Vitis seeds (Vitaceae) from the late Neogene Gray Fossil Site, northeastern Tennessee, U.S.A.

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ABSTRACT
This study focuses on morphometric and systematic analyses of the fossil Vitis seeds, recovered from the Gray Fossil Site (7–4.5 Ma, latest Miocene–earliest Pliocene), northeastern Tennessee, U.S.A. A multivariate analysis based on eleven measured characters from 76 complete fossil seeds recognizes three morphotaxa. Further comparisons with both selected modern and fossil vitaceous specimens confirm that these morphotaxa represent three new species, viz. Vitis grayensis sp. nov., Vitis lanatoides sp. nov., and Vitis latisulcata sp. nov. Furthermore, the close resemblance of the first two fossil grapes (V. grayensis and V. lanatoides) with two East Asian Vitis species provides further support concerning a strong eastern Asian aspect of the Gray fossil biota in the late Neogene southeastern North America, as previously evidenced by both animals (e.g. Pristinaiaurus bristoli [red panda]) and other plants (e.g. Sinomenium and Sargentodoxa).

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1. Introduction

Vitis L, including about 60 species, is one of the 14 genera of Vitaceae. Although one species extends into South America, all the other species of this genus are distributed in temperate to warm climate zones of the Northern Hemisphere (Soejima and Wen, 2006). The genus is phytogeographically important for its disjunct distribution between eastern Asia and eastern North America (Chen and Manchester, 2007). An earlier molecular phylogenetic analysis of plastid rbcL DNA sequences found Vitis to be paraphyletic with Cyphostemma and Pfunteniussusted 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 which includes Ampelocissus, Pterisanthes, and Nothocissus (Soejima and Wen, 2006). Another study, based on nuclear GAI1 gene sequences, also supports the monophyly of Vitis; although Nothocissus is not placed within its sister clade (Wen et al., 2007).

Morphologically, species of Vitis are defined by leaf-opposed tendrils, climbing habit, presence of “pearl” glands on leaves, polygamodioecious reproductive biology, calyxpetals, and special seed characters (Soejima and Wen, 2006; Wen et al., 2007). Two subgenera are commonly accepted in the genus Vitis. The subgenus Vitis is recognized by the shreddy bark on old stems, lenticels inconspicuous, pith interrupted by diaphragms within the nodes, and 2–3 forked tendrils; while the subgenus Muscadinia possesses prominent lenticels, pith continuous through nodes and simple tendrils (Soejima and Wen, 2006). The seed morphological characters in Vitaceae, such as the nature of dorsal chalaza and the cause of a pair of ventral infolds, have been systematically studied (Tiffney and Barghoorn, 1976; Chen and Manchester, 2007) and are confirmed to be diagnostically valuable at the generic or sometimes specific level (Chen and Manchester, 2007). Generally speaking, Vitis seeds can be identified by a centrally positioned chalaza connected with a conspicuous chalaza-apex groove and short linear ventral infolds (Manchester, 1994; Chen and Manchester, 2007). Furthermore, the smooth surface of subgenus Vitus and the furrowed or rugose dorsal face of subgenus Muscadinia are also proposed to separate seeds of these two subgenera (Tiffney and Barghoorn, 1976).

Fossil seeds of Vitaceae have been commonly discovered in Cenozoic floras of the Northern Hemisphere, although none of them has been known from southeastern North America (Kirchheimer, 1939, 1957; Tiffney and Barghoorn, 1976). The present study focuses on the systematic of fossil Vitis seeds using morphometric analyses in order to capture the variation and grouping (morphotaxa) of these seeds. Morphometrics have been successfully applied to studies such as fossil foliar morphology (e.g. Hill, 1980; Thiébaut, 2000, 2002; Habyl and Thiébaut, 2002; Tamás and Habyl, 2005) and fossil wood (Oakley and Falcon-Lang, 2009). There have been applications of morphometric studies on the seeds of Vitaceae, e.g. modern cultivated and wild Vitis seeds (Rivera et al., 2007) and modern and fossil Ampelocissus seeds (Chen and Manchester, 2007).

In the present study, using different multivariate analyses (PCA, cluster and discriminant analysis), we find patterns in the data and...
group fossil Vitis seeds into morphotaxa. Then we compare the fossils with modern species on the morphological characters proven diagnostic from morphometrics to infer the most similar species. Finally, phytogeographic implications of these seeds will also be discussed.

2. Material and methods

2.1. Fossil and extant material and its preparation

The fossil seeds used for the morphometric study were collected from the Gray Fossil Site in Washington County, northeastern Tennessee (36.5°N, 82.5°W) (Fig. 1). The site was exposed during a highway construction in May of 2000. The site is usually interpreted as the fills of several paleosinkholes within the Cambrian/Ordovician Knox Group (Wallace and Wang, 2004; Whitelaw et al., 2008). The deposits extend laterally ~2.6 ha (150 m N–S by 175 m E–W). According to Shunk et al. (2006), the lacustrine sediments are buried beneath the subaerial suite consisting of greater than 5 m of alluvium and colluviums, and divided into two parts, viz. 1) the basal graded facies, a 15 m-thick section of lacustrine sediments below 496 m elevation, which consists of mm to cm-thick, normally graded layers of primarily locally derived terrigenous silts and fine sands with low organic content; 2) the laminated facies, between 501.5 and 504.8 m elevation, which is characterized by mm 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 Vitis seeds were collected from this layer. The 5 m-thick transitional interval between 496.5 and 501.5 m elevation is marked by quasi-rhythmic alternation between laminated and graded facies depositional patterns (Shunk et al., 2006) (Fig. 2). Many of the fossil Vitis seeds were mummified with a 3-D preservation, while some are slightly compressed due to fossilization and paleo-forest fire. The deformation of the seeds is so light that our eleven measurements for morphometrics are not affected.

A diverse and well preserved fauna, including Tapiravus, Plionarctos, Pristinailurus, Arctoneles, etc. and flora, including abundant acorns of Quercus and nuts of Carya, appear to indicate a forest surrounding the former ‘pond’ (Wallace and Wang, 2004; DeSantis and Wallace, 2008; Hulbert et al., 2009). The overlapping stratigraphic range of the rhino Teleoceras and the short-faced bear Plionarctos suggests an age between 7 and 4.5 Ma (latest Miocene to earliest Pliocene) (Wallace and Wang, 2004; Shunk et al., 2006; Hulbert et al., 2009).

The preparation of fossil materials follows procedures summarized by Tiffney (1990). The organic-rich blocks of matrix, collected from the Gray Fossil Site, was soaked under water to disaggregate. Next, the 1.7 mm mesh box screen 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) (Tiffney and Barghoorn, 1976; Chen and Manchester, 2007). Seventy six complete fossil seeds of Vitis were measured for this study. Extant comparative specimens, representing 95 species from nine genera of Vitaceae, were borrowed from herbaria of Harvard University (HUH), Missouri Botanical Garden (MO) and East Tennessee State University (ETSU). Fifty-seven specimens of these extant species belong to Vitis, including all the North and South American species listed on the PLANTS database of USDA-NRCS (available: http://plants.usda.gov/java/profile?symbol=VITIS) and about half of the Asian species (Gong, 2009). Preparation of the extant seeds follows Tiffney and Barghoorn (1976) by boiling in 10% NaOH for 5–10 min to remove the outer membrane and adherent pieces of berry. Gong (2009) systematically surveyed all of these extant Vitis species in terms of morphological variations at both the interspecific and intraspecific levels (Tables 4 and 5 in Gong, 2009), on which the comparisons in the
present study is based. Digital images of both dorsal and ventral views of the seeds were recorded with MicroFire (Optronics) digital camera attached to OLYMPUS-SZX12 stereomicroscope. Measurements of seeds on digital images were made using Imagej (version 1.40g) (Rasband, 1997–2009).

2.2. Measurements and morphometric analysis

Eleven continuous variables were chosen for morphometric analysis and measured from the digital images (Table 1 and Fig. 3). 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), which 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, Thiebaut (2002) indicated that PCA is effective for grouping specimens, when it keeps a maximum of total variability; while cluster analysis is appropriate for grouping specimens, when it keeps a large number of 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, which 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 the eleven characters was performed to find the linear combinations of characters, which are shown as canonical discriminant functions, from which discriminant scores for each specimen were also calculated.

2.3. Terminology

The terminology of vitaceous seed characters (Fig. 3) is after Tiffney and Barghoorn (1976), with the exception that both chalaza-apex and chalaza-base grooves are changed to indicate opposite parts as to those in Tiffney and Barghoorn (1976), suggested by Manchester (1994).

3. Results

3.1. Morphometric study

3.1.1. 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 2). With the exception of distance between apexes of the two infolds and distance between bases of the two infolds, all the other nine characters show significant correlations with each other.

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 identity matrix and supports that the data structure fulfills the conditions of PCA. Three principal components were extracted from the data after rotation, which explain 75.78% of total variance (Table 3). The rotated component matrix after rotation demonstrates

### Table 1

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

### Table 2

<table>
<thead>
<tr>
<th>Character</th>
<th>M1 (mm)</th>
<th>M2 (mm)</th>
<th>M3 (mm)</th>
<th>M4 (mm)</th>
<th>M5 (mm)</th>
<th>M6 (mm)</th>
<th>M7 (mm)</th>
<th>M8 (mm)</th>
<th>M9 (mm)</th>
<th>M10 (mm)</th>
<th>M11 (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.934</td>
<td>0.551</td>
<td>0.661</td>
<td>0.497</td>
<td>0.326</td>
<td>0.735</td>
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<tr>
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<td>0.365</td>
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<tr>
<td>M9</td>
<td>0.656</td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
<td>M10</td>
<td></td>
<td>0.656</td>
<td>0.476</td>
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</table>

Fig. 3. Morphological terminology of vitaceous seed (after Tiffney and Barghoorn, 1976; revised by Manchester, 1994) and seed characters measured for morphometrics (see Table 1 for character descriptions).
important characters for each component, and the component score coefficient matrix displays the most important characters for each component (Table 3).

The first principal component (PC1) accounts for 33.89% of variance after rotation, and is highly weighted on four characters, which are characters to reflect seed lengths with and without beak, ventral infold length, and length between seed apex and chalaza base. According to the component score coefficients, ventral infold length 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, which are the characters focusing on seed width, beak width, distance between apexes of the two infolds, distance between bases of the two infolds and vertical distance from infold apexes to seed apex. Distance between bases of the two infolds contributes the highest coefficient score, while distance between apexes of the two infolds also shows a coefficient score close to the score of distance between bases of the two infolds, which indicated that distances between two ventral infolds are important characters for PC2. The third principal component (PC3) accounts for 14.97% of the variance after rotation. The chalaza length and width are the most important characters for PC3, and component score shows chalaza length is more important than chalaza width for PC3.

3.1.2. Grouping specimens

Hierarchical cluster analysis was applied to group specimens into morphotaxa. The dendrogram (Fig. 4) shows a large gap between rescaled distance 10 and 15, which suggests three distinctive clusters at the rescaled distance of about 15, each of which is considered as a morphotaxon. Among 76 specimens morphometrically measured in the study, morphotaxon 1 is represented by 31, morphotaxon 2 by 19, and morphotaxon 3 by 26, respectively. Morphotaxon 1 is generally characterized by low values for features grouped by PC1 and PC2. Although morphotaxon 2 is also characterized with low values of features grouped by PC1, it is distinguished with high values of PC2 features. All the features that are grouped by PC1 and PC2 generally show high values in morphotaxon 3. More detailed analyses of the three groups will be provided in the next section.

Canonical discriminant analysis were performed to find out linear combination of the characters that best summarizes 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, which meets the condition of discriminant analysis. Canonical discriminant analysis presents two discriminant functions. Function 1 explains 79.8% of variance, and function 2 explains 20.2% of variance. Two discriminant scores for each specimen were also calculated from those two functions. A plot based on discriminant scores of the 76 fossil seeds was built (Fig. 5). Except a few specimens showing transitional distribution, the three morphotaxa recognized from cluster analysis are separated from the discriminant analysis plot, which supports the three morphotaxa are successfully distinguishable based on the 11 characters (Table 1). Next, canonical 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 results, two seeds (Specimen Label Number (SLN) 68, 76) of the morphotaxon 1 from the cluster analysis are classified into predicted group 2, and three seeds (SLN 4, 37 and 55) of the morphotaxon 1 from the cluster analysis are classified into predicted group 3. The group described in discriminant analysis is conceptually the same as the cluster indicated by cluster analysis. Probabilities of these five misclassified specimens being placed in the predicted groups and original groups are listed (Table 4). After further checking morphological characters of these five seeds, we followed their position in the dendrogram of cluster analysis.

Based on our analysis, the three morphotaxa are characterized both quantitatively (Table 5) and qualitatively (Table 6).

3.2. Systematic description

The fossil seeds studied here are characterized by the presence of central positioned chalaza on dorsal surface, obvious visible chalaza-apex groove, and short linear ventral infolds, perfectly corresponding to the genus *Vitis* (Tiffney and Barghoorn, 1976; Chen and Manchester, 2007). In addition, due to the occurrence of smooth surface of the fossil seeds from Gray, they can be further identified into the subgenus *Vitis* (Tiffney and Barghoorn, 1976). *Muscadina*, the other subgenus in *Vitis*, has seeds with furrowed to striated dorsal surface (Tiffney and Barghoorn, 1976). To further recognize the specific differences of these fossil seeds, we compared other seed characters, such as seed form and size, chalaza grooves, beak, apical notch, and raphe ridge, suggested by others (e.g., Chandler, 1961, 1962, 1963, 1964; Tiffney and Barghoorn, 1976; Tiffney, 1979; Manchester, 1994). With a combination of the results from both morphometric and comparative morphological studies, three species are identified as follows.

Order Vitales Burnett
Family Vitaceae Jussieu
Genus *Vitis* Linnaeus
Subgenus *Vitis* Planchon

*Vitis graysiensis* Gong, Karsai, et Liu sp. nov. (Plates I, 1–8)

*Holotype*: ETMNH 8144 (Plate I, 1, 2).

*Paratypes*: ETMNH 8089 (Plate I, 3, 4); ETMNH 8116 (Plate I, 5, 6); ETMNH 8122 (Plate I, 7, 8).

*Repository*: East Tennessee State University and General Shale Natural History Museum Fossil Collections.

*Type locality*: The Gray Fossil Site, Washington County, northeastern Tennessee, USA (36.5°N, 82.5°W).

*Horizon*: Near the top layer of the laminated facies.

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

*Etymology*: The specific epithet *graysiensis* refers to the Gray Fossil Site from where the fossil seeds were collected.


*Specific diagnosis*: Seeds obovoid in outline on dorsal and ventral views; surface smooth; beak trapezoidal, its outline continuing the general outline of the seed seen on both dorsal and ventral views of
the beak; chalaza narrow elongate to elliptical, centrally positioned on the dorsal face; chalaza-apex groove narrow, obviously visible; chalaza-base groove narrow, slightly visible to faint; ventral infolds linear, straight, short, about 2/5–3/5 seed length, apically divergent; raphe ridge narrow.

Description: Seeds are obovoid in outline on both dorsal and ventral views (Plate I, 1–8). Seed surface is smooth. The average size of seed is $3.99 \times 3.03$ mm, based on measurements of 31 complete specimens (length range from min. 3.39 mm to max. 4.58 mm, standard deviation = 0.34; width range from min. 2.32 mm to max. 3.69 mm, standard deviation = 0.31). The obviously trapezoidal-shaped beak continues the outline of seed shown on both dorsal and ventral views (Plate I, 1–8). The narrowly elongate to elliptical chalaza, slightly or not concave, is centrally positioned on the dorsal face (e.g. Plate I, 1, 1–8).
The narrow and shallow chalaza-apex groove is obviously visible, forming a shallow to deep apical notch in its passage to the ventral face (Plate I, 1, 5). Some specimens maintain a raphe in the chalaza-apex groove, extending from the chalaza apex to the apical notch (Plate I, 5). The narrow chalaza-base groove is slightly visible to faint (e.g. Plate I, 1, 3). The linear, straight ventral infolds are short and about 2/5 of the seed, extending to the apical 1/3–2/5 of seed, and slightly or noticeably diverging apically (Plate I, 2, 4, 6, 8). The shallow infold cavities show a clear boundary from the raphe ridge and a faint boundary from the ventral surface (e.g. Plate I, 2). The narrow raphe ridge rises slightly from the ventral surface, and slightly or markedly narrows towards the seed base (Plate I, 2, 4, 6, 8).

Comparison: The present species is characterized by the ovoid outline on both of the dorsal and ventral views and narrowly elongate to elliptical chalaza. Characters, such as seed size, outline view, narrow chalaza shape, obviously narrow chalaza-apex groove and slightly visible to faint chalaza-base groove, are closely comparable with two modern species, *Vitis balanseana* and *V. thunbergii*. However, some differences exist in the beak shape, i.e. triangular in *V. thunbergii* and cylindrical in *V. balanseana*. Furthermore, both of the modern species possess much deeper infold cavities, showing a clear boundary between infold cavities and ventral surface, than the fossil species. This character should be treated with caution in comparison, as fossilization might affect it. The triangular beak outline on both dorsal and ventral views in *V. thunbergii* continues the general outline of the seed, while the prominent cylindrical beak of *V. balanseana* shows clear boundaries with the seed body on ventral face-view, which implies that the beak shape might be a useful seed character in separating species in *Vitis*. Judging from the relationship between the outline of beak and seed body, and the presence of trapezoidal beak in our fossil species, we believe that *Vitis grayensis* more resembles *V. thunbergii* than *V. balanseana*.

*Vitis thunbergii* currently distributes from warm to temperate regions of East Asia (Chen et al., 2007). The fossil seeds closely resembling this species have been reported from the upper Neogene in Japan (Miki, 1956, p. 265, fig. 15) and Pliocene of France (Reid, 1923, pp. 338–339, plate 11, figs. 3–4). Furthermore, one fossil species from Europe, *V. teutonica* (Czeczott et al., 1959, p. 102, plate 16, figs. 3, 6–7), appears closely similar to *V. grayensis* in many features except that the European species has a wedge-shaped basis. Biogeographically, it appears that *V. thunbergii*-like species covered a much wider distribution area in the North Hemisphere during the Neogene than today.

Table 4
The five misclassified specimens given by canonical discriminant analysis. Percentages of each specimen in the highest group (predicted group) and the second highest group (original group) are listed.

<table>
<thead>
<tr>
<th>SLN</th>
<th>Original group</th>
<th>Highest group</th>
<th>Second highest group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicated group (%)</td>
<td></td>
<td>Group (%)</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>3’</td>
<td>0.517</td>
</tr>
<tr>
<td>37</td>
<td>2</td>
<td>3’</td>
<td>0.679</td>
</tr>
<tr>
<td>55</td>
<td>2</td>
<td>3’</td>
<td>0.462</td>
</tr>
<tr>
<td>68</td>
<td>1</td>
<td>2’</td>
<td>0.556</td>
</tr>
<tr>
<td>76</td>
<td>1</td>
<td>2’</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Fig. 5. Score plot of the two canonical discriminant functions of the discriminant analysis. Specimen label number (SLN) of each specimen as in Fig. 4 is shown.

Holotype: ETMNH 8088 (Plate I, 9, 10).
Paratypes: ETMNH 8111 (Plate I, 11, 12), ETMNH 8113 (Plate II, 1, 2), ETMNH 8121 (Plate II, 3, 4).
Repository: East Tennessee State University and General Shale Natural History Museum Fossil Collections.
Type locality: The Gray Fossil Site, Washington County, northeastern Tennessee, USA (36.5°N, 82.5°W).

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

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


Specific diagnosis: Seed shape subglobose; outline on both dorsal and ventral views round; surface smooth; beak cylindrical, prominent; chalaza round to tear-shaped, positioned centrally on the dorsal face; chalaza-apex groove 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 outline is round on both dorsal and ventral views (Plate I, 9–12; Plate II, 1–4). Seed surface is smooth. Some seeds are subglobose in form (Plate I, 9–12; Plate II, 1–2), while the other are flattened to a certain extent (Plate II, 3–4). The average size is 4.36 ± 3.47 mm based on the measurements of 19 complete seeds (length range 3.9–5.19 mm, standard deviation = 0.32; width range 2.73–3.9 mm, standard deviation = 0.29). The beak is prominently cylindrical (e.g. Plate II, 1). The round to tear-shaped chalaza, slightly concave, is positioned centrally on the dorsal face (e.g. Plate I, 9; Plate II, 3), but several seeds possess chalaza much closer to the seed apex (Plate I, 11). The narrow chalaza-apex groove is shallow, linear, and more or less visible (Plate I, 9, 11; Plate II, 1, 3). The narrow chalaza-base groove is faintly visible (e.g. Plate I, 9). The apical notch is not distinct (Plate I, 9–12; Plate II, 1–4). 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 (Plate I, 10, 12; Plate II, 2, 4). The ventral infold cavities are deep, with clear boundaries from the ventral surface (e.g. Plate I, 10). The raphe ridge faintly or slightly rises from the ventral surface, and narrows towards the seed base (Plate I, 10, 12, Plate II, 2, 4).

Comparison: The present fossil seed species is distinguished by subglobose form and round to tear-shaped chalaza. In Vitaceae, subglobose seeds are also seen in genera other than *Vitis*, such as...
Comparison of the morphological characters of the three morphotaxa (clusters), discriminated by relative morphometric characters used in this study.

### Table 5

<table>
<thead>
<tr>
<th>Character Cluster 1 (N = 31)</th>
<th>Character Cluster 2 (N = 19)</th>
<th>Character Cluster 3 (N = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 3.39</td>
<td>M1 3.904</td>
<td>M1 4.534</td>
</tr>
<tr>
<td>M2 2.916</td>
<td>M2 4.083</td>
<td>M2 4.288</td>
</tr>
<tr>
<td>M3 2.311</td>
<td>M3 3.739</td>
<td>M3 3.739</td>
</tr>
<tr>
<td>M4 0.6</td>
<td>M4 0.882</td>
<td>M4 0.882</td>
</tr>
<tr>
<td>M5 0.828</td>
<td>M5 1.447</td>
<td>M5 1.447</td>
</tr>
<tr>
<td>M6 0.848</td>
<td>M6 1.081</td>
<td>M6 1.081</td>
</tr>
<tr>
<td>M7 1.703</td>
<td>M7 2.687</td>
<td>M7 2.687</td>
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<tr>
<td>M8 1.262</td>
<td>M8 2.115</td>
<td>M8 2.115</td>
</tr>
<tr>
<td>M9 0.801</td>
<td>M9 1.493</td>
<td>M9 1.493</td>
</tr>
<tr>
<td>R10/M10</td>
<td>R10/M10</td>
<td>R10/M10</td>
</tr>
<tr>
<td>R11/M11</td>
<td>R11/M11</td>
<td>R11/M11</td>
</tr>
<tr>
<td>R12/M12</td>
<td>R12/M12</td>
<td>R12/M12</td>
</tr>
</tbody>
</table>

### Table 6

Comparison of the morphological characters of the three morphotaxa (clusters), discriminated by relative morphometric characters used in this study.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Relative morphometric characters</th>
<th>Morphotaxon 1 (Cluster 1)</th>
<th>Morphotaxon 2 (Cluster 2)</th>
<th>Morphotaxon 3 (Cluster 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed size</td>
<td>M1, M2, M3</td>
<td>Small</td>
<td>Medium</td>
<td>Big</td>
</tr>
<tr>
<td>Seed shape</td>
<td>R1 (= M3/M1)</td>
<td>Narrow</td>
<td>Close to round</td>
<td>Narrow</td>
</tr>
<tr>
<td>Beak</td>
<td>M4 M6, M5</td>
<td>Narrow</td>
<td>Medium</td>
<td>Broad</td>
</tr>
<tr>
<td>Chalaza size</td>
<td>M5, M6</td>
<td>Narrow</td>
<td>Big</td>
<td>Big</td>
</tr>
<tr>
<td>Chalaza shape</td>
<td>R2 (= M6/M5)</td>
<td>Center of dorsal face</td>
<td>Center of dorsal face</td>
<td>Center of dorsal face</td>
</tr>
<tr>
<td>Chalaza position</td>
<td>R3 (= M7/M2)</td>
<td>Center of dorsal face</td>
<td>Center of dorsal face</td>
<td>Center of dorsal face</td>
</tr>
<tr>
<td>Ventral infolds length</td>
<td>MB, BR (= MB/M2)</td>
<td>About 2.5–3/5 seed length</td>
<td>About 1/3–2/2 seed length</td>
<td>About 1/3–2/2 seed length</td>
</tr>
<tr>
<td>Ventral infolds position</td>
<td>R5 (= M11/M2)</td>
<td>About 1/3–2/5 to seed apex</td>
<td>Broad toward seed apex</td>
<td>Broad toward seed apex</td>
</tr>
<tr>
<td>Ventral inffolds size</td>
<td>R4 (= M10/M0)</td>
<td>Broad toward seed apex</td>
<td>Broad toward seed apex</td>
<td>Broad toward seed apex</td>
</tr>
<tr>
<td>Raphe ridge width</td>
<td>M9, M10</td>
<td>Narrow</td>
<td>Medium</td>
<td>Broad</td>
</tr>
</tbody>
</table>

**Parthenocissus and Ampelopsis** ([e.g. P. angustiscutata, Scott, 1954], p. 81, plate 16, fig. 14; Manchester, 1994, p. 95, plate 45, figs. 6–7; A. roseae, Manchester, 1994, p. 94, plate 44, figs. 6–10; A. rotundata, Reid and Chandler, 1933, p. 386, plate 19, figs. 13–17; and A. crenula, Reid and Chandler, 1933, p. 383, plate 19, figs. 11–12). However, a combination of other seed characters does not support the inclusion of our fossils into either of these genera. For example, Parthenocissus has long ventral inffolds extending from the base to apex of seed and Ampelopsis lacks chalaza-apex groove.

Among the species of *Vitis*, subglobose shape with a prominent cylindrical beak, dorsal centrally positioned round chalaza, and short apical divergent ventral inffolds of *V. lanatoides* are essentially comparable with those of the modern species *V. labrusca*, currently distributed in the subtropical regions of East to South Asia (Chen et al., 2007). The main differences are that the modern species has much wider raphe ridge and broader infold cavities than our fossils.

*Chandler (1962)* described one fossil species, *V. glabra*, from the lower Tertiary floras of southern England (*Chandler, 1962, p. 103, plate 14,* figs. 13–14). We tend to believe that *V. glabra* is more comparable with another fossil *V. tiffneyi* described by Tiffney and Barghoorn (1976), which is considered another member of this fossil group.

*Vitis latisulcata* Gong, Karsai, et Liu sp. nov. (Plate II, 5–12)

**Holotype:** ETMNH 8079 (Plate II, 5, 6)

**Paratypes:** ETMNH 8074 (Plate II, 7, 8), ETMNH 8077 (Plate II, 9, 10), ETMNH 8100 (Plate II, 11, 12).

**Repository:** East Tennessee State University and General Shale Natural History Museum Fossil Collections.

**Type locality:** The Gray Fossil Site, Washington County, northeastern Tennessee, USA (36.5°N, 82.5°W).

**Horizon:** Near the top layer of the laminated facies.

**Age:** Late Hemphillian (7.45 Ma, latest Miocene to Earliest Pliocene).

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

**Material:** ETMNH 8074–8075, 8077, 8079–8080, 8082, 8086, 8091–8092, 8094–8096, 8098, 8100–8102, 8104, 8108, 8110, 8118, 8126, 8136, 8139, 8143, 8145, 8147.
Specific diagnosis: Seed 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 ventral; infold in- folds linear, straight to slightly curved, short, about 2/3–1/2 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 outline on both dorsal and ventral views of this species is ovate-elliptical to rectangular (e.g. Plate II, 5, 11). The surface of the seed is smooth. The average length of 26 complete seeds is 5.08 mm (range 4.53–5.7 mm, standard deviation = 0.36) and the average width is 3.48 mm (range 2.87–4.32 mm, standard deviation = 0.3). A cylindrical beak projects from the seed base and shows clear boundaries with the seed body (e.g. Plate II, 10). Some seeds possess beak with a flared tip (e.g. Plate II, 11). The pyriform to spatulate chalaza is positioned centrally on the dorsal face (e.g. Plate II, 5, 9). In some seeds, the chalaza was lost to form a hole on the center of chalaza position (Plate II, 7). The broad deep chalaza-apex groove obviously extends from the chalaza apex to the seed apex, and then forms the obvious deep apical notch, which extends to the ventral face and forms a narrow “V-shape” groove at top of the raphe ridge (e.g. Plate II, 5–8). The broad chalaza-base groove is slightly visible to faint (e.g. Plate II, 5, 9). The straight or slightly curved ventral in- folds are short and about 2/5–1/2 of the seed length, extending to the apical 1–3/2/5 of seed, and diverging apically (Plate II, 6, 8, 10, 12). The shallow infold cavities are broad linear on face-view, with clear to faint boundaries from the ventral surface (e.g. Plate II, 6). The raphe ridge slightly rises from the ventral surface, and narrows towards the seed base (Plate II, 6, 8, 10, 12).

Comparison: The unique combination of characters, such as large seed, pyriform to spatulate chalaza shape, deep broad chalaza-apex groove, and “V-shape” groove at top of the raphe ridge, distinguish V. latisulcata from other vitaceous seeds from the Gray site. The pyriform to spatulate chalaza, broad deep chalaza-apex groove and the short apically divergent ventral in- folds of the present fossil species are closely comparable with two modern North American species, e.g. V. candidans and V. labrusca. Due to the presence of shallow chalaza-base groove, V. latisulcata is closer to V. candidans than to V. labrusca. On the other hand, based on the cylindrical beak and the flared tip on the beak, V. latisulcata is closely comparable with V. labrusca. By contrast, V. candidans has a triangular to trapezoidal beak. Both of these two modern species are much bigger in size (about 6×4 mm) than the present fossil. This size difference is too great to be an effect of desiccation or taphonomy. Furthermore, the “V-shape” groove at the top of raphe ridge in the fossil appears not to occur in these two modern species. Both Vitis candidans and V. labrusca have been classified into the same series Labruscae based on many other morphological characters (Moore, 1991). The Gray site is located at 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. candidans (Moore, 1991). The similar seed characters among these 3 species, taxonomic close relationship between V. candidans and V. labrusca, and relative geographic ranges of the three species suggest their close relationships.

One fossil species, 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 possesses some characters similar to V. latisulcata, including seed and beak shape, seed size, and ventral in- folds 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) which shares some features with V. labrusca, but that species was suggested to be much closer 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 lots of different characters from our fossil species. The chalaza hole should be excluded as an important character from identifying fossil vitaceous seeds, because those holes may be caused by fossilization.

On the study of Vitis eolabrusca, Tiffney and Barghoorn (1976) listed other fossil species possessing similar characters with it, such as Vitis cf. silverstris (Czezott et al., 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 same characters which could be comparable with 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 occurred at a later geological age than V. eolabrusca, however the similar features might suggest the continuation of this lineage.

4. Discussion

4.1. Morphometrics of fossil Vitis seeds

Due to the exquisite preservation of the fossil seeds from the Gray site, we chose eleven characters for a morphometric analysis, which

Plate I.

1–12. Scale bar = 1 mm.
1–3. Fossil seeds of Vitis gruyensis sp. nov.
1–2. Holotype, ETMNH 8144.
1. Dorsal view showing narrow elliptical chalaza, obvious chalaza-apex groove, and slightly visible chalaza-base groove.
2. Ventral view showing linear straight ventral in- folds and infold cavities with a clear boundary from the raphe ridge and a faint boundary from the ventral surface.
3. Dorsal view showing the faint chalaza-base groove.
4. Ventral view.
5–6. Paratype, ETMNH 8116.
5. Dorsal view showing the elongate chalaza and the raphe remain at top of the chalaza.
6. Ventral view.
7–8. Paratype, ETMNH 8122.
7. Dorsal view.
8. Ventral view.
9–12. Fossil seeds of Vitis lanatoides sp. nov.
9. Dorsal view showing the round chalaza and faintly visible chalaza-base groove.
10. Ventral view showing the deep infold cavities with clear boundaries from the ventral surface.
11–12. Paratype, ETMNH 8111.
11. Dorsal view showing chalaza close to seed apex.
12. Ventral view.
could help us study the seed morphological variation using objective quantitative statistical methods. 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 to define seeds in the Vitaceae (Tiffney and Barghoorn, 1976; Chen and Manchester, 2007). Correlation analysis shows that several variables are highly correlated with each other. By using PCA, we were able to point out several important variables among them. The First Principal Component commonly shows high correlation with all variables and is considered to represent a general “size,” therefore the other components commonly represent different shape components that are unrelated to size. In our study this did not happen, but the first component correlated highly with only 4 variables. We think this happened because in our data the range in size was very moderate and we used Varimax rotation to find the best possible factors. On the PCA analysis, characters indicating chalaza size consist of the third Principal component (PC3), and are independent from other measurements. This result appears to suggest that the chalaza size should have no direct relationship with the seed size. On the other hand, although correlation analysis indicates that the distances between two ventral infolds are independent from the infold length, PCA suggests that the ventral infold length is relative to the seed length, while the distances between two infolds are relative to the seed width.

Both the large rescaled distance gap between 10 and 15 among the three clusters in the dendrogram of hierarchical cluster analysis (Fig.4) and the separation of the three groups in the discriminant plot (Fig.5) suggest the 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, canonical discriminant analysis shows that five specimens were misclassified (Table 5). Three of these five specimens (SLN 4, 55 and 68) possess a close probability in their predicted groups and the original groups, which indicate that these seeds might be transitional forms or they were slightly distorted during fossilization. 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 follows the diagnostic characters of its original group V. grayensis, so the classification of these 3 specimens follows the results of the cluster dendrogram (Fig.4). The other two (SLN 37 and 76), while different in some respects, may be confidently assigned to one of the three groups. The morphology of the specimen SLN 37 corresponds extremely closely to 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. According to its morphological characters, we 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, tear-shaped chalaza and cylindrical beak, are much closer to its predicted groups V. lanatoides and this is coincident with its high probability of V. lanatoides in the discriminant analysis. However, characters of its ventral infold accord with its original group V. grayensis in the dendrogram. This seed displays distortion to a certain extent, which might lead to the conflict in its classified position. Here, we still accept the result of cluster analysis and regard this specimen as a seed of V. grayensis.

Furthermore, most of the characters concluded by descriptive statistics are listed (Table 6), and these characters are all important for studies of vitaceous seed morphology within a single 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 suggests that they are diagnostic characters for the genus Vitis.

Lastly, some characters are important in the study of fossil vitaceous seeds, but could not be measured directly and were therefore excluded from morphometrics. Those characters include shapes of seed, beak, chalaza, and ventral infold. They present little variation within each of the three species identified in the present study. Although seed shape shows some variation among Vitis latisulcata (such as ETMNH 8079 and ETMHN 8100, Plant II, 5–6, 11–12), 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. It should conclude that the seed shape is less important than the characters such as chalaza and ventral infold to identify vitaceous seeds.

4.2. Phytogeographical significance

The earliest seed fossils of Vitaceae are late Paleocene, and by the early Eocene the fossil seeds indicate that the family was diverse and widespread (Chen and Manchester, 2007). Among the seed fossils in the family, both the most common species and the highest number of seed fossils are represented by the genus Vitis. The Paleogene Vitis seeds show a wide distribution in the North Hemisphere, from North America (e.g. Manchester, 1994; Manchester and McIntosh, 2007) to Europe including western Siberia (e.g. Reid and Chandler, 1933; Dorofeev, 1957; Chandler, 1957, 1960, 1961, 1962, 1963; Dorofeev, 1963; Chandler, 1964). During the Neogene, while fossil Vitis seeds were still common in the floras of North America (e.g. Tiffney and Barghoorn, 1976; Tiffney, 1979) and Europe (e.g. Reid, 1923; Dorofeev, 1957, 1963; Czezzott et al., 1959; Fairom-Demaret and Smith, 2002), they also became common in the Miocene and Pliocene
floras of eastern Asia (Miki, 1956), which indicates 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 actual lack of fossil Vitis in Paleogene floras or a bias of insufficient research there. As no evidence of phylogeographic history of Vitis is yet available from molecular data, it would be difficult to interpret the history of the genus in the Asian region from the limited fossil record.

Species of Vitis are now commonly distributed in North America and East to South Asia, forming a disjunct distribution between these two continents (Chen and Manchester, 2007). There is no wild record from Europe except for the cultivated species, Vitis vinifera (Webb, 1968; Punt et al., 2003). It is suggested that the final disappearance of Vitis from Europe may have resulted from the successive climatic cooling trend during the late Cenozoic (Manchester, 1999; Wen, 1999).

Of the three fossil species of Vitis recognized at the Gray site, only one, V. latissulcata, shows a close affinity with the local species L. labruscana in North America. The other two species, V. grayensis and V. lanatoidea, display close relationships with fossil grapes from Eurasia and modern grapes in Asia. Through morphological comparisons, we concluded that V. grayensis shows close relationships with V. thumbergii, an extant East Asian species recorded in the late Neogene in Japan (Miki, 1956) and the Pliocene in Europe (Reid, 1923; Czeczott et al., 1959), and the Pliocene V. teutonica from Europe (Reid, 1923; Czeczott et al., 1959). The second fossil grape from the Gray site, Vitis lanatoidea, closely resembles V. lanata, an extant grape from East to South Asia. The presence of the two fossil grapes at the Gray site showing close affinities with Asian grapes provides further evidence of the eastern Asian aspects of the biota from southeastern North America as late as the late Neogene, shown by other fossils from the same site at Gray, e.g. red panda (Wallace and Wang, 2004), Sinomenium (Menispermaceae) (Liu and Jacques, 2010), and Sargentodoxa (Lardizabalaceae) (Liu et al., 2007).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.revpalbo.2010.05.005.

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