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Research Article

Phylogenetic relationships, hybridization events, and drivers of diversification of East Asian wild grapes as revealed by phylogenomic analyses

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Abstract East Asian wild grapes show a high level of species diversity and have been widely recognized as important germplasm resources of wine, table grapes, and resistance to biotic and abiotic stresses for the grape industry. However, the deeper phylogenetic relationships and drivers of diversification of East Asian *Vitis* remain poorly understood. Hybridization and introgression events of East Asian *Vitis* are not well investigated, particularly at the genome-wide scale. The phylogenetic relationships of East Asian *Vitis* are herein explored using nuclear and plastid genome data based on target enrichment (Hyb-Seq). Seven major clades are recognized for East Asian *Vitis* based on the nuclear phylogenetic trees, but there is topological incongruence between concatenated and coalescent analyses. Furthermore, significant cytonuclear discordance is observed within East Asian *Vitis*. Species network analyses identified several hybridization events within East Asian *Vitis*. These interspecific hybridization events may have caused the topological discordances and relatively low support detected in our analyses. Ecological niche modeling shows that most of the diversification of East Asian *Vitis* species is driven by temperature and precipitation environmental variables. Sympatric parallel diversifications of major clades also may have facilitated the rich diversity in East Asian *Vitis*.

Key words: East Asia, geographic isolation, hybridization, phylogenomics, sympatric parallel diversifications, *Vitis*, wild grapes.

1 Introduction

The domesticated grape (*Vitis vinifera* L.) is well-known for its agricultural and economic significance among important fruit crops (Wen, 2007; Myles et al., 2011; Wen et al., 2018). Wild grapes are important germplasm resources for enhancing the resistance of the domesticated grape to biotic and abiotic stresses (Zhang et al., 2012; Gerrath et al., 2015; Bhattarai et al, 2021; Wang et al, 2021b). The grape genus *Vitis* L. consists of ca. 75 species with two intercontinental distribution centers of diversity in the Northern Hemisphere: North America and Eurasia (Galet, 1988; Chen et al., 2007; Wen et al., 2007; Wan et al., 2013; Liu et al., 2016; Moore & Wen, 2016; Ma et al., 2018a). There are two subgenera in the grape genus: subgenus *Muscadinia* (Planch.) Rehder ($2n = 40$) with two species from North America to Mexico and the Caribbean, and the more species-rich subgenus *Vitis* ($2n = 38$) widely distributed in the Northern Hemisphere, but with one species extending to northern South America (Moore, 1991; Wen, 2007; Moore & Wen, 2016; Wen et al., 2018). Corresponding to the geographic distributions, there are three main clades of subgenus *Vitis*: the North American, the European, and the East Asian clades. These have been supported by several studies using molecular evidence (Tröndle et al., 2010; Péros et al., 2011; Zecca et al., 2012; Aradhya et al., 2013; Miller et al., 2013; Wan et al., 2013; Liu et al., 2016; Klein et al., 2018; Ma et al., 2018a; Wen et al., 2018). There is a high diversity of wild grape germplasm resources in East Asia with ca. 60% of the *Vitis* species in the world found in East Asia (Chen et al., 2007; Wan et al., 2008). However, a few nuclear and plastid markers have not been adequate to resolve the phylogenetic relationships among species within East Asian *Vitis* (Tröndle et al., 2010; Péros et al., 2011; Zecca et al., 2012; Aradhya et al., 2013; Miller et al., 2013; Wan et al., 2013; Liu et al., 2016). The phylogenetic relationship of *Vitis* at the generic level has been reconstructed with genomic, enriched nuclear loci, and chloroplast genomes (Liang et al., 2019; Nie et al., 2019; Liu et al., 2021). For example, a phylogenetic study

of subgenus *Vitis* by Ma et al. (2018a) using 2068 single-copy orthologous genes suggested that East Asian *Vitis* contained at least four clades. However, these studies had limited sampling of East Asian *Vitis* and showed that the deeper phylogenetic relationships and the species delimitations of East Asian *Vitis* remained poorly understood.

Introgressive hybridization is considered as an important driving force of angiosperm evolution (Ellstrand & Schierenbeck 2000; Linder & Rieseberg, 2004; Vriesendorp & Bakker, 2005; Soltis & Soltis, 2009; Harrison & Larson, 2014; Vargas et al., 2017). Hybridization often leads to chromosomal rearrangements, whole-genome duplication, and differential gene expression, some of which have played an important role in plant speciation and adaptive radiations (Linder & Rieseberg, 2004; Seehausen, 2004; Baack & Rieseberg, 2007; Alix et al., 2017, Feliner et al., 2020). Interspecific hybridization has been estimated to have occurred in at least 25% of flowering plant species (Mallet, 2005, 2007). However, signals of genome-wide introgression are difficult to detect, especially in older interspecific hybridization events (Soltis & Soltis, 2009). Thus, more robust data and analyses are required to explore hybridization events, and to distinguish incomplete lineage sorting (ILS) from hybridization (both can lead to admixture signals and incongruence between nuclear and plastid phylogenies) (Sun et al., 2015; Vargas et al., 2017). An increasing number of natural hybridization or introgression events have been identified in crops and wild species with genomic analyses recently (Rieseberg et al., 2007; Eaton & Ree, 2013; Penjor et al., 2016; Yang et al., 2016; Vargas et al., 2017; Morales-Briones et al., 2018; Lee-Yaw et al., 2019; Reichelt et al., 2021; Rose et al., 2021; Wang et al., 2021a). For example, ancient and recent hybridization events in the genus *Lachemilla* (Focke) Rydb. were identified based on significant phylogenetic discordance as well as results of species network analyses using targeted enrichment capture analyses (Morales-Briones et al., 2018). An extensive organellar introgression in wild annual sunflowers (*Helianthus* L.) was suggested as the driver of cytonuclear discordance based on plastome sequence and nuclear data (Lee-Yaw et al., 2019). Rose et al. (2021) explored the evolutionary history of the genus *Polemonium* L. and showed that the cytonuclear discordance in this recently radiated genus was caused by both ILS and reticulate evolution using phylogenomic data.

Gene flow among North American *Vitis* species has been reported by several previous studies (Péros et al., 2011; Miller et al., 2013; Wan et al., 2013; Moore & Wen, 2016; Nie et al., 2019; Zecca et al., 2019; Morales-Cruz et al., 2021). Three North America taxa, *Vitis* × *champinii* Planch., *Vitis* × *doaniana* Munson ex Viala, and *Vitis* × *novae-angliae* Fernald, have been recognized as natural hybrid species based on morphological evidence (Galet, 1988; Moore, 1991; Moore & Wen, 2016). These hybrid hypotheses had been supported by molecular evidence in recent years (Péros et al., 2011; Nie et al., 2019). Wen et al. (2020) revealed the hybrid origin of the widely cultivated Concord grape using plastid and nuclear data that supported this popular grape cultivar in North America as a complex product of hybridization between *Vitis labrusca* L. and *V. vinifera*. Furthermore, hybridization events of other North American grapes, such as among *Vitis shuttleworthii* House, *Vitis mustangensis* Buckley, and *Vitis cinerea* (Engelmann) Millardet species complexes, have been identified based on phylogenomic evidence (Nie et al., 2019). The East Asian subgenus *Vitis* represents an additional excellent model for exploring speciation via hybridization in a rapidly radiated group (Ma et al., 2018a, 2018b, 2020; Wen et al., 2018). However, hybridization and introgression events of East Asian *Vitis* have not been as well investigated as North American *Vitis*, particularly at the genomic level, although Ma et al. (2018b) identified reticulate evolution among several East Asian taxa from northern China using RAD-seq data.

Ecological niche divergence is suggested as a driver for promoting diversification among closely related species and at higher levels (Castro-Insua et al., 2018; Duno De Stefano et al., 2020). Ecological niche modeling is a method for evaluating the impacts of climate factors on species divergence and predicting potential distributions of species, which is widely used in diverse biodiversity studies (Pearson & Dawson, 2003; Rissler & Apodaca, 2007).

Targeted enrichment approaches, such as the Hyb-Seq method, are reliable tools to explore phylogenetic and evolutionary history in flowering plants based on hundreds of nuclear loci with reducing genomic complexity and sequencing costs (Weitemier et al., 2014; Vatanparast et al., 2018; Dodsworth et al., 2019; Mandel et al., 2019; Lichter-Marck et al., 2020; Watson et al., 2020; Ma et al., 2021). Targeted enrichment provides a way to get genomic regions of interest by hybridization to design specific probe sets and target sequencing. Recently, target probe sets of grapes had been developed for investigations of phylogenetic relationships and introgressions of *Vitis* (Nie et al., 2019; Liu et al., 2021; Ma et al., 2021). In this study, we aim to reconstruct the phylogenetic relationships of East Asian *Vitis* using nuclear Hyb-Seq data and nearly complete plastome sequences. More systematic studies on introgression and gene flow of East Asian *Vitis* will enhance our understanding of the evolutionary history and speciation processes of East Asian grapes.

2 Material and Methods

2.1 Taxon sampling and DNA extraction

We sampled a total of 69 accessions of *Vitis* in this study (Table S1), including 63 accessions from East Asian *Vitis*, which represent >70% of taxonomic diversity in the East Asian clade of subgenus *Vitis*. Two European and four North American species were treated as outgroups in this study (Ma et al., 2018a, 2021; Nie et al., 2019). Publicly available Hyb-Seq and resequencing data of 58 accessions of *Vitis* were used in this study (Table S1). For the remaining 11 newly sampled accessions of subgenus *Vitis* from East Asia, DNA was extracted from silica gel dried leaves with a modified CTAB protocol (Table S1) (Doyle & Doyle, 1987; Lodhi et al., 1994). We obtained the target 346 nuclear genes from our earlier Hyb-Seq and resequencing studies (Ma et al., 2018a, 2021; Nie et al., 2019) using the workflow of capturing single-copy nuclear genes in Liu et al. (2021).

2.2 Library preparation and target capture sequencing

Extracted DNAs were quantified with a Qubit 4.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), either diluted with 1X TE buffer or concentrated via vacuum centrifugation, to yield appropriate DNA concentrations for library preparation. Extracted DNAs were sheared by sonication (QSonica Q800RS) to an average sheared size of 500 bp. DNA libraries were constructed using a NEBNext Library Preparation Kit for Illumina following the manufacturer's protocol. Target enrichment baits for *Vitis* that generate 346 nuclear genes were designed based on our previous studies (Wen et al., 2013; Ma et al., 2021) and a comparison to the complete *Vitis vinifera* genome (Jaillon et al., 2007). Solution-based hybridization with an MYBaits target enrichment system (MYcroarray, Ann Arbor, MI, USA) followed a standard protocol (Weitemier et al., 2014). About 40% of unenriched libraries were mixed with target-enriched libraries for recovering the chloroplast sequences as by-products. Pooled libraries were then sequenced on an Illumina HiSeq. 4000 platform with 150 paired-end reads. Phred scores were used to evaluate sequencing quality (Ewing & Green, 1998).

2.3 Sequence filtering and assembly

Raw reads were trimmed using Trimmomatic version 0.39 (Bolger et al., 2014) with a sliding window of 4 bp. Then FASTQC version 0.11.8 (Andrews, 2010) was then used to evaluate the filtered reads. The HybPiper pipeline was used to assemble the dataset and quality-trimmed reads were mapped to the reference sequence of the target nuclear loci (Johnson et al., 2016). Plastid genomic data were assembled by BWA v.0.7.17 (Li & Durbin, 2009) with the complete chloroplast genome of *V. vinifera* (NC_007957) as the reference.

2.4 Phylogenetic analyses

HybPiper-assembled nuclear sequences were aligned with MAFFT version 7.407 (Katoh & Standley, 2013), and trimmed using trimAL version 1.3 (Capella-Gutiérrez et al., 2009). All alignments were concatenated into a supermatrix using AMAS (Borowiec, 2016) for phylogenetic reconstruction with RAxML version 8.2.12 with a GTR + GAMMA model and 100 bootstrap replicates (Stamatakis, 2014). Individual gene trees for each of the target nuclear loci were inferred separately using RAxML version 8.2.12 (Stamatakis, 2014), and the best partitioning scheme and substitution model were selected by using PartitionFinder version 2.1.1 (Lanfear et al., 2012). From these, a coalescent phylogeny was conducted using ASTRAL-III with a standard parametric model (Zhang et al., 2017). Chloroplast sequences of 66 of the 69 accessions (excepting *Vitis ficifolia* Wen13234, *Vitis flexuosa* Wen13281, and *V. flexuosa* Wen13672 due to significant missing data) were aligned with MAFFT version 7.407 (Katoh & Standley, 2013) and then concatenated into a supermatrix for reconstructing a phylogenetic tree with RAxML version 8.2.12 with a GTR + GAMMA model and 100 bootstrap replicates (Stamatakis, 2014).

2.5 Inference of hybridization events

To explore possible reticulate evolution occurrences in East Asian *Vitis*, we employed a species network method SNaQ (Solís-Lemus & Ané, 2016; Solís-Lemus et al., 2017). SNaQ was implemented in the Julia package PhyloNetworks, to generate a phylogenomic network based on hundreds of nuclear loci. Considering the balance of computational cost and the representation of taxonomic diversity, we sampled 20 accessions across *Vitis* for the SNaQ analyses. We inferred the fit of network models allowing from one to nine reticulation events (h_{max}) with 50 search replicates. For each value of h_{max}, we selected the network with the lowest log-likelihood scores as the best result (Solís-Lemus et al., 2017).

2.6 Environmental variables and niche analysis

The occurrence records of East Asian grapes were collected from the global biodiversity information facility (GBIF, www.gbif.org), the national specimen information infrastructure (NSII), the Chinese virtual herbarium (CVH, www.cvh.org.cn), herbaria (US, PE, and CCAU), and fieldworks. These records were filtered to remove duplicates and low-resolution coordinates. Only species with more than five distribution records were used for subsequent analyses (Table S2).

A total of 35 environmental factors, including 19 bioclimatic, 15 soil, and 1 elevation variables, were selected for identifying potential contributions to the diversity pattern of East Asian *Vitis* (Table S3). Bioclimatic and elevation variables with 30 arc seconds (ca. 1 km) were downloaded from the WorldClim (www.worldclim.org/), and soil variables were used from the Harmonized World Soil Database version 1.2 with 30 arc seconds (HWSD, www.fao.org). For all variables, the Pearson correlation coefficient (r) was calculated to avoid over-fitting and improve the accuracy of the model prediction. After removing the highly correlated ones ($|r| > 0.8$), the remaining factors were used for the next analysis. The niche suitability of East Asian grapes was estimated by calculating the current ecological niche model (ENM) in MAXENT 3.4.4 (Phillips & Dudik, 2008). In order to evaluate the models, a jack-knife test and the area under the receiver operating characteristic (ROC) curve (AUC) value were calculated in MAXENT.

3 Results

3.1 Phylogenetic inferences using nuclear data

For the newly generated Hyb-Seq data of East Asian *Vitis* (Table S4), we obtained 16.4 Gb of raw data. The average number of reads per sample was ca. 4 964 098 with an average quality of 93.99% of bases that were ≥Q30. The raw sequencing data of this study are available in the National Center for Biotechnology Information (NCBI) under the BioProject PRJNA868429. Both nuclear concatenated and coalescent inferences supported the monophyly of East Asian *Vitis* (Fig. 1). The concatenated tree supported seven main clades within East Asian *Vitis* (Fig. 1A). The phylogenetic relationships with coalescent analyses based on 346 nuclear gene trees using ASTRAL-III (Zhang et al., 2017) also suggested the seven clades recovered by the concatenated tree (Fig. 1B). The identified seven clades of East Asian *Vitis* are: (1) the *Vitis davidii* clade including two taxa: *V. davidii* (Roman. du Caill.) Foex. and *Vitis wilsoniae* Veitch; (2) the *Vitis flexuosa* clade including two taxa: *V. flexuosa* Thunb. and *Vitis yunnanensis* C. L. Li; (3) the *Vitis hancockii* clade including

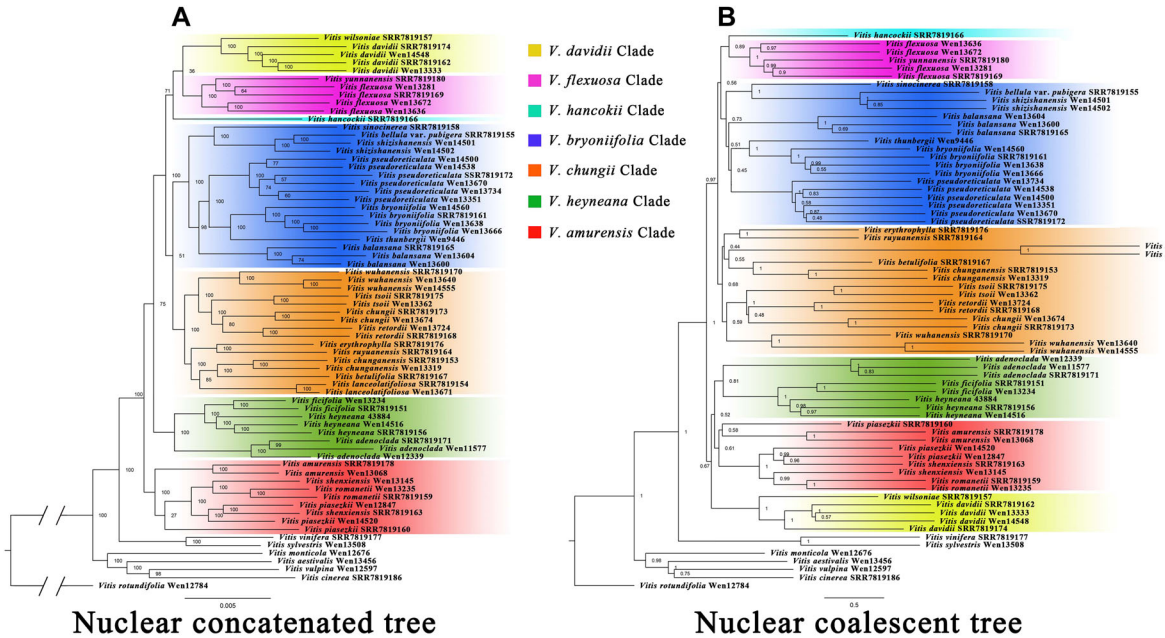

Fig. 1. Phylogenetic relationships of East Asian *Vitis*. **A**, Concatenated tree of East Asian *Vitis* based on RAxML analyzed using nuclear data. **B**, Coalescent tree of East Asian *Vitis* based on ASTRAL-III analyzed for 346 nuclear sequences. The names of clades are represented with the color font matching the central color scheme. The outgroup taxa (New World and European *Vitis*) are shown in black.

only one species: *V. hancockii* Hance; (4) the *Vitis bryoniifolia* clade containing seven taxa: *Vitis sinocinerea* W. T. Wang, *Vitis bellula* (Rehd.) W. T. Wang var. *pubigera* C. L. Li, *Vitis parvifolia* (Roxb.) Gagnep., *Vitis pseudoreticulata* W. T. Wang, *V. bryoniifolia* Bunge, *Vitis thunbergii* Sieb. & Zucc., and *Vitis balansana* Planch.; (5) the *Vitis chungii* clade including nine taxa: *Vitis wuhanensis* C. L. Li, *Vitis tsoii* Merr., *V. chungii* Metcalf, *Vitis retordii* Roman. du Caill. ex Planch., *Vitis erythrophylla* W. T. Wang, *Vitis ruyuanensis* C. L. Li, *Vitis chunganensis* Hu, *Vitis betulifolia* Diels & Gilg, and *Vitis lanceolatifoliosa* C. L. Li; (6) the *Vitis heyneana* clade containing three taxa: *V. heyneana* Roem. & Schult., *Vitis ficifolia* (Bge.) C. L. Li, and *Vitis adenoclada* Hand.-Mazz.; and (7) the *Vitis amurensis* clade including four taxa: *V. amurensis* Rupr., *Vitis shenxiensis* C. L. Li, *Vitis romanetii* Roman. du Caill. ex Planch., and *Vitis piasezkii* Maxim (Fig. 1).

The concatenated analysis robustly supported that the *V. amurensis* clade was sister to the remaining East Asian *Vitis* (Fig. 1A), while, coalescent methods suggested that the *V. amurensis* clade grouped with the *V. davidii* clade and the *V. heyneana* clade with relatively low support (Fig. 1B). The phylogenetic relationships of the *V. davidii* clade and the *V. heyneana* clade were not well supported by either analysis (Fig. 1).

3.2 Phylogenetic analyses based on plastid data

The plastid tree strongly supported a monophyletic East Asian *Vitis* (Fig. S1). However, there were significant topological differences between the plastid and nuclear trees for East Asian *Vitis* (Figs. 1, S1). The seven main clades of nuclear trees were not supported by the plastid analysis, and a widespread cytonuclear discordance was detected at the individual level (Fig. S1). For example, *V. hancockii*, which was treated as a monotypic clade due to its unique morphological characteristics, was sister to *V. thunbergii*, while, *Vitis pesudoreticulata* with multiple individuals sampled was inferred to be non-monophyletic in the plastid tree (Fig. S1).

3.3 Inference of hybridization events

The occurrence of one to nine reticulation events was explored with SNaQ analyses (Text S1). Our results suggested that the best network model with the highest log-likelihood scores in SNaQ inferences was $h_{max} = 5$ with a maximum number of five hybridization events allowed (−ploglik = 2335, Fig. 2). The inheritance probability in the reticulation event was estimated by the parameter gamma (γ), which measured the proportion of the genome contributed from each parent. Three reticulation events with significant γ inheritance values were detected in our result (Fig. 2). Hybrid edges were annotated with their γ inheritance values and indicated that there was gene flow between *V. shenxiensis* and *V. piasezkii* ($\gamma = 0.394$), between *V. adenoclada* and the most recent common ancestor of *V. heyneana* and *V. ficifolia* ($\gamma = 0.218$), and between *V. bryoniifolia* and *V. retordii* ($\gamma = 0.259$), respectively (Fig. 2).

3.4 Effects of environmental variables for distributions of East Asian *Vitis*

The current potential distributions of 25 East Asian *Vitis* species were predicted with MAXENT 3.4.4 (Fig. S2) and our results indicated that distributions of most of the East Asian *Vitis* species were mainly impacted by temperature and precipitation environmental variables, although a few species

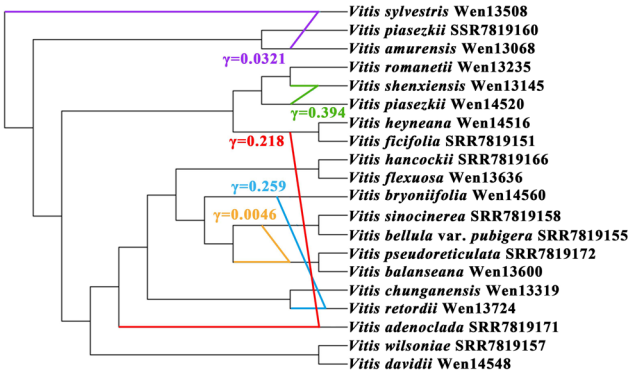

Fig. 2. Optimal SNaQ network using 20 East Asian *Vitis* species with $h_{max} = 5$ based on the highest pseudo-likelihood score (−ploglik = 2335). The parameter γ indicating the proportion of the genome involved in the reticulation.

also were influenced by elevation range and soil environmental variables (Tables S3, S5). Environmental variables with relative contributions of more than 10% were treated as major variables in the model. Four major temperature and precipitation environmental variables, BIO2 = mean diurnal range (mean of monthly [max temp − min temp]), BIO6 = min temperature of the coldest month, BIO17 = precipitation of driest quarter, and BIO18 = precipitation of warmest quarter, were detected, which affected multiple species of East Asian *Vitis* (Fig. 3).

4 Discussion

4.1 Phylogenetic relationships of East Asian *Vitis*

The backbone phylogeny of subgenus *Vitis* has been investigated by previous studies that suggested three major clades in subgenus *Vitis*: North American, European, and East Asian clades corresponding to their large-scale geographic distribution (Tröndle et al., 2010; Péros et al., 2011; Zecca et al., 2012; Aradhya et al., 2013; Miller et al., 2013; Wan et al., 2013; Liu et al., 2016; Klein et al., 2018; Ma et al., 2018a; Wen et al., 2018; Nie et al., 2019; Liu et al., 2021). However, the deep phylogenetic relationships of *Vitis* based on limited gene sequences were poorly resolved due to the lack of sufficient informative characters. Wen et al. (2018) sequenced whole plastomes of 53 accessions of *Vitis* to reconstruct the chloroplast phylogeny of the North American *Vitis*. Furthermore, phylogenetic analyses of North American subgenus *Vitis* with 300 accessions were constructed using thousands of single nucleotide polymorphism (SNP) markers, primarily for nuclear DNA by Klein et al. (2017). Zecca et al. (2019) reconstructed the phylogenetic relationships of 31 North American wild *Vitis* species with genome-wide SNPs. Our previous study showed a broader phylogenetic framework of East Asian subgenus *Vitis* using thousands of single-copy orthologous genes (Ma et al., 2018a). However, the phylogenomic analysis by Ma et al. (2018a) again only sampled one individual per species. Our present results herein using multiple individuals per species support a novel taxonomic hypothesis for East Asian *Vitis* and identify seven main clades based on the phylogenetic trees derived from the nuclear Hyb-Seq data (Fig. 1). Most taxa sampled with multiple individuals are found to be monophyletic in both the concatenation and the coalescent analyses of the nuclear data (Fig. 1). Phylogenomic analysis based on concatenated datasets suggests that the *Vitis amurensis* clade, including four taxa from northern China, was sister to the other grapes in East Asia: the *Vitis hancockii* clade, the *Vitis davidii* clade, the *Vitis flexuosa* clade, the *Vitis bryoniifolia* clade, and the *Vitis chungii* clade, which are mainly distributed in southern China to Southeast Asia (Fig. 1A). This result also supports the importance of northern China/northeastern Asia in the early evolution of East Asian *Vitis* (Fig. 1A) (Ma et al., 2018a).

Although the plastid analyses support the East Asian *Vitis* as a monophyletic group, there is cytonuclear discordance within East Asian *Vitis* (Fig. S1). Some taxa sampled with multiple individuals are inferred to be non-monophyletic in the plastid phylogeny (Fig. S1). Discordance between nuclear and plastid genomes has been observed to be prevalent in many plant lineages, which may be due to hybridization, ILS, horizontal gene transfer, and/or gene duplication (Rieseberg & Soltis, 1991; Soltis & Soltis, 2009; Arcila et al., 2017; Morales-Briones et al., 2018). Given that East Asian *Vitis* has undergone a rapid radiation (Ma et al., 2018a), rapid haplotype fixation in plastid genomes of East Asian *Vitis* may have occurred (Rieseberg & Soltis, 1991; Moore, 1995).

4.2 Hybridization events in East Asian *Vitis*

Natural hybridizations and introgressions have been observed and confirmed among species of subgenus *Vitis*, which may be caused by incomplete reproductive isolation mechanisms (Péros et al., 2011; Aradhya et al., 2013; Miller et al., 2013; Moore & Wen, 2016; Ma et al., 2018b; Zecca et al., 2019; Morales-Cruz et al., 2021; Wen & Ma, 2021). However, there are very limited studies documenting the hybridization and introgression of *Vitis* taxa from East Asia. Hybridizations among *V. amurensis*, *Vitis romanetii*, *Vitis shenxiensis*, and *Vitis piasezkii* have been supported at the population level based on morphological, biogeographic, and molecular evidence (Ma et al., 2018b). The SNaQ results show that *V. shenxiensis* is a hybrid with a 60.6% proportion sister to *V. romanetii* and a 39.4% proportion sister to *V. piasezkii* Wen14520 (Fig. 2). The network analyses further confirm the hybrid origin of *V. shenxiensis* with *V. romanetii* and *V. piasezkii* as its likely parental species (Fig. 2). Admixture signals have been found between some samples of

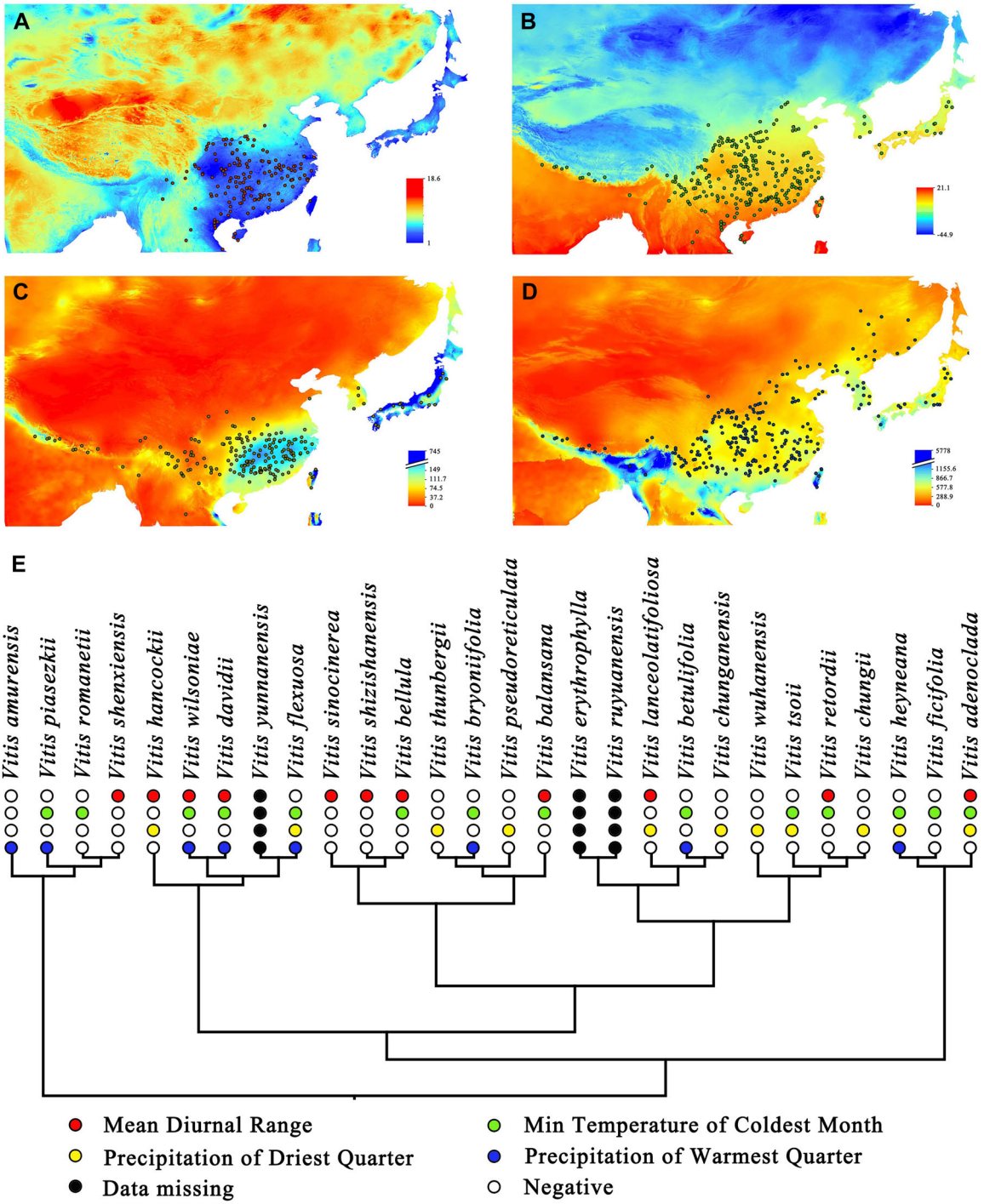

Fig. 3. Four major temperature and precipitation environmental variables with relative contributions of more than 10% are shown for multiple species. BIO2 = mean diurnal range (mean of monthly [max temp − min temp]), BIO6 = min temperature of the coldest month, BIO17 = precipitation of driest quarter, and BIO18 = precipitation of warmest quarter. **A**, Distribution of 11 *Vitis* species with the mean diurnal range as the major environmental variable. **B**, Distribution of 14 *Vitis* species with a minimum temperature of the coldest month as the major environmental variable. **C**, Distribution of 11 *Vitis* species with precipitation of the driest quarter as the major environmental variable. The excessively long scale is interrupted. **D**, Distribution of eight *Vitis* species with precipitation of the warmest quarter as the major environmental variable. Excessively long scale is interrupted. **E**, Different major environmental variables for *Vitis* species within and across clades, red circles represent the mean diurnal range (see A), green circles represent the mean minimum temperature of the coldest month (see B), yellow circles represent precipitation of the driest quarter (see C), blue circles represent precipitation of the warmest quarter (see D).

V. piasezkii, such as the accession *V. piasezkii* SSR7819160, and *V. amurensis*, which may be caused by ILS or hybridization events (Ma et al., 2018a, 2018b). Our result supports that the accession *V. piasezkii* SSR7819160 has a close relationship with *V. amurensis* (Fig. 2). A hybrid origin for *Vitis adenoclada* has been proposed due to the similar morphological characteristics, and suggests that *Vitis heyneana* is one of the parental species of *V. adenoclada* (Fig. S3) (Wen & Ma, 2021). The SNaQ result supports gene flow between *V. adenoclada* and the most recent common ancestor of *V. heyneana* and *Vitis ficifolia* (Fig. 2). Considering the red-purple glandular trichomes on the branches of *V. adenoclada* as the most important morphological character to distinguish this taxon from *V. heyneana*, species with glandular or modified glandular trichomes, such as *V. davidii* and *V. romanetii*, are suggested as the other potential parent (Ma et al., 2016; Wen & Ma, 2021). Specifically, our results suggest *V. davidii* or the recent common ancestor of *V. davidii* and *V. wilsoniae* as the most likely parental species for *V. adenoclada* (Figs. 2, S3). These ancient interspecific hybridization events may have caused the topological discordances and low support present in our analyses of nuclear and chloroplast genomes (Figs. 1, S1). A hybridization scenario as an important driver in the speciation of the East Asian *Vitis* provides important insights into our understanding of the evolutionary diversification of the Old World grapes.

4.3 Diversification of subgenus *Vitis* in East Asia

Investigating the roles of geographic, ecological, and species-specific biological traits has been recommended for exploration of patterns of species diversification and drivers of adaptive radiations (Siqueira et al., 2020). Although parallel radiations usually happen in different locations due to the intense competitive interactions, parallel diversifications of closely related clades had been reported, which might also occur in sympatry (Kozak et al., 2009; Hipp et al., 2018). The pattern of sympatric parallel diversifications of two major oak clades in the Americas was observed as a driver to shape species diversity (Hipp et al., 2018). Adaptive morphological traits similarities among distinctly related *Vitis* species were observed as a result of parallel evolution (Ickert-Bond et al., 2018; Ma et al., 2020). East Asian *Vitis* has been inferred to have undergone rapid adaptive radiations after species migrations from northeastern Asia to south East Asia. This hypothesis is supported by the observation of higher species diversity in southern China than in northern China (Ma et al., 2018a). We examined correlations between the phylogenetic relatedness within East Asian grapes, geographic distributions of *Vitis* species, and niche factors across environmental gradients. The well-resolved phylogenomic framework demonstrates sympatric parallel diversification among major clades of the East Asian subgenus *Vitis* (Figs. 1, 3, S4). Shifts in geographic distributions and ecological types have also driven the rapid diversification of the East Asian subgenus *Vitis* and are responsible for a major North/South divide in species richness within Asian *Vitis* subgenus *Vitis* (Figs. 3, 4; Table S5). The distinct diversifications within and among the seven main clades of the East Asian subgenus *Vitis* can be explained by geographic isolation and niche differentiation, even though there are overlapping distributions (Figs. 3, 4, S4; Table S5). Significant differences in geographic distributions of five species-pairs (*Vitis shizishanensis–Vitis bellula*, *Vitis thunbergii–Vitis bryoniifolia*, *Vitis erythrophylla–Vitis ruyuanensis*, *Vitis betulifolia–-Vitis chunganensis*, and *Vitis chungii–Vitis retordii*) and two species group (the *V. amurensis* clade and the *V. heyneana* clade) in four main clades of the East Asian *Vitis* have been shown, which indicate that geographic isolation among closely related species within clades plays an important role in speciation of the East Asian *Vitis* (Fig. 4).

Our results also indicate that most of the East Asian *Vitis* species are mainly driven by temperature and precipitation environmental variables (Fig. 3; Table S5). The mean diurnal range as the major environmental variable influences 11 *Vitis* species across six clades with relative contributions of more than 10%, which mainly occur in central and southern China (Figs. 3A, 3E; Table S5). Extreme climate variables, such as the minimum temperature of the coldest month (BIO6) and precipitation of the driest quarter (BIO17), are significantly correlated with distributions of many *Vitis* species in multiple clades (Figs. 3B, 3C, 3E; Table S5). The remarkable impact and distribution limitation of relatively high precipitation of the driest quarter for some species distributed in southern China have been shown (Fig. S5). Several East Asian *Vitis* species with wide distributions are driven by precipitation of the warmest quarter, which suggests that the East Asian monsoon climate with synchronization of heat and rain has a significant impact on occurrences of East Asian *Vitis* and prevents widely distributed grapes to expand to the more arid inland areas of Asia. (Figs. 3D, 3E, S4). On the other hand, soil-related factors also influence the distributions of the East Asian *Vitis* species, such as available water capacity in soil (AWC) (Table S5). Especially, the distribution of *V. chungii* is mainly influenced by the cation exchange capacity of the clay fraction in topsoil (T CEC CLAY) (Table S5). The elevation range shows a correlation with distributions of four species with relative contributions of more than 10%, that is, *Vitis pseudoreticulata*, *V. shizishanensis*, *Vitis wuhanensis*, and *V. hancockii*, which indicate that those species adapt to the relatively low-elevation niches and habitats (Table S5). Various clades of East Asian *Vitis* species occupy diverse habitat types and ecological conditions with special physiological and morphological traits (Ma et al., 2018a). Moderate to high climbing ability has been inferred to be plesiomorphic in subgenus *Vitis* (Ma et al., 2020), while the diversification of plant stature and climbing ability is identified in two major lineages in sympatry: the *V. bryoniifolia* clade and the *V. chungii* clade, which showed different ecological preferences (Chen et al., 2007; Ma et al., 2018a, 2020). The high-climbing grapes usually occur in forest habitats with large leaves, strong stems, and well-developed vascular bundles for obtaining light on the forest canopy and meeting the demand of water and nutrient transport (He et al., 1994; Ma et al., 2018a). Some sprawling to low climbing species distributed in southern China with small leaves, slender stems, and fewer vascular bundles, such as *Vitis sinocinerea* and *V. bellula* in the *V. bryoniifolia* clade, and *V. wuhanensis*, *V. chungii*, and *V. ruyuanensis* in the *V. chungii* clade, generally occur in shrublands or open habitats (He et al., 1994; Chen et al., 2007; Ma et al., 2018a). Our results support that both sympatric parallel

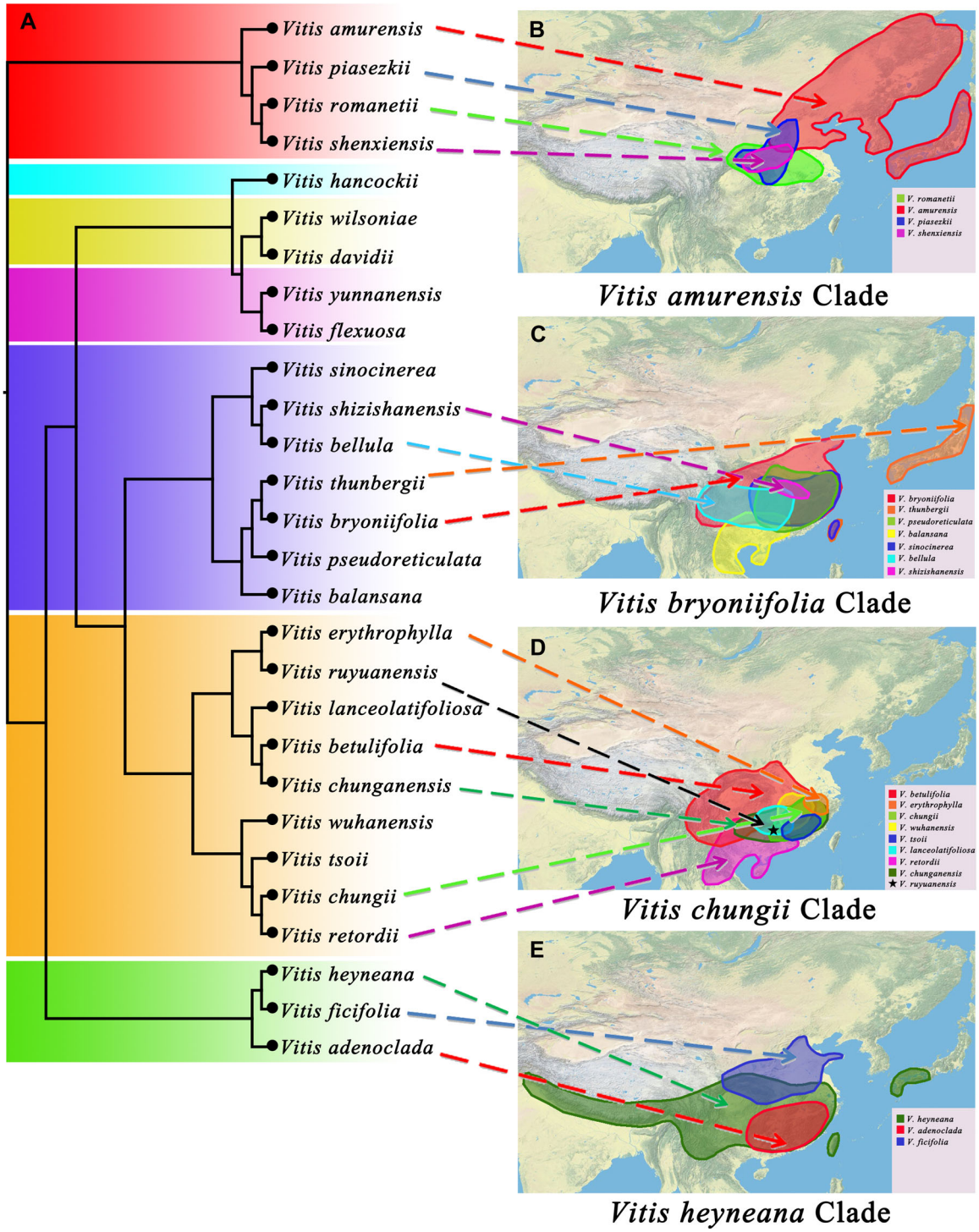

Fig. 4. Geographic distributions of four main clades of East Asian *Vitis*. **A**, Phylogenetic relationships of East Asian *Vitis* based on a concatenated tree with nuclear data. **B**, Distribution of species in the *Vitis amurensis* clade. **C**, Distribution of species in the *Vitis bryoniifolia* clade. **D**, Distribution of species in the *Vitis chungii* clade. **E**, Distribution of species in the *Vitis heyneana* clade. Dotted lines indicate distributions of five species-pairs and two species-complexes in the four main clades.

diversification of major clades and ecological niche divergences within clades have served as drivers of the East Asian *Vitis* species diversity. Integrative analyses on species adaptations are needed for the East Asian *Vitis* (Anderson & Song, 2020).

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Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12918/suppinfo:

Text S1. The best networks from 1 to 9 reticulation events (h_{max}) of SNaQ analyses.

Fig. S1. The maximum likelihood phylogeny of East Asian *Vitis* based on plastid data.

Fig. S2. Ecological niche modeling of East Asian *Vitis*. The color gradient indicates the degree of suitable niches for a species. All occurrence records were available in Table S2.

Fig. S3. Leaves and stems of *Vitis heyneana*, *Vitis adenoclada*, and *Vitis davidii*. **A**, Leaves of *V. heyneana*. **B**, Leaves of *V. adenoclada*. **C**, Leaves of *V. davidii*. **D**, Young branch of *V. heyneana*. **E**, Young branch of *V. adenoclada* with red-purple glandular trichomes. **F**, Young branch of *V. davidii* with prickles.

Fig. S4. Geographic distributions of East Asian *Vitis*. **A**, Distribution of seven main clades. **B**, Distribution of species in the *Vitis davidii* clade. **C**, Distribution of species in the *Vitis flexuosa* clade. **D**, Distribution of species in the *Vitis hancockii* clade. **E**, Distribution of species in the *Vitis bryoniifolia* clade. **F**, Distribution of species in the *Vitis chungii* clade. **G**, Distribution of species in the *Vitis heyneana* clade. **H**, Distribution of species in the *Vitis amurensis* clade.

Fig. S5. Excluding the distributions of two widespread species *Vitis flexuosa* and *Vitis heyneana*, ones of East Asian *Vitis* species with precipitation of the driest quarter as the major environmental variable, see Table S5.

Table S1. *Vitis* species sampled for analyses in this study.

Table S2. Occurrence records of East Asian *Vitis*.

Table S3. Summary of the base information for environmental variables.

Table S4. Statistics of species sampled for sequencing in this study.

Table S5. The environmental variables for East Asian grapes.