

A molecular phylogeny of the *Vitis-Ampelocissus* clade of the grape family (Vitaceae) based on plastid and nuclear sequences



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INTRODUCTION

- Vitaceae (the grape family) consist of of 14 genera and about 900 species primarily distributed in tropical regions in Asia, Africa, Australia, the Neotropics, and the Pacific islands.
- The family is an economically important, containing several agronomic crop species (e.g. *Vitis vinifera* L.) and ornamentals (e.g. *Parthenocissus quinquefolia* L.).
- Recent molecular analysis support the Vitaceae, along with the closely related Leeaceae as sister to all other rosids (Jansen et al., 2006).
- Vitaceae are typically woody or herbaceous climbing vines and occasionally succulent trees.
- Morphological characters of the family include leaf-opposed tendrils, presence of epidermal pearl glands, and calcium oxalate crystals contained in the ground tissue.
- Generic classifications within the Vitaceae has been problematic, with the number of recognized genera increasing from two at the time of Linnaeus' recognition, to ten based on morphological characters. Current molecular and morphological data suggests at least 14 genera.
- A recent molecular phylogeny of the Vitaceae provide support for a *Vitis-Ampelocissus* clade consisting of the genera *Vitis*, *Ampelocissus*, *Nothocissus*, and *Pterisanthes* (Ren et al., 2011).
- The *Vitis-Ampelocissus* clade possesses most of the Northern Hemisphere Vitaceae species with some members disjunct between Asia and North America (Soejima et al., 2006).
- The *Vitis-Ampelocissus* clade remains largely under-sampled and unresolved in the large family-wide phylogeny.

PURPOSE

- The purpose of this study was to construct the phylogeny of the *Vitis-Ampelocissus* clade of the Vitaceae using both plastid and nuclear markers. The resulting phylogeny will then be used to:
- Compare phylogenetic relationships with those determined from prior studies.
 - Estimate divergence times of major clades within the *Vitis-Ampelocissus* clade.

MATERIALS AND METHODS

Sampling, DNA Isolation, and Sequencing

- Study sampled 68 accessions representing 54 Vitaceae species and 5 Leeaceae species. 43 of the taxa represent the *Vitis-Ampelocissus* clade.
- Total genomic DNAs were extracted from silica-dried-material or herbarium material using the DNeasy Plant Minikit protocol.
- DNA was amplified by polymerase chain reaction (PCR) and sequenced via Sanger-method sequencing for four non-coding chloroplast loci (*trnH-psbA*, *trnC-petN*, *rps16*, and *atpB-rbcl*) and two low-copy nuclear loci (*adh1*, *rpb2*).
- DNA sequences were assembled in the program Geneious version 5.4.5

Phylogeny Reconstruction and Analysis

- Sequence alignment was first performed using the program MAFFT version 6.857 and then manually adjusted in the program Se-AL version 2.0.
- Sequences generated were concatenated into a family-level matrix for the four plastid markers and a matrix for *Vitis* alone with the two nuclear markers. Indels were excluded from subsequent analyses.
- Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference (BI) methods.
- Family and clade divergence times were based off the four plastid sequences and were estimated using the program BEAST, based on a partitioned analysis of the plastid four-marker data set. A "relaxed clock" model was used to enable substitution rates associated with each branch to be modeled independently.
- Fossil calibrations were set to 85 ± 4 MA million years ago (MA) for the root Vitaceae and 58.5 MA as a minimum age constraint (using a lognormal distribution, offset 58.5 MA) for the stem lineage of the *Vitis-Ampelocissus* clade (Nie et al., 2010).



Figure 1: Vitaceae taxa, as photographed by Dr. Jun Wen, found in the field. Down each column from , from left to right: *Vitis cinerea*, *Vitis amurensis*, *Ampelopsis arborea*, *Parthenocissus heptaphylla*, *Ampelopsis glandulosa*, *Vitis aestivalis*, *Vitis rotundifolia*, *Vitis riparia*, *Vitis flexuosa*, *Vitis arizonica*, *Vitis chunganensis*, *Vitis mustangensis* (x2).

RESULTS

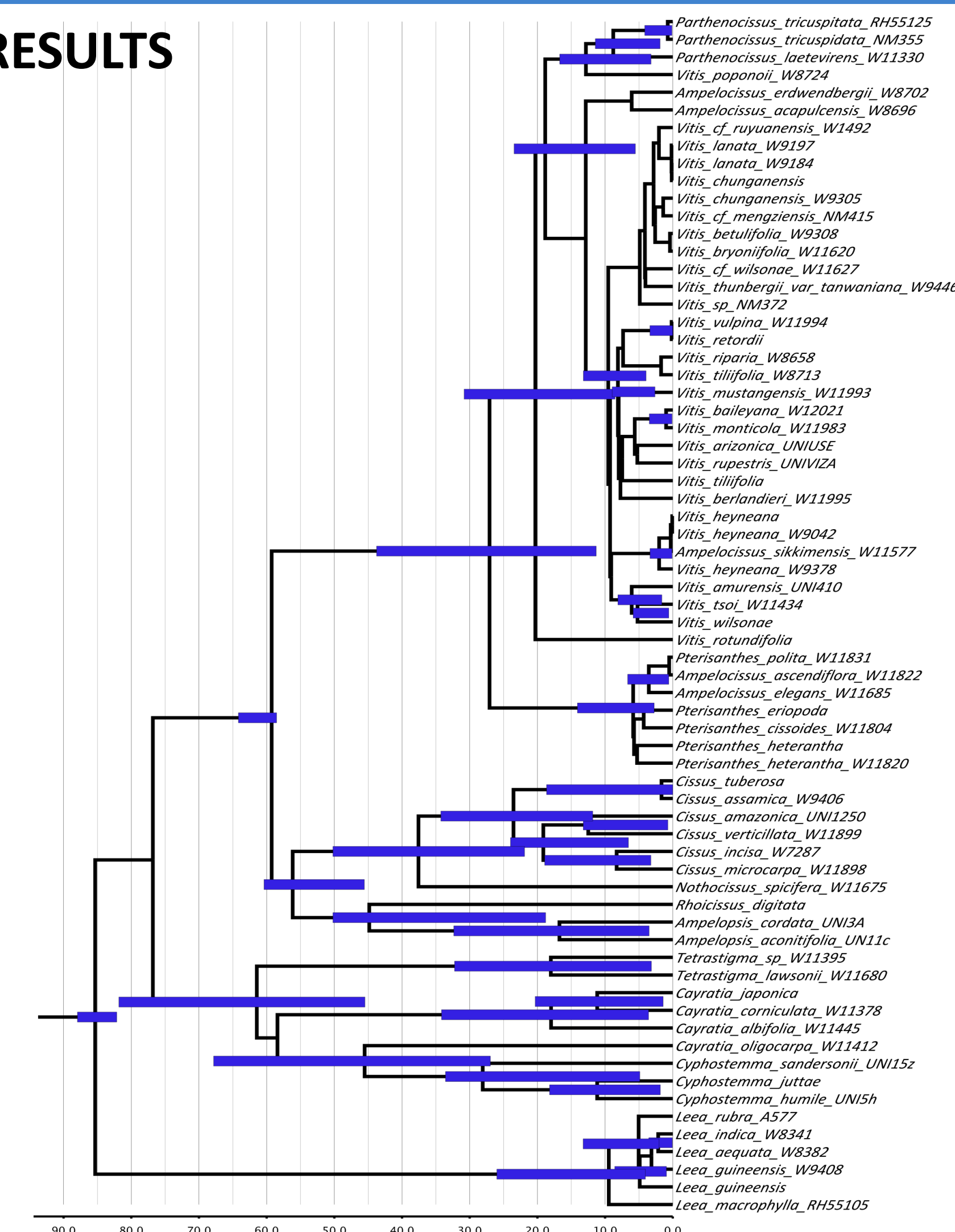


Figure 2: Chronogram inferred from a BEAST analysis of the *Vitis-Ampelocissus* clade and other Vitaceae using a multigene (*trnH-psbA*, *trnC-petN*, *rps16* and *atpB-rbcl*) plastid data matrix. Blue bars represent 95% highest posterior density credibility interval for node ages. Chronogram was calibrated at the Vitaceae-Leeaceae split and a fossil constraint on the stem of the *Vitis-Ampelocissus* clade.

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RESULTS

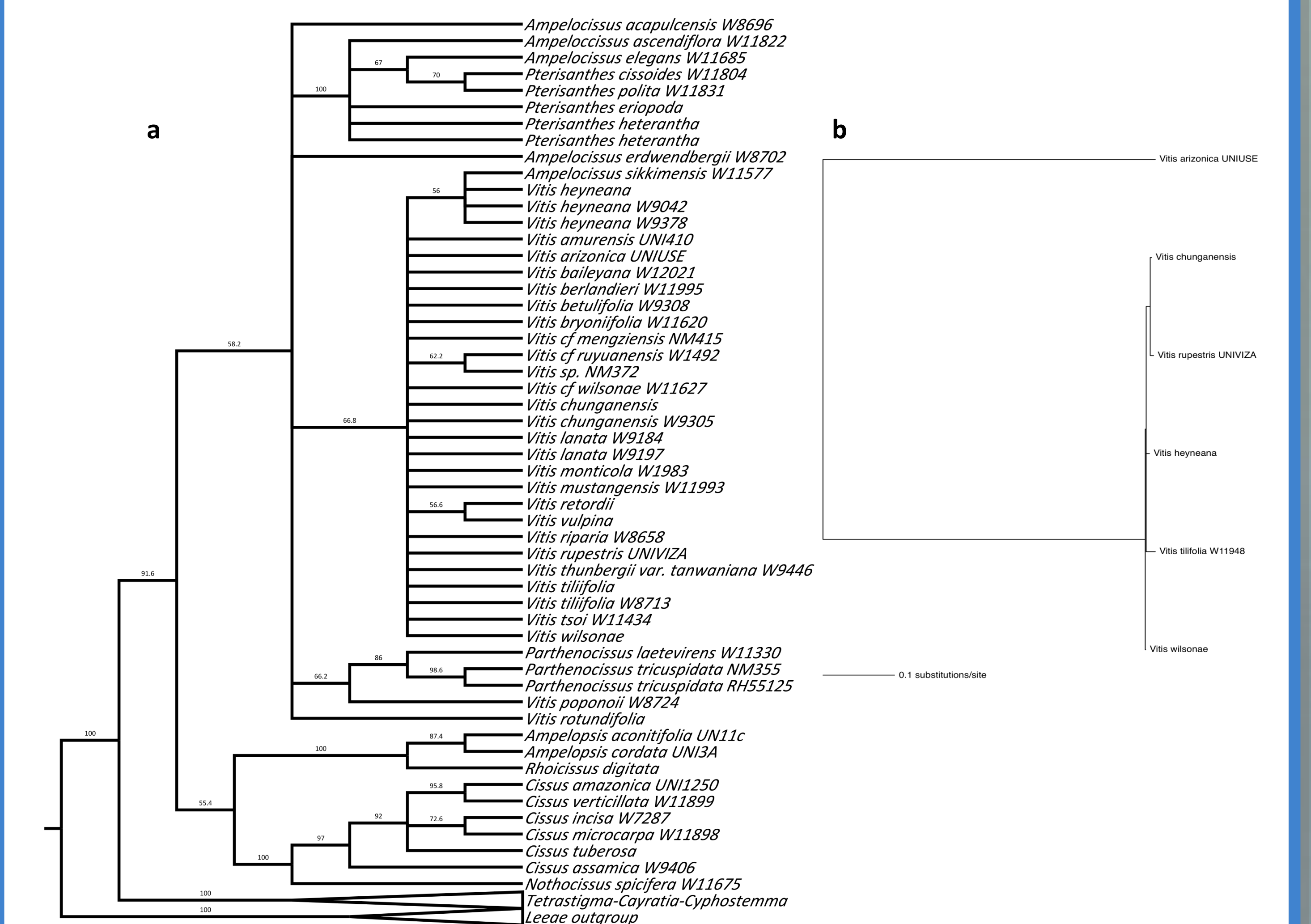


Figure 3a: Majority-rule consensus tree of 500 maximum likelihood bootstrap replicates of the combined plastid *trnH-psbA*, *trnC-petN*, *rps16* and *atpB-rbcl* dataset for 68 accessions. The *Leea* outgroup and the *Tetragymna-Cayratia-Cyphostemma* clade were strongly supported and thus collapsed. Numbers above branches are bootstrap values. **Figure 3b:** Optimal tree from a maximum likelihood analysis of the combined *adh1* and *rpb2* dataset for 6 *Vitis* accessions.

DISCUSSION AND FUTURE DIRECTIONS

- Very strong support for Leeaceae as sister group to the Vitaceae and for a clade consisting of *Tetragymna*, *Cayratia*, and *Cyphostemma*. (see Fig. 3a)
- Moderate support for the *Vitis-Ampelocissus* clade containing *Vitis*, *Ampelocissus*, and *Pterisanthes*.
- *Nothocissus* does not resolve within the *Vitis-Ampelocissus* clade as suggested by other studies. (see Fig. 3a)
- *Ampelocissus* is resolved as a paraphyletic group, as in other studies (Ren et al., 2011).
- BEAST results indicate early diversification of the *Vitis-Ampelocissus* clade at 12-44 MA, from Mid-Miocene to Eocene. (see Fig. 2)
- This suggests initial radiation of the *Vitis-Ampelocissus* clade occurred during warmer climates and that the group was probably an element of the boreotropical flora of the Eocene (34-56 MA), as suggested by fossil seed discoveries of the group.
- Subsequent major radiations may have occurred during warm paleoclimates of the Miocene (5-23 MA) according to our results.
- Further development of the nuclear *adh1* and *rpb2* markers.
- Incorporation of additional non-coding plastid loci in order to help resolve interspecific relationships within *Vitis*.

REFERENCES

- Janssen, R.K., et al. 2006. Phylogenetic analyses of *Vitis* (Vitaceae) based on complete chloroplast genome sequences: effects of taxon sampling and phylogenetic methods on resolving relationships among rosids. *BMC Evol. Biol.* 6: 32.
- Nie, Z., et al. 2010. Molecular phylogeny and biogeographic diversification of *Parthenocissus* (Vitaceae) disjunct between Asia and North America. *Am. Jo. Bot.* 8:97.
- Ren, H., et al. 2011. Phylogenetic analysis of the grape family (Vitaceae) based on the noncoding plastid *trnC-petN*, *trnH-psbA*, and *trnL-F* sequences. *Taxon.* 3:60.
- Soejima et al. 2006. Phylogenetic analysis of the grape family (Vitaceae) based on three chloroplast markers. *Am. Jo. Bot.* 2:93.