

TAXONOMY AND PHYLOGENY OF THE FLOWERING PLANT GENUS *DIERVILLA*  
(DIERVILLACEAE)

A thesis submitted to the faculty of the Graduate School of Western Carolina University in  
partial fulfillment of the requirements for the degree of Master of Science in Biology

By

Hannah Elise Meeler

Director: Dr. Katherine Mathews  
Associate Professor and Herbarium Director  
Biology Department

Committee Members: Dr. Beverly Collins, Biology  
Dr. James Costa, Biology

August 2018

## ACKNOWLEDGEMENTS

I want to thank my committee members, Katherine Mathews, Beverly Collins, and Jim Costa for their endless help, support, and encouragement throughout this research process. I would also like to thank my mother, Mary Ellen Lewis. I could not have done this without them. They all deserve to be canonized for their patience and understanding with me over the past few years.

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	i
LIST OF TABLES .....	iii
LIST OF FIGURES.....	iv
ABSTRACT .....	v
CHAPTER 1: INTRODUCTION .....	1
Taxonomy.....	1
Morphology .....	3
Biogeography.....	6
Phylogeny.....	10
CHAPTER 2: METHODS.....	14
Morphological and Environmental Study.....	14
Phylogenetic Study .....	18
CHAPTER 3: RESULTS .....	21
Morphological and Environmental Study.....	21
Phylogenetic study.....	29
CHAPTER 4: DISCUSSION.....	32
Species Co-occurrence.....	32
Utility of Morphological Characters .....	33
Phylogenetic Relationships .....	33
Conclusions and Further Research.....	34
LITERATURE CITED .....	36

## LIST OF TABLES

Table 1. A comparison of morphological characters of <i>D. lonicera</i> , <i>D. rivularis</i> , and <i>D. sessilifolia</i> .....	5
Table 2. A comparison of distinguishing morphological characters of <i>D. sessilifolia</i> and <i>D. rivularis</i> .....	6
Table 3. A comparison of habitat descriptions for <i>D. lonicera</i> , <i>D. sessilifolia</i> , and <i>D. rivularis</i> ...	9
Table 4. Unique derived mutations of <i>Diervilla</i> based on nuclear ribosomal ITS sequences.....	12
Table 5. Sampling locations of <i>D. lonicera</i> , <i>D. rivularis</i> , and <i>D. sessilifolia</i> .....	14

## LIST OF FIGURES

Figure 1. Taxonomy of <i>Diervilla</i> over time.....	3
Figure 2. Distribution of <i>D. lonicera</i> in eastern Canada and the United States.....	8
Figure 3. Distribution of <i>D. sessilifolia</i> in the southeastern United States.....	8
Figure 4. Distribution of <i>D. rivularis</i> in the southeastern United States.....	9
Figure 5. Maximum likelihood tree of <i>Weigela</i> and <i>Diervilla</i> based on ITS sequences.....	12
Figure 6. Sampling Locations for <i>Diervilla</i> .....	15
Figure 7. Cluster diagram of <i>Diervilla</i> field samples based on morphological data.....	20
Figure 8. Cluster diagram of <i>Diervilla</i> field samples based on environmental data.....	21
Figure 9. PCA results for field samples based on morphological data (Axis 1 & Axis 2).....	22
Figure 10. PCA results for field samples based on morphological data (Axis 1 & Axis 3).....	23
Figure 11. PCA results for field samples based on environmental data (Axis 1 & Axis 2).....	24
Figure 12. PCA results for field samples based on environmental data (Axis 1 & Axis 3).....	25
Figure 13. PCA results for field and herbarium samples based on morphological data (Axis 1 & Axis 2).....	26
Figure 14. PCA results for field and herbarium samples based on morphological data (Axis 1 & Axis 3).....	27
Figure 15. Single most parsimonious tree based on trnS-G sequence data.....	28

## ABSTRACT

### TAXONOMY AND PHYLOGENY OF THE FLOWERING PLANT GENUS *DIERVILLA* (DIERVILLACEAE)

Hannah Elise Meeler, M. S. in Biology

Western Carolina University (August 2018)

Director: Dr. Katherine Mathews

My research consists of a taxonomic and phylogenetic analysis of the genus *Diervilla* (Bush honeysuckle), containing three eastern North American species, *Diervilla lonicera*, *Diervilla rivularis*, and *Diervilla sessilifolia* (Diervillaceae). Because there is a large amount of morphological variation found within each species, taxonomic boundaries are unclear. *Diervilla lonicera* has the largest coarse geographic range, which spans from the southern Appalachians to northeastern Canada. *Diervilla sessilifolia* and *Diervilla rivularis* are endemic to a few states in the southern Appalachians. My research has three main components: 1) a study of morphological variation and historical range information using herbarium specimens; 2) a multivariate ecological field study of all three species in the locations where their coarse ranges overlap in the southeastern United States; and 3) a phylogenetic analysis of multiple populations of the three species and their outgroup, *Weigela*. There was no evidence of the three taxa growing together across their range. The multivariate analysis results suggest that morphological data can be helpful to separate the taxa, but only on a small geographic scale. The environmental data from the ecological field study were inconclusive, with locality being the strongest factor. The phylogenetic analysis showed that the three taxa share a few derived genetic mutations, but overall, there is very little sequence variation. Based on my analyses, I cannot conclude that *D. lonicera*, *D. sessilifolia*, and *D. rivularis* are indeed three distinct taxa.

## CHAPTER 1: INTRODUCTION

I conducted a taxonomic study and phylogenetic analysis of three native species of bush honeysuckle, *Diervilla lonicera*, *D. rivularis*, and *D. sessilifolia* (Diervillaceae). These three species of *Diervilla* are the only known species in the genus (Weakley 2015). Because there is a large amount of morphological variation found within each species, particularly within *D. sessilifolia*, taxonomic boundaries are unclear. *Diervilla lonicera* has the largest coarse geographic range, which spans from the southeastern United States to northeastern Canada (Weakley 2015). *Diervilla sessilifolia* and *D. rivularis* are endemic to a few states in the southeastern United States, where they broadly over-lap (Weakley 2015).

### Taxonomy

*Diervilla* is a genus of deciduous shrubs containing three described species that are found in eastern North America (Weakley 2015). *Diervilla* along with its sister taxon, *Weigela*, are the only two genera in the eastern N. American-eastern Asian disjunct family Diervillaceae (Backlund & Pyck 1998; Weakley 2015). Backlund and Pyck moved *Diervilla* and *Weigela* from Caprifoliaceae to Diervillaceae (1998) based on phylogenetic evidence and morphological differences. Miller first described the genus *Diervilla* in 1754 including one species, *D. lonicera* Miller (= *Lonicera Diervilla* L.) (Fig.1). Buckley described a second species, *Diervilla sessilifolia*, in 1843. Gattinger described a third species, *Diervilla rivularis*, in 1888 based on a specimen collected in 1880 at Lula Falls on Lookout Mountain in Georgia, just over the Tennessee line. Gattinger distinguished *D. rivularis* from *D. sessilifolia* by: the whole plant being hirsutely pubescent, the leaves being subsessile, the flowers being larger than those of *D. sessilifolia*, “lemon yellow” flowers versus “sulfur or greenish yellow” flowers of *D. sessilifolia*,

and *D. sessilifolia* having more regular flowers (Gattinger 1888). It is not completely clear what Gattinger (1888) meant by “more regular flowers;” but he most likely meant more regular symmetry. In his description Gattinger wrote, “*D. rivularis* is a handsomer plant.” Ahles reduced *D. rivularis* to a variety of *D. sessilifolia* in 1964 (Hardin 1968). Hardin pointed out in his revision of the genus (1968) that Ahles did not give an explanation for this action nor why he made it a variety of *D. sessilifolia* instead of *D. lonicera*. Hardin wrote, “Unfortunately his transfer of rank was effected only by the legal minimum of citing the basionym...” (Hardin 1968). Hardin also noted “the only clue to why he chose *D. sessilifolia* [instead of *D. lonicera*] is in his key to the species (in Radford et al. 1964) in which var. *rivularis* (a *nomen nudum* in the Guide) is keyed with *D. sessilifolia* on the basis of having ‘Leaves, at least the upper, sessile’” (Hardin 1968). Hardin treats *D. rivularis* as a separate species in his revision (1968) and so does Weakley in his Flora of the Southern and Mid-Atlantic States (Weakley 2015). All three species have a chromosome count of  $2n=36$  (Index to Plant Chromosome Numbers 1979). The taxonomic history of these three species is of particular interest because the two southern endemic species, especially *D. rivularis*, show a large amount of intraspecific morphological variation across their geographic ranges (Hardin 1968), and this variation may be a cause for the changes in the status of *D. rivularis*. In this study, I hope to elucidate the nature of the variation across the ranges of *D. rivularis* and *D. sessilifolia* by qualitative and quantitative means.



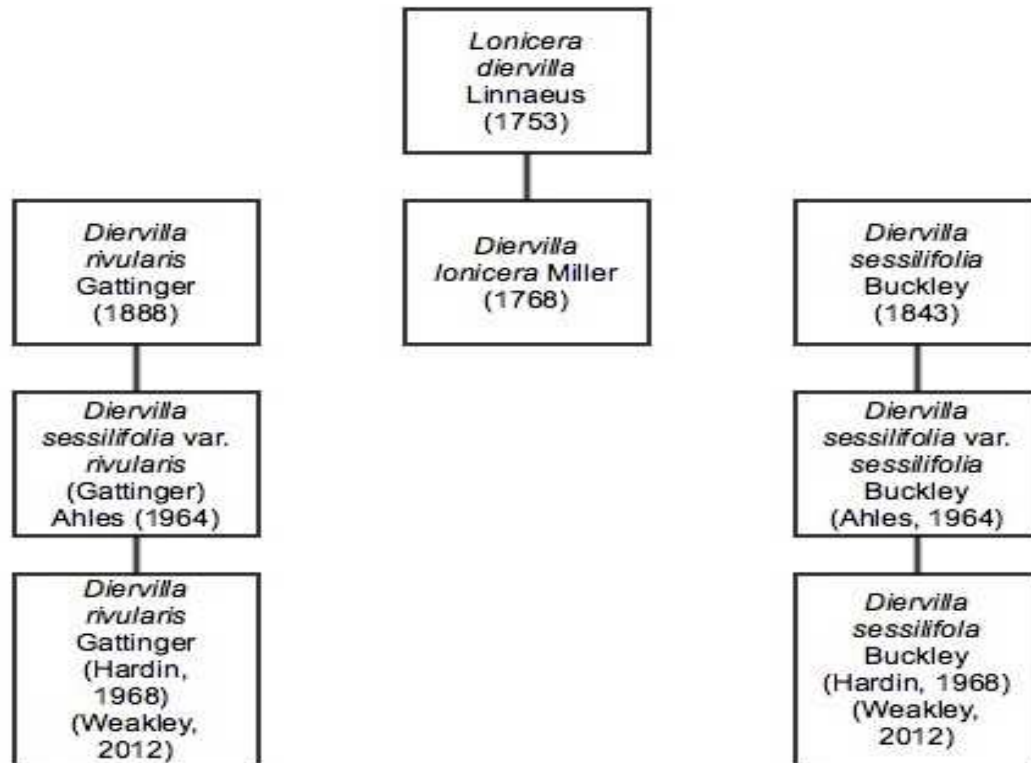


Fig. 1 – Taxonomy of *Diervilla* over time

### Morphology

All species of *Diervilla* have opposite leaves, are generally stoloniferous, and grow ca. 0.6—1.5 m. tall (Ferguson 1966). *Diervilla lonicera* is distinguished in the literature from *D. sessilifolia* and *D. rivularis* purely by vegetative characters: e.g., its petiolate leaves (but the other two can have petioles up to 5 mm long) (Ferguson 1966). *Diervilla lonicera* also has ciliate leaf margins (vs. not ciliate in the other taxa) and twigs that are terete in cross-section (vs. square in the other two) (Weakley 2015 and Table 1). Notable morphological variation that I have noticed in *D. lonicera* from herbarium specimens and field observations include, pedicel length, petiole length, leaf shape, overall pubescence of the plant, leaf length, and leaf width. One floral character that appears to be unique to northern specimens of *D. lonicera* is fewer

flowers per inflorescence (personal observation by Katherine Mathews). The variation in leaf length and leaf width could be driven by the amount of shade that the plant growing under (Goulet & Bellefleur 1986), but unfortunately this information is not provided on many herbarium specimens.

The primary morphological traits that distinguish *D. rivularis* from *D. sessilifolia* in keys are also vegetative: pubescence and calyx length (Radford et al. 1964; Weakley 2015). *Diervilla rivularis* has, “Branchlets, leaves pedicels, and calyx densely pubescent, sepal lobes less than 2 mm long” (Weakley 2015). *Diervilla sessilifolia* is glabrous, “except for hairs on the twig angles” (Weakley 2015) and the sepal lobes may be 2-3 mm long (Weakley 2015 & Table 2).

The three species are not reliably distinguishable by floral characters (aside from calyx pubescence and sepal lobe length) or fruit characters (Ferguson 1966; Weakley 2015), thus the basis for their distinction as species is based on characters that are less susceptible to selection for reproductive success (Farris & Lechowicz 1990) and which may be environmentally variable (Schlichting 1986). Hairiness in particular is known to vary in some plant species depending on the amount of sun exposure (Nikolić 1991). My personal observations in the field and of herbarium specimens reveal a large amount of intraspecific morphological variation in *D. rivularis* and *D. sessilifolia*. It is not known whether the large amount of variation observed is being driven by environmental factors or genetic differences.

There are no known natural hybrids between any of the three species of *Diervilla* (Hardin 1968). Hardin reported specimens from the over-lapping northern extent of the ranges of *D. sessilifolia* and *D. rivularis* that were more or less intermediate between the two, but noted that they were usually identified as *D. rivularis* (Hardin 1968). He suggested that this could have been the basis for Ahles demoting *D. rivularis* to a variety of *D. sessilifolia* (Hardin 1968). He

suggests that it could be a case of reticulate evolution (Hardin 1968). Hardin mentions another *Diervilla*, *Diervilla x splendens*, which is a garden hybrid between *D. lonicera* and *D. rivularis* (Hardin 1968). *Diervilla x splendens* has “an intermediate petiole length, but lacks the pubescence of *D. rivularis*” (Hardin 1968).

A morphological species concept may be most useful to define the species boundaries of *Diervilla*. A morphological species concept implies that the species have “morphological differences typical of what we think of as species” (Wiley & Lieberman 2011). The morphological species concept distinguishes species by morphological and anatomical differences (like plant structure, leaf arrangement, and floral characters) under the assumption that some morphological differences reflect genetic differences (De Queiroz 2007). However, a morphological species concept may not account for the intraspecific variance that is observed with these taxa. One way to account for the intraspecific variation is to record and quantify it. These data can be used to determine if there is any clinal variation present (Montesinos-Navarro, et al. 2010). Clinal variation is a form of geographic variation in which a species has variable characteristics, often gradually changing, throughout its geographic range (Endler 1977).

Table 1. A comparison of morphological characters of *D. lonicera*, *D. rivularis*, and *D. sessilifolia* (Weakley 2015)

	Height	Petiole Length	Leaves Ciliate	Twig Cross-section
<i>D. lonicera</i>	0.9 m	5-8 mm	Ciliate on margins	Terete
<i>D. sessilifolia</i>	0.6-1.5 m	0-5 mm	Eciliate	Roughly square
<i>D. rivularis</i>	0.6-1.5 m	0-5 mm	Eciliate	Roughly square

Table 2. A comparison of distinguishing morphological characters of *D. sessilifolia* and *D. rivularis* (Weakley 2015)

	Branchlets	Leaves	Pedicels	Calyx	Twig Angles	Sepal Lobe Length
<i>D. sessilifolia</i>	Glabrous	Glabrous	Glabrous	Glabrous	Pubescent	2-3 mm
<i>D. rivularis</i>	Densely pubescent	Densely pubescent	Densely pubescent	Densely pubescent	Densely pubescent	Less than 2 mm

### Biogeography

*Diervilla lonicera* occurs from “Newfoundland to Saskatchewan and southward in the Appalachians to North Carolina and Tennessee” (Hardin 1968 & Fig. 2). It generally occurs, in dry woods, along creek sides, on rocky slopes, on mountain summits, and on rock outcroppings at higher elevations (greater than 4000 feet) (Hardin 1968). It is currently listed as “Threatened” in Tennessee and Indiana (Tennessee Natural Heritage Program 2002; Division of Nature Preserves 2002). One of the distinguishing characters of *D. lonicera* is that it extends much further north into colder climates than *D. sessilifolia* and *D. rivularis* (Ferguson 1966). *Diervilla sessilifolia* is found in the upper elevations of the southern Appalachians (greater than 3000 feet) (Hardin 1968 & Fig. 3). It occurs in northern Georgia, northeastern Alabama, western South Carolina, a few counties in eastern Tennessee, and western North Carolina (Hardin 1968). It is endemic to these few southeastern states and seems to be found along stream banks, high slopes, and rock outcrops (Hardin 1968). *Diervilla rivularis* is also endemic to the southeastern United States and is listed as “Threatened” in Tennessee (Tennessee Natural Heritage Program 2002). Specifically, it occurs in one county in North Carolina, six counties in Tennessee, one county in

Georgia, and four counties in Alabama (USDA PLANTS Database website & Fig. 4). This species tends to be found in rocky woods and along stream banks and has an even smaller range than *D. sessilifolia* (Hardin 1968). In general, *D. sessilifolia* tends to be more common in Tennessee and North Carolina, while *D. rivularis* is more common in northeast Alabama and northwest Georgia (Hardin 1968). Even though the ranges of all three species broadly overlap in the southern Appalachians and they can occupy similar habitats, they have not been reported as growing together (Hardin 1968 & Table 3). An ecological study on these taxa would be useful to understand how sympatric the three species are, if they are sympatric at all. Hardin (1968) suggests that the species could be ecologically allopatric, since they have not been reported as growing together (at least on a local scale).

Since *D. lonicera* extends north and becomes more widespread and still maintains some overlap with the southeastern endemics, one question is whether the two southern endemics are post-glacial derivatives of the widespread *D. lonicera*? Hardin (1968) asked, “Could *D. rivularis* be a disjunct and divergent part of *D. lonicera* which is in the southern Appalachians as a relic from a once continuous and more southern extension of *D. lonicera* possibly during the Pleistocene? And could *D. rivularis* have been the product of reticulate evolution when the glaciers pushed *D. lonicera* into sympatry with *D. sessilifolia*?” (p. 32).

In her study on high-elevation rock outcrop communities in the southern Appalachians (1994), Wiser lists *D. lonicera* and *D. sessilifolia* as two endemics that were found in these communities. *Diervilla sessilifolia* was far more common than *D. lonicera* on the rock outcrops and *D. rivularis* was not listed at all (Wiser 1994). Wiser (1994) states that “some of the narrow endemics are likely to have had ancestors that were part of a Pleistocene Appalachian alpine

flora” and that these populations “deserve further study to clarify the origins of the species that provide high-elevation outcrop communities their distinctive character” (p. 94).

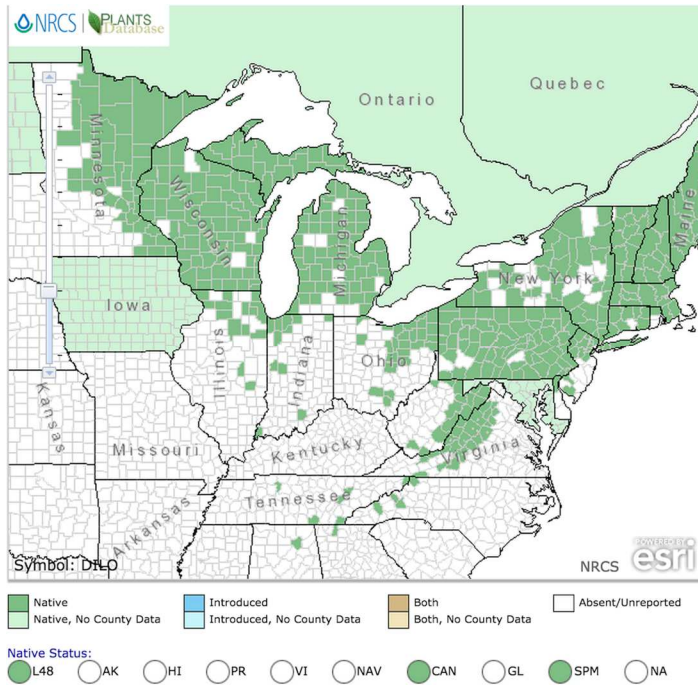


Fig. 2. Distribution of *D. lonicera* in eastern Canada and the United States (USDA, NRCS. 2017. The PLANTS Database (<http://plants.usda.gov>, 5 November 2017). National Plant Data Team, Greensboro, NC 27401-4901 USA.)

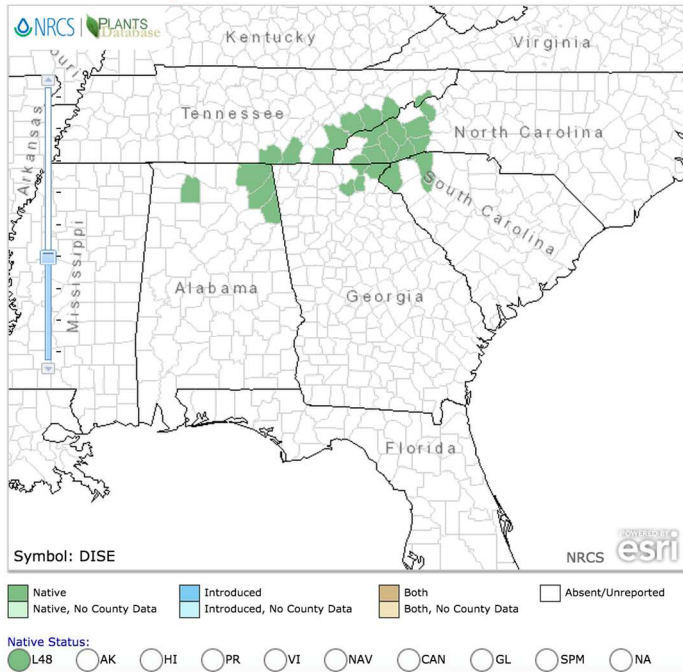


Fig. 3. Distribution of *D. sessilifolia* in the southeastern United States (USDA, NRCS. 2017. The PLANTS Database (<http://plants.usda.gov>, 5 November 2017). National Plant Data Team, Greensboro, NC 27401-4901 USA.)

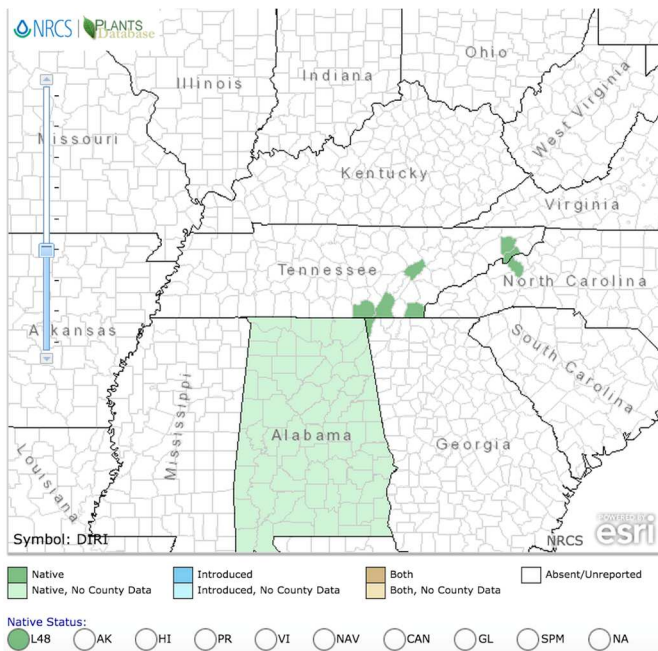


Fig. 4. Distribution of *D. rivularis* in the southeastern United States (USDA, NRCS. 2017. The PLANTS Database (<http://plants.usda.gov>, 5 November 2017). National Plant Data Team, Greensboro, NC 27401-4901 USA.)

Table 3. A comparison of habitat descriptions for *D. lonicera*, *D. sessilifolia*, and *D. rivularis* (Radford et al., 1964; Hardin, 1968; Weakley, 2015)

	Radford et al., 1964	Hardin, 1968	Weakley, 2015
<i>D. lonicera</i>	Woodlands, rocky bluffs	Dry woods, along creeks, rocky slopes, mtn. summits above 4000 feet elev.	Rock outcrops and ridges at high elevations
<i>D. sessilifolia</i>	Woodlands, bluffs, road banks	Rocky summits, slopes, stream banks above 3000 feet elev.	Rock outcrops, ridges, landslide scars, trail margins, other rocky open places, stream banks, at moderate to high elevations
<i>D. rivularis</i>	Woodlands, bluffs	Rocky woods and stream banks	Rock outcrops, ridges, stream banks at moderate to high elevations

### Phylogeny

Hardin (1968) used morphological traits to hypothesize relationships among three species of *Diervilla*. Hardin's (1968) study primarily compared previous descriptions of *Diervilla* (Ferguson 1966 & Small 1933) and herbarium specimens of populations in the southeast to ask whether *D. rivularis* is more closely related to *D. sessilifolia* as indicated by the more or less sessile leaves and general sympatric distribution, or whether *D. rivularis* was derived from a *D. lonicera* ancestor during or after the Pleistocene. He found that *D. rivularis* was unique in extreme pubescence and shorter sepal lobes, intermediate between the other two in stem cross-section and petiole length, and more similar to *D. lonicera* in leaf pubescence (Hardin 1968). Hardin also examined leaf anatomy to try to determine the evolutionary relationships of the taxa (Hardin 1968). The leaf anatomy characters included, serration size, serration frequency, ultimate venation pattern, vein islet size, veins per unit area, upper and lower epidermal cell anatomy, stomate shape, and stomate frequency (Hardin 1968). He found that *D. lonicera* was



distinctly different from *D. sessilifolia* and *D. rivularis* in leaf anatomy, but that *D. sessilifolia* and *D. rivularis* were very similar (Hardin 1968). He suggested that the differences in leaf anatomy indicate a genotypic difference between the taxa, rather than an ecotypic difference (Hardin 1968).

Hardin concluded that the three taxa should be treated as distinct species and that the two southern endemics, *D. rivularis* and *D. sessilifolia*, are more closely related to each other than they are to *D. lonicera* (Hardin 1968). While this study is very helpful, it does not provide definitive explanations for the morphological variation that is observed in these species. It also does not indicate how many herbarium specimens were used in the study and it does not provide genetic evidence to determine the evolutionary relationships between the species nor use a cladistic approach to phylogenetic analysis. A study that examines variation between and among populations of all three taxa along with a modern phylogenetic analysis at the population level would help confirm the taxonomic status and may provide more insight into the evolutionary relationships of these taxa. I will be combining genetic and morphological data to test his morphologically based hypotheses.

More recently, numerous broad molecular phylogenetic studies have included a single representative of *Diervilla* (e.g., Donoghue/Bell et al. 2001; Tank & Donoghue 2010; Jacobs et al. 2009; Howarth & Donoghue 2006; Burgess et al. 2011). The gene regions that were sequenced for at least one species of *Diervilla* include: *ndhF*, *trnK*, *rbcL*, *matK*, *atpF*, *atpH*, *rpoC1*, *trnH*, *psbA*, *rpoC2*, *rps4*, *psbB*, *psbT*, *psbN*, *psbH*, *atpB*, *trnL*, *rps16*, *atpE* (chloroplast); ITS1, ITS2, 26S, 18S (nuclear ribosomal); *nad5*, *cox3*, *cox1* (mitochondrial); and RAD1B, RAD2A, RAD2B, DIV3B, DIV3A, DIV2B, DIV1B, DIV1A, CYC3A, CYC2B, CYC2A, CYC1 (nuclear protein coding) (Pyck & Smets 2000; Jacobs et al. 2010, Burgess et al. 2011, Tank &

Donoghue 2010; Bell 2004; Bremer et al. 2002; Kim & Kim 1999; Winkworth et al. 2008; Boyden et al. 2008; Howarth & Donoghue 2006.) Only one study has included all three species and their outgroup, *Weigela* (Kim & Kim 1999). Focusing on relationships within the family Diervillaceae, Kim & Kim (1999) used ribosomal DNA internal transcribed spacers (ITS 1 and 2) and found that the region was not variable enough to resolve relationships among the species of *Diervilla* (Fig. 5) However, Kim & Kim (1999) do differentiate *D. rivularis* from *D. sessilifolia* and *D. lonicera* in their phylogenetic tree (Fig. 5). Kim & Kim (1999) do not explain in depth why they differentiate *D. rivularis* from the other *Diervilla* taxa, but they do list a few unique derived mutations in the nuclear ribosomal ITS sequences that they found (Table 4). It should be noted that Table 4 represents one taxon for each species of *Diervilla*, as the study only used three samples of *Diervilla* (one representing each taxon) (Kim & Kim 1999).

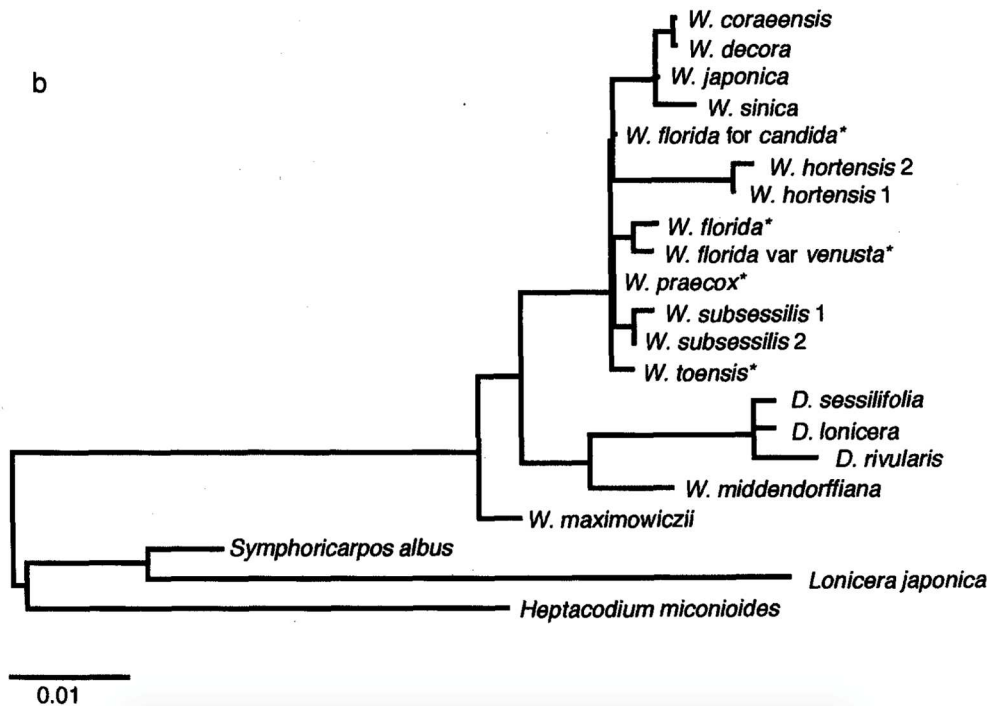


Fig. 5. Maximum likelihood tree of *Weigela* and *Diervilla* based on ITS sequences (Kim & Kim 1999)

Table 4. Unique derived mutations of *Diervilla* based on nuclear ribosomal ITS sequences (Kim & Kim 1999)

Taxon	Aligned Position	aut base	anc base	Gene Region
<i>rivularis</i>	103	t	c	ITS 1
<i>sessilifolia</i>	202	t	a	ITS 1
<i>rivularis</i>	408	t	c	ITS 2
<i>rivularis</i>	429	a	g	ITS 2
<i>lonicera</i>	608	t	c	ITS 2

With this study, I set out to answer three main questions: (1) Do the putative species of *Diervilla* co-occur in the southern Appalachians and if so, do they have different specific habitats? (2) How is morphological variation structured among populations of the three taxa and can it be used to determine species boundaries? And (3) what are the phylogenetic relationships among the three taxa of *Diervilla* and are their populations monophyletic?

## CHAPTER 2: METHODS

### **Morphological and Environmental Study**

I requested herbarium specimens of *D. lonicera* (the southern part of its range), *D. rivularis*, and *D. sessilifolia* from the herbaria at The University of North Carolina at Chapel Hill (NCU), North Carolina State University (NCSC), Jacksonville State University (JSU), and the University of Tennessee at Knoxville (TENN). I used the specimens from these herbaria along with the *Diervilla* specimens from the Western Carolina University herbarium (WCUH) to make a spreadsheet for the *Diervilla* records. Overall, 464 specimens were observed and examined, but only a subset of these (90) were used in the morphological Principal Components Analysis (PCA). Specimens used are listed in Appendix A. The spreadsheet includes information about the specimens, including information about where it was collected and what habitat it was growing in (if provided). The spreadsheet also includes a section on morphological traits for each herbarium specimen. I examined the morphological traits that are given in Weakley's Flora of the Southern and Mid-Atlantic States (2015) to distinguish these three species from each other. These traits include, petiole length, leaf margin shape, ciliate leaf margin present or absent, twig cross section shape, pubescence on branchlets, leaves, pedicels, and/or calyx, sepal lobe length, leaf length, leaf shape, the presence or absence of twig angle hairs, floral characters (corolla length and pubescence), and flower color (Weakley 2015; Radford et al. 1964). After observing many specimens, I added two traits that I thought were variable among the species: capsule length and "beak length," which is an elongation of the carpel/fruit between the inferior ovary and the sepals. Because the beak is accrescent, I measured only fully mature capsules, as they would have a different beak length than early capsules (Schoen 1977).

I collected three specimens of *D. lonicera*, thirteen specimens of *D. sessilifolia*, and one specimen of *D. rivularis* from various locations in North Carolina, Tennessee, and Georgia. I was not able to obtain a permit to collect samples in Alabama in time, so I did not make collections in this part of the over-lapping range. I based my sampling locations on data from the herbarium specimens. My sampling locations did not align with the USDA PLANTS distribution maps because in some cases, herbarium specimens were misidentified. For instance, many specimens labeled as *D. rivularis* appeared to be *D. sessilifolia*. In other cases, I visited a locality to find and sample a species, but I could not successfully locate the population. This does not mean that the population is no longer present, but that I was not able to locate it from limited location information provided on the herbarium sheet label. It should also be noted that I did not have the resources or the time to visit every possible population listed for *Diervilla*. The county-level sampling locations for each state are listed in Table 5 and Figure 6.

Table 5. Sampling locations of *D. lonicera*, *D. rivularis*, and *D. sessilifolia*

Sample #	Taxa	State	County	Latitude	Longitude
01	<i>sessilifolia</i>	NC	Jackson	35.2821	-83.1382
02	<i>sessilifolia</i>	NC	Swain	35.609167	-83.434167
03	<i>sessilifolia</i>	NC	Swain	35.595833	-83.458611
04	<i>sessilifolia</i>	NC	Swain	35.541747	-83.493239
05	<i>sessilifolia</i>	TN	Monroe	35.344917	-84.235517
06	<i>rivularis</i>	GA	Walker	34.93125	-85.373817
07	<i>lonicera</i>	NC	Haywood	35.584283	-83.060517
08	<i>sessilifolia</i>	NC	Haywood	35.32395	-82.877083
09	<i>sessilifolia</i>	NC	Haywood	35.321967	-82.850467
10	<i>sessilifolia</i>	NC	Jackson	35.163067	-83.027367
11	<i>lonicera</i>	NC	Yancey	35.860167	-82.2591
12	<i>sessilifolia</i>	TN	Polk	35.040556	-84.466667
13	<i>sessilifolia</i>	NC	Buncombe	35.7383	-82.197233
14	<i>lonicera</i>	NC	Buncombe	35.7242	-82.285383
15	<i>sessilifolia</i>	NC	Buncombe	35.704267	-82.373833
16	<i>sessilifolia</i>	NC	Buncombe	35.704267	-82.373833
17	<i>sessilifolia</i>	TN	Monroe	35.3461	-84.2426

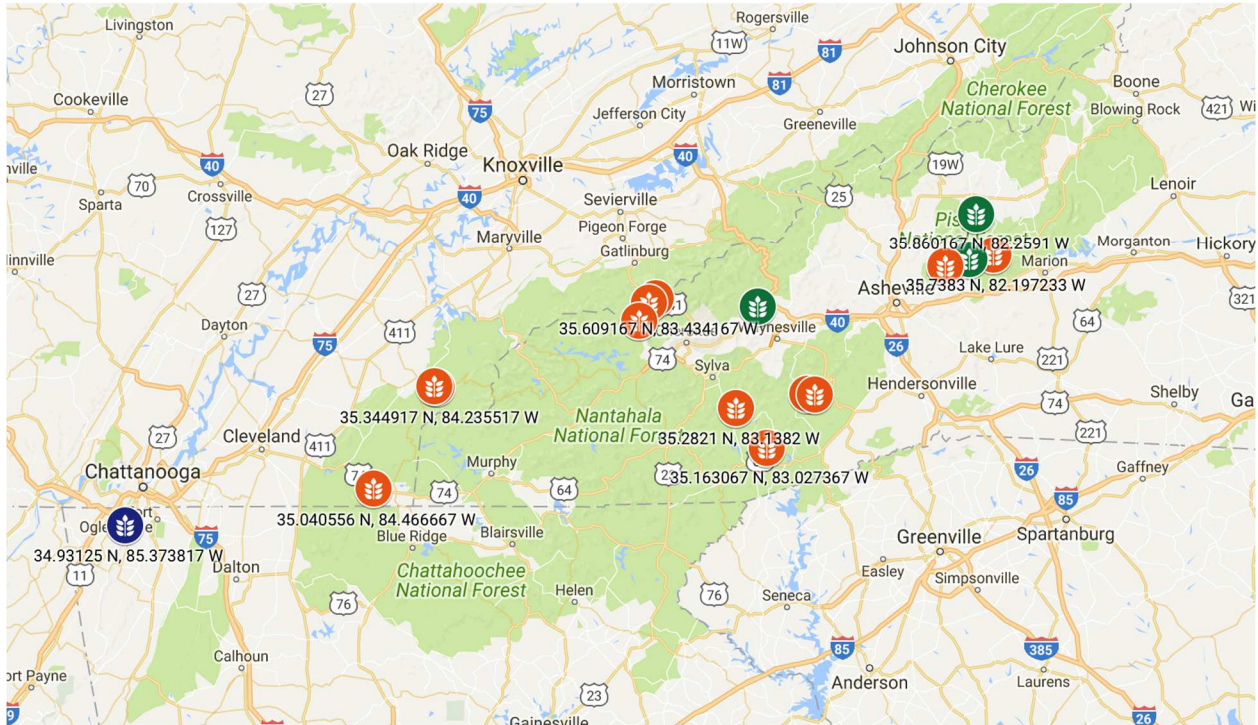


Figure 6. Sampling Locations for *Diervilla* (*D. lonicera*=green, *D. sessilifolia*=orange, *D. rivularis*=blue)

I also attempted to sample a few locations where *Diervilla* was supposed to be found according to herbarium records, but I was not able to successfully relocate the populations. These locations are not included on the map, but are listed as follows: along the Nolichucky River and surrounding areas in Tennessee, Roan Mountain State Park (TN), Caesar's Head State Park (SC), Table Rock State Park (SC), the Mountain Bridge Wilderness Area (SC), and Sassafras Mountain (SC). *D. rivularis* and *D. sessilifolia* were supposed to be located in various locations along the Nolichucky River in Tennessee. *D. sessilifolia* was supposed to be located in Roan Mountain State Park and the sites in South Carolina, aside from Sassafras Mountain. I decided to add Sassafras Mountain to the list because it is the highest point of elevation in South Carolina (Darton 1908). Most of the localities were vague and these were very large areas to

cover, so it is very possible that these populations are still there. In addition to this, many of the herbarium specimens from these areas were 20 to 50 years old and some of the areas have changed a lot since then, so it is also possible that some populations have been extirpated, primarily along the Nolichucky River.

For each population sampled, I collected leaf material for DNA extraction and a small branchlet from one of the individuals to make a voucher for the sample. The leaves used for DNA extraction were frozen in liquid nitrogen upon collection. I also collected a couple of extra leaves from each sample and preserved them in silica gel as a precautionary measure. Five-by-five meter plots were established around each clump of *Diervilla* plants sampled for environmental data. The environmental data I collected at each site included, latitude and longitude as taken with a GPS reading on site, elevation, aspect, soil depth, canopy photos to analyze light penetrating the canopy, percent ground cover, soil substrate type, plant clump measurements in meters, and a list of associated species growing near the population. I sampled my plants in five by five meter plots to be consistent with the environmental data collection. I estimated the percent ground cover within the plot and the amount that was bare soil or rock, etc. Soil substrate type was classified as soil or bare rock because these were the only two general types my samples were growing in. I did not include the associated species information in my analysis because they were characteristic of the habitats they were growing in and not necessarily indicative of *Diervilla* being present.

I used the GPS coordinates from my samples to get data on, maximum soil depth, hydrologic soil group, parent material name, pH, and higher soil classification. Data were obtained from the Web Soil Survey tool provided by the United States Department of Agriculture and Natural Resources in my analyses to have additional environmental parameters

(Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at the following link: <https://websoilsurvey.sc.egov.usda.gov/>. Accessed [Nov/09/2017]). I used the maximum soil depth in my analysis instead of the soil depth I measured in the field because it was more consistent and more representative of the plant community that *Diervilla* is growing in.

I used Gap Light Analyzer (GLA) (Frazer et al. 1999) software to analyze the canopy photo from each plot. This program uses canopy photos to measure gap light transmission through the canopy. I took my photos from the top of the plant or clump of plants that I was measuring. The photos were taken over the months June, July, and August. I used the multivariate analysis program PC-Ord v. 6 (McCune & Mefford 1999) to analyze the rest of the data I collected. I did a cluster analysis on both the measurements from field collections and the environmental data I collected for them separately. I did this analysis initially to see if any morphological traits or environmental variables grouped together. I then did a Principal Components Analysis (PCA) on both sets of data to see how similar these species are to each other based on both morphological and environmental parameters. I also did a PCA on the morphological data from herbarium specimens and the plants I collected in the field to have a larger sample size. I did not have environmental data for the herbarium specimens, so I did not do a larger PCA with the environmental data.

### **Phylogenetic Study**

I used the leaf material of *Diervilla* collected from my field study as well as leaf material collected from a *Weigela* cultivar to represent the outgroup. I extracted total genetic DNA by grinding leaf material frozen with liquid nitrogen using a mortar and pestle and then extracting with the DNeasy Plant Mini Kit (Qiagen, Germantown, MD). I tested multiple genetic regions



for variation, including chloroplast noncoding (*trnL-F*, *trnH-psbA*, *trnD-T*), nuclear (*WAXY*, *CLIP1*, *g3pdh*), and mitochondrial (*ATP9*), using a subset of the taxa. Only one cpDNA region, *trnS-G*, showed promising variation and was sequenced for all of the samples. Polymerase Chain Reaction (PCR) was done with the primers for the *trnS-G* region on an Eppendorf thermocycler in 25 $\mu$ L volumes with the following reaction components: 1 $\mu$ L of template DNA (~10-100ng), 5x buffer, 1 $\mu$ mol/L each of dNTP, 1.25mmol/L MgCl<sub>2</sub>, 1 $\mu$ mol/L of each primer, 9.625 $\mu$ L dH<sub>2</sub>O, 5 $\mu$ L of Taq Master, and 0.125 units of Taq (Go Taq) following the protocol of Shaw et al. (2005). The amplification conditions are listed as follows: initial denaturation step (80°C, 5 min.); number of repetitions of the amplification cycle [30x (denaturation 95°C, 1 min.; primer annealing 65°C, 1 min.; chain extension 66°C for 3 min.)]; final extension step (72°C, 2 min.) (Shaw et al., 2005). All reactions ended with a final 4°C holding step (Shaw et al., 2005). The PCR reactions were visualized on agarose gel to test for successful amplification and the PCR products were cleaned using ExoSAP-IT™ (Applied Biosystems, Foster City, CA). They were then cycle sequenced in both directions using the PCR primers with the Big Dye (Applied Biosystems, Foster City, CA) sequencing kit, following the manufacturer's protocol but using one-quarter volume reactions. Sequencing reactions were purified using either columns or the Agencourt CleanSEQ Sequencing Reaction Clean-Up system, which uses paramagnetic beads to remove excess dye terminators (Agencourt Bioscience Corp., Beverly, MA) and then electrophoresed on a Genetic Analyzer 3730 Dye (Applied Biosystems, Forester City, CA). The resulting sequences were imported into Sequencher (Gene Codes Corp., Ann Arbor, MI) for viewing, trimming, and creating consensus sequences from the complementary strands for each sample. These sequences were aligned using Sequencher and then exported in NEXUS format. PAUP\* 4.0a (Swofford 2001) was used to do a heuristic parsimony search of trees (TBR branch

swapping, 100 random taxon addition replicates) (Donoghue et al. 2001). We did not perform a bootstrap analysis of the data due to the low number of informative characters.

## CHAPTER 3: RESULTS

### Morphological and Environmental Study

The cluster diagrams (Figs. 7 & 8) show how similar the field samples of *Diervilla* are to each other.

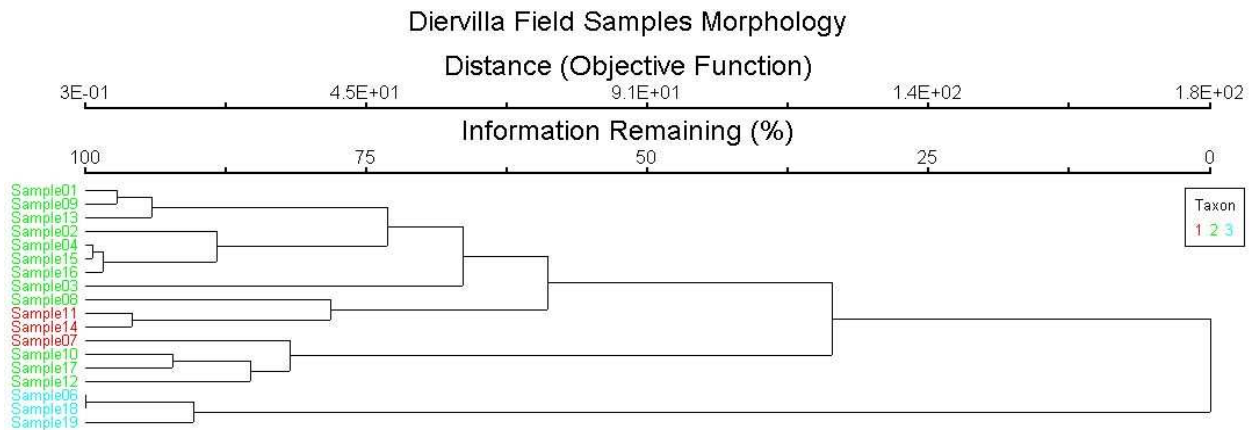


Figure 7. Cluster diagram of *Diervilla* field samples based on morphological data (Taxon 1=*D. lonicera*, Taxon 2=*D. sessilifolia*, and Taxon 3=*D. rivularis*)

The dendrogram of the cluster analysis for the field samples morphology data (Fig. 7) shows that all three samples of *D. rivularis* are more similar to each other than to the samples of *D. sessilifolia* and *D. lonicera*. Note that two of the samples (18 & 19) were not actually part of my field collections, but were taken from herbarium specimens to see if the field sample of *D. rivularis* I collected would group with other *D. rivularis* samples. *Diervilla lonicera* samples 11 and 14 grouped together, but *D. lonicera* sample 07 was more similar to *D. sessilifolia* samples 10, 17, and 12.

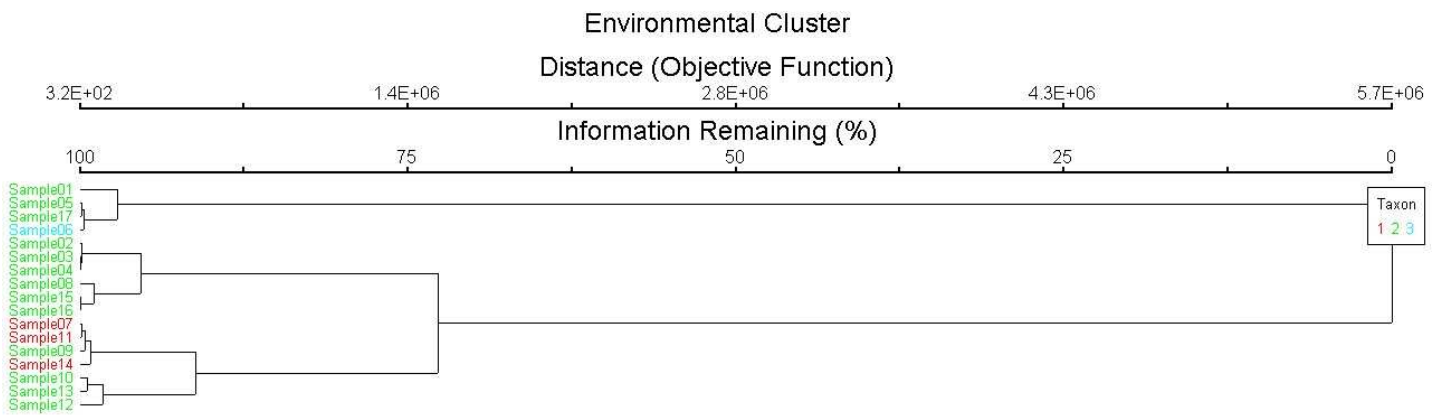


Figure 8. Cluster diagram of *Diervilla* field samples based on environmental data (Taxon 1=*D. lonicera*, Taxon 2=*D. sessilifolia*, and Taxon 3=*D. rivularis*)

The dendrogram for the environmental data (Fig. 8) showed that the field sample of *D. rivularis* (Sample 06) was most similar to *D. sessilifolia* samples 17 and 15. The *D. lonicera* samples were most similar to each other and *D. sessilifolia* sample 09.

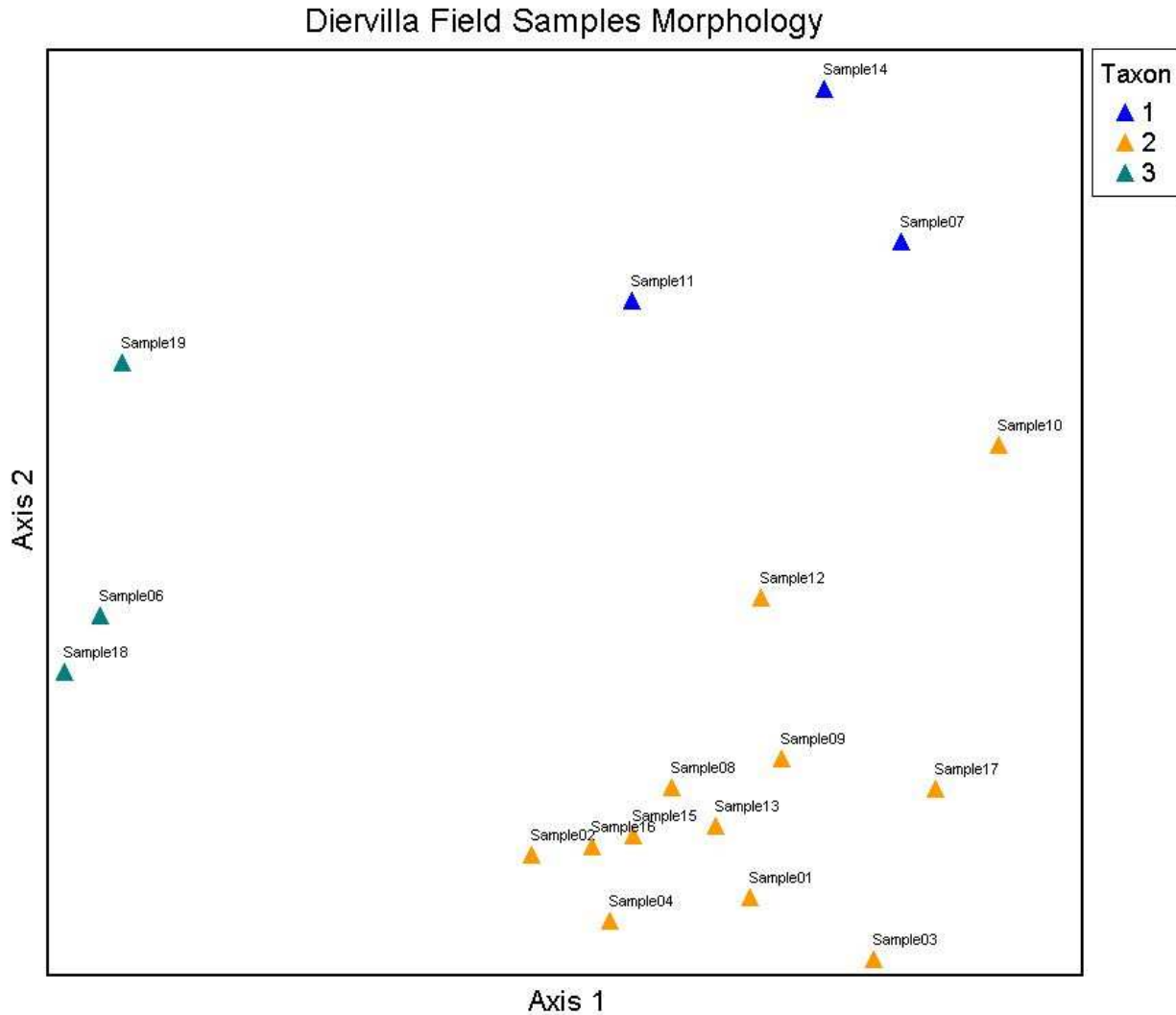


Figure 9. PCA results for field samples based on morphological data (Taxon 1=*D. lonicera*, Taxon 2=*D. sessilifolia*, and Taxon 3=*D. rivularis*). Axis 1 explains 23.834% of sample variation and Axis 2 explains 17.175% of sample variation.

The Principal Components Analysis for the morphological data on the field samples showed the three different species grouping with each other, overall. The first graph (Figure 9) plots the samples along Axis 1 and Axis 2. Axis 1 increases along two main character traits, “beak” length and petiole length and decreases along hairiness traits, the strongest being pedicel pubescence. Axis 2 increases along plant size traits, the strongest being plant height and

decreases along hairiness traits. It makes sense that the three species groups would group together by these traits: *D. rivularis* samples group together because they are much more hairy than *D. sessilifolia* and *D. rivularis*. *D. lonicera* has more characters that are categorized as “hairiness,” but it also has longer petioles and “beaks” than both *D. rivularis* and *D. sessilifolia*. *D. sessilifolia* sorts toward the lower right corner of the graph. Though the two highest positive eigen values for Axis 1 were beak length and petiole length, respectively, petiole length would be more representative for *D. sessilifolia* because it has sessile leaves.

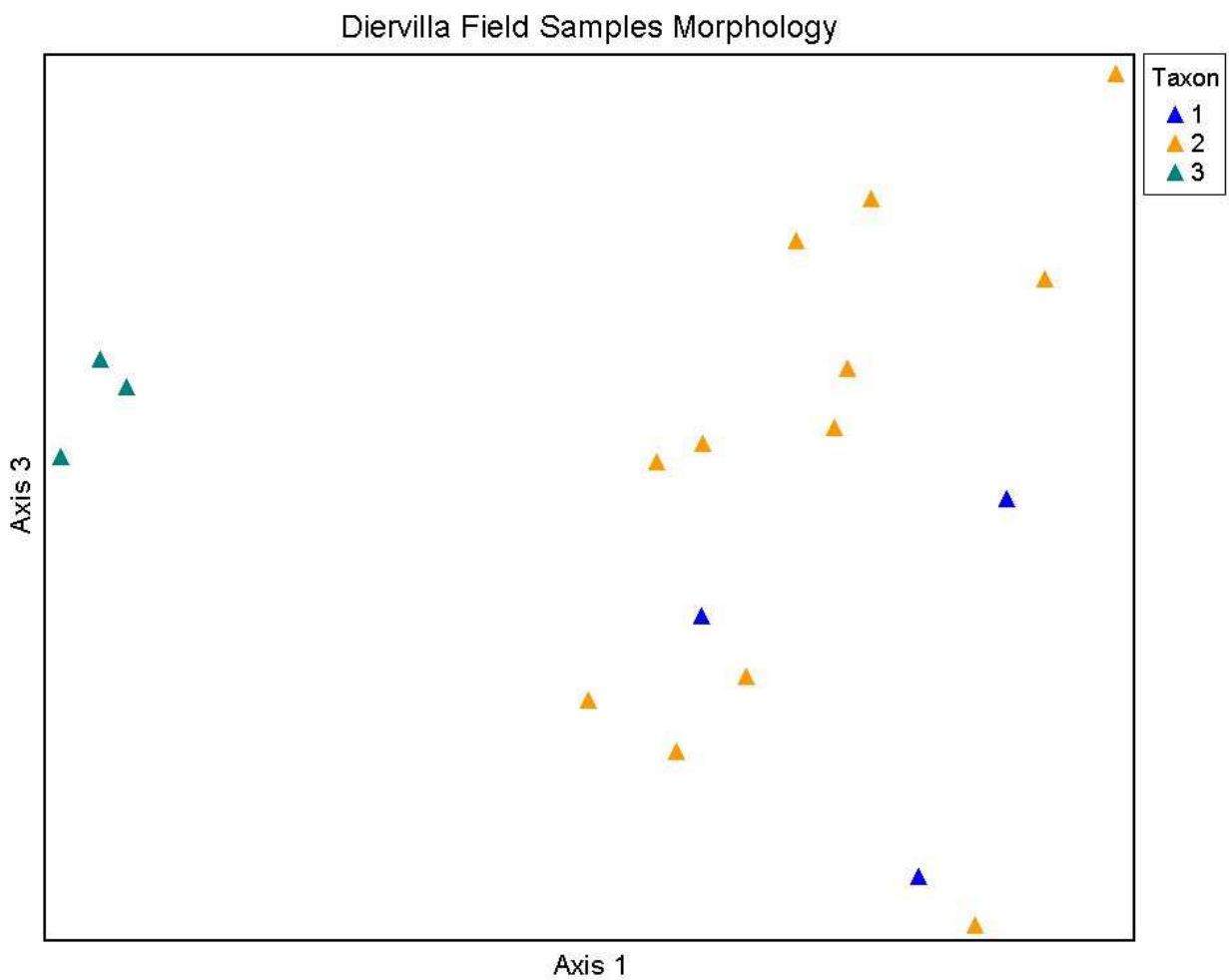


Figure 10. PCA results for field samples based on morphological data (Taxon 1=*D. lonicera*, Taxon 2=*D. sessilifolia*, and Taxon 3=*D. rivularis*). Axis 1 explains 23.834% of sample variation and Axis 3 explains 14.676% of sample variation.

The second graph (Fig. 10) plots the samples along Axis 1 and Axis 3, which increases along sepal lobe length and decreases along twig cross-section shape. Once again, the *D. rivularis* samples are grouped together and far from the other samples based on their hairiness and longer sepal lobes. The *D. lonicera* samples are scattered within the larger *D. sessilifolia* cluster, but they group together more because it would appear they have shorter sepal lobes compared to *D. rivularis* and *D. sessilifolia*.

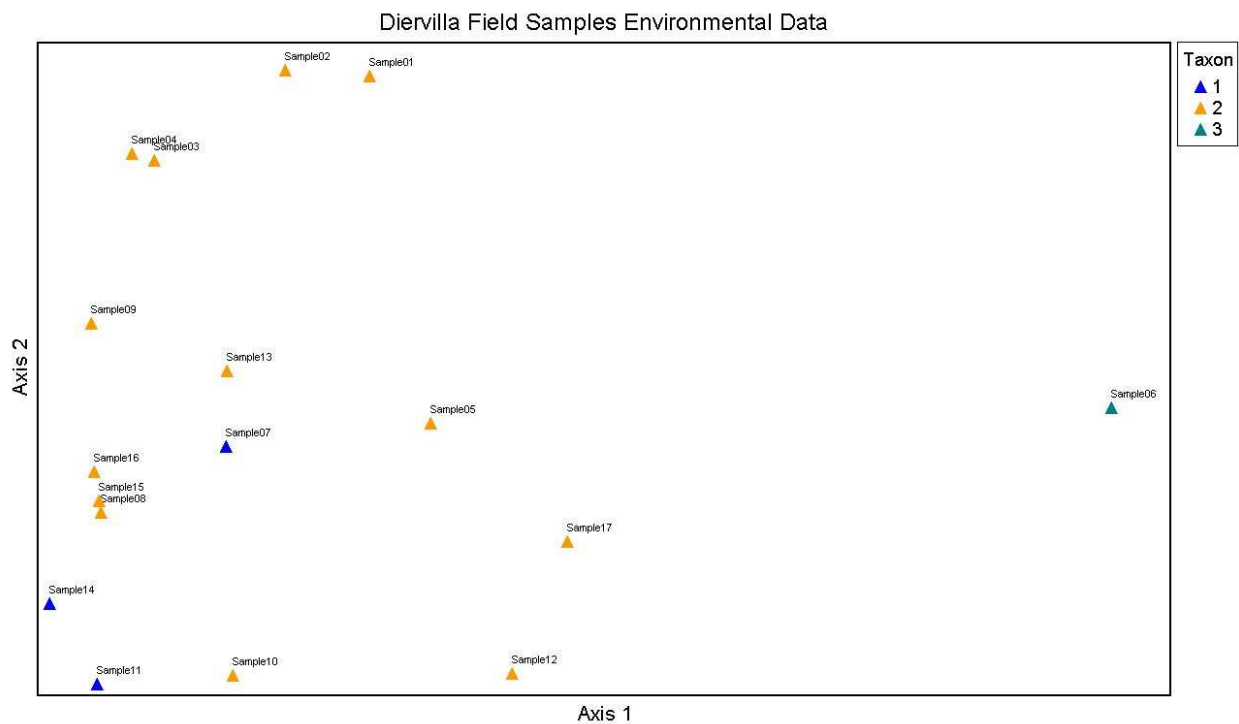


Figure 11. PCA results for field samples based on environmental data (Taxon 1=*D. lonicera*, Taxon 2=*D. sessilifolia*, and Taxon 3=*D. rivularis*). Axis 1 explains 34.209% of sample variation and Axis 2 explains 19.979% of sample variation.

The first graph of environmental data (Fig. 11) plotted Axis 1 against Axis 2. Axis 1 increases along elevation and decreases along maximum soil depth, while Axis 2 increases along the amount of light reaching the plant and decreases along the slope aspect the plant is growing on. *D. rivularis* is separate from *D. sessilifolia* and *D. lonicera*, and *D. lonicera* is grouped closely together.

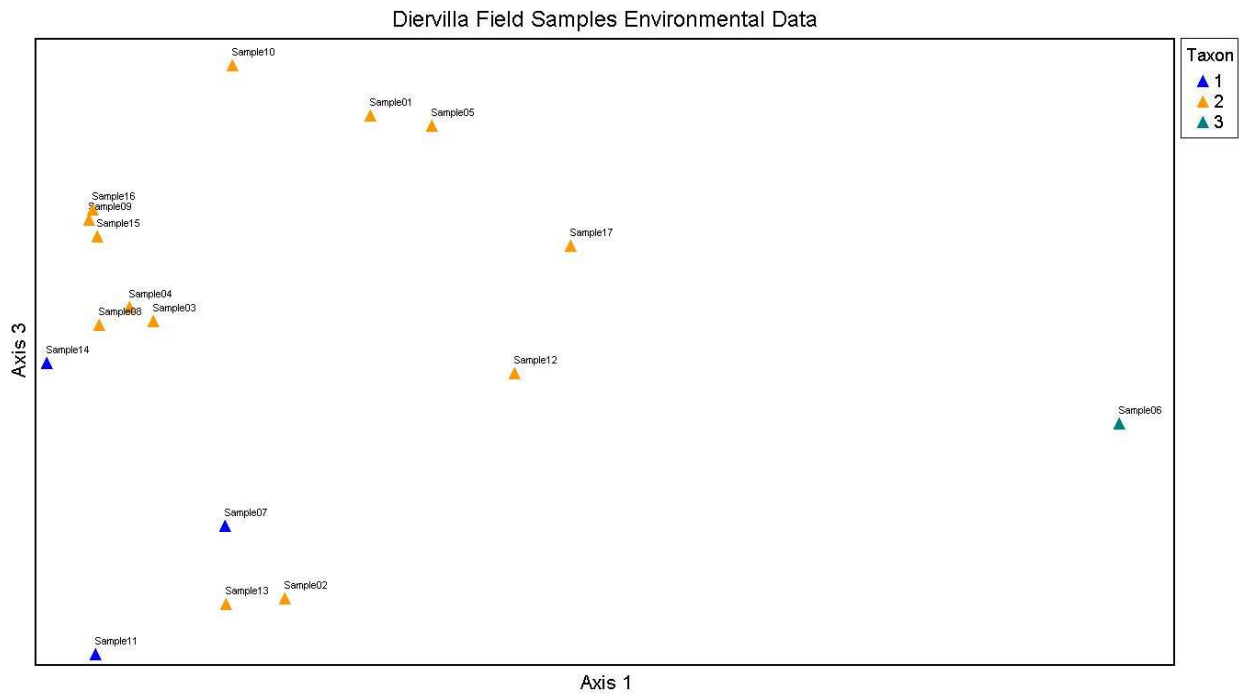


Figure 12. PCA results for field samples based on environmental data (Taxon 1=*D. lonicera*, Taxon 2=*D. sessilifolia*, and Taxon 3=*D. rivularis*). Axis 1 explains 23.834% of sample variation and Axis 3 explains 14.616% of sample variation.



The second graph (Fig. 12), which plots Axis 1 against Axis 3 (highest eigen value is percent vegetation cover and lowest eigen value is latitude), looks very similar to Figure 11.

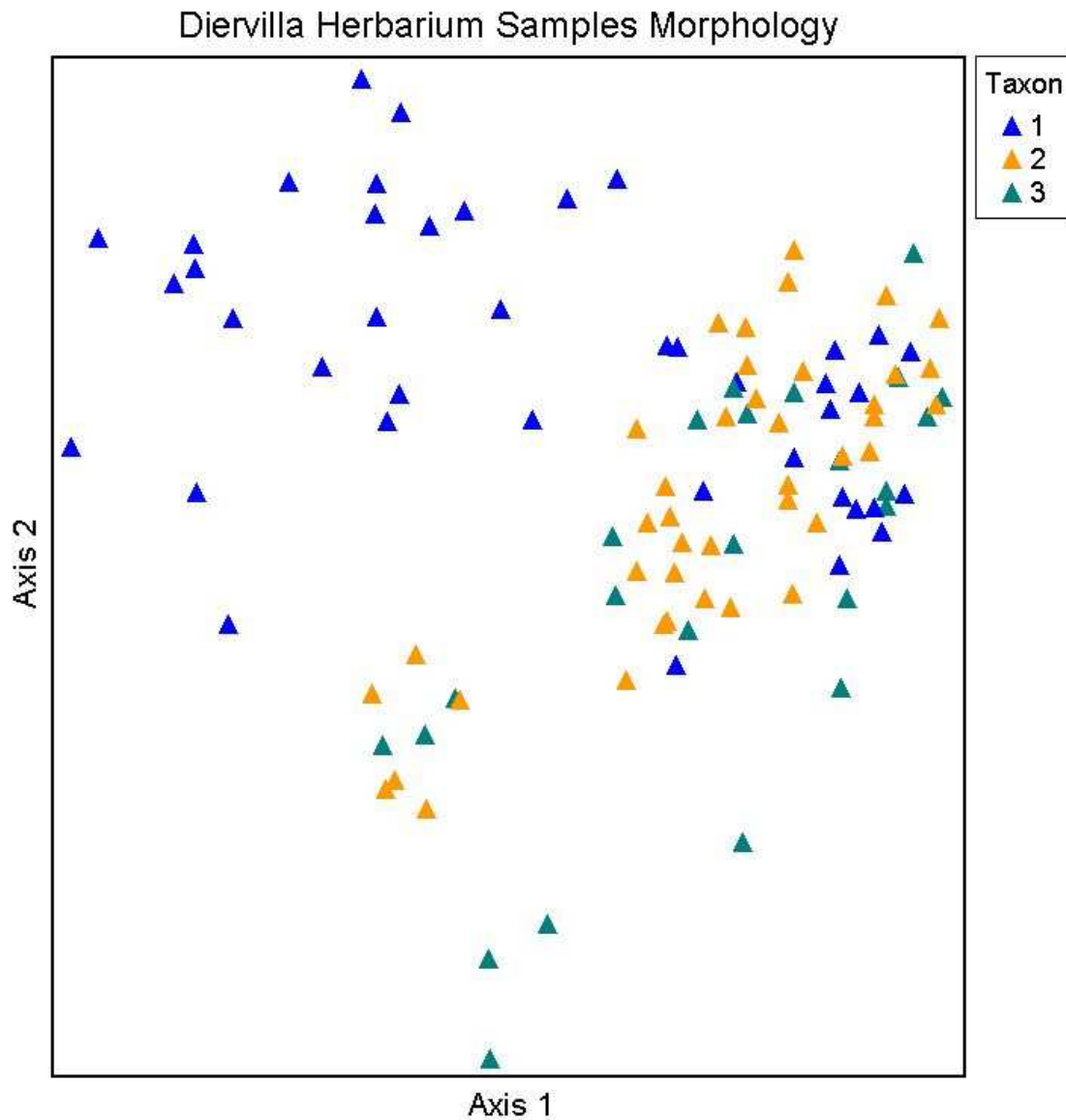


Figure 13. PCA results for field and herbarium samples based on morphological data (Taxon 1=*D. lonicera*, Taxon 2=*D. sessilifolia*, and Taxon 3=*D. rivularis*). Axis 1 explains 23.909% of sample variation and Axis 2 explains 15.834% of sample variation.

Figure 13 shows the PCA results for the morphological data collected from the herbarium specimens. Axis 1 increases strongest along the trait leaf shape, followed by petiole length and it decreases along twig pubescence followed by degree of twig pubescence. Axis 2 increases almost equally along twig shape and petiole length and decreases strongest along degree of petiole pubescence followed by pedicel pubescence.

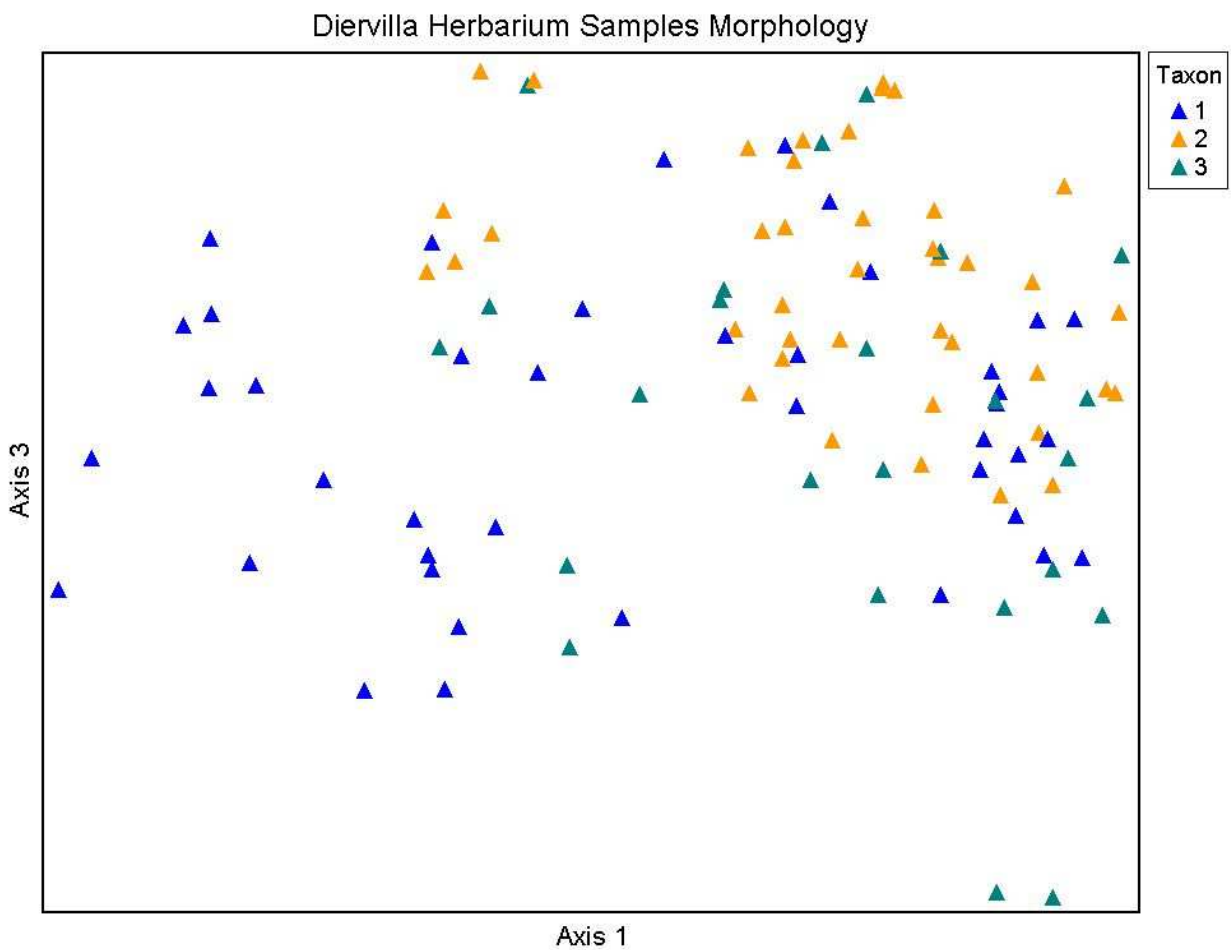


Figure 14. PCA results for field and herbarium samples based on morphological data (Taxon 1=*D. lonicera*, Taxon 2=*D. sessilifolia*, and Taxon 3=*D. rivularis*). Axis 1 explains 23.834% of sample variation and Axis 3 explains 9.214% of sample variation.

As in Figure 13, Axis 1 of Figure 14 increases strongest along the trait leaf shape, followed by petiole length and it decreases along twig pubescence followed by degree of twig pubescence. Axis 3 increases most strongly along the traits ciliate leaf margin and petiole length and decreases along degree of pedicel pubescence, though the decreasing values are not very strong. The increase in sample size shows a greater overlap between *D. sessilifolia* and *D. rivularis*.

### **Phylogenetic study**

The results of the maximum parsimony phylogenetic analysis are shown below in Figure 15.

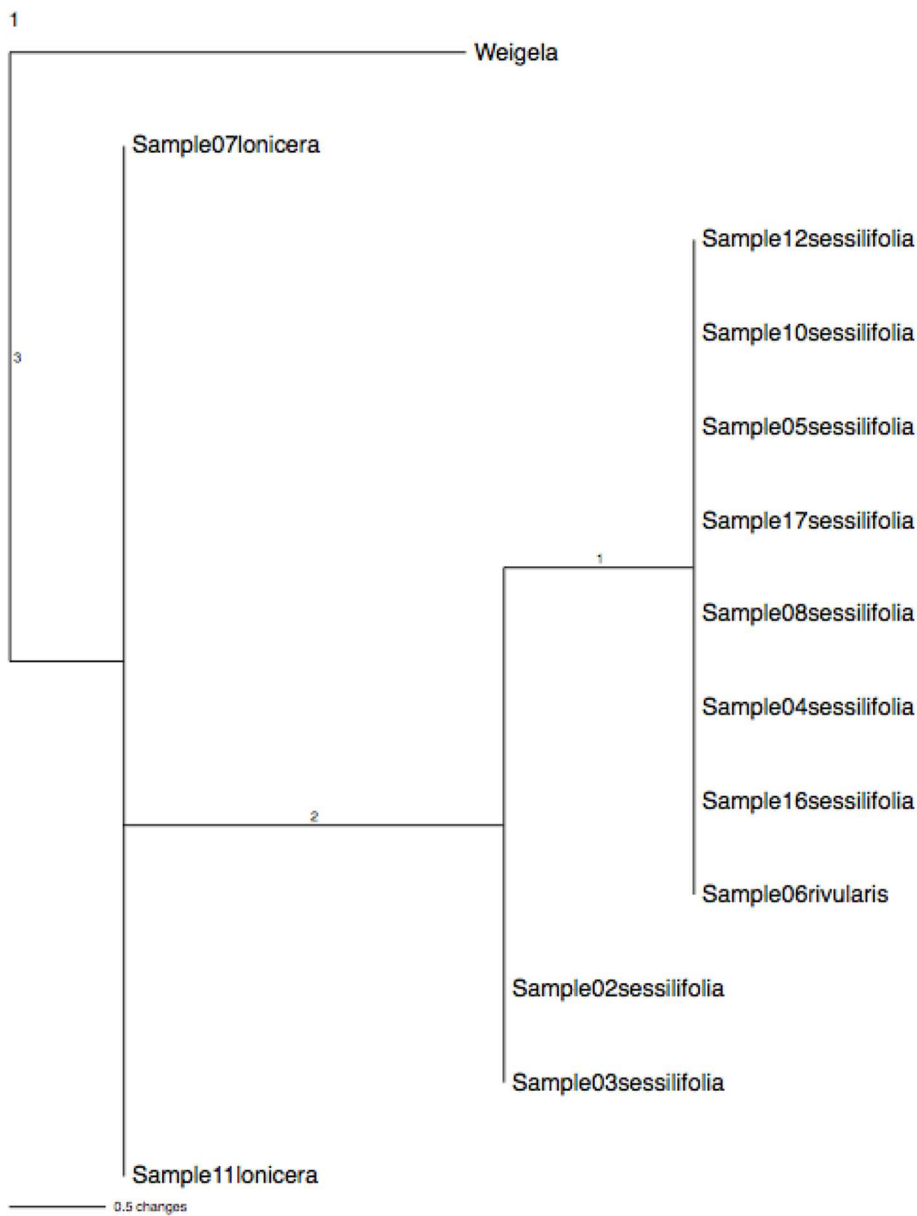


Figure 15. Single most parsimonious tree based on trnS-G sequence data (tree length=6)

The maximum parsimony analysis of cpDNA returned a single shortest tree of length 6, with CI excluding uninformative characters of 1.0000. The number of parsimony-informative characters was 3. Figure 15 shows where the tree separates the taxa. The first split in the tree

separates Sample 07 and Sample 11 from the rest of the taxa. These were two of the samples that are considered to be *Diervilla lonicera* and they are unresolved outside of the main *Diervilla* clade. The next split in the tree separates Sample 02 and Sample 03 from the remaining samples. Sample 02 and Sample 03 are both samples of *D. sessilifolia* and were collected in similar areas of Great Smoky Mountains National Park. The last clade has a mixture of *D. sessilifolia* and *D. rivularis*. Sample 06 is *Diervilla rivularis* and was collected from Lula Falls, where Gattinger described the species in 1888. Overall, there are a few shared derived mutations, but very little sequence variation. It is possible that there could be more variation in northern populations of *D. lonicera*, but they were not included in this study.

## CHAPTER 4: DISCUSSION

This research addressed three main questions: (1) Do the putative species of *Diervilla* co-occur in the southern Appalachians and if so, do they have different specific habitats? (2) How is morphological variation structured among populations of all three taxa and can it be used to determine species boundaries? And (3) what are the phylogenetic relationships among the three taxa of *Diervilla* and are their populations monophyletic?

### Species Co-occurrence

I did not find any evidence that the species of *Diervilla* co-occur in the southern Appalachians during my field research. All of the populations that I collected samples from were distinctly *D. lonicera*, *D. rivularis*, or *D. sessilifolia*. The only exception was one population of *D. sessilifolia* (sample 12) along the Ocoee River in Tennessee. Despite this sample looking slightly different from other populations of *D. sessilifolia* (leaves not sessile, more pubescent overall), it was not genetically distinct from other populations of *D. sessilifolia* and the population of *D. rivularis* in the trnS region (Figure 15). All of the populations that I visited were comprised of only one species of *Diervilla* and I did not find any other species of *Diervilla* growing anywhere near them. There is not much historical biogeographical information in the literature about *Diervilla*, but in his 1968 paper, Hardin also reports that the ranges of the three species do not appear to over-lap. In my research, Principal Components Analyses of field specimens based on environmental data (Figs. 11 & 12) show over-lap between populations of *D. sessilifolia* and *D. lonicera*. The sample of *D. rivularis* (sample 06) sorts out very distinctively from all other samples (Figs. 11 & 12), but overall the environmental data were inconclusive. The environmental characters that are most strongly influencing this are elevation, latitude, and longitude (locality).

## Utility of Morphological Characters

Principal Components Analyses on the specimens collected in the field, suggest that morphological characters may be useful to determine species boundaries (Figs. 9 & 10), but only on a small geographic scale (NC-GA). The field collections group together by species based on morphological characters such as degree of leaf and twig pubescence and petiole length (Figs. 9 & 10). However, there is still some over-lap here between *D. sessilifolia* and the other species *Diervilla* that could be a result of *D. sessilifolia* populations being more variable across their geographic range. When the sample size was increased using morphological measurements collected from herbarium specimens, the species overlap increases more (Figs. 13 & 14), which suggests that these morphological characters are not as helpful at a broader geographic scale. It is also possible and worth noting that some of the herbarium samples may be misidentified.

## Phylogenetic Relationships

Phylogenetic Analysis does agree with *Diervilla* being a monophyletic group (Fig. 15). The samples of *D. lonicera* separated into a branch together, but whether or not there are significant genetic differences between populations of *D. sessilifolia* and *D. rivularis* is still unclear. Two samples of *D. sessilifolia* (sample 02 & sample 03) were grouped together separate from the remaining samples of *D. sessilifolia* and *D. rivularis*. I am not sure why these two samples of *D. sessilifolia* separated from the other samples in the parsimony tree (Fig. 15). It is likely not caused by environmental factors as another sample collected nearby (sample 04) sorted into the larger group of *D. sessilifolia* and *D. rivularis* (Fig. 15). These results, combined with the original lack of differences among the sequences (~6 differences between *D. lonicera* and *D. sessilifolia* and *D. rivularis* in the trnS sequence and no differences between *D. rivularis* and *D. sessilifolia*), suggest that all three of these species are still very similar and closely related

genetically and that there is not a significant genetic difference between populations of *D. sessilifolia* and *D. rivularis*. This indicates that *D. rivularis* and *D. sessilifolia* are perhaps not separate species after all and should be synonymized.

### Conclusions and Further Research

Based on my analyses, I cannot conclude that *D. lonicera*, *D. sessilifolia*, and *D. rivularis* are indeed three distinct taxa and it is still not clear why the genus has such high variability, particularly *D. sessilifolia*. One possible explanation could be that the three species have only recently diverged. It is possible that *Diervilla* could have split from *Weigela* during the Oligocene to Miocene (24-38 mya), when the migratory routes between Asia and North America were cut off due to climate change (Wiser 1994). It appears that the divergence of the *Diervilla* species may have been much more recent because of their lack of genetic variation and morphological differentiation. Two hypotheses for *Diervilla* speciation are: (1) a single common ancestor existed throughout eastern North America prior to glaciation. The populations contracted southward during the Pleistocene ice ages and divergence into southern and northern species began around 12,000 years ago, as populations began to move back northward after the retreat of the glacier. Or (2) *Diervilla* diverged into northern and southern species prior to glaciation. *D. lonicera* could have been pushed southward and upward during the Pleistocene ice ages and then recolonized the north. This would mean that the current southern populations of *D. lonicera* are glacial relicts. Genetic mixing could have occurred through hybridization among the species in the southern refugium. This is quite possible as the southern Appalachians were glacial refugia for many plant species (Wiser 1994).

Based on my analyses, *D. sessilifolia* and *D. rivularis* are most similar and at least appear to be sister species, diverging from each other more recently than the split from *D. lonicera*, but



much more work needs to be done to support this. A much larger field study with many more samples and sequencing other variable genetic markers would be required to test among these possibilities. Another goal is to define better groups of recognition characters and species ranges. In addition, a common garden experiment or reciprocal transplant study could be insightful to see if these plants are still very similar genetically, but highly plastic depending upon the environment they are growing in (Thompson et al. 1991).

## LITERATURE CITED

- Backlund, A. K. and N. Pyck. 1998. "Diervillaceae and Linnaeaceae, two new families of Caprifolioids." *Taxon*, 47(3): 657-661.
- Bell, C. D. 2004. "Preliminary phylogeny of Valerianaceae (Dipsacales) inferred from nuclear and chloroplast DNA sequence data." *Molecular Phylogenetics and Evolution*, 31(1): 340-350.
- Bell, C. D.; E. J. Edwards; S.-T. Kim; M. J. Donoghue. 2001. "Dipsacales phylogeny based on chloroplast DNA sequences." *Harvard Papers in Botany*, 6(2): 481-499.
- Boyden, G. S., M. J. Donoghue, and D. G. Howarth. 2012. "Duplications and expressions of *RADIALIS*-like genes in Dipsacales." *International Journal of Plant Sciences*, 173: 971-983.
- Bremer, B., K. Bremer, N. Heidari, P. Erixon, R. G. Olmstead, A. A. Anderberg, M. Kallersjo, and E. Barkhordarian. 2002. "Phylogenetics of Asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels." *Molecular Phylogenetics and Evolution*, 24(2): 274-301.
- Buckley, S. B. 1843. "Description of some new species of plants." *American Journal of Science and Arts*, 45: 170-177.
- Burgess, K. S.; A. J. Fazekas; P. R. Kesanakurti; S. W. Graham; B. C. Husband; S. G. Newmaster; D. M. Percy; M. Hajibabaei; S. C. H. Barrett. 2011. "Discriminating plant species in a local temperate flora using the rbcL and matK DNA barcode." *Methods in Ecology and Evolution*, 2(4): 333-340.
- Darton, N. H. 1908. "The highest points in states in the United States." *Bulletin of the American Geographical Society*, 40(6): 332-340.
- De Queiroz, K. 2007. "Species concepts and species delimitation." *Systematic Biology*, 56(6): 879-886.
- Division of Nature Preserves. 2002. Endangered, threatened, and rare vascular plant species documented from Indiana (20 October 2002). Indiana Department of Natural Resources, Indiana.
- Donoghue, M. J., C. D. Bell, and J. Li. 2001. Phylogenetic patterns in northern hemisphere plant geography. *The International Journal of Plant Science*, 162(6 Suppl.): 541-552.
- Donoghue, M. J.; T. Eriksson; P. A. Reeves; R. G. Olmstead. 2001. "Phylogeny and phylogenetic taxonomy of Dipsacales, with special reference to *Sindoxa* and *Tetradoxa* (Adoxaceae)." *Harvard Papers in Botany*, 6(2): 459-479.
- Endler, J. A. 1977. "Geographic variation, speciation, and clines." Princeton, NJ, USA: Princeton University Press.
- Farris, M. A. and M. J. Lechowicz. 1990. "Functional interactions among traits that determine success in a native annual plant." *Ecology*, 71(2): 548-557.
- Ferguson, I. A. 1966. "The genera of *Caprifoliaceae* in the southeast United States." *Journal of the Arnold Arboretum*, 47: 33-59.
- Frazer, G.W., Canham, C. D., Lertzman, K. P. 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-color fisheye photographs. Copyright 1999: Simon Fraser University, Burnaby, BC, and the Institute of Ecosystem Studies, Millbrook, New York; (<http://www.rem.sfu.ca/forestry/index.htm> or <http://www.ecostudies.org>).
- Gattinger, A. 1888. "*Diervilla rivularis*." *Botanical Gazette*, 13(7): 191.

- Goulet, F. and P. Bellefleur. 1986. "Leaf morphological plasticity in response to light environment in deciduous tree species and its implication on forest succession." *Canadian Journal of Forest Research*, 16(6): 1192-1195.
- Hardin, J. W. 1968. "*Diervilla* (Caprifoliaceae) of the southeastern U. S." *Castanea*, 33(1): 31-36.
- Howarth, D. G. and M. J. Donoghue. 2006. "Phylogenetic analysis of the 'ECE' (CYC/TB1) clade reveals duplications predating the core eudicots." *Proceedings of the National Academy of Sciences*, 103(24): 9101-9106.
- Howarth, D. G. and M. J. Donoghue. 2009. "Duplications and expression of *DIVARICATA*-like genes in Dipsacales." *Molecular Biology and Evolution*, 26(6): 1245-1258.
- Index to plant chromosome numbers. 1979--. P. Goldblatt and D. E. Johnson, eds. Missouri Botanical Garden, St. Louis.
- Jacobs, B., S. Huysmans, and E. Smets. 2010. "Evolution and systematic value of fruit and seed characters in Adoxaceae (Dipsacales)." *Taxon*, 59(3): 850-866.
- Jacobs, B. ; F. Lens; E. Smets. 2009. "Evolution of fruit and seed characters in the *Diervilla* and *Lonicera* clades (Caprifoliaceae, Dipsacales)." *Annals of Botany*, 104: 253-276.
- Kim, Y.-D. and S.-H. Kim. 1999. "Phylogeny of *Weigela* and *Diervilla* (Caprifoliaceae) based on nuclear rDNA ITS sequences: biogeographic and taxonomic implications." *Journal of Plant Research*, 112(3): 331-341.
- McCune, B. and M. J. Mefford. 1999. PC-Ord: Multivariate analysis of ecological data; Version 4 for Windows.
- Miller, P. 1768. *The Gardeners Dictionary*:...eighth edition.
- Montesinos-Navarro, A., J. Wig, F. X. Pico, and S. J. Tonsor. 2010. "*Arabidopsis thaliana* populations show clinal variation in a climatic gradient associated with altitude." *New Phytologist*, 189(1): 282-294.
- The NCBI handbook [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2002 Oct. Chapter 18, The Reference Sequence (RefSeq) Project. Available from <http://www.ncbi.nlm.nih.gov/books/NBK21091/>
- Nikolić, T. 1991. "Phenotypic plasticity and taxonomic significance in *Thalictrum minus*-complex." *Feddes Repertorium*, 102(5-6): 345-349.
- Pyck, N. and E. Smets. 2000. "A search for the phylogenetic position of the seven-son flower (*Heptacodium*, Dipsacales): combining molecular and morphological evidence." *Plant Systematics and Evolution*, 225(1): 185-199.
- Radford, A.E.; Ahles, H.E.; Bell, C.R. 1968. *Manual of the vascular flora of the Carolinas*. Chapel Hill, NC: University of North Carolina Press.
- Schlichting, C. D. 1986. "The evolution of phenotypic plasticity in plants." *Annual Review of Ecology and Systematics*, 17: 667-693.
- Schoen, D. J. 1977. Floral biology of *Diervilla lonicera* (Caprifoliaceae). *Bulletin of the Torrey Botanical Club*, 104(3): 234-240.
- Small, J. K. 1933. *Manual of the southeastern flora*. NY: the author.
- Tank, D. C. and M. J. Donoghue. 2010. "Phylogeny and phylogenetic nomenclature of the Campanulidae based on an expanded sample of genes and taxa." *Systematic Botany*, 35(2): 425-441.
- Tennessee Natural Heritage Program. 2002. Rare plant list (20 October 2002). Department of Environment and Conservation, Tennessee.

- Thompson, J. D., T. McNeilly, and A. J. Gray. "Population variation in *Spartina anglica* C. E. Hubbard. I. Evidence from a common garden experiment." *The New Phytologist* 117, no. 1 (1991): 115-28. <http://www.jstor.org/stable/2556790>.
- USDA, NRCS. 2017. The PLANTS Database (<http://plants.usda.gov>, 5 November 2017). National Plant Data Team, Greensboro, NC 27401-4901 USA.)
- USDA Web Soil Survey. Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at the following link: <https://websoilsurvey.sc.egov.usda.gov/>. Accessed [November/09/2017].
- Weakley, A. S. 2015. *Flora of the southern and mid-Atlantic states*. Working draft, Chapel Hill, NC: University of North Carolina at Chapel Hill.
- Wiley, E. O. and B. S. Lieberman. 2011. *Phylogenetics: theory and practice of phylogenetic systematics*, second edition. Hoboken, NJ: John Wiley & Sons, Inc.
- Winkworth, R. C., C. D. Bell, and M. J. Donoghue. 2008. "Mitochondrial sequence data and Dipsacales phylogeny: mixed models, partitioned Bayesian analyses, and model selection." *Molecular Phylogenetics and Evolution*, 46(3): 830-843.
- Wiser, S. K. 1994. "High-elevation cliffs and outcrops of the southern Appalachians: vascular plants and biogeography." *Castanea*, 59(2): 85-116.