Splitting the *Vitis cinerea* Species Complex (Downy Grapes)

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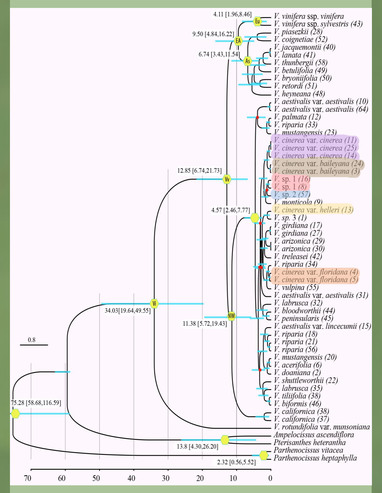

Figure 1. Minimum clade credibility tree of Vitis crown group. Scale bellow tree in millions of years, scale left of tree representing branch lengths (Wen et al., 2018).

Introduction

Vitis, the grape genus, is the most commercially important fruit group. Although most North American species are not commercially harvested, they have economic importance to viticulture because they can be used as rootstocks for commercial species like *V. labrusca* or *V. vinifera* and confer resistance to common pests and diseases. Undomesticated species in *Vitis* have also been used in hybridizations with commercial strains to create hardier grape cultivars. However, despite the genetic contributions of these species, their taxonomy and phylogeny have remained poorly understood.

Vitis cinerea is known as the downy, sweet winter, or graybark grape. *Vitis cinerea* has been generally recognized as a single wide-ranging but highly variable species (Moore & Wen, 2016). Recent molecular evidence has shown that the species is not monophyletic. A study of chloroplast phylogenomics in *Vitis* subgenus *Vitis* suggests splitting *V. cinerea* into at least four distinct species (Wen et al., 2018).

The goal of this study was to examine morphological evidence of this species complex. Specifically, seeds and leaf epidermal characters are taxonomically useful and we focused on these two suites of characters.

Methods

We collected seeds of *Vitis cinerea* and its varieties from the US National Herbarium. We took photos of multiple seeds of each specimen. For each variety, we sampled from several locations across its range. Once we made a pass through the catalog, we went back and took images of cross-sections and pictures without the outer seed coat (sarcotesta). We organized the seed morphological data based on species and varieties recognized in Moore & Wen (2016). We examined most species in North America to assess the taxonomic delimitation of the varieties and species.

We measured five seed characters: seed length (bottom to apex), distance between chalaza and apex (top of chalaza to apex), chalaza length (bottom of chalaza to apex), chalaza width, and seed width. We then compared those measurements among the varieties and species we examined.

We used the scanning electron microscope (SEM) to document leaf epidermal characters (trichomes and stomate morphology) on the abaxial (upper) and adaxial (lower) of each leaf sample. We measured the absence, presence, and / or general density of trichomes. Unlike the two previous ways we compared seeds, the trichome characters have binary character states.

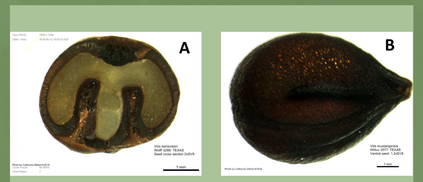

Figure 2. General morphology of a *Vitis* seed. A: cross-section of *V. cinerea* var. *helleri*, showing M-shaped endosperm. B: ventral view of *V. mustangensis* seed, showing ventral infolds. C: key to measured characters using a *V. cinerea* var. *cinerea* seed as an example. Photos taken by Catherine Gilbert.

Results

Seed morphological characteristics were illustrated in Figure 3.

Vitis cinerea var. *cinerea* (purple) has a thicker chalaza, shallow, scooped ventral inverts in photos with sarcotesta, and higher-reaching ventral infolds in cross-section.

Vitis cinerea var. *floridana* (orange) has a longer body, narrower ventral inverts, thinner seed apex, and a wedge-shaped cross-section.

"Vitis cinerea" from Louisiana sp. nov. 1 (red) has a ridged seed apex, round body, centered chalaza, and elongated hilum.

Vitis cinerea var. helleri (yellow) has a short chalaza, round body, and blunted, short base.

"Vitis cinerea" from the District of Columbia sp. nov. 2 (blue) has a grooved seed apex, thin, high chalaza, and elongated hilum.

The seed of *Vitis cinerea var. baileyana* (brown) is much smaller and more spherical in shape than any of the other varieties.

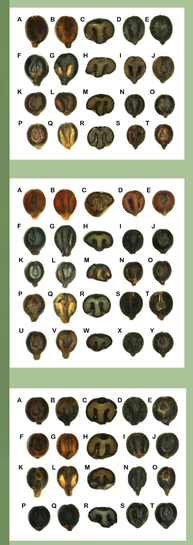

Figure 3. Each row corresponds to the species / variant labeled on the right. From left to right for each row: chalaza side, ventral side, cross-section, debrided ventral side, debrided chalaza side. Plates above composited from photographs taken by Catherine Gilbert.

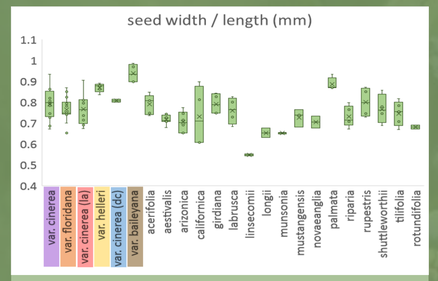

Figure 4. Histogram of seed width / length ratio in mm for all specimens.

The differences among varieties of *Vitis cinerea* that we observed in our study were shown in the measurements we made (Figures 3 - 4). Distinct differences can be seen between the measurements of our four varieties and two potential new taxa, most prominently in the dimensions of seed width / length, chalaza width, chalaza length, and chalaza placement.

The data shows some level of overlapping among the varieties' ranges of measurement. However, this does not discredit the differences we've found. *Vitis labrusca* and *Vitis mustangensis* have highly distinctive seed morphologies, but most species within the Vitis genus have overlapping ranges of measurement. *Vitis cinerea* var. *baileyana*, one of the four *V. cinerea* varieties, was actually one of the most extreme in the genus in the dimensions of width / length and chalaza length.

Table 1. The distribution of epidermal characters in *Vitis.* Dark green: dense trichomes present. Light green: character present. Red: character absent. Grey: character unobserved.

sample #	ID #	species name	upper ribbon-like trichomes	upper simple short trichomes	upper apical stomates	striated upper stomates	lower ribbon-like trichomes	lower simple short trichomes	lower stomates	striated lower stomates
1	12533	*V. sp. 2*								
2	13467	*V. cinerea*								
3	13503	*V. vinifera*								
4	13504	*V. sylvestris*								
5	13493	*V. californica*								
6	5713	*V. tiliifolia*								
7	12680	*V. linsecomii*								
8	12761	*V. cinerea* var. *floridana*								
9	12631	*V. palmata*								
10	12662	*V. monticola*								
11	12653	*V. cinerea* var. *helleri*								
12	13850	*V. arizonica*								
13	12713	*V. girdiana*								
14	12681	*V. mustangensis*								
15	12676	*V. longii*								
16	12689	*V. cinerea* var. *cinerea (TX)*								
17	12623	*V. cinerea* var. *cinerea (IL)*								
18	13501	*V. californica*								
19	13789	*V. cinerea* var. *baileyana*								

We discovered that *Vitis* leaves have two types of trichomes: long hairs, which coil like ribbons and can be found anywhere on the surface of a leaf, and short simple hairs, which resemble small cones and are generally found on the veins or margins of the leaf. Both types of trichomes are generally more dense on the abaxial side of the leaf. We discovered differences in the presence, absence, and density of these two types of hairs between *Vitis cinerea* varieties (Table 1). For example, *Vitis cinerea* var. *cinerea* has very dense long hairs on the adaxial side of its leaves, while *Vitis cinerea var. baileyana* lacks them completely.

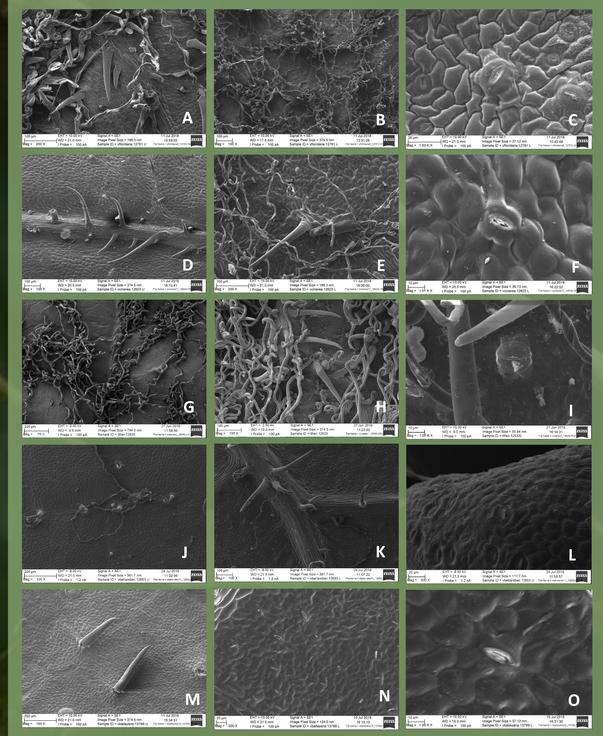

Figure 5. Morphology of epidermal characters of the *Vitis cinerea* complex. A – C: *V. cinerea* var. *floridana, Wen 12761, FL.* D – F: *V. cinerea* var. *cinerea, Wen 12623, IL.* G – I: *V. sp. 2, Wen 12533, DC.* J – L: *V. cinerea* var. *helleri, Wen 12653, TX.* M – O: *V. cinerea* var. *baileyana, Wen 13789, KY.* First column is adaxial surface, second column is abaxial surface, and third column is stomates of abaxial surface.

Conclusions

The micromorphological data that we have obtained show that the *Vitis cinerea* species complex can be separated into several distinct species that show differences in seed and trichome morphologies. The differences that we've observed support the molecular findings of Wen et al., 2018. We found that Vitis mustangensis and Vitis labrusca have highly distinctive seed morphologies. Several characters such as presence/absence of seed apex grooves, seed shape, and ventral infolds shape can be of significance in delimiting species.

Our study is the first attempt to comprehensively document the seed morphology of North American grapes, which have a rich fossil record. The baseline data from this study will help accurately place fossils into species groups.

We only detected two types of trichomes in North American grapes. Ma et. al 2016 reported a third type of trichome (glandular trichomes) in Asian grapes. The functional adaptation of the lack of glandular trichomes in North American grapes should be explored in future work.

Trichomes can be taxonomically useful in North American *Vitis*. We found that the adaxial surface of *Vitis cinerea* var. *baileyana* has only simple trichomes (lacking ribbon trichomes), distinct from all other varieties of the *Vitis cinerea* complex.

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