AN INVESTIGATION OF POTENTIAL ADDITIONAL SPECIES AND HYBRIDIZATION IN EARLY SAXIFRAGE (*MICRANTHES VIRGINIENSIS*)

A thesis presented to the faculty of the Graduate School of
Western Carolina University in partial fulfillment of the
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By

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ABSTRACT

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Micranthes virginiensis (Saxifragaceae) is an herbaceous, flowering plant native to Eastern North America with a range extending from Arkansas into Maine and Canada. This broad range, known from previous studies to contain individuals with varying chromosome numbers and morphological variation outside of the current formal description, indicates the need for a reexamination of the taxonomy of this species. Some populations in Southeastern Appalachia display intermediate traits between *M. virginiensis* and *M. careyana* and have unresolved phylogenetic placement, raising the possibility of hybridization. This study aimed to define the identity of *M. virginiensis* by comprehensively sampling populations from throughout this species' range. Specifically, this study will explore hypotheses of hybridization and cryptic or undescribed species within *M. virginiensis* based on morphometric and chromosome data collected from voucher specimens across Eastern North America. Floral, fruit, and leaf measurements were analyzed to investigate morphological variation across the species' range. Chromosome counts from *M. careyana*, *M. palmeri*, and *M. petiolaris* populations all showed diploidy (2n = 20), representing the first known chromosome counts for these species. Tetraploidy and unique floral morphology in the Gap Creek and Wadakoe Mountain escarpment

populations, where the distribution of *M. virginiensis* and *M. careyana* overlap, indicate a new species of hybrid origin which could be described in a future publication. Other tetraploid populations in the Southeast have no known morphological differences from diploid *M. virginiensis*, suggesting autopolyploidy. Additionally, the diploid Polk Co., NC escarpment populations exhibit unique floral morphology with spreading and somewhat reflexed petals and may represent a separate lineage.

CHAPTER ONE: INTRODUCTION

Introduction & Significance

Geographically widespread species often encompass considerable genetic and morphological variation as a result of adaptations to factors such as ecological differences across the range, genetic isolation and drift, migration, selection, and genetic sorting (Wang et al. 2020; Karron 1987; Soltis & Soltis 1991). In some cases, this variation is artefactual, representative of multiple undescribed species (i.e., Nesom 2021; Judd et al. 2007; Ji et al. 2020; Diaz-Tapia et al. 2018). One example of a geographically widespread species is *Micranthes virginiensis* (Michx.) Small (Saxifragaceae), an Eastern North American flowering plant often described as polymorphic (Engler 1872; Johnson 1923) and highly variable (Small 1986; Bush 1928; Lord 1960). *Micranthes virginiensis* has had many taxonomic synonyms and subtaxa described and has had multiple ploidy levels reported, leading to the question of whether it contains more than one species. In this study, I will consider the variations in morphology and chromosome number and perform species diagnoses to determine if there has been significant differentiation throughout the range to constitute multiple lineages.

Micranthes Haw. includes ~85 species in Saxifragaceae and is found from polar regions at sea level to mountainous regions throughout the Northern Hemisphere (Tkach et al. 2015). It is primarily comprised of scapose herbs with leaves in a basal rosette, a cyme or thyrse inflorescence of small, white flowers, and a bilobed/partially apocarpous ovary maturing into two dehiscent follicles (eFloras 2021). Although originally recognized as a genus by Haworth in 1812, some investigators (i.e., Engler 1872, Engler & Irmscher 1916, Gornall 1987) continued to classify *Micranthes* as a subgroup or section of genus *Saxifraga* L. due to similar morphology.

Other taxonomists (i.e., Small 1903) viewed the two groups as distinct genera. Currently, *Micranthes* is recognized as a genus separate from *Saxifraga* following molecular phylogenetic analyses which revealed their distinctiveness (Soltis et al. 1996). *Micranthes*, containing North American, South American, and Eurasian species, was placed in the Heucheroid clade of Saxifragaceae, while *Saxifraga*, containing predominantly Eurasian species, was separated into its own clade, *Saxifraga* s. str. (Soltis et al. 2001).

Past dramatic fluctuations in climate have led to many range changes and disproportionate species richness in cold areas of high elevation and latitude, resulting in evolutionary complexities within *Micranthes* that remain difficult to disentangle, including hybridization, cryptic species, and variation in chromosome number (Stubbs et al. 2020a). For example, the Pacific Northwestern *Micranthes hitchcockiana* (Elvander) Brouillet & Gornall (*n* = 38) is believed to be of hybrid origin between *M. rufidula* Small (*n* = 19) and *M. oregana* (Howell) Small (*n* = 19) based on chromosome number and apparent morphological intermediacy (Elvander 1984). The most common chromosome numbers in this genus are 2*n* = 20, 38, and 56, with many species exhibiting several different counts, indicating that aneuploidy and polyploidy are rife throughout the genus (Stubbs et al. 2020a). The multiple instances of 2*n* = 20, 38 chromosomes in *Micranthes* may be a result of chromosome fusions followed by tetraploidization (Stubbs et al. 2020a). *Micranthes virginiensis* faces similar complexities to other members of this genus, thus a study that would clarify morphological and cytological variation within the geographic range of this taxon and investigate potential cryptic lineages or hybrid populations would improve our understanding of the evolutionary history and taxonomy of this genus and species.

Micranthes virginiensis is found on rock outcrops, moist alluvial and slope forests, streambanks, and riverbanks, and has a broad distribution from southeast Canada throughout the eastern United States into Louisiana and Arkansas (Weakley 2020; Fig. 1). It grows at an elevation of 0-1500m (eFloras 2021), and thus is restricted to escarpment regions in the Southern Appalachians. Lord (1960) noted that this species has long been known to exhibit variations in its physical characteristics, particularly with regard to leaf shape and margin type, distribution of pubescence, follicle number, and scape branching pattern. After examining herbarium specimens from across the range, she determined that all individuals are consistent in their presence of a hypanthium (a cup-like structure surrounding the ovary) and short stamen length relative to petal length when compared to sympatric *Micranthes* species. However, populations inconsistent with these characters have recently been discovered (Lanning & Mathews 2019), adding to the question of potential undescribed species encompassed under the name *M. virginiensis*.

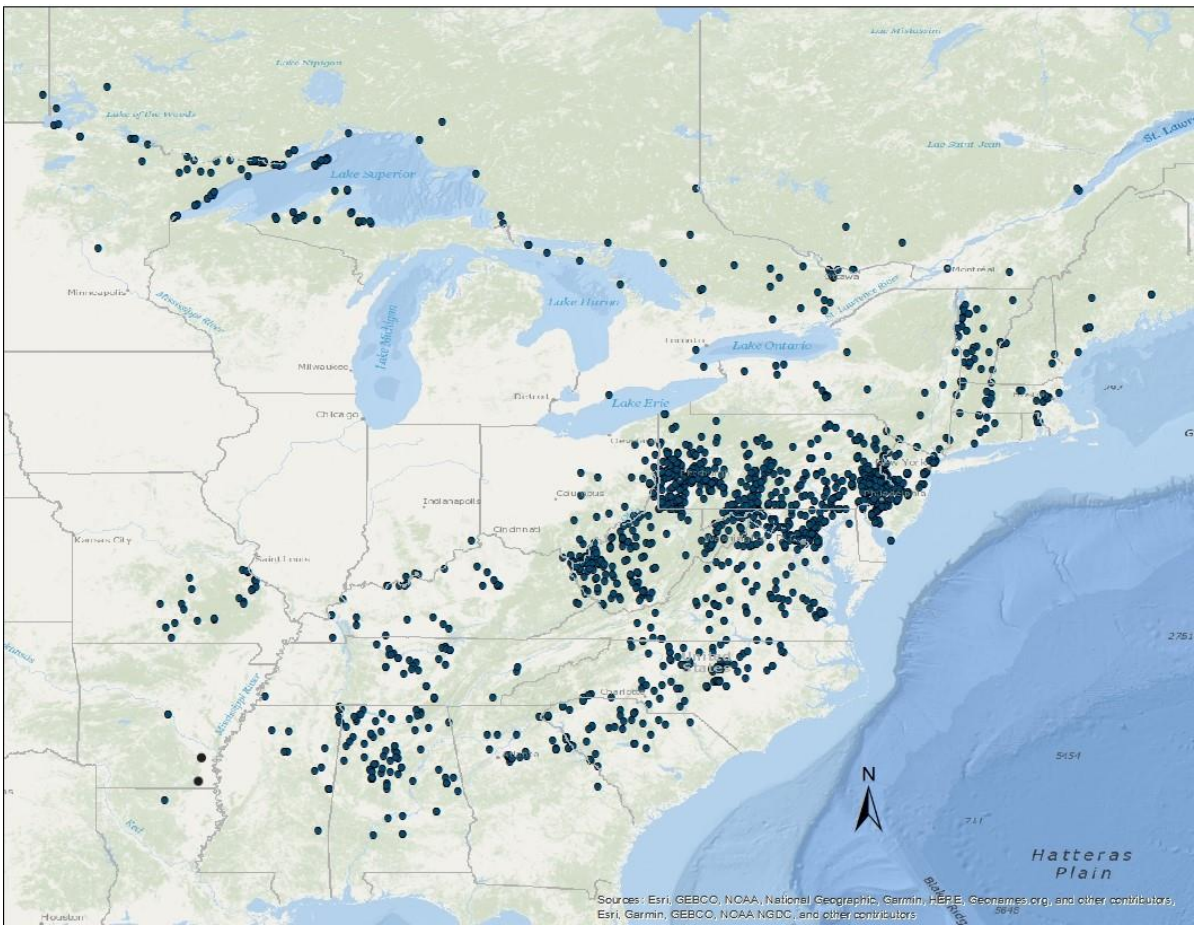

Figure 1: Distribution map of *Micranthes virginiensis.* Locality information obtained from SERNEC, herbarium records, and iNaturalist. Map created with ArcGIS.

Taxonomic History of M. virginiensis

André Michaux described *Saxifraga virginiensis* in his *Flora Boreali-Americana* (1803) prior to the first description of *Micranthes* (Haworth 1812). Translated from Latin, he described it as follows: "The whole thing is a little pubescent; leaves oval, obtuse, somewhat petiolate, crenate; scape mostly aphyllous, paniculate, its branches with subsessile alternating flowers; calyx erect." The range was listed as rocky Pennsylvania, Virginia, and the mountains of the Carolinas. Michaux cited a drawing in Plukenet's *Phytographia* (1691) as the type but stated that the panicle was not yet fully developed. Notably, Michaux's description does not mention the

stamens. The stamens are also not visible in the flowers of the type illustration, though this may be intentional as the stamens of *M. virginiensis* are often short enough to be hidden within the hypanthium. Stamen size and shape are known to be important characters in delimiting Southern Appalachian *Micranthes* (Lanning & Mathews 2019), so the omission of stamen traits in the description and type illustration leaves some ambiguity regarding this important flower part in this species.

Interestingly, there is an herbarium specimen of *Saxifraga virginiensis* collected by Michaux and housed in the National Museum of Natural History in Paris, France (Fig. 3; P00709241) that I have determined contains individuals of both *M. virginiensis* and the Southern Appalachian endemic *Micranthes careyana* (A. Gray) Small (1843) based on stamen length. This suggests that Michaux did not recognize the presence of two distinct species, emphasizing their similarity. Though the only locality information given is "Amériq Sept" (= North America), the individuals of this specimen were most likely collected in an area of the Southern Appalachian escarpment, such as eastern Tennessee or western North Carolina, as these are the only areas where both species occur (Fig. 9).

Figure 2: Type specimen of *Saxifraga virginiensis* found in *Phytographia* (1691) by Leonard Plukenet. Designated as the type by André Michaux in *Flora Boreali-Americana* (1803) in his original description of *S. virginiensis*. https://bibdigital.rjb.csic.es/records/item/13656-phytographia-pars-tertia

Figure 3: (a) Specimen labeled as *Saxifraga virginiensis* collected in North America by A. Michaux (n.d.). (b) Magnified image of flowers on leftmost plant in Image a. Short stamens indicate *Micranthes virginiensis.* (c)

Magnified image of flowers on center plant in Image a. Long stamens indicate *M. careyana* misidentified here as *S. virginiensis.* MNHN-P-P00709241

Some authors have recognized segregate taxa from *M. virginiensis* based on perceived morphological differences associated with particular geographic areas (i.e., Hooker 1833; Sternberg 1810; Bush 1928), though these heterotypic species names have all subsequently been synonymized with *M. virginiensis* based on overlap in the characters. Below is a summary of these taxonomic synonyms.

Willdenow (1803) described *Saxifraga vernalis* as synonymous with *S. virginiensis* in his original description, automatically rendering the former name as illegitimate in accordance with Article 52 of the International Code of Nomenclature for algae, fungi, and plants (ICN, Turland et al. 2018; i.e., it was superfluous at its time of publication, because the taxon (as represented by the type) already has a name; see Fig 4a). However, Hooker (1833) recognized *S. vernalis* as distinct on the basis of differences in the inflorescence — the flower arrangement of *S. vernalis* forms an imperfect corymb or thyrse that contrasts with the sessile, alternate, and somewhat unilateral flowers on the branches of the panicle of *S. virginiensis*. Yet, when examining the type illustration of *S. virginiensis,* the panicle appears to match Hooker's description and type illustration of *S. vernalis.* In the original description, Willdenow states the range of *S. vernalis* as Pennsylvania, Virginia, and the Carolina mountains, whereas Hooker ascribes this species to Canada without mention of the range stated by Willdenow, and he reports that he received samples of *S. virginiensis* mixed with *S. vernalis* (Hooker 1833). *Saxifraga vernalis* was not considered distinct from *S. virginiensis* by Torrey & Gray (1840) in their flora of North America, as they noted that they perceived no differences between the two taxa. Hooker (1847) later relegated this taxon to a variety of *S. virginiensis*.

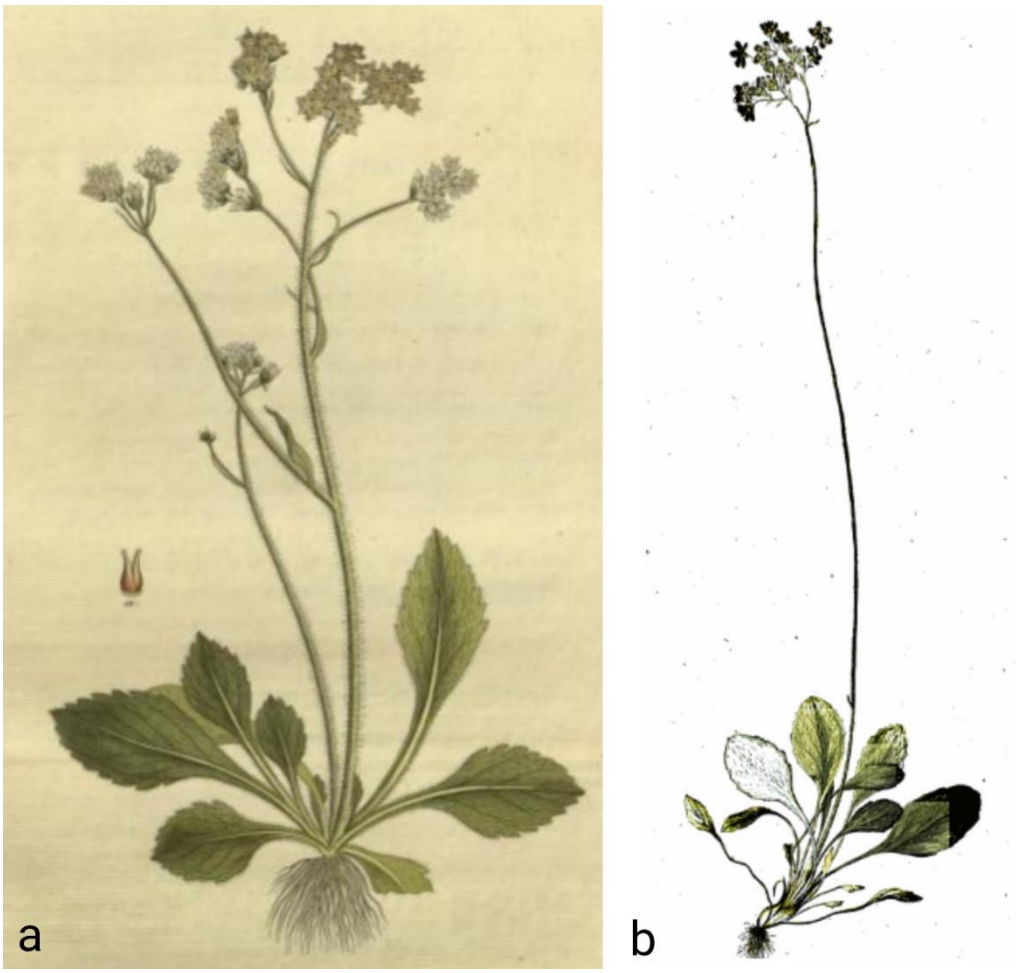

Figure 4: (a) Type illustration of *Saxifraga vernalis* Willd. in *Hortus Berolinensis* (1803). Accessed via biodiversitylibrary.org. (b) Type illustration of *Saxifraga elongata*, described by Sternberg (1810). Accessed via books.google.com. These taxon names are currently considered subordinate to *Micranthes virginiensis*.

Sternberg (1810), following Willdenow's description of *Saxifraga vernalis* as synonymous with *S. virginiensis,* described *S. elongata* Sternb. as different from *S. vernalis* based on differences in the inflorescence. *Saxifraga elongata* (Fig. 4b), occurring in the Carolinas, has an elongated, unbranched scape with a cluster of small branches at the apex that contrasts with the branching scape of *S. vernalis* (Fig. 4a). Hooker (1833) considered *S. elongata* a variety of *S. vernalis.*

Haworth (1803) first described *Saxifraga pilosa* (Haw.) Bush as a distinct species based on the pilose nature of the entire plant and the obtusely dentate leaves, and he later recognized *S. vernalis* and *S. virginiensis* as subordinate to this species (Haworth 1821) though he never indicated a type specimen. Bush (1928) recognized *S. pilosa* as separate from *M. virginiensis* based on morphological and geographic differences. He described *M. virginiensis* as northeastern, possessing a cyme inflorescence, sharply serrated leaves, and multiple scapes, while the southern and midwestern *S. pilosa* was described as racemose, with obtusely dentate leaves and a short, solitary scape (Bush 1928). Steyermark (1959) opposed this split, arguing that though there is variation in many characters of *M. virginiensis,* there is intergradation of the characters between the two regions and thus there is no justification for splitting the species. *Saxifraga vernalis, S. elongata,* and *S. pilosa* are all currently considered subordinate names to *Micranthes virginiensis.*

There are no currently recognized varieties of *M. virginiensis* (FNA vol. 8, 2009), though numerous varieties have been described. *Saxifraga virginiensis* var. *cicinnata* Engl. (1872) is described from Pennsylvania and Canada reporting the fruiting plants as loosely paniculate, with elongate secondary branches surpassing the terminal flower and flowers in a cicinnate inflorescence (Engler 1872). However, Engler lists this variety as a synonym of *S. virginiensis,* so it is not entirely clear how it differs from the nominal variety of *S. virginiensis*. Another variety, *Saxifraga virginiensis* var. *cuneata* Farw. (1944) is described based on an apparent discrepancy in the descriptions of *S. virginiensis* in *Gray's New Manual* (1908) and *North American Flora,* 22 (1905) — *Gray's New Manual* describes obovate or oval-spatulate leaves and purplish follicles, whereas *North American Flora* describes ovate, oval, or oblong leaves and green follicles. Farwell (1944) observed a population in Keeweenaw Co., Michigan that he felt

best aligned with the *Gray's New Manual* description and designated this *S. virginiensis* var. c*uneata* (Fig. 5).

Figure 5: (a) Type of *Saxifraga virginiensis* var. *cuneata*, designated by Farwell (1944). This taxon name is currently considered subordinate to *Micranthes virginiensis*. (b) Magnified image of the head of the inflorescence. (c) Herbarium label. Accessed via plants.jstor.org.

While there are no currently recognized varieties, there are two recognized species that have previously been considered varieties of *M. virginiensis* due to morphological similarities. *Micranthes californica* (Greene) Small (1905) (=*Saxifraga virginiensis* var. *californica* (Greene) Jeps. (1901)), is a Western North American species that is geographically disjunct from the rest of *M. virginiensis* and morphologically distinguished by differences in petal and sepal shape and orientation (Small 1896). *Micranthes palmeri* Bush (1928) (=*Saxifraga virginiensis* var. s*ubintegra* Goodman (1950)) occurs in Arkansas and Oklahoma, overlapping with the range of *M. virginiensis* (Fig. 8). It is distinguished from *M. virginiensis* by the entire leaf margins, as

opposed to toothed margins, lack of glands on the inflorescence pubescence, and glabrous pedicels (Steyermark 1959). Molecular phylogenetic analyses have also revealed these three taxa to be distinct lineages (Stubbs et al. 2020b). *Micranthes palmeri* is the sister taxon of *M. virginiensis*, while *M. californica* is less closely related, though still within the core Micranthes clade (Stubbs 2020b).

Multiple forms of *M. virginiensis* have been described in New England. Within Essex Co., Massachusetts, three forms have been reported to exist in addition to typical *M. virginiensis*, two of which are only known to this county. *Saxifraga virginiensis* f. *chlorantha* (Oakes) Fernald (1917) has pale green petals contrasting with the typical white flowers as well as short hairs on the margins and backs of the petals. This form was found in Topsfield, Mass. and only known from a short description in an 1842 publication that designated no type specimen (Oakes 1847; Fernald 1917). *Saxifraga virginiensis* f. *pentadecandra* (Sterns) Fernald (1917) is apetalous and possesses 15 stamens, as opposed to 10, with five stamens taking the positions of the petals (Sterns 1870; Fernald 1917). It was first described on Manhattan Island in New York, but was reported in Essex Co., Mass. as well. *Saxifraga virginiensis* f. *glomerulata* Fernald (1917) is distinguished from typical *M. virginiensis* by a lack of pedicels that cause the flowers to form glomerules and was described based on three collections by A. S. Pease in Andover, Mass. from 1901-1902 (Fernald 1917; Fig. 6). In addition to the Essex Co. forms, *Saxifraga virginiensis* f. *plena* Eames differs from typical *M. virginiensis* only in the doubled number of petals (Eames 1931). This form is described from one location in Litchfield County, Connecticut, though Eames noted other reports of double-flowered plants from Massachusetts, Pennsylvania, and New York in his original description. Sterns (1887) suggested that these forms were likely teratological phenomena.

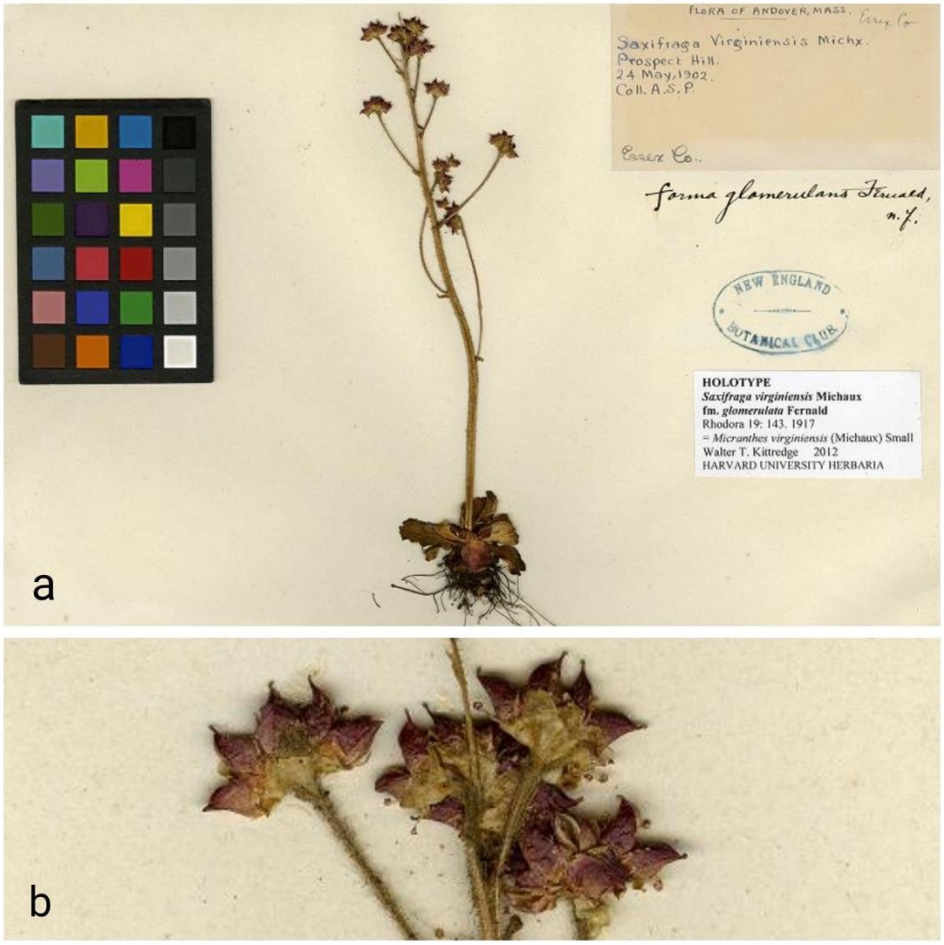

Figure 6: (a) Type specimen of *Saxifraga virginiensis* f. *glomerata*, described by Fernald (1917). This taxon name is currently considered subordinate to *Micranthes virginiensis*. (b) Zoomed in image of sessile fruits in glomerules. NEBC00348793

Chromosome Counts

Although numerous subordinate taxa have been described, none of them correspond to populations with known differences in chromosome number. However, polyploidy may have taxonomic significance for this species. *M. virginiensis* is reported to have 2*n* = 20 chromosomes in specimens from Massachusetts, Missouri, Tennessee, northern and western Virginia, and Canadian populations, and 2*n* = 38 chromosomes in specimens from North Carolina and eastern Virginia populations, the latter indicative of tetraploidy (2*n* = 40) followed by an aneuploid reduction (Soltis 1983, Fig. 7). Though allopolyploidy, referring to chromosome duplication due

to hybridization, is well known as a catalyst for speciation (Mallet 2007), autopolyploidy, which results from chromosome duplication within a species, has often been considered by taxonomists to represent cytotypes of a single species rather than multiple species due to convention and morphological similarity (Soltis et al. 2007). However, it has been argued that autopolyploids can represent distinct evolutionary lineages that can fulfill requirements of multiple species concepts, including biological, taxonomic, diagnosability, apomorphic, and evolutionary, despite often exhibiting high morphological similarity to their diploid progenitors (Soltis et al. 2007). Soltis et al. (2007) suggested that in these cases, autopolyploids should be considered separate species to accurately reflect evolution and facilitate conservation. The populations of *M. virginiensis* that exhibit polyploidy could be reproductively isolated or associated with unique morphological or ecological characteristics, indicating potential undescribed or cryptic species as in *Tolmiea* (Judd et al. 2007). A variable number of supernumerary chromosomes was also reported by Soltis (1983), which could be of taxonomic significance (D. Poindexter, pers. comm.), further evidencing the complexity of this species. Sister species of *M. virginiensis* with overlapping distributions (*Micranthes careyana, M. caroliniana* (A. Gray) Small, and *M. palmeri*, Fig. 8) have not had chromosome counts reported, and the ability for hybridization between *M. virginiensis* and its sister taxa is not known, therefore polyploidy in this species is in need of further investigation and will be a focus of this study.

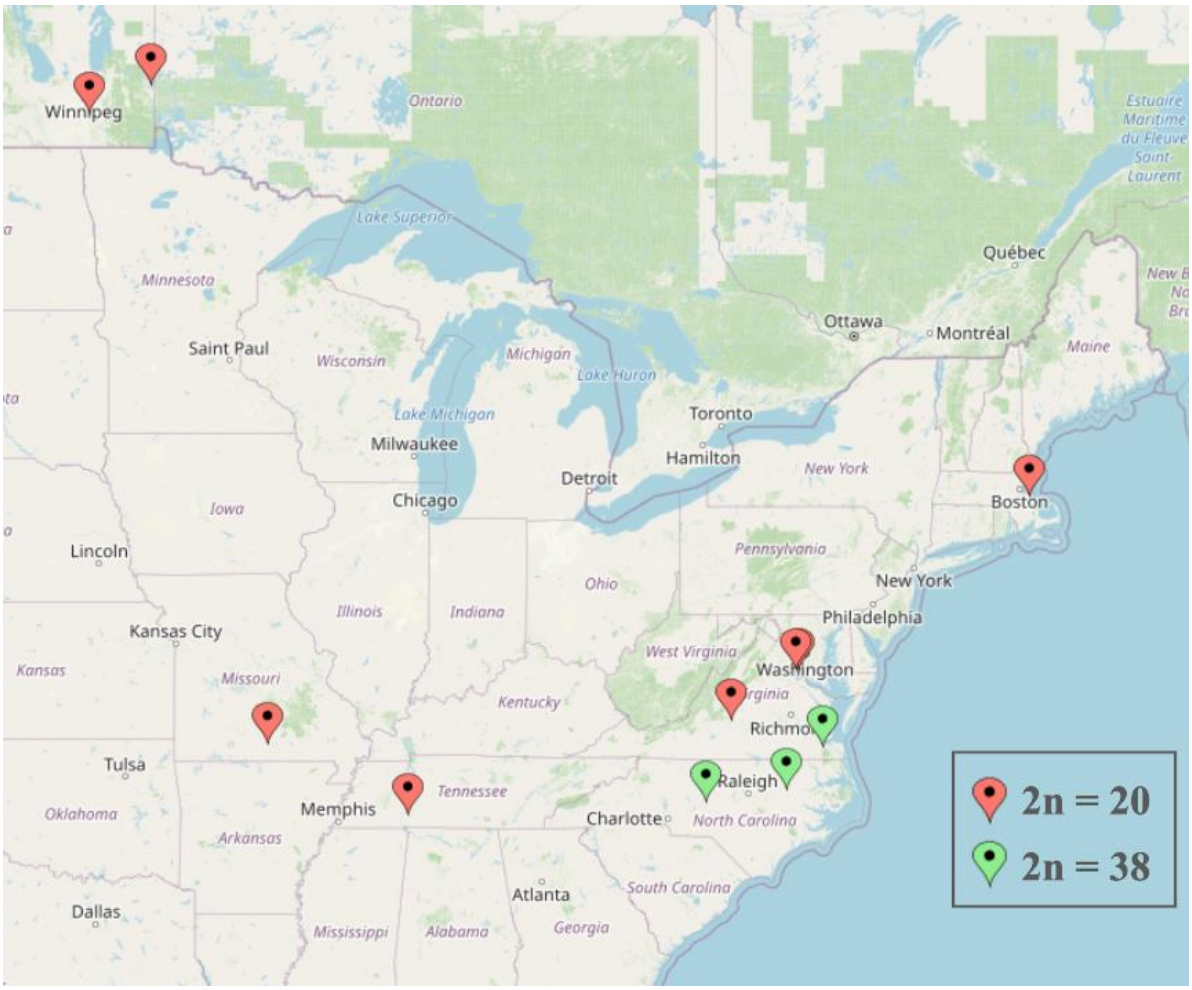

Figure 7: Map depicting the locations of previously reported chromosome counts for *Micranthes virginiensis* populations from Soltis (1983), Löve & Löve (1982), Kovanda (1978), Hill (1989) and Löve & Ritchie (1966). Red indicates 2n = 20; green indicates 2n = 38. All counts originally reported as 2n = 28 were determined by Soltis (1983) to be 2n = 20 (+ 8 supernumerary) and were thus treated as 2n = 20 here. Map created with MapCustomizer.

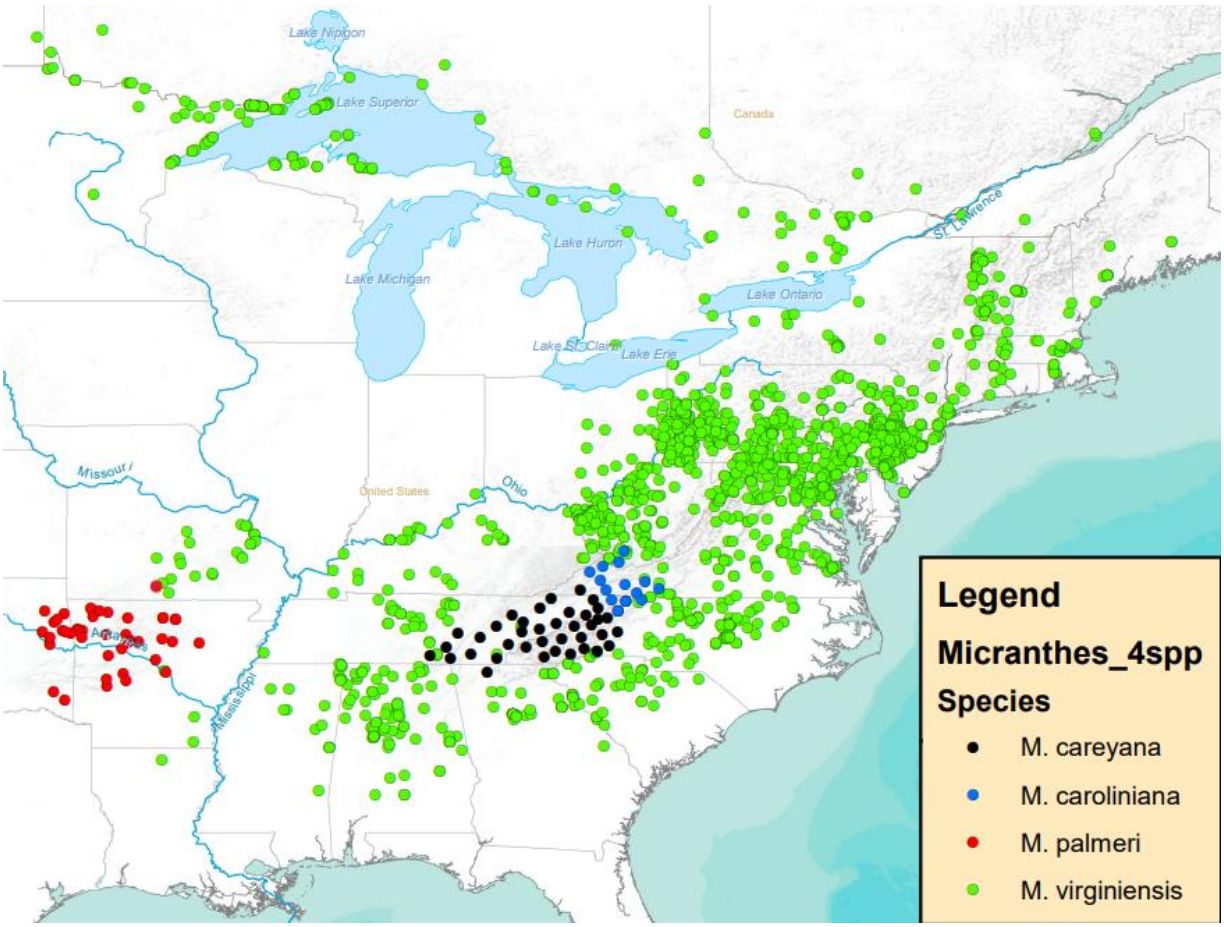

Figure 8: Distribution map of *Micranthes virginiensis* and its three most closely related sister species, *M. careyana*, *M. caroliniana*, and *M. palmeri*. Locality information obtained from SERNEC, herbarium records, and iNaturalist. Map created with ArcGIS.

Species Diagnosability

A clear definition of a species should help determine whether polyploidy and other conditions indicate the presence of undescribed species within *M. virginiensis*. However, though many species concepts have been proposed and employed, the clarification of a universal species concept has long been a point of contention in the scientific community (Sites & Marshall 2004; de Queiroz 2007). Historically, there have been many proponents of concepts based on morphology or reproductive compatibility (i.e., biological species concept, Mayr 1963), yet these concepts pose issues for the taxonomy of organismal groups that hybridize or reproduce asexually and often lead to nonmonophyletic groupings that misrepresent evolutionary history

(Donoghue 1985, Wheeler 1999, de Queiroz & Donoghue 1988). Concepts focusing on monophyly (i.e., phylogenetic species concept, Donoghue 1985), can address these issues with trait-based concepts by better accounting for hybridization and asexual reproduction. However, those who prefer trait-based concepts argue that the ranking of a phylogenetic species based on monophyly does not have a discrete criterion when compared to the delimitation of taxa at other ranks, such as genus or subspecies, and thus the species rank is not special (Baum & Smith 2013). Also, paraphyly is not uncommon in the early stages of speciation, so many biologists consider it acceptable to delimit a paraphyletic species based on other data.

In an effort to resolve the longstanding species concept debate, de Queiroz (2007) proposed a unified species concept whereby the only requirement for species status is existence as a separately evolving metapopulation lineage. In this concept, the different properties (i.e., reproductive isolation, monophyly, etc.) typically required in well-known but less inclusive concepts serve as lines of evidence of lineage separation. When interpreted correctly, it can be used to delimit species, though no specific property is recognized as necessary for species status (de Queiroz 2007). This separates the issues of species concept and species delimitation, providing one unified species definition while allowing various properties to validly delimit species. Though under this concept only one property is needed to demonstrate a separate lineage, the use of multiple lines of evidence is seen as providing a higher degree of corroboration. An extension of this would include evidence of an ecological role for species delimitation, as advocated by Freudenstein et al. (2017), although that is not the focus of this study. The use of multiple properties as evidence of novel taxa is valuable in delimiting species within evolutionarily complex groups that have undergone rapid radiation, such as *Micranthes* (Stubbs et al. 2020b).

Cryptic and Undescribed Species in Saxifragaceae

Consideration of multiple lines of evidence is particularly useful in a variable species like *M. virginiensis*, as the morphological, cytological, and molecular variations noted in previous studies (Lord 1960; Soltis 1983; Lanning & Mathews 2019) indicate a potential for undescribed and/or cryptic species which may be difficult to confirm through the use of just one property. Cryptic species are taxa that have been incorrectly identified as a single species as they are morphologically difficult or impossible to distinguish (Beheregaray & Caccone 2007), and the mounting array of DNA sequencing methods has fueled an exponential increase in the identification of cryptic species (Bickford et al. 2007).

There are numerous cases where previously undescribed or cryptic species in Saxifragaceae have been identified. Several studies in recent years have delimited new taxa in multiple genera through morphological, molecular, and cytogenetic means. *Tiarella* L. (1753), a genus long considered to represent only one eastern USA species, *T. cordifolia* L. (Weakley 2020), was recently split into five species based on previously unnoticed morphological differences in stolon presence, leaf shape, and stem leaves/bracts (Nesom 2021). In a study examining the utility of nuclear ribosomal DNA sequences for delimiting species, Okuyamo & Kato (2009) found evidence of at least three cryptic species in *Mitella* and suggested that many other angiosperm lineages contain cryptic species that could be discerned with molecular methods. Additionally, the genus *Tolmiea* held one species, *T. menziesii* (Pursh) Torr. & A. Gray, containing both diploid and autotetraploid populations, and though the two cytological conditions were not easily morphologically distinguishable from one another, Judd et al. (2007)

separated the diploid entity as a unique species, *T. diplomenziesii,* due to the different geographic distributions and apparent reproductive isolation determined through artificial crossing studies.

Hybridization leading to allopolyploidy could result in hybrid speciation, in which the hybrid offspring are able to persist and maintain a stabilized hybrid lineage generally recognized as species — in the case of allopolyploids, this often includes reproductive isolation (Mallet 2007). A recent systematic study of *M. caroliniana* and *M. careyana*, two Southern Appalachian endemics, found these species to be phylogenetically closely related and morphologically similar to *M. virginiensis* (Lanning & Mathews 2019), and these relationships were later confirmed by Stubbs (2020b), although they are not known to have the ability to hybridize. During Lanning's field collections, several putative *M. virginiensis* populations were observed to have some floral characteristics more consistent with *M. careyana,* including long stamens and large fruits. However, Lanning and Mathews (2019) did not sample throughout the range of *M. virginiensis*. *Micranthes virginiensis* characteristically displays partially fused sepals that form an adnate hypanthium (Weakley 2020). Notably, a Polk County, NC population (hereafter referred to as "Melrose") had unfused sepals and reflexed petals. Other morphological characteristics were consistent with *M. virginiensis,* and phylogenetic analyses of this population agreed with this placement. A population in Greenville County, SC (hereafter referred to as "Gap Creek") was previously identified as *M. virginiensis,* though phylogenetic analysis yielded mixed results, suggesting a possible hybrid origin. This population, along with another South Carolina population (hereafter referred to as "Wadakoe Mountain"), was difficult to confirm as *M. virginiensis* due to a late observation of the flowering state. These three populations are all found in the Blue Ridge escarpment region where *M. virginiensis* and *M. careyana* are sympatric.

The Southern Blue Ridge Province, spanning across the Appalachian Mountains of Virginia to Georgia, is known for high rates of endemism and species diversity, a mixture of tropical and alpine species, and populations at the periphery of many Northern and Western species ranges (Pittillo et al. 1998). This is due to the unique microhabitats created by the geomorphic structure of this mountain range formed over hundreds of millions of years, as well as the glacial advance and retreat cycle of the past 2 Ma (Pittillo et al. 1998; McMillan et al. 2018). Endemic species, disjunct distributions, and morphological oddities are commonplace in this region, particularly in the escarpment (i.e., Gray 1879; Wagner 1965; Billings & Anderson 1966; McMillan et al. 2018). In fact, a new variety of *Micranthes petiolaris* (Raf.) Bush was recently described in this area (*M. petiolaris* var. s*healyi*; Cushman et al. 2020).

Only one of the four populations of *M. virginiensis* sampled in Lanning & Mathews' study, from the Piedmont region near Birmingham, AL, exhibited all characteristics typical of this species, indicating that a more thorough study of this species is warranted and raising the possibility that there are hybridizing populations or undescribed species within this group (Lanning & Mathews 2019). Furthermore, because taxa with cosmopolitan geographic distributions have been frequently shown to hold cryptic diversity (i.e., Nesom 2021; Whittall et al. 2020; Wu et al. 2018; Diaz-Tapia et al. 2018), the latitudinal and climatic variation in the distribution of *M. virginiensis* across the eastern United States makes it a likely candidate for having such undescribed species.

Value of Peripheral Populations

Because some peripheral populations of *M. virginiensis* located in the escarpment region of the Southern Appalachians exhibit unique morphology and unclear phylogenetic placement (Lanning & Mathews 2019), a more thorough study of the peripheral populations across this

species range is of particular importance. Peripheral populations, or populations along the outskirts of a species' geographic range, have previously been undervalued in their role in the preservation of biodiversity. This resulted from assumptions that peripheral populations are less likely to survive and have lower genetic diversity than those in the center of the range, and conservation decisions have consequently diverted resources and support away from fringe populations (Channell 2004). However, there have been studies demonstrating that peripheral or disjunct populations can aid in the persistence of a species amid anthropogenic disturbance (Channell & Lomolino 2000). Phenotypes or genotypes of peripheral populations can vary from those in central populations, and these genetically unique populations can be better suited to the geographic range changes that will occur as a result of global climate change (Steen & Barrett 2015). Protecting peripheral populations of species can help preserve genetic diversity and therefore species richness, indicating that these populations should not be overlooked when investigating species and making conservation decisions.

Objectives

In light of the above discussion of questions pertaining to the number of species within this widespread and variable taxon, this study further examines *M. virginiensis* to determine if cryptic species or undescribed variation that may represent distinct species occurs throughout its range or if any subordinate taxa should be elevated to species status or recognized as a legitimate variety. In line with the unified species concept (de Queiroz 2007), multiple lines of evidence are used to delimit species, including cytological data from new meiotic chromosome counts and morphological data analyzed using multivariate analyses while sampling populations throughout the species' known geographic range, focusing on peripheral populations and populations with known cytological and morphological variation, examining specimens from the geographic

regions of the subordinate taxa to determine if the documented variation is representative of species-level differences, investigate the reported variations in the inflorescence, and identify any patterns in the morphological variation throughout the range. This study aims to (1) clarify the taxonomic boundaries of *M. virginiensis* and (2) unveil any undescribed species or hybrid populations existing within this taxon with particular focus on the Blue Ridge escarpment populations in order to assist botanists in field identification of *M. virginiensis*, contribute to the existing body of knowledge surrounding Southern Appalachian and Eastern North American biodiversity, and aid in informed management and conservation decisions.

CHAPTER TWO: METHODS

Plant Collections

In late winter and early spring of 2022 and 2023, I collected living plant specimens in pre-flowering condition (overwintering rosettes) from 37 populations (at least two individuals per population) of *Micranthes virginiensis* (including the populations identified by Lanning & Mathews (2019) in the Blue Ridge escarpment). I also collected from sympatric or peripatric populations of morphologically similar and closely related *Micranthes* species (Stubbs et al. 2020a): *M. careyana* and *M. palmeri*, and from more distantly related species: *M. micranthidifolia* (Haw.) Small and *M. petiolaris* to use as outgroups in the phylogenetic analyses (Fig. 9; Suppl. Table 1). Plants were grown in the Western Carolina University greenhouse until flowering stalks formed. I removed the young buds and placed them in vials containing Carnoy's solution (3:1 95% ethanol:glacial acetic acid) for 24 hours, after which I replaced the solution with 70% ethanol. Vials were placed in a -20°C freezer for later use in anther squashes for chromosome counts. The potted plants were grown until full anthesis and pressed as voucher specimens. From late winter to summer of 2022 and 2023, I obtained two living specimens from at least one population of *M. virginiensis* in full anthesis or in fruit from each U.S. state in the range and two Canadian provinces, Quebec and Ontario (Suppl. Table 1). Southern populations of *M. virginiensis* are known to flower earlier due to the earlier onset of warm weather, so collections began further south and moved northward throughout the field season to ensure full reproductive characteristics were available for accurate identification. I obtained collecting permits from the appropriate agencies as necessary. I pressed one voucher herbarium specimen

from each sampled population and deposited it into the Western Carolina University herbarium (WCUH).

Newly collected vouchers and herbarium specimens accessed via the Southeast Regional Network of Expertise and Collections (SERNEC) were georeferenced by an herbarium assistant, Caroline Witherspoon. I then created a distribution map for *M. virginiensis* in ArcMap using all georeferenced specimens available as of February 2023, supplementing regions containing no georeferenced SERNEC specimens with locality information from iNaturalist. Due to the volume of SERNEC specimens (1,721 individuals as of May 2023), not every specimen was examined; however, all specimens in peripheral areas of the range and in unexpected locations were thoroughly examined to confirm the identity. Misidentified specimens were annotated and *M. virginiensis* specimens with inaccurate locality information were not included in the distribution map or other analyses.

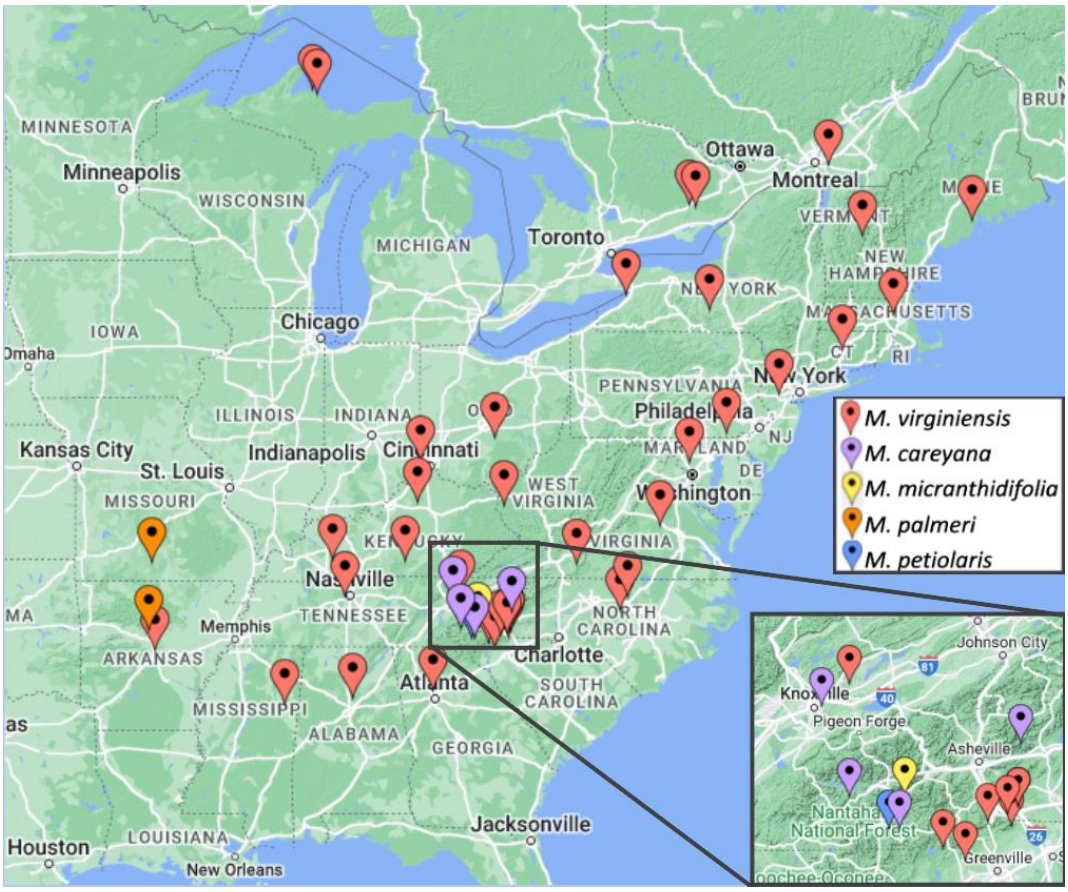

Figure 9: Locality information for voucher specimens of five Eastern North American *Micranthes* species collected for use in this study. Map created with Google Maps.

Morphological Analysis

I dissected live flowers from at least one individual from each sampled population of *M. virginiensis*, *M. careyana*, and the putative hybrids from the Blue Ridge escarpment region and imaged them using a Leica M205 C microscope and Leica Application Suite X software.

Leaf, flower, and fruit characters were measured from living and pressed specimens of *M. virginiensis*, the Gap Creek and Wadakoe Mountain populations, and *M. careyana* to compare the morphology of known taxonomically informative characters (i.e., hypanthium length, stamen length, pedicel glands, anther color, and fruit size) and search for additional characters that may be informative to determine if *M. virginiensis* is comprised of one species and if the escarpment

populations identified in Lanning & Mathews (2019) are of hybrid origin. Characters measured from live flowers included hypanthium length, pistil length, stamen length, petal length, petal width, presence of glands on pedicel pubescence, and anther color. I used the largest leaf from each pressed voucher specimen to measure blade length, blade width, petiole length, leaf margin type, blade circularity, and blade area. I obtained measurements of fruit length, distance between fruit horns, inflorescence number, internode number and lengths, and leaf margin type from images of 92 specimens in fruit from other herbaria accessed via SERNEC (Suppl. Table 2). Efforts were made to measure the largest flower and fruit from each specimen. All measurements of continuous characters were conducted in ImageJ (Schneider et al. 2012) from images containing scalebars for accuracy. Leaf characters were measured with the LeafJ plugin (Maloof et al. 2013). Non-continuous characters, including branching pattern, inflorescence type, and leaf margin type from 155 SERNEC specimens were examined.

All multivariate analyses were conducted in R Studio (vers. 4.2.3; R Core Team 2023). I conducted principal components analyses (PCAs) using the *princomp*() function in the stats package (R Core Team 2023) to explore if the specimens form distinct clusters based on morphological characteristics, identify components responsible for the greatest amount of variation in the dataset, and determine if any traits are associated with particular geographic regions. Five PCAs were conducted from the multivariate data matrix: 1) using all continuous floral and vegetative characters for the *M. virginiensis* populations, excluding the putative hybrids from Gap Creek and Wadakoe Mountain, to look for any indication of morphologically distinct clusters based on a wide range of characters, 2) using only continuous floral characters and plant height (excluding vegetative characters as previous research has indicated that these are not taxonomically informative in distinguishing *M. virginiensis* from *M. careyana* and *M.*

caroliniana; Lanning & Mathews 2019) for *M. virginiensis* populations to determine if there are any floral differences that were masked in the first PCA due to similarity in leaf morphology, 3) using only continuous floral characters and plant height (excluding leaf characters) for *M. virginiensis* and the Gap Creek and Wadakoe Mountain populations to determine if individuals from these two escarpment populations would cluster together distinct from *M. virginiensis*, 4) using only continuous floral characters and plant height (excluding leaf characters) for *M. virginiensis*, the Gap Creek and Wadakoe Mountain populations, and *M. careyana* to determine if all three groups would cluster separately based on these characters, and 5) using fruit characters for *M. virginiensis* specimens accessed via SERNEC to determine if fruit size and shape could reveal multiple clusters of *M. virginiensis*. Two-tailed t-tests were used to determine if the most informative characters identified in the PCAs were significantly different between *M. virginiensis* and the Gap Creek and Wadakoe Mountain populations to investigate the utility of those characters for field identification.

I conducted linear discriminant analyses (LDA) using the *lda*() function in the MASS package (Venables & Ripley 2002) to determine if the samples of *M. virginiensis*, *M. careyana*, and the Gap Creek and Wadakoe Mountain populations can be discriminated based on the measured characters and identify the characters that best discriminate the samples. I conducted analyses of variance (ANOVA) with the *aov*() function in the stats package (R Core Team 2023) using the LDA scores as latent variables to determine if the groups were significantly different. I used the *adonis2*() function in the vegan package (Oksanen et al. 2022) to conduct a permutational multivariate analysis of variance (PERMANOVA) using the morphological data matrix to determine if the three groups are significantly morphologically different. Because PERMANOVA cannot distinguish between dispersion and location (Anderson 2017), further

analyses were required. A dissimilarity matrix was calculated from the data set with *vegdist*() and then tested using *betadisper*() to see if dispersion differed significantly among groups. I used the ggord package (Beck 2022) to create all figures for the PCAs and LDA.

Chromosome Counts

I determined chromosome counts following the procedures outlined in Windham et al. (2020). I removed the young flower buds that had been fixed in Carnoy's solution and stored in 70% ethanol from the -20°C freezer and placed them on a petri dish slightly submerged in 70% ethanol. Under a dissecting microscope, I removed the anthers and broke them open with a needle tip. I separated the tissues from the anthers and used this as material for the chromosome counts. Material was stained on a clean slide with acetocarmine stain and crushed with a dissecting needle with extra acetocarmine added as needed to ensure the sample did not dry out. I added a drop of Hoyer's solution to increase chromosome visibility and reduce cover slip movement. To finish preparing the slides, I placed a cover slip on and pressed straight down with high pressure for 15 seconds on each corner of the slip as well as the edges. I examined prepared slides with the 65x oil immersion lens using a Leica Stellaris 5 confocal microscope. I took stacked images of cells with countable chromosomes and recorded chromosome counts for *M. virginiensis, M. careyana, M. palmeri, M. petiolaris,* and *M. micranthidifolia* to report new chromosome counts, confirm previously reported counts (Soltis 1983; FNA vol. 8 2009), detect any geographical patterns of variation in number, and compare chromosome numbers in *M. virginiensis* to those from related species and putative hybrid populations. Though attempts were made, I was not able to collect the buds of *M. caroliniana* due to its rarity and inaccessibility of sites, thus its chromosome number remains unknown.

Utilizing the root tip squash method, Soltis (1983) noted that, due to the similar size and shape of supernumerary chromosomes and A chromosomes, supernumerary chromosomes of *M. virginiensis* could only be identified during prophase of mitosis, as they appear much darker than A chromosomes. Effort was made in the present study to distinguish between A chromosomes and supernumerary chromosomes, yet it was found that the anther squash method did not allow for supernumerary chromosomes to be distinguished from A chromosomes in any stage of meiosis in these species. As Soltis (1983) reported counts of 2n = 20 or 38 chromosomes and 1-6 (and potentially up to eight) supernumerary chromosomes in *M. virginiensis,* I considered any *M. virginiensis* sample with a meiotic count of n = 10-18 to be diploid (2n = 20 + 0-8 supernumerary) and any meiotic count of n = 19-27 to be tetraploid (2n = 38 + 0-8 supernumerary).

CHAPTER THREE: RESULTS

Morphological Analysis

The principal components analysis (PCA) conducted with *M. virginiensis* samples from populations across the range of this species does not show clusters forming among these samples based on the measured floral and leaf characters (Fig. 10). This PCA also indicates that the floral character vectors are synergistic, and mostly opposed to most leaf character vectors. Both categories of traits are positively correlated with PC1, indicating that these traits increase with size. However, vegetative traits are somewhat negatively correlated based on PC2, indicating a potential trade-off between flower and leaf size in this species. All traits are negatively correlated with blade circularity along PC1, indicating that as plants get larger, the leaves become less circular. There is an outlier, data point 12, that is from a West Virginia population that exhibited typical floral and leaf morphology for *M. virginiensis* with the exception of being comprised of very large individuals (Fig. 10, Table 1). As this sample has a much higher score for PC1, which is associated with size, it is likely that the large size of the individual explains its separation from the other samples.

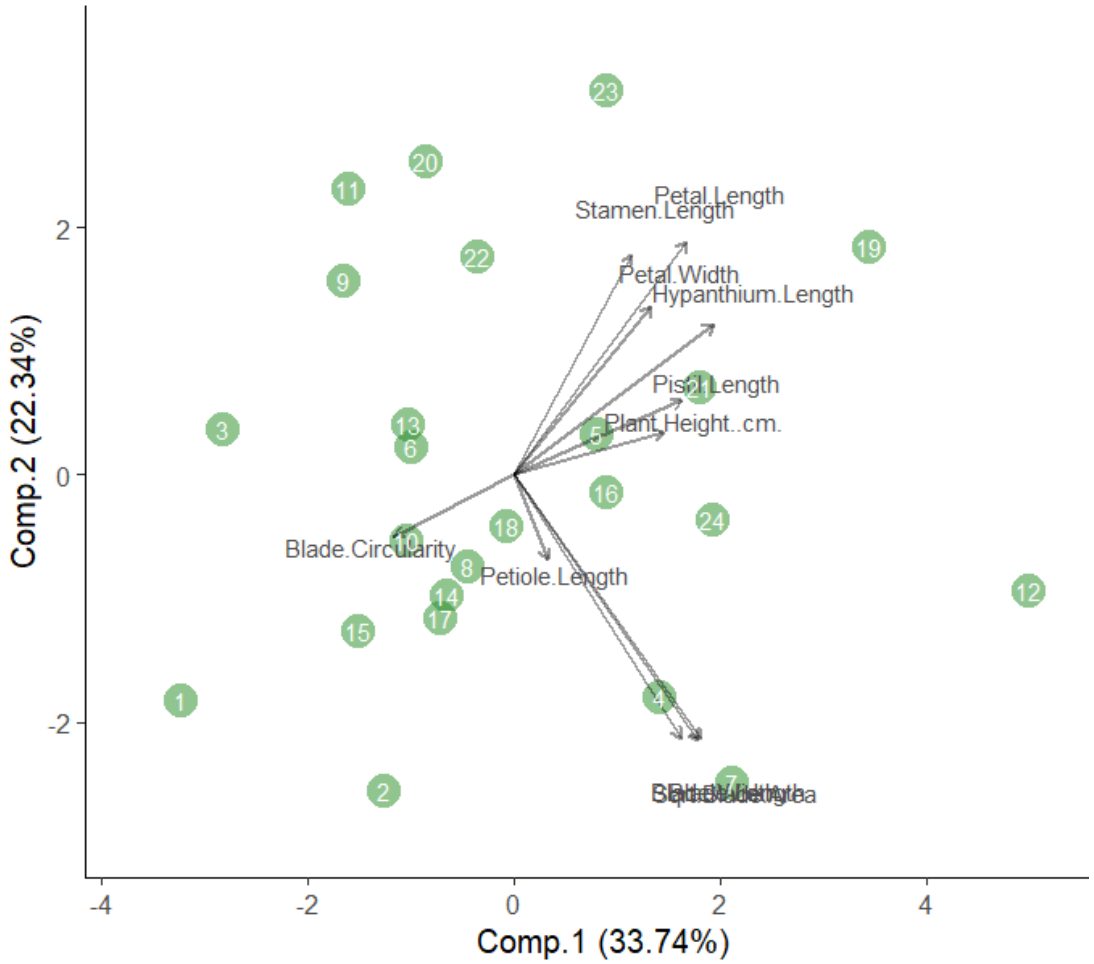

Figure 10. Principal Components Analysis of specimens from *Micranthes virginiensis* populations based on morphological data from leaves, flowers, and plant height.

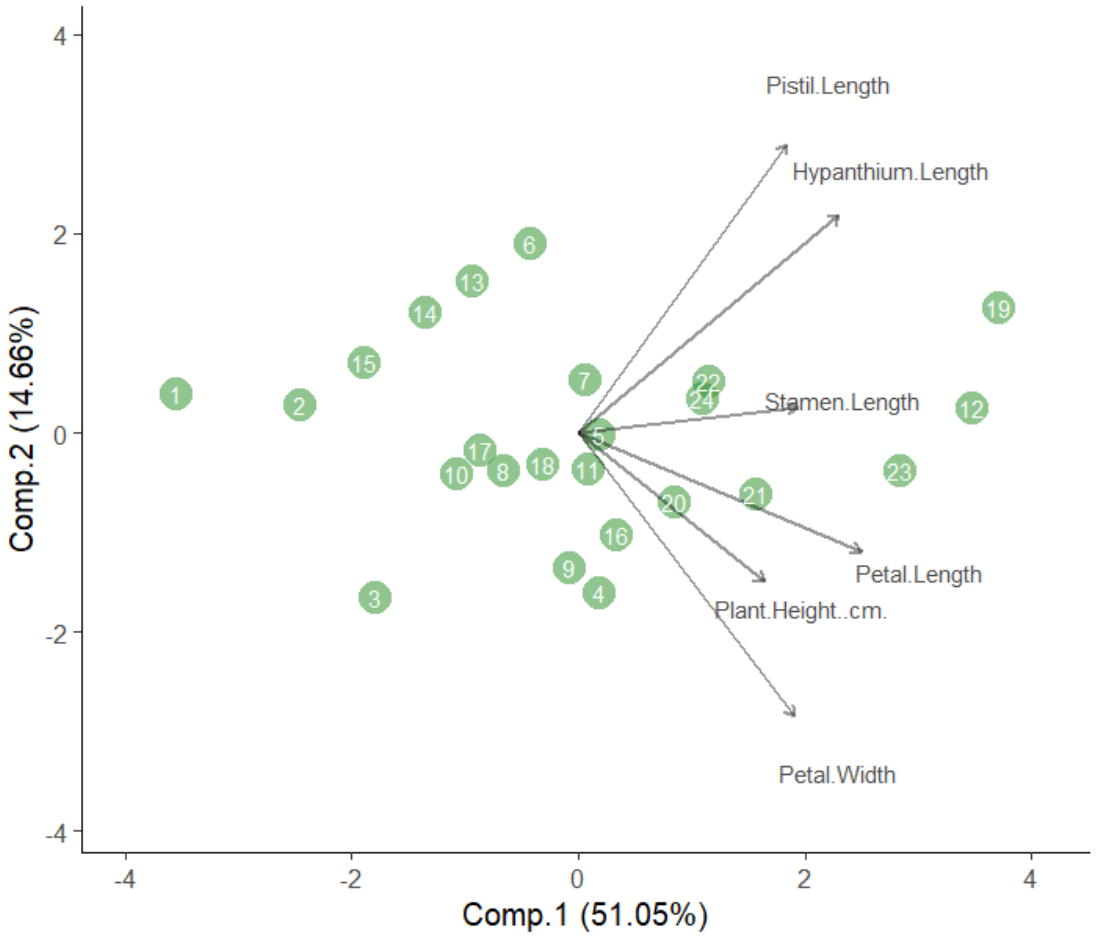

Figure 11. Principal Components Analysis of specimens from *Micranthes virginiensis* populations based on morphological data from flowers and plant height (excluding leaf characters).

Table 1. Voucher specimens for populations of *M. virginiensis* included in Figure 10, PCA using leaf and flower measurements and Figure 11, PCA using flower measurements. Voucher specimens are deposited in WCUH.

PCA #	Coll. Date	Country	State/ Province	County	Latitude	Longitude	Elev. (m)	Coll. #
1	27-Mar-23	USA	NC	Polk	35.272053	-82.216317	727.04	2
2	14-Mar-23	USA	NC	Polk	35.221517	-82.305908	406.8	1
3	6-Apr-23	USA	SC	Spartanburg	35.140617	-82.278862	352.76	19
4	14-Apr-23	USA	OH	Hamilton	39.124675	-84.782745	218.64	26
5	15-Apr-23	USA	OH	Fairfield	39.63158	-82.647383	263.43	29
6	14-May-23	USA	NY	Erie	42.700905	-78.904725	205.45	45
7	14-Apr-23	USA	KY	Franklin	38.218933	-84.847183	167.45	25
8	21-Mar-23	USA	AL	Jefferson	33.703495	-86.692383	182.64	9
9	11-Mar-23	USA	MS	Clay	33.537987	-88.633453	435.2	11
10	27-Mar-23	USA	NC	Polk	35.272053	-82.216317	727.04	2
11	21-Mar-23	USA	VA	Powhatan	37.682778	-77.938333	61.0	7
12	15-Apr-23	USA	WV	Wayne	38.146458	-82.382308	201.71	28
13	18-May-23	USA	MI	Marquette	46.761716	-87.73377	330.0	39
14	18-May-23	CAN	ONT	Lennox	44.560672	-77.116398	202.8	35
15	18-May-23	CAN	ONT	Lennox	44.53755	-76.92789	185.5	33
16	13-Apr-23	USA	TN	Jefferson	36.101922	-83.627633	270.36	24
17	13-Apr-23	USA	KY	Clinton	36.871587	-85.192345	205.31	23
18	11-May-23	USA	MA	Middlesex	42.278647	-71.343405	67.54	40
19	11-May-23	USA	CT	New Haven	41.55783	-72.759383	196.38	41
20	12-May-23	USA	ME	Knox	44.254487	-69.095772	49.42	42
21	28-Mar-23	USA	AR	Pulaski	34.801828	-92.32132	87.31	6
22	27-Mar-23	USA	NC	Durham	36.072998	-78.864864	84.11	5
23	13-May-23	CAN	QBC	Le Haut-Richelieu	45.354428	-73.150506	100.29	44
24	14-Mar-23	USA	SC	Pickens	34.9005288	-82.659331	404.1	4

Given that leaf morphology in this species is known to have high variability not associated with any particular geographic area and that leaf characters cannot reliably distinguish between *M. virginiensis* and multiple closely related species, a second PCA was conducted on the same dataset to determine if any clustering would occur based on floral morphology alone that may have been masked by the inclusion of the leaf characters (Fig. 11). Once again, no clear clusters form among the samples, though the amount of variation explained by PC1 (size)

increased from 33.74% (Fig. 10) to 51.05% (Fig. 11) and the amount of variation explained by PC2 (shape) decreased from 22.34% (Fig. 10) to 14.66% (Fig. 11). This indicates a wide range of sizes of the flowers between different individuals, with flower size increasing from left to right, though the flowers generally have similar shape. The specimens do not consistently cluster based on geographic location, though interestingly, data points 1, 2, and 3, the individuals with the smallest flower size, are from populations in the Southern Appalachian escarpment region (not the Gap Creek or Wadakoe Mountain populations, which were excluded from this analysis). Other individuals with small flowers came from Ontario, Michigan, and New York (Table 1). Notably, the West Virginia population that was much larger than other individuals in Figure 10 now falls more within the main group. This indicates that the West Virginia population likely had much larger leaves than a typical individual of this species. Other individuals with relatively large flowers were from Connecticut, Quebec, and Arkansas (Table 1), indicating that both small-flowered and larger-flowered populations are found throughout the range and are not associated with a particular geographic location. Only two tetraploid individuals (Table 4) were included in this PCA, from Glassy Mountain, Pickens Co., South Carolina (data point 24), and Durham Co., NC (data point 22). These two points clustered together, though their flowers appear to be of average size and shape compared to diploid individuals.

There are no known leaf characters that can reliably distinguish among *M. virginiensis*, *M. careyana*, and *M. caroliniana*, and preliminary analysis (not shown) indicated that PCAs cluster *M. virginiensis* and *M. careyana* individuals together when leaf characters are included despite their well-documented and consistent differences in floral morphology. Because leaf characters appear insufficient to distinguish closely related Southern Appalachian *Micranthes* species, the remainder of the multivariate morphological analyses will exclude leaf characters.

A third PCA was conducted with floral characters to determine if individuals from the Gap Creek and Wadakoe Mountain populations would be morphologically distinct from the rest of *M. virginiensis* (Fig. 12). Two clusters are observed, one comprised of only samples of typical *M. virginiensis* and one comprised of the Gap Creek and Wadakoe Mountain populations. The Gap Creek and Wadakoe Mountain populations are most associated with the vectors for pistil length, stamen length, and plant height, though the plant height vector is much smaller than the other vectors lengths. This indicates that these plants have longer stamens and pistils and are slightly taller. Though separation between the clusters is visible, there is nearly complete overlap on PC1 and slight overlap on PC2, indicating that, while somewhat different, the two groups are still very morphologically similar.

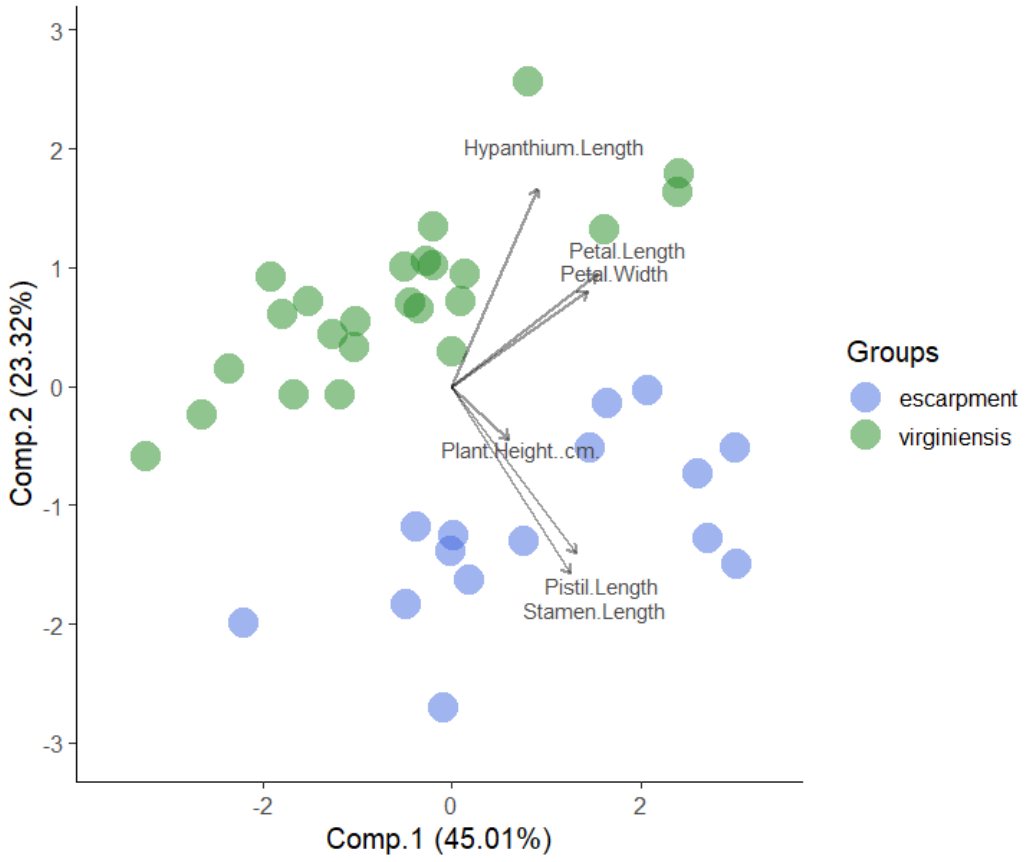

Figure 12. Principal Components Analysis of specimens of *Micranthes virginiensis* and the Gap Creek and Wadakoe Mountain populations ("escarpment") based on morphological data from flowers and plant height.

The LDA (Fig. 13) and subsequent ANOVA conducted using individuals from *M. virginiensis* and the Gap Creek and Wadakoe Mountain populations indicate that the measured characters are sufficient to sort the samples into the *a priori* groups (LD1, $F = 342.5$, $p < 0.001$). All samples were correctly sorted by the model, with the stamen length being the most influential character in discriminating the two groups, followed by hypanthium length (Table 2; Table 3). In *M. virginiensis*, hypanthium length ranged from 0.603-1.79 mm (mean = 1.22 mm), compared to the hypanthia of the Gap Creek and Wadakoe Mountain populations, which were smaller on average (0.7-1.36 mm, mean=1.03 mm). The stamen measurements showed no overlap, with lengths of 0.908-2.04 mm (mean = 1.59 mm) for *M. virginiensis* and 2.24-3.57 mm (mean = 2.90 mm) for Gap Creek and Wadakoe Mountain. Two-tailed t-tests indicated that the two groups have significantly different hypanthium and stamen lengths ($p = 0.03$ and $p < 0.001$ respectively).

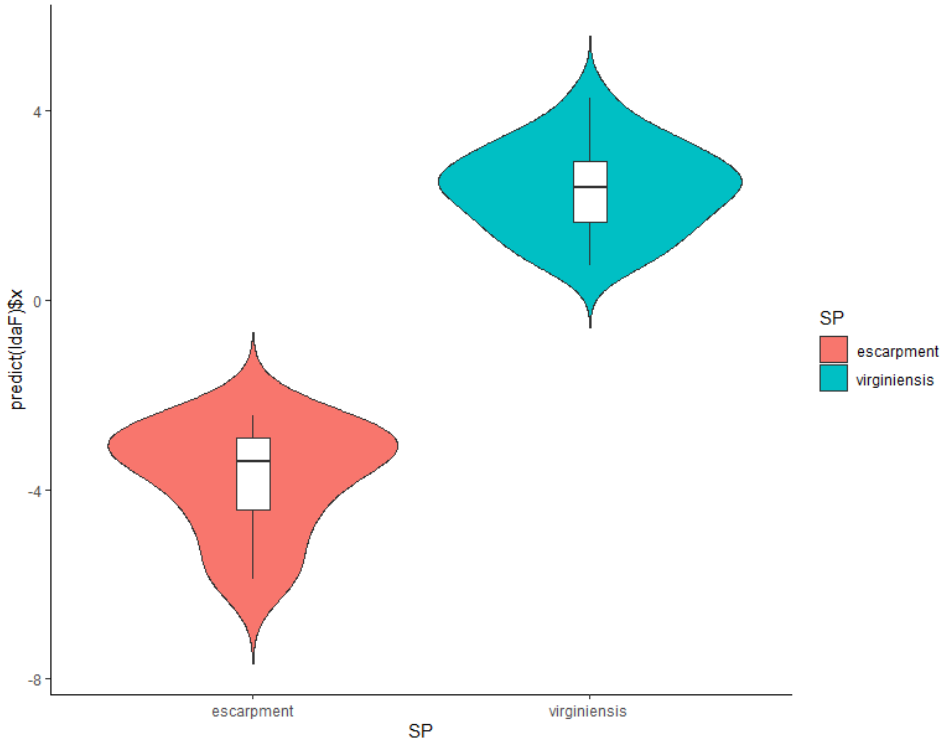

Figure 13. Violin plot depicting results of a linear discriminant analysis of specimens of *Micranthes virginiensis* and the Gap Creek and Wadakoe Mountain populations ("escarpment") based on morphological data from flowers and plant height.

Table 2: Predicted classifications of each sample based on a linear discriminant analysis of individuals from *M. virginiensis* and the Gap Creek and Wadakoe Mountain escarpment populations.

	escarpment	*virginiensis*
escarpment	15	0
virginiensis	0	24

Table 3: Factor loadings for each character used in a linear discriminant analysis of individuals from *M. virginiensis* and the Gap Creek and Wadakoe Mountain escarpment populations.

Characters	**Loadings**
Hypanthium Length	1.09
Stamen Length	-2.35
Petal Length	0.2
Petal Width	-0.03
Pistil Length	-0.71
Plant Height	-0.29

To visualize clustering between *M. virginiensis*, the Gap Creek and Wadakoe Mountain populations, and the other putative parent species, *M. careyana*, a PCA was conducted with individuals from the three groups using floral characters (Fig. 14). Though there is much overlap along PC1, the groups are somewhat separated by PC2. One individual from the Wadakoe Mountain population is negatively correlated with the size of the flower parts, while the other individuals from Gap Creek and Wadakoe Mountain are somewhat to very positively correlated with flower size. *Micranthes virginiensis* individuals had a larger hypanthium and are negatively correlated with stamen length, while the inverse tends to be true for individuals of *M. careyana.* Individuals of the Gap Creek and Wadakoe Mountain populations tend to fall between the *M. virginiensis* and *M. careyana* clusters on PC2, though there is some overlap along this axis between *M. careyana* and the escarpment individuals. It is worth noting that *M. careyana* has distinct yellow/green petal spots, while the other two groups do not (Fig. 15). As this is a presence/absence trait and not continuous, it is not accounted for in the multivariate analyses conducted in this study.

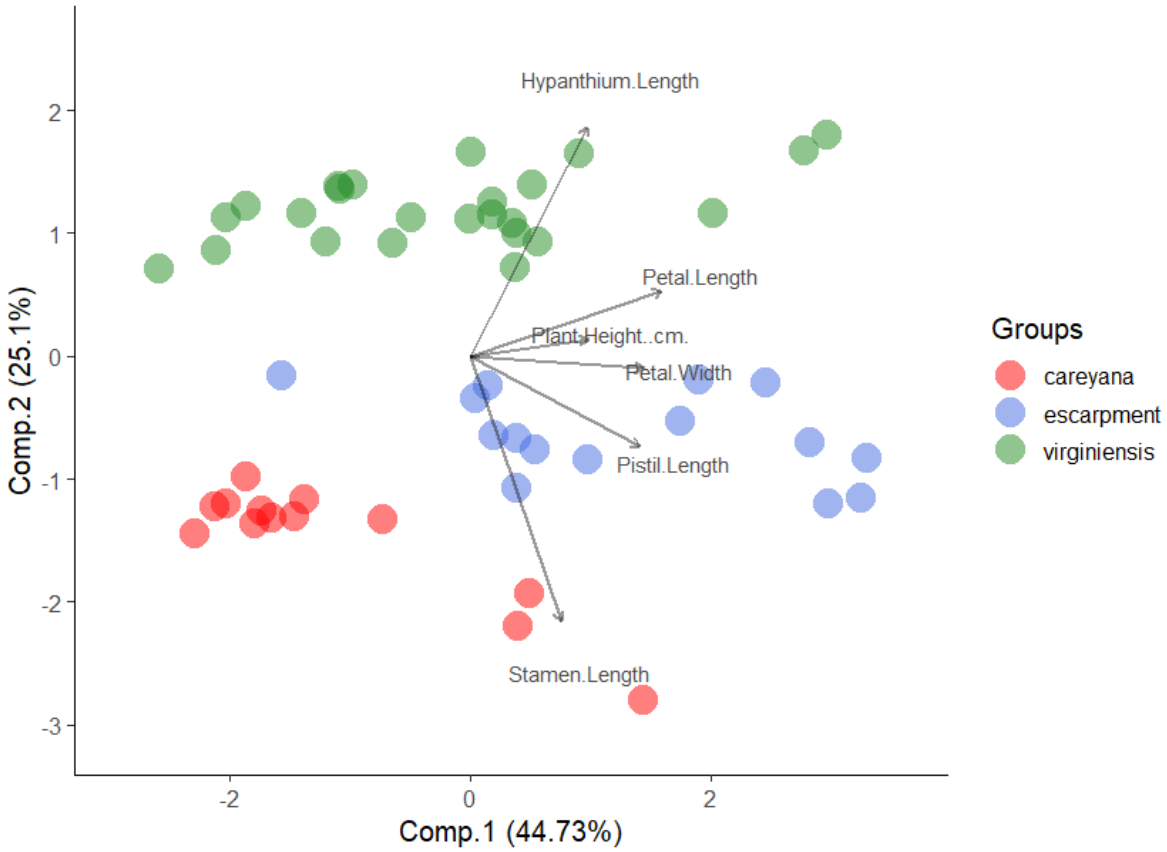

Figure 14. Principal Components Analysis of specimens of *Micranthes virginiensis, M. careyana*, and the Gap Creek and Wadakoe Mountain populations ("escarpment") based on morphological data from flowers and plant height.

Figure 15. (a) Typical *Micranthes virginiensis* flower. Glassy Mountain, SC. (b) *Micranthes* sp. Flower. Gap Creek, Greenville Co., SC. (c) *Micranthes* sp. Flower. Wadakoe Mountain, Pickens Co., SC. (d) Typical *M. careyana* flower. Swain Co., NC.

The LDA (Fig. 16) and subsequent ANOVAs conducted using individuals from *M. virginiensis, M. careyana,* and the Gap Creek and Wadakoe Mountain populations indicate that

the measured characters are sufficient to sort the samples into the *a priori* groups (LD1, F = 335.4, $p < 0.001$; LD2, F = 38.97, $p < 0.001$). The samples are best sorted based on hypanthium length, which separates *M. careyana* from the other two groups, and stamen length, which separates the Gap Creek and Wadakoe Mountain escarpment populations from *M. virginiensis*. These two characters sort the samples along the first and second axes (LD1 = 89.59%; LD2 = 10.41%), though they are negatively correlated with each other. Pistil length somewhat sorts the escarpment individuals from the other groups on the second axis, and the other characters (petal length, petal width, and plant height) do not sort the samples at all. The model was able to accurately predict the group for each sample (Table 4). The null hypothesis of PERMANOVA, that the centroid and dispersion are equal among groups, was rejected ($p = 0.001$), indicating that the centroid and/or the dispersion are different among groups. The dispersion test found no significant differences in the dispersion of values among groups ($p = 0.94$), indicating that the groups are significantly different from one another based on location.

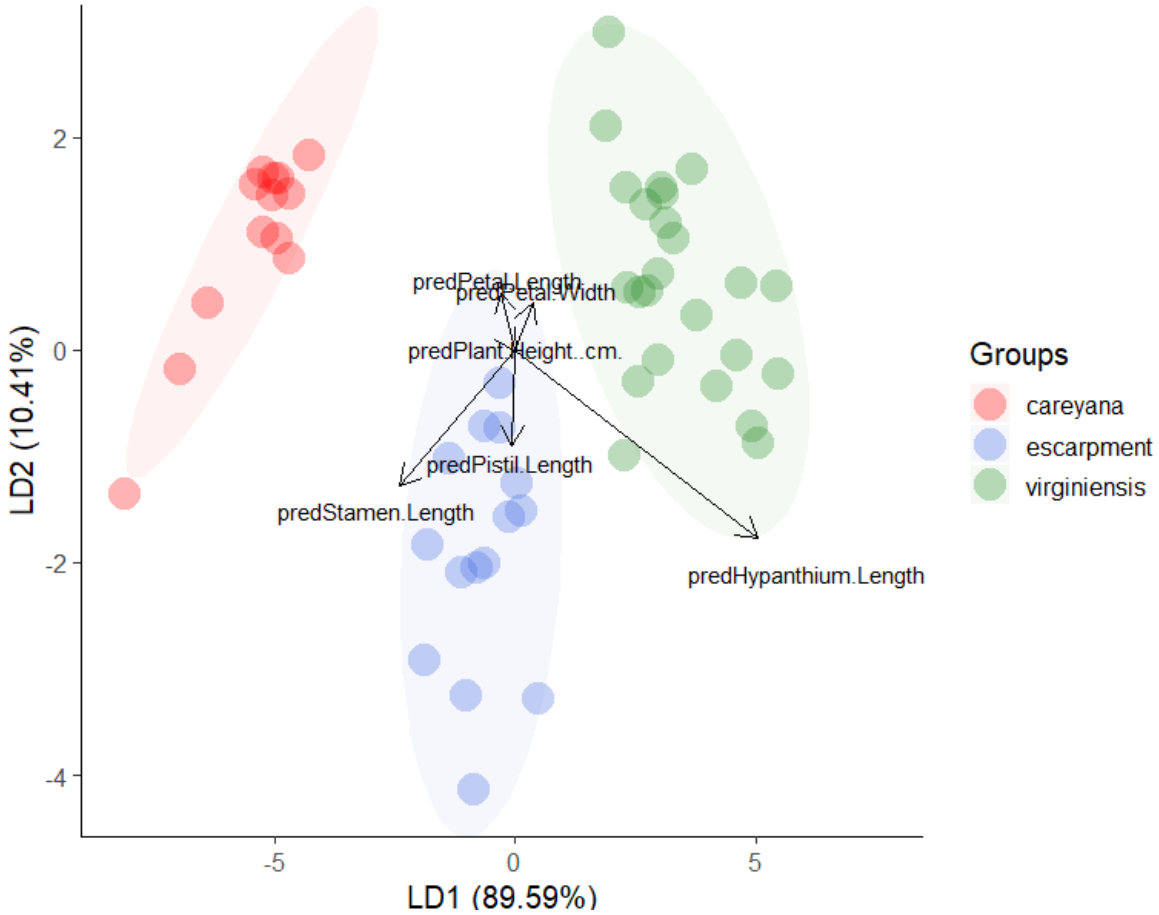

Figure 16. Linear Discriminant Analysis of specimens of *Micranthes virginiensis*, *M. careyana*, and the Gap Creek and Wadakoe Mountain populations ("escarpment") based on morphological data from flowers and plant height.

Table 4: Predicted classifications of each sample based on a linear discriminant analysis of individuals from *M. virginiensis*, *M. careyana*, and the Gap Creek and Wadakoe Mountain escarpment populations.

	careyana	escarpment	*virginiensis*
careyana	13	0	0
escarpment	0	15	0
virginiensis	0	0	24

The PCA conducted with fruit characters and plant height from *M. virginiensis* samples from SERNEC indicated minimal to no relationship between the size of the fruit and plant height (Fig. 17). There was no clustering observed among the samples, though there were a few

outlying individuals. Data point 58 is from a Butler Co., PA population that appears to have atypically small fruits. However, the fruit size of 1.89 mm still falls within the previously reported range of 1-3 mm (Lord 1960). The other two outliers are from a New Castle Co., DE population (data point 12) and a Stokes Co., NC population (data point 51). Data from other individuals from these three populations and counties were incorporated into this PCA, and those data points all fell within the primary cluster, indicating that the outliers may just be errant individuals. Fruit lengths ranged from 1.89-5.59 mm with an average of 3.51 mm, and over half the fruits were larger than the reported maximum of 3 mm for this species (Lord 1960; Suppl. Table 5). Notably, there is no gap in the values for fruit length (that might indicate multiple distinct groups) and fruit size does not appear to be related to geographic area (Suppl. Table 5).

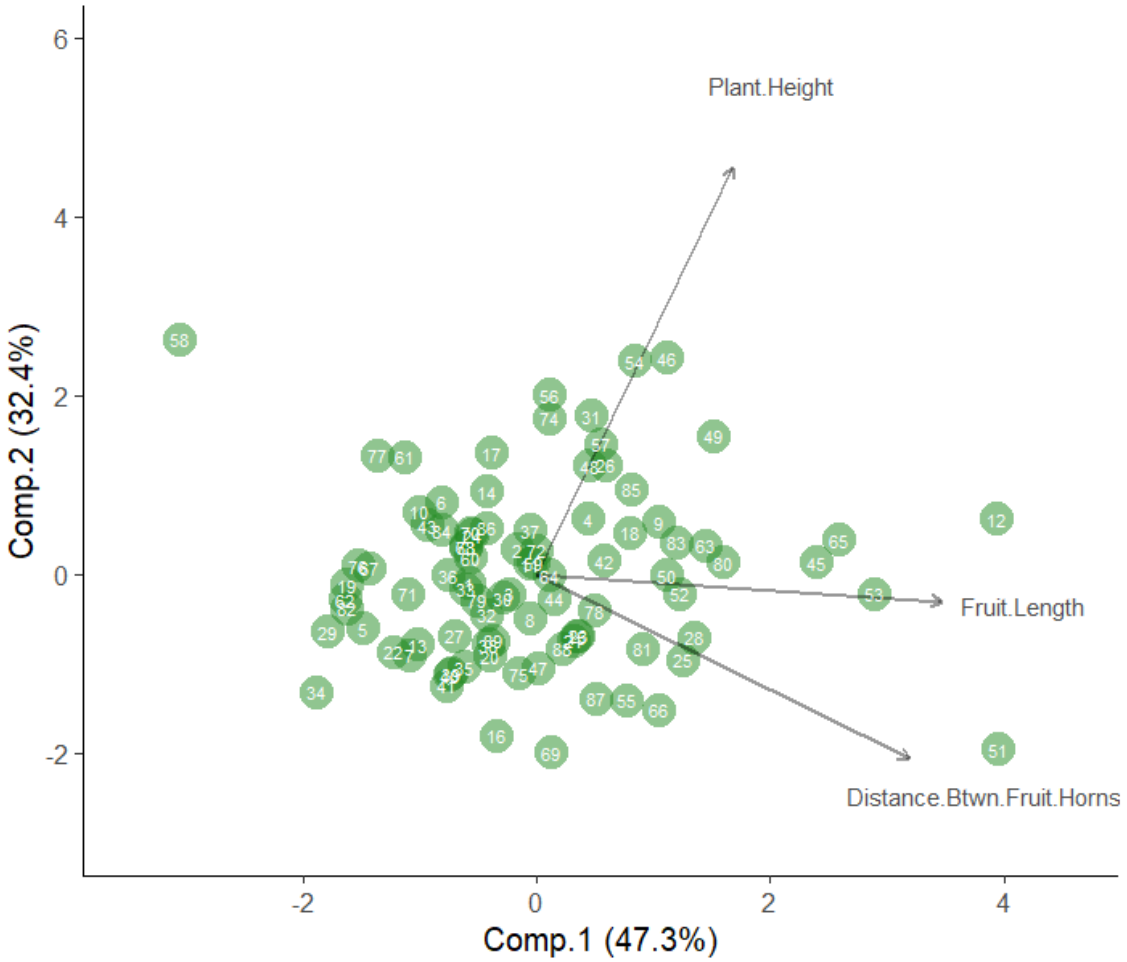

Figure 17. Principal Components Analysis of SERNEC specimens of *Micranthes virginiensis* based on fruit characters and plant height.

My data indicate that the Gap Creek and Wadakoe Mountain individuals typically have significantly larger fruits than *M. virginiensis* (2.51-5.56 mm, mean = 4.26 mm vs. 1.89-5.59 mm, mean = 3.51 mm; $p = 0.01$), however, there is overlap among the samples (Fig. 18). I found *M. careyana* to have fruits of 2.68-4.96 mm (mean = 3.83) and all values fell within the normal range for *M. careyana* (3-5 mm, mean = 3.61 mm; Lanning & Mathews 2019). Lanning & Mathews (2019) found that the fruits of *M. careyana* were larger than the fruits of *M. virginiensis*, however, they did not sample from throughout the range of the species. My data indicate the contrary — *M. virginiensis* fruits are often the same size or even larger than *M.*

careyana. The fruit sizes of *M. careyana* and the Gap Creek and Wadakoe Mountain individuals were not significantly different ($p = 0.294$).

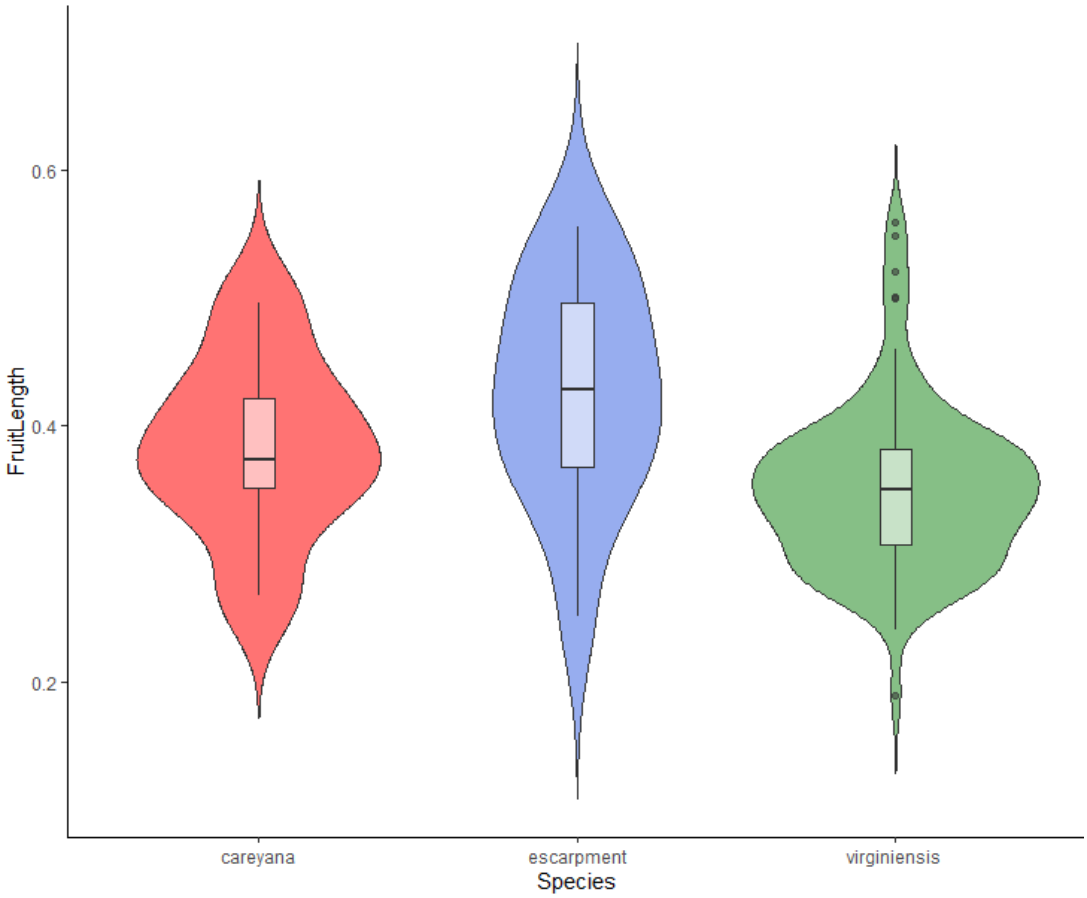

Figure 18. Violin plot comparing fruit lengths of *Micranthes virginiensis*, *M. careyana*, and the Gap Creek and Wadakoe Mountain populations ("escarpment").

In all multivariate analyses, individuals from regions where the previously recognized separate species or varieties of *M. virginiensis* have been described from were found to fall within the primary cluster of *M. virginiensis* samples. However, many of these taxa were described based on non-continuous characters that were not included in the multivariate analyses, such as branching pattern. After examining non-continuous characters on herbarium

specimens from throughout the range, including all regions where the subordinate taxa were described from, I found no evidence for the recognition of the subordinate taxa (Suppl. Table 3).

Chromosome Counts

Chromosome counts were obtained from meiotic pollen mother cells for individuals from 24 populations of *M. virginiensis*, three populations of *M. careyana*, two populations of *M. palmeri*, two populations of *M. petiolaris*, and one population of *M. micranthidifolia* (Table 5, Fig. 19). The four populations of *M. virginiensis* sampled from the North Carolina Piedmont region are tetraploid (x = 19, Fig. 20). In the Blue Ridge escarpment region, the morphologically distinct Gap Creek and Wadakoe Mountain populations are tetraploid (x = 19), as is a population consistent with the morphology of typical *M. virginiensis*, the Glassy Mountain, SC population (Fig. 20). In the same region, three morphologically typical *M. virginiensis* populations from this region are diploid (x = 10). Sampled individuals from populations from all other geographic regions throughout the range of this species are diploid (Table 5; Fig. 20). In most *Micranthes* populations, supernumerary chromosomes were not observed, though I counted supernumeraries in some populations of *M. virginiensis*, *M. careyana*, *M. petiolaris*, and *M. micranthidifolia* (Table 5). With the exception of the Gap Creek and Wadakoe Mountain populations, no morphological differences were observed between diploid and tetraploid *M. virginiensis* in qualitative observations and PCAs (Fig. 10-12, Fig. 17).

Table 5: Meiotic chromosome counts for populations of different *Micranthes* species done in this study.

Species	Voucher	Country	State/ Province	County	Chromosome Count [x (+ supernumeraries)]
Micranthes virginiensis					
	Hall 9	USA	AL	Jefferson	10
	Hall 6	USA	AR	Pulaski	10
	Hall 41	USA	CT	New Haven	10
	Hall 10	USA	GA	Fulton	10
	Hall 22	USA	KY	Todd	10
	Hall 38	USA	MD	Montgomery	10
	Hall 39	USA	MI	Marquette	10
	Hall 11	USA	MS	Clay	10
	Hall 1	USA	NC	Polk	10
	Hall 2	USA	NC	Polk	10
	Hall 46	USA	NC	Polk	10
	Hall 5	USA	NC	Durham	19
	Hall 34	USA	NC	Chatham	19
	Hall 50	USA	NC	Mecklenburg	19
	Hall 51	USA	NC	Montgomery	19
	Hall 12	USA	NJ	Somerset	10
	Hall 4	USA	SC	Pickens	19 (+3)
	Hall 19	USA	SC	Spartanburg	10
	Hall 21	USA	TN	Davidson	10 (+1)
	Hall 35	CAN	ONT	Lennox	10
	Hall 7	USA	VA	Powhatan	10
	Hall 36	USA	VA	Floyd	10
Micranthes sp.	Hall 3	USA	SC	Greenville (Gap Creek)	19 (+0-4)
	Hall 14	USA	SC	Pickens (Wadakoe Mtn.)	19
M. careyana					
	Hall 31	USA	NC	Macon	10
	Hall 20	USA	TN	Knox	10 (+3)
	Hall 32	USA	NC	McDowell	10
M. palmeri					
	Hall 8	USA	AR	Conway	10
	Hall 16	USA	MO	Douglas	10
M. petiolaris					
	Hall 30	USA	NC	Macon	10 (+0-3)
	Hall 47	USA	NC	Ashe	10
M. micranthidifolia					
	Hall 15	USA	NC	Jackson	11 (+0-1)

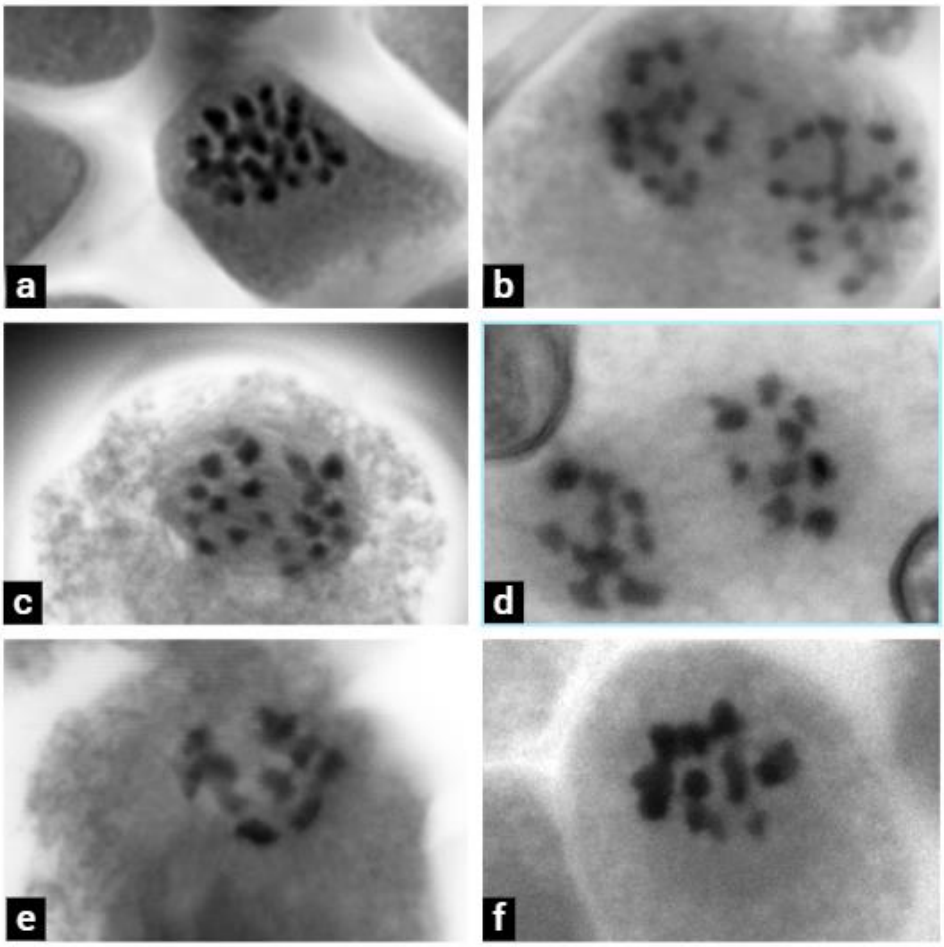

Figure 19. Images of chromosomes in various stages of meiosis from six *Micranthes* populations. (a) *Micranthes* sp. x = 19 (+1). Greenville Co., SC (Gap Creek). Prophase I. (b) *M. virginiensis* x = 19. Chatham Co., NC. Prophase II. (c) *M. petiolaris* x = 10. Macon Co., NC. Anaphase I. (d) *M. virginiensis* x = 10. Polk Co., NC (Melrose). Anaphase I. (e) *M. palmeri* x = 10. Conway Co., AR. Prophase I. (f) *M. careyana* x = 10 (+3). Knox Co., TN. Prophase I. Images are from just one plane of focus, and thus relative sizes of chromosomes may not be accurately reflected in each image.

I am reporting the first counts for *M. careyana* and *M. petiolaris* as x = 10 (+ 0-3 supernumerary) and *M. palmeri* as x = 10 (Table 5). For *M. micranthidifolia,* I observed x = 11 (+ 0-1 supernumerary) chromosomes (Table 5), agreeing with the previously reported count of 2n = 22 (FNA vol. 8 2009). Given that many *Micranthes* species are x = 10 and that only one population of *M. micranthidifolia* was sampled in this study, it is possible that this species is actually x = 10 and that the count reported in this present study and in FNA vol. 8 (2009) have mistaken a supernumerary chromosome for an A chromosome. However, since there are now

multiple accounts of x = 11 chromosomes, this should remain the accepted count for *M. micranthidifolia*, though further investigation may be warranted.

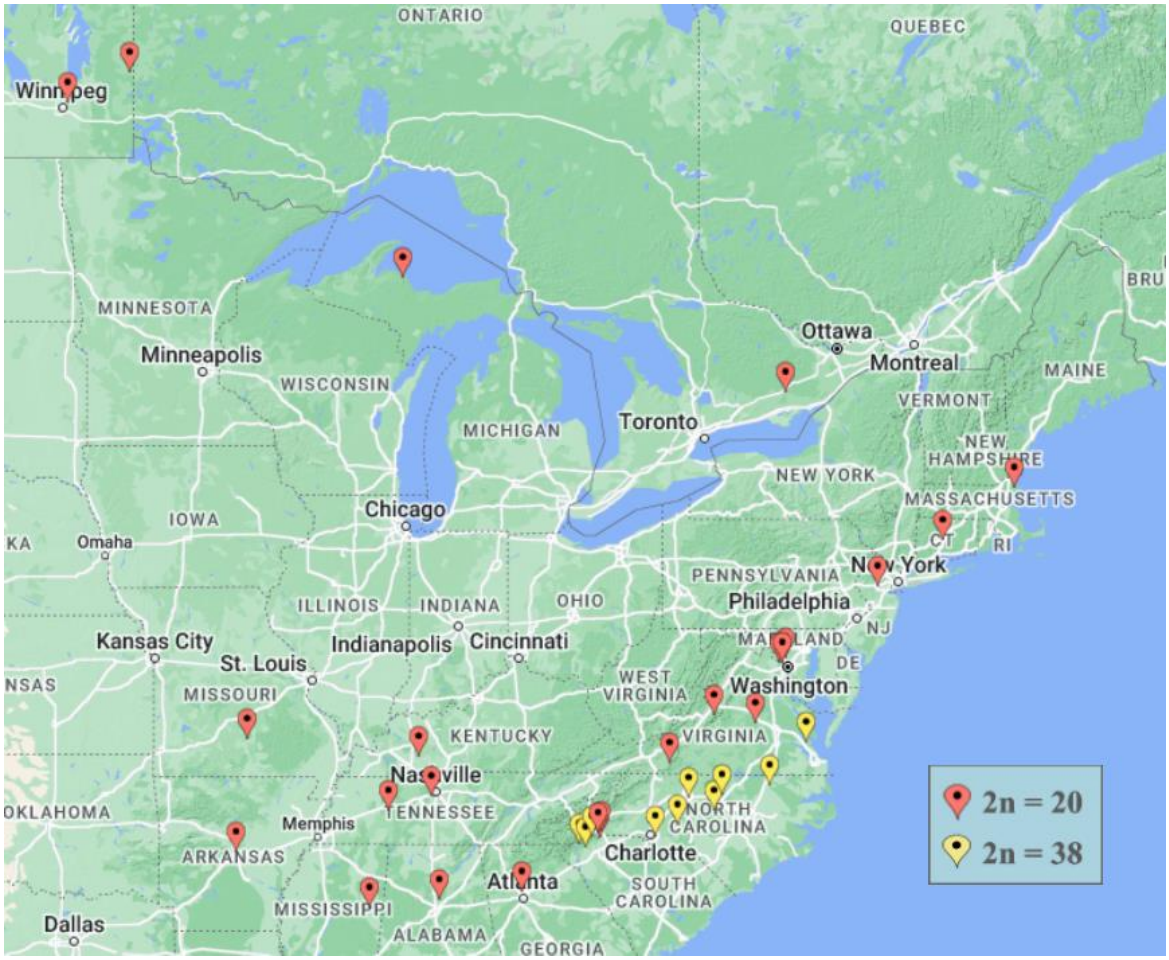

Figure 20: Map depicting chromosome counts for *Micranthes virginiensis* (including Gap Creek and Wadakoe Mountain) populations from this present study as well as Soltis (1983), Löve & Löve (1982), Kovanda (1978), Hill (1989) and Löve & Ritchie (1966). Map created with Google Maps.

CHAPTER FOUR: DISCUSSION

The results of this study indicate that, while geographically widespread and morphologically variable, *Micranthes virginiensis* is not comprised of multiple morphologically distinct species, excepting the Gap Creek and Wadakoe Mountain populations (to be discussed in detail below). The various separate taxa and varieties that have been recognized by numerous authors throughout the history of this species should remain subordinate to *Micranthes virginiensis.* Both multivariate analysis of quantitative traits and analysis of qualitative characters on living and herbarium specimens indicate that the morphological variation within many of the leaf and inflorescence characters used to describe the subordinate taxa are not consistent geographically and rather occur throughout the range. I have observed that even within one population, characters like leaf shape, margin type, and branching pattern can vary wildly, indicating that these are not reliable characters to use when attempting to delimit species from *M. virginiensis.* The PCAs (Fig. 10; Fig. 11; Fig. 17) indicate that there are no distinct clusters forming among samples of *M. virginiensis* from all throughout Eastern North America based on the measured leaf, flower, and fruit characters. Clustering would be indicative of morphologically distinct groups, so the absence of separate clusters indicates one species. There also does not appear to be any consistent geographic cline among the specimens regarding flower and fruit size. Populations in the periphery of the range of *M. virginiensis* (i.e., Arkansas, Maine, etc.) do not have consistently distinguishable morphology from populations in the middle of the range.

The direction of the vectors in the PCA containing leaf and floral characters from *M. virginiensis* specimens (Fig. 10) indicates a trade-off between leaf size and flower size in this

species. This mirrors a trade-off between somatic growth and reproductive allocation that has been observed in other plants (i.e., Thorén et al. 1996; Hemborg & Karlsson 1998; Zhang et al. 2015). For example, a study of eight subarctic plant species in the Swedish Lapland found a general trade-off between allocation of resources to somatic (including growth and storage) and reproductive functions (Hemborg & Karlsson 1998). Another study observed the flower/leaf size trade-off in *Stellera chamaejasme* populations of the northern Qilian Mountains (Zhang et al. 2015). Both of these studies found that reproductive investment tended to increase with elevation, though no elevational pattern was observed in my dataset (elev. range of sampled populations: 49.42–727.04 m), though this may be due in part to the climatic differences across the broad geographic range from which my samples come compared to the smaller ranges of these other studies. Notably, other studies have found a positive correlation between the size of floral and vegetative characters rather than a trade-off (i.e., E-Vojtkó et al. 2022). Therefore, the leaf-flower size trade-off observed in *M. virginiensis* is not observed in all plant species, but also is not unusual. Further studies should be conducted to see if this resource allocation trade-off is a pattern across other *Micranthes* species.

There are some populations that, though not distinct enough based on my results for me to confidently consider an additional species, may warrant further investigation. The *M. virginiensis* populations of Polk Co., NC (at Melrose Falls, Pearson's Falls, and Shunkawauken Falls) all appear to share similar floral oddities — the petals are much more spreading, somewhat reflexed in some individuals, and the hypanthium is smaller than typical *M. virginiensis* (Fig. 21). This is similar to *M. careyana* (Fig. 15d), though the flowers of these Polk Co. populations do not have petal spots or long stamens, indicating *M. virginiensis* (Fig. 15a). They also differ from the Gap Creek and Wadakoe Mountain escarpment populations, which have long stamens

and red-orange anthers (Fig 15b, 15c). This odd morphology is more pronounced in some individuals than others, and I have never observed the spreading petals in any other region of this species' range. These Polk Co. populations are diploid, whereas most other populations in North and South Carolina are tetraploid, though there is a nearby diploid population in Spartanburg Co., SC that has normal floral morphology (Table 4; Fig. 20).

A previous phylogenetic study included one of these populations, from Melrose Falls, and found that it clustered with an Alabama population of *M. virginiensis* and the Wadakoe Mountain population in both nrITS and *trn*L-F cpDNA analyses (Lanning & Mathews 2019). As the morphological results of my study indicate that the Wadakoe Mountain population is distinct from typical *M. virginiensis*, it is possible that these gene regions are not sufficient for species delimitation of these unique Southern Appalachian *Micranthes.* To obtain phylogenetic results that accurately reflect evolutionary history and determine if the Polk Co. populations are a distinct species, a molecular analysis based on more variable characters or a genome-wide molecular representation, such as RAD-Seq, is needed. I have successfully extracted DNA from populations of *M. virginiensis* from across the geographic range, as well as from both closely related and outgroup *Micranthes* species, and these samples can be sequenced and analyzed in the future to answer many of the lingering questions.

Figure 21: Longer and somewhat reflexed petals of the Melrose population of *M. virginiensis* from Polk Co., NC. Photo by Ken Borgfeldt, https://wcbotanicalclub.org/20180402_early-saxifrage-micranthes-virginiensis-02/.

Though there are not multiple morphologically distinct species throughout most of the range of *M. virginiensis,* the Southern Appalachian escarpment populations at Gap Creek and Wadakoe Mountain do appear to form a distinct species under the unified species concept. Since their discovery, it is believed that these populations are in some ways different from typical *M. virginiensis*, though it has been unclear if they represent a new species, a variety, a hybridization event with a nearby *M. careyana*, or simply normal variation within *M. virginiensis.* Using multiple lines of evidence, including morphology and chromosome count, I have determined the Gap Creek and Wadakoe Mountain populations to be a distinct species, most likely of hybrid origin between *M. virginiensis* and *M. careyana.* This new species does not correspond with any of the previously recognized species, varieties, or forms of *M. virginiensis,* and thus will require a name and formal description in the future. Other species in *Micranthes* have been determined to be a result of allopolyploidy, such as *M. hitchcockiana* (Elvander) Brouillet & Gornall (*n* = 38), a species likely resulting from hybridization between *M. rufidula* Small (*n* = 19) and *M. oregana* (Howell) Small (*n* = 19) (Elvander 1984), so a hybrid origin seems most likely for the

morphologically intermediate Gap Creek and Wadakoe Mountain populations. It is not surprising that a new species should be discovered in the Southern Appalachian escarpment as the prevalence of unique microhabitats has allowed for high rates of biodiversity, endemism, and disjunct populations in this region (Pittillo et al. 1998). Wadakoe Mountain, much of which is a South Carolina Department of Natural Resources heritage preserve, is known for its diverse assemblage of uncommon plants due to the underlying amphibolite and circumneutral pH of the soils (SCDNR 2016). The Gap Creek site is located at the eastern edge of the Mountain Bridge Wilderness area, managed by the South Carolina Department of Parks, Recreation, and Tourism.

All multivariate analyses (including PCA, LDA, and PERMANOVA) indicate that the Gap Creek and Wadakoe Mountain populations have morphology that is intermediate between *M. careyana* and *M. virginiensis*. Specifically, the stamens are more than half the length of the petals in both *M. careyana* and the Gap Creek and Wadakoe Mountain populations, whereas the stamens are less than half the length (and often only around a quarter of the length) of the petals in *M. virginiensis.* Additionally, the hypanthia of the Gap Creek and Wadakoe Mountain populations are generally of intermediate length between the absent hypanthium of *M. careyana* and the distinct hypanthium of *M. virginiensis*, though there is much overlap between the hypanthium lengths of individuals from the Gap Creek and Wadakoe Mountain populations and *M. virginiensis.* The flowers of *M. virginiensis* and the Gap Creek and Wadakoe Mountain populations lack petal spots, while *M. careyana* has yellow/green spots on the flower petals. While the pistil length of the Gap Creek and Wadakoe Mountain populations tended to be greater than the other two species, this trait was not consistent enough to be useful for species delimitation. Fruit size also showed considerable overlap between the three groups and is thus also an uninformative taxonomic character. The hybrid origin hypothesis could be further tested

through molecular analyses (such as STRUCTURE), which would indicate if these populations contain admixture between alleles from both *M. virginiensis* and *M. careyana*. It could also be tested through artificial crosses that could reveal if *M. virginiensis* and *M. careyana* can hybridize and if those offspring possessed the same unique characters as the Gap Creek and Wadakoe Mountain populations.

Table 6: Diagnostic morphological characters used to distinguish among *M. virginiensis*, *M. careyana*, and the Gap Creek and Wadakoe Mountain populations.

Taxon	Stamen:Petal	Stamen Length (mm)	Hypanthium (mm)	Petal Spots	Anther Color
M. virginiensis	< ½ petal length	0.908-2.04	Present (0.603-1.79)	Absent	Yellow
Gap Creek/Wadakoe	> ½ petal length	2.24-3.57	Present (0.7-1.36)	Absent	Red-orange
M. careyana	> ½ petal length	2.557-3.894	Absent	Present	Red-orange

Multiple other tetraploid populations have been discovered in the Southeast beyond the Gap Creek and Wadakoe Mountain populations. These tetraploid populations are not indicated by any of my analyses to be morphologically distinct from diploid *M. virginiensis*, and thus it is likely that they result from an instance of autotetraploidy, or chromosome duplication within a species. Based on the distribution of the tetraploids across eastern Virginia, North Carolina, and northern South Carolina (Fig. 20), it seems likely that a spontaneous autotetraploid population persisted and spread throughout the region. Soltis et al. (2007) advocates for autotetraploids to be recognized as a distinct species in cases where multiple species concepts are fulfilled even if they are not morphologically distinct, so these tetraploid *M. virginiensis* populations may represent a novel species separate from diploid *M. virginiensis* despite their lack of known morphological differences.

Notably, the autotetraploid populations are mostly geographically separate from the diploid populations, with some overlap in the escarpment region (Fig. 20). This geographic separation is a strong indicator of a distinct evolutionary lineage. This lineage may have existed long enough to evolve ecological niche differences that could also be used as evidence of speciation. Though ecological factors were not explicitly measured in this study, the habitats of the presumed autotetraploid populations seemed usual for *M. virginiensis.* One autotetraploid population occurred in a mossy seep on a granite bald on Glassy Mountain, Pickens Co., SC and another occurred in Durham Co., NC on the bank of the Eno River. Three additional autotetraploid populations were included in this study (but not in the PCAs) — from Chatham Co., NC found on the bank of the Haw River, from Montgomery Co., NC on the bank of the Yadkin River, and from Mecklenburg Co., NC in a mossy area of a forest. Typical diploid *M. virginiensis* is found on rock outcrops, moist alluvial and slope forests, streambanks, and riverbanks (Weakley 2020), so this is in line with the habitats of the autotetraploid populations. However, there could be niche differences that are not readily apparent. Visger et al. (2016) found that the spatial segregation between the morphologically similar *Tolmiea diplomenziesii* (diploid) and *T. menziesii* (autotetraploid) was accompanied by climatic niche differentiation and corresponding physiological divergence. Though there are cases where autotetraploids and their diploid progenitors do not exhibit niche differentiation (i.e., Godsoe et al. 2013), niche modeling and common garden experiments similar to the methods of Visger et al. (2016) would be beneficial next steps in determining if niche differentiation exists between diploid and autotetraploid *M. virginiensis.* Currently, it is not known if the autotetraploid populations are reproductively isolated, as this was outside the scope of this study. However, no triploid populations, which would be expected from a tetraploid-diploid cross, have been found to date

despite the peripatry of the tetraploid and diploid populations, suggesting they may be reproductively isolated. Examining potential reproductive isolation through a cross-pollination study and determining the chromosome counts of more populations would also be important next steps in determining if autotetraploid *M. virginiensis* is deserving of species status.

Based on clear and consistent morphological differences in the floral morphology, I have determined that the Gap Creek and Wadakoe Mountain populations in the Southern Appalachian escarpment region are worthy of recognition as a distinct species of putative hybrid origin between diploid *M. virginiensis* and *M. careyana*. I have also identified two other potential species contained within the *M. virginiensis* taxon, the diploid Polk County, NC populations and the autotetraploid populations, though further study is needed before strong conclusions can be formed regarding the taxonomic status of these two groups. After examination of a wealth of living plants and herbarium specimens from all regions where *M. virginiensis* occurs, no evidence was found for any other additional species beyond those previously mentioned in the Southeastern USA. Future work that could address the lingering questions includes a robust molecular analysis that could confirm any independent lineages or hybridization, artificial crossing experiments to determine if hybridization is possible between diploid *M. virginiensis* and *M. careyana*, the Gap Creek and Wadakoe Mountain populations, or the autotetraploid populations, and morphological analysis of characters not investigated in this study (i.e., seed coat characters, guard cell size) that could reveal cryptic differences between diploid and autotetraploid *M. virginiensis*. The recognition of at least one (and perhaps multiple) distinct species within *M. virginiensis* allows for improved understanding of biodiversity and can incentivize others to revisit geographically widespread species to look for other instances where cryptic or morphologically similar species have been overlooked.

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APPENDIX: SUPPLEMENTARY TABLES

Supplementary Table 1: Voucher specimens. All specimens are deposited at WCUH.

Species	Year	Coll. Date	Country	State	County	Latitude	Longitude	Chrom. #	Hall Coll. #	Permit #
careyana	2022	1-Apr	USA	NC	Swain	35.33403	-83.624428		13	
careyana	2023	16-Apr	USA	NC	McDowell	35.700737	-82.19558	10	53	
careyana	2022	23-Apr	USA	NC	Macon	35.117584	-83.270458	10	31	
careyana	2022	23-Apr	USA	NC	McDowell	35.700737	-82.19558		32	File Code: 2720
careyana	2022	10-Apr	USA	TN	Knox	35.955775	-83.863	10	20	
micranthidifolia	2022	11-Apr	USA	NC	Jackson	35.345428	-83.164555	12	15	
palmeri	2022	6-Apr	USA	AR	Conway	35.288696	-92.483929	10	8	
palmeri	2022	1-Apr	USA	MO	Douglas	36.82929	-92.4244	10	16	No #
petiolaris	2022	23-Apr	USA	NC	Macon	35.117584	-83.270458	10-13	30	
petiolaris	2023	11-Jun	USA	NC	Ashe	36.406256	-81.467033	10	47	
virginiensis	2022	21-Mar	USA	AL	Jefferson	33.703495	-86.692383	10	9	
virginiensis	2022	28-Mar	USA	AR	Pulaski	34.801828	-92.32132	10	6	
virginiensis	2022	11-May	USA	CT	New Haven	41.55783	-72.759383	10	41	
virginiensis	2022	30-Mar	USA	GA	Fulton	33.882412	-84.440262	10	10	
virginiensis	2022	13-Apr	USA	KY	Clinton	36.871587	-85.192345		23	
virginiensis	2022	13-Apr	USA	KY	Todd	36.921097	-87.285705	10	22	
virginiensis	2022	14-Apr	USA	KY	Franklin	38.218933	-84.847183		25	
virginiensis	2022	11-May	USA	MA	Middlesex	42.278647	-71.343405		40	
virginiensis	2022	10-May	USA	MD	Montgomery	39.088158	-77.124992	10	38	No #
virginiensis	2022	12-May	USA	ME	Knox	44.254487	-69.095772		42	
virginiensis	2022	18-May	USA	MI	Marquette	46.761716	-87.73377	10	39	
virginiensis	2022	18-May	USA	MI	Marquette	46.854202	-87.859005		43	
virginiensis	2022	11-Mar	USA	MS	Clay	33.537987	-88.633453	10	11	
virginiensis	2022	14-Mar	USA	NC	Polk	35.221517	-82.305908	10	1	
virginiensis	2023	16-Mar	USA	NC	Mecklenburg	35.152347	-80.736725	19	50	
virginiensis	2022	27-Mar	USA	NC	Polk	35.272053	-82.216317	10	2	
virginiensis	2022	27-Mar	USA	NC	Durham	36.072998	-78.864864	19	5	
virginiensis	2022	6-Apr	USA	NC	Polk	35.272053	-82.216317	10	46	
virginiensis	2022	4-May	USA	NC	Chatham	35.736786	-79.112814	19	34	
virginiensis	2023	16-Mar	USA	NC	Montgomery	35.40607	-80.09268	19	51	
virginiensis	2022	28-Mar	USA	NJ	Somerset	40.584764	-74.559456	10	12	via email

virginiensis	2022	27-Apr	USA	NY	Tompkins	42.399242	-76.53585		27	via email
virginiensis	2022	14-May	USA	NY	Erie	42.700905	-78.904725		45	
virginiensis	2022	14-Apr	USA	OH	Hamilton	39.124675	-84.782745		26	
virginiensis	2022	15-Apr	USA	OH	Fairfield	39.63158	-82.647383		29	
virginiensis	2022	10-May	USA	PA	Chester	39.727925	-76.073142		37	22-827
virginiensis	2022	14-Mar	USA	SC	Pickens	34.9005288	-82.6593307	22	4	
virginiensis	2022	6-Apr	USA	SC	Spartanburg	35.140617	-82.278862	10	19	
virginiensis	2022	10-Apr	USA	TN	Davidson	36.053741	-86.91092	11	21	
virginiensis	2022	13-Apr	USA	TN	Jefferson	36.101922	-83.627633		24	
virginiensis	2022	21-Mar	USA	VA	Powhatan	37.682778	-77.938333	10	7	PW-RCP-020822
virginiensis	2022	9-May	USA	VA	Floyd	36.803506	-80.341778		36	BLRI-2022-SCI-0017
virginiensis	2022	27-Apr	USA	VT	Orange	43.919051	-72.210572		18	
virginiensis	2022	15-Apr	USA	WV	Wayne	38.146458	-82.382308		28	
virginiensis	2022	18-May	CAN	ONT	Lennox	44.53755	-76.92789		33	
virginiensis	2022	18-May	CAN	ONT	Lennox	44.560672	-77.116398	10	35	
virginiensis	2022	13-May	CAN	QBC	Le Haut-Richelieu	45.354428	-73.150506		44	
virginiensis (Gap Creek)	2022	14-Mar	USA	SC	Greenville	35.164122	-82.475519	19	3	N-2-23
virginiensis (Wadakoe Mtn.)	2022	26-Mar	USA	SC	Pickens	34.98221	-82.84356	19	14	SC-92-2022

Supplementary Table 2: *M. virginiensis* specimens used for PCA conducted with fruit characters.

Specimen ID	Year	Date	State	County	Latitude	Longitude
UNA00034416	1982	16-Apr	AL	Wilcox	31.908333	-87.380556
UNA00034491	1982	9-Apr	AL	Dallas	32.32	-83.03
UNA00034496	1982	7-Apr	AL	Lowndes	32.353611	-86.690833
NCU00090765	1967	16-Apr	AL	Randolph	33.279788	-85.645296
UNA00014924	1977	2-Apr	AL	Walker	33.614444	-87.363333
UNA00065282	2003	9-Apr	AL	Lawrence	34.395833	-87.215278
NCU00090772	1967	30-Apr	AR	Franklin	35.673382	-93.699275
NCU00090770	1967	31-Mar	AR	Cleburne	35.459218	-92.03591
ANHC009866	2016	9-Apr	AR	Pulaski	34.80187	-92.32307
UVMVT068793	1974	19-May	CT	Hartford	41.65711	-72.66329
NCU00090930	1897	7-May	DC	Washington, D.C.	38.895112	-77.036366
PH00498081	1881	10-May	DE	New Castle	39.739001	-75.635761
NCU00090787	1964	23-Apr	GA	Walton	33.765827	-83.852404
CLEMS0066960	1978	4-Apr	GA	Elbert	34.2575	-82.747778
GA035914	1986	21-Apr	GA	Cherokee	34.317418	-84.645479
NCU00090982	1949	26-Apr	IL	Hardin	37.560192	-88.120932
IND-0046733	1927	24-Apr	IL	Crawford	38.182216	-86.381489
IND-0046741	1929	5-May	IN	Spencer	37.886783	-87.046321
IND-0046743	1941	15-Apr	KY	Warren	37.083509	-86.579398
NCU00090791	1963	5-May	KY	Henry	38.364896	-84.880879
MARY1018304	1966	15-May	MD	Baltimore	39.443768	-76.510269
DOV0036330	1997	17-May	MD	Allegany	39.665667	-78.462833
NCU00090907	1969	16-May	MD	Washington	39.637175	-78.329943
MARY1018308	1980	17-May	MD	Allegany	39.693337	-78.451993
HUDC00009878	1967	27-May	MD	Allegany	39.636887	-78.457557
MARY1018360	1947	11-May	MD	Montgomery	39.152383	-77.120321
UVMVT144783	1984	10-May	MD	Prince Georges	38.473681	-77.013484
4737	1914	30-May	MA	Worcester	42.5834	-71.8023
IND-0046746	1905	28-May	MA	Middlesex	42.345801	-71.450001
1465255	1970	16-Jun	MI	Keweenaw	48.099098	-88.601638
1465257	1930	30-Jun	MI	Keweenaw	48.12023	-88.53492
1477443	1958	30-May	MI	Ontonagon	46.693192	-89.732307
1465249	1957	6-Jul	MI	Keweenaw	48.044027	-88.701576
1465270	1958	30-May	MI	Ontonagon	46.76667	-89.75
1465253	1979	18-Jun	MI	Chippewa	46.075773	-83.666114
UNCC_45631	1984	10-Apr	MS	Tishomingo	34.6024	-88.1938
MMNS006411	1979	9-Apr	MS	Tishomingo	34.93403	-88.17902
59163	1963	20-Apr	MO	Douglas	39.4	-93.8167
ANHC010841	2009	22-Apr	MO	Shannon	37.11615	-91.19997
NCU00090991	1887	14-Apr	MO	Jefferson	38.261071	-90.537689
UVMVT068805	1969	12-Jun	NH	Strafford	43.44935	-71.00751
PH00498301	1936	24-May	NJ	Cape May	38.987613	-74.95323
PH00498241	1922	22-May	NJ	Monmouth	40.106692	-74.518673
1246908	1892	1-May	NY	Bronx	40.856767	-73.875414
SIM0003763	1885	21-Jun	NY	Richmond	40.625278	-74.095833
NCU00088179	1957	25-Apr	NC	Vance	36.324846	-78.375974
NCU00088161	1958	6-Apr	NC	Lee	35.575831	-79.201701

NCU00088181	1938	11-Apr	NC	Wake	35.830113	-78.638615
NCU00088142	1958	22-May	NC	Caswell	36.28707	-79.221237
NCU00088173	1992	24-Apr	NC	Surrey	36.55013	-80.908687
NCU00088176	1974	21-Apr	NC	Stokes	36.429051	-80.298403
NCU00088173	1992	24-Apr	NC	Surrey	36.55013	-80.908687
NCU00088177	1958	4-May	NC	Stokes	36.429951	-80.288942
NCU00090960	1959	10-Jul	ONT	Thunder Bay	48.751251	-87.975253
PH00498115	1947	5-Jun	PA	Wayne	41.610825	-75.060752
PH00497956	1923	30-May	PA	Bucks	40.387142	-75.181386
PH00498196	1957	23-May	PA	Clearfield	41.069224	-78.367683
PH00498109	1937	17-May	PA	Butler	40.855111	-80.098514
PH00498199	1946	5-Jun	PA	Indiana	40.869762	-79.094215
PH00498182	1946	11-May	PA	Franklin	40.151347	-77.715536
PH00497914	1921	8-May	PA	Lehigh	40.560523	-75.572792
IND-0046755	1917	1-Jun	QBC	Cap-a-la-Branche	47.384136	-70.429108
PBRU00056700	2016	12-May	RI	Providence	41.91861	-71.44625
CLEMS0067009	1992	20-Apr	SC	Newberry	34.497412	-81.58919
USCH0057789	2012	11-Apr	SC	McCormick	33.6863	-82.1697
CLEMS0067011	1987	25-Apr	SC	Oconee	34.757904	-83.197198
CLEMS0067017	1974	22-Mar	SC	Richland	34.096051	-81.126246
CLEMS0067014	2002	19-Apr	SC	Pickens	34.980175	-82.843122
WCUH0024319	2008	19-Apr	SC	Pickens	34.98221	-82.84356
CLEMS0067008	1978	9-Apr	SC	Laurens	34.498504	-82.139386
CLEMS0067012	1986	26-Apr	SC	Oconee	34.757904	-83.197198
NCU00090720	1957	14-Apr	SC	York	34.904823	-81.461335
APSC0003160	2010	15-Apr	TN	Jackson	36.4239	-85.6497
NCU00090910	1935	24-Mar	TN	Davidson	36.16589	-86.784443
WCUH0024320	1973	12-Apr	TN	Smith	36.14249	-85.823105
UVMVT068755	1908	1-Jun	VT	Bennington	43.25879	-73.05147
UVMVT068741	1959	30-May	VT	Chittenden	44.53826	-72.88613
UVMVT068723	1892	15-May	VT	Chittenden	44.48735	-73.23124
UVMVT068708	1967	13-May	VT	Bennington	42.79188	-73.21203
18702	1975	2-May	VA	New Kent	37.485776	-76.784858
NCU00092555	1966	7-May	VA	Rockingham	38.302073	-78.622517
1400883	2011	10-May	VA	Patrick	36.606569	-80.449547
WVA-V-0068747	1984	27-Apr	WV	Summers	37.587332	-80.745956
WVA-V-0025989	2014	17-May	WV	Calhoun	38.828533	-81.147217
WVA-V-0068718	1952	10-May	WV	Pocahontas	38.90027778	-78.15916667
WVA-V-0068668	1985	24-Apr	WV	Fayette	38.15	-81.2
WVA-V-0068728	1998	3-Jun	WV	Pendleton	38.826779	-79.29143
WVA-V-0068706	2001	26-Apr	WV	Monongalia	39.55	-80
WVA-V-0068654	1891	1-Apr	WV	Fayette	37.97161	-81.154165
WVA-V-0068674	1939	6-May	WV	Jefferson	39.492599	-77.780272
WVA-V-0068669	2008	2-May	WV	Boone	38.155429	-81.644985

Supplementary Table 3: All *M. virginiensis* SERNEC specimens examined in this study.

Specimen ID	Year	Date	State/Province	County	Latitude	Longitude
TROY000042226	2012	15-Mar	AL	Butler	31.916959	-86.688774
UNA00034491	1982	9-Apr	AL	Dallas	32.32	-83.03
TENN-V-0229552	1993	5-Apr	AL	Jefferson	33.772864	-86.841349
UNA00065282	2003	9-Apr	AL	Lawrence	34.395833	-87.215278
UNA00034496	1982	7-Apr	AL	Lowndes	32.353611	-86.690833
UNA00014923	1979	15-Mar	AL	Marshall	34.41	-86.39
NCU00090765	1967	16-Apr	AL	Randolph	33.279788	-85.645296
UNA00014924	1977	2-Apr	AL	Walker	33.614444	-87.363333
UNA00034416	1982	16-Apr	AL	Wilcox	31.908333	-87.380556
UNA00065427	2005	19-Apr	AL	Winston	34.09	-87.61
NCU00090770	1967	31-Mar	AR	Cleburne	35.459218	-92.03591
ANHC007463	2006	28-Mar	AR	Drew	33.73613	-91.62441
NCU00090772	1967	30-Apr	AR	Franklin	35.673382	-93.699275
276702	2016	6-Apr	AR	Pulaski	34.8018	-92.3213
ANHC009866	2016	9-Apr	AR	Pulaski	34.80187	-92.32307
UVMVT068793	1974	19-May	CT	Hartford	41.65711	-72.66329
NCU00090930	1897	7-May	DC	Washington, D.C.	38.895112	-77.036366
PH00498083	1897	29-Apr	DE	New Castle	39.788368	-75.636863
PH00498081	1881	10-May	DE	New Castle	39.739001	-75.635761
GA035914	1986	21-Apr	GA	Cherokee	34.317418	-84.645479
WCUH0024322	2006	16-May	GA	Cobb	33.953698	-84.592143
CLEMS0066961	1978	4-Apr	GA	Elbert	34.052856	-82.645015
CLEMS0066960	1978	4-Apr	GA	Elbert	34.2575	-82.747778
NCU00090787	1964	23-Apr	GA	Walton	33.765827	-83.852404
IND-0046733	1927	24-Apr	IL	Crawford	38.182216	-86.381489
NCU00090982	1949	26-Apr	IL	Hardin	37.560192	-88.120932
IND-0046734	1934	29-Apr	IN	Dearborn	38.987077	-85.022697
IND-0046741	1929	5-May	IN	Spencer	37.886783	-87.046321
NCU00090789	1955	6-Apr	KY	Fayette	37.902746	-84.397728
MUHW031902	1937	14-May	KY	Hancock	37.896533	-86.755675
NCU00090791	1963	5-May	KY	Henry	38.364896	-84.880879
HTTU034555	1998	28-Mar	KY	Metcalfe	36.977	-85.696167
IND-0046743	1941	15-Apr	KY	Warren	37.083509	-86.579398
NCU00090798	1959	6-Mar	LA	Union	32.729938	-92.405699
IND-0046746	1905	28-May	MA	Middlesex	42.345801	-71.450001
4737	1914	30-May	MA	Worcester	42.5834	-71.8023
DOV0036330	1997	17-May	MD	Allegany	39.665667	-78.462833
MARY1018308	1980	17-May	MD	Allegany	39.693337	-78.451993
HUDC00009878	1967	27-May	MD	Allegany	39.636887	-78.457557
MARY1018304	1966	15-May	MD	Baltimore	39.443768	-76.510269
MARY1018360	1947	11-May	MD	Montgomery	39.152383	-77.120321
UVMVT144783	1984	10-May	MD	Prince Georges	38.473681	-77.013484
NCU00090907	1969	16-May	MD	Washington	39.637175	-78.329943
UVMVT068804	1999	18-May	ME	Androscoggin	44.09146	-70.16808
1465248	1985	26-May	MI	Chippewa	46.07868	-83.644765
1465253	1979	18-Jun	MI	Chippewa	46.075773	-83.666114
1465255	1970	16-Jun	MI	Keweenaw	48.099098	-88.601638

1465257	1930	30-Jun	MI	Keweenaw	48.12023	-88.53492
1465249	1957	6-Jul	MI	Keweenaw	48.044027	-88.701576
1477443	1958	30-May	MI	Ontonagon	46.693192	-89.732307
1465270	1958	30-May	MI	Ontonagon	46.76667	-89.75
906510	2008	18-Jun	MN	Cook	47.895	-90.56
178406	1894	10-Aug	MN	Lake of the Woods	49.353711	-95.002845
928696	2010	10-Jun	MN	Saint Louis	47.8002778	-92.0622222
59163	1963	20-Apr	MO	Douglas	39.4	-93.8167
NCU00090991	1887	14-Apr	MO	Jefferson	38.261071	-90.537689
ANHC010841	2009	22-Apr	MO	Shannon	37.11615	-91.19997
UNCC_45631	1984	10-Apr	MS	Tishomingo	34.6024	-88.1938
MMNS006411	1979	9-Apr	MS	Tishomingo	34.93403	-88.17902
NCU00088139	1958	2-May	NC	Alleghany	36.571007	-81.2
NCU00088142	1958	22-May	NC	Caswell	36.28707	-79.221237
NCU00088145	1960	18-Apr	NC	Catawba	35.604469	-80.943845
NCU00088147	1956	22-Mar	NC	Cleveland	35.201363	-81.665131
NCU00088150	1958	23-Apr	NC	Edgecombe	35.959087	-77.781101
NCU00088152	1958	17-May	NC	Forsyth	36.183674	-80.073653
NCU00088156	1956	26-Apr	NC	Granville	36.194807	-78.582936
NCU00088162	1958	15-Apr	NC	Lee	35.580857	-79.154666
NCU00088161	1958	6-Apr	NC	Lee	35.575831	-79.201701
CLEMS0066966	1958	18-Apr	NC	Mecklenburg	35.500153	-80.832807
NCU00088176	1974	21-Apr	NC	Stokes	36.429051	-80.298403
NCU00088177	1958	4-May	NC	Stokes	36.429951	-80.288942
NCU00088173	1992	24-Apr	NC	Surrey	36.55013	-80.908687
NCU00088178	1956	16-Apr	NC	Surry	36.277438	-80.770647
NCU00088179	1957	25-Apr	NC	Vance	36.324846	-78.375974
NCU00088181	1938	11-Apr	NC	Wake	35.830113	-78.638615
UVMVT068805	1969	12-Jun	NH	Strafford	43.44935	-71.00751
CM453972	1923	28-Apr	NJ	Burlington	39.912305	-74.810137
PH00498301	1936	24-May	NJ	Cape May	38.987613	-74.95323
PH00498241	1922	22-May	NJ	Monmouth	40.106692	-74.518673
PH00498313	1938	1-May	NJ	Somerset	40.448585	-74.756494
PAC0042447	1927	16-May	NY	Albany	42.604802	-73.769566
1246908	1892	1-May	NY	Bronx	40.856767	-73.875414
KHD00051421	1939	11-May	NY	Monroe	43.173105	-77.709017
SIM0003761	1881	1-May	NY	Richmond	40.635638	-74.092162
SIM0003763	1885	21-Jun	NY	Richmond	40.625278	-74.095833
CM396722	1993	7-May	NY	Ulster	41.989476	-74.244577
NCU00090960	1959	10-Jul	ON	Thunder Bay	48.751251	-87.975253
CM051049	1951	12-May	PA	Armstrong	40.877908	-79.440748
CM537111	2016	21-May	PA	Bedford	39.81707	-78.40278
MOAR0015267	2005	5-Apr	PA	Bucks	40.515294	-75.09192
PH00497956	1923	30-May	PA	Bucks	40.387142	-75.181386
PH00498109	1937	17-May	PA	Butler	40.855111	-80.098514
CM051032	1924	1-May	PA	Chester	39.895387	-75.734384
PH00498196	1957	23-May	PA	Clearfield	41.069224	-78.367683
CM051038	1970	6-May	PA	Clinton	41.277475	-77.885329
CM469925	2005	8-Jun	PA	Erie	42.01745	-80.390603

PH00498182	1946	11-May	PA	Franklin	40.151347	-77.715536
CM495302	1996	10-May	PA	Fulton	39.73333	-78.33333
CM050960	1952	13-May	PA	Huntingdon	40.228339	-78.050899
PH00498199	1946	5-Jun	PA	Indiana	40.869762	-79.094215
PH00498076	1960	2-May	PA	Lancaster	40.178826	-76.082168
CM050986	1952	3-May	PA	Lawrence	40.856203	-80.315999
PH00497914	1921	8-May	PA	Lehigh	40.560523	-75.572792
PH00498136	1937	22-May	PA	Schuylkill	40.641123	-76.600521
PH00498115	1947	5-Jun	PA	Wayne	41.610825	-75.060752
IND-0046755	1917	1-Jun	QB	Cap-a-la-Branche	47.384136	-70.429108
129120	1957	23-May	QB	Charlevoix	47.4384	-70.4631
PBRU00056700	2016	12-May	RI	Providence	41.91861	-71.44625
ASU0131689	1957	12-Apr	SC	Laurens	34.454803	-82.198702
CLEMS0067008	1978	9-Apr	SC	Laurens	34.498504	-82.139386
USCH0057789	2012	11-Apr	SC	McCormick	33.6863	-82.1697
CLEMS0067009	1992	20-Apr	SC	Newberry	34.497412	-81.58919
CLEMS0067011	1987	25-Apr	SC	Oconee	34.757904	-83.197198
CLEMS0067012	1986	26-Apr	SC	Oconee	34.757904	-83.197198
CLEMS0067016	1974	22-Mar	SC	Richland	34.099859	-81.111838
CLEMS0067017	1974	22-Mar	SC	Richland	34.096051	-81.126246
NCU00090724	1975	6-Apr	SC	York	34.8625	-81.09492
NCU00090720	1957	14-Apr	SC	York	34.904823	-81.461335
NCU00090913	1961	18-Apr	TN	Cheatham	36.246058	-87.017755
276703	2016	12-Apr	TN	Coffee	35.4856	-86.1062
NCU00090914	1964	14-Apr	TN	Davidson	36.095409	-86.533971
NCU00090910	1935	24-Mar	TN	Davidson	36.16589	-86.784443
HTTU016081	1999	16-Apr	TN	Dekalb	36	-85.666
APSC0003160	2010	15-Apr	TN	Jackson	36.4239	-85.6497
GA155493	1949	31-Mar	TN	Polk	35.219754	-84.519161
WCUH0024320	1973	12-Apr	TN	Smith	36.14249	-85.823105
NCU00092526	1975	24-Apr	VA	Carroll	36.892739	-80.712262
57763	1991	4-May	VA	Greene	38.378211	-78.511065
WVA-V-0016587	1964	11-Apr	VA	James City	37.145595	-76.733115
GMUF-0042091	2017	2-Apr	VA	Loudoun	39.2895	-77.737189
18702	1975	2-May	VA	New Kent	37.485776	-76.784858
1400883	2011	10-May	VA	Patrick	36.606569	-80.449547
NCU00092553	1966	30-Apr	VA	Prince Edward	37.25815	-78.414276
NCU00092555	1966	7-May	VA	Rockingham	38.302073	-78.622517
ODU00024347	1992	24-Apr	VA	Surry	36.55013	-80.908687
Benn-2188	1975	19-May	VT	Bennington	42.793086	-73.255313
UVMVT068755	1908	1-Jun	VT	Bennington	43.25879	-73.05147
UVMVT068708	1967	13-May	VT	Bennington	42.79188	-73.21203
UVMVT068741	1959	30-May	VT	Chittenden	44.53826	-72.88613
UVMVT068723	1892	15-May	VT	Chittenden	44.48735	-73.23124
UVMVT068751	1977	1-May	VT	Westmore	44.76016	-72.02697
UVMVT068717	1937	16-May	VT	Williston	44.43457	-73.08868
WVA-V-0068669	2008	2-May	WV	Boone	38.155429	-81.644985
WVA-V-0025989	2014	17-May	WV	Calhoun	38.828533	-81.147217
WVA-V-0068668	1985	24-Apr	WV	Fayette	38.15	-81.2

WVA-V-0068654	1891	1-Apr	WV	Fayette	37.97161	-81.154165
NCU00090921	1970	4-May	WV	Hampshire	39.222223	-78.845537
WVA-V-0068674	1939	6-May	WV	Jefferson	39.492599	-77.780272
WVA-V-0068712	2002	16-Apr	WV	Mason	38.685413	-82.034138
WVA-V-0068710	2002	10-Apr	WV	McDowell	37.4566	-81.882144
WVA-V-0068706	2001	26-Apr	WV	Monongalia	39.55	-80
WVA-V-0068728	1998	3-Jun	WV	Pendleton	38.826779	-79.29143
WVA-V-0068734	2013	17-Apr	WV	Pleasants	39.41505	-81.08415
WVA-V-0068718	1952	10-May	WV	Pocahontas	38.90027778	-78.15916667
WVA-V-0068747	1984	27-Apr	WV	Summers	37.587332	-80.745956

Supplementary Table 4: Leaf and floral measurements used in multivariate analyses. “escarpment” = Gap Creek and Wadakoe Mountain.

Species	Hypanthium Length (mm)	Stamen Length (mm)	Petal Length (mm)	Petal Width (mm)	Pistil Length (mm)	Plant Height (cm)
virginiensis	1.668	1.574	6.356	2.316	3.782	24
virginiensis	0.777	1.279	4.873	1.638	1.243	16.2
virginiensis	1.749	1.802	6.654	1.937	4.036	21.4
virginiensis	0.87	1.49	5.3	2.13	2.251	16.1
virginiensis	1.145	1.532	5.392	2.11	2.422	9
virginiensis	1.1	1.37	4.657	2.312	2.068	21.4
virginiensis	1.375	1.417	4.648	1.625	2.292	21.9
virginiensis	0.603	0.908	2.876	1.57	2.612	7.4
virginiensis	1.012	1.466	4.619	1.8	2.275	14.5
virginiensis	1.211	1.449	4.467	1.953	3.059	13.2
virginiensis	1.112	1.517	4.499	1.471	1.634	16.1
virginiensis	0.91	1.12	3.21	1.32	2.218	16.5
virginiensis	1.707	1.841	5.12	2.511	1.904	12.3
virginiensis	1.189	1.731	4.724	2.147	1.944	16.8
virginiensis	1.454	1.447	3.939	1.135	2.981	16.9
virginiensis	1.547	2.025	5.285	2.429	2.952	22.3
virginiensis	1.203	1.831	4.739	2.313	2.426	15.3
virginiensis	1.35	2	5.1	1.69	2.685	16.4
virginiensis	1.068	1.801	4.167	1.466	2.03	22.1
virginiensis	1.25	2.04	4.66	1.82	2.828	17.9
virginiensis	1.073	1.626	3.499	1.33	1.976	9.7
virginiensis	1.413	1.593	3.426	1.451	2.06	8.6
virginiensis	1.085	1.769	3.749	1.386	1.818	20.6
virginiensis	1.36	1.59	4.078	1.445	2.545	6.5
escarpment	1.2	2.89	5.61	2.19	3.56	16
escarpment	1.14	2.99	5.72	2.52	3.13	20.5
escarpment	1.15	3.27	6	2.68	4.53	16
escarpment	0.78	2.62	4.64	1.81	3.79	12.5
escarpment	1.11	2.88	4.91	2.42	3.82	15
escarpment	0.89	2.32	3.92	1.37	3.75	25
escarpment	0.87	2.59	4.29	1.69	2.98	18.1
escarpment	0.9	2.65	4.35	1.73	4.42	13.5
escarpment	1.36	3.12	5.06	2.43	4.935	18.5
escarpment	0.98	3.17	5.14	1.7	3.74	14.5
escarpment	1.03	2.83	4.43	1.58	3.86	12
escarpment	1.13	3.57	5.556	2.36	4.6	19.4
escarpment	1.32	3.54	5.29	2.19	5.34	22.5

escarpment	0.7	2.24	2.97	1.11	2.19	26
escarpment	0.893	2.81	3.538	1.374	4.78	22.2
careyana	0	2.66	4.4	1.69	2.66	15
careyana	0	2.65	4.37	1.56	1.99	12.5
careyana	0	2.33	3.73	1.63	1.94	12
careyana	0	2.6	3.93	1.58	2.08	10.5
careyana	0	2.55	3.79	1.55	2.53	8.5
careyana	0	2.64	3.83	1.83	1.93	9
careyana	0	2.6	3.64	1.54	1.69	12.5
careyana	0	3.23	4.46	2.22	2.84	19
careyana	0	2.54	3.4	1.58	1.81	12
careyana	0	2.557	3.385	1.517	2.345	19.5
careyana	0	2.8	3.58	1.67	1.56	6.5
careyana	0	3.466	4.266	2.022	2.968	21
careyana	0	3.894	4.638	2.194	3.968	20.7

Species	Blade Length (cm)	Blade Width (cm)	Petiole Length (cm)	Sqrt Blade Area (cm2)	Blade Circularity
virginiensis	4.419	2.444	4.37	2.912215651	0.351
virginiensis	1.171	1.038	0.845	0.976729236	0.43
virginiensis	2.591	1.686	1.035	1.852565788	0.289
virginiensis	1.277	0.837	0.656	0.916515139	0.433
virginiensis	0.809	0.759	1.344	0.694262198	0.353
virginiensis	3.249	2.503	1.29	2.527449307	0.407
virginiensis	2.797	1.183	0.478	1.564928113	0.186
virginiensis	1.746	1.359	1.736	1.365283853	0.412
virginiensis	2.864	1.402	2.215	1.775387282	0.414
virginiensis	4.08	2.555	1.772	2.861642885	0.359
virginiensis	2.168	1.538	1.476	1.618641406	0.395
virginiensis	2.658	1.907	1.949	1.994993734	0.4
virginiensis	2.261	2.099	2.993	1.930802942	0.299
virginiensis	2.89	1.684	1.838	1.954993606	0.299
virginiensis	2.4	0.524	8.64	0.643428318	0.3733
virginiensis	1.474	0.886	0.487	1.012916581	0.395
virginiensis	1.128	0.724	0.918	0.800624756	0.349
virginiensis	1.712	1.045	0.475	1.185326959	0.456
virginiensis	2.849	1.324	1.968	1.72133669	0.358
virginiensis	3.046	2.119	1.644	2.25166605	0.315
virginiensis	2.435	1.688	1.496	1.665232716	0.418
virginiensis	2.712	1.552	1.379	1.818515878	0.338
virginiensis	2.67	1.623	2.329	1.844722201	0.473

virginiensis	2.083	1.039	1.634	1.280234354	0.289

Supplementary Table 5: *M. virginiensis* fruit measurements used in PCA. All measurements in cm.

PCA Number	Specimen ID	Plant Height	Distance Between Fruit Horns	Fruit Length
1	UNA00034416	21.7	0.475	0.285
2	UNA00034491	25.49	0.516	0.285
3	UNA00034496	22.848	0.555	0.271
4	NCU00090765	26.391	0.43	0.382
5	UNA00014924	15.017	0.355	0.294
6	UNA00065282	27.698	0.463	0.241
7	NCU00090772	15.18	0.45	0.282
8	NCU00090770	17.741	0.405	0.39
9	ANHC009866	29.181	0.553	0.362
10	UVMVT068793	25.226	0.388	0.274
11	NCU00090930	23.382	0.468	0.331
12	PH00498081	33.845	0.673	0.549
13	NCU00090787	16.045	0.449	0.285
14	CLEMS0066960	26.651	0.367	0.332
15	GA035914	19.886	0.557	0.339
16	NCU00090982	8.687	0.42	0.398
17	IND-0046733	30.121	0.384	0.311
18	IND-0046741	29.015	0.589	0.321
19	IND-0046743	17.95	0.338	0.276
20	NCU00090791	16.21	0.479	0.326
21	MARY1018304	18.361	0.503	0.371
22	DOV0036330	14.006	0.388	0.306
23	NCU00090907	18.31	0.483	0.386
24	MARY1018308	22.681	0.343	0.352
25	HUDC00009878	18.826	0.567	0.424
26	MARY1018360	30.386	0.413	0.386
27	UVMVT144783	16.179	0.413	0.333
28	4737	22.352	0.647	0.375
29	IND-0046746	14.387	0.346	0.273
30	1465255	19.053	0.398	0.365
31	1465257	33.195	0.368	0.384
32	1477443	16.781	0.357	0.384
33	1465249	19.348	0.388	0.339
34	1465270	9.229	0.332	0.296
35	1465253	13.698	0.412	0.353
36	UNCC_45631	20.592	0.4	0.313
37	MMNS006411	23.363	0.343	0.395
38	59163	15.059	0.385	0.379
39	ANHC010841	14.695	0.483	0.301
40	NCU00090991	15.14	0.509	0.284
41	UVMVT068805	13.367	0.467	0.311
42	PH00498301	25.175	0.52	0.354
43	PH00498241	24.849	0.42	0.266
44	1246908	21.645	0.506	0.339
45	SIM0003763	24.807	0.459	0.559
46	NCU00088179	39.099	0.411	0.395
47	NCU00088161	16.1247	0.509	0.35
48	NCU00088181	29.534	0.38	0.394
49	NCU00088142	37.342	0.602	0.342
50	NCU00088173	26.267	0.609	0.354
51	NCU00088176	20.484	0.893	0.501
52	NCU00088173	24.96	0.611	0.369
53	NCU00088177	25.974	0.612	0.521
54	NCU00090960	36.761	0.321	0.427

55	PH00498115	15.836	0.591	0.38
56	PH00497956	35.634	0.413	0.316
57	PH00498196	32.488	0.435	0.361
58	PH00498109	31.678	0.12	0.189
59	PH00498199	24.095	0.493	0.317
60	PH00498182	20.38	0.319	0.374
61	PH00497914	27.901	0.317	0.286
62	IND-0046755	16.412	0.319	0.292
63	PBRU00056700	24.349	0.403	0.5
64	CLEMS0067009	21.828	0.431	0.373
65	USCH0057789	31.721	0.69	0.424
66	CLEMS0067011	15.826	0.618	0.393
67	CLEMS0067017	19.927	0.36	0.274
68	CLEMS0067008	22.66	0.384	0.326
69	CLEMS0067012	11.412	0.593	0.34
70	NCU00090720	23.566	0.375	0.33
71	APSC0003160	18.218	0.366	0.31
72	NCU00090910	21.095	0.309	0.429
73	WCUH0024320	23.48	0.426	0.3
74	UVMVT068755	32.849	0.371	0.351
75	UVMVT068741	15.735	0.515	0.334
76	UVMVT068723	19.911	0.355	0.267
77	UVMVT068708	26.677	0.263	0.298
78	18702	21.036	0.512	0.371
79	NCU00092555	17.499	0.34	0.382
80	1400883	29.314	0.689	0.343
81	WVA-V-0068747	16.935	0.451	0.46
82	WVA-V-0025989	16.566	0.359	0.272
83	WVA-V-0068718	25.504	0.45	0.447
84	WVA-V-0068668	22.000	0.303	0.351
85	WVA-V-0068728	31.526	0.548	0.332
86	WVA-V-0068706	23.051	0.33	0.368
87	WVA-V-0068654	16.038	0.6	0.35
88	WVA-V-0068674	18.068	0.521	0.354
89	WVA-V-0068669	18.592	0.532	0.29