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Vitis Seeds (Vitaceae) from the Late Neogene Gray Fossil Site, Northeastern Tennessee, USA.

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Vitis Seeds (Vitaceae) from the Late Neogene Gray Fossil Site, Northeastern Tennessee, USA

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by

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ABSTRACT

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This study focuses on the morphometric and systematic studies of fossil vitaceous seeds recently recovered from the Gray Fossil Site (7-4.5 Ma, latest Miocene-earliest Pliocene) northeastern Tennessee. Morphologically, all fossil seeds correspond to the extant subgenus *Vitis* (genus *Vitis*) of the Vitaceae based on the smooth dorsal surface with a centrally positioned chalaza connected with a conspicuous chalaza-apex groove and short linear ventral infolds that are slightly diverged apically. A multivariate analysis based on 11 measured characters from 76 complete seeds identified three types of seeds, each representing a distinct morphotaxon. Based on comparison with modern and fossil vitaceous specimens, three new species were recognized: *Vitis grayana* sp. nov., *Vitis lanatoides* sp. nov., and *Vitis latisulcata* sp.nov. The close resemblance between the first two fossil grapes (*Vitis grayana* and *Vitis lanatoides*) with extant eastern Asian *Vitis* provides further evidence that the eastern Asian floristic elements existing in the southeastern North American flora continued to as late as late Neogene.

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CHAPTER 1

INTRODUCTION

The Gray Fossil Site

The Gray Fossil Site was discovered during highway construction in Washington County, northeastern Tennessee (36.58°N, 82.58°W, elevation 490-510m) in May 2000 (Figure 1). Its deposits extend laterally ~2.6 ha (150 m N–S by 175 m E–W) and up to 40m thick (Smith 2003; Wallace and Wang 2004; Clark et al. 2005; Shunk et al. 2006). The Gray Fossil Site is now interpreted as the fill of a paleosinkhole whose deposits consist of finely laminated clays, silts, and fine sands with intermixed gravel lenses buried beneath >5m of alluvium and colluviums (Wallace and Wang 2004; Shunk et al. 2006; DeSantis and Wallace 2008). The predominant bedrock lithologies in the fossil site area are limestones and dolostones of the Cambrian-Ordovician Knox Group (Wallace and Wang 2004; Clark et al. 2005, p84, Fig. 2; Shunk et al. 2006, p267, Fig. 1). The high-resolution gravity study of the Gray Fossil Site indicates that the overall sinkhole basin consists of 11 deep sinkholes formed within the Knox Group carbonates, which range between 20 and 44m in depth and are aligned northwest and northeast trending linear features that correlate to structural features formed during the Appalachian orogenies (Whitelaw et al. 2008). According to Shunk et al. (2006), the lacustrine sediments include two parts: the basal graded facies, a 15m-thick section of lacustrine sediments below 496m elevation that consists of millimeter to centimeter-thick, normally graded layers of primarily locally derived terrigenous silts and fine sands with low organic content; the laminated facies, between 501.5 and 504.8 m elevation that is characterized by millimeter thick, non-graded "A–B couplets" of abundant macerated terrestrial organic matter and fine to coarse quartz sand (A), alternating

with quartz and carbonate silt (B). The laminated facies is the fossil-bearing horizon, and all the fossil plant materials were collected from this layer. The 5m-thick transitional interval between 496.5 and 501.5m elevation is marked by quasi-rhythmic alternation between laminated and graded facies depositional patterns (Shunk et al. 2006). The laminated facies is capped by a subaerial suite of sediment that consists of greater than 5m of dominantly gravelly alluvium and colluviums within which multiple paleosols are developed (Smith 2003). The uniform sediment composition and grain size for both the *laminated* and graded facies suggest that the sources of sediment did not change significantly through time, and chert and dolostone rock fragments both indicate local derivation from the Cambrian-Ordovician Knox Group. The presence of monocrystalline quartz grains with resorption rims and metamorphic polycrystalline quartz grains indicate that the Gray Fossil Site sediment was also derived from crystalline basement bedrock sources >50km to the east in southwestern Virginia or northwestern North Carolina (Smith 2003; Shunk et al. 2006). All these geological studies indicate that the provenance for the Gray Fossil Site sediment was both intrabasinal and extrabasinal.

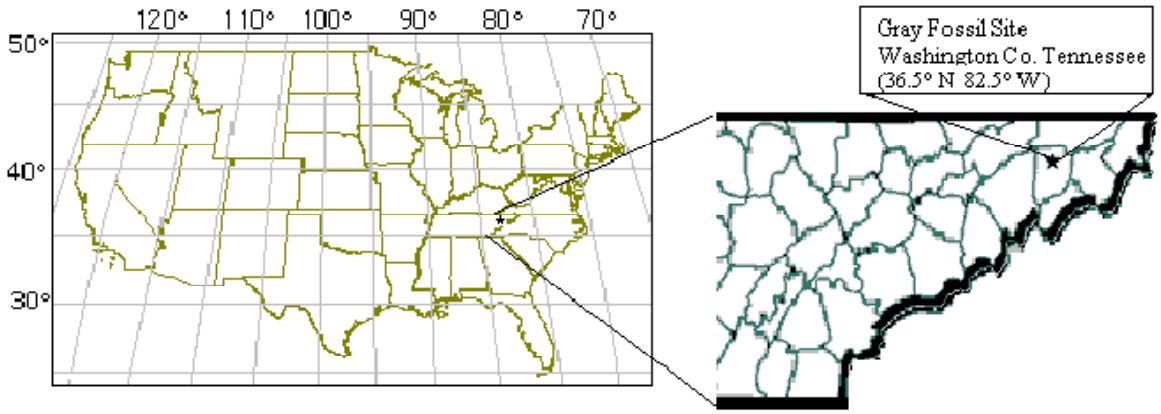

Figure 1 Location of the Gray Fossil Site, Washington County, Northeastern Tennessee, USA (36.5 °N, 82.5°W).

The biochronology data suggest the geological age of the Gray Fossil Site is latest Miocene to earliest Pliocene (4.5-7 Ma) (Wallace and Wang 2004; Shunk et al. 2006). Firstly, occurrences of *Tapirus* (cf. *T. polkensis*), *Teleoceras* sp., a small *Megalonyx* sp. (or *Plimetanastes* sp.), and cf. *Catagonus* sp. from the site indicate that the mammals can be assigned to the Hemphillian Land Mammal Age (late Miocene-early Pliocene; >4.5Ma) (Parmalee et al. 2002; Shunk et al. 2006). Secondly, the stratigraphic range of a short-faced bear *Plionarctos* sp. uncovered from the site suggests that the site is Late Hemphillian, with a maximum age of 7 Ma (Hunt 1998; Wallace and Wang 2004). Furthermore, one feature of the rhino *Teleoceras* sp. from the Gray Fossil Site, the presence of a medial projection on the posterior processes of the unciforms, also supports the Late Hemphillian age (7-4.5 Ma) of this site, because this feature is only common at the end of the *Teleoceras* lineage in the latest Miocene to earliest Pliocene (Harrison and Manning 1983; Shunk et al. 2006). In addition, paleomagnetic investigations were also used to secure an absolute date for the site and concluded that the lacustrine sediments of the site contain both normal and reverse polarities (Smith 2003). The interpretations of the paleomagnetic data indicate that the reverse polarity component as confirmation that the lacustrine sediments of the Gray Fossil Site are older than 1.0 Ma (Smith 2003; Whitelaw 2005). The reversal cannot be further constrained in time (Shunk et al. 2006). Therefore, the age of latest Miocene –earliest Pliocene (7-4.5 Ma) appears more acceptable and is used in this study.

A diverse, well preserved biota has been discovered from the Gray Fossil Site (Table 1). Abundant terrestrial animal bone and teeth fossils (tapirs, rhinos, red panda, badger, etc.) and aquatic vertebrate fossils (fish, turtles, crocodilians, etc.) uncovered from the site suggest the Gray Fossil Site was formally an open lacustrine environment (Wallace and Wang 2004; Shunk

et al. 2006). Furthermore, abundant plant remains including leaves, stems, seeds, fruits, and pollen were also uncovered from the laminated facies. Pollen records indicate that *Quercus* and *Carya* were the dominant plants, which constitute about 70% of initial pollen samples (Wallace and Wang 2004). Both the animal fossil such as the red panda (*Pristinailurus bristoli*) (Wallace and Wang 2004) and plant fossils such as *Sinomenium* (Menispermaceae) and *Sargentodoxa* (Lardizabalaceae) (Liu et al. 2007) show elements of extant eastern Asian biota. As the only late Miocene-early Pliocene fossil site in the south Appalachian Mountains (Boardman 2009), the discovery of the Gray Fossil Site has important significance for the study of the late Neogene fauna and flora of southeastern North America.

Table 1 Biota from the Gray Fossil Site (compiled from Clark et al. 2005; Liu et al. 2007; DeSantis and Wallace 2008).

Fauna		Flora	
Osteichthyes	Mammalia	Conifers	Herbs
Amphibia	Soricidae	*Tsuga*	*Ambrosia*-type
Anura	Talpidae	*Pinus*	Cyperaceae
Plethodontidae	Lagomorpha	Deciduous	Gramineae
Ambystoma sp.	Rodentia	*Quercus*	Umbelliferae
Reptilia	Xenarthra	*Carya*	Caryophyllaceae
Chrysemys sp.	Gomphotheriidae	*Ulmus*	*Ephedra*
Trachemys sp.	*Tapirus polkensis*	*Betula*	Vines
Terrapene sp.	*Teleoceras* cf. *T. hicksi*	*Fraxinus*	*Vitis*
Chelydridae	Tayassuidae	*Celtis*	*Sinomenium*
Alligator sp.	cf. *Megatylopus* sp.	*Corylus*	*Sargentadoxa*
Viperidae	Canidae	Shrubs	
Colubridae	Mustelidae	*Alnus*	
Aves	cf. *Machairodus* sp.	*Salix*	
Passeriformes	*Pristinailurus bristoli*		
	Plionarctos sp.		
	Arctomeles dimolodontus		

Taxonomy of Vitaceae

Vitaceae (the grape family) contains approximately 14 genera and 900 species (Table 2) (Soejima and Wen 2006; Wen 2007; Wen et al. 2007). The precise phylogenetic position of

Vitaceae within the Eudicots is uncertain (Judd and Olmstead 2004; Soltis and Soltis 2004). A recent study based on the complete chloroplast genome sequence, as represented by *Vitis vinifera* L., strongly supports the position of Vitaceae as the earliest diverging lineage of rosids (Jansen et al. 2006). APG II (2003) added Vitaceae to the rosids but left it unassigned to order. Traditionally, the Vitaceae was placed in the order Rhamnales along with Rhamnaceae (e.g., Cronquist 1981, 1988). But, some recent studies have considered it as one family of the order Vitales (e.g. Takhtajan 1997; Wen 2007).

Species of the Vitaceae are usually woody climbers or herbaceous vines, rarely small succulent trees (Wen 2007). Other important diagnostic characters include leaf-opposed tendrils, which are considered to be modified shoots or inflorescences (Tucker and Hoefert 1968; Gerrath et al. 2001), "pearl" glands on leaves, which are multicellular, stalked, caduceus spherical structures (Wen 2007; Wen et al. 2007), and a suite of unique seed morphological characters (Tiffney and Barghoon 1976; Soejima and Wen 2006; Chen and Manchester 2007; Wen 2007; Wen et al. 2007). Vitaceous seeds have a thin sarcotesta that are composed of several layers of parenchyma cells and an inner lignified endotesta composed of columnar cells (Periasamy 1962; Corner 1976; Chen and Manchester 2007). The endosperm is ruminate with a pair of infolds of the endotesta on the ventral face of the seed. The vascular strand of the raphe extends from the hilum passing medially along the ventral face and over the apex terminating as an enlarged lignified chalaza on the dorsal face of the seed (Chen and Manchester 2007). Usually, the sarcotesta and the vascular strand are not preserved in fossil seeds, and the external fossil seed morphology is mirrored in the underlying endotesta, the surface of which varies from smooth to rugose (Chen and Manchester 2007). The combination of a pair of ventral infolds and the dorsal chalaza is unique to the grape family, and it is commonly applied in recognizing the family and

component genera (Tiffney and Barghoorn 1976; Chen and Manchester 2007). Some species of Menispermaceae, tribe Tinosporeae possess a pair of cup-shaped indentations on the ventral face of a compressed seed, but they never show a chalaza on the dorsal face (Chen and Manchester 2007).

The grape family is mostly distributed in the pantropical areas in Asia, Africa, Australia, the neotropics, and the Pacific islands, with a few genera in temperate regions (*Vitis* L., *Parthenocissus* Planch., and *Ampelopsis* Michx.) (Table 2) (Soejima and Wen 2006; Wen 2007; Wen et al. 2007). The distribution of Vitaceae illustrates important phytogeographical significance. Species of *Ampelopsis* (~25 spp.), *Parthenocissus* (~15 spp.), and *Vitis* (~60 spp.) show disjunct distributions between eastern Asia and eastern North America (Soejima and Wen 2006; Chen and Manchester 2007).

Table 2 Generic Diversity and Distribution of Vitaceae (adapted from Soejima and Wen 2006).

Genus	No. of species	Distribution
Acareosperma Gagnepain	1	Laos
Ampelocissus Planch.	95	Africa, tropical Asia, and Australia with only four species in Central America and the Caribbean
Ampelopsis Michx.	25	Temperate to subtropical Asia (ca. 20 spp.) and North and Central America (3 spp.) and 2 in West Asia
Cayratia Juss.	63	Tropical and subtropical Asia, Africa, Australia, and the Pacific Islands
Cissus L.	350	All tropical regions with a few extending into the temperate zone
Clematicissus Planch.	1	Western Australia
Cyphostemnia (Planch.) Alston	200	Mainly in Africa and Madagascar with a few species in India and Sri Lanka extending into Thailand
Nothocissus (Miq.) Latiff	5	Peninsular Malaysia, Sumatra, Bangka, Borneo, and Papua New Guinea.
Parthenocissus Planch.	15	12 in East Asia with one species extending into the western Ghats, India and three in North America
Pterisanthes Blume	20	Malay Peninsula, Borneo, Sumatra, Java, Philippines, and peninsular Thailand.
Rhocissus Planch.	12	Tropical and South Africa
Tetrastigma (Miq.) Planch.	95	Primarily in tropical and subtropical Asia with five species in Australia
Vitis L.	60	Mostly temperate regions of the northern hemisphere, 1 sp. extending into South America.
Yua C. L. Li	3	Subtropical China, India (Assam) and central Nepal

Vitis L. including about 60 species is one of the 14 genera of Vitaceae. A molecular phylogenetic analysis of plastid *rbcL* DNA sequences found that *Vitis* to be paraphyletic with

Cyphostemma and *Parthenocissus* nested within it (Ingrouille et al. 2002). However, a recent phylogenetic study based on three chloroplast markers (the *trnL-F* region, the *atpB-rbcL* spacer, and the *rps16* intron) supports *Vitis* as a monophyletic group within a clade that includes *Ampelocissus* Planch., *Pterisanthes* Blume, and *Nothocissus* Latiff. (Figure 2) (Soejima and Wen 2006). Furthermore, the study based on the nuclear *GAI1* gene sequences confirms the monophyly of *Vitis*; however, *Nothocissus* is not placed within its sister (Wen et al. 2007). Morphologically, in addition to the morphological synapomorphies of Vitaceae, species of *Vitis* are also defined by their polygamodioecious reproductive biology, calyptrate petals, and five-merous flowers (Soejima and Wen 2006; Wen et al. 2007). Two subgenera are commonly accepted in *Vitis*. The subgenus *Vitis* is recognized by the shreddy bark on old stem, lenticels inconspicuous, pith interrupted by diaphragms within the nodes, and 2-3 forked tendrils. The subgenus *Muscadinia* possesses prominent lenticels, pith continuous through nodes, and simple tendrils (Soejima and Wen 2006).This genus occurs mainly in temperate to warm regions of the Northern Hemisphere (Table 1) (Soejima and Wen 2006). About 40 species of *Vitis* occur in eastern to southern Asia (Chen et al. 2007), and about 20 species are native to North America (Rogers and Rogers 1978; Moore 1991), with only one species (*V. tifiifolia*) extends into South America (Lombardi 2007). Only the cultivated grape (*V. vinifera*) exists in Europe (Webb 1968; Punt et al. 2003). In addition, about 5 species from 2 subgenera of *Vitis* (subgenus *Vitis*: *V. aestivalis*, *V. cinerea*, *V. labrusca*, and *V. vulpina*; subgenus *Muscadinia*: *V. rotundifolia*) occur in the northeastern Tennessee area (Chester et al. 1997). Fossil records of vitaceous seeds, the most common species and the highest number of seeds belonging to *Vitis,* have been commonly discovered from the Paleogene and Neogene floras of the Northern Hemisphere as reviewed by Kirchemimer (1939, 1957) and Tiffney and Barghoon (1976). The fossil vitaceous seed from

Gray Fossil Site is the first discovery of this family from latest Miocene to earliest Pliocene of southeastern North America due to the general absence of late Neogene fossil records in this region (Liu et al. 2007).

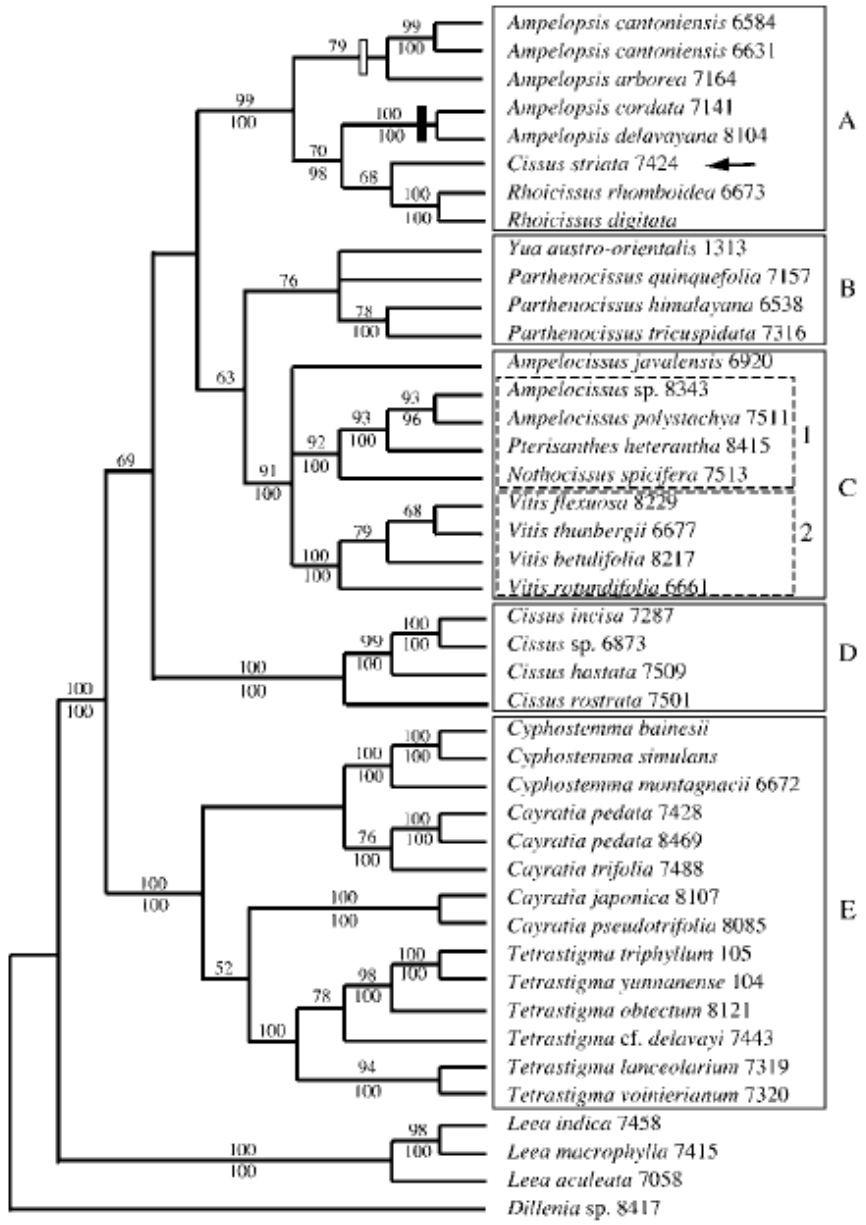

Figure 2 The Combined Chloroplast (the *trnL-F* Region, the *atpB-rbcL* Spacer, and the *rps16* Intron) Strict Consensus Tree of Vitaceae (adapted from Soejima and Wen 2006). It shows *Vitis* as a monophyletic group and forming a clade with *Ampelocissus*, *Pterisanthes*, and *Nothocissus* (Rectangle C).

Morphometerics in Paleobotany

In the present work, I chose morphometrics as part of the research methods. Morphometrics are the quantitative description, analysis, and interpretation of shape variance in biology (Rohlf 1990). In paleobotanical research it has been commonly used on the foliar morphological study (Hill 1982; Thiebaut 2000, 2002; Hably and Thiebaut 2002; Tamas and Hably 2005) and woody fragments study (Oakley and Falcon-Lang 2009). Morphometrics also have been used in the morphological study of vitaceous seeds such as the study on cultivated and wild *Vitis* seeds (Rivera et al. 2007) and the study on modern and fossil *Ampelocissus* seeds (Chen and Manchester 2007). These two studies mainly used morphometrics to examine the variation of seed characters for specimens already determined to species (Rivera et al. 2007; Chen and Manchester 2007). Here, morphometrics are used to distinguish taxonomically undetermined vitaceous seeds from the Gray Fossil Site.

Objectives

The first objective of this study was to identify the fossil vitaceous seeds from the Gray Fossil Site at the generic level using original observations along with previous vitaceous seed morphology studies (Tiffney and Barghoon 1976; Chen and Manchester 2007).

A second objective is to use multivariate analyses to group the vitaceous seed remains from the Gray Fossil Site into morphospecies.

A systematic morphological study focusing on modern *Vitis* seeds was also performed to investigate the seed morphological variance at the interspecific and intraspecific level.

Lastly, I examined the biogeographical patterns associated with distribution of fossil and modern *Vitis* species and the possible relationship of the Gray fossil *Vitis* specimens.

CHAPTER 2

MATERIALS AND METHODS

Fossil and Extant Materials and Preparation

Fossil seed materials used in present study were collected from the laminated facies horizon of the Gray Fossil Site (see Introduction). The preparation of the fossil materials follows Tiffney (1990). The organic-rich blocks of matrix were collected from the Gray Fossil Site and returned to the laboratory of the on-site East Tennessee State University and General Shale Brick Nature History Museum (ETMNH). Then the matrix was soaked under water to disaggregate. Next, the 1.7mm mesh box screen (Boardman 2009) was used to separate the organic materials and the fine clays. After that, the vitaceous seeds were picked out from the fossil plant remains based on the unique characters (a pair of infolds on ventral face and the chalaza on dorsal face) and stocked in Paleobotany lab of Department of Biological Sciences, ETSU for the subsequent studies. Seventy-six complete fossil seeds were measured for the morphometric study.

Seed specimens from extant species were obtained for comparative studies. Seeds representing 95 species from 9 genera of the Vitaceae were loaned from the Herbaria of Arnold Arboretum (A) and Gray Herbarium (GH) of Harvard University, John C. Warden Herbarium of East Tennessee State University (ETSU), and Missouri Botanical Garden (MO). Preparation of the extant seeds follows Tiffney and Baeghoon (1976) by boiling in 10% NaOH for 5-10 min to remove the outer membrane and adherent pieces of berry.

Digital images of both dorsal and ventral views of the fossil and extant seeds were recorded with a MicroFire (Optronics) camera attached to the OLYMPUS-SZX12

stereomicroscope. Measurements of the digital images were taken using the program ImageJ (version 1.40g) (Rasband 1997-2009).

Measurements and Morphometric Analysis

Eleven continuous variables were chosen for morphometric analysis and were measured from the digital images (Table 3 and Figure 3).

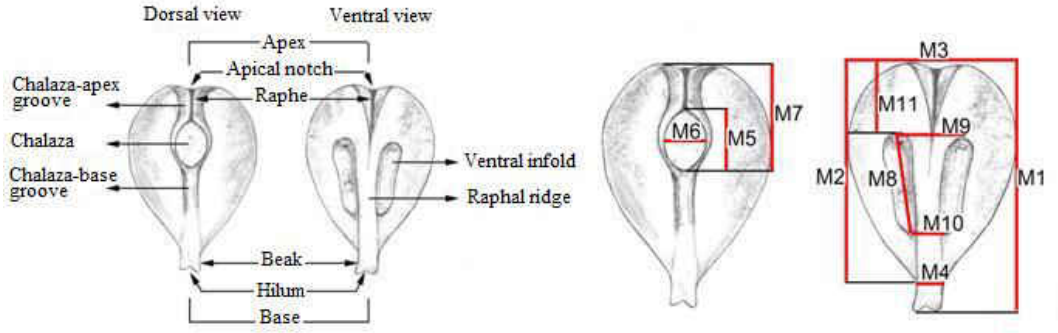

Figure 3 Morphological Terminology of Vitaceous Seed (compiled from Tiffney and Barghoorn, 1976; revised by Manchester 1994) and Seed Characters Measured for Morphmetrics (See Table 3 for Character Descriptions).

Table 3 List of Morphometric Characters (M= Measurement) Used in the Present Study. The measurement points for the characters (M1-M11) are shown in Figure 3.

Character (mm)	Description
M1	Seed length with beak
M2	Seed length without beak
M3	Seed width
M4	Beak width at the juncture with seed body
M5	Chalaza length
M6	Chalaza width
M7	Distance from chalaza base to seed apex
M8	Ventral infold length
M9	Distance between apexes of the two infolds
M10	Distance between bases of the two infolds
M11	Vertical distance from infold apexes to seed apex

Data processing was performed using SPSS 16.0 (SPSS Inc. 2008). Frequency histograms were used to examine the variation and normal distribution of the measured

characters. The Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO Test) and Bartlett's Test of Sphericity were applied to test the condition of principal component analysis (PCA) that was used to study the relationships among the measured characters. In PCA, eigenvalues were computed from the raw data and data after Varimax rotation with Kaiser Normalization, and then eigenvectors and component score coefficient for each principal component were calculated after rotation. PCA enables us to describe the relationship of the measured variables in the multidimensional space. Although PCA is also a common method for grouping specimens, Thiébaut (2002) proposed that PCA is a good tool when it keeps a maximum of total variability; on the other hand, cluster analysis is more appropriate with many taxa. In this study hierarchical cluster analysis was carried out to calculate and graph the multidimensional distance among the specimens studied. Similarities of specimens were calculated by squared euclidean distances. These computed distances were graphed on a dendrogram using furthest neighbor cluster method that calculates the distance between two clusters as the distance between their two furthest points and standardizes the measured characters in the range 0 to 1. Box's M value test was performed to check the condition of canonical discriminant analysis, and then discriminant analysis using all 11 characters was performed to find the linear combinations of characters that are shown as canonical discriminant functions from which discriminant scores for each specimen are also calculated. Statistic descriptive and independent sample t-test were also performed to examine variance between difference clusters.

Terminology

The terminology of vitaceous seed characters (Figure 3) is after Tiffney and Barghoorn (1976) with the exception that the terms chalaza-apex and chalaza-base grooves are reversed (Manchester 1994).

CHAPTER 3

RESULTS

Seed Morphology of Vitaceae

The vitaceous seeds are distinguished from seeds of other families by the combination of paired ventral infolds and the dorsal chalaza (Figure 3) (Chen and Manchester 2007). For better understanding the seed morphological range of all genera of the Vitaceae, I examined vitaceous seeds representing 95 species from 9 genera of the Vitaceae by myself (Table 4; Figure 4; Figure 5) and consulted the previous studies (Tiffney and Barghoon 1976; Chen and Manchester 2007; Wen 2007). In general, the vitaceous seeds can be distinguished to at least the generic level by a combination of certain morphological characters, especially seed surface characters, chalaza shape and position, and shape of ventral infolds. Based upon these examinations, I have prepared a new dichotomous key to the genera of Vitaceae based upon seed morphology.

Taxonomic key to 12 genera of the Vitaceae based on seed morphology:

1a. Chalaza central on dorsal surface, chalaza-apex groove slightly or obviously visible..... 2

2a. Ventral infolds cup-shape or long linear, extending from the seed base to the seed apex……... *Ampelocissus* (*Nothocissus*)

2b. Ventral infolds linear, not extending to the seed apex…….. 3

3a. Dorsal and ventral surfaces obviously rugose with deep furrow…….. *Yua*

3b. Dorsal and ventral surface extremely smooth or furrowed to striated…….*Vitis*

1b. Chalaza near the apical notch or starting from apical notch and central on dorsal surface, chalaza-apex groove not visible…… 4

4a. Ventral infolds cup-shape……. 5

5a. Chalaza linear to elongate……..…….. *Cayratia*

5b. Chalaza oval…… *Pterisanthes*

4b. Ventral infolds linear…… 6

6a. Chalaza starting from the ventral face and crossing the apical notch

…… *Cissus* or *Cyphostemma*

6b. Chalaza starting or near the apical notch…….. 7

7a. Ventral infolds short linear or small pit, not extending to the

seed apex……. *Ampelopsis*

7b. Ventral infolds long linear, extending from the seed base to the

seed apex…….. 8

8a. Seed surface smooth...……..... *Parthenocissus*

8b. Seed surface rugose……... 9

9a. Pattern of the rugose surface marked by

horizontal furrows……... *Tetrastigma*

9b. Pattern of the rugose surface irregular…… *Rhoicissus*

Among of the 14 genera of Vitaceae, *Acareosperma* Gagnepain comprises only one species from Laos (Table 2). Because no specimen collections or data on seed morphology were available for *Acareosperma*, I exclude it here. *Clematicissus* Planch. is another genus represented by only one species. Although I could not obtain specimen for direct observation, Chen and Manchester (2007) indicate that seeds of *Clematicissus* possess only one linear long infold on ventral surface, which is totally different from other vitaceous seeds. In addition, *Nothocissus* (Miq.) Latiff is another small genus including only 5 species, which was usually considered as one section of *Ampelocissus* (reviewed by Chen and Manchester 2007). Seeds of *Nothocissus*

also show some close similarity with seeds of some *Ampelocissus* species that is consistent with the inclusion of *Nothocissus* with *Ampelocissus*. I do not separate these two genera in the basis of seed characteristics. *Cyphostemnia* (Planch.) Alston (~200 spp.) is the second largest genus in the Vitaceae. I was unable to obtain specimens of *Cyphostemnia*. According to Chen and Manchester (2007), seeds of *Cyphostemnia* share some characteristics with *Cissus* L. (~300 spp.), the largest genus of Vitaceae. Considering the range of these two genera and lacking more data on seed morphology, I put them together in the taxonomy key as well.

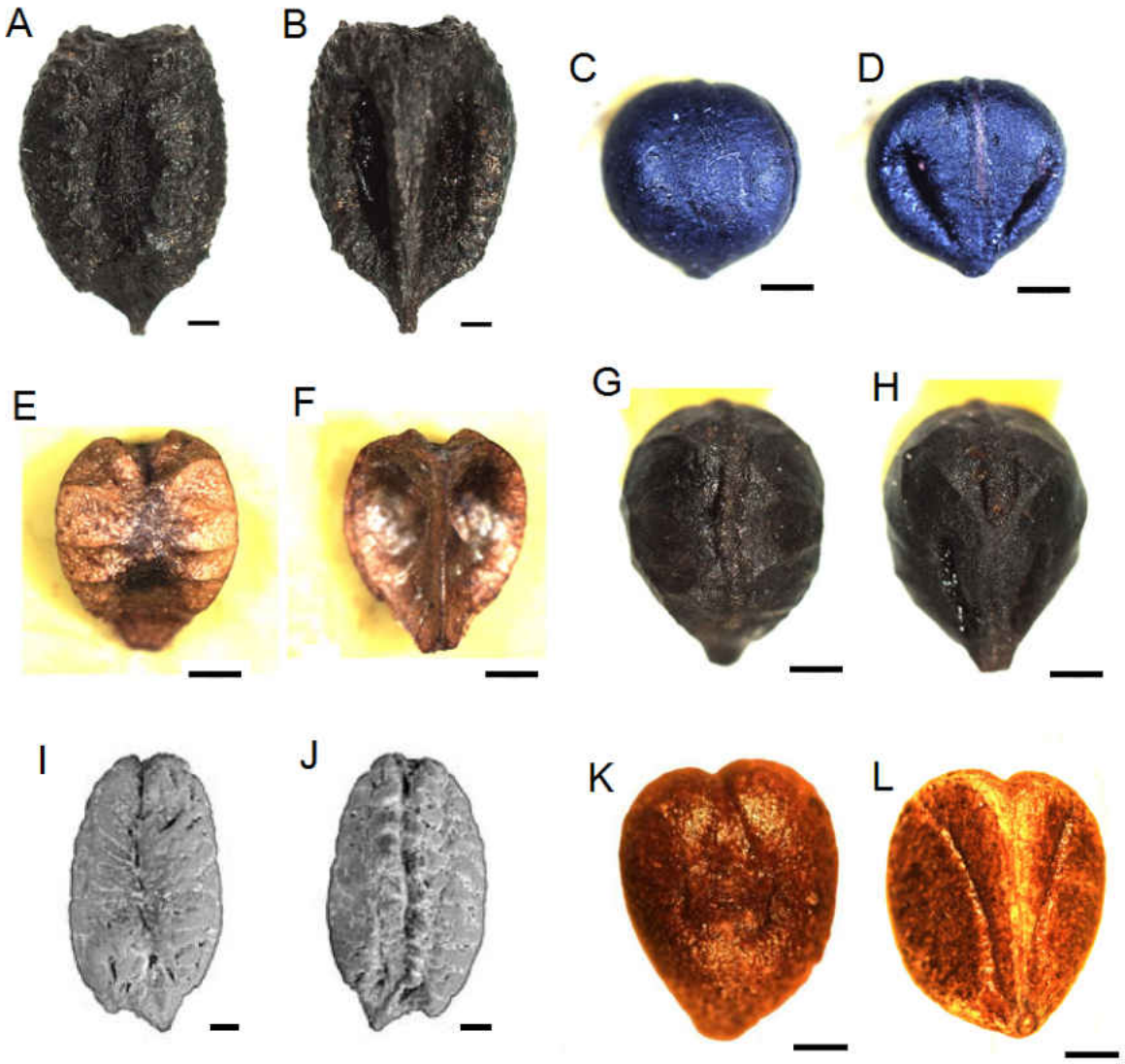

Figure 4 Representative Seeds for Genera of Vitaceae. Scale bar =1mm. A-B. Dorsal and ventral views of *Ampelocissus acapulcensis*; C-D. Dorsal and ventral views of *Ampelopsis brevipedunculata*; E-F. Dorsal and ventral views of *Cayratia japonica*; G-H. Dorsal and ventral views of *Cissus incisa*; I-J. Dorsal and ventral views of *Nothocissus spicifera*, (adapted from Chen and Manchester 2007); K-L. Dorsal and ventral views of *Parthenocissus quinquefolius*.

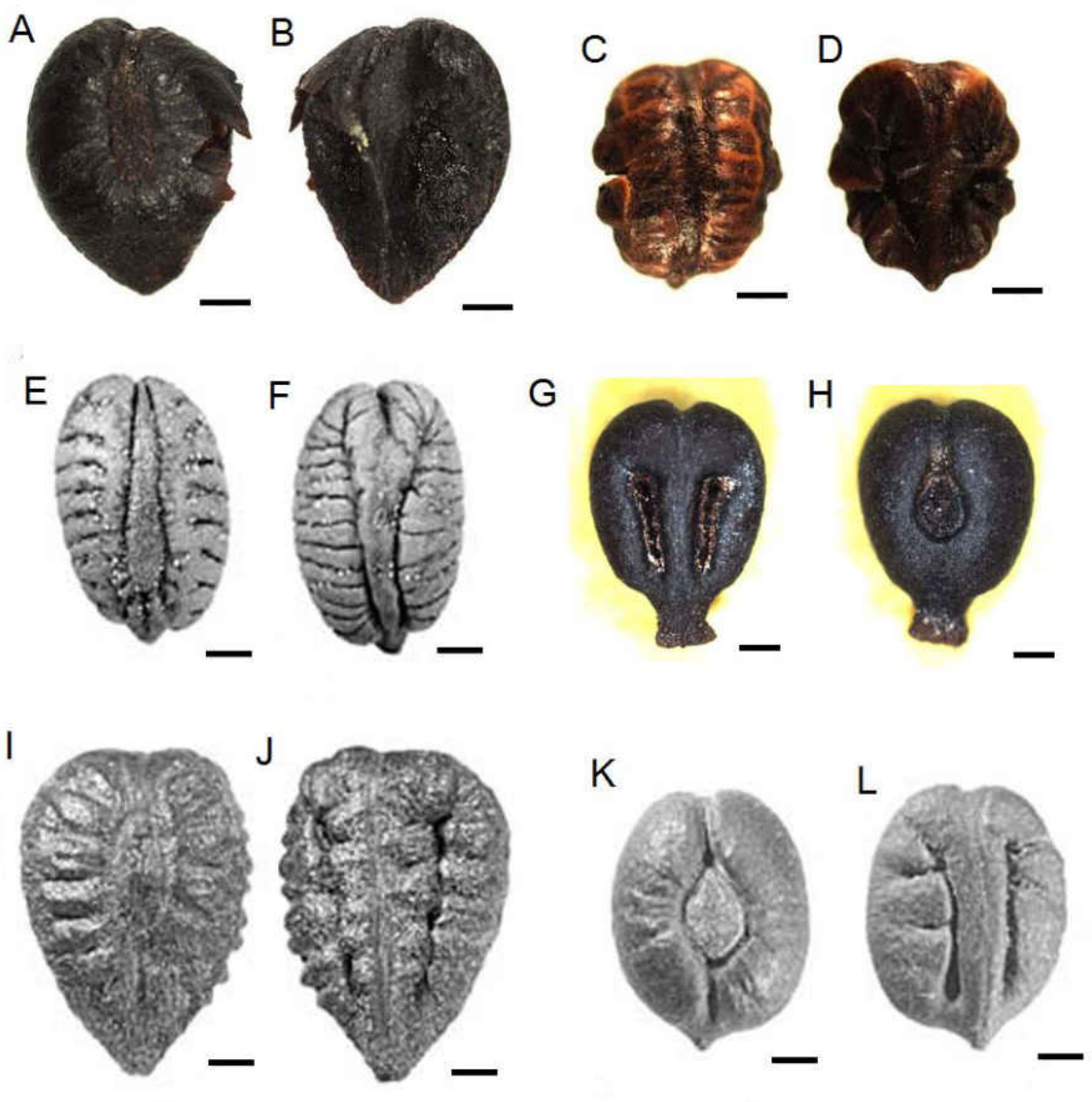

Figure 5 Representative Seeds for Genera of Vitaceae (Continued). Scale bar = 1mm. A-B. Dorsal and ventral views of *Pterisanthes cissoides*; C-D. Dorsal and ventral views of *Rhoicissus tridentata*; E-F. Dorsal and ventral views *Terastigma kwangsiensis,* (adapted from Chen and Manchester 2007); G-H. Dorsal and ventral views of *Vitis labrusca*; I-J. Dorsal and ventral views of *Yua austro-orientalis*, (adapted from Chen and Manchester 2007); K-L. Dorsal and ventral views of *Vitis rotundifolia*, (adapted from Chen and Manchester 2007).

Table 4 Some Important Morphological Characters Distinguishing Seeds of Genera of Vitaceae. Data from my observation and complied from Tiffney and Barghoon (1976); Chen and Manchester (2007); Wen (2007).

Genus	Surface	Shape and position of ventral Infolds	shape and position of Chalaza	Chalaza-apex groove	representative species
Acareisperma	no data				
Ampelocissus	finely to obvious rugose	long linear, cup-shaped, or dish like; parallel to slight diverged apically	round to oval; central on dorsal surface	obvious visible	*Ampelocissus acapulcensis* (Figure 4, A-B)
Ampelopsis	smooth to rugose	short, broad, linear; parallel to slight diverged apically	variance; near the shallow apical notch	not existence	*Ampelopsis brevipedunculata* (Figure 4, C-D)
Cayratia	smooth to rugose	short, small pit to cup-shaped; central	linear to elongate; starting from apical notch and central on dorsal surface	not existence	*Cayratia japonica* (Figure 4, E-F)
Cissus	smooth to rugose	short, linear, closely spaced and parallel	linear to elongate; starting from apical end of ventral infolds; central on dorsal surface	not existence	*Cissus incisa* (Figure 4, G-H)
Clematicissus	no data	only one infold; central	pyriform; central on dorsal surface	no data	no specimen observed
Cyphostemma	rugose	short, linear, covered by extra lignified testa; closely spaced and parallel,	continuous from ventral side and central on dorsal surface	not existence	no specimen observed
Nothocissus	rugose	long, linear; parallel	linear to oval; central on dorsal surface	slight visible	*Nothocissus spicifera* (Figure 4, I-J)
Parthenocissus	smooth	long, linear, extending from base to apex; diverged apically	variance; near the deep apical notch	not existence	*Panthenocissus quinquefolius* (Figure 4. K-L)
Pterisanthes	smooth	long, cup-shaped; central	oval; central on dorsal surface	not existence	*Pterisanthes cissoides* (Figure 5,A-B)
Rhoicissus	rugose	long, linear; diverged apically	linear to elongate; starting from apical notch and central on dorsal surface	not existence	*Rhoicissus tridentata* ((Figure 5, C-D)
Terastigma	rugose	long, linear;closely spaced and parallel, or divergent in Y-or U-shape	linear to elongate; starting from apical notch and central on dorsal surface	not existence	*Terastigma Kwangsiense* (Figure 5, E-F)
Vitis	slight rugose or smooth	short, linear; parallel to diverged apically	variance; central on dorsal surface	obvious visible	*Vitis labrusca* (Figure 5, G-H); *V. rotundifolia* (Figure 5,K-L)
Yua	rugose	long, linear; parallel	oval; central on dorsal surface	obvious visible	*Yua austro-orientalis*(Figure 5, I-J)

Seed Morphology of *Vitis* L.

All fossil vitaceous seeds from the Gray Fossil Site that are characterized by the combination of the central positioned chalaza on dorsal surface, obvious visible chalaza-apex groove, and short linear ventral infolds correspond to the genus *Vitis*. Moreover, all the fossil seeds collected from the Gray Fossil Site show a smooth surface, which is one common character of the subgenus *Vitis*; while seeds of the other subgenus *Muscadinia* (Figure 5, K-L) show furrowed to striated dorsal surface (Tiffney and Baghoon 1976). For better understanding of the seed morphological variance at the interspecific and intraspecific levels, a systematic study on *Vitis* seed morphology based on 57 specimen collections representing by 41 extant species of subgenus *Vitis* was performed (Table 5). These species include all North and South American species and about half of the Asian species. Names used in this study are those currently accepted by the USDA PLANTS Database (USDA NRCS 2009) and Germplasm Resources Information Network (GRIN) (USDA ARS National Genetic Resources Program 2009). Besides the chalaza position and shape and ventral infolds shape (important characters for identification of vitaceous seeds at the generic level, Table 4), I also considered other characters including beak shape and size, chalaza-apex and -base grooves, ventral infold position on ventral surface, apical notch, etc. to describe the seed morphology at the specific level. Although only a few species (*V. palmata*, *V. riparia*, *V. novae-angliae* etc.) could be easily identified by one or two distinct characters (listed in bold on Table 5), seeds of majority of *Vitis* species could be identified to specific level by a combination of several seed morphological characters especially beak shape and size, chalza-base groove, ventral infold length and position on ventral face. Nevertheless, seed morphology of several species (Figure 6, *V. candicans*, *V. palmata*, *V. labrusca*, *V. lanata*, etc.) is morphologically indistinguishable with the exception of the

cultivated grape *V. vinifera*. This result means that most species of *Vitis* could be identified at the specific level based only on seed morphological characters. This result is very important for the study of fossil *Vitis* because fossil seeds are the most common remains of this genus.

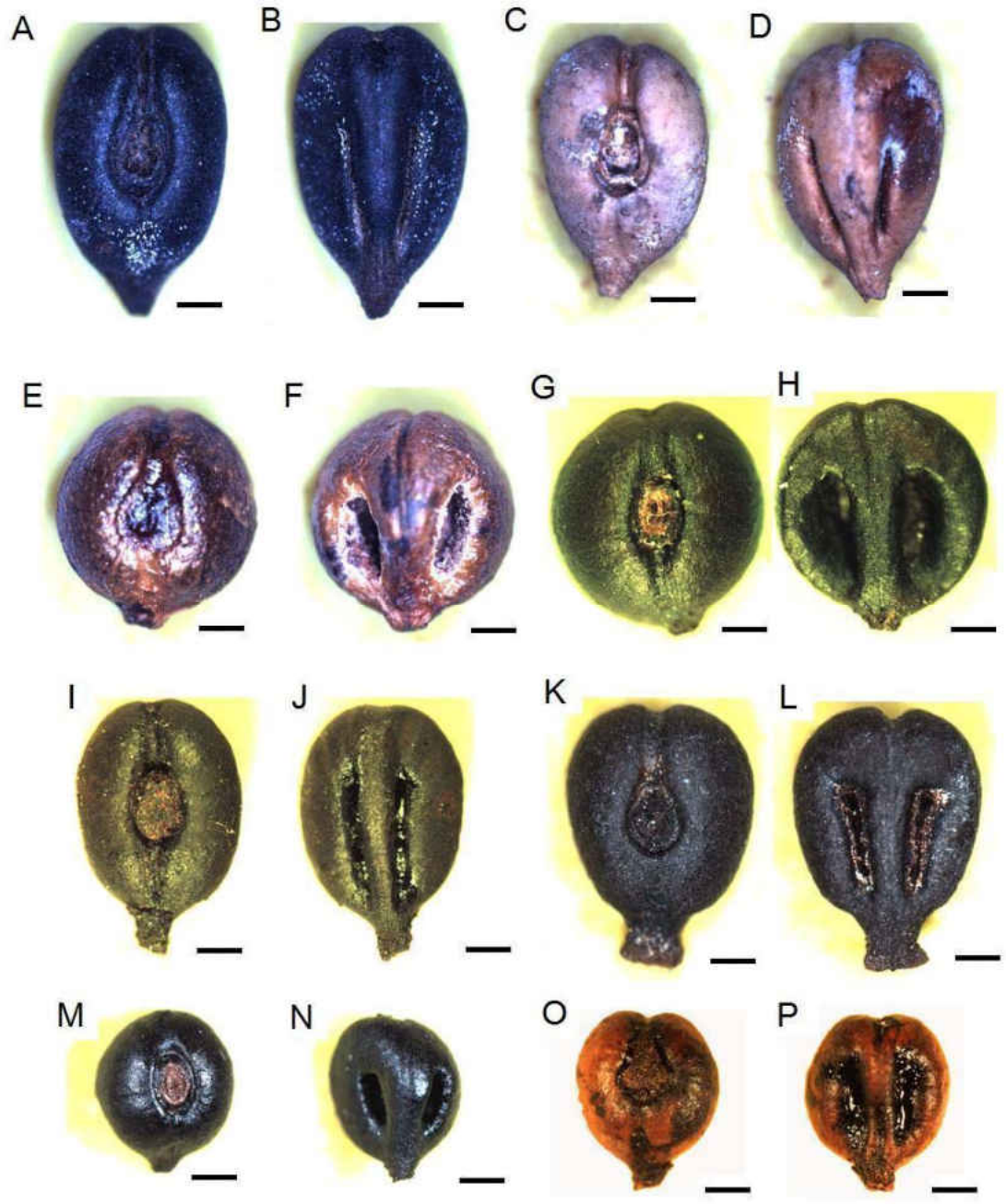

Figure 6 *Vitis* Seeds Showing Intraspecific Morphological Uniformity Based on Same Species Possessing Different Collections. Scale bar = 1mm. A-B. Dorsal and ventral views of *Vitis candicans* (GH, Munson 1891, North Texas); C-D. Dorsal and ventral views of *V. candicans* (GH, Goodman 5858, Oklahoma); E-F. Dorsal and ventral views of *V. palmata* (AA, C.C. Deam 33065, Indiana); G-H. Dorsal and ventral views of *V. palmata* (MO, W. J. Faircloth 4646, Georgia); I-J. Dorsal and ventral views of *V. labrusca* (MO, J. R. Churchill S.N., Pennsylvania); K-L. Dorsal and ventral views of *V. labrusca* (GH, M. L. Femald & Bayard Long 9876, Massachusetts); M-N. Dorsal and ventral views of *V. lanata* (AA, S.Sasaki 21614, Taiwan); O-P. Dorsal and ventral views of *V. lanata* (AA, R. N. Parner, no num, India). Specimen organization of information is as follow: Herbarium, Voucher specimen, Locality.

Table 5 Some Important Seed Characters Distinguishing Extant Species of Subgenus *Vitis* (Only Showing Distinct Characters to Identify Each Species). Bold descriptions show the only one character to identify seeds of the relative *Vitis* species. (N.S. = number of specimen collection)

Species	Synonyms	Distribution	N.S.	Beak	Chalaza	Chalaza-apex groove	Chalaza- base groove	Ventral infolds	Apical notch
V. labrusca		North America	3	cylindrical, extremely prominent	pyriform to spatulate	broad deep	faint		distinct
V. cinerea var. baileyana	*V. baileyana*	Eastern USA	1		elongate to elliptical		no		
V. cinerea var. cinerea		USA	1	small triangle	round	deep	no	broad, deep	
V. cinerea var. floridana	*V. simpsonii*	southeastern USA	1				obviously visible	**very close to seed base**	
V. riparia		North America	1		**narrow spatulate to linear**				
V. palmata		USA	2	slightly prominent				**Broad to semicircle**	
V. rupestria		USA	1	slightly prominent		faint	no		no
V. vulpina	*V. cordifolia*	USA	2	slightly prominent	round			close to seed base	
V. monticola		south-central USA	1				no	short, close to seed base	
v. acerifolia	*V. solonis; V. longii*	central USA	2	prominent, triangular					
V. mustangensis	*V. candicans*	central USA	2	trapezoidal					
V. virginiana		Eastern USA	1	round	deep	broad deep		obviously apical divergent, close to seed base	
V. aestivalia var aestivalia	*V. smalliana, V. rufotomentosa*	USA	3	cylindrical	pyriform to round	broad deep	no	slightly curved, broad	distinct
V. aestivalia var. lincecumii	*V. lincecumii*	USA	1		small round			long, extending to seed base	
V. aestivalia var. bicolor	*V. argentifolia*	North America	2				obviously visible	long, extending to seed base	
V. novae-angliae		northeastern USA	1		**very small round**				
V. californica		Western USA	1	small, triangle	stilliform				
V.girdiana		Western USA Mexico	1		elongate to elliptical		visible	wrinkle margin	

Table 5 Continued

Species	Synonyms	Distribution	N.S.	Beak	Chalaza	Chalaza-apex groove	Chalaza- base groove	Ventral infolds	Apical notch
V. arizonica		Western USA, Mexico	1		small round	broad deep	obviously visible	obviously apical divergent, close to seed base	
V. blancoi		Mexico	1	slightly prominent	Small round, concave	deep	no	obviously apical divergent	
V. amurensis		eastern Asia	1	slightly prominent	elongate	deep	obviously visible		
V. balanseana		south China, southeastern Asia	1	cylindrical, extremely prominent					
V. betulifolia		south China	1	slightly prominent	big round			very broad	
v. piasezkii		China	1	obviously prominent	big round			very broad	
V. wilsonae		China	1	obviously prominent	big elliptical		no		
V. thunbergii	*V. ficifolia, V. kaempferi*	eastern Asia	2	triangular, prominent	elliptical		slightly visible		
V. araneosa		southeastern Asia	1	prominent	big round		slightly visible		
V. flexuosa	*V. parvifolia*	eastern, southeastern south Asia	1	extremely prominent	elliptical		no		
V. lanata		eastern to southern Asia	2	prominent	round		slightly visible	short	
V. heyneana	*V.quinquangularis*	eastern, southeastern, south Asia	1	slightly prominent	elongate			short	no
V. chungii		China	1	short, cylindrical		deep		short broad	deep
V. chunganensis		China	1	prominent	close to seed apex				
V. saccharifera		Japan	1	prominent, triangle	elliptical		no	obviously apical divergent	
V. sinensis		China	1		big elongate		no		
V. tiliifolia	*V. tiliaefolia; V. caribaea*	southern America	4	prominent					

Table 5 Continued

Species	Synonyms	Distribution	N.S.	Beak	Chalaza	Chalaza-apex groove	Chalaza- base groove	Ventral infolds	Apical notch
Questionable specimens									
V. vinifera	*V. sylvestris*	western Asia	3	cultivated all over the world, seed morphology variance					
V. retordii		China	1	seed specimens checked in this study showing morphology not corresponding to *Vitis*					
V. boorquiniana			1	cultivated					
Vitis sicyoides			1	transferred to *Cissus*					
Vitis capensis			1	transferred to *Rhoicissus*					
Vitis japonica			1	transferred to *Cayratia*					

Morphometric Study

Relationships of Variables

Principal component analysis (PCA) using a correlation matrix was performed to examine the relationships between each pair of measured characters and among all the characters. Correlation coefficients between each pair of measured characters were calculated on the raw data matrix (Table 6). With the exception of M9 and M10 (see Table 3), all the other nine characters show significant correlations with each other.

Table 6 Correlation of the Measured Characters. Correlation coefficients are shown in bold if $p<0.05$.

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
M1		**0.934**	**0.551**	**0.661**	**0.497**	**0.426**	**0.735**	**0.753**	**0.304**	**0.272**	**0.759**
M2			**0.543**	**0.479**	**0.427**	**0.402**	**0.713**	**0.813**	**0.238**	0.177	**0.717**
M3				**0.564**	**0.361**	**0.529**	**0.365**	**0.277**	**0.505**	**0.409**	**0.654**
M4					**0.329**	**0.382**	**0.412**	**0.287**	**0.375**	**0.446**	**0.624**
M5						**0.481**	**0.609**	**0.357**	**0.289**	**0.296**	**0.385**
M6							**0.347**	**0.26**	**0.31**	**0.336**	**0.423**
M7								**0.636**	**0.194**	0.147	**0.49**
M8									0.08	-0.107	**0.338**
M9										**0.656**	**0.476**
M10											**0.428**
M11											

KMO Test gives a value 0.776, which indicates that the data from the measured characters are acceptable for PCA (Kaiser 1974). The Bartlett's Test of Sphericity showed a P-value less than 0.001, which rejects the hypothesis that the correlation matrix from the raw data is an identifying matrix and supports that the data structure fulfills the conditions of PCA. Three principal components were extracted from the data after rotation that explain 75.78% of total variance (Table 7). The rotated component matrix after rotation demonstrates important

characters for each component, and the component score coefficient matrix displays the most important characters for each component (Table 7).

Table 7 The Rotated Component Matrix (Bold Numbers Showing Characters Significantly Loaded to Principal Components) and the Component Score Coefficient Matrix (Bold Numbers Indicating the Most Important Character for Each Principal Component) for the First Three Principal Components (PCs). Percentages (%) of variance explained by each PC are listed on the rotated component Matrix.

Characters	Rotated Component Matrix			Component Score Coefficient Matrix		
	PC1(33.89 %)	PC2 (26.92 %)	PC3 (14.97 %)	PC1	PC2	PC3
M2	**.914**	.235	.180	.297	-.022	-.121
M1	**.885**	.346	.207	.267	.027	-.111
M8	**.870**	-.125	.201	**.310**	-.189	-.011
M7	**.709**	.068	.495	.147	-.147	.274
M10	-.114	**.823**	.231	-.190	**.344**	.075
M9	.004	**.788**	.193	-.129	**.324**	.015
M3	.388	**.678**	.192	.037	.234	-.065
M11	.592	**.651**	.078	.153	.226	-.223
M4	.471	**.643**	.043	.114	.242	-.224
M5	.281	.165	**.851**	-.131	-.136	**.712**
M6	.190	.380	**.652**	-.136	.016	.497

The first principal component (PC1) accounts for 33.89% of variance after rotation and is highly weighted on four characters that are characters to reflect seed length (M1, M2), ventral infold length (M8), distance from seed apex to chalaza base (M7). According to the component score coefficients, ventral infold length (M8) contributes the highest coefficient score for PC1, which indicates that it is the most important character for PC1. The second principal component (PC2) accounts for 26.92% of variance after rotation and is highly weighted on five characters that are the characters focusing on seed width (M3), beak width (M4), distances between apexs of the two infolds (M9), distance between bases of the two infolds (M10) and vertical distance from infold apexes to seed apex (M11). Distance between bases of the two infolds (M10) contributes the highest coefficient score, while distance between apexes of the two infolds (M9) also show a coefficient score close to the score of distance between bases of the two infolds

(M10), which indicated that distances between two ventral infolds (M9, M10) are important characters for PC2. The third principal component (PC3) accounts for 14.97% of the variance after rotation. The chalaza length (M5) and width (M6) are the most important characters for PC3, and component score shows chalaza length (M5) is more important than chalaza width (M6) for PC3.

Hierarchical Cluster Analysis

The hierarchical cluster analysis was carried out to group specimens into morphotaxa. The result was shown as a dendrogram (Figure 7), which was built following the agglomeration schedule table (Table 8). There are two different ways to define the different clusters of the dendrogram: firstly, looking for "gaps" between joinings along the horizontal axis of the dendragram; secondly, finding the sudden jump (gap) in the distance coefficient from the agglomeration schedule table (SPSS Inc. 2008). The dendrogram (Figure 7) shows a large gap between rescaled distance 10 and 15, which suggests 3 distinctive clusters at the rescaled distance of about 15. Then, the sudden jump (gap) in the distance coefficient firstly appears as stage 74 of the agglomeration schedule table (Table 8), which suggests that the two clusters represented by specimen 1 and 4 could be considered as different clusters. Following that, three clusters are also showed on the dendrogram. According to these analyses hierarchical cluster analysis suggests three distinctive clusters each of which can be considered as a morphotaxon.

Table 8 Agglomeration Schedule Table for Hierarchical Cluster Analysis. The bold numbers show the sudden jump (gap) of the distance coefficient from stage 73 to stage 74.

Stage	Cluster Combined		Coefficients
	Cluster 1	Cluster 2	
1	21	56	0.075
2	47	61	0.101
3	19	26	0.118
4	32	38	0.118
5	68	73	0.121
6	13	50	0.128
7	8	36	0.129
8	49	51	0.138
9	6	47	0.144
10	24	30	0.148
11	4	18	0.151
12	27	66	0.155
13	12	33	0.157
14	45	65	0.168
15	58	76	0.171
16	37	55	0.180
17	17	31	0.183
18	5	75	0.190
19	10	46	0.191
20	69	72	0.198
21	42	48	0.201
22	7	23	0.208
23	25	44	0.213
24	35	62	0.230
25	21	70	0.230
26	6	40	0.251
27	34	49	0.253
28	22	29	0.255
29	9	21	0.263
30	19	24	0.264
31	11	57	0.281
32	8	14	0.294
33	32	64	0.300
34	68	69	0.331
35	12	52	0.332
36	9	13	0.333
37	20	22	0.337
38	2	28	0.344

Continued.

Stage	Cluster Combined		Coefficients
	Cluster 1	Cluster 2	
39	1	17	0.345
40	42	63	0.348
41	35	41	0.352
42	4	6	0.355
43	67	74	0.372
44	3	5	0.372
45	7	8	0.379
46	15	53	0.392
47	59	68	0.422
48	10	20	0.437
49	4	34	0.477
50	27	58	0.488
51	1	25	0.500
52	19	67	0.501
53	45	60	0.519
54	4	16	0.548
55	37	39	0.553
56	2	32	0.556
57	12	59	0.566
58	35	43	0.626
59	3	7	0.638
60	11	27	0.664
61	9	45	0.705
62	4	35	0.839
63	3	10	0.844
64	1	42	0.897
65	9	12	1.007
66	19	71	1.011
67	1	11	1.019
68	3	19	1.180
69	2	54	1.214
70	4	15	1.327
71	1	9	1.396
72	4	37	1.759
73	2	3	**2.176**
74	1	4	**3.652**
75	1	2	5.753

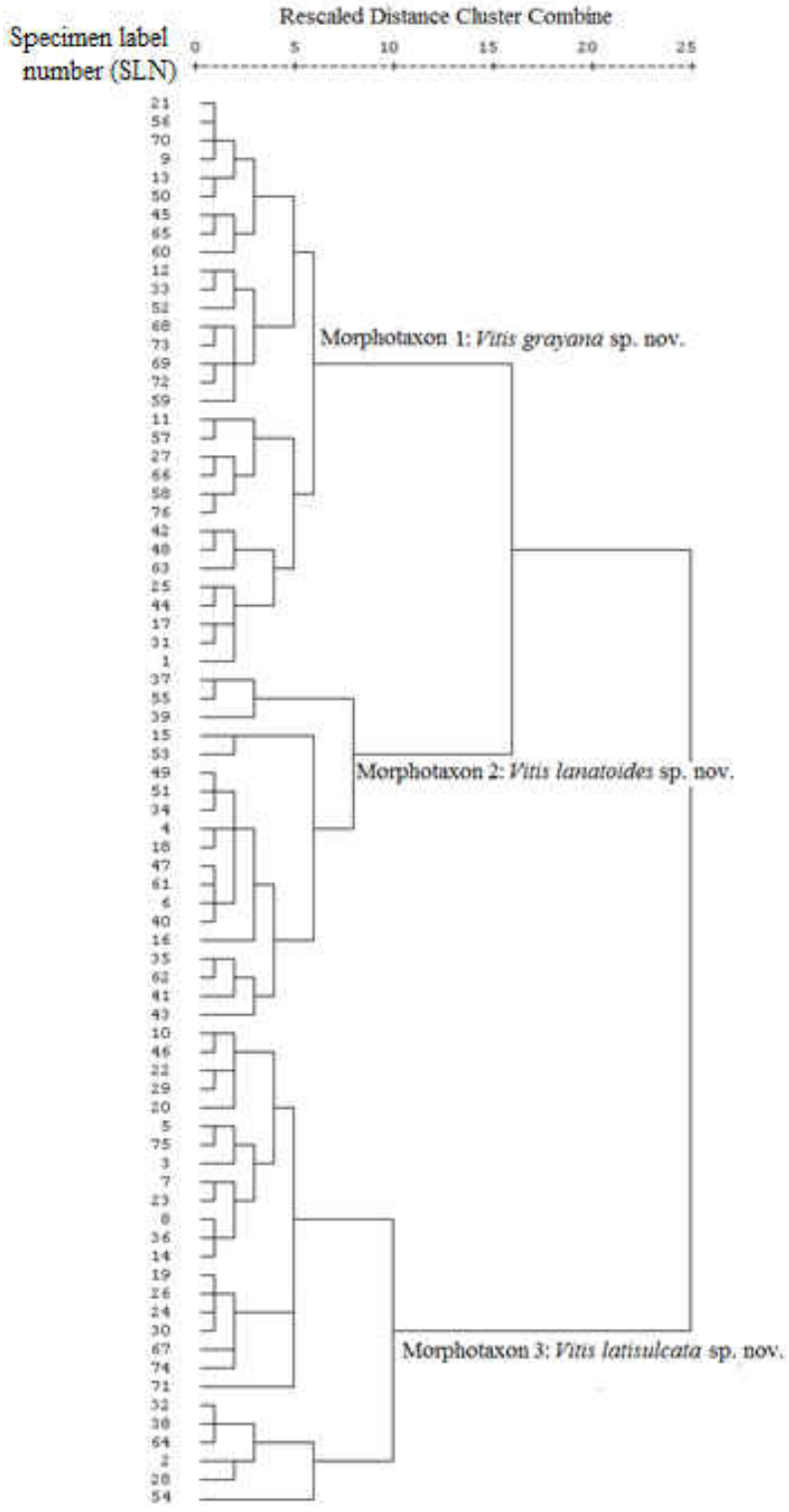

Figure 7 Dendrogram of Fossil Seeds from Hierarchical Cluster Analysis. Three clusters are clearly separated at the rescaled distance of 15 each of which would be considered as one morphotaxon. The specimen label number (SLN) is given by this study (Appendenix).

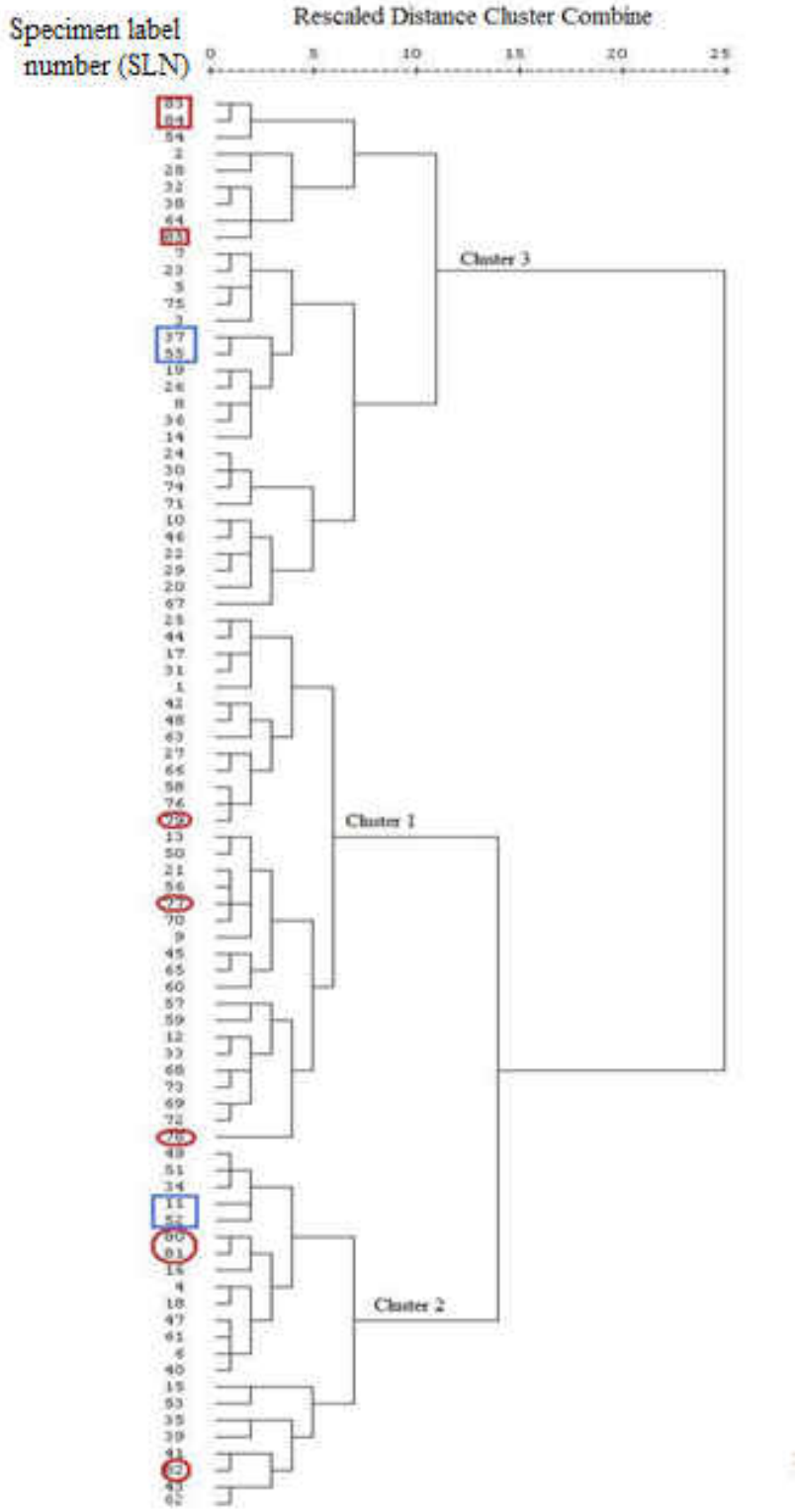

Figure 8 Dendrogram of Fossil and Extant Seeds from Hierarchical Cluster Analysis.

Figure 8 Dendrogram of Fossil and Extant Seeds from Hierarchical Cluster Analysis. The three clusters correspond to the morphotaxa in Figure 7. The red rectangles (SLN 83, 84, 85) indicate extant *Vitis labrusca*: SLN 83, 84 (*V. labrusca*: MO, J. R. Churchill S.N., Pennsylvania), SLN 85 (*V. labrusca*: GH, M. L. Femald & Bayard Long 9876, Massachusetts); the red ellipses (SLN 77, 78, 79) indicate extant *Vitis thunbergii*: A, A. Muroi 6706, Japan; and the red circles (SLN 80, 81, 82) indicate extant *Vitis lanata*: SLN 80,81 (*V. lanata*: A, S.Sasaki 21614, Taiwan), SLN 82 (*V. lanata*: A, R. N. Parner, no num, India). The blue rectangles (SLN 11, 37, 52, 55) indicate the 4 fossil seeds positioned in different clusters of Figure 7. Specimen organization of information is as follow: Herbarium, Voucher specimen, Locality.

Based on the morphological characters of these three morphotaxa, we chose three extant *Vitis* species (*V. thunbergii, V. lanata*, and *V. labrusca*) that separately show closest morphological characters (seed size and shape, chalaza position and shape, chalaza grooves, beak shape, ventral infolds position, length, etc.) with the three morphotaxa to perform the second hierarchical cluster analysis using modern and fossil specimens together. Besides four fossil seeds (SLN 11, 37, 52, 55), the three clusters resulted from the dendrogram of the first step (Figure 7) are also distinct separated on this dendrogram (Figure 8). The specimens of the three extant *Vitis* species are also separately distributed into the three clusters that are coincident with the morphological observation: *Vitis thunbergii* with Cluster 1; *V. lanata* with Cluster 2; *V. labrusca* with Cluster 3.

Canonical Discriminant Analysis

In this step canonical discrimimant analysis were performed to find linear combinations of characters that best summarize the differences among the three morphotaxa and calculate probabilities of misclassification in each morphotaxon. The Box's M value test results a p-value >0.05 that meets the condition of discriminant analysis. Canonical discriminant analysis presents two canonical discriminant functions. Function 1 explains 79.8% of variance, and function 2 explains 20.2% of variance. Two discriminant scores for each specimen are also

calculated from those two functions. A plot based on discriminant scores of the 76 fossil seeds was built (Figure 9). Except a few seeds showing transitional distribution, the three morphotaxa recognized from the cluster analysis are separated from the discriminant analysis plot, which supports the three morphotaxa are successfully distinguishable based on the 11 characters (Table 3). Next, discriminant analysis was performed to calculate probabilities of misclassification in each morphotaxon. Its result shows 93.4% of specimens were originally classified correctly. According to the classification result, two seeds (SLN 68, 76) of the morphotaxon 1 from the cluster analysis are classified into predicted group 2 (groups described in the discriminant analysis are equal to clusters indicated by the cluster analysis), and three seeds (SLN 4, 37, 55) of the morphotaxon 1 from the cluster analysis are classified into predicted group 3. Probabilities of these five misclassified specimens being placed in the predicted groups and original groups are listed (Table 9). After further checking morphological characters of these five seeds, we followed their position in the dendrogram (Figure 7) of cluster analysis.

Table 9 The Five Misclassified Specimens Indicated by the Canonical Discriminant Analysis. Percentages of each specimen in the highest group (predicted group) and the second highest group (original group) are listed.

Specimen label number (SLN)	Original group	Highest group		Second highest group	
		Predicted group	%	Group	%
4	2	3’	0.517	2	0.423
37	2	3’	0.679	2	0.319
55	2	3’	0.462	2	0.459
68	1	2’	0.556	1	0.437
76	1	2’	0.790	1	0.210

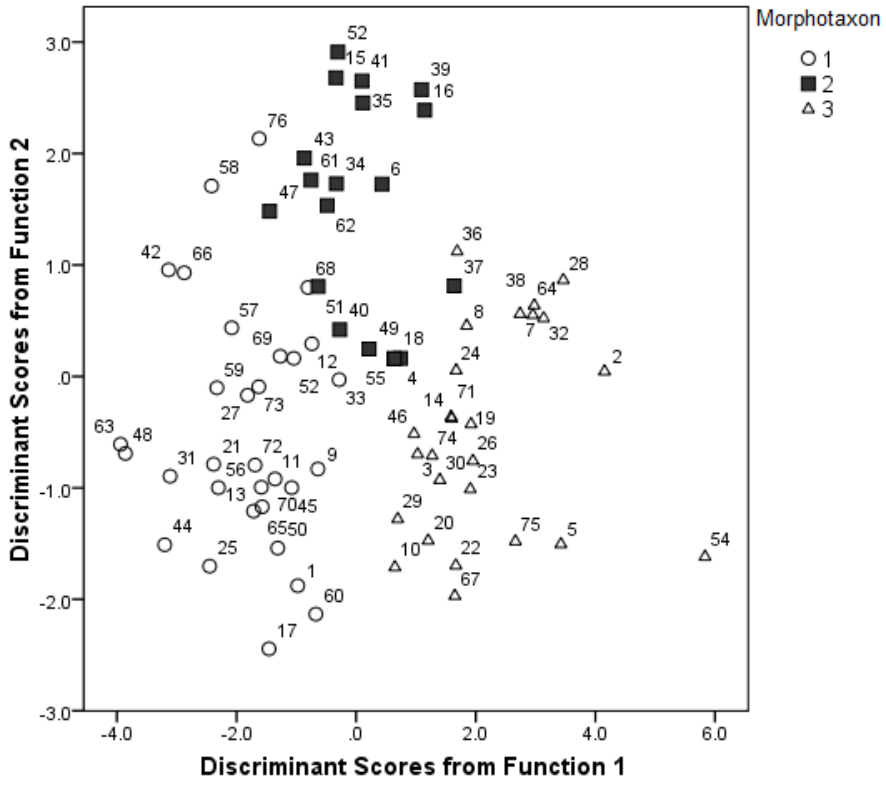

Figure 9 Score Plot of the Two Canonical Discriminant Functions of Discriminant Analysis. Specimen label number (SLN) of each specimen as in Figure 7 is shown.

Descriptive Statistics and Independent Sample t-test

Descriptive statistics of all 11 characters were calculated separately for those 3 clusters (Table 10, Figure 10). Then independent sample t-test for equality of means was performed to test the difference of the 11 characters for each cluster pair (Table 11). During the t-test, Levene's Test for equality of variances was used to examine the conditions of t-test. Based on the t-test result, 9 characters are different significantly between cluster 1 and 2, and all 11 characters are different significantly between cluster 1 and 3 at the level $p<0.05$, which means that cluster 1 is clearly distinguishable from the other two clusters. Six characters from measurements of seed size and ventral infolds show significant difference between clusters 2 and

3 at the level $p<0.05$, while the chalaza size and beak width are indistinguishable between these two clusters. However, considering the previous analysis and morphological characters, I still can identify these two clusters as distinct morphotaxa.

Table 10 Descriptive Statistics of the Eleven Characters and Six Ratios for the Three Clusters from Hierarchical Cluster Analysis.

Character	Cluster1 (N=31)				Cluster 2 (N=19)				Cluster 3 (N=26)			
	Min	Max	Mean	S.D.	Min	Max	Mean	S.D.	Min	Max	Mean	S.D.
M1	3.390	4.580	3.992	0.337	3.904	5.187	4.363	0.315	4.534	5.700	5.082	0.361
M2	2.916	4.059	3.489	0.318	3.320	4.083	3.739	0.212	4.020	5.288	4.389	0.318
M3	2.317	3.686	3.030	0.307	2.729	3.896	3.470	0.287	2.869	4.324	3.484	0.297
M4	0.600	1.065	0.774	0.101	0.614	1.252	0.882	0.172	0.607	1.222	0.979	0.153
M5	0.828	1.314	1.069	0.126	0.980	1.447	1.208	0.129	1.012	1.493	1.215	0.135
M6	0.484	0.817	0.619	0.101	0.669	1.081	0.801	0.092	0.584	1.089	0.806	0.112
M7	1.703	2.729	2.173	0.264	1.905	2.667	2.300	0.221	2.193	3.180	2.633	0.224
M8	1.262	2.224	1.719	0.204	1.206	2.115	1.710	0.208	1.774	2.520	2.095	0.178
M9	0.801	1.525	1.158	0.186	1.140	1.956	1.481	0.209	0.927	1.828	1.337	0.240
M10	0.482	0.887	0.669	0.105	0.669	1.353	0.878	0.170	0.436	1.089	0.781	0.157
M11	0.902	1.602	1.213	0.175	1.081	1.859	1.455	0.201	1.213	2.038	1.617	0.209
R1(M3/M1)	0.567	0.995	0.764	0.095	0.650	0.981	0.798	0.079	0.578	0.783	0.687	0.051
R2(M5/M6)	0.425	0.807	0.583	0.094	0.498	0.902	0.673	0.123	0.472	0.829	0.667	0.087
R3(M7/M2)	0.518	0.820	0.624	0.069	0.512	0.724	0.616	0.055	0.480	0.737	0.602	0.055
R4(M10/M9)	0.381	0.981	0.590	0.127	0.441	0.866	0.597	0.105	0.428	0.764	0.585	0.073
R5(M11/M2)	0.264	0.429	0.348	0.040	0.293	0.547	0.390	0.061	0.286	0.474	0.368	0.040
R6(M8/M2)	0.418	0.576	0.493	0.044	0.355	0.542	0.456	0.416	0.430	0.551	0.478	0.339

Table 11 Independent Sample t-test for Equality of Means between Each Cluster Pair. The bold numbers show significant difference between cluster pairs at $p<0.05$.

	Cluster 1-2		Cluster 1-3		Cluster 2-3	
Variables	t	p-value	t	p-value	t	p-value
M1	-3.940	**0.000**	-11.776	**0.000**	6.961	**0.000**
M2	-3.029	**0.004**	-10.631	**0.000**	7.723	**0.000**
M3	-5.033	**0.000**	-5.633	**0.000**	0.157	0.876
M4	-2.492	**0.019**	-5.835	**0.000**	1.995	0.052
M5	-3.782	**0.000**	-4.248	**0.000**	0.174	0.862
M6	-6.406	**0.000**	-6.635	**0.000**	0.156	0.877
M7	-1.756	0.085	-7.072	**0.000**	4.963	**0.000**
M8	0.156	0.877	-7.345	**0.002**	6.676	**0.000**
M9	-5.677	**0.000**	-3.170	**0.003**	-2.086	**0.043**
M10	-4.848	**0.000**	-3.101	**0.000**	-1.986	0.053
M11	-4.481	**0.000**	-7.392	**0.001**	2.610	**0.012**

Lastly, the morphological characters of the three clusters, which could be reflected by relative morphometric characters used in this study, were compared (Table 12). Based on my analysis, the three morphotaxa are characterized both quantitatively (Table 10) and qualitatively (Table 12).

Table 12 Comparison of the Morphological Characters of the Three Clusters Based on Relative Morphometric Characters Used in This Study.

Characters	Relative morphometric characters	Cluster1 (morphotaxon 1)	Cluster2 (morphotaxon 2)	Cluster 3 (morphotaxon 3)
Seed size	M1, M2, M3	Small	Medium	Big
Seed shape	R1 (=M3/M1)	Narrow	Close to round	Narrow
Beak	M4	Narrow	Medium	Broad
Chalaza size	M5, M6	Small	Big	Big
Chalaza shape	R2 (=M6/M5)	Narrow	Nearly round	Nearly round
Chalaza position	R3 (=M7/M2)	Center of dorsal face	Center of dorsal face	Center of dorsal face
Ventral infolds length	M8, R6 (=M8/M2)	About 2/5-3/5 seed length	About 1/3-1/2 seed length	About 2/5-1/2 seed length
Ventral infolds position	R5 (=M11/M2)	About 1/3-2/5 to seed apex	About 1/3-1/2 to seed apex	About 1/3-2/5 to seed apex
Ventral infolds shape	R4 (=M10/M9)	Broaden toward seed apex	Broaden toward seed apex	Broaden toward seed apex
Raphe ridge width	M9, M10	Narrow	Medium	Broad

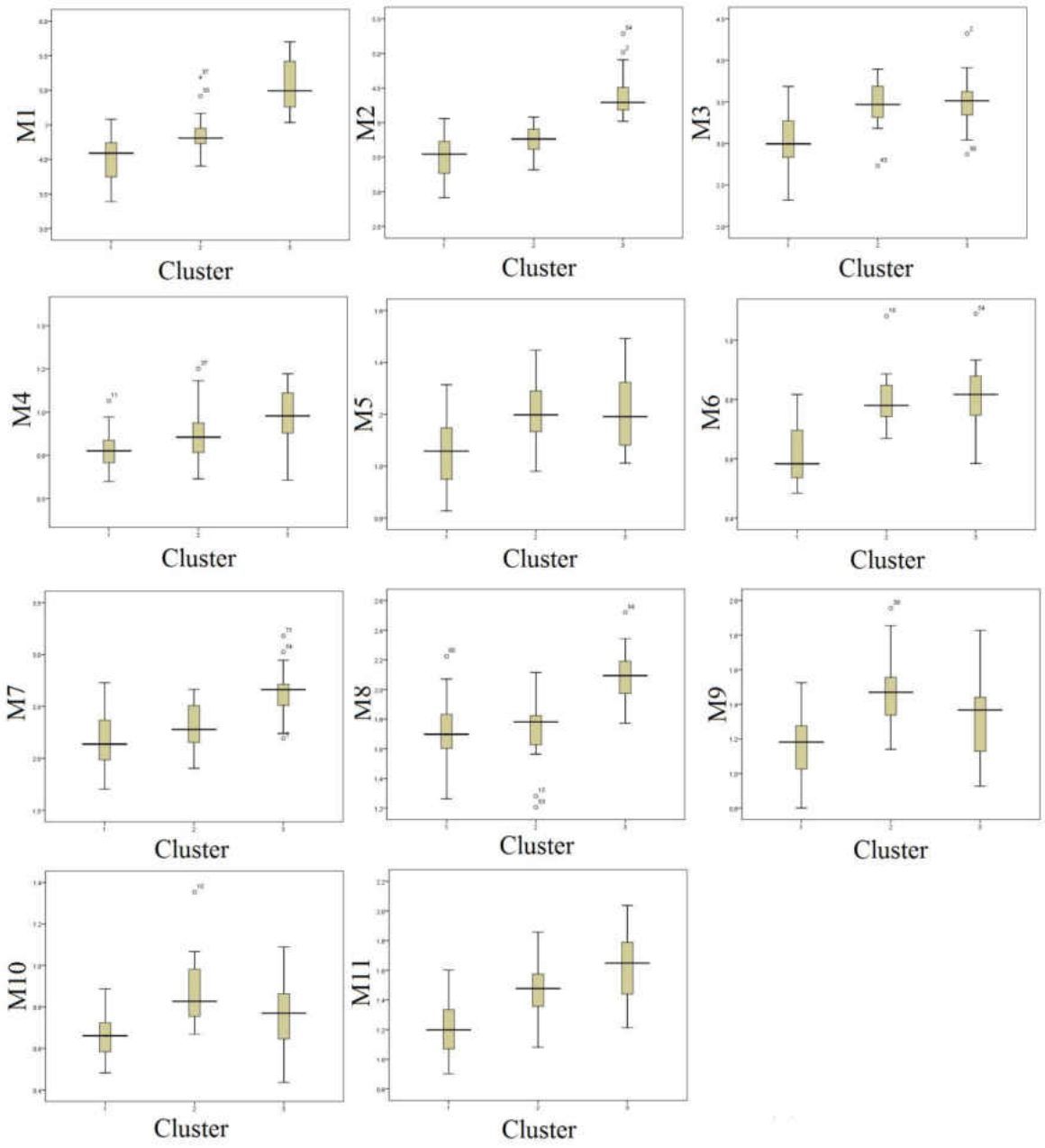

Figure 10 Boxplots Showing Variation in the Eleven Characters (Table 1) Based on the Three Clusters (Morphotaxa) from the Dendragram of Hierarchical Cluster Analysis (Figure 7).

Systematic Description

Based on my survey of seed morphology at the general level based on 95 extant species representing 9 genera of the Vitaceae from HUH and MO (Table 4) with additional information from published studies on extant vitaceous seed morphology (Tiffney and Barghoorn 1976; Chen and Manchester 2007), I conclude that the fossil vitaceous seeds from the Gray Fossil Site that are characterized by the combination of the central positioned chalaza on dorsal surface, obvious visible chalaza-apex groove, and short linear ventral infolds correspond to the genus *Vitis*. On the basis of the smooth surface of these fossil seeds, I further place them in the subgenus *Vitis*. Seeds of another subgenus *Muscadinia* show furrowed to striated dorsal surface (Tiffney and Baghoon 1976). Furthermore, morphometric study identifies three morphotaxa (Figure 7). Here, I considere them as three new *Vitis* species.

Order: Vitales Burnett

Family: Vitaceae Jussieu

Genus: *Vitis* Linnaeus

Subgenus: *Vitis* Planchon

Taxonomic key to the fossil seed of the three *Vitis* species from the Gray Fossil Site:

1a. Seed size >5x4mm, chalaza pyriform to spatulate, chalaza-apex groove broad and deep……..... *Vitis latisulcata* sp. nov.

1b. Seed size <5x4mm, chalaza round, elongate to elliptical, chalaza-apex groove narrow and shallow to deep……..……... 2

2a. Seed shape outline subglobose, chalaza round……...….. *Vitis lanatoides* sp. nov.

2b. Seed shape outline on dorsal and ventral views obovoid, chalaza elongate to elliptical……..…… *Vitis grayana* sp. nov.

Species: *Vitis grayana* Gong et Liu, sp. nov. (Figure 11 A-H)

Specific diagnosis (The following are specific charactertics of *Vitis grayana*): Seed shape outline on dorsal and ventral views obovoid; surface smooth; beak trapezoidal, outline on dorsal and ventral views of the beak continuing the general outline of the seed; chalaza narrow elongate to elliptical, centrally positioned on the dorsal face; chalaza-apex groove narrow, obviously visible; chalaza-base groove narrow, slightly visiable to faint; ventral infolds linear, straight, short, about 2/5-3/5 seed length, apically divergent; raphe ridge narrow.

Description:

The seed shape outline on both dorsal and ventral views is obovoid. Seed surface is smooth. The mean length of the 31 complete specimens is 3.99mm (range 3.39-4.58mm), while the mean width is 3.03mm (range 2.32-3.67mm). The outline on dorsal and ventral views of the obviously trapezoidal-shape beak continues the outline of the seed. The narrow elongate to elliptical chalaza is centrally positioned on the dorsal face and slightly or not concave to the seed surface. The narrow and shallow chalaza-apex groove is obviously visible forming a shallow to deep apical notch in its passage to the ventral face. Some specimens maintain a raphe in the chalaza-apex groove extending from the chalaza apex to the apical notch. The narrow chalaza-base groove is slightly visible to faint. The linear, straight ventral infolds are short and about 2/5-3/5 length of the seed extending to the apical 1/3-2/5 of seed and slightly or noticeably diverging apically. The shallow infold cavities show a clear boundary from the raphe ridge and a faint

boundary from the ventral surface. The narrow raphe ridge rises slightly from the ventral surface and slightly or markedly narrows towards the seed base.

Holotype: ETMNH-8144 (Figure11, A- B).

Paratypes: ETMNH-8089 (Figure 11, C- D); ETMNH-8115 (Figure 11, E-F); ETMNH-8122 (Figure 11, G-H).

Etymology: The specific epithet *grayana* refers to the Gray Fossil Site where specimens were collected.

Type locality: The Gray Fossil Site, Washington County, northeastern Tennessee, USA (36.58°N, 82.58°W).

Horizon: Near the top layer of the laminated facies.

Age: Late Hemphillian (7-4.5 Ma, latest Miocene to earliest Pliocene).

Material: ETMNH-8073, ETMNH-8081, ETMNH-8083, ETMNH-8084, ETMNH-8085, ETMNH-8089, ETMNH-8093, ETMNH-8097, ETMNH-8099, ETMNH-8103, ETMNH-8105, ETMNH-8114, ETMNH-8116, ETMNH-8117, ETMNH-8120, ETMNH-8122, ETMNH-8124, ETMNH-8128, ETMNH-8129, ETMNH-8130, ETMNH-8131, ETMNH-8132, ETMNH-8135, ETMNH-8137, ETMNH-8138, ETMNH-8140, ETMNH-8141, ETMNH-8142, ETMNH-8144, ETMNH-8145, ETMNH-8148.

Comparison:

This present species is characterized by the obovoid seed shape outline at both the dorsal and ventral views and narrow elongate to elliptical chalaza. The seed size, outline views, narrow

chalaza shape, obvious narrow chalaza-apex groove, and slightly visible to faint chalaza-base groove are closely comparable to two modern species *Vitis balanseana* and *V. thunbergii*. Differences exist in the triangular beak shape of *V. thunbergii* and cylindrical beak shape of *V. balanseana* and much deep infold cavities of these two modern species that show clear boundaries between infold cavities and ventral surface. Both of these two modern species show deep infold cavities that differ from *V. grayana*; however, considering the taphonomy, one would not suggest the depth of infold cavities as important characters to identify vitaceous seeds. The outline on dorsal and ventral views of the triangular beak of *V. thunbergii* continues the general outline of the seed, while the prominent cylindrical beak of *V. balanseana* shows clear boundary with the seed body on ventral face-view, which implies that the beak shapes should be useful characters to identity species of *Vitis*. Considering the relationship between the outline of the beak and the seed body and that the trapezoidal beak of our fossil species might be formed from one triangular beak destroyed during the fossilization, *Vitis grayana* could be most similar to the modern species *V. thunbergii* than *V. balanseana*.

Vitis thunbergii is currently distributed in warm to temperate regions of East Asia (Chen et al. 2007). Its fossil seeds were reported from the late Neogene in Japan (Miki 1956, p.265, fig. 15) and the Pliocene of France (Reid 1923, pp.338-339, plate 11, figs.3-4). Furthermore, one fossil species *V. teutonica* (Czeczott 1959, p.102, plate 16, figs.3, 6-7) also shows similar features with *V. grayana* except for the wedge-shaped basis of this European fossil species. All this evidence suggests that this kind of *V. thunbergii*-like fossil species possessed a wide distribution in the Neogene of North Hemisphere.

Species: *Vitis lanatoides* Gong et. Liu sp. nov. (Figure11 I-L; Figure12 A-D)

Specific diagnosis (The following are specific charactertics of *Vitis lanatoides*): Seed shape outline on both dorsal and ventral views round; surface smooth; beak cylindrical, prominent; chalaza round, positioned centrally on the dorsal face; chalaza-apex grooves narrow, shallow; chalaza-base groove narrow slightly visible to faint; apical notch not distinct; ventral infolds straight, short, about 1/3-1/2 length of the seed, divergent apically; ventral infold cavities narrow, deep, with clear boundaries from the ventral surface.

Description:

The seed shape outline on both dorsal and ventral views is round. Seed surface is smooth. Some seeds have subglobose shape, while the other are flattened to a certain extent. The mean length of the 19 complete seeds is 4.36mm (range 3.9-5.19mm). The mean width is 3.47mm (range 2.73-3.9mm). The cylindrical beak is prominent from the seed base. The round chalaza is positioned centrally on the dorsal face and slightly concave to the seed surface. A few seeds possess chalaza much closer to the seed apex. The narrow chalaza-apex groove is shallow, linear, and slightly to obviously visible. The narrow chalaza-base groove is faintly visible. The apical notch is not distinct. The straight narrow linear ventral infolds are short and about 1/3-1/2 of the seed length extending to the apical 1/3-1/2 of seed and diverging apically. The ventral infold cavities are deep with clear boundaries from the ventral surface. The raphe ridge faintly or slightly rises from the ventral surface and narrows towards the seed base.

Holotype: ETMNH-8088 (Figure 11, I- J).

Paratypes: ETMNH-8111 (Figure 11, K- L), ETMNH-8113 (Figure 12, A- B), ETMNH-8121 (Figure 11, C-D).

Etymology: The specific epithet *lanatoides* refers to a close resemblance of this fossil seed to seeds of the extant *V. lanata* Roxburgh.

Type locality: The Gray Fossil Site, Washington County, northeastern Tennessee, USA (36.58°N, 82.58°W).

Horizon: Near the top layer of the laminated facies.

Age: Late Hemphillian (7-4.5Ma, latest Miocene to Earliest Pliocene).

Material: ETMNH-8076, ETMNH-8078, ETMNH-8087, ETMNH-8088, ETMNH-8090, ETMNH-8106, ETMNH-8107, ETMNH-8109, ETMNH-8111, ETMNH-8112, ETMNH-8113, ETMNH-8115, ETMNH-8119, ETMNH-8121, ETMNH-8123, ETMNH-8125, ETMNH-8127, ETMNH-8133, ETMNH-8134.

Comparison:

This present species is distinguished by the subglobose seed shape and the round chalaza. Some modern and fossil species from *Parthenocissus* and *Ampelopsis*, e.g. *P. angustisucata* (Scott 1954, p.81, plate 16, fig. 14; Manchester 1994, p.95, plate 45, figs. 6-7), *A. rooseae* (Manchester 1994, p.94, plate 44, figs. 6-10), *A. rotundata* (Reid and Chandler 1933, p.386, plate 19, figs. 13-17), and *A. crenulata* (Reid and Chandler 1933, p.385, plate 19, figs. 11-12) are also subglobose in shape. However, *Parthenocissus* can be distinguished by its long ventral infolds extending from the base to apex of seed, and *Ampelopsis* can be distinguished by the lack of a chalaza-apex groove, which excludes my current fossil species from those two genera. Among the species of *Vitis* subglobose shape with a prominent cylindrical beak, dorsal centrally positioned round chalaza, and short apical divergent ventral infolds of *V. lanatoides* are

essentially the same as in the modern species *V. lanata*, which is distributed in the subtropical regions of East to South Asia (Chen et al. 2007). That species differs in the much wider raphe ridge and broader infold cavities than in the fossil species. Chandler (1962) described that one fossil species *V. glabra* from the lower Tertiary floras of southern England (Chandler 1962, p.103, plate 14, figs.49-53) and compared it with the extant *V. lanata*. Judging from the seed shape and chalaza of *V. glabra* illustrated by Chandler (1962), we tend to believe that *V. glabra* is more comparable with another extant *V. labrusca* than *V. lanata*.

One fossil species *Vitis tiffneyi* (Manchester 1994) from the Nut beds, Clarno Formation, Oregon, also shows similar characters with *V. lanatoides* including subglobose shape, round central chalaza, and short straight ventral infolds. But the dorsal surface of *V. tiffneyi* is concave on the position of chalaza and chalaza grooves. In addition, other characters of *V. tiffneyi* such as the parallel ventral infolds, much narrower raphe ridge, and obvious groove on surface of raphe ridge are also different from *V. lanatoides*. Another fossil species possessing subglobose shape and similar size with *V. lanatoides* is *V. subglobosa* from London Clay (Reid and Chandler 1933, p.379, plate 18, figs.34-37; Chandler 1961 p.245, plate 24, figs.14-17). Tiffney and Barghoorn (1976) concluded that some vitaceous fossil species with short and wide deep ventral infolds including *V. rostrata*, (Tiffney and Barghoorn 1976), *V. subglobosa*, *Ampelopsis crenulata*, *A. rotundata* (Reid and Chandler 1933; Chandler 1961), *V. platyformis*, *V. rectisulcata*, *Palaeovitis paradoxa*, and *V. obovoidea* (Chandler 1960) should be considered as an unrecognized modern form or an extinct lineage in the genus *Vitis*. *Vitis lanatoides* possess similar ventral infolds characters with those fossil species, but infold cavities are narrower. It should be considered another member of this fossil group.

Species: *Vitis latisulcata* Gong et. Liu sp. nov. (Figure12 E- L)

Specific diagnosis (The following are specific charactertics of *Vitis latisulcata*): Seed shape outline on both dorsal and ventral views ovate-elliptical to rectangular; surface smooth; beak cylindrical, extremely prominent from the seed base; chalaza pyriform to spatulate; chalaza-apex groove broad and deep; chalaza-base groove broad, slightly visible to faint; ventral infolds linear, straight to slightly curved, short, about 2/5-1/2 seed length, apically divergent; infold cavities broad, shallow; apical notch deep, forming a "V-shape" groove at top of the raphe ridge.

Description:

The seed shape outline on both dorsal and ventral views of this species is ovate-elliptical to rectangular. The surface of the seed is smooth. The mean length of 26 complete seeds is 5.08mm (range 4.53-5.7mm) and the mean width is 3.48mm (range 2.87-4.32mm). A cylindrical beak projects from the seed base and shows clear boundary with the seed body. Some seeds possess beak with a flared tip. The pyriform to spatulate chalaza is positioned centrally on the dorsal face. In some seeds, the chalaza was lost to form a hole on the center of chalaza position. The broad deep chalaza-apex groove obviously extends from the chalaza apex to the seed apex and then forms the obvious deep apical notch that extends to the ventral face and forms a narrow "V-shape" groove at top of the raphe ridge. The broad chalaza-base groove is slightly visible to faint. The straight or slightly curved ventral infolds are short and about 2/5-1/2 of the seed length extending to the apical1/3-2/5 of seed and diverging apically. The shallow infold cavities are broad linear on face-view, with clear to faint boundaries from the ventral surface. The raphe ridge slightly rises from the ventral surface and narrows towards the seed base.

Holotype: ETMNH-8079 (Figure 12, E-F)

Paratypes: ETMNH-8074 (Figure 12, G-H), ETMNH-8077 (Figure 12, I-J), ETMNH-8100 (Figure 12, K-L).

Etymology: The specific epithet *latisulcata* refers to the broad chalaza grooves of this species.

Type locality: The Gray Fossil Site, Washington County, northeastern Tennessee, USA (36.58°N, 82.58°W).

Horizon: Near the top layer of the laminated facies.

Age: Late Hemphillian (7-4.5 Ma, latest Miocene to Earliest Pliocene).

Material: ETMNH-8074, ETMNH-8075, ETMNH-8077, ETMNH-8079, ETMNH-8080, ETMNH-8082, ETMNH-8086, ETMNH-8091, ETMNH-8092, ETMNH-8094, ETMNH-8095, ETMNH-8096, ETMNH-8098, ETMNH-8100, ETMNH-8101, ETMNH-8102, ETMNH-8104, ETMNH-8108, ETMNH-8110, ETMNH-8118, ETMNH-8126, ETMNH-8136, ETMNH-8139, ETMNH-8143, ETMNH-8145, ETMNH-8147.

Comparison:

The large size of *Vitis latisulcata*, the pyriform to spatulate chalaza shape, the deep broad chalaza-apex groove, and the "V-shape" groove at top of the raphe ridge distinguish this species from other vitaceous seeds of the Gray Fossil Site. The pyriform to spatulate chalaza, broad deep chalaza-apex groove, and the short apically divergent ventral infolds of this fossil species are closely comparable to two modern North American species *V. candicans* and *V. labrusca*. Another character of *V. latisulcata* similar with that of *V. candicans* is the slightly to obviously

shallow chalaza-base groove that could often not be found in *V. labrusca*. The cylindrical beak and the flared tip on the beak of *V. latisulcata* are very close to the beak of *V. labrusca*, although the latter is somewhat broader than the former. Furthermore, both the beak characters of the fossil *V. latisulcata* and the modern *V. labrusca* are different to the triangular to trapezoidal beak of *V. candicans*. However, both of these two modern species are much bigger in size (about 6 X 4mm) than the present fossil species. This size differences is too great to be an effect of desiccation or tapophomy. The "V-shape" groove at top of the raphe ridge of the fossil *V. latisulcata* is also not distinct in these two modern species. Both *Vitis candicans* and *V. labrusa* have been classified into the same series Labruscae based on many other morphological characters (Moore 1991). The Gray Fossil Site is located in about the southern limit of the present geographic range of *V. labrusca* and close to the eastern limit of the present geographic range of *V. candicans* (Moore 1991). The similar seed characters among these 3 species, taxonomic close relationship between *V. candicans* and *V. labrusca*, and relative geographic ranges of the three species suggest their close relationships.

One fossil species named *Vitis eolabrusca* (Tiffney and Barghoorn 1976, p.179, plate 2, figs. A and C) from the early Miocene Brandon Lignite shares many features with *V. labrusca. Vitis eolabrusca* also shares some characteristics with *V. latisulcata* including these for seed and beak shape, seed size, and ventral infolds features. Differences are mainly in the round chalaza, narrow chalaza-apex groove, and faint chalaza-base groove of *V. eolabrusca*. Miki (1956) described one species from the Miocene and Pliocene of Japan named *V. labruscoidae* (Miki 1956, pp.262-263, fig.12 A-D) that shared some similar features with *V. labrusca*, but that species was suggested to be much closed to *V. coignetiae,* an Asian species, by Tiffney and Barghoorn (1976). In the same paper Miki (1956) described another species named *V. rotundata*

showing a small hole in the central chalaza. But *Vitis rotundata* shows many different characters from our fossil species. The chalaza hole should be excluded as an important character to identify fossil vitaceous seeds because those holes may be formed during fossilization.

On the study of *Vitis eolabrusca*, Tiffney and Barghoorn (1976) listed other fossil species possessing similar characters such as *Vitis cf. silverstris* (Czeczott 1959, p.102, plate 16, figs. 1-2), *V. silvestris* (Szafer 1961, p.72, plate 18, figs. 18-20), *V. glabra* (Chandler 1962, p.103, plate 14, figs.49-53), and *V. tomskiana* (Dorofeev 1963, pp.214-215, plate 38, figs.11-12). All those fossil species show some characters that could be comparable to *V. latisulcata*. Tiffney and Barghoorn (1976) indicated that all these species show similar characters with modern species *V. coignetiae* and *V. labrusca* and then suggested that they would represent the Tertiary parental stock of both *V. coignetiae* and *V. labrusca*. *Vitis latisulcata* presented at a later geological age than *V. eolabrusca*; however, the similar features might suggest the continuation of this lineage.

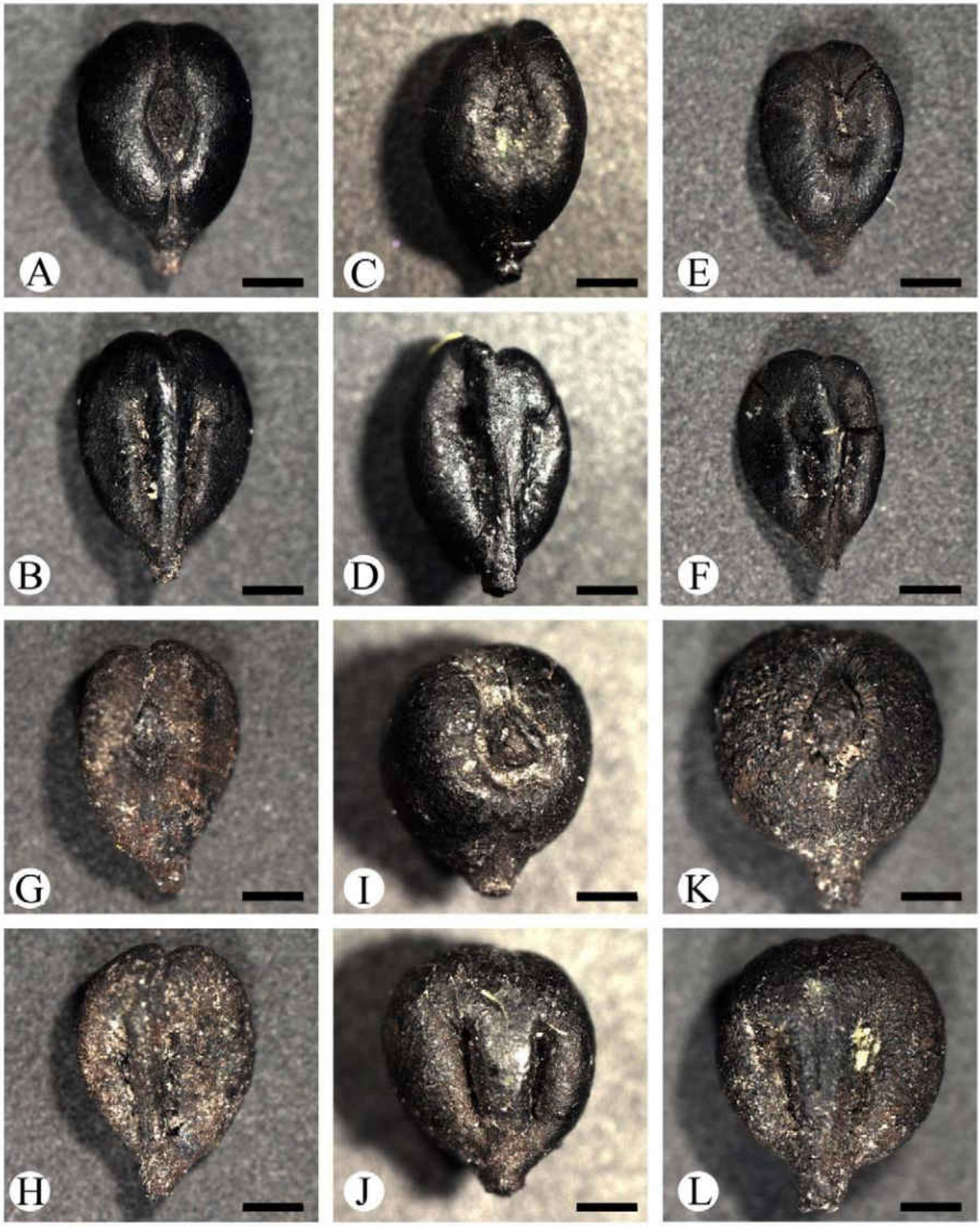

Figure 11 A-H. Fossil seeds of *Vitis grayana* sp. nov., I-L. Fossil seeds of *Vitis lanatoides* sp. nov.

Figure 11 A-H. Fossil seeds of *Vitis grayana* sp. nov., I-L. Fossil seeds of *Vitis lanatoides* sp. nov. Scale bar =1 mm. A. Dorsal view of specimen no. ETMNH-8144. B. Ventral view of specimen no. ETMNH-8144. C. Dorsal view of specimen no. ETMNH-8089. D. Ventral view of specimen no. ETMNH-8089. E. Dorsal view of specimen no. ETMNH-8115. F. Ventral view of specimen no. ETMNH-8115. G. Dorsal view of specimen no. ETMNH-8122. H. Ventral view of specimen no. ETMNH-8122. I. Dorsal view of specimen no. ETMNH-8088. J. Ventral view of specimen no. ETMNH-8088. K. Dorsal view of specimen no. ETMNH-8111. L. Ventral view of specimen no. ETMNH-8111.

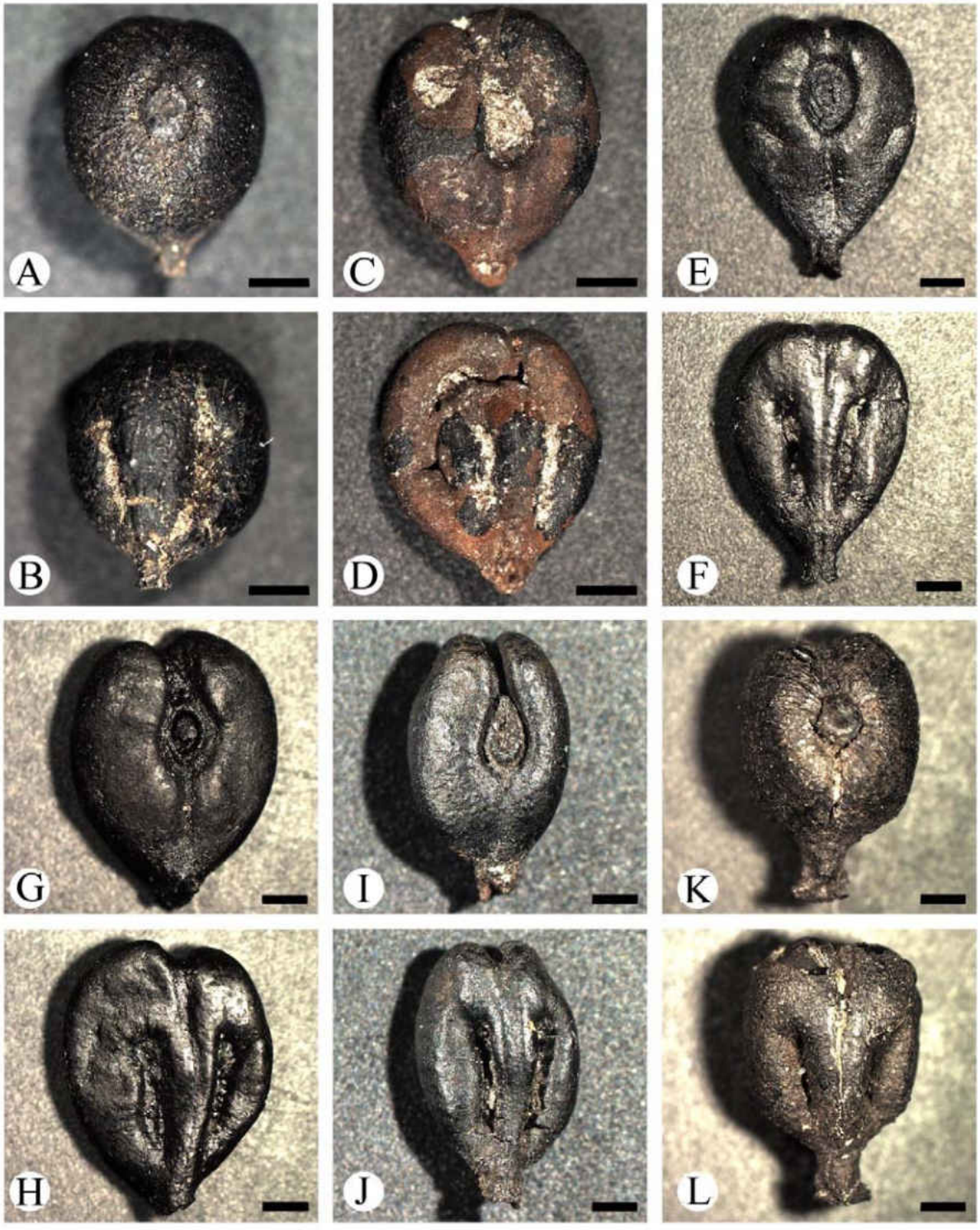

Figure 12 A-D. Fossil seeds of *Vitis lanatoides* sp. nov., E-L. Fossil seeds of *Vitis latisulcata* sp. nov.

Figure 12 A-D. Fossil seeds of *Vitis lanatoides* sp. nov., E-L. Fossil seeds of *Vitis latisulcata* sp. nov. Scale bar =1 mm. A. Dorsal view of specimen no. ETMNH-8113. B. Ventral view of specimen no. ETMNH-8113. C. Dorsal view of specimen no. ETMNH-8121. D. Ventral view of specimen no. ETMNH-8121. E. Dorsal view of specimen no. ETMNH-8079. F. Ventral view of specimen no. ETMNH-8079. G. Dorsal view of specimen no. ETMNH-8074. H. Ventral view of specimen no. ETMNH-8074. I. Dorsal view of specimen no. ETMNH-8077. J. Ventral view of specimen no. ETMNH-8077. K. Dorsal view of specimen no. ETMNH-8100. L. Ventral view of specimen no. ETMNH-8100.

CHAPTER 4

DISCUSSION

Morphometrics on *Vitis* Seed Study

To avoid the subjective error from the morphological observation and improve the classification for these fossil seeds, I chose 11 size characters for a morphometric analysis that could help me to study the seed morphological variance with objective statistical method. The characters that were chosen for this analysis mainly focus on the dimensions of seed body, beak, chalaza, and ventral infolds, all of which are important characters in the study of fossil vitaceous seeds (Tiffney and Barghoorn 1976; Chen and Manchester 2007). According to the study of the relationships among these characters, the correlation analysis (Table 6) shows that several variables are highly correlated with each other, and PCA results distinguish the most important variables among them (Table 7).

In the specimen grouping study both the large rescaled distance gap between 10 and 15 among the three clusters in the dendrogram of hierarchical cluster analysis (Figure 7; Figure 8) and the separation of majority of fossil seeds from the three clusters in the discriminant plot (Figure 9) support conclusion that three *Vitis* morphotaxa exist in the Gray fossil flora. Furthermore, the diagnostic differences among the three species investigated in the systematic description also support this conclusion. However, the discriminant analysis show that five specimens were misclassified (Table 9). Three of the five specimens (SLN 4, 55, 68) possess close probabilities in their predicted groups and the original groups, which indicate that these seeds might be transitional forms. After further checking their morphological characters, the first two specimens (SLN 4 and 55) follow the diagnostic characters of their original group *V.*

lanatoides, and SLN 68 specimen follows the diagnostic characters of its original group *V. grayana*, so the classification of these 3 speciemens follows the results of the cluster dendrogram (Figure 7). With the exception of these three specimens, the other two (SLN 37 and 76) show much higher probabilities in predicted groups than in original groups. The morphology of the SLN 37 specimen corresponds extremely closely to its original group *V. lanatoides*, and it is probable that its much bigger size, which lies in the size range of its predicted group *V. latisulcata*, caused its misclassification. Considering its morphological characters, I still maintain its position in the dendrogram and consider it as *V. lanatoides*. Some characters of the SLN 76 specimen such as the subglobose shape, round chalaza, and cylindrical beak are much closer to its predicted groups *V. lanatoides* and this is coincident with its high probability of placement in *V. lanatoides* in the discriminant analysis. However, characters of its ventral infold accord with its original group *V. grayana* in the dendrogram. This seed displays distortion to a certain extent, which might lead to the conflict in its classified position. Here, I still consider the result of the cluster analysis correct and consider this specimen as one seed of *V. grayana*.

Most of the characters included in the descriptive statistics are listed in the comparsion among the three species (Table 12), and these characters are all important for studies of vitaceous seed morphology in the same genus. On the other hand, the quantitative results of chalaza position and the ventral infold length relative to seed length appear similar for the three species, which should suggests that they are diagnostic characters for the genus *Vitis*.

Lastly, some characters that are also used as important characters in the study of fossil vitaceous seeds but could not be measured directly and were excluded from morphometrics present little intraspecific variation. Those characters include seed shape, beak shape, chalaza shape, and ventral infold shape among others. Although seed shape shows some variation among

Vitis latisulcata (such as ETMNH-8079 and ETMHN-8100, Figure 12, E-F, J-L), the chalaza, chalaza-apex groove, apical notch, and ventral infolds show consistent characters, which suggests that *Vitis latisulcata* is a reliable and well marked species. Considering this result and the morphological study of extant *Vitis* species (Table5, Figure 6), I concluded that the seed shape is less important than the chalaza and ventral infold characters to identify vitaceous seeds.

Ecological Consideration

Considering the fossil *Vitis* species would have similar ecological habits with their nearest living relatives, one can make some inferences on the ecology of the Gray Fossil Site based on the three extant *Vitis* species similar with the fossil *Vitis* species discovered from the site. One of the fossil *Vitis* species from the Gray Fossil Site, *V. latisulcata,* shows close seed morphological similarity with one extant North American species *V. labrusca*. The Gray Fossil Site is located near the southern limit of the present geographic range of *V. labrusca* (Moore 1991). *Vitis labrusca* inhabits a very wide variety of sites: both upland, well drained areas and lowland, poorly drained areas including intermittently flooded bottomland (Moore 1991). This does not exclude the previous interpretation of the Gray Fossil Site as a lacustrine environment; however, the wild variation of the habitat and environment of *V. labrusca* precludes any clear interpretation of ecological environment of the Gray Fossil Site area in latest Miocene-earliest Pliocene. The other two fossil *Vitis* species from the Gray Fossil Site also show resemblance to two modern Asian *Vitis* species: *V. thunbergii* and *V. lanata*. *Vitis thunbergii* inhabits in forests, shrublands, and hillsides (elevation 100-1300m) of east China, extending to Japan, while *Vitis lanata* inhabits in forests, shrublands, hillsides, valleys (elevation 100-3200m) of East and South Asia (southeastern China, Taiwan, Bhutan, India, Nepal) (Chen et al. 2007). According to the habitats of these two Asian Vitis species, the ecological environment may be consistent with

forests, shrublands, and hillsides. The areas of cooccurrence of *V. thunbergii* and *V. lanata* lie in the warm-temperate zone to subtropics of southeastern China that are located in a lower latitude than the Gray Fossil Site. The alliance of parts of *Vitis* species from the Gray Fossil Site with these two extant *Vitis* species of eastern Asia support to the previous suggestion that the climate of the Gray Fossil Site was warm-temperature during the Late Neogeone (Shunk et al. 2006; Liu and Zavada 2009).

Phytogeographical Significance

The seed fossil record of Vitaceae appears to start in the late Paleocene and is diverse and widespread by the early Eocene (Chen and Manchester 2007). Among the fossil vitaceous seeds, the commonest species and the highest number of seeds belong to *Vitis*. The Paleogene *Vitis* seed records show a wide distribution in both North America (e.g. Manchester 1994; Manchester McIntosh 2007) and Europe to West Siberia (e.g. Reid and Chandler 1933; Chandler 1957, 1960, 1961, 1962, 1963, 1964; Dorofeev 1957, 1963). Chen and Manchester (2007) mentioned that the North Atlanta Land Bridges should play the key role for expanding of *Vitis* and other genera of Vitaceae between these two continents. In the Neogene, while fossil *Vitis* seeds were still commonly uncovered from floras of North America (e.g. Tiffney and Barghoorn 1976; Tiffney 1979) and Europe (e.g. Reid 1923; Czeczott et al. 1959; Dorofeev 1957, 1963; Fairon-Domaret and Smith 2002), the fossil *Vitis* seeds were also described from the Miocene and Pliocene floras of eastern Asia (Miki 1956) indicating that the distribution of *Vitis* expanded to the whole North Hemisphere during the Neogene. The absence of Paleogene *Vitis* records in eastern Asia may have resulted from either the lacking of fossil *Vitis* records in Paleogene floras of this area or the later occurrence of *Vitis* in eastern Asia than in North America and Europe. However, lacking evidence from phylogenetic analysis based on molecular data, it would be biased to deduce the

evolution of *Vitis* only according to its fossil records. Nowadays, the *Vitis* is still commonly distributed in North America and East to South Asia and dispalys a disjunct distribution between these two continents (Chen and Manchester 2007); however, it has no wild records from Europe (Webb 1968; Punt et al. 2003). The distribution change of *Vitis* in present Europe could be the result of the climatic changes of the Pleiostocene (Manchester 1999; Wen 1999).

Among the three fossil *Vitis* species from the Gray Fossil Site, only *V. latisulcata* shows much close morphological characters with the local species *V. labrusca* in North America. *Vitis grayana* shows a close relationship with one modern and late Neogene species *V. thunbergii* (Miki 1956) from East Asia and two Pliocene species *V. thunbergii* and *V. teutonica* from Europe (Reid 1923; Cezeczott et al. 1959). *Vitis lanatoides* shows similar characters to one East to South Asian species, *V. lanata*. Although present day *Vitis* show a disjunct distribution between eastern Asia and North America, the similarity and close relationships of fossil and modern *Vitis* between eastern Asia and eastern North America has been mentioned by previous studies (Mike 1956; Tiffney and Barghoon 1976). Together with the fossil red panda (Wallace and Wang 2004) and other plant fossils such as *Sinomenium* (Menispermaceae) and *Sargentodoxa* (Lardizabalaceae) (Liu et al. 2007) from the Gray Fossil Site, the *Vitis* species uncovered from the Gray Fossil Site provides further evidence that the eastern Asian floristic elements existing in the southeastern North American flora continued to as late as the late Neogene.

The disjunct distribution between eastern Asia and eastern North America of many flowering plants (about 65 genera) represents one of the most prominent intercontinental disjunctions of closely related species and has been comprehensively studied based on evidence from paleobotany, geology, climate, and molecular phylogenetic analysis (e.g. Tiffney 1985a,

1985b; Tiffney and Manchester 2001; Manchester 1999; Manchester and Tiffney 2001; Wen 1999, 2001; Xiang and Soltis 2001; Xiang et al. 1998, 2000). In general these disjunct plants are interpreted as relicts of the continuous mesosphytic forests in the Northern Hemisphere during the Paleogene and Neogene, with both the North Atlantic and Bering land Bridges involved (Tiffney 1985a, 1985b; Wen 1999) and with the forests subsequently reduced as the climatic and geological changes occurred through the late Neogene and Quaternary (Graham 1972; Wen 1999; Xiang et al. 2000). With the North Atlantic Land Bridges broken in the early Eocene (Tiffney 1985b, 2000; Tiffeny and Manchester 2001), the Bering Land Bridge became the primary connection between eastern Asia and North America in the Neogene until its closure in the late Neogene (about 7.4 to 4.8 Ma) (Tiffney and Manchester 2001). However, the disjucnt plants between these two continents show a complicated array of divergence times. Tiffney (1985b) proposed two major disjunct periods/patterns (Miocene and late Neogene-Quaternary) in the Neogene. Based on molecular data from 11 eastern Asian-eastern North American disjunct species pairs, Xiang et al. (2000) estimated divergence times of these species pairs with a range from less than 0.31 to 12.40 (± 4.30 Ma) coinciding to the mid-Miocene to Quaternary periods. After considering a factor of 1/2 in calculating the divergence times (Li 1997), these results (Xiang et al. 2000) shorten by about half the previously estimated range of divergence times (about 2-25 Ma, early Miocene to Quaternary) reviewed by Wen (1999).

The geological age of the Gray Fossil Site is about latest Miocene to earliest Pliocene(7-4.5 Ma) (Wallace and Wang 2004; Shunk et al. 2006), which falls within or a little earlier than the divergence time ranges of the majority of the disjunct species pairs studied by Xiang et al. (2000). Examples include *Campsis* (Bignoniaceae, 3.62 ± 2.10 Ma), *Caulophyllum* (Berberidaceae, 2.38 ± 2.07 Ma), *Cornus* (Cornaceae, 4.88±2.46 Ma), *Decumaria*

(Hydrangeaceae, 2.38±1.69 Ma), *Liriodendron* (Magnoliaceae, 4.19±2.26Ma), *Mitchella* (Rubiaceae, 5.89±2.38Ma), *Penthorum* (Penthoraceae, 4.88± 2.46Ma), and *Podophyllum* (Berberidaceae, 6.71±3.08Ma). In addition the divergence time of *Symplocarpus* and *Lysichiton* (Araceae, the former 4.49±1.69 or 6.88±4.18 Ma, and the latter 4.02±1.60 or 7.18±4.33 Ma) (Nie et al. 2006a), *Phryma* (Phrymaceae, 3.68±2.25 or 5.23±1.37 Ma) (Nie et al. 2006b), also correspond to the range of the divergence time mentioned above and to the geological time of the Gray Fossil Site. These validated the conclusion that many migrations still occurred between eastern Asia and eastern North America during the latest Miocene and earliest Pliocene via the Bering Land Bridge. This could explain the eastern Asian elements of the Gray fossil flora, and the close resemblance of parts of *Vitis* species from the Gray Fossil Site with extant East Asian *Vitis*.

CHAPTER 5

CONCLUSION

Numerous vitaceous seeds were uncovered from the late Neogene Gray Fossil Site. Based on morphological study, all the vitaceous seeds were identified into the genus *Vitis*, subgenus *Vitis*.

Eleven characters measured from 76 complete fossil seed were chosen for a morphometric study. Two seperate multivariate analyses support the recognition of three different morphotaxa of *Vitis* occurring in the Gray fossil flora.

Ninety-five extant species from 11 genera of Vitaceae were used for comparative morphological study. A systematic study on 41 species of subgenus *Vitis* indicated that *Vitis* seed morphology show same intraspecific consistency, which is important for studies of the fossil *Vitis* seeds at the specific level.

After comparison with modern and fossil vitaceous specimens, the three morphotaxa recognized by the morphometric study were defined into three new taxa: *Vitis grayana* sp. nov., *Vitis lanatoides* sp.nov., and *Vitis latisulcata* sp. nov. A systematic treatment for these three species was presented.

The broad range of the ecological conditions for the modern *Vitis* specimens closed to the Gray fossil *Vitis* species suggest that vegetational conditions at the Gray Fossil Site may be consistent with forest, shrublands, and hillsides. The alliance of two *Vitis* species from the Gray Fossil Site with two extant eastern Asian *Vitis* species supports the previous suggestion that the climate of the Gray Fossil Site was warm-temperature during the Late Neogeone.

The close resemblance between parts of the fossil *Vitis* from the Gray Fossil Site with extant eastern Asian *Vitis* provides further evidence of the eastern Asian floristic elements of the flora from southeastern North America continued to as late as the late Neogene. The dates for the Vitis seeds from the Gray Fossil Site coincide well with established biogeographical time windows for plant migrations between North America, Europe, and Asia.

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APPENDIX

Specimen Label Number and Catalogue Number

The Specimen Label Number (SLN) for each fossil specimen used in this study and the corresponding specimen catalogue number (Catalogue No.) in the East Tennessee State University and General Shale Brick Natural History Museum (ETMNH) are listed.

SLN	Catalogue No.	SLN	Catalogue No.	SLN	Catalogue No.	SLN	Catalogue No.
Vitis grayana nov. sp.		*Vitis grayana* sp. nov. (continued)		*Vitis lanatoides* sp. nov. (continued)		*Vitis latisulcata* sp. nov. (continued)	
1	ETMNH-8073	59	ETMNH-8131	39	ETMNH-8111	20	ETMNH-8092
9	ETMNH-8081	60	ETMNH-8132	40	ETMNH-8112	22	ETMNH-8094
11	ETMNH-8083	63	ETMNH-8135	41	ETMNH-8113	23	ETMNH-8095
12	ETMNH-8084	65	ETMNH-8137	43	ETMNH-8115	24	ETMNH-8096
13	ETMNH-8085	66	ETMNH-8138	47	ETMNH-8119	26	ETMNH-8098
17	ETMNH-8089	68	ETMNH-8140	49	ETMNH-8121	28	ETMNH-8100
21	ETMNH-8093	69	ETMNH-8141	51	ETMNH-8123	29	ETMNH-8101
25	ETMNH-8097	70	ETMNH-8142	53	ETMNH-8125	30	ETMNH-8102
27	ETMNH-8099	72	ETMNH-8144	55	ETMNH-8127	32	ETMNH-8104
31	ETMNH-8103	73	ETMNH-8145	61	ETMNH-8133	36	ETMNH-8108
33	ETMNH-8105	76	ETMNH-8148	62	ETMNH-8134	38	ETMNH-8110
42	ETMNH-8114	*Vitis lanatoides* sp. nov.		*Vitis latisulcata* sp. nov.		46	ETMNH-8118
44	ETMNH-8116	4	ETMNH-8076	2	ETMNH-8074	54	ETMNH-8126
45	ETMNH-8117	6	ETMNH-8078	3	ETMNH-8075	64	ETMNH-8136
48	ETMNH-8120	15	ETMNH-8087	5	ETMNH-8077	67	ETMNH-8139
50	ETMNH-8122	16	ETMNH-8088	7	ETMNH-8079	71	ETMNH-8143
52	ETMNH-8124	18	ETMNH-8090	8	ETMNH-8080	74	ETMNH-8145
56	ETMNH-8128	34	ETMNH-8106	10	ETMNH-8082	75	ETMNH-8147
57	ETMNH-8129	35	ETMNH-8107	14	ETMNH-8086		
58	ETMNH-8130	37	ETMNH-8109	19	ETMNH-8091		

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and seed remains. Abstracts of the 42nd annual meeting of the American Association of Stratigraphic Palynologists, September 27-30, 2009, Kingsport, Tennessee.

Gong, F., I. Karsai, and Y.S. Liu. 2009. Grape seeds (*Vitis*) from the late Neogene Gray Fossil Site, northeastern Tennessee. Abstracts for the Annual Meeting of the Botanical Society of America. July 25-29, 2009. Snowbird, Utah.

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