**Phylogeny of the *Ampelocissus-Vitis* clade in Vitaceae supports the New
World origin of the grape genus**

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Abstract

The grapes and the close allies in Vitaceae are of great agronomic and economic importance. Our previous studies showed that the grape genus *Vitis* was closely related to three tropical genera, which formed the *Ampelocissus-Vitis* clade (including *Vitis*, *Ampelocissus*, *Nothocissus* and *Pterisanthes*). Yet the phylogenetic relationships of the four genera within this clade remain poorly resolved. Furthermore, the geographic origin of *Vitis* is still controversial, because the sampling of the close relatives of *Vitis* was too limited in the previous studies. This study reconstructs the phylogenetic relationships within the clade, and hypothesizes the origin of *Vitis* in a broader phylogenetic framework, using five plastid and two nuclear markers. The *Ampelocissus-Vitis* clade is supported to be composed of five main lineages. *Vitis* includes two described subgenera each as a monophyletic group. *Ampelocissus* is paraphyletic. The New World *Ampelocissus* does not form a clade and shows a complex phylogenetic relationship, with *A. acapulcensis* and *A. javalensis* forming a clade, and *A. erdvendbergiana* sister to *Vitis*. The majority of the Asian *Ampelocissus* species forms a clade, within which *Pterisanthes* is nested. *Pterisanthes* is polyphyletic, suggesting that the lamellate inflorescence characteristic of the genus represents convergence. *Nothocissus* is sister to the clade of Asian *Ampelocissus* and *Pterisanthes*. The African *Ampelocissus* forms a clade with several Asian species. Based on the Bayesian dating and both the RASP and Lagrange analyses, *Vitis* is inferred to have originated in the New World during the late Eocene (39.4 Ma, 95% HPD: 32.6- 48.6 Ma), then migrated to Eurasia in the late Eocene (37.3 Ma, 95% HPD: 30.9-45.1 Ma). The North Atlantic land bridges (NALB) is hypothesized to be the most plausible routes for the *Vitis* migration from the New World to Eurasia, while intercontinental long distance dispersal (LDD) can not be eliminated as a likely mechanism.

Keywords: *Ampelocissus*; *Vitis*; grapes; Vitaceae; biogeography

1. Introduction

The grape family (Vitaceae) has been widely recognized for its agronomic and economic importance as sources of grapes, wine, and raisins (Wen, 2007). It includes about 15 genera and ca. 900 species mostly in pantropical regions of Asia, Africa, Australia, the Neotropics, and the Pacific islands, with a few genera (*Vitis* L., *Parthenocissus* Planch. and *Ampelopsis* Michx.) in temperate regions of the Northern Hemisphere (Wen, 2007; Wen et al., 2007, 2013a, 2014). The phylogeny of Vitaceae has caught the attention of several teams of workers in recent years (Ingrouille et al., 2002; Liu et al., 2013; Lu et al., 2013; Ren et al., 2011; Rodrigues et al., 2014; Rossetto et al., 2002, 2007; Soejima and Wen, 2006; Trias-Blasi et al., 2012; Wen et al., 2007, 2013b). The family is morphologically unique, especially in having leaf-opposed tendrils, an unusual axile placentation with incompletely fused septa in a bicarpellate gynoecium, multicellular, stalked, caducous spherical structures known as "pearl" glands on various organs of the plant, and a suite of unique seed characters (Chen and Manchester, 2011; Gerrath and Poluszny, 2007; Ickert-Bond et al., 2014; Süssenguth, 1953; Wen, 2007; Wilson and Posluszny, 2003; Zhang et al., 2015).

Several studies focused on the relationship of the economically important genus *Vitis* as well as other genera in Vitaceae. Ingrouille et al. (2002) suggested that *Vitis* formed a clade with *Cayratia* Juss., *Cyphostemma* (Planch.) Alston, *Parthenocissus* and *Tetrastigma* (Miq.) Planch., but the result was based on a limited number of species (20 species) and markers (only plastid *rbcL*) sampled. In the context of resolving *Cissus* phylogeny, Rossetto et al. (2002) reported that *Vitis* was associated with several *Cissus* species endemic to Australia. Rossetto et al. (2007) later concluded that these few Australian *Cissus* species were intermediate between *Vitis* and *Ampelopsis* mainly based on review of Australian Vitaceae. Soejima and Wen (2006) resolved five major clades of the family based on three chloroplast

1 markers for 37 taxa. *Vitis* was shown to form a clade with *Ampelocissus*, *Nothocissus* and
2 *Pterisanthes*, which formed the *Ampelocissus-Vitis* clade. This clade was strongly supported
3 by the nuclear *GAIH* sequences with 95 Vitaceae taxa sampled (Wen et al., 2007) and by
4 three chloroplast markers based on 114 samples representing 12 genera (Ren et al., 2011).
5 This clade was also strongly supported by five plastid markers based on an 174 samples (Liu
6 et al., 2013) and by sequences of 417 orthologous genes extracted from the transcriptome
7 data of 15 species of Vitaceae (Wen et al., 2013b). So far, the *Ampelocissus-Vitis* clade has
8 been confirmed to be composed of four genera, but the phylogenetic relationships within the
9 clade have not been well resolved.

10 *Vitis* includes ca. 70 species mostly in the temperate regions of the Northern Hemisphere
11 (Chen et al., 2007; Moore and Wen, in press; Wen, 2007; Zecca et al., 2012). Several recent
12 studies have reconstructed the phylogeny and/or hypothesized on the origin of *Vitis* (Miller et
13 al., 2014; Péros et al., 2011; Tröndle et al., 2010; Wan et al., 2013; Zecca et al., 2012).
14 Ingrouille et al. (2002) and Pelsy (2007) reported that *Vitis* was paraphyletic, whereas later
15 studies supported its monophyly (Soejima and Wen, 2006; Tröndle et al., 2010; Wan et al.,
16 2013; Wen et al., 2007; Zecca et al., 2012). Two subgenera of *Vitis* have been commonly
17 recognized. Subg. *Vitis* includes the majority of species with a wide distribution in the
18 Northern Hemisphere, and subg. *Muscadinia* Planch. (2 species) is restricted to the
19 southeastern United States, the West Indies and Mexico (Brizicky, 1965; Wen, 2007). The
20 recognition of the two distinct subgenera is still debated (Zecca et al., 2012). While some
21 phylogenetic analyses (Ingrouille et al., 2002; Pelsy, 2007) did not retain two separate clades,
22 others robustly supported the placement of subg. *Muscadinia* as sister to subg. *Vitis* (Aradhya
23 et al., 2008; Tröndle et al., 2010; Wan et al., 2013; Wen et al., 2007; Zecca et al., 2012). The
24 phylogenetic relationships within subg. *Vitis* remain controversial. Galet (1988) classified 59
25 species of subg. *Vitis* into 11 series mainly based on morphological traits but also included

habitat and biogeography. Galet's classification scheme was partly supported by Péros et al. (2011) and Wan et al. (2013), but strongly rejected by Zecca et al. (2012) based on molecular data. Recent phylogenetic reconstructions reflected intercontinental disjunctions of the subgenus, but criticized a strict correspondence between phylogenetic and geographic groups (Aradhya et al., 2008; Di Gaspero et al., 2000; Pelsy, 2007; Tröndle et al., 2010; Zecca et al., 2012). In fact, the species delimitation within subg. *Vitis* is difficult and questioned due to hybridization or clinal variation within species (Comeaux et al., 1987; Moore, 1991; Péros et al., 2011; Wan et al., 2013; Zecca et al., 2012). Péros et al. (2011) suggested that subg. *Vitis* originated in Asia, and then dispersed to Europe and North America based on the ancestral chloroplast haplotypes. Zecca et al. (2012), however, questioned the inference and pointed out that the North American species might be older than the Asian ones, but the origin of *Vitis* was inconclusive based on chloroplast and the nuclear *RPB2* gene sequences. Wan et al. (2013) argued for the origin of *Vitis* in North America, with subsequent migration to Asia and Europe.

Ampelocissus includes ca. 95 species mostly from Africa, tropical Asia, and Australia, with only six species known from Central America (Chen and Manchester, 2007; Galet, 1967; Lombardi, 1997, 1999, 2000, 2005; Planchon, 1887; Süssenguth, 1953; Wen, 2007). The genus is characterized by inflorescences subtended by a tendril, a prominent floral disc usually with ten linear marks on its side, and the frequent association of rusty arachnoid hairs in young parts of the plant (Chen and Manchester, 2007). Based on inflorescence structure as well as leaf and seed morphology, Planchon (1887) recognized four sections, viz. sect. *Euampelocissus* Planch. (= sect. *Ampelocissus*), sect. *Nothocissus* (Miq.) Planch., sect. *Kalocissus* (Miq.) Planch. and sect. *Eremocissus* Planch. (Wen, 2007). Latiff (2001a) recognized a new section (sect. *Ridleya* Latiff) in which the inflorescence branches of the species became flattened similar to the lamellae of the *Pterisanthes* inflorescence (Latiff,

1982a).

Nothocissus was elevated from sect. *Nothocissus* of *Ampelocissus* by Latiff (1982b). *Nothocissus* was initially monotypic, with only *N. spicifera* (Griff.) Latiff (Latiff 1982b), and was similar to *Ampelocissus* sect. *Kalocissus* from the Malesian region in its floral structure and seeds (extremely rugose) (Latiff, 1982b). Latiff (2001b) expanded the generic concept of *Nothocissus* and transferred five species of *Cissus* (*C. hypoglauca* A. Gray, *C. sterculiifolia* (F. Muell. ex Benth.) Planch., *C. penninervis* F.v. Muell., *C. acrantha* Lauterb. and *C. behrmannii* Lauterb.) from Papua New Guinea and Australia to *Nothocissus*. However, recent studies cast doubt on three of these new combinations from Australia based on inflorescence morphology (Chen and Manchester, 2007) and molecular data (Rossetto et al., 2002, 2007; J. Wen, unpublished). Chen and Manchester (2007, 2011) recognized that the seeds of three Australian species (*C. hypoglauca*, *C. penninervis* and *C. sterculiifolia*) were not similar to those of *N. spicifera*. Liu et al. (2013) showed that the Australian *C. hypoglauca* formed a clade with the Neotropical *C. trianae* Planch. and another Australian species *C. antarctica* Vent., rather than showing a close relationship with *N. spicifera*. These studies indicate *Nothocissus* as defined by Latiff (1982b; 2001b) is clearly not monophyletic.

Pterisanthes (ca. 20 species) from the Malay Peninsula, Borneo, Sumatra, Java, the Philippines, and peninsular Thailand, has seeds very similar to those of *Ampelocissus*, but is characterized by the unusual applanate or laminar structure of its inflorescence (Chen and Manchester, 2007; Latiff, 1982c; Wen, 2007; Ickert-Bond et al., submitted). The morphological similarity between *Ampelocissus*, *Nothocissus* and *Pterisanthes* has long been recognized (reviewed in Ickert-Bond et al., submitted; Chen and Manchester, 2007; Latiff, 1982c), and recent molecular data have shown that *Nothocissus* and *Pterisanthes* are nested within *Ampelocissus* (Liu et al., 2013; Ren et al., 2011; Soejima and Wen, 2006; Wen et al.,

2007). Chen and Manchester (2007) designated *Ampelocissus*, *Nothocissus* and *Pterisanthes* as *Ampelocissus* s.l, which is distinguished from all other genera in Vitaceae by its seeds with long, parallel ventral infolds and a centrally positioned oval chalazal scar (Chen and Manchester, 2011).

In the context of inferring the origin of *Vitis*, previous studies (e.g., Péros et al., 2011; Wan et al., 2013; Zecca et al., 2012) had very limited sampling of non-*Vitis* taxa in Vitaceae. We herein expand the sampling scheme in the closely related genera of *Vitis* and employ sequences of five plastid (*rps16*, *trnL-F*, *atpB-rbcL*, *trnH-psbA* and *trnC-petN*) and two nuclear (*GAII* and ITS) markers. The objectives of this study are to: (1) resolve the phylogenetic relationships within the *Ampelocissus-Vitis* clade; and (2) hypothesize the origin of *Vitis* in a broader phylogenetic framework.

2. Materials and methods

2.1. Sampling, DNA isolation and sequencing

The study sampled 111 accessions representing the *Ampelocissus-Vitis* clade including 70 accessions of *Vitis* (38 spp.), 31 of *Ampelocissus* (20 spp.), eight of *Pterisanthes* (5 spp.) and two of *Nothocissus* (1 sp.), and generated sequences for five plastid (*trnL-F*, the *rps16* intron, *atpB-rbcL*, *trnH-psbA* and *trnC-petN*) and two nuclear (*GAII* and ITS) markers (Table S1). The sampling covers the geographic and morphological diversity of the clade. Six species of *Parthenocissus* were selected as outgroup of the clade, as the genus had been shown to be sister to the *Ampelocissus-Vitis* clade (Ren et al., 2011; Wen et al., 2013b). In the dating analysis, we also used 55 representative taxa of other genera in Vitaceae and Leeaceae (*Leea* van Royen ex L. as the outgroup of Vitaceae).

Total DNAs were extracted from silica gel dried leaves using the DNeasy Plant Mini Kit

(QIAGEN, California, USA) following the manufacturer's protocol. The sequences of five plastid DNA markers (*trnL-F*, *rps16*, *atpB-rbcL*, *trnH-psbA*, and *trnC-petN*) were amplified and sequenced following previous methods (Anderssons and Rova, 1999; Chen et al., 2011a; Lee and Wen, 2004; Oxelman et al., 1997; Ren et al., 2011; Soejima and Wen, 2006; Taberlet et al., 1991). For the nuclear *GAIL* gene, PCRs were first carried out using primers (1F, 1R, 2F, and 2R) of Wen et al. (2007) to amplify *GAIL* sequences. In the cases where amplification was not successful, we designed several pairs of primers to amplify the gene. These were forward primers 83F (5'-GCT TCC GAG ACT GTT CAT TAC -3'), 350F (5'-GGT AAG GCT CTY TAT TCC CAT-3'), or 496F (5'-TGA AGC CCA CAA CTT CAG CT-3') and reverse primer 1520R (5'-CTT CGC AGG CCA CCA CGT T-3'). For ITS, PCR amplification used the forward primers N-nc18S10 and ITS1 and reverse primers C26A and ITS4 (Wen and Zimmer, 1996; White et al., 1990). For *GAIL* and ITS, most amplification products were sequenced directly after purification using the QIAquick PCR Purification kit (QIAGEN, California, USA). For those PCR products that were weakly amplified and difficult to be sequenced directly, we used the QIAGEN PCR Cloning Kit (QIAGEN, California, USA) to clone and sequence at least eight clones. If more than one copy was isolated in one sample, we first constructed a phylogeny including all the copies. If multiple copies from the same sample grouped together, one copy was randomly selected in further analysis. DNA sequences were assembled using the program Sequencher version 5.0.1 (Gene Codes Corp., Ann Arbor, Michigan, USA).

2.2. Sequence alignment and phylogenetic analysis

Sequence alignment was initially performed using the program MUSCLE 3.8.31 (Edgar, 2004) in multiple alignment routine, followed by manual adjustment with the program Se-Al version 2.0a11 (Rambaut, 2002).

Phylogenetic trees of the plastid data and the combined matrix of the five plastid and two nuclear markers were reconstructed using maximum parsimony (MP; Fitch, 1971), maximum likelihood (ML) and Bayesian inference (BI) (Mau et al., 1999; Rannala and Yang, 1996). MP analyses were conducted under the heuristic search option using 10 random stepwise additions and tree-bisection-reconnection (TBR) branch swapping in PAUP* version 4.0 b10 (Swofford, 2003). Zero-length branches were collapsed and gaps were treated as missing data or coded as simple indels (Simmons and Ochoterena, 2000) using the program SeqState (Müller, 2005). Parsimony bootstrap analyses (Felsenstein, 1985) with 1,000 replicates were subsequently performed under the option fast and stepwise addition to evaluate the robustness of the MP trees.

MrModeltest 2.3 (Nylander, 2004) was used to determine the best available model for nucleotide substitutions using the Akaike Information Criterion (AIC). The chloroplast genome is generally considered as one unit without combination although there had been reports of recombination in the chloroplast genome (Stein et al., 1986). Therefore, we combined the five chloroplast data (*rps16*, *trnL-F*, *atpB-rbcL*, *trnH-psbA* and *trnC-petN*). The most appropriate nucleotide substitution model (GTR + G) was determined by MrModeltest 2.3 using AIC for each of five chloroplast data and the combined dataset. A partitioned Bayesian analysis of the five chloroplast data was then implemented by applying the GTR + G model as determined above. The SYM + G and GTR + I + Γ models were determined as the most appropriate nucleotide substitution ones for ITS and *GAIL* data, respectively. In the following ML and BI analysis the substitution models and parameters were adjusted according to the estimates of MrModeltest.

Bayesian inference was used to estimate the posterior probabilities of phylogenetic trees by employing an analysis of 5 million generations Metropolis-coupled Markov chain Monte

Carlo (MCMC) with MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). For analyses of the concatenated datasets, all datasets were partitioned with unlinked substitution models as estimated before. The sampling rate of the trees was 1,000 generations. Runs were repeated twice to confirm results. After discarding the trees saved prior to this point as burn-in, the remaining trees were loaded into PAUP*, and a 50%-majority rule consensus tree was computed to obtain posterior probabilities of the clades. Results were considered reliable once the effective sampling size (EES) for all parameters exceeded 200 as suggested by the program manual (Drummond et al., 2007).

2.3. Bayesian dating and fossil calibration

Representatives of the entire grape family plus *Leea* were sampled to help date the ages with both fossils and secondary calibrations in Viales. We used the Bayesian dating method based on a relaxed-clock model to estimate divergence times (Drummond et al., 2006; Thorne et al., 1998; Thorne and Kishino, 2002). The Bayesian coalescent approach to estimate the times and their credibility intervals was implemented in the Program BEAST 1.7.0 (Drummond and Rambaut, 2007), which employed a Bayesian MCMC to co-estimate topology, substitution rates and node ages. After optimal operator adjustment as suggested by the output diagnostics from several preliminary BEAST runs, two final independent runs (each 50 million generations) were performed on a cluster of Mac XServes for analysis of biological data at the Smithsonian Institution. Convergence between runs was assessed with MrBayes using Tracer version 1.5. After discarding the first 10% samples as burn-in, the trees and parameter estimates from the two runs were combined by LogCombiner 1.6.1 (Drummond and Rambaut, 2007). Results were considered reliable once the effective sample size (ESS) for all parameters exceeded 200 as suggested by Drummond et al. (2007). The samples from the posterior were summarized on the posterior probabilities on its internal

nodes (Drummond et al., 2007) using the program TreeAnnotator version 1.6.1 (Drummond and Rambaut, 2007) with posterior probability limit set to 0.5 and summarizing mean node heights. These were visualized using the program FigTree version 1.2.2 (Drummond *et al.*, 2007). Mean and 95% highest posterior density (HPD) of age estimates were obtained from the combined outputs using Tracer version 1.5.

In the Paleobiological Database (2010), fossils with a Vitaceae affinity date back to as early as the early Cenomanian (99.6-93.5 Ma), but the taxonomic affinities of the oldest fossils are very controversial (Chen and Manchester, 2007; Chen, 2009; Manchester et al., 2013). Vitaceae fossils are available from sediments from the late Cretaceous to the Pleistocene and include leaves, pollen, stems, and seeds (Chen and Manchester, 2007; Greguss, 1969; Manchester et al., 2013; Tiffney and Barghoorn, 1976; Wheeler and Lapasha, 1994). Nevertheless, the seed record of the family is potentially more informative for addressing questions of evolutionary and phytogeographic divergence, because these fossils can be differentiated at the generic level (Chen, 2009; Chen and Manchester, 2007, 2011). The oldest confirmed Vitaceae fossil (66 million years old) is *Indovitis chitaleyae* Manchester, Kapgate & J.Wen from the late Cretaceous of India (Manchester et al., 2013). But it seems not suitable for our calibration strategy due to the two alternative phylogenetic positions for *Indovitis* either sister to the *Ampelocissus-Vitis* clade or sister to the *Ampelopsis-Clematicissus-Rhoicissus* clade (Manchester et al., 2013).

The second oldest confirmed seed fossil is undoubtedly assigned to *Ampelocissus* s.l. (*A. parvisemina* Chen & Manchester) and dates back to the late Paleocene (56.8-62.0 Ma) in North Dakota of North America (Chen and Manchester, 2007). We used *Ampelocissus parvisemina* as our first fossil calibration to estimate the divergence time of *Vitis*, similar to the dating analyses of *Parthenocissus* (Nie et al., 2010), *Tetrastigma* (Chen et al., 2011b),

Ampelopsis (Nie et al., 2012), *Cissus* (Liu et al., 2013), and *Vitis* (Zecca et al., 2012) in Vitaceae. Because the fossil seed was assigned with certainty to the genus *Ampelocissus* (Chen and Manchester, 2007), Liu et al. (2013) assigned it as representing an early member of the *Ampelocissus-Vitis* clade. We ran the analysis constraining the crown age of this clade, with a lognormal prior distribution (mean: 58.5 Ma; log (stdev: 0.03); offset: 0 Ma; mean in real space) approximately corresponding to the time span of the late Paleocene. Fossil seeds of *Vitis macrochalaza* Tiffney from the early Miocene from the Brandon Lignite in Vermont, northeastern North America, are strikingly similar to those of the modern species *Vitis rotundifolia* Michx. (Tiffney, 1979, 1994; Tiffney and Barghoorn, 1976). Thus the fossil was assigned to the early members of subg. *Muscadinia* and used as the second calibration point of the *Vitis* divergence. We fixed the fossil to the crown of subg. *Muscadinia* with a lognormal prior distribution (mean: 20 Ma; log (stdev: 0.07); offset: 0 Ma; mean in real space) approximately corresponding to the upper and lower bounds of the early Miocene.

The late Eocene *Vitis glabra* Chandler from the lower Bagshot beds of the London Clay of southern England, is the most reliable among the earliest fossils of subg. *Vitis* based on the fossil seed morphology described in the literature (Chandler, 1957, 1960, 1961, 1962, 1963, 1964; Collinson, 1983; Mai, 2000; Manchester, 1994; Miki, 1956; Reid and Chandler, 1933; Tiffney and Barghoorn, 1976). So, this fossil was assigned to the early members of subg. *Vitis* and used as the third calibration point of the *Vitis* divergence. We ran the analysis constraining the crown age of subg. *Vitis* with a lognormal prior distribution (mean: 35 Ma; log (stdev: 0.02); offset: 0 Ma; mean in real space) approximately corresponding to the time span of the late Eocene. Gong et al. (2010) reported several fossil *Vitis* seeds including three morphotaxa from the Gray fossil site in northeastern Tennessee (7-4.5 Ma, latest Miocene to earliest Pliocene). Based on their preservation, the fossil seeds were included as members of subg. *Vitis* and described as *V. grayensis* Gong, Karsai & Liu, *V. lanatoides* Gong, Karsai &

Liu and *V. latisulcata* Gong, Karsai & Liu, most closely comparable to modern *V. balanseana* Planch. and *V. thunbergii* Sieb. & Zucc. from Asia, modern *V. lanata* Roxb. from Asia, and modern *V. labrusca* L. from North America, respectively (Gong et al., 2010). Since there is uncertainty of the phylogenetic position of the fossil species from Tennessee, we did not include these fossil seeds as calibration points. Herrera et al. (2012) described a new genus *Saxuva* Herrera, Manchester & Jaramillo based on Vitaceae fossil seeds from the late Eocene of Panama. Its type species *S. draculoidea* Herrera, Manchester & Jaramillo was described as having tetramerous flowers. The taxon exhibited characters seen in three modern genera *Cayratia*, *Cissus* and *Cyphostemma* (Herrera et al., 2012) which were placed in different clades in the Vitaceae phylogeny (Liu et al., 2013; Wen et al., 2013b), and thus it did not seem suitable for our calibration strategy.

For the root age of Vitaceae, Nie et al. (2010) fixed the split between Vitaceae and *Leea* as 85 ± 4.0 Ma based on the estimated age of 78-92 Ma by Wikström et al. (2001). Magallón and Castillo (2009) reported a pre-Tertiary origin at 90.65 to 90.82 Ma for Vitaceae. The estimated ages from Magallón and Castillo (2009) and Wikström et al. (2001) are close, but the latter was criticized for using nonparametric rate smoothing and for calibrating the tree using only a single calibration point (Nie et al., 2012). Bell *et al.* (2010) suggested a time ranging from 48 to 65 Ma for the stem age of Vitaceae. Although their estimates were based on 36 fossil calibrations in dating 567 taxa of angiosperms, they obviously underestimated the age for Vitaceae because the ages were younger than the age suggested by fossil evidence (e.g., in Chen and Manchester, 2007). We herein used the estimate from Magallón and Castillo (2009) and set the normal prior distribution of 90.7 ± 1.0 Ma for the stem age of the family (Liu et al., 2013).

2.4. Ancestral area reconstruction

To reconstruct the geographic origin of *Vitis*, a dispersal-vicariance analysis was conducted (Ronquist, 1997) using the software RASP v2.1 (Reconstruct Ancestral State in Phylogenies, available online at <http://mnh.scu.edu.cn/soft/blog/RASP>), a modified version of S-DIVA (Statistical Dispersal-Vicariance Analysis; Yu et al., 2010) using a Bayesian binary MCMC (BBM) approach. The method calculates the optimized areas over a set of trees, thus taking into account topological uncertainty (Thiv et al., 2011). We used the 7,500 trees retained from the BEAST analysis of the combined data set. The ancestral area of *Vitis* was also reconstructed by maximum likelihood (ML) optimization in Lagrange version 20120508 (Ree and Smith, 2008). The program Lagrange not only finds the most likely ancestral area at a node and the split of the areas in the two descendant lineages, it also calculates the probabilities of these most-likely areas at each node (Ree and Smith, 2008), using an ultrametric tree combining the ML topology with internal node age estimated from a BEAST analysis based on the combined data set. In our case, all combinations of areas were allowed in the adjacency matrix, and baseline rates of dispersal and local extinction were estimated. The maximum number of areas in the ancestral ranges was limited to two in RASP and Lagrange, as no species of Vitaceae is distributed in more than two areas of endemism. Four areas of endemism were defined according to the distribution of the clade of grapes and their allies: A, Southern and eastern Asia, Malesia and Northern Australasia; B, North and Central America, and the northern border of South America (with *Vitis tiliifolia* extended southward); C, Sub-Saharan Africa and Madagascar; and D, Mediterranean and Western Asia (only *Vitis vinifera*).

3. Results

3.1. Phylogenetic analyses

We used the combined matrix of all the plastid data (*rps16*, *trnL-F*, *atpB-rbcL*, *trnH-*

$psbA$ and $trnC-petN$) in our analysis. The aligned matrix of combined plastid DNA data has 6,701 characters including 1,312 parsimony-informative sites (consistency index $CI=0.60$, retention index $RI=0.82$). The aligned ITS data matrix comprises 927 characters including 533 parsimony-informative sites ($CI=0.39$, $RI=0.64$). The aligned *GAIL* data matrix has 1,493 characters including 454 parsimony informative sites ($CI=0.61$, $RI=0.83$). Two datasets of ITS and *GAIL* resulted in five major clades (A., B., C., D., E.; Fig. S2–3) with high values of Bayesian posterior probabilities ($PP>0.95$) and MP bootstrap support ($BS>0.50$). The combined chloroplast datasets support the four clades (A., C., D., E.), while clade B is not supported (Fig. S1): *A. acapulcensis* (Kunth) Planch. is sister to clade A with low supporting values ($BS<50\%$, $PP=0.74$), and *A. javalensis* (Seemann) Stevens & Pool is sister to clade E with low supporting values ($BS<50\%$, $PP=0.90$), respectively. Two clades show low PP and BS values: (1) clade E based on chloroplast sequences ($BS<50\%$, $PP=0.66$, Fig. S1), and (2) clade A based on ITS sequence data ($BS<50\%$, $PP=0.77$, Fig. S2). All of three datasets (ITS, *GAIL* and the combined plastid matrix) support that clade A includes three subclades, and that clade E includes two subclades (Fig. S1–3), except for the ITS dataset due to missing sequences (Fig. S2). We combined these three datasets based on the very similar topologies among the major clades in the current case. The concatenated data clearly show improved phylogenetic resolution of the major clades ($PP=1.00$, $BS>85\%$) (Fig. 1).

The combined matrix of the three datasets is 8,491 bp in length, containing 2,299 parsimony-informative sites. Trees generated with different methods (MP, ML, and BI) are consistent with respect to the *Ampelocissus-Vitis* clade. Therefore, only the BI strict consensus tree with MP bootstrap support (BS) and Bayesian posterior probabilities (PP) is shown (Fig. 1). The parsimony search of the combined dataset yielded more than 100,000

most parsimonious trees (CI= 0.51, RI= 0.76). The strict consensus tree of the combined dataset corresponds to the majority-rule consensus of 7,500 trees (10,000 trees minus 2,500 as burn-in) derived from the BI analysis (Fig. 1).

In the combined analysis of cpDNA, *GAIL* and ITS data, the *Ampelocissus-Vitis* clade is well supported with BS = 100% and PP = 1.00. Five clades (Fig. 1) are contained. Clade A (BS=96%; PP=1.00) consists of *Vitis* and the New World *Ampelocissus erdvendbergiana* Planch., with the latter sister to the former. *A. erdvendbergiana* forms a polytomy with both subclades of *Vitis* in *GAIL* analyses (Fig. S3). *Vitis* is supported as a monophyletic genus (BS=84%; PP=1.00). Within *Vitis*, subg. *Muscadinia* (BS=97%; PP=1.00) and subg. *Vitis* (BS=91%; PP=1.00) are each supported to be monophyletic. Within subg. *Vitis*, two groups are weakly supported, corresponding to their biogeographic distribution in the New World and Eurasia, respectively (Fig. 1). *Ampelocissus* is paraphyletic, with *Vitis*, *Nothocissus* and *Pterisanthes* nested within it (Fig. 1). Taxa of *Ampelocissus* are primarily placed in three clades (B, C and D; Fig. 1) except for the species of *A. erdvendbergiana* from the New World. Clade B consists of two New World species, *A. javalensis* and *A. acapulcensis*, forming a well supported clade (BS=90%; PP=1.00) based on the combined data (Fig. 1). These results are identical to the *GAIL* result (Fig. S3) with two exceptions, *A. javalensis* and *A. acapulcensis*. But *A. javalensis* and *A. acapulcensis* have different placements in the chloroplast tree with low support (Fig. S1): *A. javalensis* is sister to clade E (BS < 50%, PP=0.90), and *A. acapulcensis* is sister to clade A (BS < 50%, PP=0.74), respectively. We failed to obtain the ITS sequence of *A. javalensis* (Fig. S2). Clade C (BS=94%; PP=1.00) consists of the majority of the Asian *Ampelocissus* species (corresponding to *Ampelocissus* sect. *Kalocissus*) in which *Pterisanthes* from tropical Asia is nested. Clade D (BS=100%; PP=1.00) includes one species, *N. spicifera*, which is sister to clade C (BS=94%; PP=1.00). Clade E includes the African *Ampelocissus* with several Asian species (BS=88%, PP=1.00),

and is divided into two subclades (BS=51%, PP=1.00; and BS=55%; PP=1.00 respectively). Clade E roughly corresponds to *Ampelocissus* sect. *Ampelocissus* (except for species from the New World).

3.2. Divergence times of the *Ampelocissus*-*Vitis* clade

Bayesian estimation of divergence times of the *Ampelocissus*-*Vitis* clade is presented in Figure 2. The clade is estimated to have diverged from the closest relative in Vitaceae (the *Parthenocissus*-*Yua* clade) in the late Cretaceous (70.5 Ma, 95% HPD: 57.9- 82.0 Ma; node 1 in Fig. 2). The Mexican species *A. erdvendbergiana* is estimated to have split from the *Vitis* clade at in the late Eocene (39.4 Ma, 95% HPD: 32.6-48.6 Ma; node 3 in Fig. 2). The split of the two subgenera of *Vitis* is estimated to have occurred in the late Eocene (37.3 Ma, 95% HPD: 30.9-45.1 Ma; node 3 in Fig. 2).

3.3. Biogeographic origin of *Vitis*

The ancestral area of *Vitis* is reconstructed to be in the New World according to the two analyses of RASP (B (0.69) / AB (0.31), posterior probabilities = 1.00, node a in Fig. 3) and Lagrange (B|B and B|AB, with 0.83 and 0.14 relative probabilities, respectively, node a in Fig. 3). The ancestral area of subg. *Vitis* is inferred in the New World by RASP (B (0.72)/ AB (0.28), posterior probabilities = 0.83, node b in Fig. 3) and Lagrange (B|B and B|AB, with 0.77 and 0.18 relative probabilities, respectively, node b in Fig. 3).

4. Discussion

4.1. Phylogenetic relationships

Our phylogenetic results supports five major clades (A., B., C., D., E; Fig. 1) within the *Ampelocissus*-*Vitis* clade in Vitaceae. Clade A consists of *Vitis* and *Ampelocissus*

erdvendbergiana (BS=96%; PP=1.00; Fig. 1), which shows a disjunct distribution in the Neotropics, Eurasia and North America. Taxa in clade A bear cordiform seeds with short, linear, and parallel ventral infolds with round to linear cavities (Chen and Manchester, 2007, 2011). Clade A is divided into three subclades (Fig. 1). The first subclade includes only one species, *A. erdvendbergiana* from Central America (BS=100%; PP=1.00; Fig. 1). This study suggests that *A. erdvendbergiana* is most closely related to *Vitis*, and confirms that *Vitis* is monophyletic, consisting of two subgenera. The second subclade includes the only two species of *Vitis* subg. *Muscadinia*: *V. popenoei* J.H. Fennel. from Mexico and *V. rotundifolia* Michx. from the southeastern U.S.A. (BS=97%; PP=1.00; Fig. 1). Taxa of subg. *Muscadinia* have 40 chromosomes, simple tendrils, larger fruits and seeds with longer ventral infolds than the rest of species in *Vitis* (Chen and Manchester, 2011). The third subclade consists of subg. *Vitis* with 38 chromosomes, and bifurcate or trifurcate tendrils. Subg. *Vitis* includes species from temperate North America (BS<50%; PP=1.00) and their sister group from temperate Asia and Europe (BS<50%; PP=0.71; Fig. 1). Our result confirms the biogeographic disjunctions of subg. *Vitis* between North America and Eurasia (Miller et al., 2014; Péros et al., 2011; Tröndle et al., 2010; Zecca et al., 2012). Wan et al. (2013) suggested that the clades of Eurasian species were nested within the North American grade of subg. *Vitis*, but our result supports the sister relationships between the North American and Eurasian subclades (BS=91%; PP=1.00; Fig. 1) within subg. *Vitis*. Our resolution within subgen. *Vitis* is low, probably due to our marker choice, as well as recent divergences, hybridization among species, and clinal variation within species (Comeaux et al., 1987; Péros et al., 2011; Zecca et al., 2012). In our study, several species are not supported as monophyletic, because they may be more appropriately viewed as ecospecies or ecotypes rather than biological species (Zecca et al., 2012). The taxonomy of *Vitis* clearly needs to be critically assessed.

Ampelocissus is paraphyletic (Figs. 1-3). Taxa in this genus are placed in three clades

(B., C., E.; Fig. 1) except for *A. erdvendbergiana*. The three Central American *Ampelocissus* species do not form a clade (Fig. 1). *Ampelocissus acapulcensis* and *A. javalensis* constitute a clade B (BS=90%; PP=1.00; Fig. 1), whereas *A. erdvendbergiana* is sister to *Vitis* based on the combined data (Fig. 1). Clade B is strongly supported by the *GAI* as well (Fig. S3), but the cpDNA sequences do not resolve the clade (Fig. S1). The two species in clade B have oval or pyriform seeds with broad ventral infolds (Chen and Manchester, 2007). In fact, the phylogenetic positions of the New World *Ampelocissus* species have been uncertain for a long time. *Ampelocissus javalensis* was placed in *Vitis* (Seemann, 1869), *Cissus* (Planchon, 1887) or *Ampelocissus* (Lombardi, 1999). Planchon (1887) treated *A. acapulcensis* and *A. erdvendbergiana* as members of sect. *Ampelocissus*, together with species from the Old World. Our results suggest that *A. erdvendbergiana* is perhaps best treated as a member of *Vitis*, and *A. acapulcensis* and *A. javalensis* should be placed in a new genus.

Clade C consists of nine species of *Ampelocissus* and six species of *Pterisanthes* sampled from tropical Asia (BS=94%; PP=1.00; Fig. 1). These taxa have a paniculate thyrses of spikes or a thyrses with specialized lamellate inflorescence branches, mostly with sessile flowers (Ickert-Bond et al., submitted). The seeds of Asian *Ampelocissus* in clade C are oval, highly compressed dorsiventrally, smooth, flattened and with broad ventral infolds. The seeds of *Pterisanthes* are rotund and have cup-shaped and wide ventral infolds, which are very similar to those of *Ampelocissus* of clade C (Chen and Manchester, 2007). All taxa of *Ampelocissus* in clade C are distributed in the lowland forests of tropical Asia and correspond to sect. *Kalocissus* recognized by Planchon (1887). Our phylogenetic results support that *Pterisanthes* is nested within Asian *Ampelocissus* and has a very close relationship with *Ampelocissus* sect. *Kalocissus* (BS=94%; PP=1.00; Fig. 2). *Pterisanthes* differs from *Ampelocissus* morphologically mainly because of its unique lamellate inflorescence branches, but the similarities in seed morphology, petiole anatomy, indumentum types and

differentiation of the primary branch of the inflorescence into tendrils show a close relationship with *Ampelocissus* species in clade C (also see Latiff, 1982c). Van Steenis and Bakhuizen van den Brink (1967) suggested that *Pterisanthes* might be an artificial genus consisting of an assemblage of *Ampelocissus* because the former might have originated from the latter via inflorescence specialization. Latiff (2001a, 2001b) recognized *Ampelocissus* sect. *Ridleya*, including three species (*A. pterisanthella* Ridley, *A. complanata* Latiff and *A. madulidii* Latiff), which showed flattened inflorescence branches simulating the lamellae of *Pterisanthes* (Latiff, 1982a, d). However, we failed to sample the three species included in sect. *Ridleya* in our study. Our results show the polyphyly of *Pterisanthes* (Figs. 1-3), supporting the notion by van Steenis and Bakhuizen van den Brink (1967) on the artificial nature of *Pterisanthes*. We suggest new combinations need to be made to transfer these species of *Pterisanthes* to *Ampelocissus*.

Clade D includes only *Nothocissus spicifera* (Fig. 1), which is sister to the clade containing most Asian *Ampelocissus* and *Pterisanthes* (BS=94%; PP=100; Fig. 1, Clade C). Previous abovementioned studies (Chen and Manchester, 2007, 2011; Liu et al., 2013; Rossetto et al., 2007) indicated that *Nothocissus* as defined by Latiff (1982b; 2001b) was clearly not monophyletic. Latiff (2001b) transferred five species of *Cissus* to *Nothocissus*, but these new combinations (Latiff, 2001b) were questionable. Based on the sister phylogenetic relationships of the type species *N. spicifera* and *Ampelocissus* sect. *Kalocissus* (Fig. 1, Clade C) in this study as well as the similarity of their floral structure and seeds (extremely rugose) (Latiff, 1982b), we suggest *N. spicifera* need to be best transferred to *Ampelocissus*, whereas the other “*Nothocissus*” species likely form a new genus associated with Neotropical *C. trianae* according to their close phylogenetic relationship (Liu et al., 2013) or maintaining them as part of *Cissus* s.l. (J. Wen, unpublished data).

Clade E consists of species of *Ampelocissus* sampled from Africa and a few taxa from tropical Asia, including the type species of *Ampelocissus*, *A. latifolia* (BS=88%; PP=1.00; Fig. 1). These taxa possess thyrsoid inflorescences with pedicellate flowers. Their seeds are oval, more or less compressed, rugose, flattened and with wide or linear ventral infolds, except for *A. martini*, which has relatively smooth seeds (Chen and Manchester, 2007). These taxa are mainly distributed in dry and open areas in Africa and Southeast Asia, and correspond to *Ampelocissus* sect. *Ampelocissus* recognized by Planchon (1887) excluding the Central American species. We have not sampled the Australian *Ampelocissus* species, but they should be part of clade E based on the morphology of inflorescences and seeds (Planchon, 1887; Jackes, 1984; Chen and Manchester, 2007).

4.2. The New World origin of *Vitis*

Our dating analyses suggest that the *Ampelocissus-Vitis* clade have its origin in the late Cretaceous (70.5 Ma, 95% HPD: 57.9- 82.0 Ma; node 1 in Fig. 2). *Vitis* is estimated to have split from its close relatives from Central America in the late Eocene (39.4 Ma, 95% HPD: 32.6- 48.6 Ma; node 2, Fig. 2). The diversification of the *Vitis* crown group occurred in the late Eocene (37.3 Ma, 95% HPD: 30.9-45.1 Ma; node 3 in Fig. 2), with the differentiation of subg. *Muscadinia* from the New World and subg. *Vitis* from the Old and the New World. Our estimated divergence time of *Vitis* is earlier than that of Wan et al. (2013), which suggests the crown age of *Vitis* at 28.32 Ma. In subg. *Vitis*, the Eurasian lineage is estimated to have split from the New World clade at least at 35 ± 2.0 Ma (late Eocene) (Fig. 2), because *Vitis glabra* from the late Eocene of London Clay is the most reliable fossil record of an early member of subg. *Vitis* (Chandler, 1962). Our RASP and Lagrange analyses suggest that *Vitis* originated in the New World (nodes a and b in Fig. 3). Péros et al. (2011) suggested that subg. *Vitis* had its origin in Asia, and then dispersed to Europe and North America because the Asian

chloroplast haplotypes were ancestral. However, their phylogenetic relationships within subg. *Vitis* were not well supported based on their consensus Bayesian tree of combined chloroplast data. Zecca et al. (2012) suggested that the North American species of *Vitis* were older than the Asian ones, however, their result was not conclusive. In their study, the divergence time of two the subgenera of *Vitis* in the early Miocene (from 18.60 to 19.05 Ma) was much later than the fossil record of subg. *Vitis* (*Vitis glabra*) from the London Clay in the late Eocene (Chandler, 1962). Wan et al. (2013) suggested that the Eurasian species were nested within the North American *Vitis* and the divergence of the Eurasian and North American taxa occurred at 11.12 Ma. This divergence time was also much later than the fossil record of the Eurasian *Vitis* (*Vitis glabra*) (Chandler, 1962). In the abovementioned studies, the sampling of non-*Vitis* taxa in Vitaceae was limited. Our study estimates that *Vitis* originated in the New World in the late Eocene, which is consistent with the fossil record. The seed fossil records show *Vitis* species were widely distributed from the Eocene to the Pliocene of the Tertiary in North America and Europe including western Siberia (Chandler, 1957, 1962, 1963, 1964; Tiffney and Barghoorn, 1976; Tiffney, 1979; Manchester, 1994; Fairon-Demaret and Smith, 2002; Manchester and McIntosh, 2007; Gong et al., 2010). The oldest confirmed fossil of the *Ampelocissus-Vitis* clade is *Ampelocissus parvisemina*, which dates back to the late Paleocene in western North America (Chen and Manchester, 2007). The fossil record of *Vitis* (for example, the fossil seeds of *Vitis thunbergii* from the Pliocene in Japan) in eastern Asia (Miki, 1956) is much younger, which seems to argue against the *Vitis* migration route from America to Asia and Europe of Wan et al. (2013). The fossil record is consistent with the biogeographic scenario that *Vitis* originated in the New World, then dispersed to Europe, and finally to Asia, i.e., the “Out of Americas” hypothesis (Miller et al., 2011). Recent studies showed that many plant lineages originated in the New World, with subsequent dispersal to the Old World (Davis et al., 2002, 2004; Jeandroz et al., 1997; Nie et al., 2006, 2012;

Schultheis and Donoghue, 2004; Tu et al., 2010; Wen, 2011; Wen et al., 2010; Xie et al., 2009, 2010; Zhou et al., 2012). Nevertheless, eastern Asia has been suggested to be the ancestral area for many eastern Asian – eastern North American disjunct groups (Donoghue and Smith, 2004; Milne, 2006; Wen, 1999; Wen et al., 2010).

Wan *et al.* (2008) suggested that a region in China might be one of the major centers of *Vitis* diversity in Asia with over 30 species. This region lies in the Qingling-Bashan Mountains and the provinces of Jiangxi, Hubei, Hunan, and Guangxi in China. The extinction rate of species was reported to be lower in Asia, higher in North America, and the highest in Europe among Northern Hemisphere continents (Donoghue et al., 2001; Pérois et al., 2011; Ricklefs, 2005). The modern distribution pattern of *Vitis* with higher species richness in eastern Asia probably resulted from climatic fluctuations and paleogeographic changes during the late Tertiary and the Quaternary periods, which might have caused the range reduction and widespread extinction of *Vitis* members in the northern latitudes and pushed taxa southward (Aradhya et al., 2008). Detailed analyses of *Vitis* based on phylogenetic results and niche modeling (also see Wen et al., 2013c) might shed insights into the distributional dynamics of *Vitis* in the Tertiary and the Quaternary.

Three major hypotheses have been proposed to explain the intercontinental vicariance/migrations between North America and Eurasia. The North Atlantic land bridges (NALB) across the north end of the Atlantic Ocean linking northern Canada to Europe via Greenland have been viewed as a principal route for the intercontinental spread of thermophilic boreotropical flora between the Old and the New Worlds in the early Tertiary (Davis et al., 2002, 2004; Fritsch and Cruz, 2012; Tiffney, 1985a, b; Tiffney and Manchester, 2001; Wen, 1999). NALB is speculated to have existed from the early Eocene until the late Miocene, although it was possibly interrupted during the Oligocene (Manchester, 1999). The

most drastic cooling after the thermal maximum did not occur until the beginning of the Oligocene (Wolfe, 1975; Zachos et al., 2001). Boreotropical vegetation existed at much higher latitudes (50-60°N) and floristic exchanges might have occurred frequently between the Old and the New World in the Northern Hemisphere during that time (Tiffney and Manchester, 2001). In the current study, the Eurasian *Vitis* lineage is estimated to have split from that of the New World at least during the late Eocene (37.3 Ma, 95% HPD: 30.9-45.1 Ma; node 3 in Fig. 2). During the Eocene, many fossils of thermophilic taxa were recovered in the Northern Hemisphere (Reid and Chandler, 1933; Chandler, 1964; Wolf, 1975; Tiffney, 1985b), which indicated that climates during that time could support the existence of thermophilic vegetation at high latitudes (Tiffney, 1985a, b). Based on the New World origin of *Vitis* as well as the *Vitis* fossil records older in Europe than those in Asia, we suggest that *Vitis* migrated from North America firstly to Europe, then to Asia. Although most modern species of *Vitis* are distributed in the temperate regions of the Northern Hemisphere, *Vitis* species are also extending to the Neotropics; for example, *Vitis tiliifolia* extends southward to Central America and the northern border of South America and *V. popenoei* is native to Central America. *Vitis* thus contains thermophilic elements of the boreotropical flora. The NALB might be the plausible migration route of *Vitis* from North America to Eurasia. Another possible migration route was the Bering land bridge (BLB), which connected northeastern Asia and northwestern North America (Emadzade et al., 2011; Riggins and Seigler, 2012; Tiffney and Manchester, 2001; Wen, 1999). BLB has been suggested to be open to terrestrial organisms from the early Paleocene until its closure between 7.4 and 4.8 Ma (Tiffney and Manchester, 2001). Donoghue and Smith (2004) favored BLB over NALB as the primary pathway between eastern Asia and the New World, because many temperate forest plant groups dispersed to the New World from the Old World mostly during the last 30 Ma (also see Wen et al., 2010). If *Vitis* migration was via BLB, the

most parsimonious scenario might be that *Vitis* species migrated from North America to eastern Asia first, subsequently reaching Europe. Such scenario is inconsistent with the fossil records that European fossils are older than the eastern Asia ones (Chandler, 1962; Miki, 1956), which indicates that migration via BLB is less likely. A third possible explanation of the North American-Eurasian disjunction was intercontinental long distance dispersal (LDD). Although LDD is considered as an *ad hoc* explanation when a disjunct distribution can not be explained by other factors (Erkens et al., 2009), it has been mainly viewed as a dominant mechanism of distribution of many very young plant groups (Lavin et al., 2004; Renner, 2004; Clayton et al., 2009; Bartish et al., 2011). If the *Vitis* migration was via LDD, the migration route might be that *Vitis* dispersed directly from eastern North America to western Europe, and subsequently reaching Asia. Although the dispersal of *Vitis* via LDD does not seem to a parsimonious scenario because the migration from North America to Europe needs to span the broad North Atlantic region, LDD can not be eliminated as a likely mechanism. In conclusion, the fossil evidence combined with phylogenetic and dating results indicates that *Vitis* migration between the New and the Old Worlds was most likely via NALB or LDD.

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Fig. 1. Phylogenetic relationships of grapes and their close allies using the combined datasets of chloroplast, ITS and *GAIL* sequences based on the BI strict consensus cladogram with MP bootstrap support (numbers below branches) and Bayesian posterior probabilities (numbers above branches). Bold branches represent PP=1.00 and BS >90%.

Fig. 2. Chronogram of *Vitis* based on the combined plastid and nuclear datasets inferred from BEAST. Blue bars represent the 95% highest posterior density credibility interval for node ages. Calibration points are indicated with stars. Estimated divergence times of grapes and their close allies are indicated at the nodes (nodes 1–3) using circles and the estimated ages are shown on the left.

Fig. 3. Ancestral area reconstruction of the *Ampelocissus-Vitis* clade based on RASP and Lagrange analyses. The tree was based on a 50% majority-rule consensus tree of a Bayesian Markov chain Monte Carlo (MCMC) analysis of the combined dataset. The four areas of endemism are: A, Southern and eastern Asia, Malesia and Northern Australasia (blue circle); B, Northern and Central America and Northern South America (pink circle); C, Sub-Saharan Africa and Madagascar (brown circle); and D, Mediterranean and Western Asia (black circle). Painted areas in the map represent distributions of different genera: green, *Vitis*; yellow, *Ampelocissus*; orange, *Nothocissus*; and red, *Pterisanthes*. Colored circles at the tip of the nodes in the tree indicate species distributions as seen in the map below. Results of Lagrange and RASP analyses were at the upper and lower sides of a short line, respectively. For the Lagrange results, a slash indicates the split of areas into two daughter lineages, i.e., left/right, where “up” and “down” are the ranges inherited by each descendant branch; the values in brackets represent relative probabilities. For the RASP ones, the values in brackets represent S-DIVA support. Nodes a and b in the tree show the ancestral areas of the different clades. Red and purple dashed arrows in the map indicate *Vitis* migration route via NALB or LDD between the New and the Old Worlds, respectively.

Figure 1

Figure 2

Figure 3

Pterisanthes stoei

Ampelocissus martini

Vitis chunganensis