Keys

Identification of seeds was completed using the USDA's "Family Guide for Fruits and Seeds," a free identification program based on seed characteristics (Kirkbride *et al.* 2006; Wright 2009). Palynomorph identifications were done using comparisons with published modern and paleopalynological keys and personal communications with other professional palynologists. Ronald Kapp's (1969) book *How to Know Pollen and Spores* published by the William C. Brown Company was the key source for paleopalynological identifications. *The Pollen Morphology and Plant Taxonomy* series of books by Gunnar Erdtman published by the Hafner Publishing Company, Incorporated was a helpful aid in clarifying pollen to specific families and genera. McAndrews, Berti, and Norn's (1973) "Key to the Quaternary Pollen and Spores of the Great Lakes Region" was a helpful visual aid for comparisons to other Pleistocene pollen samples and palynomorph average measurements during this time period. Alfred Traverse's (2007) book *Paleopalynology* 2nd Edition published by Springer was an invaluable source for understanding basics of identification procedures of palynomorphs and their general morphology, and was also a key aid in identifying Pleistocene specific/dominate species. Dr. Martin Farley of the University of North Carolina at Pembroke, Department of Geology, provided expertise on protocol procedures and identifications of specific species.

C. Confocal Microscopy

A Zeiss LSM 710 confocal microscope was used to take confocal and differential interference contrast (DIC) images of the palynomorphs. This microscope is a laser scanning confocal, which is capable of creating Z-stacks and quasi-3D representations of the palynomorphs. A 458 nm, 488 nm, 514 nm multiline argon laser was used to scan the

palynomorphs without fluorescent dyes. The palynomorphs were found to be autofluorescent. Dr. Eva Johannes, the Interim Director of the Cellular and Molecular Imaging Facility at North Carolina State University, was consulted on slide preparations and auto florescent techniques.

D. Scanning Electron Microscopy

A JEOL 5900 LV scanning electron microscope was used to analyze cross sections of *Taxodium* seeds. Seeds were cleaned in H₂O₂ and HCL, and allowed to dry under pressure for one day. Seed cross sections were sputter coated in gold/palladium at 20KA thick on the sides and 30KA thick on the top. Images were taken at 15kv.

E. Statistical Evaluations

SAS version 9.2 was used to evaluate the statistical significance of specific characteristics of *Taxodium* seeds. A discriminate analysis was conducted. Dr. Consuelo Arellano of North Carolina State University's Department of Statistics, was consulted to develop a specific SAS protocol for this evaluation.

PALYNOLGICAL ASSESSMENT

Background Information

A. History of the Pleistocene

The Pleistocene is a period known for its increased rate in climatic changes with the onset of glacial periods followed by warm interglacials. The Pleistocene experienced up to seven major glacial-interglacial cycles (Figure 2.1), with each varying in atmospheric CO₂ composition, longevity, and expansion across North America (Delcourt and Delcourt 1991; Ruen 2005). North America experienced at least five glacial maximums with the Illinois and the Wisconsin being the latter two occurrences, both separated by the Sangamon interglacial. The Wisconsin has loosely been marked as ending with the beginning of the Holocene (10,000 yrs B.P.), though this point is still in argument (Bryant and Holloway 1985). Increasing evidence suggest that around 16-18,000 yrs B.P., vegetation changes were occurring, especially in the southern United States (Bryant and Holloway 1985; Delcourt and Delcourt 1985). The Late-Pleistocene is well documented but few pollen records from early pre-Wisconsin glacial cycles are described (Bryant and Holloway 1985). Pre-Sangamon records exist but none for the southeastern United States (Bryant and Holloway 1985).

Research of Late Pleistocene floras in the southeastern United States have yielded conflicting results about vegetation and climatic conditions, because of an increase in varied results and habitats (LaMoreaux *et al.* 2009). Holocene records from North Carolina are also limited to a few key studies but none that is considered modern (Frey 1951; Frey 1953; Frey 1955; Whitehead 1964; Whitehead 1981). However, there is paleoecological evidence during the Quaternary that shows how change in climatic conditions can affect plant ecosystems, including the glacial cycles of the Pleistocene (Delcourt and Delcourt 1991).

Species richness and diversity, migration patterns, community composition, and dispersion patterns on a species by species level has been shown to change or adapt from climate change during the Quaternary (Delcourt and Delcourt 1991). Climatic shifts that have a lasting range from hundreds to thousands of years can produce instability even at the species level (Delcourt and Delcourt 1991). As with the Pleistocene, sudden and dramatic change in climate affected species distribution and migration. More evidence has shown that areas known as refugia are becoming increasingly common, along with more evidence of stable or few vegetational changes further south during the Wisconsin glacial maximum (Frey 1953). Refugia locations, where the topography or abiotic conditions remain stable, have protected some species from dropping temperatures and climatic affects along the edge of the Laurentide ice sheet (Delcourt and Delcourt 1991). Overall, the Pleistocene was a period of dramatic change and offers a perfect case study of how plant communities are affected by their climate.

B. Other Analyses in the Southeast and North Carolina

Relatively few palynological studies have been conducted in North Carolina and the southeastern United States. Some key studies have been conducted which the author has used as a basis for his palynological comparisons and understanding of the changing flora during the Late Pleistocene and Early Holocene. Frey (1951, 1953, 1955) in his evaluation of Singletary Late, NC described the pollen flora from the early Holocene to Pre-Wisconsin glacial. Frey also noted that his age estimates for the middle and bottom sediment layers probably date back to Sangamon or later ages. Frey (1953) proposed a generalized pollen diagram for the region that can be found in Figure 2.2. From his overall study, and corrections about the age of the locality, it was shown that climatic changes did change

community composition; however the flora was shown to rebound back into similar states during interglacials. Frey (1951, 1953) also noted that some key species were indicators of glacial or cooler climates; these include *Isoetes*, *Tsuga*, and abundant *Picea* pollen.

Whitehead (1964, 1981) analyzed full-glacial vegetation in southeastern North Carolina, including Singletary Lake. Whitehead showed that during a full-glacial period in North Carolina, Pine-Spruce-Fir forest were dominant, along with abundant *Isoetes* spores that were not affected by increases in Pine-Spruce-Fir abundance. Hussey (1993) studied the 20,000 year history of Clear Pond in Myrtle Beach, SC. He came to the same conclusions as Whitehead and Frey, but noted that pollen abundance can differ based on the habitat of the site.

Paleofloristics of the Martin Marietta Locality

A. Approach

A presence-absence approach was considered the best method to conduct for this study. This approach determines the palynoflora and the paleoclimate of this specific habitat and North Carolina during this time period. From the palynoflora we can make inferences about habitat type and climate, and we can determine a relative age of this locality. With few palynological examples and geologic uncertainty, it was considered optimal to construct a community flora using presence-absence until more information is gained from the geology and exact dating of the site. Following a presence-absence survey, a relative abundance was conducted using counts of three slides. This second approach is incomplete and represents a work in progress.

B. Results of Pollen Analysis

A palynological assessment of the sediment shows a wide range in diversity. Results of the analysis can be found in Table 1.1. Palynomorphs found include, but are not limited to, *Pinus*, *Taxodiaceous* types, *Picea*, *Alnus*, *Liquidambar*, *Betula*, *Quercus*, *Ilex*, *Nyssa*, Monolete Fern types, *Poaceae*, *Ambrosia spp.*, and other *Compositae*. Fungal, diatom, and protozoan dinoflagellate palynomorphs are also present, providing evidence of a wetland or riverine setting. From the analysis it was easily determined that *Pinus* was the dominant genus followed by *Quercus* and then grass species. More importantly was the abundance of wetland species including *Taxodiaceae (Taxodium)* and *Nyssa* pollen types, along with Fern and *Sphagnum* spores. A relative abundance was estimated based on counts of 3 slides. Though incomplete for the flora, this relative abundance gives a basic idea to species richness and diversity found at this site.

With these species we can infer what the habitat was like at the Martin Marietta Locality or surrounding communities. The first habitat identified was a wetland community. This assessment is supported by the geology of the site as a lacustrine deposit, or riverine habitat. Wetland species common in this palynoflora are comparable to modern wetland species found in areas such as the Carolina Bays, low level swamps, and river banks. Also seen in this flora is abundant grass species, along with *Pinus* species. This is characteristic of Pine-savanna habitats which also have wetland associations such as pocosins and swamps. The last major habitat type found would be characteristic of a mixed mesic deciduous forest, with *Quercus* being the dominant genus. Examples of pollen types found can be seen in Figure 2.3.

Dating the Locality

From the palynology and the geology of the site, we provide an estimated date for the locality. For exact dating, radiocarbon analysis, thermoluminescence dating, or other isotopic methods are required (Delcourt and Delcourt 1985). A relative date can be obtained from the site geology and palynology. Some pollen or spores can be used as indicator species to determine climate. *Taxodium* pollen is a warm, wet climate indicator (Frey 1951). While on the other hand *Isoetes* spores are typical of cooler, wet climates associated with glacial periods (Frey 1951). Using these indicators, along with the abundance of deciduous species it can be determined that this site is interglacial. The presence of mesophytic types indicates that the climate was moist and warm. Grasses, composites, and sedge pollen percentages are less than the total tree pollen types, indicating that the surrounding area was forested rather than open or prairie (Frey 1951). The absence of *Isoetes* is important, not only as an indicator of cooler climates, but also as a species not associated with brown, or acidic waters common in North Carolina (Frey 1951).

The site is clearly interglacial, but determining which interglacial becomes complicated. The geology of the site is uncertain, but it is certain that the sediment is not Holocene interglacial in age (sand based sediment). Based on the clay based sediment, plant taphonomic state, and locality information it is probable that the site is older than mid-Wisconsin. The Wisconsin glacial period reached a maximum around 23 thousand years B.P., but similar climates comparable to the palynoflora climates described from the Martin Marietta Locality last occurred over 38,000 years B.P. (Frey 1951, 1953). The locality is also similar to the reported *Taxodium* sites along the Atlantic Coast including the clay based sediments (Stahle *et al.* 1990). Stahle *et al.* (1990) argues that these sites are all correlated

to the same sea level increase during the Late Pleistocene dating as far back as 125,000 years. This would date the locality as Sangamon interglacial. Until more data are provided, we will date this locality Late-Sangamon to Early Wisconsin interglacial. An exact date will be determined at a future time.

TAXODIUM SEEDS OF THE MID-LATE PLEISTOCENE

Descriptions of Extant Species

A. Extant Species Taxonomic Debate

The current status of the species of the genus *Taxodium* has yet to be determined and the current debate is centered on the taxonomy of the three species. *Taxodium distichum* is the type species for the genus (Farjon 2005). *Taxodium ascendens* and *T. distichum* are treated as sympatric varieties commonly named *T. distichum* (L.) Rich. var. *imbricarium* and *T. distichum* var. *distichum* (Nutt.) Croom, respectively, depending on the source of classification (Weakley 2010). Farjon (2005) also argues that all three species in the genus could be treated as the same species with varieties, or subspecies. However, the status of *T. mucronatum* is especially considered since it is the national tree of Mexico, and a famous ornamental (Farjon 2005). Farjon (2005) details how there are very few morphological distinctions in cuticle morphology, cone characteristics, and plant architecture including deciduous phenology between the three species. However, Farjon conflicts with Godfrey (1988) in his treatment of *Taxodium*, which evaluated population morphology differences between *T. ascendens* and *T. distichum*. Godfrey (1988) finds *T. ascendens*, and *T. distichum* distinct, but each appears to mimic each other on some occasions, especially during juvenile years. Weakley (2010) describes instances of hybrid habitats, and pseudointermediates that cause difficulties in field identification between *T. ascendens* and *T. distichum*. *Taxodium distichum* begins to resemble *T. mucronatum* in architecture and Weakley (2010) suggests the possibility of introgression. This has led other authors to treat *T. mucronatum* as a third variety of *T. distichum*, commonly called *T. distichum* var. *mexicanum* Gordan (Weakley 2010).

Genetic analyses of *Taxodium* have been conducted and yield similar results to Farjon (2005) and his argument of varietal status for the two southern United States species. Tsumura *et al.* (1999) conducted an evaluation of the genetic differences in *T. distichum* and *T. ascendens* using cleaved amplified polymorphic sequences (CAPS). Using specific DNA markers, comparisons were conducted against *Cryptomeria japonica*, a close relative of *Taxodium*. Most of the genetic variation was found within populations (91%) rather than between different populations (4.9%) and the two test taxa (3.2%). Results of this study suggested that Pond-cypress and Bald-cypress were not distinct species but rather varieties (Tsumura *et al.* 1999). Lickey and Walker (2002) performed a similar genetic analysis using allozyme differences within populations and across populations with more than 400 individuals sampled across 21 locations. Their results agreed with Tsumura *et al.* (1999) in that Pond-cypress and Bald-cypress differed in only one locus of 11 tested. Lickey and Walker (2002) also suggest that *T. distichum* and *T. ascendens* are varieties of the same species.

The two species, *T. distichum* and *T. ascendens*, in North Carolina are distinct in their specific habitat and exhibit morphological differences. Watson (1983) expressed thoughts on speciation processes that resulted in the varietal species of bald cypress and pond cypress. Further arguments on this subject will be discussed after the evaluation of the late Pleistocene *Taxodium* species found at the Martin Marietta locality.

B. *Taxodium distichum* (Linnaeus) Richard

Species description adapted from Weakley (2010) and Watson (1993).

Taxodium distichum is commonly called the Bald-cypress tree, is found in brown and black water swamps across the southeastern United States, and is frequently found in riverine

situations giving the species a very broad range. A distribution map is shown in Figure 3.1. However, Weakley (2010) describes the species as being rare in the Piedmont and Interior Low Plateau of North Carolina as well as in its northernmost limits of Delaware. This species of tree is seasonally cladoptosic (senesced branches) and like other *Taxodium* species, it is a deciduous conifer which produces leaves in early to mid spring (Watson 1993). *Taxodium distichum* produces mature cones around late fall, typically early to mid October in central North Carolina. It is also a widely used cultivare, though when not in its natural habitat it commonly does not produce pneumatophores, simply known as knees.

Weakley (2010) describes *Taxodium distichum* as having large pneumatophores (knees) that are often more than 4 dm tall which is normally conical, mainly tapering at the tops. He also depicts the knees as having thin to shreddy bark. Watson (1993) describes the trunk as often enlarged basally, and buttressed in more frequent flooded habitats. Weakley (2010) further explains *T. distichum* as producing leafy branchlets that spread laterally from the twigs, sometimes used as a field identification characteristic, except in the crowns of mature trees that sometimes mimic that of *T. ascendens*. The crown is often monopodial and conic when young, and as Watson (1993) describes becoming irregular flattopped or deliquescent in aged specimens. Watson (1993) and Weakley (2010) further portray *T. distichum* leaves as linear to lanceolate, spirally arranged, mostly 8-20 mm long, sometimes smaller near the crown and level to flat compared to other genera species. Weakley (2010) further explains how the spiral arrangement is created by the twisting of the basal portions, creating a pseudo-distichous appearance. Occasionally, appressed leaves only on drooping branches of the crown will appear. Watson (1993) describes the shoot system as being evidently dimorphic with the long shoots being indeterminate and the short shoots being

determinate. He explains how long shoot leaves abscise in the fall along with the entire short shoots, with some cases of total shoot abscission. Watson (1993) describes *Taxodium distichum* shoot development as usually being pendent to horizontally spreading, which produce decurrent divergent leaves in two rows. Weakley (2010) illustrates that a striking characteristic of *Taxodium distichum* is the shreddy-orange brown strips from exfoliating bark, that is thin and usually less than 1 cm thick. Watson (1993) describes the pollen cones as being in pendent panicles and noticeable in winter prior to pollination. He further describes the seed cones as ranging from 1.5 cm to 4 cm in size. From the authors' current research, it has been found that mature seeds sometimes range larger in size compared to those documented (Table 2.1).

C. *Taxodium ascendens* Brongniart

Species description adapted from Weakley (2010) and Watson (1993).

Taxodium ascendens is commonly called the Pond-cypress tree, and has a more restricted range than *Taxodium distichum*. Weakley (2010) describes it as being common in ponds, clay-based Carolina Bays, wet savannas, pocosins and other wet to peaty habitats. Figure 3.2 shows the known distribution of *Taxodium ascendens*. Like *Taxodium distichum* it is also found along the shores of natural black water lakes, and along black water streams from swamps but less so in standing water (Weakley 2010). It is common across its range except for Delaware where it is rare. It produces leaves during early to mid spring and produces cones in late fall, typically early to mid October in central North Carolina. Weakley (2010) describes *T. ascendens* as one of the most widely scenic trees across the United States.

Weakley (2010) describes *T. ascendens* as producing large pneumatophores that are rarely greater than 4 dm tall. He describes the knees as being columnar and mound-like

instead of conical like *T. distichum*. Weakley (2010) also states that the pneumatophores are mostly covered with thick, compact bark on top. *Taxodium ascendens* also produces leafy branchlets ascending from the twigs where its name is derived from. The leaves are mostly ascending or uniformed vertically with sizes ranging from 3-10 mm long and in some occasions juveniles produce larger leaves (Weakley 2010). Weakley (2010) describes the leaves as being secundly (arranged to one side or angle) erect due to the curving of the leaflet base positioning the apical portion of the branchlet in a vertical plane. He further elaborates that this characteristic is sometimes absent from juvenile trees which mimic *T. distichum*. Weakley (2010) explains that the leaves are subulate unlike *T. distichum*, but are similar to *T. distichum* in that they also appear spirally arranged. However, the leaves are not spread laterally or featherlike, except on juveniles. Weakley (2010) explains that the exfoliating bark differs from *T. distichum* by having furrowed bark brown strips, and is usually greater than 1 cm thick.

Watson (1993) considers *T. distichum* and *T. ascendens* to be varieties of the same species, but pure populations of extreme characteristics can be found. He describes a characteristic extreme seen of the branchlets and leaves, as being strictly ascending, which can be synonymous with characteristics of *T. ascendens*. Watson (1993) describes that the varieties are generally indistinguishable in reproductive characteristics. Finally, Watson (1993) concedes that pure stands are morphologically and ecologically distinct.

D. *Taxodium mucronatum* Tenore

Species description adapted from Tenore (1853) and Schopmeyer (1974).

Taxodium mucronatum, commonly called the Mexican cypress, is very similar to *T. distichum* with few key differences. As with *T. distichum*, it is found in riverine habitats, but

chiefly native to Mexico and southern Texas (Farjon 2005). A distribution map can be found on Figure 3.3. *Taxodium mucronatum* differs from *T. distichum* and *T. ascendens* in leaf persistence, by being mostly evergreen to semi-evergreen (Schopmeyer 1974). Pollen cones are noticeable larger than the two northern species, while the seed cones mature and disperse during autumn rather than spring as in *T. distichum* (Schopmeyer 1974). The species is known for its age and ability to attain great size.

Descriptions of Extinct Species

A. A Brief History of the *Taxodium-Sequoia-Metasequoia* Paleobotanical Confusion

The genera *Sequoia* and *Taxodium*, before the discovery of *Metasequoia*, were shrouded in systematic and taxonomic debates and confusion. Upon the discovery of *Metasequoia*, Dr. Ralph Chaney of the University of California performed an extensive restudy of the three genera and formulated the exhaustive diagnostic characteristics to clearly identify paleobotanical species belonging to one of the groups (Chaney 1950). Chaney cataloged hundreds of fossil specimens, and reviewed the collections of many of his contemporaries and predecessors to correctly classify a century's worth of fossil plants. His research was limited to American paleobotanical collections with resources provided through the Museum of Paleontology of the University of California which had access to records across the United States, and with sparse access to European records through the collections of Dana, Hayden, Newberry, Lesquerex, and others (Chaney 1950). Chaney (1950) provided a history of *Taxodium* and *Sequoia* that showed, for over a century, the confusion associated with placing newly discovered species within these genera, and at the lack of studying fossil phyllotaxy which would have helped create the third genus *Metasequoia* before the discovery of the living Chinese species. Chaney (1950) states in his review that most records show that the

importance in aging a specimen and consideration of habitat would have alleviated some of the errors associated with identifying a *Taxodium* species. Upon completion of his review, he renamed five species of *Taxodium* to *Metasequoia*, while five species of *Sequoia* foliage were reclassified as *Taxodium* (Chaney 1950). Chaney (1950) states that the species confusion was so great, that all paleo-specimens of *Sequoia* and *Taxodium* need to be reviewed and examined using leaf based characters to determine correct identifications. In conclusion of his review, one fossil species of *Taxodium* was identified as *Taxodium dubium* (Sternberg) Heer, which includes an exhaustive list of synonymous species and a respectable amount of evaluation from him and previous authors. Also, because of this revision the paleo-species of *Taxodium* decreased from more than a dozen species to just one.

Aulenback and LePage (1998) described a new *Taxodium* found in the Upper Cretaceous called *Taxodium wallissii* Aulenback et LePage sp. nov. This species is the oldest known *Taxodium* to be discovered and the first one discovered that places *Taxodium* in the Cretaceous. Aulenback and LePage (1998) state that Chaney's focus on leaf morphology and special characteristics is important for species-level identification, whereas seed cone morphology is essential for generic-level identifications. Unfortunately, seed cones that are found intact or whole are rare compared to branches or leaves. Kunzmann *et al.*'s (2009) analysis of *Taxodium* in the Paleogene and Neogene of Central Europe agrees with Chaney and his evaluation of *Taxodium* in the American paleobotanical record. All species found in Central Europe have been designated as *Taxodium dubium*, with two additional forma designated as *Taxodium dubium* (Sternberg) Heer, forma *heerii* (Dorofeev, 1976) stat. nov. et emend, and *Taxodium dubium* (Sternberg) Heer, forma *dubium* which differs based on the lack of spines or thorns on the cone scales.

B. *Taxodium wallissii* Aulenback et LePage sp. nov

Species description adapted from Aulenback and LePage (1998).

Taxodium wallissii was found in Upper Cretaceous silicified remains in the Horseshoe Canyon Formation in Alberta, Canada (Aulenback and LePage 1998). Aulenback and LePage describe *T. wallissii* as branching alternatively with dimorphic leaves, taxodioid and cupressoid, that appear helically arranged (1998). They further state that both leaf types are persistent. The taxodioid leaves are decurrent, appearing acicular, acuminate, and linear-falcate with sizes up to 11.5 mm long and 1.0 mm wide. The cupressoid leaves are nondecurrent appearing ovate with an acute to acuminate apex ranging up to 2.0 mm long and 1.5 mm wide; however, this leaf type is rare. Aulenback and LePage (1998) state that cupressoid leaves are subtending the staminate and pistillate axes, which are persistent sometimes falcate, and range up to 5.0 mm long and 1.75 mm wide. *Taxodium wallissii* seed cones are globose in shape while erect on short-stalks, and are arranged alternately on the branches. The seed cones have been found to have up to fifteen helically arranged imbricate bract-scale complexes that are deciduous (Aulenback and LePage 1998). Seeds are found two per scale unequally three angled, with sizes ranging from 4.0-9.0 mm long to 1.9-5.5 mm wide (Aulenback and LePage 1998). *Taxodium wallissii* staminate cones are arranged in long terminal panicles with up to nine helically arranged microsporophylls which are typical of extant *Taxodium* species (Aulenback and LePage 1998). Pollen is also typical of other *Taxodium* species, only on a smaller scale. Aulenback and LePage (1998) state that the vasculature and arrangement of the resin canals in the cone scales are a unique characteristic of *T. wallissii*. The resin canals in immature bracts begin as a central canal with two lateral canals that end in the bract mucro, while mature bracts have three sets of canals to form from

the bifurcation of the lateral and central canals (Aulenback and LePage 1998). This elaborate setup produces resin canals that supply the bract and are well developed where seed attachment occurs. Aulenback and LePage state that this is more complex than structures in any extant *Taxodium* species (1998). *T. wallissii* habitat is interpreted to be from an alluvial wetland in a coastal plain that was paleoclimatically humid and frost free (Aulenback and LePage 1998).

C. *Taxodium dubium* (Sternberg) Heer

Species description adapted from Chaney (1950), which includes Heer's original description, and Kunzmann *et al.* (2009).

Taxodium dubium was first found in the Paleocene-Eocene of Alaska and Canada, and it appears to have become widespread during the Middle Tertiary, where it began migrating into the Upper and Southern United States (Chaney 1950). Chaney (1950) states that from the fossil record, the last known occurrence of *T. dubium* was in transitional age deposits of Miocene to Pliocene in the West and from Eocene to Pliocene in the southeastern United States. No Quaternary fossils of *T. dubium* have been found.

Taxodium dubium also has long shoot and short shoot organization when compared to *T. distichum*. The shoot system is dimorphic, with the leaves mostly acicular (Chaney 1950). Chaney (1950) describes long shoots as having widely spaced needles (single leaves) that are up to 1.2 mm long and 1.0 mm wide, which are spirally arranged and occasionally producing alternate short shoots in their axils. He further describes short shoots that are slender, while either straight or curving, that are up to 8 cm long and at times bearing 60 leaves helically attached at maturity in a distichous position. The leaves of the short shoots are largest at the middle, and decrease in length towards the apex (Chaney 1950). Leaf blades are linear to

lanceolate, with abrupt round to narrow and short decurrent bases that when in angle appears to have a short petiole (Kunzmann *et al* 2009, Chaney 1950). The leaf tips are mostly acute, with sizes ranging from 5 to 17 mm long, and 0.5 to 1.2 mm wide (Chaney 1950). Complete seed cones are rare, but are globose in shape, spirally attached with helically arranged bract-scale complexes that are commonly deciduous (Kunzmann *et al* 2009, Chaney 1950). Seed cone bract-scales are shield shaped with protruding margins that can vary in size.

Ornamentation of the bract-scale margins distinguishes the two forma that are found in Central Europe, although this is not the only differential characteristic (Kunzmann *et al* 2009). *Taxodium dubium* (Sternberg) Heer, forma *heerii* (Dorofeev, 1976) stat. nov. et emend has significantly more ornamentation compared to *Taxodium dubium* (Sternberg) Heer, forma *dubium* which can be either smooth to having very little ornamentation (Kunzmann *et. al* 2009). Seeds are born two per scale, and depending upon the author are either vestigial or thick winged at the three lateral ridges, but are traditionally shaped singular to triangular like extant *Taxodium* species. The staminate cones of *T. dubium* are found in panicles on short shoots, with the branches found regularly detached (Chaney 1950). The staminate cones are arranged spirally, but are not born in pairs but singly with globular to ovoid shape and up to 1 mm in diameter (Chaney 1950).

Current Knowledge of *Taxodium* Seeds

A. Background Information

Taxodium is considered a relic genus in age and in discovery. The age in which most species of the genus were described focused more on leaf characteristics, plant form, and overall plant anatomy, and less so on seed morphology and anatomy. This is reasonable since leaf morphology was the major distinguishing characteristic for the genus until recently

(Aulenback and LePage 1998). The original description of *T. dubium* seeds described the shape as being angular, heavily ribbed, with thick wings, and a general size of 12 mm long to 10 mm wide (Chaney 1950). The Aulenback and LePage (1998) description of *T. wallissii* provides the best detail of seed anatomy of the fossil species compared to the extant species. They describe *T. wallissii* seeds to be unequally three-angled and to have sizes ranging from 4.0-9.0 mm long to 1.9-5.5 mm wide. Aulenback and LePage (1998) analyzed the internal anatomy of the *T. wallissii* seeds and discovered three distinct layers of the seed coat, which could be differentiated based on cell shapes, height, and location. However, it was their study and comparison of *T. wallissii* seed anatomy to the extant species that provided a new distinguishing characteristic for the genus.

Extant species of *Taxodium* have three distinct layers of the seed coat tissue (Aulenback and LePage 1998, Werker 1997). The outer layer (exotesta) is composed of rectangular cells that form a thin single layer, while the middle layer (mesotesta) is spongy because of its composed mixture of isodiametric and papillate cells (Aulenback and LePage 1998). The outer and middle layers are the same for all three extant species, but the inner layer (endotesta) of the seed coat is the differential characteristic. *Taxodium distichum* has a thick inner layer on the abaxial and adaxial surfaces, while the lateral walls of the inner layer are thinner (Aulenback and LePage 1998). This differs from the other two extant species, *T. ascendens* and *T. mucronatum*, in that the inner layer of the seed coat is uniformly thin (Aulenback and LePage 1998). This is a new characteristic that can be added to the list of morphological differences seen among the three species, but it is also important in that it is a new differentiating characteristic between *T. ascendens* and *T. distichum*, which are more commonly seen as one species. *Taxodium distichum* seeds can be seen in Figure 3.4, *T.*

ascendens seeds can be seen in Figure 3.5, and *T. mucronatum* seeds can be seen in Figure 3.6.

One last issue to point out when talking about *Taxodium* fossil seeds, is fossil seed assemblage sorting bias in an estuarine environment. Sims and Cassara (2009) performed a field study to compare a living hard-wood forest and salt marsh community to the death seed assemblages collected in a nearby tidal estuary. Results of their study revealed that death assemblages collected from the tidal estuary differed significantly from the source communities (Sims and Cassara 2009). Their study showed that small-seed morphotypes were less represented in the collected death assemblages, and 45% of the death assemblage collected was in the life assemblages but more so only 33% of the species in the life assemblage were present in the death assemblage (Sims and Cassara 2009). Overall, the study showed that estuarine environments only collect a small representation of the local seed community, with bias likely to derive from issues of transport into natural collection areas (Sims and Cassara 2009). This also creates a problem when creating a seed-size distribution of a community, and affects *Taxodium* seeds which appear to be favored in taphonomic processes in estuarine communities or wetlands, thanks to having a relatively large seed, and being non-arborescent (Sims and Cassara 2009).

B. Results of Cross Section Analysis of the new Pleistocene *Taxodium* Seeds

Fossil seeds can be seen in Figure 3.7 unclean, and cleaned with H₂O₂ and 10% HCL in Figure 3.8. Initially, longitudinal sections were made using an IsoMet low speed saw. These sections showed that the fossil *Taxodium* seeds were very similar to the extant species. Figures 3.9 and 3.10 show how the seed coat is composed of three distinct layers. Scanning electron images were taken of cross sections of the seeds (Figure 3.11 and Figure 3.12). The

outer layer (exotesta) of the testa showed that it had a single layer of rectangular cells which is identical to the three extant species (Figure 3.13). The middle layer (mesotesta) of the testa was composed of isodiametric and papillate cells that form a spongy layer very similar to those of the extant species, and thickness of this layer is comparable also (Figure 3.13). However the inner layer (endotesta) of the testa is uniformly thin in longitudinal section and cross section (Figure 3.14 and Figure 3.15). This is identical to *T. ascendens* and *T. mucronatum* but differs from the enlarged abaxial and adaxial portions of *T. distichum*'s inner testa layer. Overall the fossil seeds are very similar to those of the extant species, but have some key differences that appear on a testa layer by layer basis (Table 2.2)

Statistical Analysis and Comparisons

A. Explanation of Methods Used

SAS version 9.2 was used to evaluate the statistical significance of specific characteristics of *Taxodium* seeds. A discriminant analysis was conducted with 290 observations on three variables of four categorical groups. The variables include three continuous, numeric variables (seed length, seed width, and seed length-seed width ratios) and four categorical groups, the three extant *Taxodium* species and the fossil *Taxodium* from the Pleistocene. For this analysis comparisons were conducted between the individual variables, conducted a multivariate analysis, and overall determine if the species were significantly different. Dr. Consuelo Arellano, of North Carolina State University's Department of Statistics, was consulted to develop a specific SAS protocol for this evaluation. Both the proc discrim and proc candisc procedures were used and yielded the same results. Both procedures produce canonical correlations, which compares sets of data and linear combinations between similar

variables. An important note is that discriminant analysis is not a cluster analysis. With discriminant analysis, prior knowledge of the classes or categorical groups is required.

B. Results

Initially, a comparison was made between the three extant species and the fossil *Taxodium* (Table 2.3). Three variables were used as discriminants and four classes which represent different species. Seed counts differed for all four species depending on availability, while most of the fossil seeds were used unless not whole or broken (Table 2.4). 290 seeds were used overall in this initial sample. Comparisons were conducted in SAS, beginning with the Seed Length and Seed Width Ratios (SLSW_Ratio) shown in Figure 3.16. This chart shows that the fossil *Taxodium* has a larger range in SLSW_Ratio compared to the extant species, and also helps demonstrate that the seeds are noticeably larger. Graphing the SLSW_Ratio compared to either the seed length or seed width shows the fossil *Taxodium* as having a wide range that overlaps and engulfs the three extant species (Figures 3.17, and 3.18). Comparing just seed length and seed width (Figure 3.19) yields similar results. A 3D representation of the SLSW_Ratio along with seed length and seed width was drawn to gather a spatial representation of the four *Taxodium* species (Figure 3.20). Both proc candisc and proc discrim yielded the same results for the multivariate test. SAS 9.2 runs four different multivariate tests simultaneously and all tests showed significant differences (Table 2.5). P values ($P < 0.001$) for all tests reject the null hypothesis that all canonical correlations are equal to zero. The P values show that the groups are significantly different from each other. Table 2.6 shows the canonical correlations that were calculated from comparing the variables and produced variable values from the categorical groups (species). Table 2.6 continued shows the eigenvalues and likelihood ratios calculated from the canonical correlations that

were adjusted and squared. The larger the canonical correlations the more significantly different the likelihood ratios will be. Likelihood ratios are a test of the null hypothesis, that the canonical correlations are zero, which were rejected by being significantly different. A cross-validation summary using the quadratic discriminant function (Table 2.7) was conducted. This procedure calculates from given variable information the most likely grouping it should occur in statistically, compared to natural groupings already predetermined. The fossil seeds were classified 60.91% of the time into their own distinct group with 20.91% placed in the *T. ascendens* group, 8.18% placed in the *T. distichum* group, and 10% placed in the *T. mucronatum* group. Comparing *T. distichum* and *T. ascendens*, over 55% and 61.67% of the time the respective seeds were placed in the correct groups but 35% and 33.33% of the time placed in the other group. *T. mucronatum* appears more distinct with 91% of its seeds placed within the correct group. It is from this data set that we can calculate error rates with the fossil *Taxodium* seeds placed in one of the extant groups 39% of the time, but compared to *T. mucronatum* with only 9% error rates (Table 2.8). It became clear that *T. mucronatum* was a distinct group from not only the other extant species but also the fossil species.

A second SAS analysis using proc discrim and proc candisc was conducted, but excluded *T. mucronatum*. Data summary can be found in Tables 2.9 and 2.10. 190 seeds were used in this analysis with 3 variables and 3 categorical groups. The multivariate tests showed significant differences (Table 2.11). P values ($P < 0.001$) for all tests reject the null hypothesis that all canonical correlations are equal to zero. The P values show that the groups are significantly different from each other. Table 2.12 shows the canonical correlations, eigenvalues, and likelihood ratios calculated from the canonical correlations that

were adjusted and squared. The likelihood ratios also proved to be significantly different. A cross-validation summary using the quadratic discriminant function (Table 2.13) was also conducted. The fossil seeds were classified 70.91% of the time into their own distinct group with 20.91% placed in the *T. ascendens* group, and 8.18% placed in the *T. distichum* group. Compared to the first analysis, some *T. mucronatum* seeds were placed into the fossil group and the *T. ascendens* and *T. distichum* ratios stayed the same. Error counts for the fossil *Taxodium* seeds was also lower at 29% (Tables 2.14). However, this time *T. ascendens* gained a lower error rate of 20% due to a 80% correct grouping. These results show that without *T. mucronatum*, *T. distichum* and *T. ascendens* become noticeably different as groups while the fossil species becomes more distinct as a separate group.

Description and Systematics of *Taxodium macrosperma* Locklear and Mickle

Compiling all of the known data available, it is clear that the fossil *Taxodium* seeds differ from the extant species based on overall gross morphology, seed coat anatomy, and are significantly different statistically. It is important to place this species on record until additional evidence surfaces to aid and better understand the species and genus overall.

Systematics

Taxodium macrosperma sp. nov. Locklear and Mickle

Diagnosis: Seeds unequally three angled, averaging 6-17mm long by 3-9 mm wide, appearing small winged to having raised edges, and dark brown to light brown in color. Hilum is large and lighter in color compared to the rest of the seed surface. Seed coat consists of three distinct layers comparable to extant and extinct species in the genus.

Collecting Locality: Martin Marietta Mine, Wilmington, New Hanover County, North Carolina. (Latitude 34° 22. 368' N, Longitude 77 ° 50.356' W)

Age: Interglacial Pleistocene, probably Late Sangamon-Early Wisconsin

Holotype: NCSU Paleobotanical Collections #1000, Figure 3.21

Description of Species

The seeds of *Taxodium macrosperma* are unequally three angled, irregular in shape, and are larger in size compared to the extant species in the genus (Figures 3.4, 3.5, 3.6, and 3.21).

The seeds average 6-17 mm long by 3-9 mm wide and the depth of the seeds vary with no clear correlation to seed length or seed width. The seeds are dark brown to light brown in color after hydrogen peroxide washes, and the hilum is lighter in color compared to the rest of the seed and occupies up to a third of the seed surface. The seed surface is not glabrous but rather rugulose and striate, and it is corky in appearance. The seed surface is porous, net-like at high magnification, and probably vascularized (Figures 3.22, and 3.23). The anatomy of the testa shows three distinct layers: an outer and thin exotesta, a middle spongy mesotesta, and a thin endotesta. The exotesta is a single layer of rectangular cells with a general uniform thickness (Figure 3.13). The mesotesta is thick, ranging over ten times to twenty times thicker than the exotesta, and is composed of isodiametric and papillate cells that form a spongy layer in appearance and function (Figure 3.13). The endotesta is uniformly thin, as seen in Figure 3.15, and is shown to separate from the mesotesta if damaged as seen in Figure 3.24. The endotesta is thinner than the exotesta. Degraded and desiccated embryos can be found in some seed cross sections, but no information can be determined from their current state as seen in Figure 3.25. The embryo is centered to irregular positioned longitudinally, but more centered in cross section. The micropyle is visible on some seeds, and can be clearly seen in a longitudinal seed split in SEM (Figure 3.26). The micropylar end of the seeds is typically attenuate, while the chalaza end of the seed is usually obtuse

(Figure 3.21). The position and anatomy of the embryo is probably similar to the extant species; a diagram of the extant species anatomy can be seen in Figure 3.27.

Discussion

The fossil *Taxodium* seeds discovered from the Martin Marietta locality have offered conflicting results. Evidence shows that the seeds differ from the three extant species, but also appears similar in many ways. These results, however, were expected. Given its occurrence during the Pleistocene, the fossil species should be very similar to the living species today. As seen with the living species and the ongoing taxonomic debates about their nomenclatural status, it is agreed that the species within the genus are very similar. This fossil specimen discovered has the potential to help clear up this debate, at least in paleobotanical terms and offers ideas about genus radiation. Once we look at the history of the genus we can see that *Taxodium* is a reserved, relic, and stable taxon with few species discovered over its 80 million year history. The discovery of an intermediate or transitional species would help explain the current debate ongoing within the genus.

Taxodium macrosperma seeds differ from extant species based on general gross morphology (seed size). The fossil seeds are larger, in comparison with the extant species, with dramatic size differences compared to *T. mucronatum* which has smaller seeds compared to *T. distichum* and *T. ascendens* (Summary in Table 2.1). A factor that must be taken into consideration is sorting bias in the paleobotanical record. However, a review of seed dispersal for the genus and an understanding of its natural habitats show that the species of *Taxodium* are adapted to wet, lacustrine, or standing water types of environments. *Taxodium* seeds have adapted to these environmental situations with thick testa and flanges for increased surface area allowing the seeds to float, also periods of water saturation are

needed for seed germination to occur (Watson 1983). *Taxodium* seed testa thickness and composition, such as a spongy or corky mesotesta, allows for the seeds to float, with varying size differences of the seeds showing no initial bias (Werker 1997, Watson 1983). Testa thickness appears to be a dispersal mechanism designed for situations where the species is in standing water, especially for *T. ascendens*, pond cypress (Werker 1997, Watson 1983). It is these details that lead me to believe that sorting bias is minimal for *Taxodium macrosperma* since seed anatomy helps prevent sorting bias in wet-flowing habitats or situations. Ranges in the seed length and seed width ratios (SL: SW) for *Taxodium macrosperma* also provides evidence of minimal sorting bias (Figure 3.16). The SL: SW ratio for *Taxodium macrosperma* has a larger range compared to the extant species. Though it is uncertain (not able to determine 100% certainty with fossil species) that all the collected seeds during deposition are of the same species, analysis of the seed anatomy helps demonstrate that the seeds are the same anatomical, which supports the claim that these seeds are from the same individual and/or species of *Taxodium*.

Taxodium macrosperma seed coat anatomy offers differing results when compared to the extant species. The exotesta appears identical in all species of *Taxodium*. The mesotesta of *T. macrosperma* also appears comparable to the extant species. It is the endotesta that offers differing results. The endotesta of *T. macrosperma* is uniformly thin as seen in Figures 3.11 and 3.12, but this differs from *T. distichum*. Aulenback and LePage (1998) show that *T. distichum* has a thickening of the endotesta at the abaxial and adaxial regions of the seed, and that the endotesta of *T. ascendens* and *T. mucronatum* is uniformly thin. A summary of these results is found on Table 2.2. It is unclear if environmental conditions have any effect on the formation of *Taxodium*'s endotesta, but generally seed characteristics

are genetically derived and are usually stable species to species (Werker 1997). The endotesta of *T. macrosperma* is similar to that of *T. ascendens* and *T. mucronatum*, but differs to *T. distichum*, the type species of the genus. The environmental setting of *T. macrosperma* would be more similar to that of *T. distichum* rather than *T. ascendens* or *T. mucronatum*. With Edward Berry (1907, 1909a, 1909b) describing *Taxodium* species of the Pleistocene as being *T. distichum*, you would expect the fossil seeds to be similar to *T. distichum* especially if little visible genetic variation has occurred. Initially the results are conflicting. However two results can be gained from the seed anatomy, 1) *Taxodium macrosperma* seeds differs from those of *T. distichum*, and 2) are anatomically similar to *T. ascendens* or *T. mucronatum*. So from these results you could argue that the seeds have to be *T. ascendens* or *T. mucronatum*, but as will be demonstrated, the seed morphology is statistically different from those two species. It can be argued that the habitat appears to be different also, with *T. macrosperma* appearing in lacustrine settings while *T. ascendens* is commonly found in stagnant or flooded water habitats.

The fossil seeds also differ statistically compared to the extant species. *Taxodium macrosperma* seeds differ from the extant species based on comparisons of the seed width, seed length, and the seed length and seed width ratios. Using discriminant statistics and creating canonical values, comparisons of sets of different variables was possible. Comparing the extant species and their respective sets of data to *Taxodium macrosperma* yielded interesting results; initial expectations included no differing results. The results showed that *Taxodium macrosperma* has a large range in seed length and seed width to that of the extant species, and, if scatter plotted, shows that *Taxodium macrosperma* morphological characteristics engulf the extant species (Figures 3.17 and 3.18). Not only is

the morphological plasticity in *Taxodium macrosperma* greater, but it is also significantly different from the extant species as a group ($P < 0.001$) and when comparing each specific morphological characteristic ($P < 0.001$). Using a cross-validation summary with the quadratic discriminate function, the *Taxodium macrosperma* seeds were naturally placed into a new group 60% of the time, while 40% of the seeds were placed into extant groupings. This is significant that over 50% of the seeds formed a new group. It is also understandable that some of the seeds would be placed into extant groups, given the wide range in morphological characteristics displayed in the fossil seeds. With the exclusion of *T. mucronatum*, the fossil seeds created a new group 71% of the time. Two key points can be learned from the statistics: 1) the fossil seeds display morphological characteristics that cover the range of morphological characteristics seen in the extant species, and 2) these results are significant with a clear cross validation of a new specific group.

With the discovery of only the fossil seeds and no other plant organs discovered so far, it is not 100% certain if this is an extant species. Thus we propose a new species, more so an organ species, for the fossil *Taxodium* seeds called *Taxodium macrosperma*. With these overall results I have come to the conclusion that *Taxodium macrosperma* is an ancestral species to the extant species found today, and a probable transitional species from *T. dubium*. I have proposed an evolution timeline for the genus in Figure 3.28. Frank Watson (1983) in his dissertation on the taxonomy of bald-cypress and pond-cypress expresses his thoughts about the genus undergoing species radiation. From the results compiled so far, it adds evidence to his ideas. With some morphological and anatomical differences along with statistical significance compared to the extant species, I would argue that *Taxodium macrosperma* represents a transitional species. This conclusion would help

with the current argument surrounding the taxonomic debate ongoing in the genus. Taking into account the history of the genus, and gathering a paleoecological perspective of Pleistocene conditions, this hypothesis seems plausible.

Looking at the history of the genus and the revisions made by Chaney (1950) along with the discovery of *Taxodium wallissii* (Aulenback and LePage 1998), our understanding of the genus is still limited. As seen in Table 2.15, we can see that detailed accounts of *Taxodium* are missing from the Pliocene, Pleistocene, and the Paleocene. No fossil evidence or detailed accounts of species from these time periods have been found or described in the literature. With the discovery of *T. wallissii* the genus was found to be older by 25 million years, and hopefully with more discovered fossil material of Paleocene age, the transition from *T. wallissii* to *T. dubium* can be analyzed. More importantly we have a gap in knowledge after the Miocene and the transition of *Taxodium dubium* into the extant species alive today. There is paleoecological evidence during the Quaternary that shows how change in climatic conditions can affect plant ecosystems, species richness and diversity, and community composition (Delcourt and Delcourt 1991). Climatic shifts that range from hundreds to thousands of years can produce instability (adaptation rates or failures) even at the species level (Delcourt and Delcourt 1991). The Pleistocene is a period known for its increased rate in climatic changes with the onset of glacial periods followed by warm interglacials. The Pleistocene experienced up to seven major glacial-interglacial cycles (Figure 2.1) with gradual cooling lasting up to 90,000 years, while some interglacial maxima lasting only 10,000 years (Delcourt and Delcourt 1991). This sudden and dramatic change in climate affected species distribution and migration; however it has been found that pockets of land became safe havens during glacial maxima. These safe havens are known as refugia,

where landscape or abiotic conditions protected some species from dropping temperatures along the edge of the Laurentide ice sheet (Delcourt and Delcourt 1991). These dramatic changes during the Pleistocene had an effect on the species of *Taxodium*. The periods of migration, areas of refugia, and areas not affected by glacial events could have initiated species radiation in the genus. In hypothesis, an ancestral species, probably *Taxodium macrosperma*, under stress from dramatic climate changes, migration up and down middle and eastern North America, stable refugia locations along the eastern seaboard, and areas unchanged by glacial events in southern Texas and Mexico, probably helped to initiate species radiation which gave rise to the three extant species today. The habitats differed and species locked in specific locations adapted to their new environment and community instability (Delcourt and Delcourt 1991). This hypothesis would also explain the current distribution of the three extant species with *T. distichum* favoring riverine and swamp habitats, with *T. ascendens* favoring ponds and areas of standing water, and *T. mucronatum* found only in riverine environments in South Texas and Mexico. This hypothesis was first proposed by Frank Watson (1983), who argued spatial isolation during the Pleistocene in response to directional selection pressures from differing habitats, initiated radiation. Watson (1983) concludes that *T. ascendens* is a semispecies of *T. distichum*, however he fails to communicate the importance of the fossil record and its lack of a complete genus history. Watson also doesn't consider a previous undiscovered species of *Taxodium* that could undergo species radiation, producing the three extant species seen today. One of Watson's arguments for his theory includes fossil evidence of leaf morphological characteristics more similar to *T. mucronatum* and *T. distichum*, rather than *T. ascendens*, in sense declaring that *T. ascendens* is the most recently derived. However, this comparison is incomplete, with

little qualitative or quantitative fossil evidence discovered and described during the Pliocene-Early Pleistocene of the extant species, where no such comparisons or evidence available of resilient leaf characters can be argued. Also, *T. dubium*, a widespread species during the Tertiary in North America, was more dominant and predominantly described from European fossils. A comparison of *T. dubium* and the extant species has not been analyzed exhaustively, but Kunzmann *et al.* (2009) and their analysis of *T. dubium* in Europe makes a significant attempt.

This improved hypothesis would help with the taxonomic debate currently ongoing with the extant species. Debates mostly focused on metrics of morphological and genetic differences that determine the distinction of species or variety, could evaluate another genus or group of species undergoing radiation and make a comparable example to *Taxodium*. Also, it must be taken into consideration that radiation in the genus may be in-process rather than complete. Another question raised is how to consider species radiation, and classification procedures for such occurrences. This is an important consideration, especially for conservation practices for these species and maintaining species diversity. Overall it is unclear if the genus is in the process of species radiation, but the discovery of *Taxodium macrosperma* adds support to Watson (1983) and his initial idea.

Future work and more evidence are needed. The discovery of different plant organs of *Taxodium macrosperma* may help cement the hypothesis that it is a transitional species. Discovery of leaf, or cone organs will allow for more detailed and diagnostics evaluations to be conducted with extant and extinct species of *Taxodium*. The Martin Marietta locality has produced abundant macrofossils, which future work will be focused on identification and evaluation of that particular flora. Further evaluation of sediment samples of the Martin

Marietta locality should help us come to a conclusion about the status of *Taxodium macrosperma*, and the *Taxodium* genus overall.

Table 1.1 – Palynological assessment results and relative abundance.

Plant Genera	Relative Abundance	Non-Plant Species
<i>Alnus</i>	3%	Diatoms
<i>Ambrosia</i> -type	5%	Dinoflagellates
<i>Betula spp.</i>	3%	Fungal
<i>Carya spp.</i>	2%	
<i>Castanea</i>	1%	
<i>Compositae</i>	5%	Plant Macrofossils not found in the Palynology
<i>Ericaceae</i>	1%	<i>Fagus</i>
<i>Ilex</i>	1%	<i>Vitis spp.</i>
<i>Liquidambar</i>	3%	<i>Ulmus</i>
Monolete Fern types	1%	
<i>Nyssa spp.</i>	3%	
<i>Picea</i>	1%	
<i>Pinus spp.</i>	40%	
<i>Poaceae</i>	5%	
<i>Quercus</i>	20%	
<i>Sphagnum</i>	1%	
<i>Taxodiaceae</i>	5%	

Table 2.1 – Summary of important morphological features of *Taxodium* species. Partly compiled from literature: Aulenbeck and LePage (1998) and Kunzmann *et al.* (2009). Author’s additions and/or modifications highlighted yellow.

Species	Locality	Seed cone diameter (mm)	Cone scale distal part width x distal part height x length (mm)	Seed length x width (mm)	Short shoot length (mm)	Leaf length x width (mm)	Stoma-apparatus, abaxial length x width (um)	Stoma-apparatus, adaxial length x width (um)	Stomatal chamber length x width (um)
<i>Taxodium wallissii</i>	Drumheller, Alberta Late Cretaceous	13	No data published	4-9 x 1.9-5.5	No data published	Taxodioid 11.5 x 1 Cupressoid 5 x 1.7	No data published	No data published	No data published
<i>Taxodium dubium</i>	Svetlogorsk, Russia Early Oligocene	33□ x 28	6-17 x 7-16 x 11.16	5.6-11 □ x 3.1-5.8	35-65	6-15 x 1-2	No data published	No data published	No data published
	Otradnoje, Russia Early Oligocene	No complete cone preserved	4-10 x 5.7-12.2 x ?	6.5-9.3 x 4.3-5.4	No data published	No data published	No data published	No data published	No data published
	Padekovo, Russia Early Oligocene	No complete cone preserved	No data published	6.5-7.6 x 3.5-4	No data published	No data published	30-40 x ?	No data published	No data published
	Haselbach, Schleenhain, Germany Early Oligocene	No complete cone preserved	6-17 x 6-17 x ~8.5	8.5-9 x 4-5.25	27-105 or longer	4-17 x 1.5-1.9	23-60 x 23.5-55.5	60-84 x 40-52	23.5-28 x 6-18
	Seifhennersdorf, Germany Early Oligocene	No complete cone preserved	7-14 x 5.5-13 x 11-15	6-10 x 3.5-8	25-145 or longer	4-14 x ~1	38 x 10-26	Not preserved	Not preserved
	Borna-Ost, Bockwitz, Germany Late Oligocene	14-16(20)	5-12 x 4-11 x 9-12	4.5-10 x 2.8-5.5	≤90 or longer	10-25 x 1-2	45-75 x 36-70	50-63 x 43-53	17-30 x 13-20
	Bilina, Czeck Republic Early Miocene	20-24.5	9-13.5 x 6-8.5 x 11.5-16	11.5 x 6	22-80 or longer	4.5-18.5 x 0.75-2	52-98 x 42-96	57-80 x 65-103	23-49 x 13-26
<i>Taxodium macrosperma</i>	Martin Marietta, Mid-Late Pleistocene	Not preserved	Not preserved	6-17 x 3-9	Not preserved	Not preserved	Not preserved	Not preserved	Not preserved
<i>Taxodium distichum</i>	North Carolina State Herbarium, Holocene	(15)-20-35-(40)	6-17 x 6-15 x 6-17	4-13 x 5-7 Mod. 10-16 x 6-9	80-100 or longer	3-17 x 0.7-1.5	N/A	N/A	N/A
<i>Taxodium ascendens</i>	North Carolina State Herbarium, Holocene	20-30	6-17 x 6-15 x 6-17	Mod. 10-15 x 9-13	80-100 or longer	3-17 x 0.7-1.5	N/A	N/A	N/A
<i>Taxodium mucronatum</i>	University of Naples Botanical Gardens, Holocene	(12)-14-25-(30)	7-12 x 5-10 x ≤12	4-9 x 3-4 Mod. 6-12 x 3-8	60-160 or longer	3-18 x 1-15	48-88 x 36-88	52-88 x 38-64	20-40 x 8-18

Table 2.2 – Anatomy of *Taxodium* seed testa layers. Data partly compiled from Aulenbeck and LePage (1998).

	<i>Taxodium wallissii</i>	<i>Taxodium dubium</i>	<i>Taxodium macrosperma</i>	<i>Taxodium distichum</i>	<i>Taxodium ascendens</i>	<i>Taxodium mucronatum</i>
Outer Layer (Exotesta)	Single layer of rectangular cells	No data published.	Single layer of rectangular cells	Single layer of rectangular cells	Single layer of rectangular cells	Single layer of rectangular cells
Middle Layer (Mesotesta)	Isodiametric and rectangular cells that form a spongy layer	No data published.	Isodiametric and papillate cells that form a spongy layer	Isodiametric and papillate cells that form a spongy layer	Isodiametric and papillate cells that form a spongy layer	Isodiametric and papillate cells that form a spongy layer
Inner Layer (Endotesta)	Uniformly thin, appearing elliptical in cross section	No data published.	Uniformly thin	Thicker on the abaxial and adaxial surfaces and thinner on the lateral walls	Uniformly thin	Uniformly thin

Table 2.3 – Data Summary of Discriminant Statistics using the Candisc and Discrim procedures. Total sample size represents total seeds measured; the three variables represent Seed Length and Seed Width Ratios along with individual Seed Widths and Seed Lengths. The four classes represent the four species of *Taxodium*.

Total Sample Size	290	DF Total	289
Variables	3	DF Within Classes	286
Classes	4	DF Between Classes	3

Table 2.4 – Class level information of the four *Taxodium* species. *T. fossil* represents the fossil *Taxodium*, *Taxodium macrosperma*. Frequency represents distinct seed measurements made for each group.

Class Level Information				
group	Variable Name	Frequency	Weight	Proportion
T.ascendens	T_ascendens	20	20	0.068966
T.distichum	T_distichum	60	60	0.206897
T.fossil	T_fossil	110	110	0.37931
T.mucronatum	T_mucronatum	100	100	0.344828

Table 2.5 – Table showing the results of the four multivariate tests run by SAS in the Discrim and Candisc procedures. This test includes the data for all four *Taxodium* species. P values for all tests reject the null hypothesis that all canonical values are equal to zero. The P values show that the groups are significantly different from each other.

Multivariate Statistics and F Approximations					
S=3 M=-0.5 N=141					
Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.22116	65.98	9	691.33	<.0001
Pillai's Trace	0.87713	39.39	9	858	<.0001
Hotelling-Lawley Trace	3.08383	97.06	9	443.11	<.0001
Roy's Greatest Root	2.93782	280.07	3	286	<.0001
NOTE: F Statistic for Roy's Greatest Root is an upper bound.					

Table 2.6 – Canonical Correlations, Eigenvalues, and Likelihood Ratios of the discriminating variables and the groupings (class levels). Results from both Candisc and Discrim procedures give identical results. There are three discriminant dimensions all of which are statistically significant.

	Canonical Correlation	Adjusted Canonical Correlation	Approximate Standard Error	Squared Canonical Correlation
1	0.863743	0.862035	0.014938	0.746052
2	0.337393	0.3278	0.052127	0.113834
3	0.131328	.	0.057809	0.017247

	Eigenvalues of Inv(E)*H = CanRsq/(1-CanRsq)				Test of H0: The canonical correlations in the current row and all that follow are zero				
	Eigenvalue	Difference	Proportion	Cumulative	Likelihood Ratio	Approximate F Value	Num DF	Den DF	Pr > F
1	2.9378	2.8094	0.9527	0.9527	0.22115844	65.98	9	691.33	<.0001
2	0.1285	0.1109	0.0417	0.9943	0.87088204	10.2	4	570	<.0001
3	0.0175		0.0057	1	0.98275293	5.02	1	286	0.0258

Table 2.7 – Cross-validation Summary using Quadratic Discriminant Function. Each results cell contains a seed count, and percent of total observations.

Number of Observations and Percent Classified into group					
From group	T.ascendens	T.distichum	T.fossil	T.mucronatum	Total
T.ascendens	11 55%	7 35%	2 10%	0 0%	20 100%
T.distichum	20 33.33%	37 61.67%	3 5%	0 0%	60 100%
T.fossil	23 20.91%	9 8.18%	67 60.91%	11 10%	110 100%
T.mucronatum	4 4%	0 0%	5 5%	91 91%	100 100%
Total	58 20%	53 18.28%	77 26.55%	102 35.17%	290 100%
Priors	0.25	0.25	0.25	0.25	

Table 2.8 – Error Counts for the Cross-validation Summary. The row labeled Rate represents the error rate, while the row labeled Priors represents the assumed error rates for each group.

Error Count Estimates for group					
	T.ascendens	T.distichum	T.fossil	T.mucronatum	Total
Rate	0.45	0.3833	0.3909	0.09	0.3286
Priors	0.25	0.25	0.25	0.25	

Table 2.9 – Data Summary of Discriminant Statistics using the Candisc and Discrim procedures. Total sample size represents total seeds measured, the three variables represent Seed Length and Seed Width Ratios along with individual Seed Widths and Seed Lengths. The three classes used are *T. ascendens*, *T. distichum*, and the fossil *Taxodium*, *Taxodium macrosperma*. *T. mucronatum* was omitted from this sample.

Total Sample Size	190	DF Total	189
Variables	3	DF Within Classes	187
Classes	3	DF Between Classes	2

Table 2.10 – Class level information of the three *Taxodium* species. *T. fossil* represents the fossil *Taxodium*, *Taxodium macrosperma*. Frequency represents distinct seed measurements made for each group. *T. mucronatum* was omitted from this sample.

Class Level Information				
group	Variable Name	Frequency	Weight	Proportion
T.ascendens	T_ascendens	20	20	0.105263
T.distichum	T_distichum	60	60	0.315789
T.fossil	T_fossil	110	110	0.578947

Table 2.11 – Table showing the results of the four multivariate tests run by SAS in the Discrim and Candisc procedures. The three classes used are *T. ascendens*, *T. distichum*, and the fossil *Taxodium*, *Taxodium macrosperma*. *T. mucronatum* was omitted from this sample. P values for all tests reject the null hypothesis that all canonical values are equal to zero. The P values show that the groups are significantly different from each other.

Multivariate Statistics and F Approximations					
S=2 M=0 N=91.5					
Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.45738	29.52	6	370	<.0001
Pillai's Trace	0.59746	26.41	6	372	<.0001
Hotelling-Lawley Trace	1.06644	32.79	6	244.9	<.0001
Roy's Greatest Root	0.9387	58.2	3	186	<.0001
NOTE: F Statistic for Roy's Greatest Root is an upper bound.					
NOTE: F Statistic for Wilks' Lambda is exact.					

Table 2.12 – Canonical Correlations, Eigenvalues and Likelihood Ratios of the discriminating variables and the groupings. Results from both Candisc and Discrim procedures give identical results. There are two discriminant dimensions both of which are statistically significant.

	Canonical Correlation	Adjusted Canonical Correlation	Approximate Standard Error	Squared Canonical Correlation
1	0.695837	0.690286	0.03752	0.48419
2	0.336563	0.332578	0.0645	0.113275

	Eigenvalues of Inv(E)*H = CanRsq/(1-CanRsq)				Test of H0: The canonical correlations in the current row and all that follow are zero				
	Eigenvalue	Difference	Proportion	Cumulative	Likelihood Ratio	Approximate F Value	Num DF	Den DF	Pr > F
1	0.9387	0.811	0.8802	0.8802	0.4573821	29.52	6	370	<.0001
2	0.1277		0.1198	1	0.8867253	11.88	2	186	<.0001

Table 2.13 – Cross-Validation Summary using Quadratic Discriminant Function. Each results cell contains a seed count, and percent of total observations. *T. mucronatum* was omitted from this sample.

Number of Observations and Percent Classified into group				
From group	T.ascendens	T.distichum	T.fossil	Total
T.ascendens	16 80%	3 15%	1 5%	20 100%
T.distichum	20 33.33%	37 61.67%	3 5%	60 100%
T.fossil	23 20.91%	9 8.18%	78 70.91%	110 100%
Total	59 31.05%	49 25.79%	82 43.16%	190 100%
Priors	0.33333	0.33333	0.33333	

Table 2.14 - Error Counts for the Cross-validation Summary. The row labeled Rate represents the error rate, while the row labeled Priors represents the assumed error rates for each group. *T. mucronatum* was omitted from this sample.

Error Count Estimates for group				
	T.ascendens	T.distichum	T.fossil	Total
Rate	0.2	0.3833	0.2909	0.2914
Priors	0.3333	0.3333	0.3333	

Table 2.15 – *Taxodium* evolutionary history with time table, along with notes about origins and discovery of species.

Period	Epoch	Notes	Evolutionary History
Quaternary	Holocene	Present	<i>T. distichum</i> , <i>T. ascendens</i> , <i>T. mucronatum</i>
	Pleistocene	<i>Taxodium macrosperma</i> discovered	<i>Taxodium macrosperma</i>
Neogene	Pliocene	No new <i>Taxodium</i> found in the fossil record.	
	Miocene	<i>Taxodium dubium</i> found in the fossil record. Forma found in Europe, with overlap occurring during the Oligocene.	
Paleogene	Oligocene		
	Eocene		
Paleogene	Paleocene	No <i>Taxodium</i> found in the fossil record so far	
	Senonian	<i>Taxodium wallissii</i> first occurred around 70-80mya	<i>Taxodium wallissii</i>



Figure 1.1 - Aerial photo of the Martin Marietta Aggregate Mine (Martin Marietta 2011)



Figure 1.2 - Spoil heaps of the Martin Marietta Locality



Figure 1.3 - Photo of the Martin Marietta Aggregate Mine

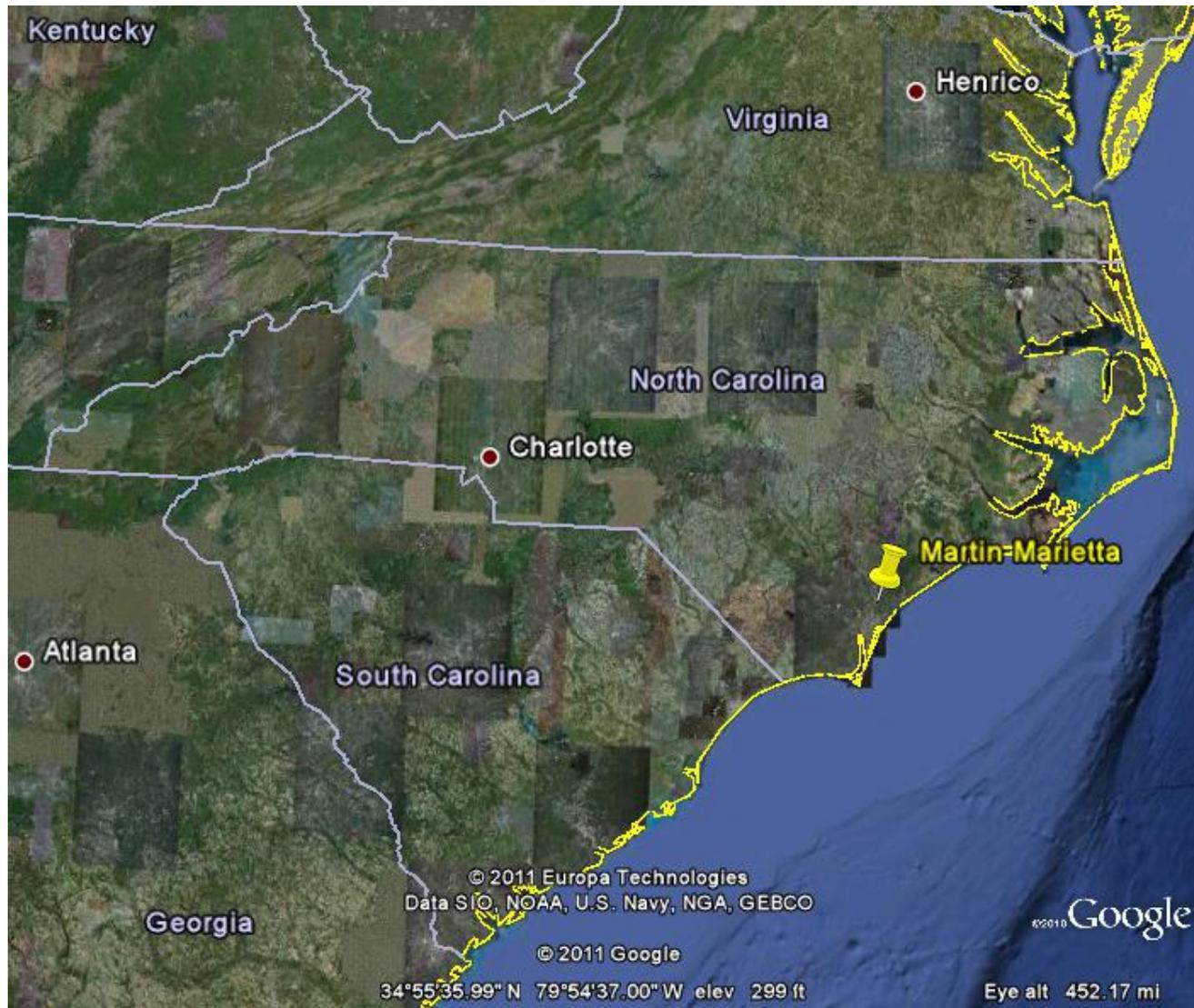


Figure 1.4 - Map of North Carolina and site location (Martin Marietta 2011)

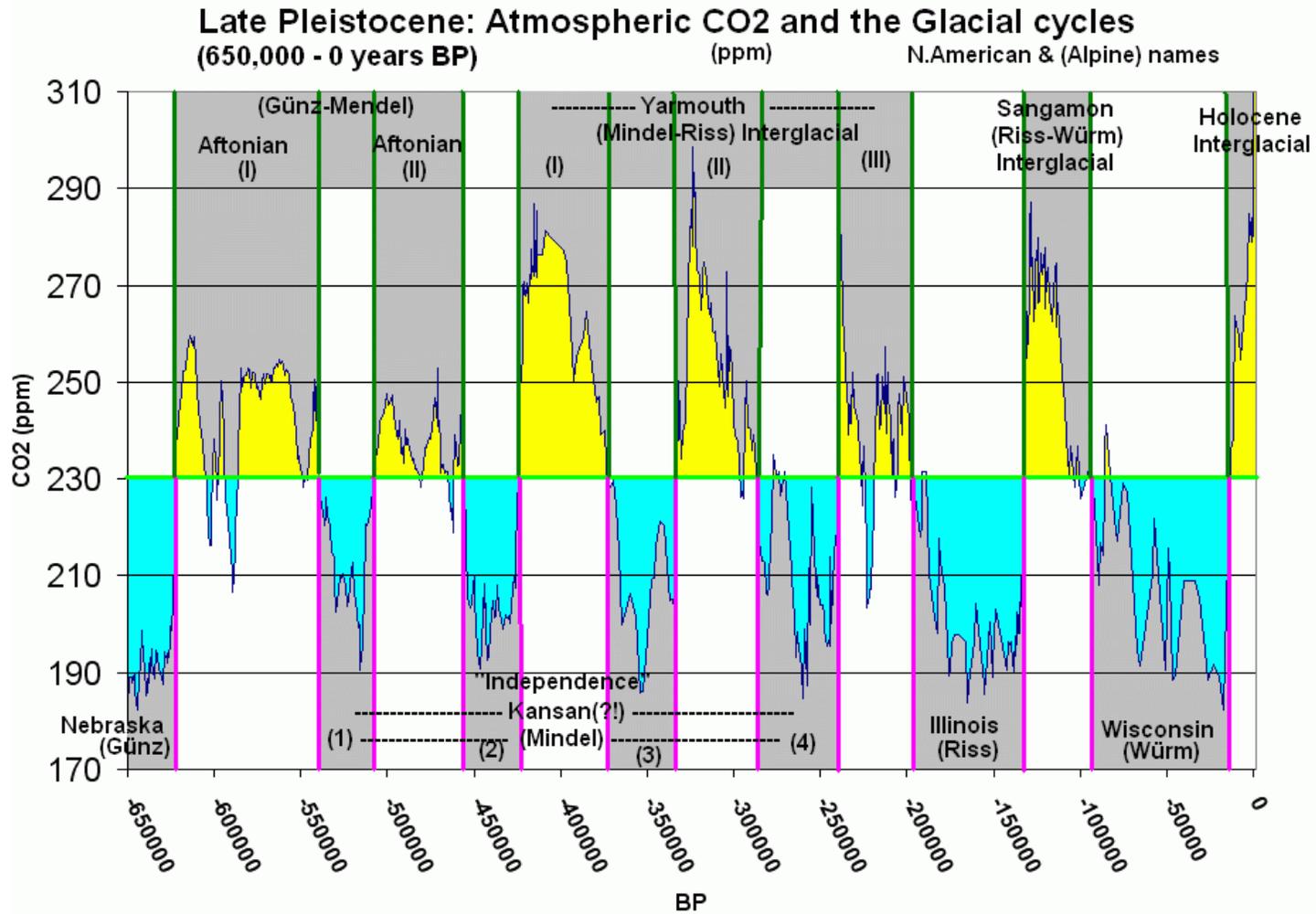
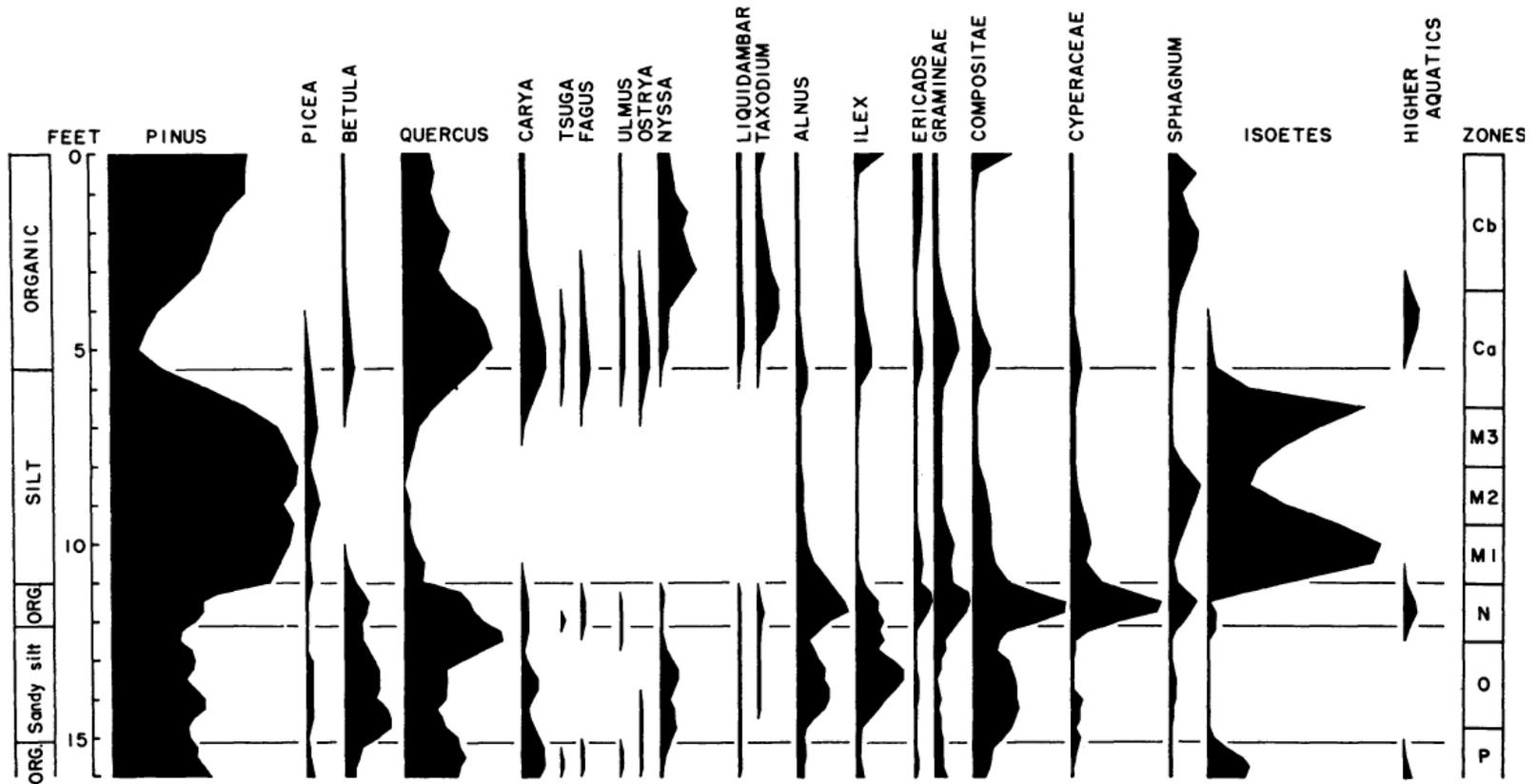


Figure 2.1 – Atmospheric CO₂ and glacial cycle of the Late Pleistocene (Ruen 2005)



GENERALIZED POLLEN DIAGRAM FOR BLADEN COUNTY, N.C.

Figure 2.2 – Generalized Pollen Diagram for Bladen County, NC (Frey, 1953). Shows relative abundance during the Wisconsin glacial period to present.

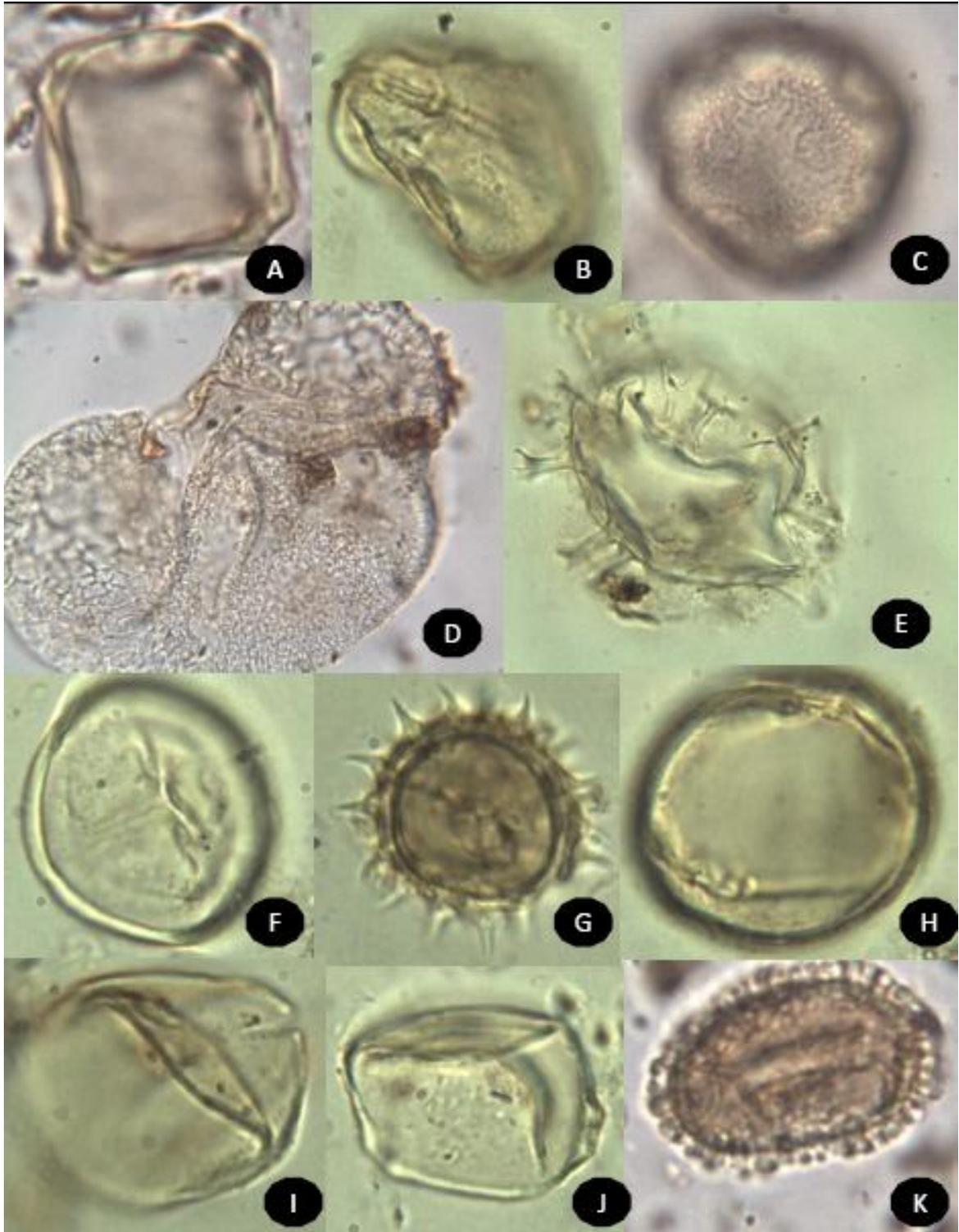


Figure 2.3 – Sample pollen found at the Martin Marietta Locality. (A) *Alnus*, (B) *Quercus*, (C) *Liquidambar*, (D) *Pinus*, (E) Dinoflagellate, (F) *Sphagnum*, (G) *Compositae*, (H) *Nyssa*, (I) Taxodiaceae, (J) *Betula*, (K) *Ilex*. (Syndicate, not to scale)



Figure 3.1 – *Taxodium distichum* distribution map. (Little 2011)



Figure 3.2 –*Taxodium ascendens* distribution map (Flora of North America Editorial Committee, eds. 1993+)



Figure 3.3 – *Taxodium mucronatum* distribution map (Little 2011)



Figure 3.4 – *Taxodium distichum* seeds



Figure 3.5– *Taxodium ascendens* seeds



Figure 3.6 – *Taxodium mucronatum* seeds



Figure 3.7 – Fossil *Taxodium* seeds (*Taxodium macrosperma*), not cleaned with any chemicals



Figure 3.8 - Fossil *Taxodium* seeds (*Taxodium macrosperma*), cleaned with H₂O₂ and then 10% HCl

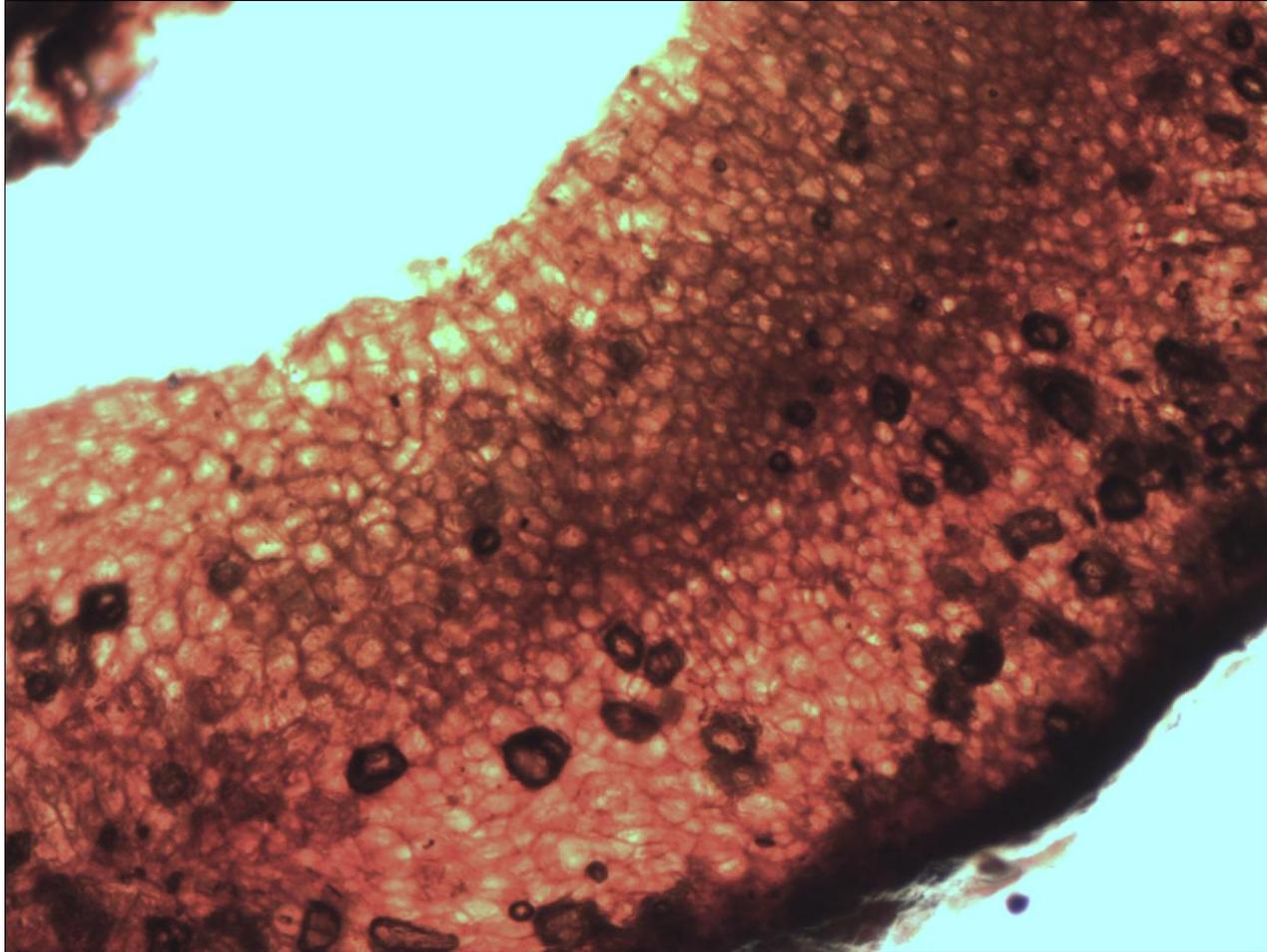


Figure 3.9 - Longitudinal cross section of fossil *Taxodium* seeds (*Taxodium macrosperma*) at 10x. Three testa layers are visible.

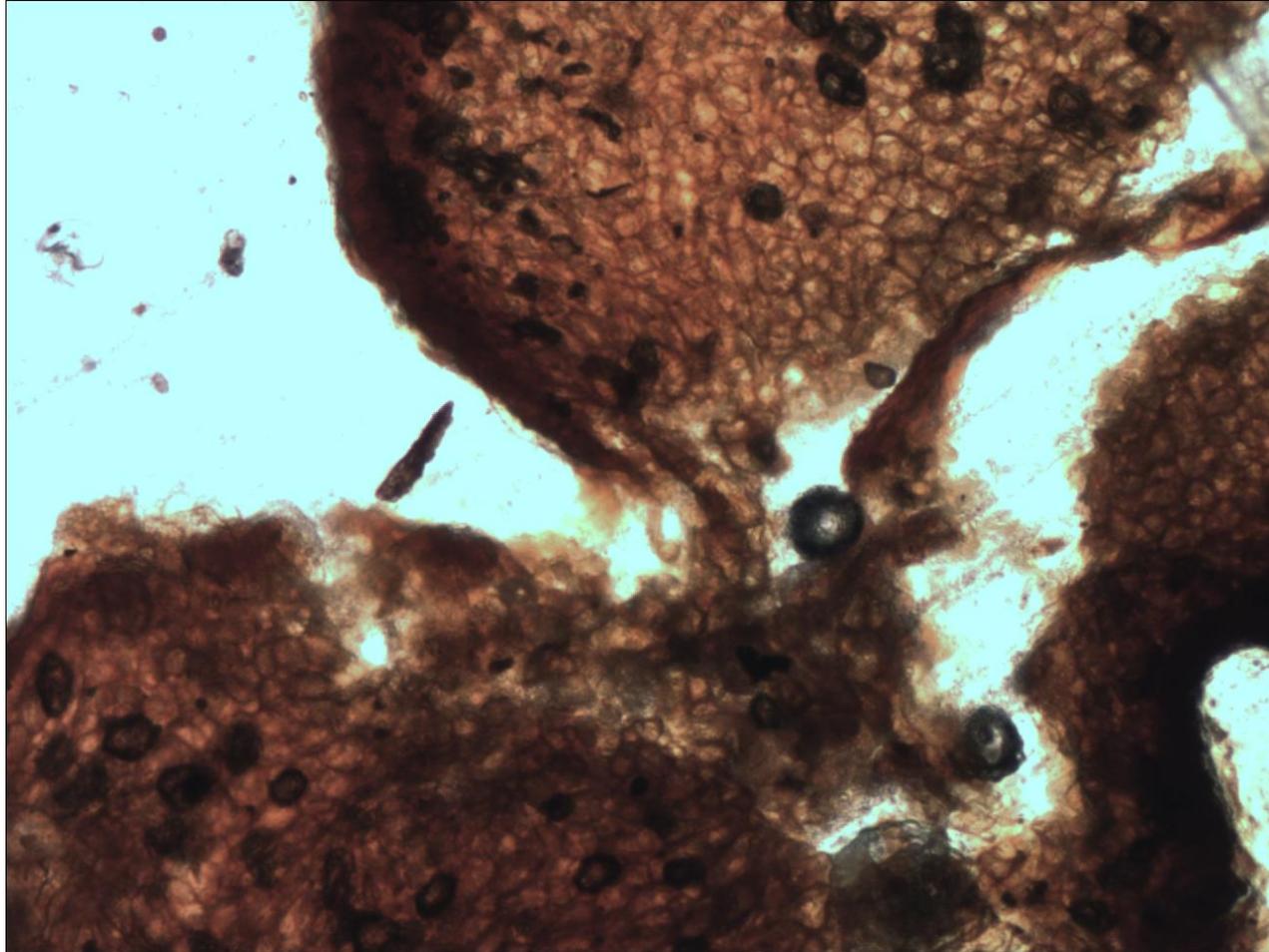


Figure 3.10 Longitudinal cross section of fossil *Taxodium* seeds (*Taxodium macrosperma*) at 5x. The opening of the microphyle is visible.

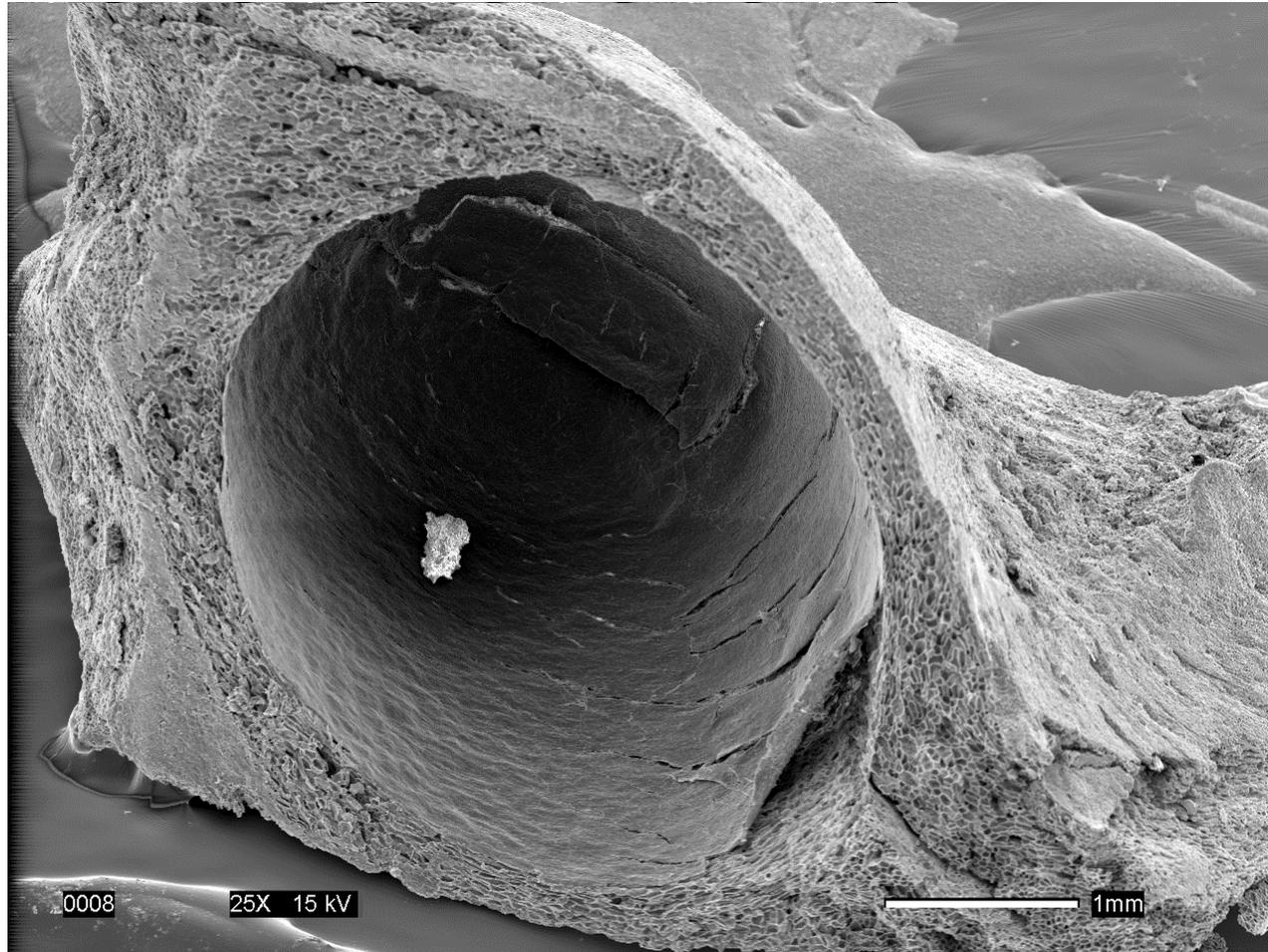


Figure 3.11- *Taxodium macrosperma* whole seed cross section (25x, 15kv). Chalaza end in the background.

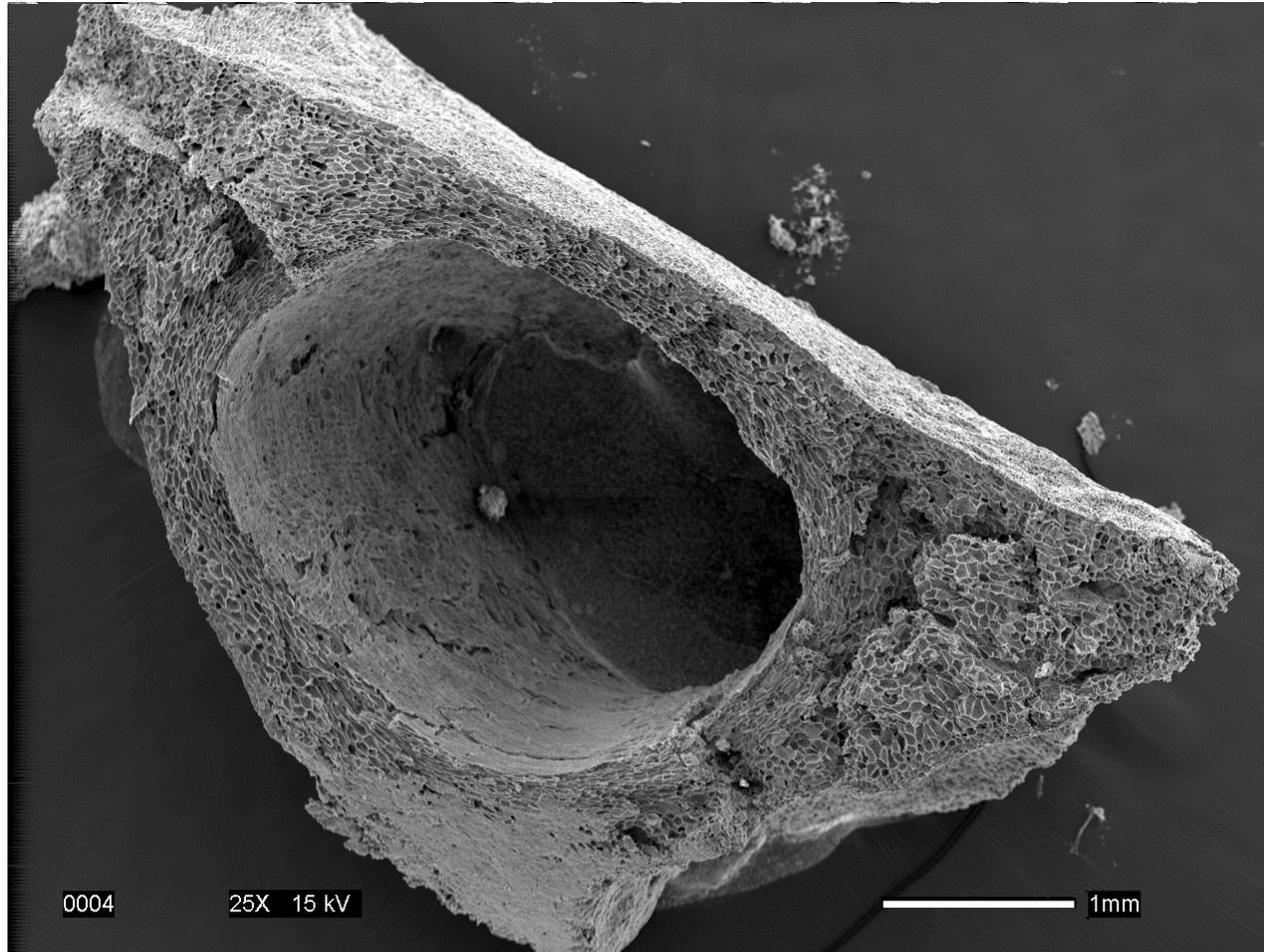


Figure 3.12 - *Taxodium macrosperma* whole seed cross section (25x, 15kv).

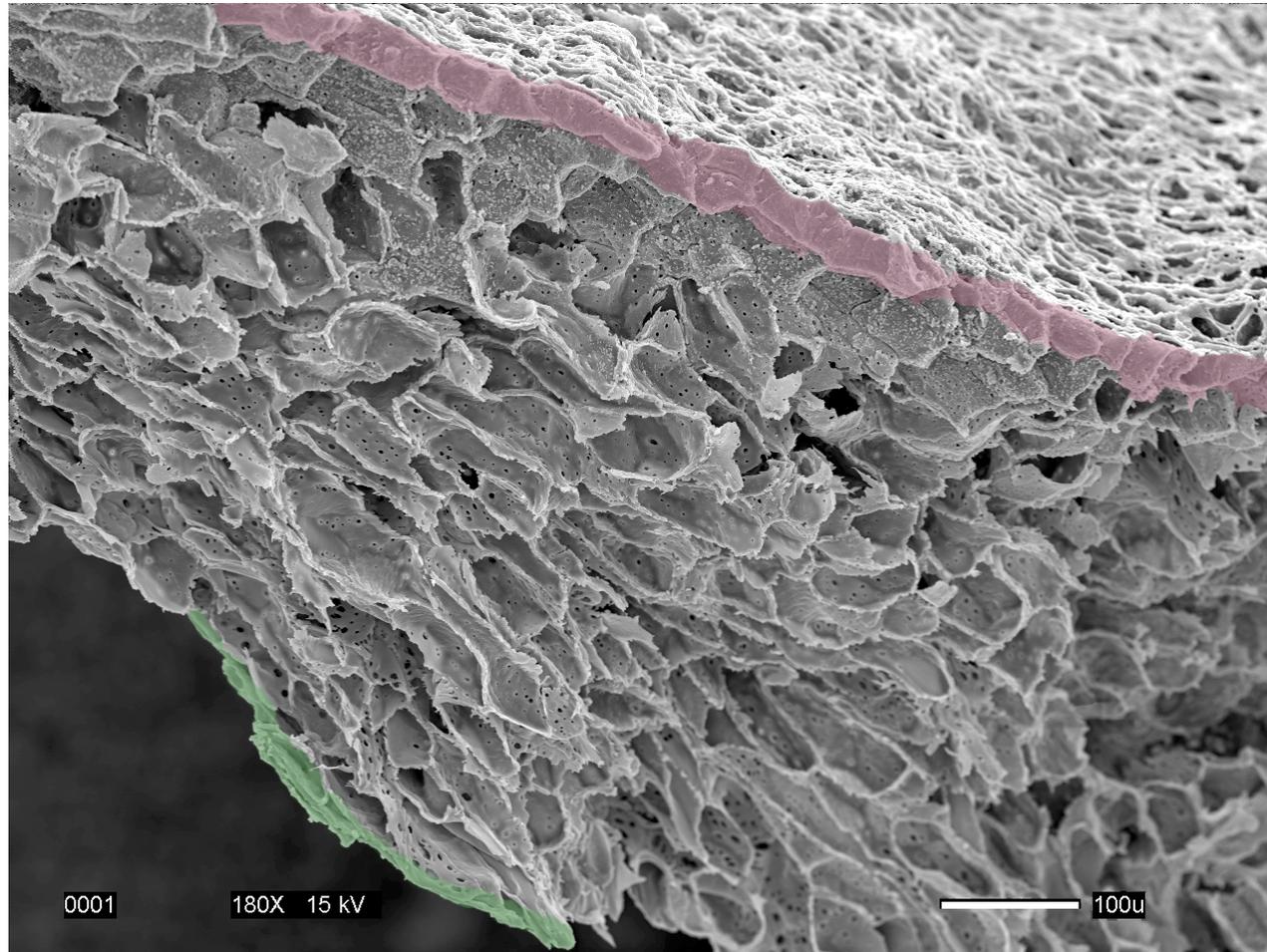


Figure 3.13 - *Taxodium macrosperma* seed wall cross section (180x, 15kv). Red layer represents the exotesta, and the green layer represents the endotesta. The mesotesta is the area in between these two layers.

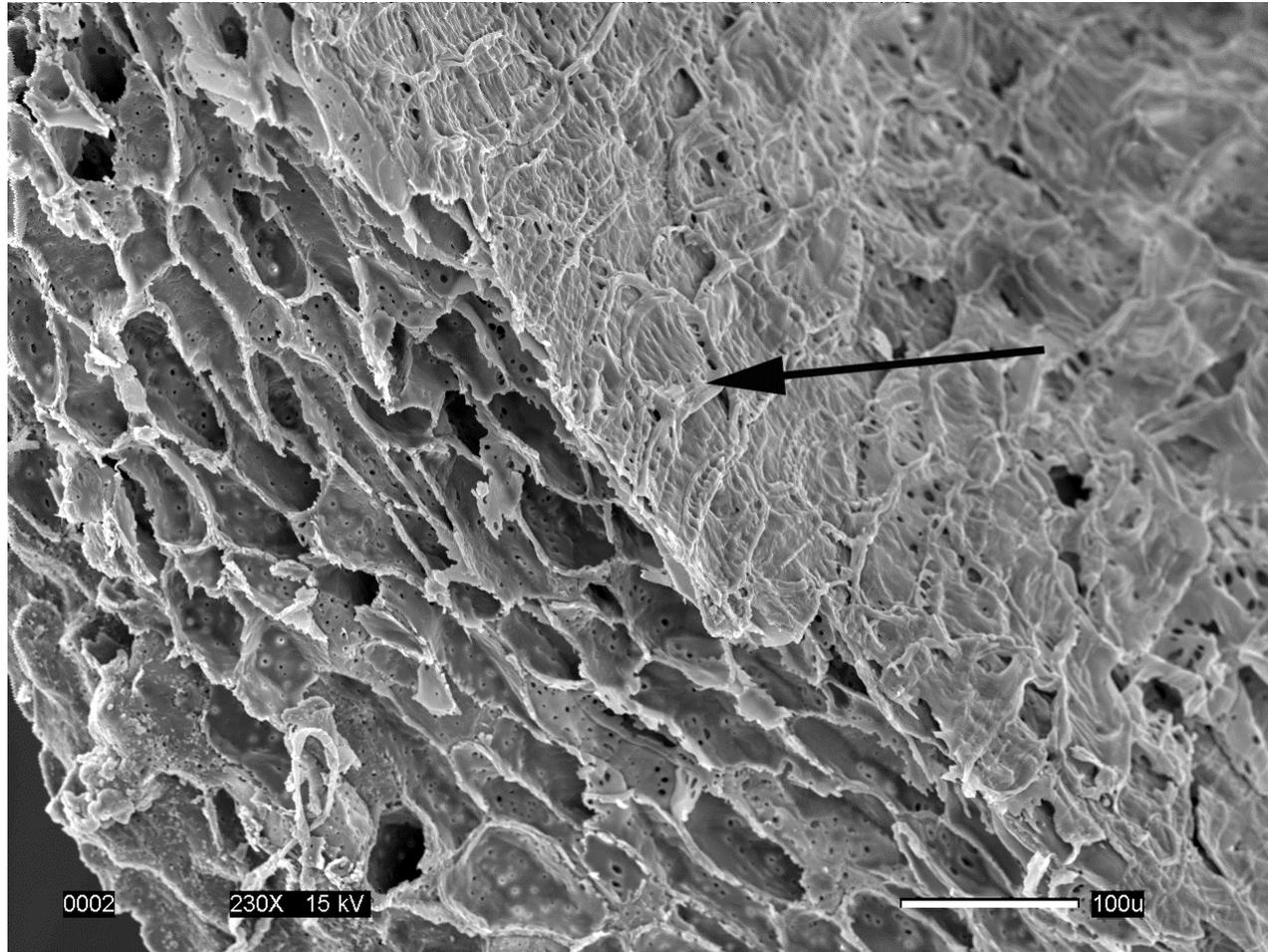


Figure 3.14 – *Taxodium macrosperma* seed cross section (230x, 15kv). Arrow is pointing to the endotesta.

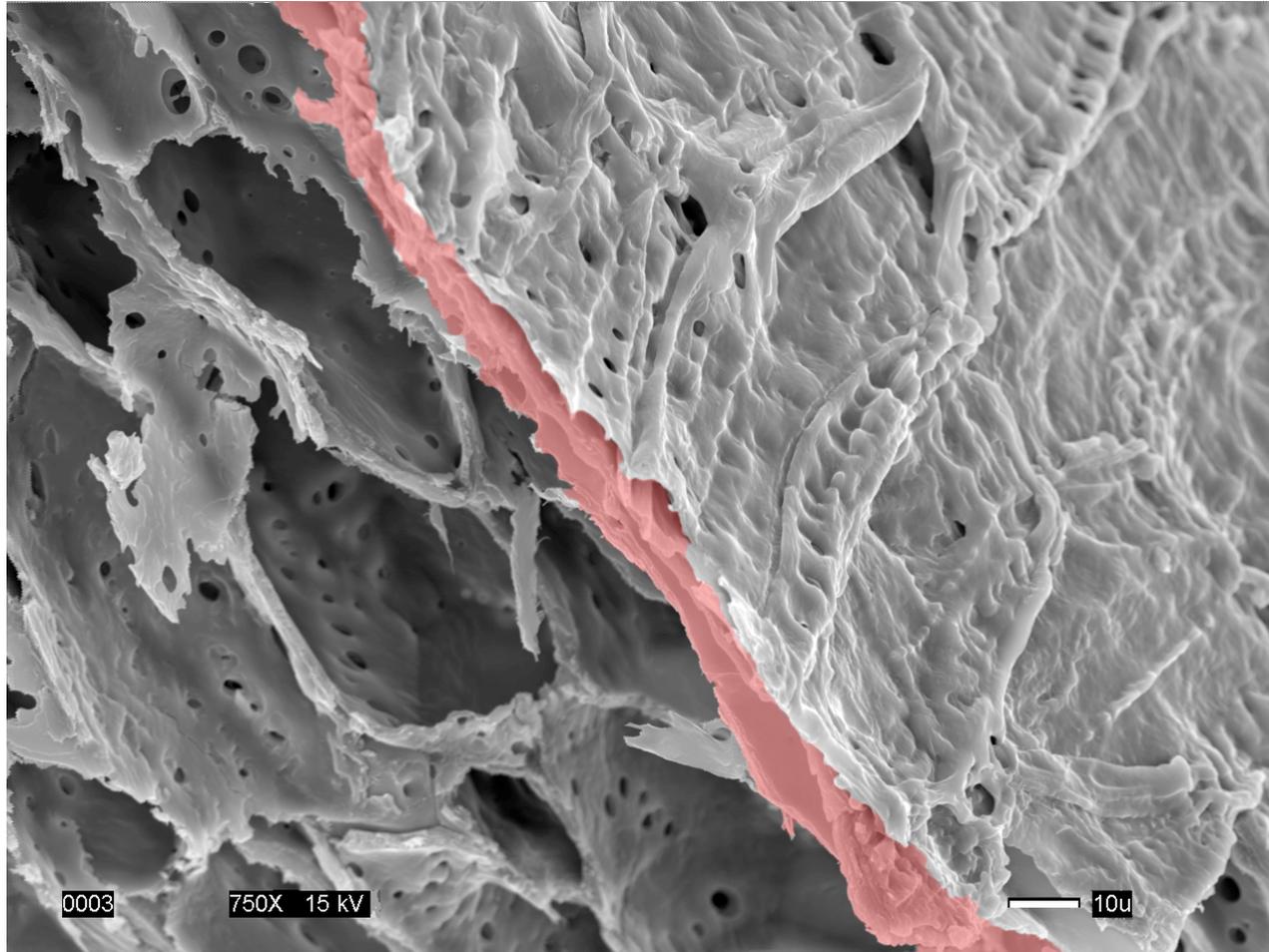


Figure 3.15 – *Taxodium macrosperma* seed cross section (750x, 15kv). Thin endotesta is red.

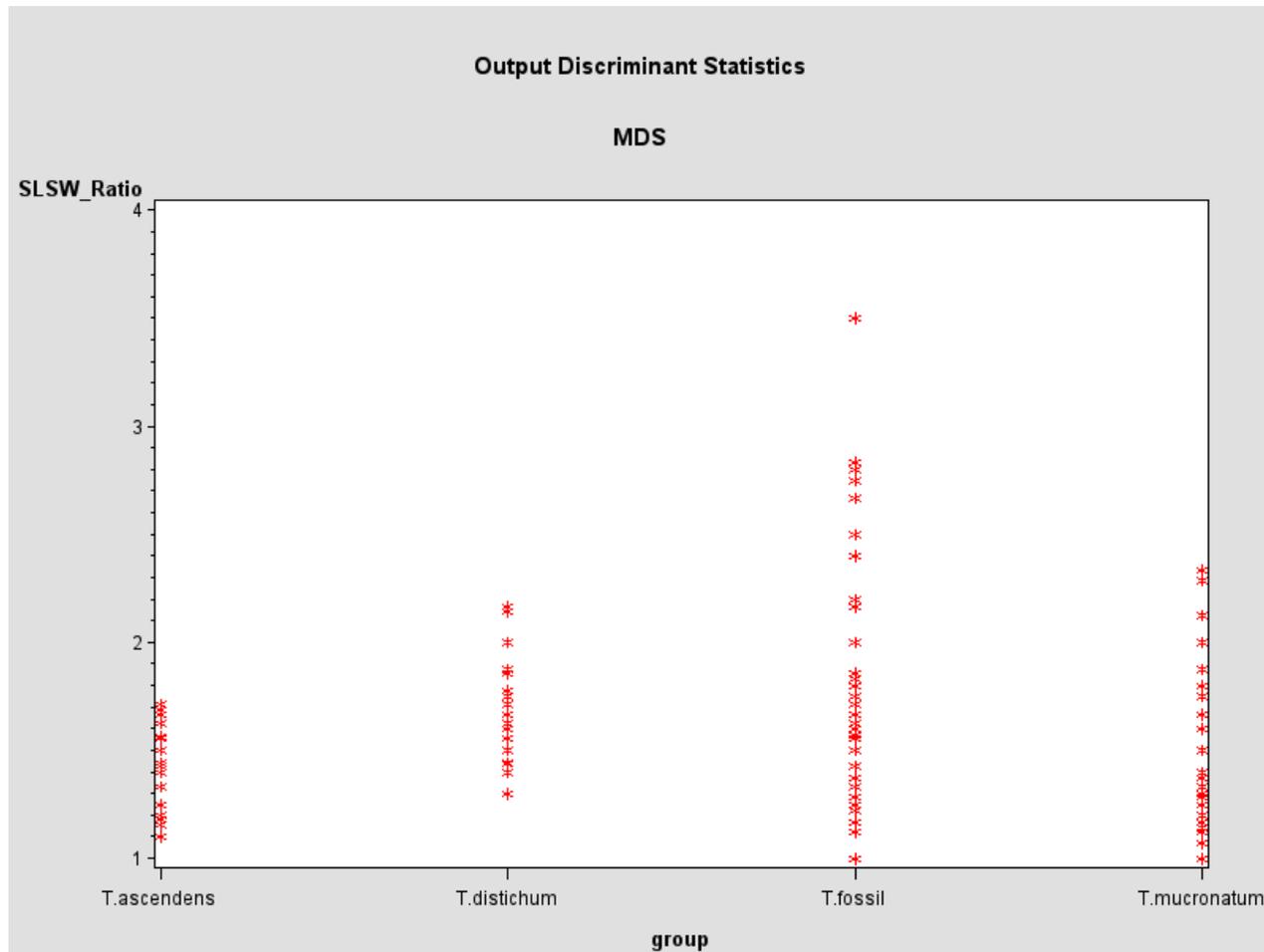


Figure 3.16 – Graph of the Seed Length and Seed Width Ratios (SLSW_Ratio) of the three extant *Taxodium* and the fossil *Taxodium*, *Taxodium macrosperma*. The fossil *Taxodium* has a larger range in SLSW_Ratio compared to the three extant species.

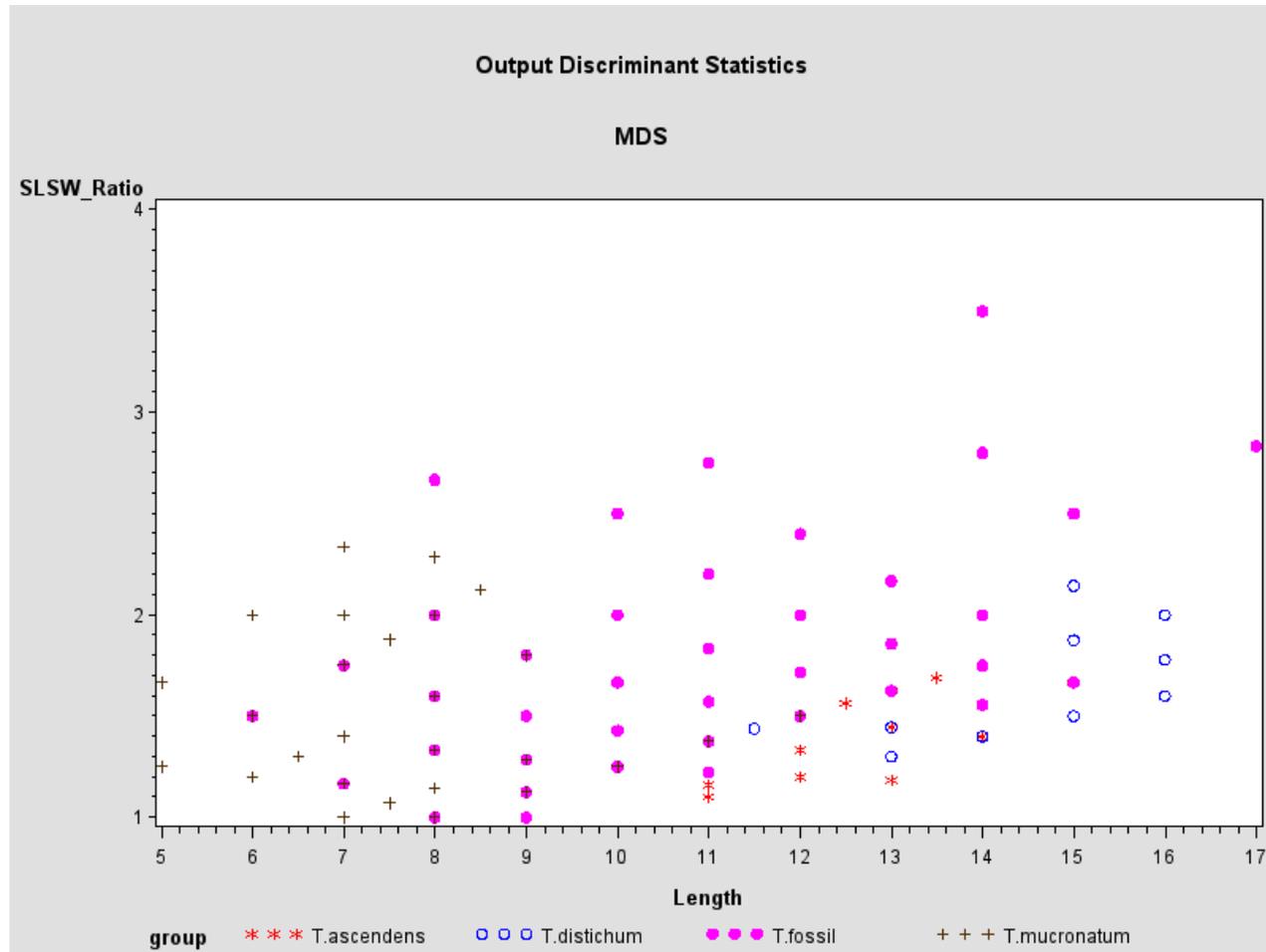


Figure 3.17 – Graph of the Seed Length and Seed Width Ratio (SLSW_Ratio) compared to Seed Length (Length) of the three extant *Taxodium* species and the fossil *Taxodium*, *Taxodium macrosperma*. The fossil *Taxodium* overlays the range of the three extant species, which has formed three distinct groups compared to the fossil species.

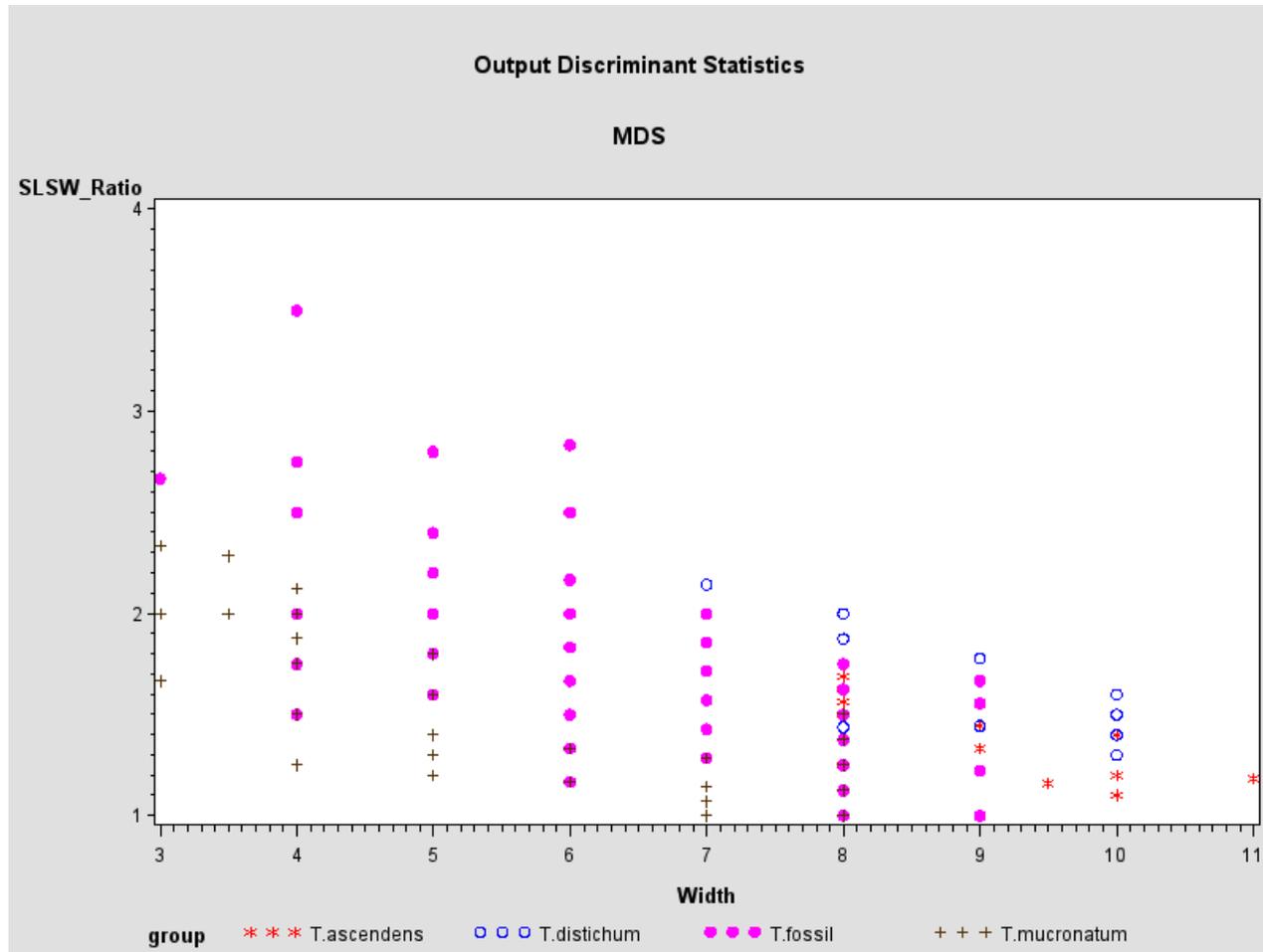


Figure 3.18 – Graph of the Seed Length and Seed Width Ratio (SLSW_Ratio) compared to Seed Width (Width) of the three extant *Taxodium* species and the fossil *Taxodium*, *Taxodium macrosperma*. The fossil *Taxodium* overlays the range of the three extant species, which has formed three distinct groups compared to the fossil species.

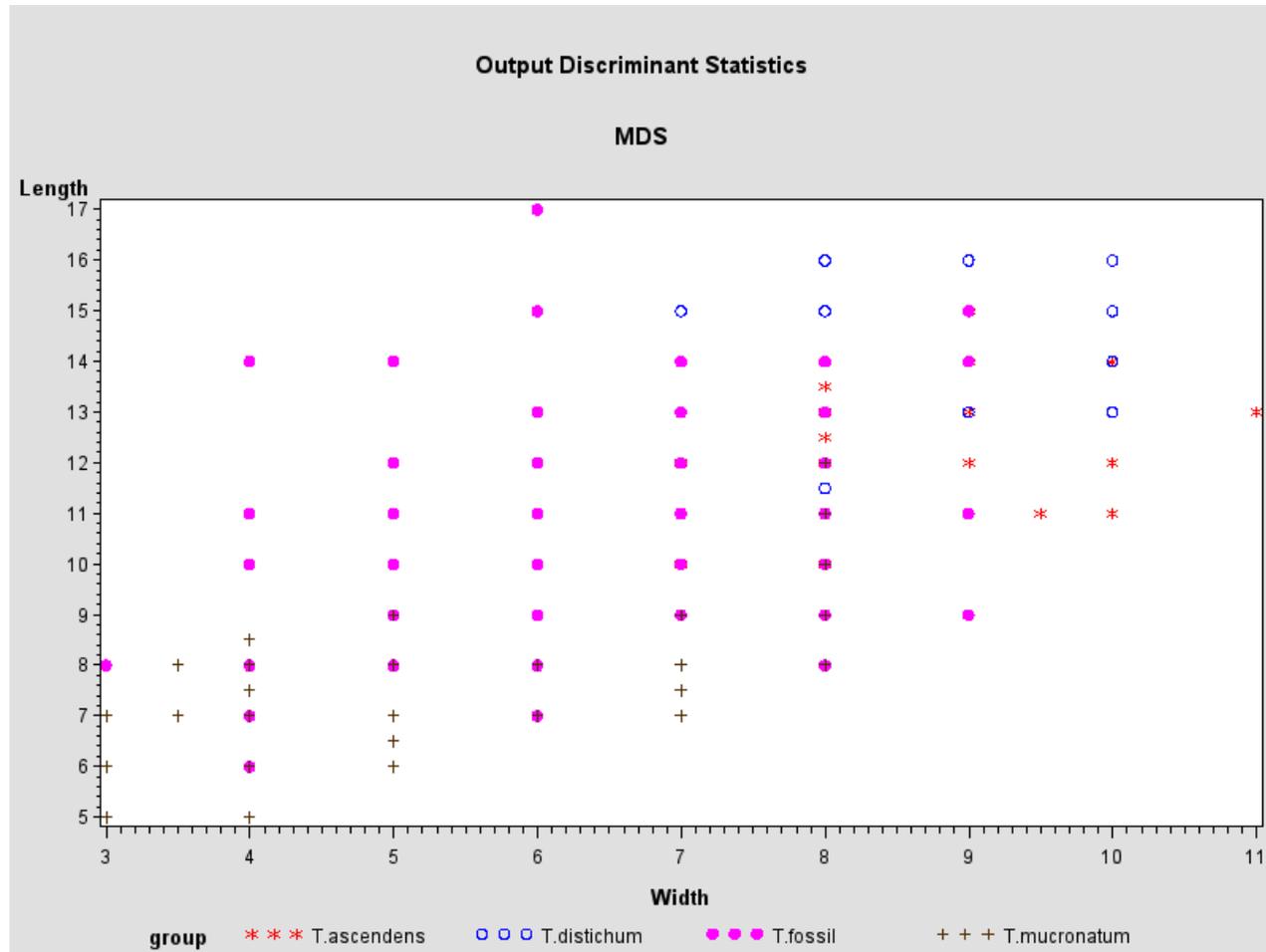


Figure 3.19 – Graph of the Seed Length (Length) and the Seed Width (Width) of the three extant *Taxodium* species and the fossil *Taxodium*, *Taxodium macrosperma*. The fossil *Taxodium* overlays the range of the three extant species, which has formed three distinct groups compared to the fossil species. *T. ascendens* and *T. distichum* groups overlap while *T. mucronatum* has a separate more distinct group. This graph also represents a scatter plot of the seed length compared to the seed width of the four species.

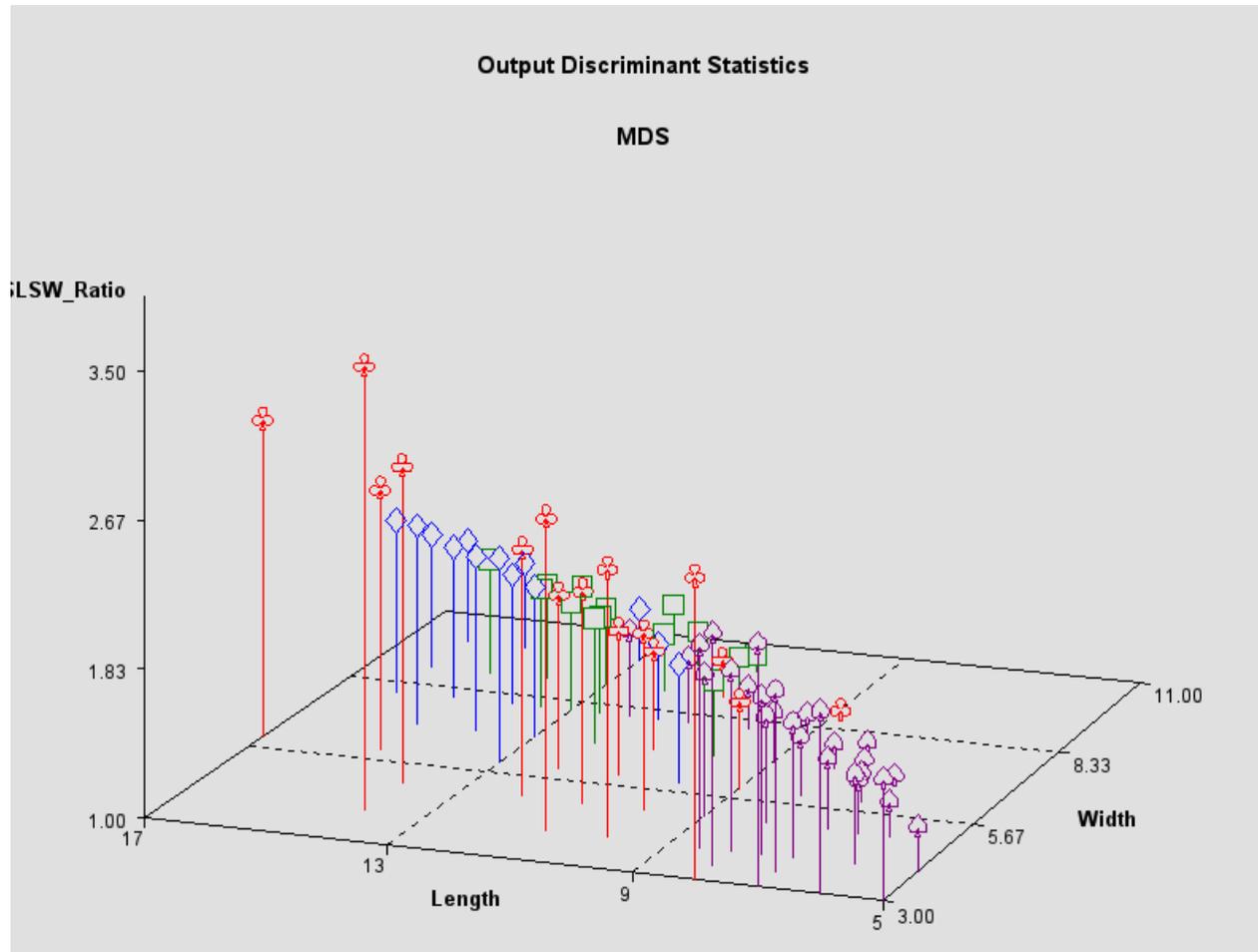


Figure 3.20 – 3D Scatter Plot of the Seed Length and Seed Width Ratios (SLSW_Ratio) by Seed Length (Length) and Seed Width (Width) of the three extant *Taxodium* species and the fossil *Taxodium*, *Taxodium macrosperma*. Legend: *T. macrosperma* (♣), *T. ascendens* (□), *T. distichum* (◆), and *T. mucronatum* (♠). The three extant species form distinct groups, while *T. macrosperma* has a range that overlaps them.

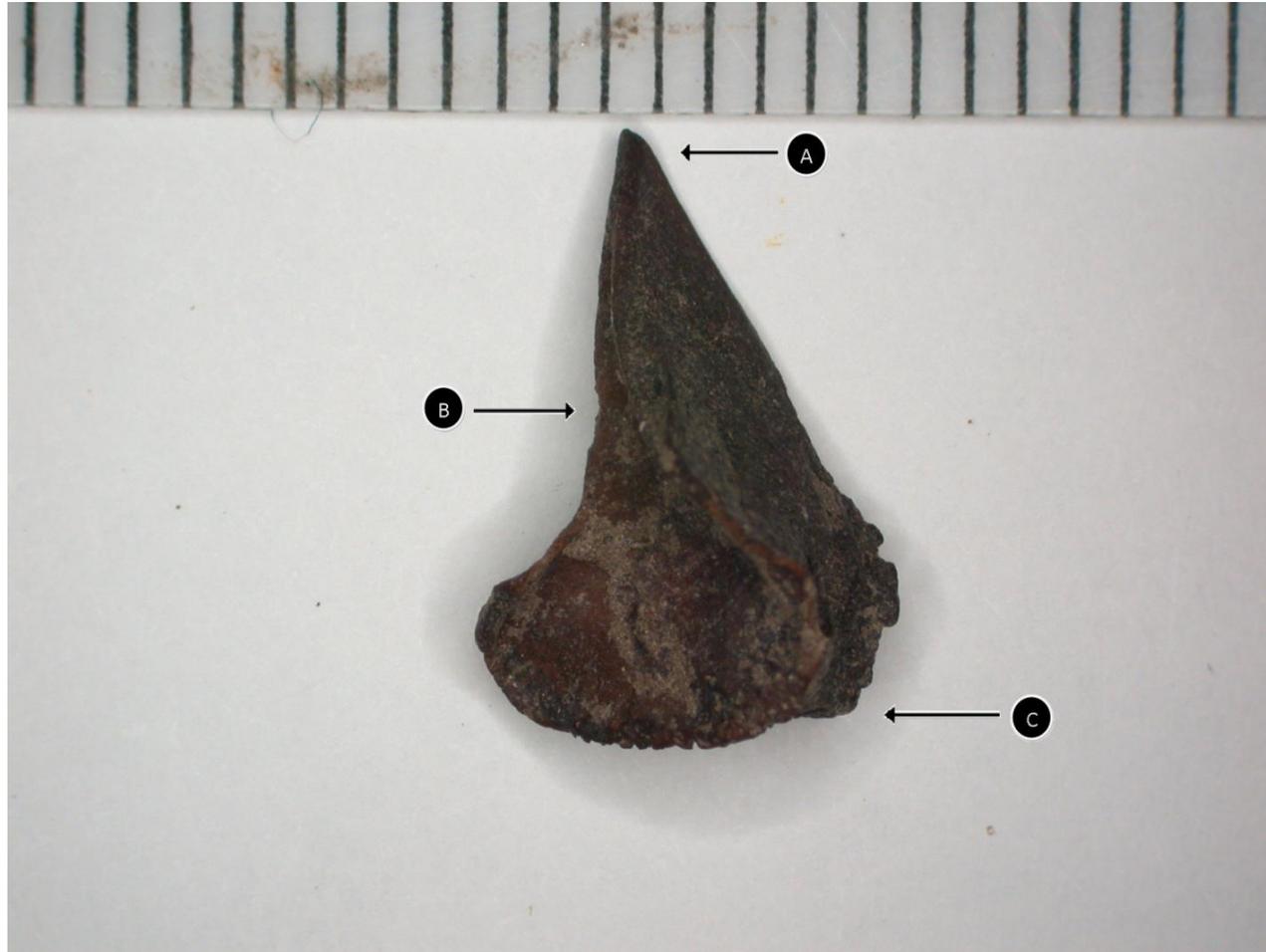


Figure 3.21 - Fossil *Taxodium* seeds (*Taxodium macrosperma*) Holotype. A) Micropylar end, B) Hilum, C) Chalaza end

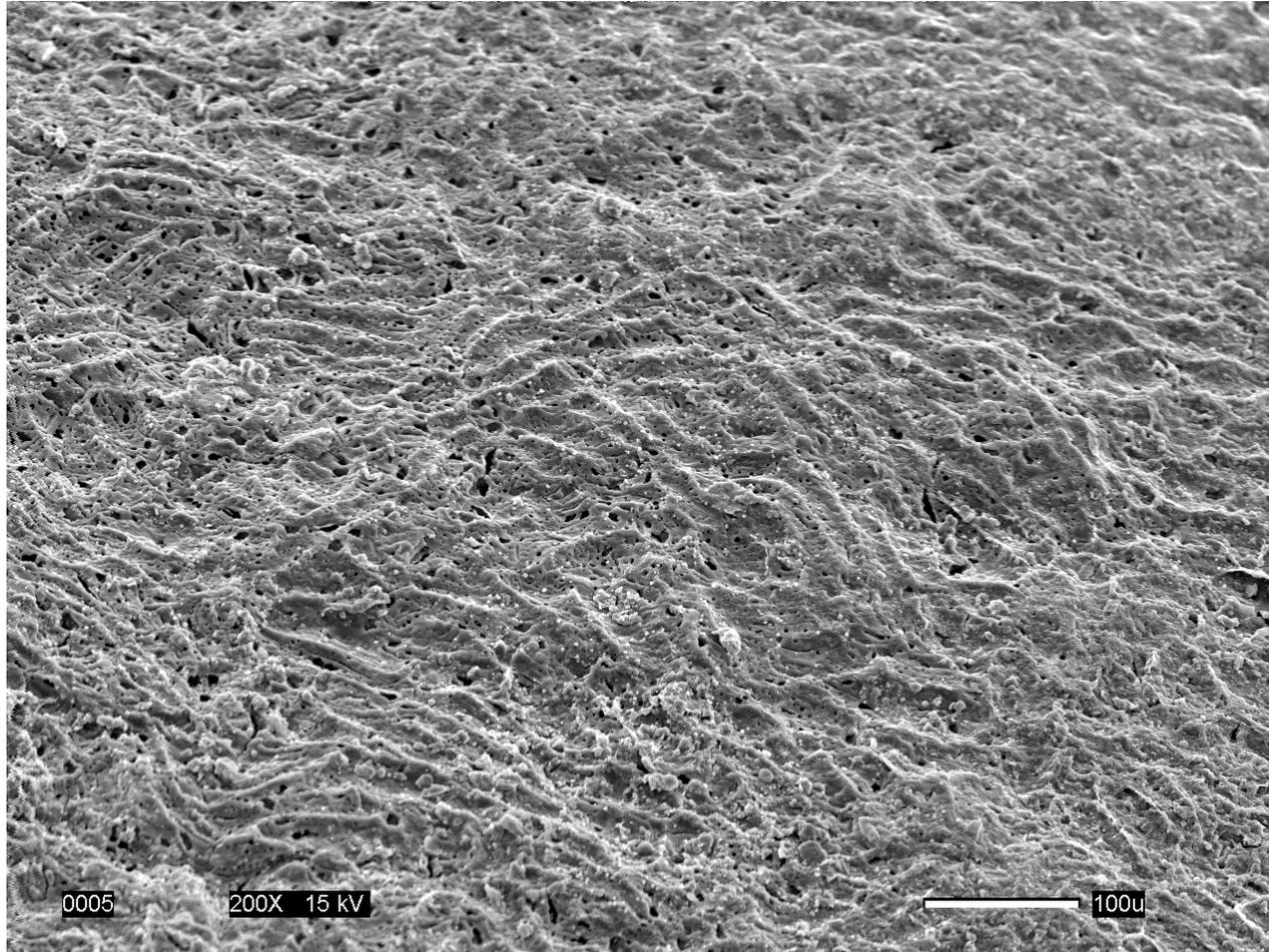


Figure 3.22 – *Taxodium macrosperma* exotesta (200x, 15kv). Patchwork-like nature of the exotesta.

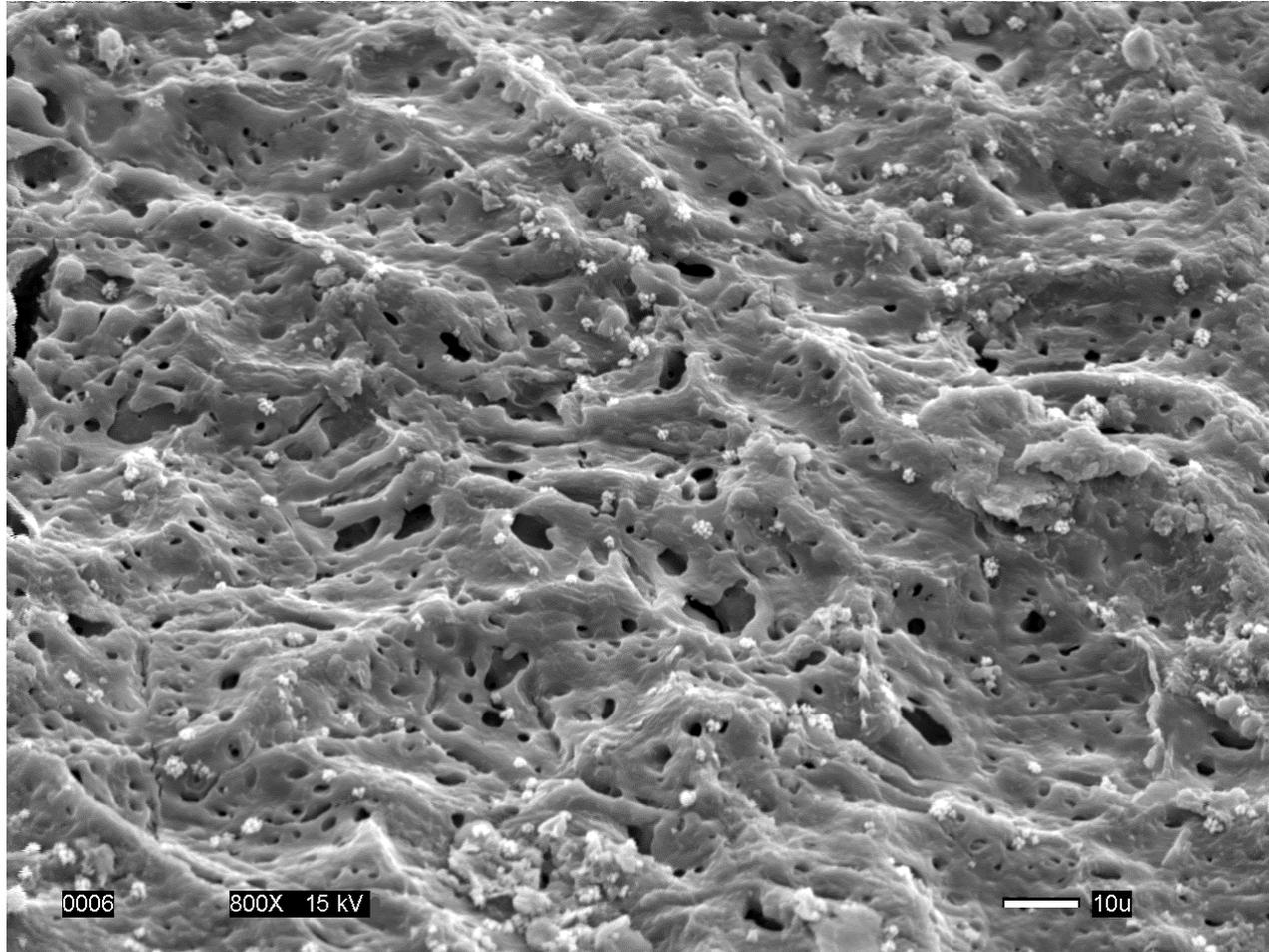


Figure 3.23 – *Taxodium macrosperma* exotesta (800x, 15kv). It appears vascularized and porous.

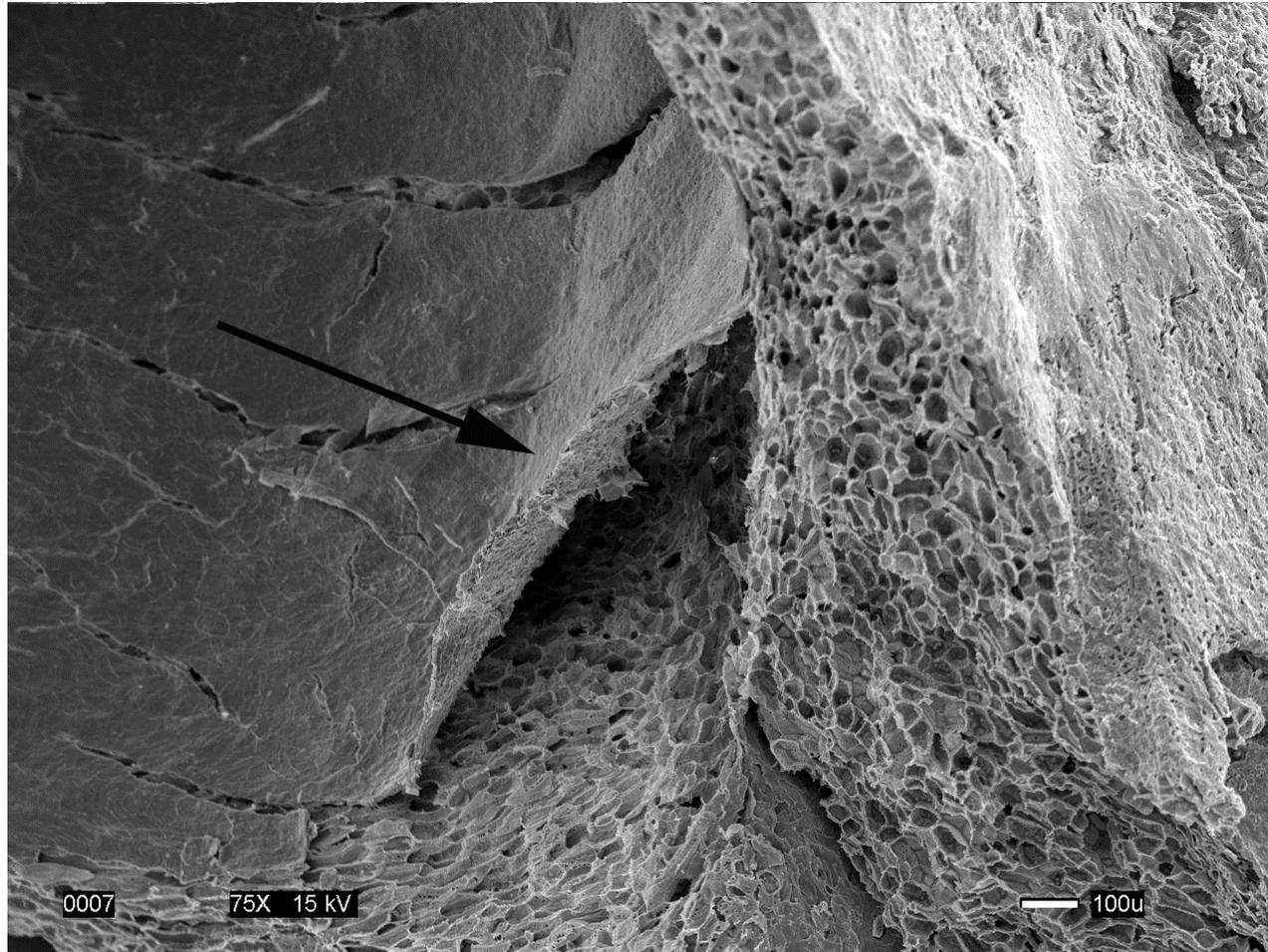


Figure 3.24 – *Taxodium macrosperma* seed cross section (25x, 15kv) with endotesta curling and clearly visible.

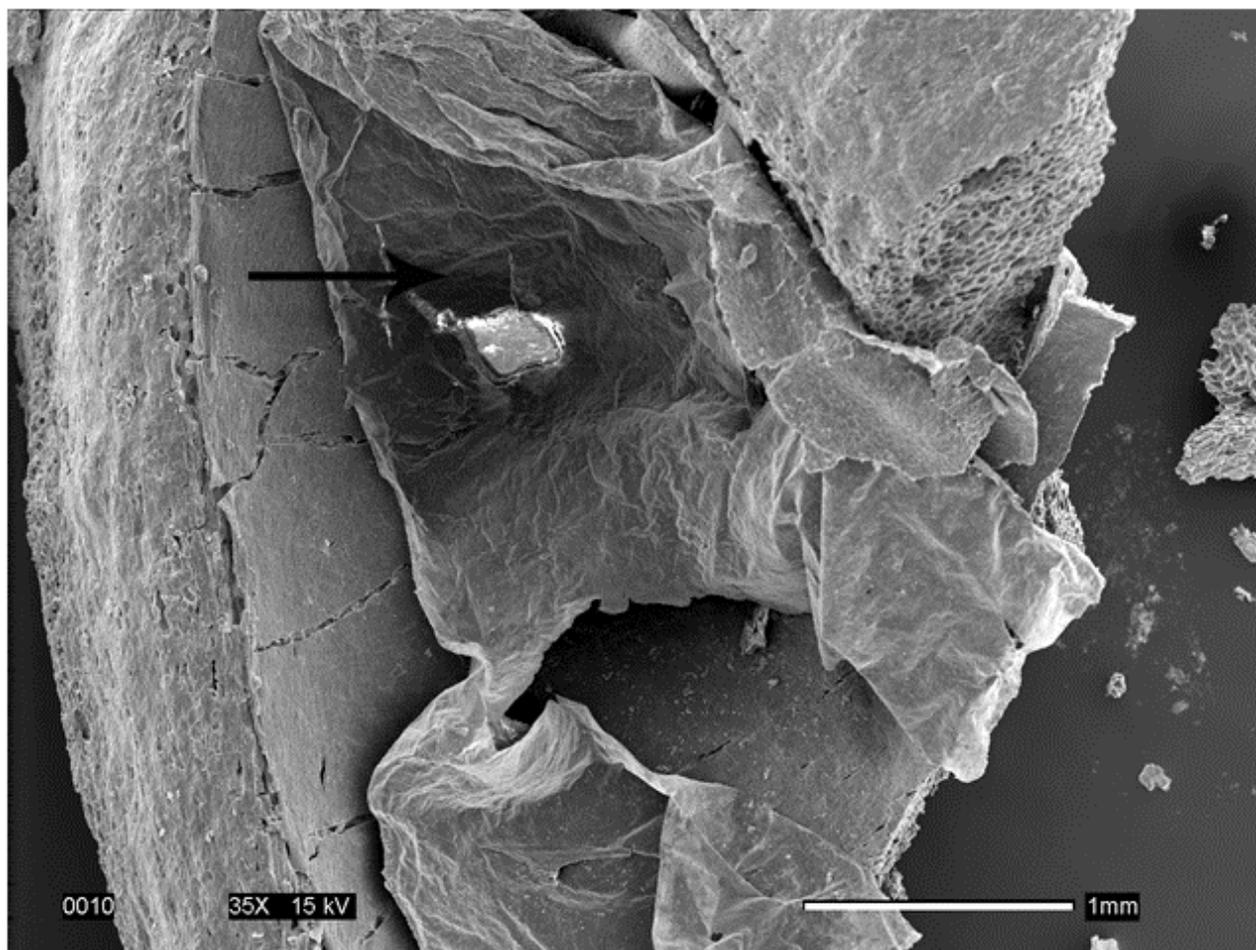


Figure 3.25 – Broken *Taxodium macrosperma* seed (35x, 15kv) with degrading embryo/nucellus, and testa layering clearly visible.

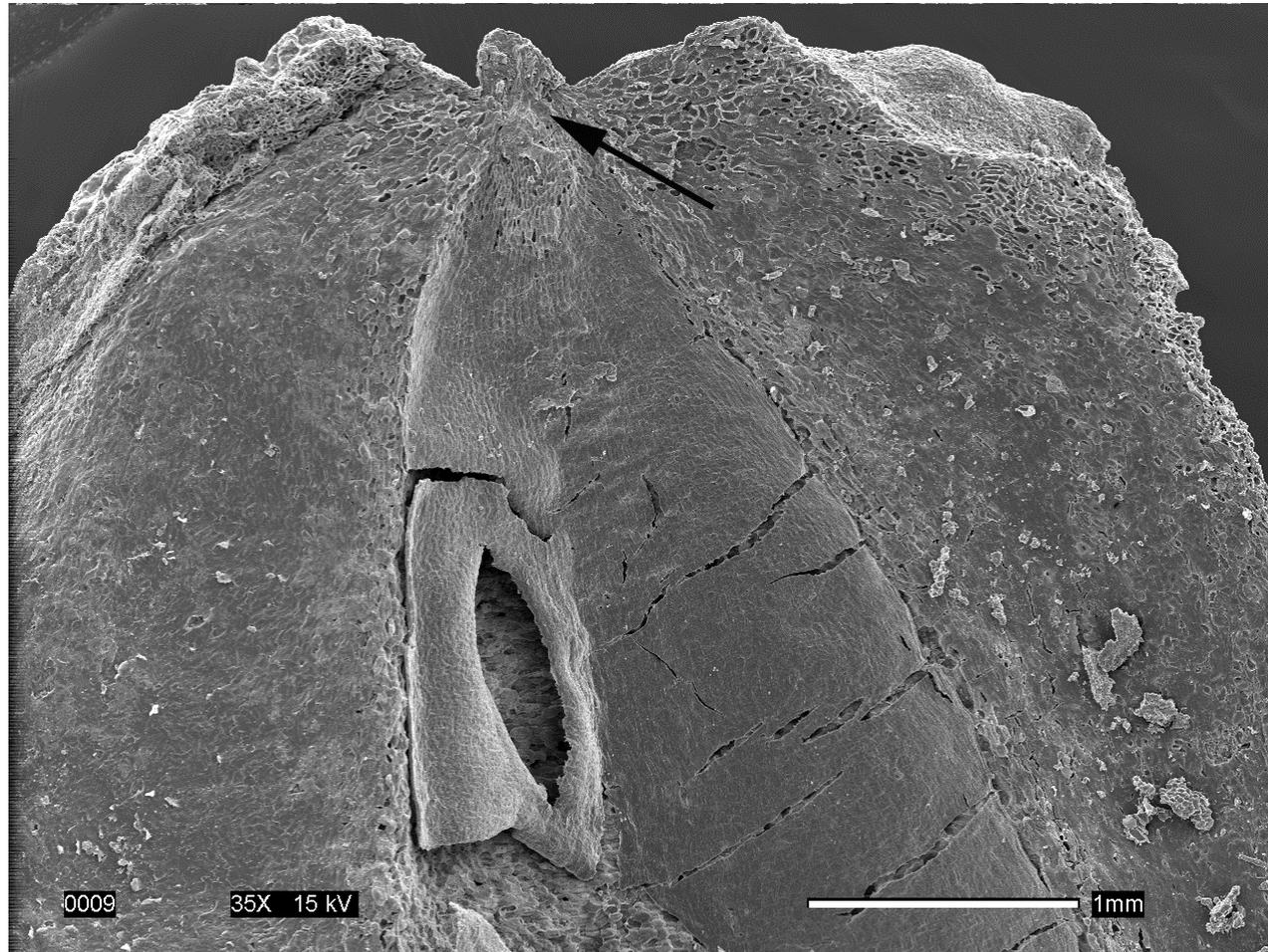


Figure 3.26 – Micropylar end of *Taxodium macrosperma* seed longitudinal section (35x, 15kv). Arrow points to micropyle.

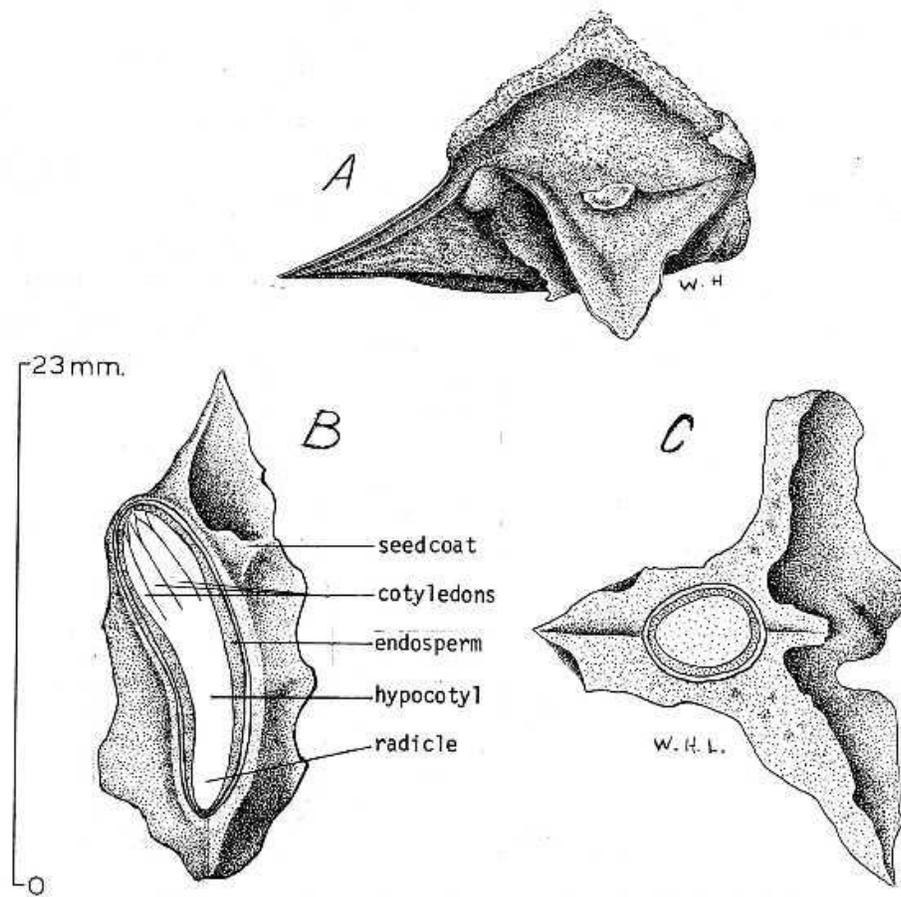


Figure 3.27 - *Taxodium distichum* seed anatomy (Plants USDA 2011)
 W.H.L. @ USDA-NRCS PLANTS Database

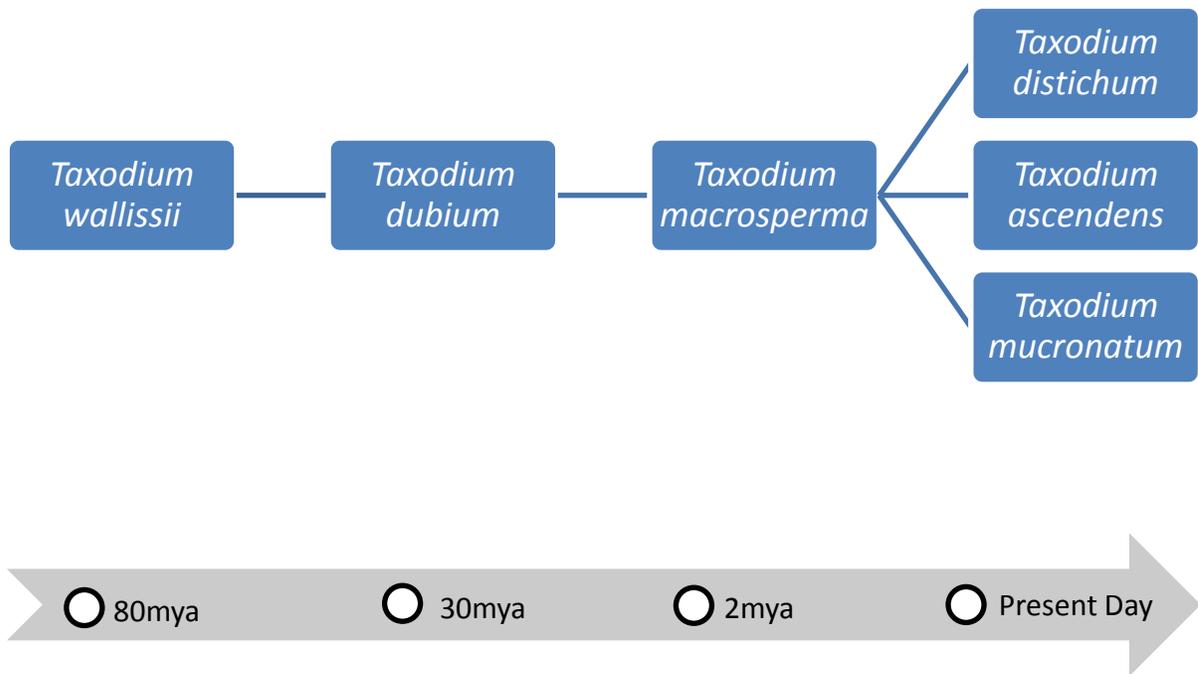


Figure 3.28 – Proposed evolutionary history of *Taxodium*.

REFERENCES

- Aulenback, K.R., and LePage, B.A., 1998. *Taxodium wallisii* sp. nov.: first occurrence of *Taxodium* from the upper Cretaceous. *International Journal of Plant Sciences*, **159** (2), 367–390.
- Baum, G. R., Harris, W. B., and Zullo, V. A., 1978. Stratigraphic revision of Eocene to middle Miocene strata of North Carolina. *Southeastern Geology*, **20**, 1-19.
- Baum, G. R., Harris, W. B., and Dres, P. E., 1985. Origin of dolomite in the Eocene Castle Hayne Limestone, North Carolina. *Journal of Sedimentary Petrology*, **55**, 506-517.
- Berry, E.W., 1907. Pleistocene Plants from Alabama. *The American Naturalist*, **41** (491), 689-699.
- Berry, E.W., 1909a. Contributions to the Pleistocene Flora of North Carolina. *The Journal of Geology*, **15** (4), 338-349.
- Berry, E.W., 1909b. Pleistocene Swamp Deposits in Virginia. *The American Naturalist*, **43** (511), 432-436.
- Bryant, V.M., Jr., and Holloway, R.G., 1985. Introduction. *Pollen Records of Late Quaternary North American Sediments*. American Association of Stratigraphic Palynologists Foundation, Texas.
- Chaney, R.W., 1950. A revision of fossil *Sequoia* and *Taxodium* in western North America based on the recent discovery of *Metasequoia*. *Transactions of the American Philosophical Society*, **40** (3), 170-263.
- Delcourt, H.R. and Delcourt P.A., 1985. Quaternary palynology and vegetational history of the southeastern United States. *Pollen Records of Late Quaternary North American Sediments*. American Association of Stratigraphic Palynologists Foundation, Texas.
- Delcourt, H.R. and Delcourt P.A., 1991. *Quaternary Ecology: A paleoecological perspective*. New York: Chapman and Hall.
- Erdtman, Gunnar., 1971. *Pollen Morphology and Plant Taxonomy*. Hafner Publishing Company Incorporated, New York, New York.
- Farjon, A., 2005. A monograph of Cupressaceae and *Sciadopitys*. The Royal Botanic Gardens, Kew.
- Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 16+ vols. New York and Oxford. Accessed May 05, 2011
<http://www.efloras.org/object_page.aspx?object_id=7080&flora_id=1>

- Frey, D.G., 1951. Pollen succession in the sediments of Singletary Lake, North Carolina. *Ecology*, **32** (3), 518-533.
- Frey, D.G., 1953. Regional Aspects of the Late-Glacial and Post-Glacial Pollen Succession of Southeastern North Carolina. *Ecological Monographs*, **23** (3), 289-313
- Frey, D.G., 1955. A Time revision off the Pleistocene pollen chronology of Southeastern North Carolina. *Ecology*, **36** (4), 762-763.
- Godfrey, R.K., 1988. *Trees, Shrubs, and Woody Vines of Northern Florida and Adjacent Georgia and Alabama*. University of Georgia Press. Athens, GA.
- Harris, W.B., Zullo, V.A., and Otte, L.J., 1986. Road log and description of field trip stops; in Textoris, D. A. (ed.), SEPM Field Guidebooks, southeastern United States Third Annual Midyear Meeting, 1986, Raleigh, North Carolina. *Society of Economic Paleontologists and Mineralogists*, p. 311-324.
- Hussey, T.C., 1993. A 20,000 year history of vegetation and climate at Clear Pond, Northeast South Carolina. University of Maine, Thesis.
- Kapp, Ronald., 1969. *How to Know Pollen and Spores*. William C. Brown Company Publishing, Dubuque, Iowa.
- Kirkbride, J.H., Jr., C.R. Gunn, and M.J. Dallwitz., 2006. Family Guide for Fruits and Seeds, vers. 1.0. Accessed June 20, 2010. <URL: <http://nt.ars-grin.gov/sbmlweb/OnlineResources/frsdfam/Index.cfm>>.
- Kunzmann, L., Kvaček, Z., Mai, D.H., and Walther, H., 2009. The genus *Taxodium* (Cupressaceae) in the Palaeogene and Neogene of Central Europe. *Review of Palaeobotany and Palynology*, **153**, 153–183.
- LaMoreaux, H.K., Brook, G.A., and Knox, J.A., 2009. Late Pleistocene and Holocene environments of the Southeastern United States from the stratigraphy and pollen content of a peat deposit on the Georgia Coastal Plain. *Paleogeography, Paleoclimatology, Paleoecology*, **280**, 300-312.
- Lickey, E.B., and Walker, G.L., 2002. Population Genetic Structure of Baldcypress (*Taxodium distichum* [L.] Rich. var. *distichum*) and Pondcypress (*T. distichum* var. *imbricarium* [Nuttall] Croom): Biogeographic and Taxonomic Implications. *Southeastern Naturalist*, **1** (2), 131-148.
- Little, E.L., Jr., Digital Representations of Tree Species Range Maps from "Atlas of United States Trees"(and other publications). U.S. Geological Survey. Last updated: April 10, 2006. Accessed May 05, 2011. <URL: <http://gec.cr.usgs.gov/data/atlas/little/index.html>>.

- Madhumi, Mitra., 2002. Paleopalynology of the Tar Heel Formation of Atlantic Coastal Plain of North Carolina, United States. North Carolina State University. PhD Diss.
- “Martin Marietta.” 34°22'27.70" N and 77°50'18.99" W. **Google Earth**. Feb 28, 2006. May 6, 2011.
- McAndrews, J.H., Berti, A.A., and G. Norris., 1973. Key to the Quaternary Pollen and Spores of the Great Lakes Region. Royal Ontario Museum, Life Sciences Miscellaneous Publications
- Ruen, Tom. 2005. Atmosphere CO₂ with glacial cycles. Wikimedia Commons. Accessed May 11, 2011.
<http://commons.wikimedia.org/wiki/File:Atmospheric_CO2_with_glaciers_cycles.gif>
- Schopmeyer, C.S., 1974. *Seeds of woody plants in the United States*. USDA Handbook no. 450. Forest Service, USDA, Washington, D.C.
- Sims, H.J., and Cassara J.A., 2009. The taphonomic fidelity of seed size in fossil assemblages: a live-dead case study. *Palaios*, **24**, 387-393.
- Stahle, D.W., Griffin, R.D., Cleaveland, M.K., and Eve, F.K., 2005. Ancient Baldcypress Forests Buried in South Carolina. University of Arkansas Tree-Ring Laboratory. <www.uark.edu/dendro/subfossil.pdf>
- Tenore, M., 1853. Index seminum quae anno 1853 in Horto Regio Neapolitano offerentur. *Annales des Sciences Naturelles; Botanique* **19** (Sér. 3), 355–356.
- Traverse, Alfred. 2007. *Paleopalynology* 2nd Edition. Topics in Geobiology Vol. 28. Springer, The Netherlands
- Tsumura, Y., Tomaru, N., Suyama, Y., and Bacchus, S., 1999. Genetic diversity and differentiation of *Taxodium* in the south-eastern United States using cleaved amplified polymorphic sequences. *Heredity*, **83**, 229-238.
- Upchurch, M. L., 1973. Petrology of the Eocene Castle Hayne Limestone at the Ideal Cement Quarry, New Hanover County, North Carolina: M.S. Thesis, University of North Carolina Chapel Hill, Chapel Hill, NC, 110 p.
- USDA, NRCS. 2011. The PLANTS Database (<http://plants.usda.gov>, 5 May 2011). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.
- Ward, L.W., and Blackwelder, B.W., 1980. Stratigraphy of Eocene, Oligocene, and lower Miocene formations - Coastal Plain of the Carolinas; in DuBar *et al.* (eds.), Cenozoic biostratigraphy of the Carolina outer Coastal Plain. *Excursions in southeastern geology, Annual Meeting of the Geological Society of America*, **1**, 190-210.

- Watson, F.D., 1983. A taxonomic study of pondcypress and baldcypress. N.C. State Univ., Raleigh, PhD Diss.
- Watson, F.D., 1993. *Taxodium* Richard. Flora of North America Editorial Committee (eds.): *Flora of North America north of Mexico*. Vol. 2, 403-404 Oxford University Press, New York.
- Weakley, A.S., 2010. Flora of the Southern and Mid-Atlantic States. UNC Herbarium, North Carolina Botanical Garden, University of North Carolina at Chapel Hill.
- Werker, Ella. 1997. *Seed Anatomy*. Handbuch der Pflanzenanatomie (Encyclopedia of plant anatomy). Berlin; Stuttgart: Borntraeger.
- Whitehead, D.R., 1964. Fossil Pine pollen and Full-Glacial vegetation in Southeastern North Carolina. *Ecology*, **45** (4), 767-777.
- Whitehead, D.R., 1981. Late-Pleistocene vegetational changes in Northeastern North Carolina. *Ecological Monographs*, **51** (4), 451-471.
- Wright, Donna., 2009. Personal communication. Multiple dates throughout 2009.
- Zullo, V.A., and Harris, W.B., 1986. Introduction: sequence stratigraphy, lithostratigraphy and biostratigraphy of North Carolina Eocene carbonates; in Textoris, D. A. (ed.), SEPM Field Guidebooks, southeastern United States Third Annual Midyear Meeting, 1986, Raleigh, North Carolina. *Society of Economic Paleontologists and Mineralogists*, p. 257-263.