BIOGEOGRAPHICAL, ECOLOGICAL, MORPHOLOGICAL, AND MICROMORPHOLOGICAL ANALYSES OF THE SPECIES IN THE *Hexastylis heterophylla* COMPLEX.

A Thesis

By

JAMES EDWARD PADGETT

Submitted to the Graduate School

Appalachian State University

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2004

Major Department: Biology

BIOGEOGRAPHICAL, ECOLOGICAL, MORPHOLOGICAL, AND MICROMORPHOLOGICAL ANALYSES OF THE SPECIES IN THE *Hexastylis heterophylla* COMPLEX.

A Thesis

By

James Edward Padgett

May 2004

APPROVED BY:

____
Dr. Zack E. Murrell
Chairperson, Thesis Committee

____
Dr. Gary L. Walker
Member, Thesis Committee

____
Dr. Ray S. Williams
Member, Thesis Committee

____
Dr. Vicki J. Martian
Chairperson, Department of Biology

____
Dr. Judith E. Domer
Dean of Graduate Studies and Research

ABSTRACT

BIOGEOGRAPHICAL, ECOLOGICAL, MORPHOLOGICAL, AND MICROMORPHOLOGICAL ANALYSES OF THE SPECIES IN THE *Hexastylis heterophylla* COMPLEX. (May 2004)

James Edward Padgett, B. S. Gardner-Webb University

M. S. Appalachian State University

Thesis Chairperson: Zack E. Murrell

The genus *Hexastylis* Raf. (Commonly known as "Wild Ginger" or "Little Brown Jugs") is comprised of nine species that are distributed throughout the southeast of North America. Blomquist (1957) and Gaddy (1987) both recognized a group of eight *Hexastylis* species as the Virginica Group. Blomquist further divided the group into three Subgroups: Virginica, Shuttleworthii, and Heterophylla. Three species have been recognized in the *Hexastylis heterophylla* complex: *H. heterophylla* (Ashe) Small, *H. minor* (Ashe) Blomquist and *H. naniflora* Blomquist. Field biologists have generally recognized considerable morphological overlap occurring within this group. *Hexastylis naniflora* is a federally threatened species and is distributed in one of the most rapidly growing urban and industrial areas of the western Piedmont of North and South Carolina. The *H. heterophylla* complex was the focus of this thesis research, with an emphasis on the biology of the imperiled species *H. naniflora*

Herbarium specimens (N=693) borrowed from 17 herbaria were used to generate distribution maps for the three species in the *H. heterophylla* complex. Elemental occurrence data from the North Carolina Natural Heritage Program and the South Carolina Heritage Trust Program were obtained to augment the distribution map for *H. naniflora*. Based upon these maps, field investigations were conducted across the geographic range of the three species within the complex. Ecological, morphological, micromorphological, soil, and pollen analyses of the *H. heterophylla* complex were conducted. Using ecological and biogeographical information obtained from the study, we located 31 new populations of *H. naniflora*. One of these populations was found in the Yadkin River drainage where *H. naniflora* was not previously known to occur. This study extended the total number of known populations of *H. naniflora* to 143.

The three species in the Heterophylla complex were subjected to biosystematic and ecological analyses to explore species boundaries in the group. Eighty-five representative populations from the three species in the *H. heterophylla* complex were examined in field investigations. Based upon the ecological comparisons, the range of *H. naniflora* appears to be restricted by soil type and/or species co-occurrence. Using Scanning Electron Microscopy (SEM), pollen characters were discovered that distinguish *H. naniflora* from other members of the subgroup. Based upon biogeographical, ecological, morphological, and micromorphological work, the results show that *H. naniflora* is a well-defined species, while *Hexastylis minor* and *H. heterophylla* exhibit considerable morphological and micro-morphological overlap, making species circumscription difficult.

ACKNOWLEDGEMENTS

There are many individuals and organizations I would like to acknowledge for both their assistance and guidance through my education and research.

The first three individuals I want to acknowledge were mentors and educators, as well as friends. Dr. Wayne Hutchings, former professor at Isothermal Community College was the first person to spark my interest in biology and later botany. Dr. Thomas H. Jones, Chair of the Biology Department at Gardner Webb University, introduced me to the world of plant systematics and taxonomy. He also encouraged me to grow as a botanist by helping me pursue a floristic study at the Broad River Greenway, where I first encountered *Hexastylis*. Finally, Dr. Zack E. Murrell, my advisor while at Appalachian State University, allowed me to take my love and interest with the rare plant *Hexastylis naniflora* (Dwarf-flowered Heartleaf) and use it as the basis for my research at ASU. His guidance and insight, as well as constructive criticism, were very valuable to me during this project.

There are a number of other individuals who were important in helping guide me during this project. My thesis committee members Dr. Gary Walker and Dr. Ray Williams were valuable for guidance and support while at ASU. Emily Parisher and Seth Peoples were of great importance to me personally, as well as to my research.

They were helpful in the field, for comic relief, or for allowing me to crash at their place when I needed a place to relax and sleep.

Emily Parisher was especially important to me as a field assistant. She traveled with me across North and South Carolina helping with vegetation surveys. She was excellent when it came to recording data and helping to identify vegetation at the many sites we surveyed. Seth Peoples was my sounding board and person I turned to when I needed a break from my research. We spent many nights listening to Led Zeppelin and talking about family and friends.

Also from ASU, were a number if individuals whom I want to acknowledge who were of great assistance in helping me in my research. They are Dr. Ruth Dewel, Dr. Mary Connell, Dr. Howard Nuefeld, Dr. Robert Creed, Ms. Emily Gillespie, Mrs. Joanne Holden, Ms. Betsy Harris, Mrs. Vickie Issacs, Ms. Karen Bost, Mr. Scott Taylor, Mr. Alex Martin, Mr. John Sealy, Ms. Kristen Bright, and Ms. Daphne Gensberg.

Other individuals, I would like to acknowledge are L. L. Gaddy from the University of South Carolina in Columbia, Dr. Gil Newberry from the University of South Carolina in Spartanburg, Mr. Henry Edwards, owner of the Kudzu Farm, Dr. Doug Rayner from Wofford College in Spartanburg, Dr. Tim McDowell from East Tennessee State University, Mrs. Jame Amoroso and Mr. Shawn Oakley from the North Carolina Natural Heritage Program, Mr. Hal Bain and Ms. Karen Lynch from the North Carolina Department of Transportation, Dr. Cecil Frost and Ms. Marj Boyer from the North Carolina Department of Agriculture, Dr. Bert Pitman, Mr. Jim Sorrow, and Ms. Mary Bunch from the South Carolina Heritage Program, Dr. Robert

Peet from The University of North Carolina at Chapel Hill, Mrs. Brownie Plaster from the Broad River Greenway Council, and Dr. Alan Ratzlaff from the United States Fish and Wildlife Service.

I would also like to acknowledge and thank my wife Joyce Padgett. It was with great sacrifice on her part that I was able to pursue my dreams and goals regarding my interest and love of science. Without her love and support none of this would have been possible. Along with my wife, the love and support of my children Jessica and Brandon cannot go unmentioned.

There are many organizations and agencies to whom I own a debt of gratitude for all the advice, permits, valuable information, and funding they provided to my research. The North Carolina Department of Transportation (NCDOT), the North Carolina Department of Agriculture Plant Protection Program, the North Carolina Natural Heritage Program, the South Carolina Natural Heritage Program, the United States Fish and Wildlife Service (USFWS), Cowpens National Battlefield, the Catawba Lands Conservancy, the United States Forest Service (USFS), Crowder's Mountain State Park, Kings Mountain State Park, Rhyne Nature Preserve, the Cratus D Williams Graduate School, Bunched Arrowhead Heritage Preserve, Peters Creek Heritage Preserve, Dan River Inc., Spartanburg County Waterworks, Broad River Greenway, Cleveland County Landfill, Clemson Soil Lab, Association of Southern Biologists (ASB), United States Department of Agriculture (USDA), ASU Biology Graduate Student Association , and the Southern Appalachian Botanical Society (SABS). Herbaria that graciously allowed me to examine specimens are listed by name and acronym: Appalachian State University (BOON), Converse College

(CONV), Duke University (DUKE), East Tennessee State University (ETSU), Gray Herbarium (GH), Gardner Webb University (GWU), Missouri Botanical Garden (MO), New York Botanical Garden (NY), North Carolina State University (NCSU), University of Georgia (UGA), University of North Carolina at Chapel Hill (UNCH), United States National Herbarium (US), University of South Carolina at Columbia (USCH), University of South Carolina at Spartanburg (USCS), University of Tennessee Knoxville (TENN), University of Wisconsin at Madison (UWI), Virginia Polytech Institute (VPI) and Wofford College (WOFF).

TABLE OF CONTENTS

	Page
Abstract	iii
Acknowledgements	v
List of Tables	xi
List of Figures	xii
Introduction	1
Methods	17
Results	33
Discussion	75
Bibliography	86
Appendix A	93
Appendix B	108
Appendix C	112
Appendix D	114
Vitae	127

LIST OF TABLES

	Page
1. *Hexastylis* groups and subgroups	14
2. List of Herbaria for annotated specimens	18
3. NCVS Sites	27
4. Soil sample sites	32
5. Species richness data	62
6. GLM results for species richness	63
7. Associate species *H. heterophylla* complex	70
8. GLM results on soils	71
8. Tukey's results on soils	72

LIST OF FIGURES

Page

1. Diagram of *Asarum* clade by L. Kelly. 13

2. Diagram showing flower measurements 23

3. Diagram of NCVS Plot 29

4. Distribution Map for *H. naniflora* 39

5. Distribution Map for *H. heterophylla* Complex 41

6. New *H. naniflora* population distribution map 43

7. River Drainage Distribution Map for *H. naniflora* 45

8. PCA results from *H. minor* and *H. naniflora* 47

9. PCA results from *H. heterophylla* and *H. naniflora* 49

10. PCA results from *H. heterophylla* and *H. minor* 51

11. PCA results from *H. heterophylla* complex 53

12. Pollen micrographs of the *H. heterophylla* complex 55

13. *H. heterophylla* pollen close up 57

14. *H. minor* pollen close up 59

15. *H. naniflora* pollen close up 61

16. Average species diversity 65

17. Sorenson's Index results 67

18. Dendrogram from results of Sorenson's analysis 69

LIST OF FIGURES (continued)

Page

19. Transplant survival 74

INTRODUCTION

Species recognition and delineation is a critical part of conservation biology. If we are to use species as our unit of conservation, we must be able to determine if the unit is a "good species." There is considerable literature addressing the question, "What is a species?" (Avise and Wollenburg 1997; Bridle and Richie 2001; Britton-Davidian 2001; Noor 2002; Orr 2001; Riesenberg and Burke 2001; Rundle 2001; Shaw 2001; Vogler 2001; Wu 2001). However, most studies are theoretical and very few address the issue of species in "real life" settings. I contend that there is a critical need to examine imperiled species to determine if they can be defended as biological units. This effort is necessary in order to show the public that the funds invested in species conservation are worth the costs as well as the effort.

There is also a critical need in conservation biology to determine the autecology of imperiled species. This effort is necessary in order to 1) maintain and/or extend the range of imperiled species in a time of ever-dwindling non-disturbed habitat, and 2) to prepare for not-too-distant future needs to reconstruct whole alliances of organisms in the face of climatic change and wholesale movement of appropriate microhabitat from current locations. If we fail to gain this information in the near future, we will not be prepared to adequately protect imperiled species through the 21st century. In my study I examined species boundaries and autecology of the federally threatened *Hexastylis naniflora* Blomquist in the family *Aristolochiaceae* (Department of the Interior 1989). This species appears to be closely related to two other species with sympatric distributions, *Hexastylis minor* and *Hexastylis heterophylla*. I used a variety of morphological and ecological analyses to elucidate relationships among these three species, to examine the rarity of *H. naniflora*, and to develop a management plan that will ensure that this species is not in danger of extinction.

The family *Aristolochiaceae*, also known as the Birthwort family, consists of eight genera with more than four hundred species (Judd et al. 2002). The distribution of *Aristolochiaceae* is primarily pantropical with a few species found in temperate regions of Asia, Europe, and North America. The two major genera in the family are *Aristolochia* with 300-350 species and *Asarum* with about 70 species (80 if *Hexastylis* and *Heterotropa* are included).

Most of the family consists of climbing woody vines in *Aristolochia*, which has a tropical distribution. *Asarum* occurs in North America and Asia and consists of herbaceous perennial and annual species. *Asarum* species often have aromatic stems or leaves, due in part to the ethereal oils many of them possess.

Depending on the authority used, the North American species of *Hexastylis* can be segregated as a separate genus or nested within *Asarum*. After the genus *Hexastylis* was first segregated from *Asarum* by Rafinesque (1825), it slowly gained general acceptance in the North American literature (Blomquist 1957; Britton and Brown 1947; Cronquist 1981; Gaddy 1981, 1986a, 1986b, 1987; Gonzalez 1972; Kral 1983; Otte 1977; Radford et al 1968; Rayner 1994; Small 1933; Wofford 1989). *Hexastylis* was segregated from *Asarum* primarily due to the persistent glabrous leaves and the unique ovary position

(Rafinesque 1825). Currently, *Hexastylis* is commonly used to describe a genus of nine species and four varieties that is endemic to the southeastern United States.

In spite of this general acceptance, several North American taxonomists refused to recognize *Hexastylis* as a genus and published their work using the genus *Asarum* (Fernald 1943, 1950; Gregory 1956; Peattie 1929, 1940; Wyatt 1955). Barringer (1993) stated that new nomenclatural combinations were needed for North American species of *Asarum* to align them with the current understanding of the genus and he revised the genus *Asarum* and placed the genus *Hexastylis* in synonymy. In transferring the species of *Hexastylis* to *Asarum*, Barringer (1993) expanded the genus *Asarum* to 80 species. Barringer (1993) noted that all of the *Asarum* are linked together by similar vegetative and reproductive characteristics, as well as having similar ethereal oils, as was first determined by Hayashi et al. (1982). Recent molecular work by Kelly (1997, 1998a, 1998b) has shown *Hexastylis* nested within the genus *Asarum*, further supporting the work of Barringer (1993).

The recent publication of the Flora of North America (FNA) again recognized *Hexastylis* as a separate genus apart from *Asarum*, but footnotes were added in both the *Hexastylis* and *Asarum* keys and descriptions to denote that some problems existed in our understanding of the phylogeny (Barringer 1993; Whittemore and Gaddy 1997).

One of the earliest descriptions and illustrations of *Asarum* was in an herbal by Dodoens (1574) who discussed the use of *Asarum* as a purgative. He noted the medicinal properties of the plant he called *Asaron* (*Asarum europaeum*). In the 17^{th} century other herbals and botanical journals described several species of *Asarum* (Parkinson 1640; Tournefort, 1694). In 1789, de Jussieau recognized a relationship between *Asarum*, a genus made up entirely of herbs, with *Aristolochia*, a large genus comprised almost entirely of woody vines. De Jussieau (1789) made these associations based on floral characteristics and plant morphology. In Species Plantarum Linnaeus (1753) described four species of *Asarum*, including two North American species, *Asarum canadense* L., and *Asarum virginicum* L. Andre Michaux (1803) published a description of a third North American *Asarum* he named *Asarum arifolium* Michx.

In 1825, when Rafinesque erected the genus *Hexastylis*, his circumscription was based on characters that were unique to the three or four known North American species of *Asarum*. Those characters used to delineate *Hexastylis* included glabrous persistent leaves, connate sepals, sessile anthers, and apical bifid styles. Based on those characters, Rafinesque (1825) segregated *Hexastylis virginica* (L.) Raf., and *Hexastylis arifolia* (Michx.) Raf., leaving *A. canadense* as a sole species in the genus *Asarum* in North America. Rafinesque's (1825) description of *Hexastylis* in Neogenyton is as follows:

> "***Hexastylis*. Cal. Tubular, trifid, cor o. anthers twelve, sessile, bilobe adnate, epigyne; pistil half free, cylindrical, and concave; styles six, lateral erect; stigmas six, truncate, oblique, bicorne; caps. Six locul. Few central seeds. Type *Asarum arifolium*, Michx."**

Morren and Decaisne (1834) erected the genus *Heterotropa*, and described the Asian species *Heterotropa asarodies* Morr. & Dec. The characters used by Morren and Decaisne (1834) to describe *Heterotropa* were very similar to those used by Rafinesque (1825) to describe *Hexastylis*. Braun (1861) was the first to recognize the similarity in the descriptions of the two genera and placed both *Hexastylis* and *Heterotropa* in synonymy within *Asarum*. Braun divided the genus *Asarum* into three sections:

Ceratasarum, *Heterotropa*, and *Euasarum* and placed *Hexastylis* within section *Ceratasarum,* which included *A. arifolium* and *A. virginicum* along with one Japanese species, *Asarum variegatum*. Duchartre (1864) closely followed Braun's treatment in A. P. de Candolle's publication Prodromus Systematis Naturalis Regni Vegetabilis which was quoted in Braun's 1861 publication.

In the late 19th century W. W. Ashe traveled extensively across much of the southeastern United States examining various plant communities. In his travels Ashe realized that separation of the species of *Asarum* was difficult and the genus exhibited considerable variation across its range. Seeing that many specimens did not fit within the circumscription for *A. virginicum,* Ashe started collecting *Asarum* throughout the southeast United States. He also made numerous notes and sketches in regards to flower and leaf morphology. Ashe (1897) described several species of *Asarum,* including two species that would eventually be placed into the Virginica group of *Hexastylis*. Ashe's (1897) description of the three new species is as follows:

> "A***sarum minus*****. Leaves solitary, glabrous, think, round cordate at base, but rarely orbicular. Tube of calyx cylindro campanulate, about 1 cm wide, about the same length, the very sort lobes spreading. Peduncle as long as flower, the large ract pointed. Projection of style very short; the seeds oblong."**

> **"*Asarum heterophyllum*. Leaf-blades orbicular, ovate or triangular in outline, cordate at base (or occasionally almost hastate), about the same size as in above. Calyx campanulate rounded at the base, the tube. 7-1 cm long, the lobes nearly equaling in length .8-1 cm wide at the base, orange-purple or purple-brown without, bright within; the very stout notched style much prolonged the much minute round stigma; capsule short, cylinderous barely as long as the stamens, scarcely distending the calyx; seeds oval."**

> **"*Asarum heterophyllum ochranthum*. Calyx yellow or orange, oblong-campanulate, the spreading lobes as long as the 1cm tube. Calyx urceolate or somewhat contracted at the mouth, the oval stigma thicker than the slender deeply 2-parted projection of the style, and placed near the base of the style."**

Along with the descriptions quoted above, Ashe (1897) also described the distributions of the three new species. He described the distribution of *A. heterophyllum* Ashe (*H. heterophylla*) as being from North Carolina, Tennessee, and Virginia. Herbarium records and recent publications show *H. heterophylla* to be found in Alabama, Georgia, Kentucky, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia. *Asarum heterophyllum ochranthum* was never well accepted and today is included within *H. heterophylla*. *Asarum minus* was described by Ashe (1897) as being from North Carolina, South Carolina and Tennessee and is presently known from Georgia, North Carolina, South Carolina, and Virginia (Amoroso 2002; Blomquist 1957; Gaddy 1987; Radford et al, 1968; McMillian 1995; Sutter et al, 1983; Wofford 1989).

The genus *Hexastylis* was not widely recognized in North American publications until J. K. Small (1933) used *Hexastylis* instead of *Asarum* to denote the genus in his Manual of Southeastern Flora. This was the first major publication of southeastern flora in the United States to recognize the genus *Hexastylis*. After Small's (1933) publication, the use of the name *Hexastylis* became widely accepted for the genus and was recognized in most North American publications separate from *Asarum*. In fact, *Hexastylis* has for the most part replaced *Asarum* in most North American publications, with very few exceptions.

In Southeast Asia, the treatment of *Hexastylis* as a separate genus is not accepted and is still not referred to except when used as a synonym or as a common name to denote

Hexastylis as a southeastern United States endemic. Schmidt (1937) recognized the same four sections within *Asarum* that were first erected by Duchartre (1864) and placed the four sections in two subgenera, *Asarum* subgenus *Heterotropa* and *Asarum* subgenus *Ceratasarum*. Schmidt (1937) also broadened the descriptions of the four sections of *Asarum* to allow for many species that had been described since Duchartre (1864) to fit within his taxonomic framework. Maekawa (1936) and Maekawa and Ono (1965) worked with Japanese flora and recognized two segregate genera for *Asarum*. Maekawa moved 45 species from *Asarum* to *Heterotropa* and described dozens of new species. Maekawa (1936) erected the genus *Asiasarum* and placed five newly named species and one variety into that genus. Included in one of those new genera was the newly described species *Asarum Japonasarum* Nakai (1936). Araki (1937, 1953) divided *Asarum* into two subgenera, *Asarum* section *Asarum*, and *Asarum* section *Asiasarum*. He further divided *Asarum* section *Asarum* into three subsections, *Euasarum, Calidasarum*, and *Japonasarum*. With *Asarum* section *Asiasarum* Araki erected the three subsections *Asiasarum*, *Hexastylis*, and *Heterotropa*.

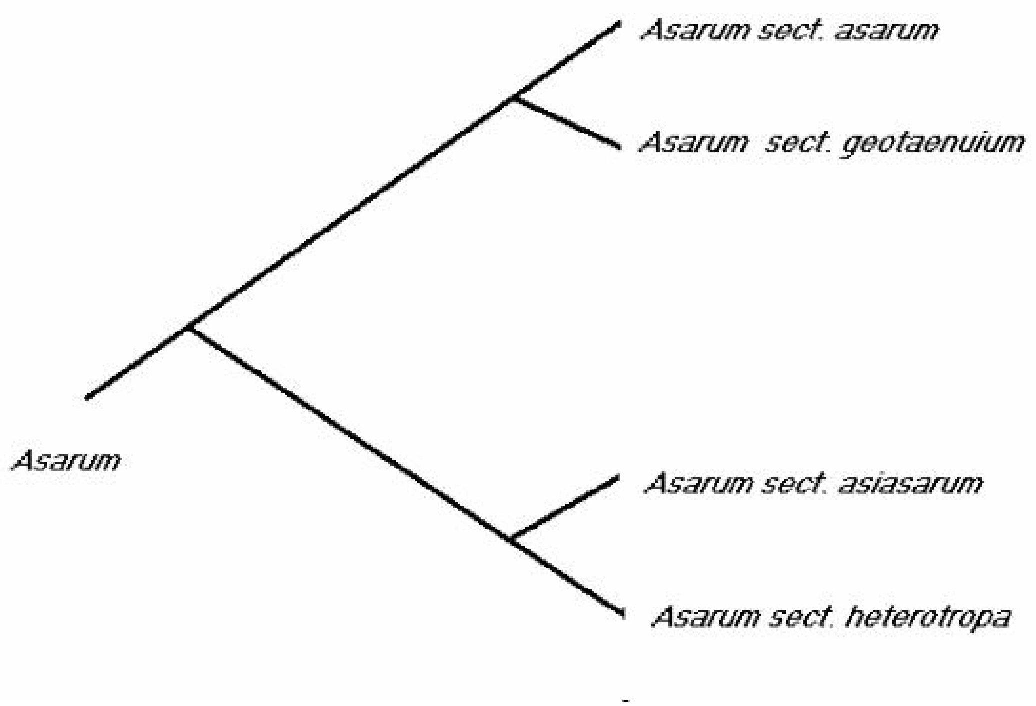
Blomquist (1957) made a complete revision of the genus *Hexastylis* in North America. Kelly (1998b) suggested that Blomquist (1957) might have overlooked the work of Araki (1953), who merged *Hexastylis* with the Japanese *Asarum*. In his treatment, Blomquist kept the genus name *Hexastylis*, divided the genus into three groups, and then recognized subgroups within these groups. Blomquist changed the specific epithet on the name *H. minus* to *H. minor*. He work described one new species, which he placed in the subgroup *Heterophylla*. *H. naniflora* was described from specimens found in three locations in

North Carolina and South Carolina. The description of *H. naniflora* by Blomquist (1957) is as follows:

> ***Hexastylis naniflora*** **sp. nov. Leaf-blades cordate to orbicular-ovate, 4-5.76 cm long by 4-5.5 cm wide, the apices obtuse, the sinuses broad to narrow, the lobes rarely overlapping, usually variegated along the principal veins. Petioles averaging 10.5 cm long. Rhizomes short and moderately branching. Calyces relatively small, brown, thr tube cylinderic, slightly narrowing upwards, 7 mm long by 6.5 mm wide in diameter, sometimes pale brown above the base, the lobes relatively large, flaring at the base, 7 mm wide at the base by 5.5 mm long, moderately spreading, without colorless spots inside. Stamens essentially sessile, those opposite the styles conspicuously shorter than the alternating ones. 1.61 mm-1.84 mm long, the anther-connective not prolonged into three appendage. Ovary ca. ½ inferior. Styles ca. 2.5 mm long, extending 0.75mm above the stigma, only notched at apex. Mature seeds not seen.**

Recent work by Kelly (1997, 1998a, 1998b, 2001, and 2002) using morphology and molecular data support the previous Asian studies, has shown that *Hexastylis* should be recognized within *Asarum* under the section *Heterotropa* (see Figure 1). Kelly (1997) conducted molecular analysis on the Internal Transcriber Space (ITS) region of a number of *Asarum* species from around the world as well as the southeastern species *A. canadense* and three species of *Hexastylis*. His work demonstrated that *Hexastylis* is rooted within *Asarum* and should be treated as *Asarum*. However, due to the localized stigma associated with the use of the genus name *Asarum* for *Hexastylis*, the species in this paper will be referred to as *Hexastylis* with the understanding that *Hexastylis* is rooted in *Asarum* and the proper treatment of the species complex is within *Asarum*. Kelly (2002) advocated the use of *Asarum* and supported the monophyletic arrangement based on morphological and molecular data. His work supports the broad treatment of *Asarum* by Araki (1937, 1953) who recognized two subgenera, *Asarum* and *Heterotropa*.

Figure 1. Phylogeny showing *Hexastylis* nested within *Asarum* based on the molecular analysis preformed by Kelly (1997). *Hexastylis* is included in *Asarum* section *Heterotropa.*


Asarum sect. asarum
Asarum sect. geotaenuium
Asarum
Asarum sect. asiasarum
Asarum sect. heterotropa

The sections *Asarum* and *Geotaenium* are included within the subgenus *Asarum* and the sections *Asiasarum* and *Heterotropa* (which includes *Hexastylis*) are placed within the subgenus *Heterotropa*.

Hexastylis heterophylla Complex

By the late 1950's, *Hexastylis* was recognized as consisting of eight species that were endemic to the southeastern United States. Blomquist (1957) established the currently recognized grouping of *Hexastylis*, and it has become widely accepted, especially by botanist from the southeastern United States.

The genus *Hexastylis*, as recognized by Blomquist, consists of three groups: Arifolia, Speciosa, and Virginica. The group Arifolia has one species comprised of three varieties. They are *H. arifolia var. arifolia, H. arifolia var. ruthii*, and *H. arifolia var. callifolia*. The second group, Speciosa, consists of a single species, *H. speciosa* that is found only in central Alabama. The third group, the Virginica group, is divided into three subgroups. Blomquist named the subgroups of this group Virginica, Shuttleworthii, and Heterophylla. The Virginica subgroup recognized by Blomquist contained only *H. virginic*a. Morphological analysis (Gaddy 1987) showed that *H. rhombiformis* is a close relative of *H. virginica* within the Virginica subgroup. Gaddy (1987) allied *H. rhombiformis* with *H. virginica* and placed it into the Virginica subgroup. The Shuttleworthii subgroup, as recognized by Blomquist, had two species, *H. shuttleworthii* and *H. lewisii*. *Hexastylis shuttleworthii* has two recognized varieties, *H. shuttleworthii var. shuttleworthii* and *H. shuttleworthii var. harperii*. The Heterophylla subgroup, as recognized by Blomquist, contains *H. heterophylla, H. minor*, and *H. naniflora*. Gaddy

(1987) suggest that *H. contracta* is allied with the Heterophylla subgroup and he subsequently placed it into this subgroup. The *Hexastylis heterophylla* subgroup has been thought to form an overlapping complex of species (Blomquist 1957; Gaddy 1987). One of the main concerns regarding this complex was the inability to distinguish between species without access to fresh flowers. Even with fresh flowers Blomquist (1957) and Gaddy (1987, 2003) still recognized considerable overlap in flower morphology in the complex making species delineation difficult (see Table 1).

Through the 1980's, Gaddy examined the groups and subgroups of *Hexastylis* erected by Blomquist (1957) in closer detail. He retained the framework of groups and subgroups described by Blomquist (1957), and he added details to the dichotomous keys to aid in the distinction of species within the genus. Along with characteristics known to exist, Gaddy also looked at biogeography and soil types in an effort to resolve species level questions, and verify the validity of species in the *H. heterophylla* complex.

Species Conservation and Conservation Recommendations

The rarity of *H. naniflora* has prompted the protection of a number of sites. The Spartanburg County Water Works in South Carolina was among the first organizations to see the significance of protecting *H. naniflora*. They placed over 1,000 plants into protection in the late 1980's. Camp Mary Elizabeth, also in Spartanburg County, is another site where a considerable number of *H. naniflora* individuals have been protected. In the early 1990's, through the efforts of Dr. Gil Newberry with the University of South Carolina at Spartanburg, *H. naniflora* was found to exist at the Cowpens National Battlefield in Cherokee County, South Carolina, around Lake Blalock, and around Lake Saranac, in Spartanburg County, South Carolina. These sites

Table 1. *Hexastylis* groups and subgroups recognized by Blomquist (1957) and Gaddy (1987).

GROUPS	**SUBGROUPS**	**SPECIES**
ARIFOLIA		*Hexastylis arifolia* var. *arifolia* (Michx.) Small *Hexastylis arifolia* (Michx.) Small var. *callifolia* (Small) Blomquist *Hexastylis arifolia* (Michx.) Small var. *ruthii* (Ashe) Blomquist
SPECIOSA		*Hexastylis speciosa* Harper
VIRGINICA	VIRGINICA	*Hexastylis virginica* (L.) Small *Hexastylis rhombiformis* Gaddy
	SHUTTLEWORTHII	*Hexastylis shuttleworthii var. shuttleworthii* (B. & B.) Small *Hexastylis shuttleworthii var. harperii* (B. & B.) Gaddy *Hexastylis lewisii* (Fernald) Blomquist and Oosting
	HETEROPHYLLA	*Hexastylis heterophylla* (Ashe) Small *Hexastylis minor* (Ashe) Blomquist *Hexastylis naniflora* Blomquist *Hexastylis contracta* Blomquist

now allow for the protection of several thousand plants. The South Carolina Department of Natural Resources in South Carolina ultimately purchased and now manages a 161-acre tract of land in Spartanburg County that was developed into the Peters Creek Heritage Preserve. This preserve contains well over a thousand *H. naniflora* plants.

In North Carolina, the Henson's Ravine Natural Heritage Site in Rutherford County received protection as a natural area as early as the mid 1980's and contains around 1,500 plants. Also located in Rutherford County, the Sandy Mush Rock Outcrop supports around 300 *H. naniflora* plants, as well as other rare and unique plant species. It has been scheduled for development into a rock quarry, but local citizens are presently fighting to have it stopped. In Cleveland County North Carolina, the Broad River Greenway Council and the North Carolina Department of Transportation (NCDOT) worked together for several years and obtained around 1500 acres along the Broad River, which houses over 5000 *H. naniflora* plants.

Research Questions

My study attempts to resolve the taxonomic confusion in the *Hexastylis heterophylla* complex, comprised of *H. heterophylla*, *H. minor* and *H. naniflora*. One rationale for the study is to gain understanding of the relationship between *H. naniflora* and the other two species in the complex. *Hexastylis naniflora* (dwarf flowered heartleaf) is a federally listed species and is recognized by the USFWS, the North Carolina Natural Heritage Program as being both a state and federally threatened species (USFWS 1990; Amoroso 2002). *Hexastylis naniflora* is known from eight counties in North Carolina and three counties in South Carolina. *Hexastylis naniflora* appears to be restricted to Pacolet sandy loam, Pacolet-Bethlehem sandy-loam, Madison gravelly sandy loam, and Musella fine sandy-loam soils (Gaddy 1981, 1987). These soils are restricted to an area near Charlotte, North Carolina west to the foot of the mountains near Rutherfordton, North Carolina, and from Hickory, North Carolina southward to just south of Spartanburg, South Carolina. This area is one of the fastest growing regions of North and South Carolina, and this plant has played a key role in several recent highway routing decisions in North Carolina. Given the rate of development within the region, it is likely that it will continue to impact highway construction projects. In order to assist the NCDOT in their efforts to protect *H. naniflora*, I conducted a study to 1) utilize morphological, micromorphological and ecological methods to examine the species boundaries of *H. heterophylla, H. minor,* and *H. naniflora* and to use this information to generate distribution maps for the three taxa, 2) conduct autecological analyses of the species using $10m^2$ plots and test soil samples to evaluate the ecology of known sites, 3) use the collected ecological and biosystematics information to search for new sites in Alexander, Iredell, Yadkin, and Gaston Counties, and 4) conduct ecological analyses at a transplant site in Caldwell County, North Carolina to determine possible active management techniques that could be used to improve the reproductive capability of the species following Newberry (unpublished, 1996) and Henderson (2001).

The objectives of this study were twofold. The first objective was to evaluate species boundaries in the *H. heterophylla* complex using morphological, micromorphological, and ecological analyses to determine if sufficant differences exist between the species in the complex that one could be used to delineate species in the group. My research focus was on the federally threatened *H. naniflora*, but data on *H. heterophylla* and *H. minor* were also gathered and used in comparison with *H. naniflora*. Secondly, we wanted to determine what conditions are optimal to maintain a population of *H. naniflora* and to test whether we can use this information to search for new populations and to relocate populations that are in danger of being eradicated by development projects in the region. The hypotheses that address the first objective of this research are:

> H_1. There is discontinuity in the variation among two, three or more groups of populations in the *H. heterophylla* complex, and two or more species can be recognized in this complex.
>
> H_1 null hypothesis. The variation in morphology, micromorphology, and ecology of the three putative species in the *H. heterophylla* complex is continuous, and no species can be delineated within the group

The hypotheses that address the second objective of this research are:

> H_2. Habitat requirements for *H. naniflora* are unique within the complex. This information can be used to locate new populations and select sites to successfully transplant populations of *H. naniflora*.
>
> H_2 null hypothesis. Habitat requirements for the establishment and maintenance of populations of *H. naniflora* are not unique to this species in the complex. There is no predictive value in the locating or transplanting of populations of *H. naniflora* based upon ecological data.

METHODS

Biogeography

Specimens were examined both from collections made in the field and from samples that were sent to Appalachian State University for identification. A total of 693 specimens of various species of *Hexastylis* were examined from 17 herbaria (Table 2) in order to retrieve habitat, locality, and phenology label data (Appendix A). I also obtained and examined type specimens from the *Hexastylis heterophylla* complex. Samples from Alabama, Georgia, eastern Kentucky, southwest Virginia, and North Carolina were examined. Locality data was compiled and used to create distribution maps for the *Hexastylis heterophylla* complex**.**

To develop a detailed map of the range of *H. naniflora*, I visited as many known populations as possible; obtained GPS data for all sites visited, and conducted field examinations at many of those populations (Appendix B). From early March to late June of 2001-2003 (three flowering/fruiting periods) I searched for new populations of *H. naniflora* throughout the eleven counties of known distribution of the species, as well as five bordering counties. I obtained location information for most of the known sites of *H. naniflora*, which had been documented through the North Carolina Natural Heritage Program and South Carolina Heritage Trust Program. I compiled all the coordinates for known sites as well as those sites that were located in this study.

Table 2. Herbaria that provided specimens for study.

Herbaria	**Location**	**Number of Specimens Examined**
BOON	Appalachian State University	77
CONV	Converse College	12
DUKE	Duke University	46
ETSU	East Tennessee State University	35
GH	Gray Herbarium	27
GWU	Gardner-Webb University	24
MO	Missouri Botanical Gardens	3
NY	New York Botanical Gardens	26
GA	University of Georgia	17
NCU	University of NC at Chapel Hill	144
US	Smithsonian Institute	5
USCH	University of SC at Columbia	106
USCS	University of SC at Spartanburg	44
TENN	University of TN at Knoxville	45
WIS	University of WI at Madison	6
VPI	Virginia Polytech Institute	60
WOFF	Wofford College	16
17		**693**

Biogeographical locality information was converted to decimal degrees on a NAD-87 topography map projection, and maps were generated from those results using Arc View (Ormsby et al 1998).

United States Department of Agriculture (USDA) soil maps were consulted for Cleveland, Henderson, Lincoln, McDowell, and Rutherford Counties, North Carolina and Cherokee, Greenville and Spartanburg Counties, South Carolina. These are counties where *H. naniflora* is known to exist and/or where we thought it might exist (USDA 1962, 1980, 1995a, 1995b, 2000a, 2000b and 2000c). Through the use of soil and stream drainage data I was able to began making predictions where *H. naniflora* localities might exist. To test those predictions, I conducted field surveys using the newly acquired data from stream drainage, distribution information, and soil maps. Field investigation sites chosen based upon soil and geographical data in the first growing season (2001) yielded nine new populations of *H. naniflora,* and also allowed me to develop strategies for use in field investigations conducted over the following two growing seasons.

After conducting field surveys for one growing season, obtaining soil data, acquiring a general understanding of the ecological requirements of the species, and collecting preliminary distribution maps from herbarium specimens and North Carolina and South Carolina Heritage database information, I gained important information that allowed me to conduct more focused field investigations the following two growing seasons. I was able to make more accurate assessments of ideal habitat and localities where new populations might exist. Field examinations in the second and third growing seasons were more efficient, allowing more time for detailed examination at those sites where the species were more likely to exist.

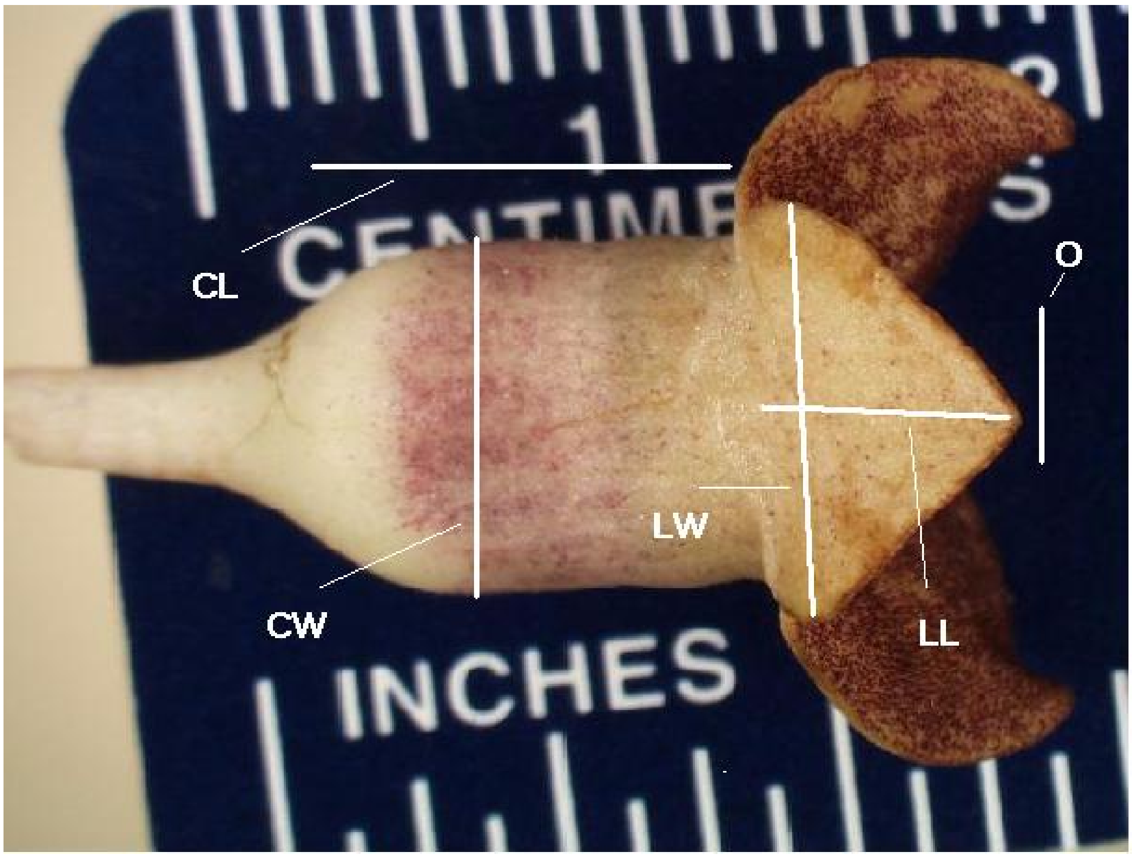
Flower Morphometrics

Federal, state and local (Federal and State Parks, County Parks, and Natural Areas) permits were obtained to collect plant materials from localities across the range of *H. naniflora*. Flowers were collected from at least three individuals from 85 different *Hexastylis* populations in North Carolina, South Carolina, and Virginia (Appendix B). The collected flowers were placed into freezer bags with collection data placed inside the bag with the flowers as well as marked on the outside of each bag. The flowers were placed on ice until they could be stored in a refrigerator at Appalachian State University (ASU). The flowers were later removed from the refrigerator and five floral measurements were recorded from each flower. The measurements included calyx length (CL), calyx width (CW), calyx lobe length (LL), calyx lobe width (LW), and calyx opening (CO) (Figure 2). Measurements were recorded for 274 flowers from the *H. heterophylla* complex. All measurement data were compiled and later subjected to statistical analyses using Statistical Analysis Systems (SAS) (Delwiche and Slaughter 2000).

Materials collected for the morphological and micromorphological analyses consisted of flower materials collected from all three species in the *H. heterophylla* complex. After being measured, they were either placed in a –80^{o} C freezer for future use or placed in a herbarium dryer at 29^{o} C (84.2^{o} F) for pollen analysis.

Inner calyx reticulations have been examined in several studies and determined to be useful distinguishing characters to delineate some of the species of *Hexastylis*. These reticulations had been thought to be of taxonomic value in the *H. heterophylla* complex (Blomquist 1957; Gaddy 1987). Most *Hexastylis* species possess a series of ridges and

Figure 2. Flower measurement taken and used for morphological analysis in the *Hexastylis heterophylla* complex. Measurements taken were Calyx length (CL), Calyx Width (CW), Calyx Lobe Length (LL), Calyx Lobe Width, (LW), and Calyx Opening (CO).


CL
CW
LW
LL
O
CENTIMETERS
INCHES
1
2

reticulations in the lower portions of the inner calyx tube of the flowers (*H. arifolia* does not possess them). Flowers taken from *H. heterophylla*, *H. minor*, and *H. naniflora* were examined using an Olympic SZX12 dissection scope and a DF PLFL 0.5X PF lens. Photographs were taken using an Olympic DP10 digital camera mounted on this dissecting scope. The inner calyx regions were photographed to compare the ridges and reticulations among the three species.

Pollen Micromorphology

Pollen was obtained from fresh flower material, as well as from dried specimens. One to three flowers were collected from 37 individuals and placed into separate plastic collection bags to avoid contamination from other flowers. The flowers were kept on ice while in the field and then transferred to refrigeration until they could be dried. The bags were placed into a plant dryer at 29^{o} C (84.2^{o} F) for three to five days to dry. After the flowers were dried, they were placed separately into paper envelopes with collection data recorded on the outside of each envelope. Pollen was also obtained from four herbarium specimens by removing one anther from a flower. All specimens from whom pollen was obtained were deposited at the ASU herbarium (BOON) except for one specimen of *H. lewisii* that was borrowed from North Carolina State University (NCSU) herbarium. Permission was obtained to remove an anther from the NCSU specimen.

The pollen was extracted and placed onto a specially designed aluminum stub made for Scanning Electron Microscope use. Each stub was prepared by adding two-sided carbon tape to the top surface of the stub. The pollen was extracted by one of two methods.

One was by the use of a makeshift miniature brush constructed from a toothpick, with the bristles of a paintbrush attached to the end with scotch tape.

The other method of pollen extraction was to remove an anther and spread pollen from the anther over the stub and carbon tape. In two instances, the entire anther was attached to the carbon tape for examination under the SEM.

When using herbarium specimens, one anther was extracted and the pollen dusted over the carbon tape. Each stub was labeled separately by using a probe and etching an identification number into the carbon tape. They aluminum stubs were mounted on a turntable located in the vacuum chamber of a FEI Philips Quanta 200 low/high vacuum SEM. Six stubs (six different individuals) were loaded at one time for examination in the SEM.

A digital camera mounted inside the vacuum chamber of the SEM was used to acquire images of the pollen. Digital photos were taken of 47 individuals that included three genera and 13 species. I examined the specimens at low vacuum mode. By using the low vacuum mode, the specimens didn’t require special preparation or sputter coating before they could be examined. I used a wide range of magnifications in order to obtain a variety of images. Magnifications ranged from 1000X to 5000X. Images captured between 2400X or 3000X were used to compare surface features as well as make size comparisons between pollen of each species examined. The digital photos were collected on a computer hard drive linked to the camera. The images were later transferred to a CD-ROM for analysis and examination.

Vegetation Survey

I examined thirteen population sites in North Carolina and South Carolina using the North Carolina Vegetation Survey (NCVS) (Peet et al 1998) to compare species richness between the three species of the *H. heterophylla* complex (Table 3). The NCVS is a flexible and inexpensive method of examining habitat species richness and percent cover of species. Three populations of *H. heterophylla,* three populations of *H. minor* and seven populations of *H. naniflora* were examined from across the range of the complex. These populations were examined to compare species richness within the *H. heterophylla* complex in order to look for marked differences among the habitats of the three species. Permanent markers were placed in eleven of the thirteen plots. Location data was recorded from the centerline of the 50 x 20 meter plot using GPS. In the two plots surveyed within the Pisgah National Forest in Madison County, North Carolina no permanent markers were installed but GPS plot locations were obtained. The 50 x 20 meter plots were erected within the *Hexastylis* populations using five 50-meter measuring tapes. The corner of the plot was marked with a flag, as was the centerline of the plot. Eleven of the plots consisted of ten 10 x 10 meter plots in two rows of five plots each. Within those ten smaller plots there were four intense sample plots (i.e. I) that were used for data collection. Two plots consisted of five 10 x 10 meter plots in a single row. These two plots had two I plots (I) for data collection. The reason for the two smaller plots was area constraints where the population was too small to fit a 50 x 20 meter plot. Thus, a 50 x 10 meter plot was used instead. This methodological alteration is in line with the parameters set forth by Peet et al. (1998) to deal with smaller areas of interest.

Table 3. Locations where North Carolina Vegetational Survey (NCVS) analyses were conducted. Species are denoted as *H. heterophylla* (HH), *H. minor* (HM) and *H. naniflora* (HN).

Species	**Location**	**Coordinates (D/M/S/)**
HH	Appalachian Trail, Hot Springs Madison County, NC	35^{o} 54’ 01” N 82^{o} 47’ 40” W
HH	Hickey’s Fork, Shelton Laurel Madison County, NC	35^{o} 59’ 31” N 82^{o} 42’ 10” W
HH	Bunker Hill Bridge, Claremont Catawba County, NC	35^{o} 43’ 12” N 81^{o} 06’ 57” W
HM	Broad River Greenway Cleveland County, NC	35^{o} 12” 01” N 81^{o} 39’ 24” W
HM	Crowder’s Mountain State Park Gaston County, NC	35^{o} 13’ 00” N 81^{o} 17’ 29” W
HM	Kings Mountain State Park York County, SC	35^{o} 09’ 01” N 81^{o} 20’ 26” W
HN	Henry Miller Bridge Road Alexander County, NC	35^{o} 31’ 34” N 81^{o} 03’ 22” W
HN	Little Gunpowder Creek Caldwell County, NC	35^{o} 45’ 09” N 81^{o} 26’ 21” W
HN	Kudzu Farm, Harris Rutherford County, NC	35^{o} 14’ 04” N 81^{o} 53’ 54” W
HN	Dan Rivers Property, Harris Rutherford County, NC	35^{o} 13’ 02” N 81^{o} 52’ 48” W
HN	Rhyne Preserve, Labatory Lincoln County, NC	36^{o} 26’ 09” N 81^{o} 14’ 55” W
HN	Cowpens National Battlefield Cherokee County, SC	35^{o} 07’ 37” N 81^{o} 48’ 34” W
HN	Peters Creek Preserve Spartanburg County, SC	34^{o} 59’ 52” N 81^{o} 52’ 00” W

In specified corners of the (I) plots, a series of nested plots, following Peet et al. (1998), were measured. Vegetation data was collected from the nested plots. These plots were 0.10 meter, 0.32 meter, 1.0 meter, 3.16 meters, and 10.0 meters square. Species in these nested plots were assigned values from 5 in the smallest nested plot of 0.10 meter to 1 in the largest nested plot of 10.0 meters respectively. These values were assigned to a species when it was first observed in the series of nested plots (Figure 3).

Percent cover data were collected and assigned numbers from 0-9 with 0 being the smallest cover class representing the smallest percent coverage and 9 representing the largest percent cover. The data were recorded on a standardized data sheet. Species found outside the intense plots were recorded as residuals and entered into the datasheet. After the data were collected from each of the thirteen plots from *H. heterophylla*, *H. minor* and *H. naniflora* localities it was converted from field forms to electronic data to be used for statistical analyses. The data were analyzed in a series of SAS statistical programs. The resulting data for species richness were then used in another series of SAS statistical programs.

Species richness metrics were used to obtain Sorenson Index values, which were in turn used to generate a dendogram that showed the differences in the plots by species numbers. The following calculations were used to calculate Sorenson's Index of Community Similarity and Coefficient of community. The following calculations follow statistics from Communities and Ecosystems, second edition (Whittaker, 1975).

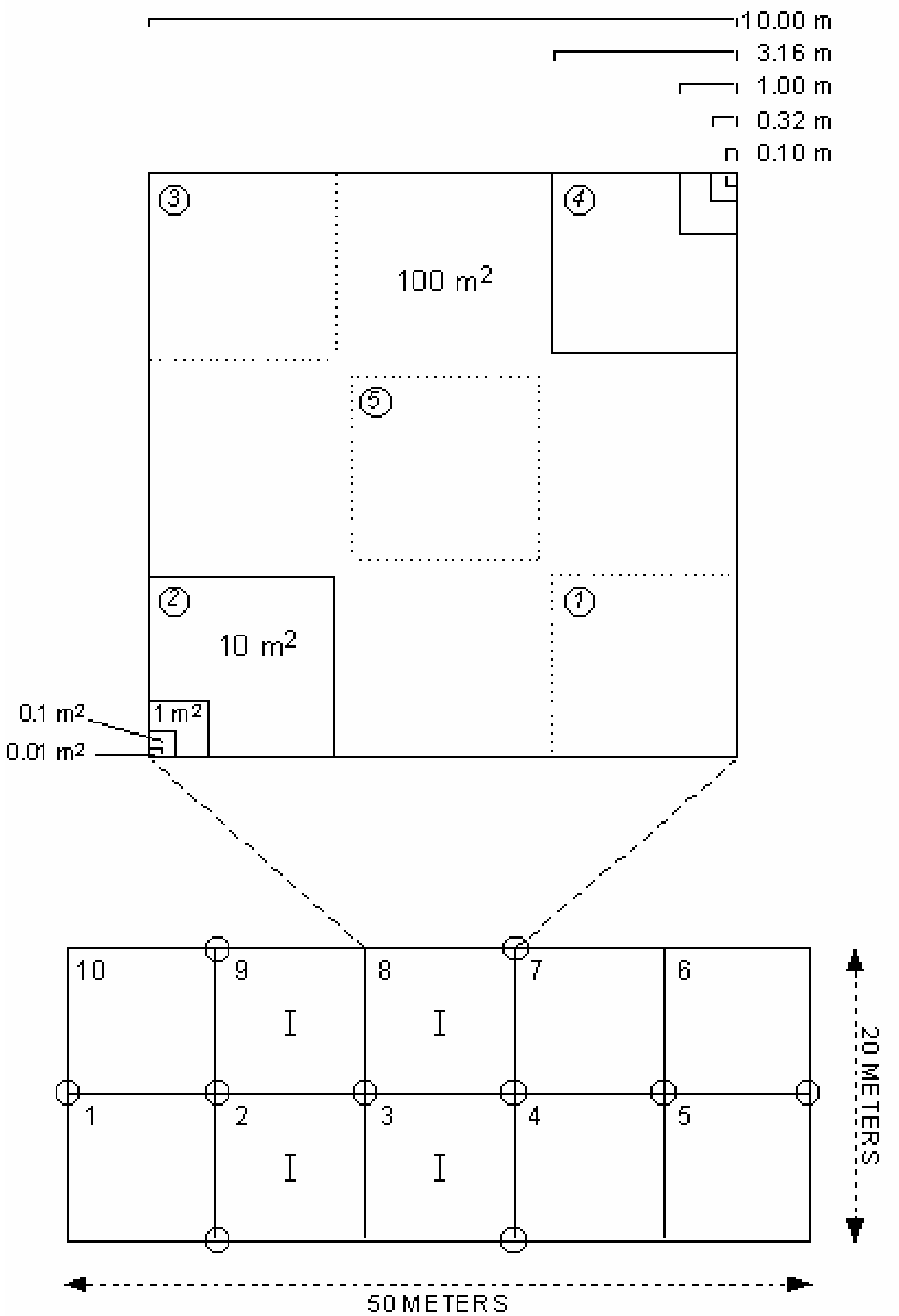
$C_S = 2j / A+B$

- C_S = Community Similarity
- 2j = Species Common To Both
- A = Species In Community A
- B = Species In Community B

$C_n = 2N_j / N_A + N_B$

- C_n= Coefficient of Community
- $2N_j$= Number Species Common To Both
- N_A= Number Species In Community A
- N_B= Number Species In Community

Figure 3. Diagram of the North Carolina Vegatational Survey (NCVS) plot configuration.


10.00 m
3.16 m
1.00 m
0.32 m
0.10 m
③
④
100 m²
⑤
②
10 m²
①
0.1 m²
1 m²
0.01 m²
10
9
8
7
6
I
I
1
2
3
4
5
I
I
20 METERS
50 METERS

Soil Analysis

Soil samples were collected from 34 *Hexastylis heterophylla* complex sites located in North Carolina, South Carolina, and Virginia, between August 2001 and February 2003 (Table 4). Samples were taken from a mid-point within a population using a standard 1” soil augur. Each sample was collected to a depth of 12” and placed into either a plastic 1” soil tube and sealed with a cap on each end, or into a new plastic storage bag, which was then sealed and tagged.

Soil samples were dried slowly at approximately 29^{o} C (84.2^{o} F). Samples were sealed and sent to the Clemson Soil Lab for analysis. Standard soil tests were performed on the collected samples. Soils were tested for pH, Buffer pH, Phosphorous (P), Potassium (K), Magnesium (Mg), Zinc (Zn), Manganese (Mn), Copper (Cu), Boron (B), Sodium (Na), Cation Exchange Capacity (CEC), Acidity, Base Saturation for Magnesium (BSMg), Base Saturation for Potassium (BSK), Base Saturation for Sodium (BSNa), Total Base Saturation (TBS). Two statistical tests were conducted to examine the soil data. These test were a General Linear Model (GLM) and Tukey’s test (SAS).

Transplant analysis

In November 2000, individuals from ASU, NCDA, and NC-DOT met at a NC-DOT bridge construction site on Cedar Valley Road (SR1108) off of US Hwy 321 near Saw Mills, in Caldwell County, North Carolina. This site would become a transplant area of *H. naniflora* individuals at a bridge construction. The plants were dug up and moved onto an adjacent conservation easement established by NC-DOT. The construction site included over two hundred *H. naniflora* plants that would have been destroyed during bridge construction if they had not been moved.

Table 4. Location of soil samples collected for analysis. *H. heterophylla* (HH) = 12, *H. minor* (HM) =7, and *H. naniflora* (HN) = 15.

COUNTY	**STATE**	**SPECIES**	**LOCATION**
CALDWELL	NC	HH	HWY 60/90 JUST ACROSS COUNTY LINE
CATAWBA	NC	HH	BUNKERHILL BRIDGE OFF US 70
IREDELL	NC	HH	HUNTER BRIDGE ROAD AT YADKIN RIVER
MADISON	NC	HH	HICKEY'S FORK ROAD IN NATIONAL FOREST
MADISON	NC	HH	OFF US 25 IN HOT SPRINGS ALONG AT
RUTHERFORD	NC	HH	LUCKADOO MT ROAD SITE 1
RUTHERFORD	NC	HH	LUCKADOO MT ROAD SITE 2
RUTHERFORD	NC	HH	PLEASANT MT CHURCH ROAD IN GOLDEN VALLEY
RUTHERFORD	NC	HH	CAMP McCALL ROAD SITE 1 OFF US HWY 226
RUTHERFORD	NC	HH	CAMP McCALL ROAD SITE 2 OFF US HWY 226
RUTHERFORD	NC	HH	OLD CC ROAD IN GOLDEN VALLEY
BUCHANNAN	VA	HH	OFF ROAD 628 ALONG CREEK
CLEVELAND	NC	HM	BROAD RIVER GREENWAY
CLEVELAND	NC	HM	BROAD RIVER GREENWAY SOUTH SIDE OF RIVER
GASTON	NC	HM	CROWDERS MOUNTAIN STATE PARK
MOORE	NC	HM	OFF US HWY 1 IN SOUTHER PART OF COUNTY
RANDOLPH	NC	HM	UHARRIE RIVER NEAR UWHARRIE GAME LANDS
RICHMOND	NC	HM	HWY 22 ALONG RIVER BANK
YORK	SC	HM	KINGS MOUNTIAN STATE PARK
ALEXANDER	NC	HN	OFF HWY 64 ON HENRY MILLER BRIDGE RD
BURKE	NC	HN	WILL HUDSON ROAD SR 1090 AT CREEK
CALDWELL	NC	HN	LITTLE GUNPOWDER CREEK OF HWY 321
CLEVELAND	NC	HN	HOUSLER PROPERTY, SANDY RUN CREEK
RUTHERFORD	NC	HN	KUDZU FARM SITE
RUTHERFORD	NC	HN	DAN RIVER PROPERTY NEAR POND HARRIS NC
RUTHERFORD	NC	HN	HENSON RAVINE OFF SR1106
RUTHERFORD	NC	HN	JEBB LAMB ROAD
RUTHERFORD	NC	HN	HENSON ROAD OFF HWY 221 AT FLOYDS CREEK
RUTHERFORD	NC	HN	HENSON RAVINE NORTH SIDE OF RIVER SR 1106
RUTHERFORD	NC	HN	DAN RIVER PROPERTY ACROSS CREEK
RUTHERFORD	NC	HN	DILLS CREEK TRIBUTARY
CHEROKEE	SC	HN	COWPENS NATIONAL BATTLEFIELD
SPARTANBURG	SC	HN	PETERS CREEK PRESERVE

The method in the transplanting of *H. naniflora* individuals was one developed by Dr. Gill Newberry (1996). The plants were dug up and placed into plastic one-gallon freezer bags. The freezer bags were used to avoid contamination from plant pathogens and to make sure that any beneficial bacterial components in the soil that might be associated with *H. naniflora* were transplanted along with the plants. After the plants were dug up and placed into the freezer bags, they were carried onto the easement site and placed into clusters for transplanting. The plants were then placed into newly dug holes and filled in around. Each transplanted individual was marked with a flag for future reference. Once the freezer bag had been used once, it was discarded to avoid contamination. Over the next three-growing/flowering seasons, the site was revisited and data were collected and recorded on those individuals that had been transplanted to determine the survival rate for transplanted individuals.

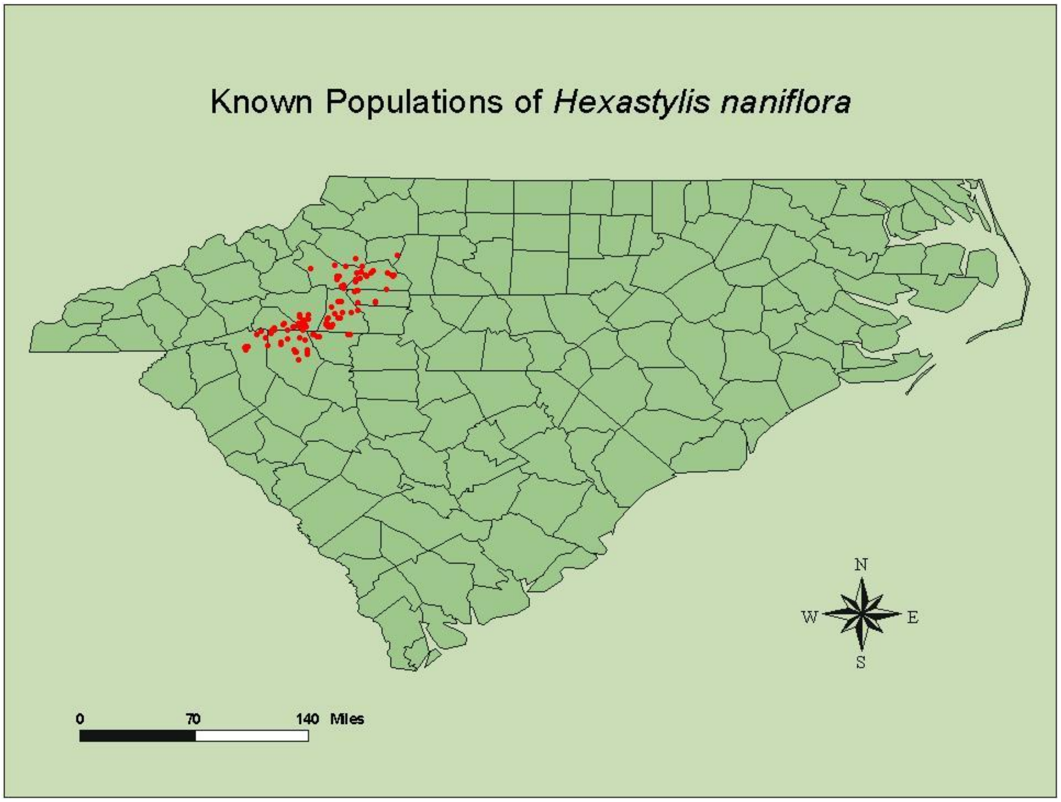
RESULTS

Biogeography

I examined and annotated a total of 693 specimens from seventeen herbaria. Ashe (1897) had reported that *Asarum minus* was located in Tennessee, however, after examining herbarium records of 17 herbaria and The Floral Atlas of Tennessee, no specimens of *A. minus* (*H. minor*) were found to be from Tennessee (Chester et al. 1997; Harvill et al. 1981). I obtained information on documented *H. naniflora* populations from Element of Occurrence (EOC) field sheets in North Carolina and South Carolina, as well as from the North Carolina Natural Heritage Program and the South Carolina Heritage Trust. I added 31 new *H. naniflora* populations located over the three growing seasons, and this information was submitted for entry into the appropriate North and South Carolina databases. I was able to obtain GPS points for 123 known *H. naniflora* populations. Some GPS points are approximations due to the lack of data available. In those instances, coordinates were drawn as close as possible to the site based on directions to site and site descriptions. Some GPS points are recorded as two or more populations; therefore, the map generated from these data of the known distribution of *H. naniflora* represents a total of 143 populations (Figure 4).

Maps were generated for the distributions of all three species in the *H. heterophylla* complex. Counties where considerable overlap occurs are denoted with color dots, which correspond to the species present in that county (Figure 5). Some information used to

Figure 4. Distribution maps showing the approximate locality of known or reported *H. naniflora* sites in North and South Carolina. All points were derived from GPS data.


Known Populations of Hexastylis naniflora
N
W
E
S
0
70
140 Miles

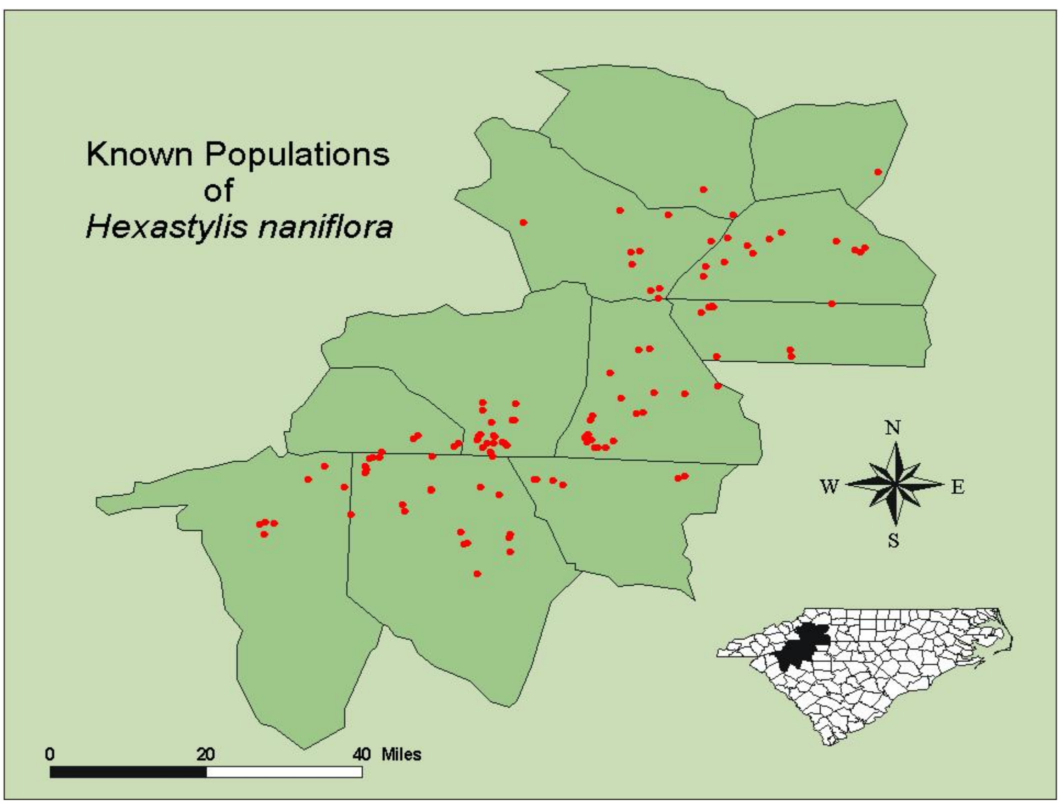
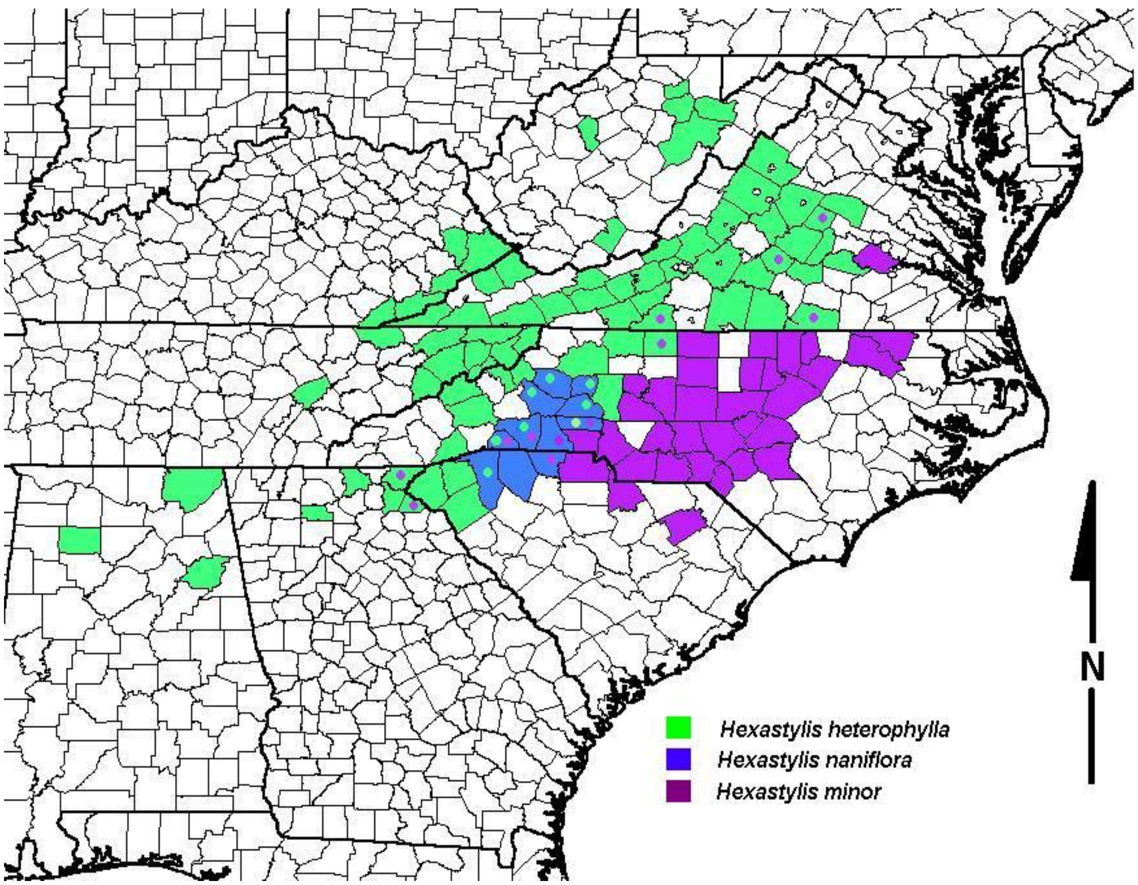

Known Populations
of
Hexastylis naniflora
N
W
E
S
0
20
40 Miles

Figure 5**.** Distribution map showing county records for the three species in the *H. heterophylla* complex. Data was gathered from herbarium specimens, Element Of Occurrence (EOC) sheets and field studies.


Hexastylis heterophylla
Hexastylis naniflora
Hexastylis minor
N

generate the distribution maps came from field collections. Voucher specimens were deposited in the Appalachian State University herbarium (BOON) in Boone, North Carolina and Gardner Webb University herbarium (GWU) in Boiling Springs, North Carolina.

Over three flowering seasons, I located 31 new *H. naniflora* populations (Figure 6). A map, generated using ArcView, shows the localities of new *H. naniflora* populations located over two growing seasons from the spring of 2001 to the summer of 2002. New populations found in 2003 (nine populations total) are not shown in that map. I located one population of *H. naniflora* in the Yadkin River drainage. Previously, *H. naniflora* was only known to exist from the Broad-Pacolet River and Catawba River drainages. After this initial discovery, we conducted numerous field surveys in the Yadkin River drainage in Iredell, Gaston, and Yadkin counties, but no other populations of *H. naniflora* were located. A map was generated showing populations of *H. naniflora* within the three river drainages (Figure 7).

Flower morphometrics

After the examination of 50 inner calyx reticulations and ridges from the three species within the *H. heterophylla* complex, it was determined that too many similarities existed between the three species of the *H. heterophylla* complex to accurately make species identification using these characters.

From the series of flower measurements taken on *H. heterophylla* complex specimens, a Principle Components Analysis (PCA) was conducted using SAS. The results showed that flower morphology can be used statistically to separate *H. naniflora* and the other two species in the complex based on the series of standardized

Figure 6. New populations of *H. naniflora* located during the field seasons of 2001-2002. Thirty-one new populations and sub-populations were located between 2001-2003. Data for the 2003 growing season are not represented in this map.

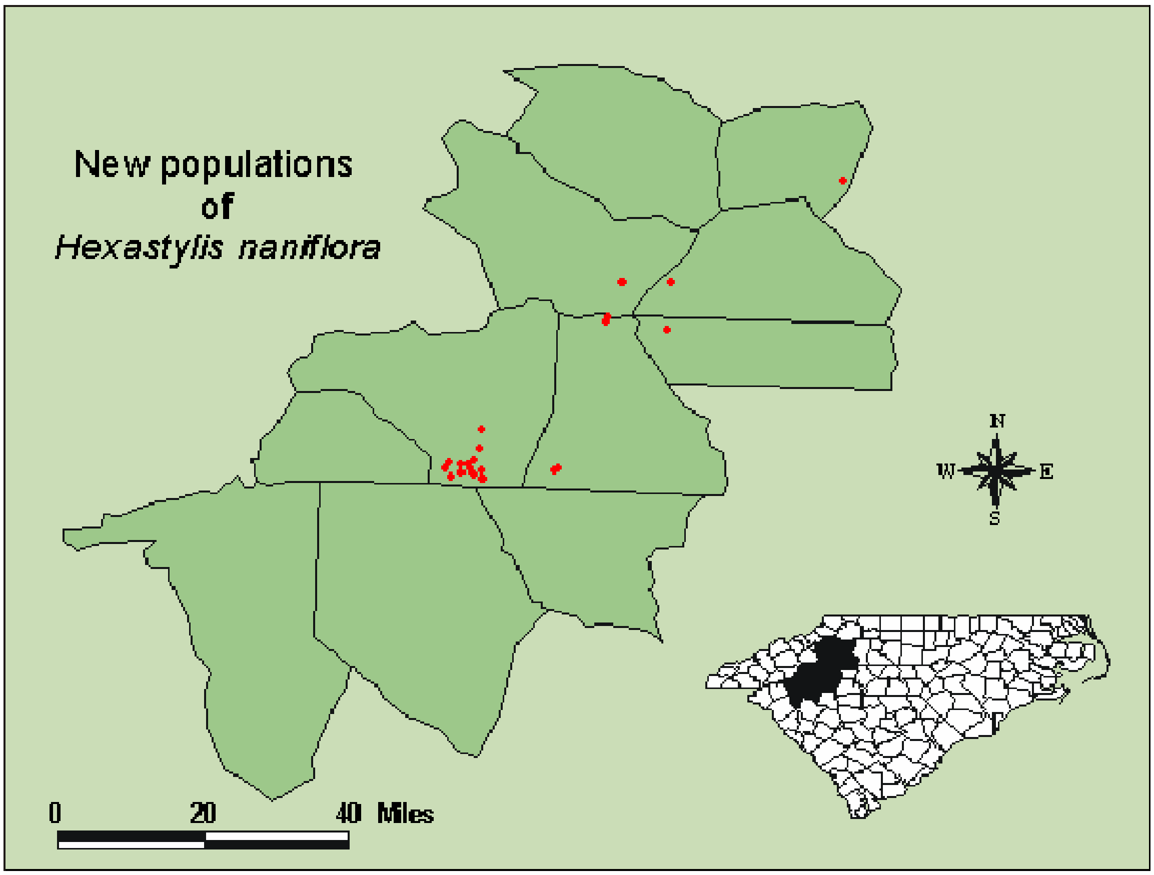
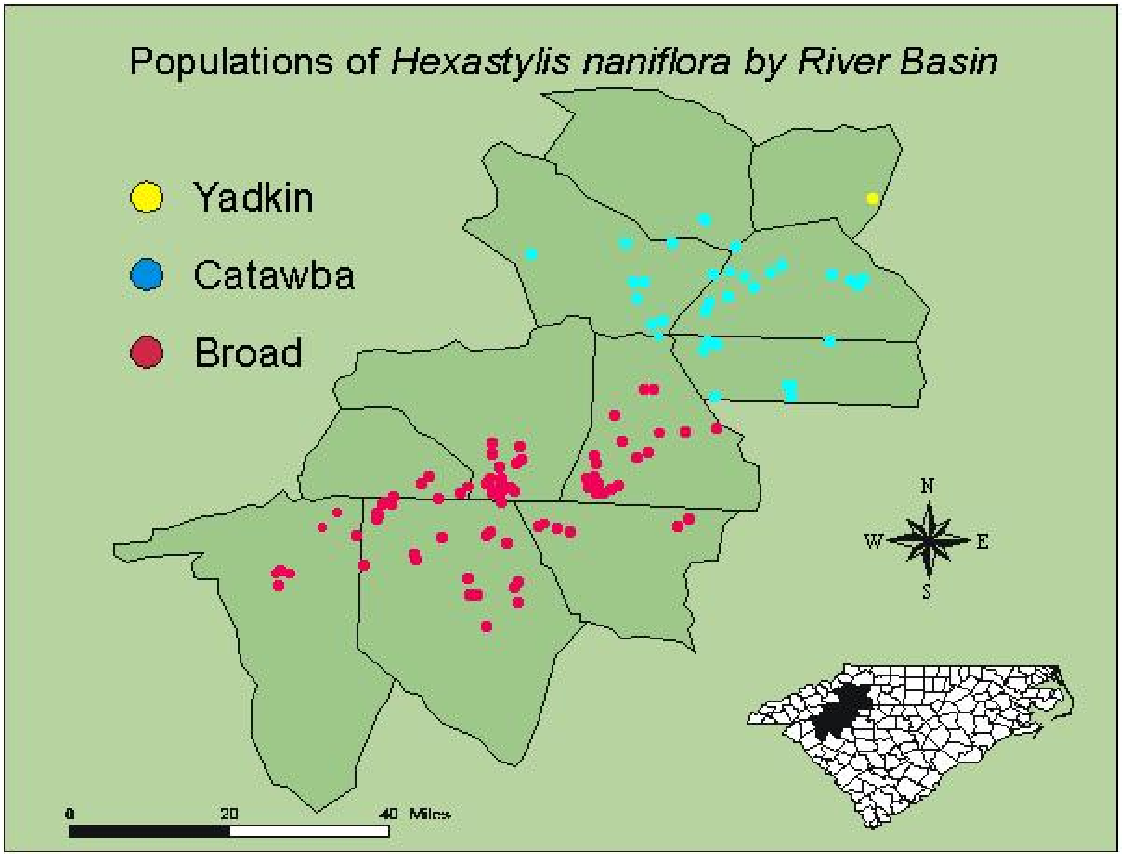

New populations
of
Hexastylis naniflora
N
W
E
S
0
20
40 Miles

Figure 7. Distribution map showing known sites of *H. naniflora* by river drainages.


Populations of *Hexastylis naniflora* by River Basin
Yadkin
Catawba
Broad
N
W
E
S
0
20
40 Miles

measurements taken of flower calyces. *Hexastylis naniflora* individuals form a more compact group in the PCA analysis than either *H. heterophylla* or *H. minor*. The results also show that separating *H. heterophylla* from *H. minor* is not possible due to the significant overlap that occurs in flower size measurement of the two species (Figures. 8-11).

Pollen micromorphology

Results from digital images taken of pollen from the *H. heterophylla* complex using SEM showed differences in the surface features of pollen from the three species. *Hexastylis heterophylla* has an exine (outer pollen surface) that contains both baculate and gemmate positive sculptural elements (positive elements extends outward from the exine) in high density, with no flat or smooth surface exine showing (Figures 12 and 13). *Hexastylis minor* has an exine of scattered gemmate sculptural elements. The remaining surface area visible on the exine appears smooth (Figures 12 and 14). *Hexastylis naniflora* has an exine that contains no positive surface elements and is rugulate across the surface (Figures 12 and 15).

Vegetation Survey

Species richness data collected using the NCVS were analyzed using a series of SAS statistical tests designed specifically for use with NCVS (Peet et al, 1998). The Means at each Depth (DMS) were computed from the nested plot 5 to nested plot 1 (see Figure 3 for nested plots) (Table 5). The DMS at each nested plot was subjected to a GLM analysis for all plots surveyed using the NCVS. The results showed no significant difference between species (Table 6). The total number of associated species for each

Figure 8. Principle Components Analysis (PCA) results comparing flower morphology measurements of *H. minor* (Circle), and *H. naniflora* (Cross).

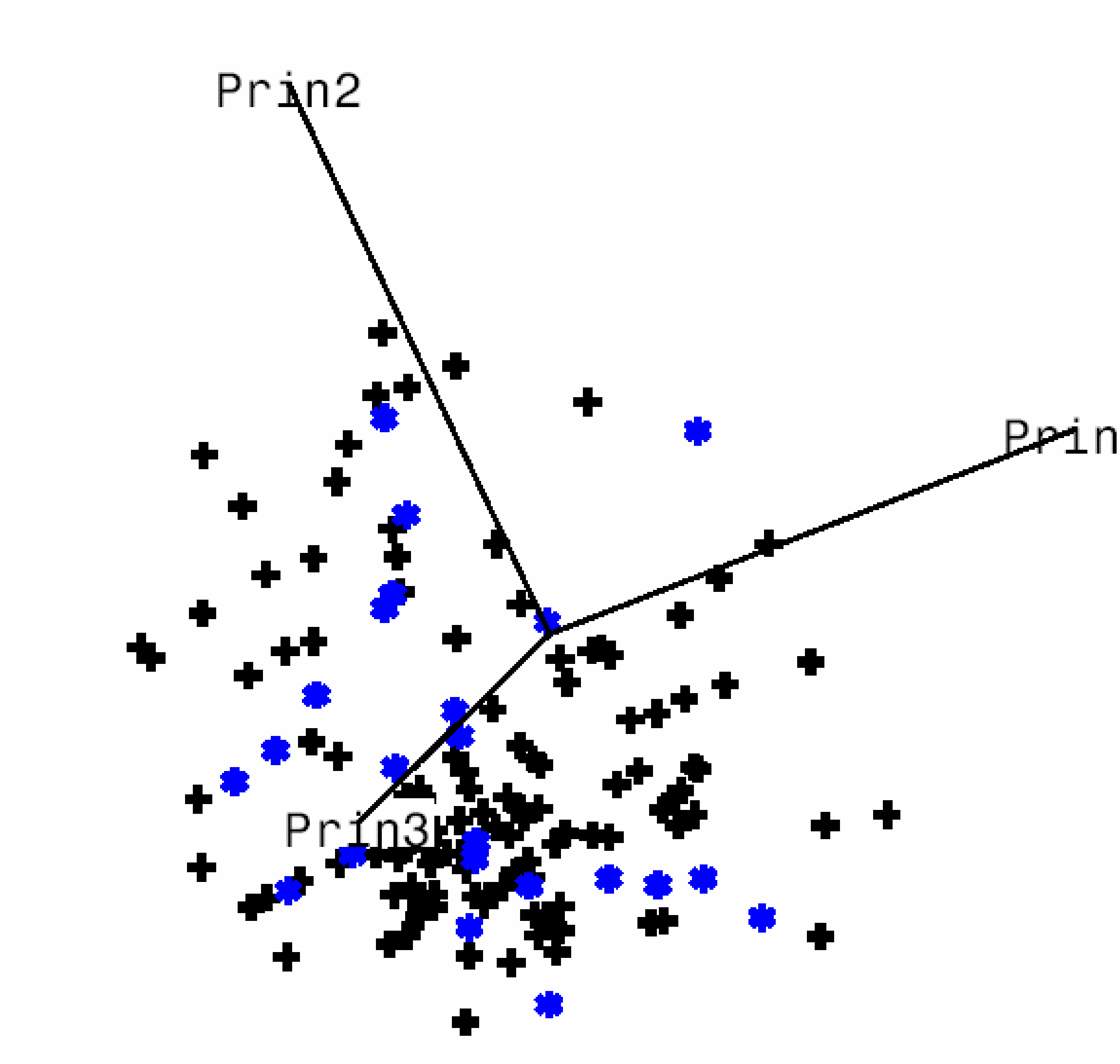

Prin2
Prin
Prin3

Figure 9. Principle Components Analysis (PCA) results comparing flower morphology measurements of *H. heterophylla* (Square), and *H. naniflora* (Cross).

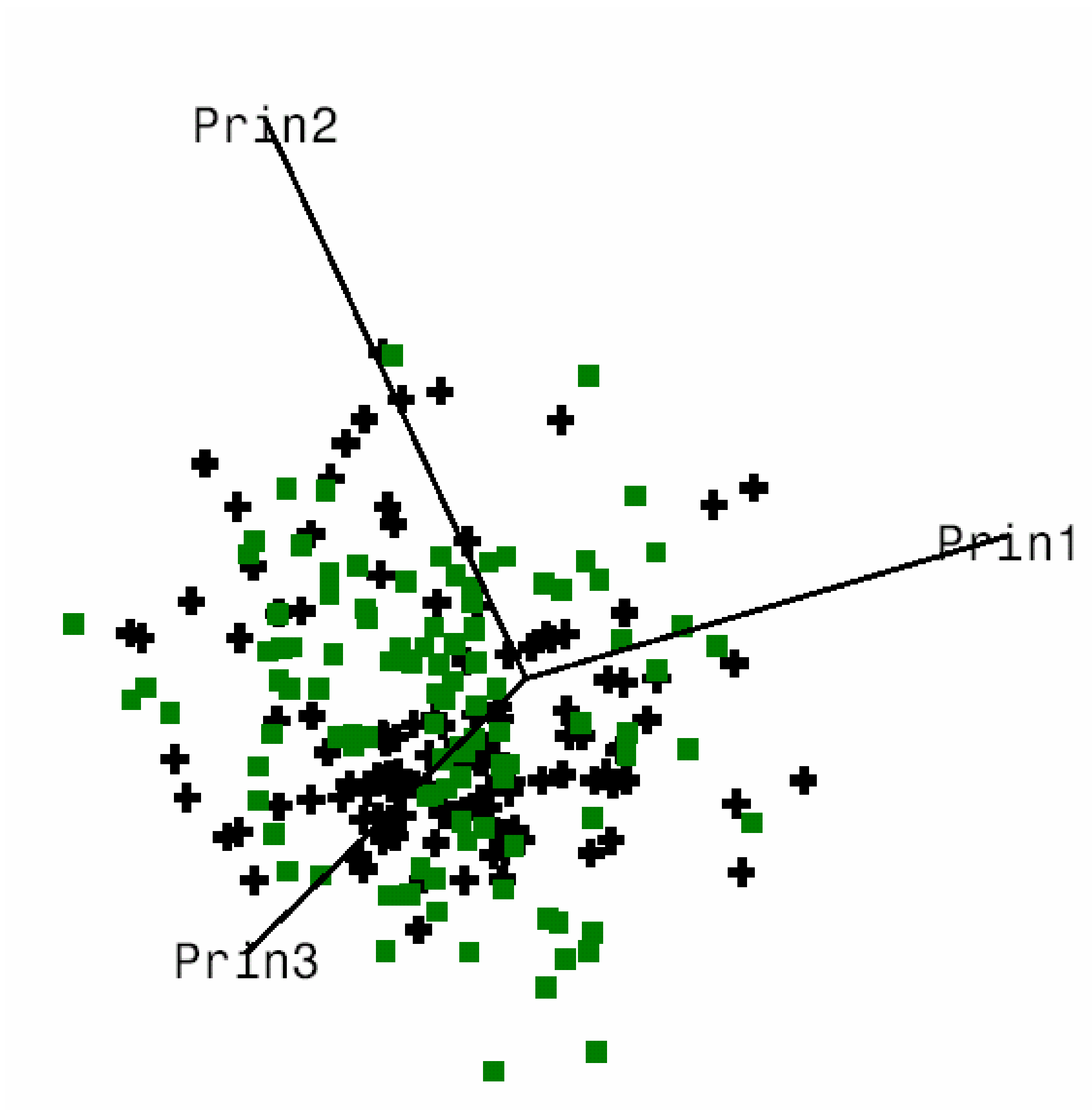

Prin2
Prin1
Prin3

Figure 10. Principle Components Analysis (PCA) results comparing flower morphology measurements of *H. heterophylla* (Square) *and H. minor* (Circle).

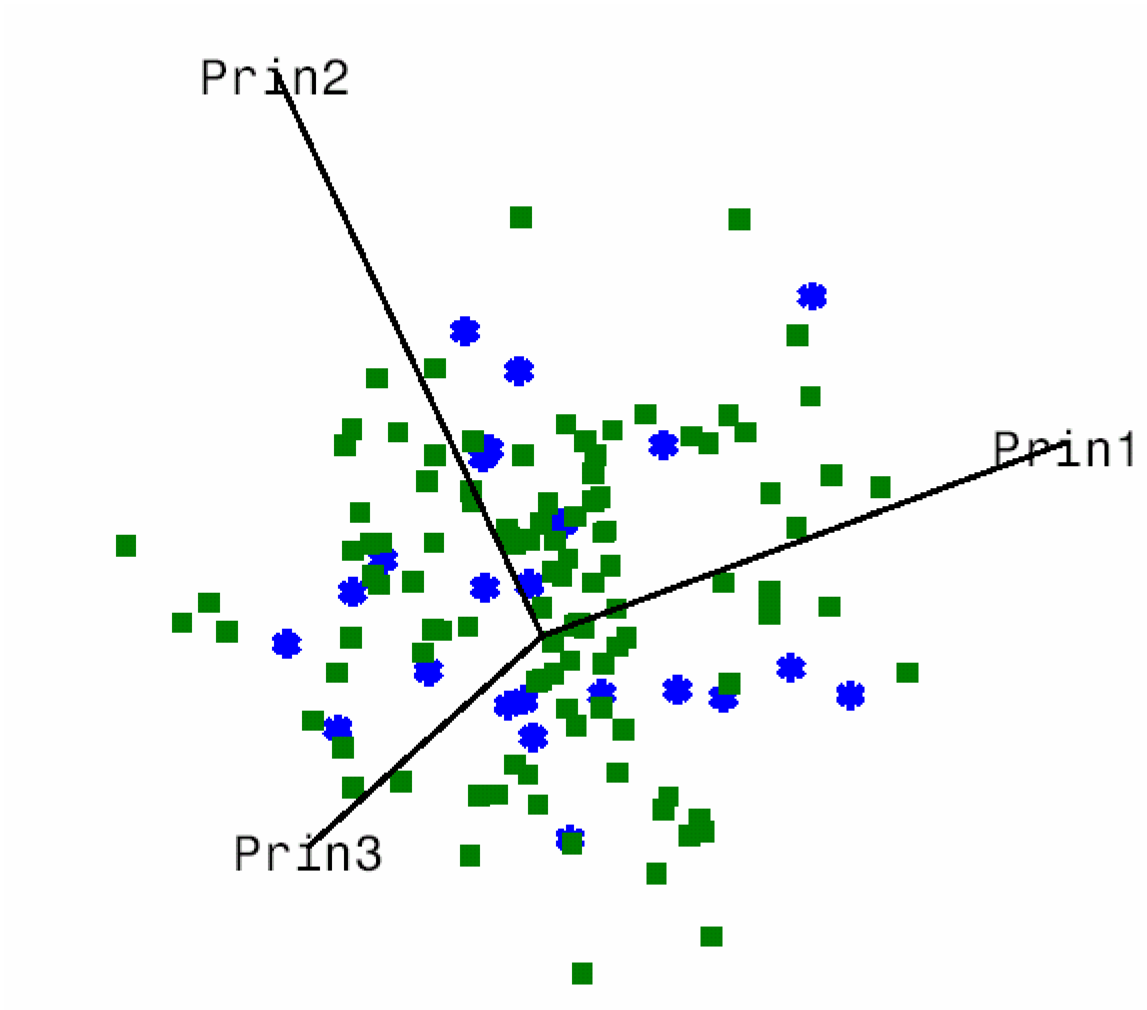

Prin2
Prin1
Prin3

Figure 11. Principle Components Analysis (PCA) results comparing flower morphology measurements of *H. heterophylla* (Square), *H. minor* (Circle), and *H. naniflora* (Cross).

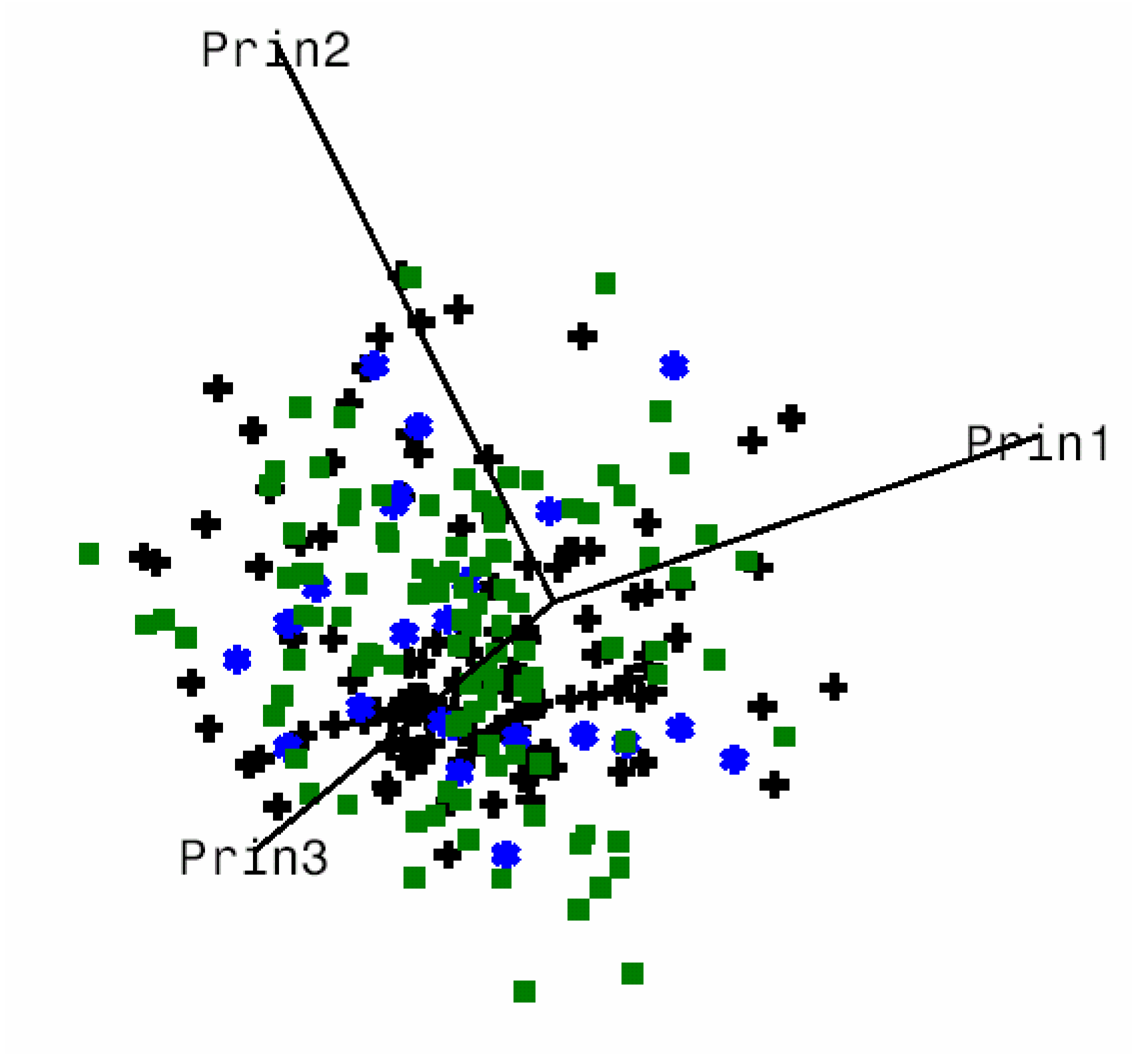

Prin2
Prin1
Prin3

Figure 12. Digital images of pollen from the three species in the *H. heterophylla* complex taken with Scanning Electron Microscope (SEM). *H. heterophylla* (A), *H. minor* (B), and *H. naniflora* (C).

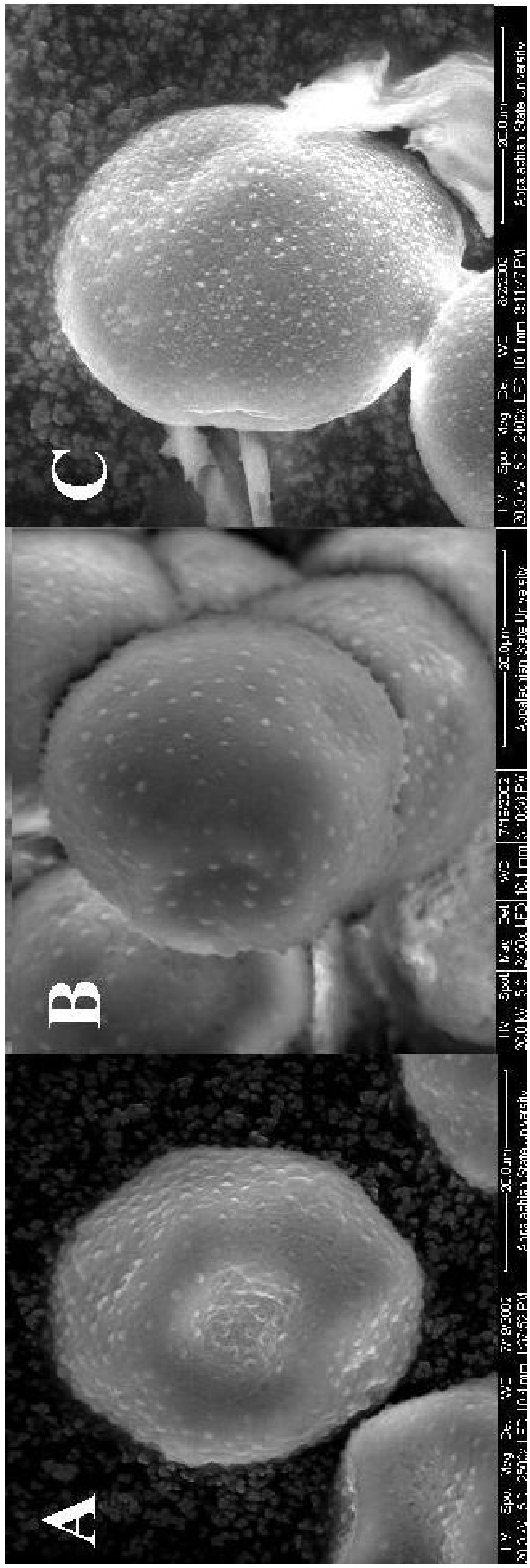

A
B
C

Figure 13. Close-up digital image of pollen from *H. heterophylla*.

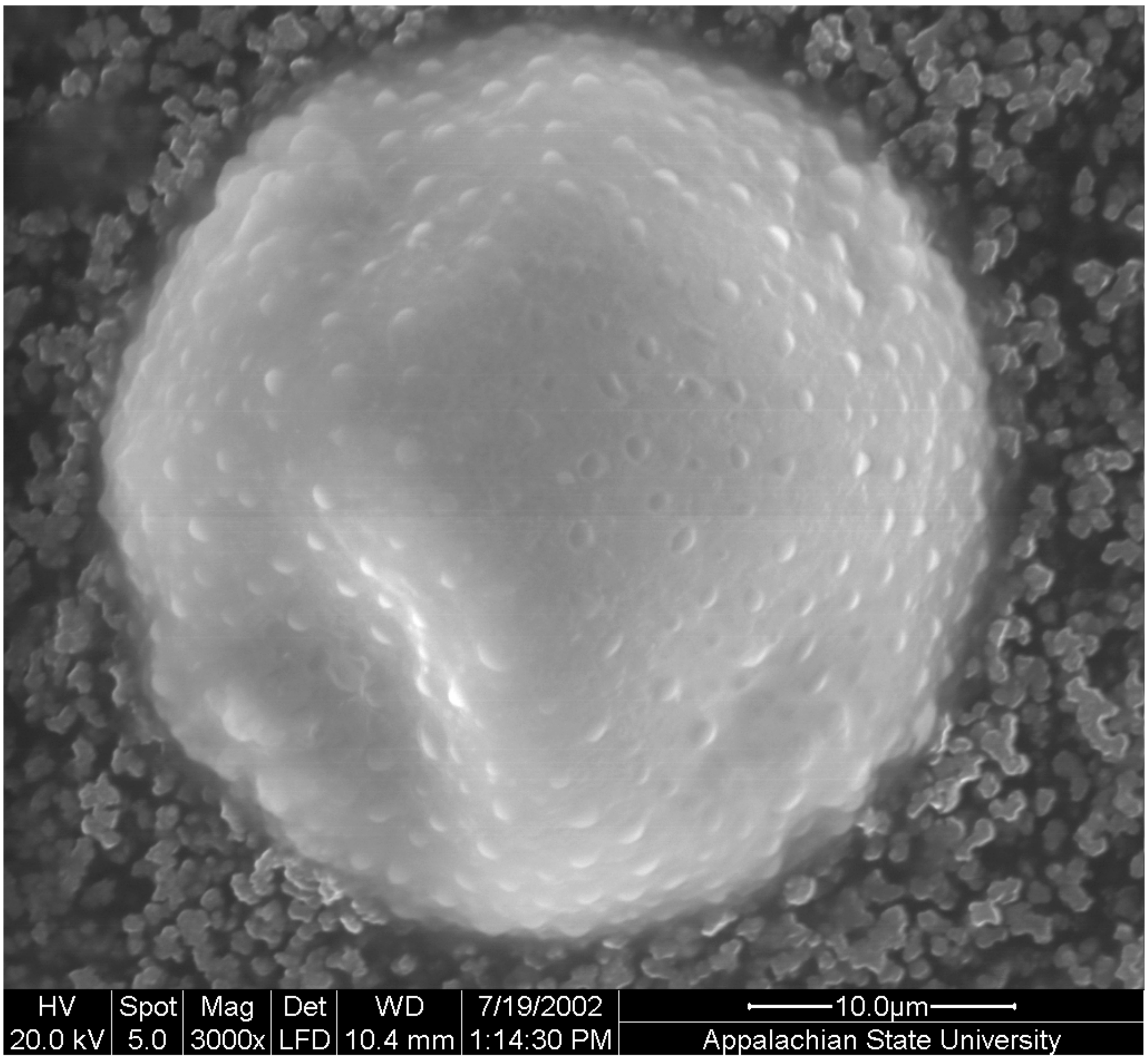

HV
Spot
Mag
Det
WD
7/19/2002
10.0µm
20.0 kV
5.0
3000x
LFD
10.4 mm
1:14:30 PM
Appalachian State University

Figure 14. Close-up digital image of pollen from *H. minor*.

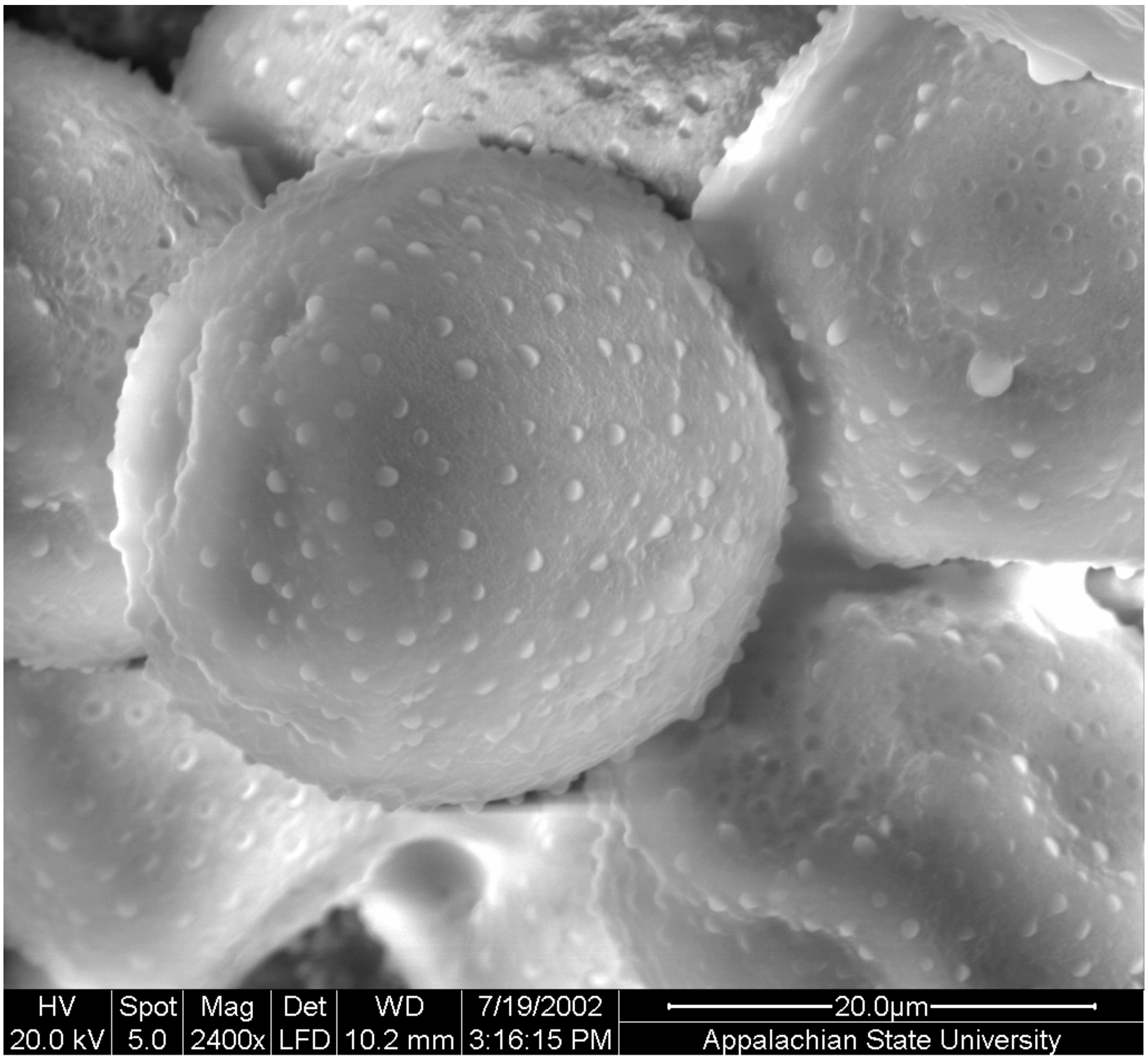

HV
Spot
Mag
Det
WD
7/19/2002
20.0µm
20.0 kV
5.0
2400x
LFD
10.2 mm
3:16:15 PM
Appalachian State University

Figure 15. Close-up digital image of pollen from *H. naniflora*.

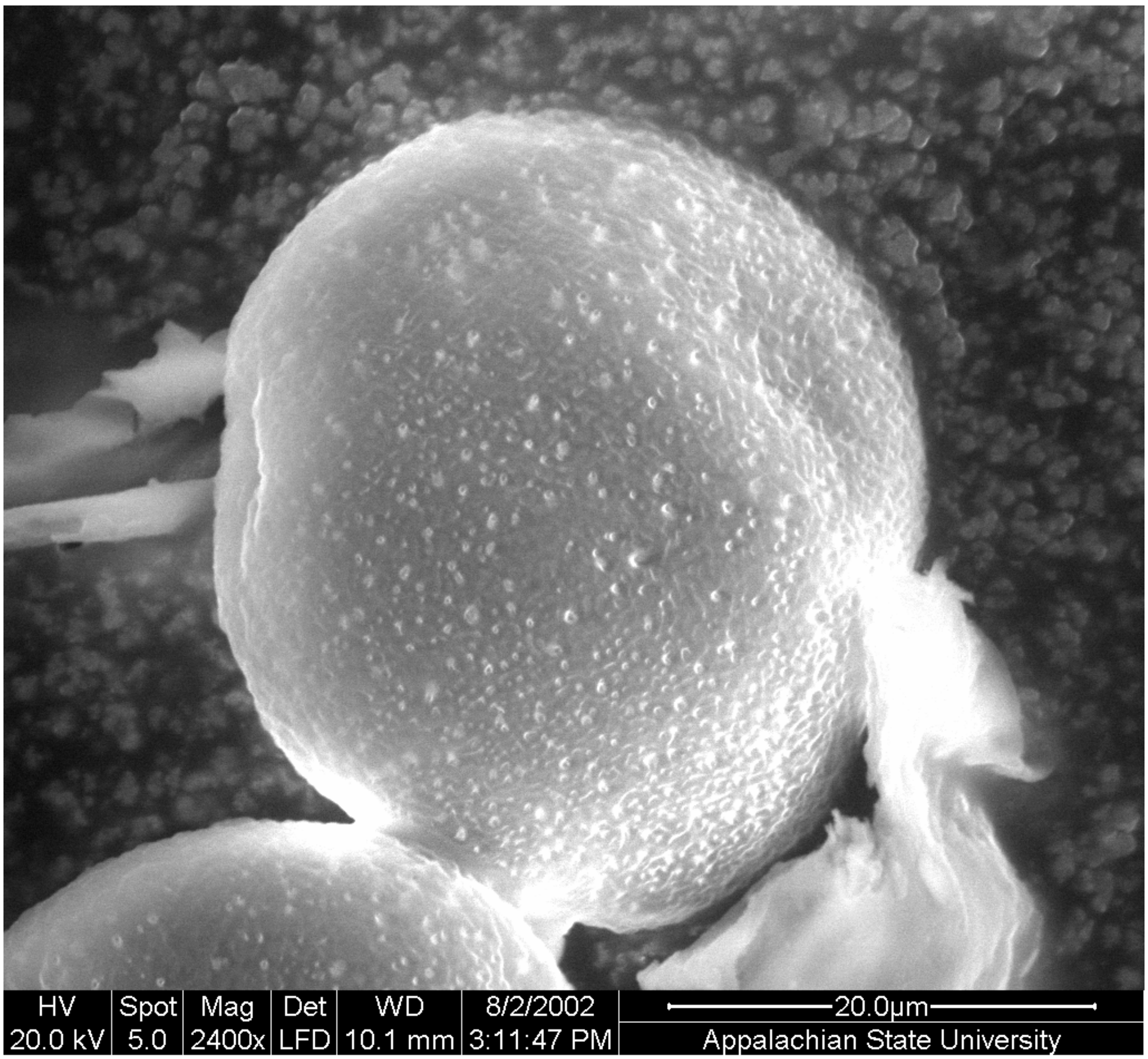

HV
20.0 kV
Spot
5.0
Mag
2400x
Det
LFD
WD
10.1 mm
8/2/2002
3:11:47 PM
20.0µm
Appalachian State University

Table 5. Species richness Depth Means (DMS) at each nested plot (5 - 1) of each study plot and the total over-all number of species found in each plot.

SPECIES	PLOT	DMS 5	DMS 4	DMS 3	DMS 2	DMS 1	TOTAL SPECIES
HM	1	0.88	2.5	6.5	15.88	28.78	55
HN	2	1	2	3.88	7.5	16	50
HH	3	0.38	1.63	6.63	15.88	30.25	63
HN	4	0.25	0.5	2.23	5.13	11.5	41
HN	5	0.5	2.38	8	18.5	36	63
HN	6	1.13	3.38	9	18.38	39	69
HN	7	0.25	0.75	3.13	7.63	14.75	43
HN	8	0.38	2.13	6.38	13.5	28	48
HM	9	0.63	1.75	5.5	16.75	35.5	71
HH	10	0.5	1.63	5.63	12.75	21.75	41
HH	11	0.38	1.13	3.63	8.88	16.5	35
HN	12	0.5	2.13	6.75	16.5	32	55
HM	13	0.38	0.75	4.13	13.38	29.25	61

Table 6. Comparison of the nested plots species richness with an alpha value at P< 0.05.

Nested Plot	Pr > F
5	0.7397
4	0.8859
3	0.9588
2	0.5542
1	0.4392
Total	0.2437

species in the *H. heterophylla* complex was averaged and the results show that species richness is lowest for *H. heterophylla* at 46.3%, with an intermediate value for *H. naniflora* at 59.9%, and the highest value for *H. minor* at 62.3% (Figure 16). This part of the vegetation analysis is confounded by the lack of inclusion of seasonal taxa. The results from the statistical analysis of species richness from the NCVS showed no significance when comparing the nested plots (Table 6).

Results of the Sorenson's Index of Community Similarity and Coefficient of Community analyses are shown in Figure 17. With an index established, I could then construct a dendrogram showing community similarity. This dendrogram shows that *H. heterophylla* and *H. minor* have more similar habitats and species richness. *Hexastylis heterophylla* is more variable in species richness across its range with the mountain populations exhibiting lower species richness, whereas the Piedmont populations have much higher species richness (Figure 18). Therefore, the Piedmont populations of *H. heterophylla* are found in habitats that are more similar to the habitats of *H. minor* and *H. naniflora*.

Most associate plant species either occurred in all or most of the plots in the *H. heterophylla* complex. However, a few occurred with only one of the species in the complex and therefore could be useful as indicator species for presence or absence of the co-occurring *Hexastylis* species. The results of the analysis of associate species are shown in Table 7. The associate species names are given using a formula devised for the NCVS, where the first four letters indicate the genus name and the last three letters indicate the specific epithet. For example, CORNFLO is the acronym for

Figure 16. Average species diversity for the three species in the *H. heterophylla* complex

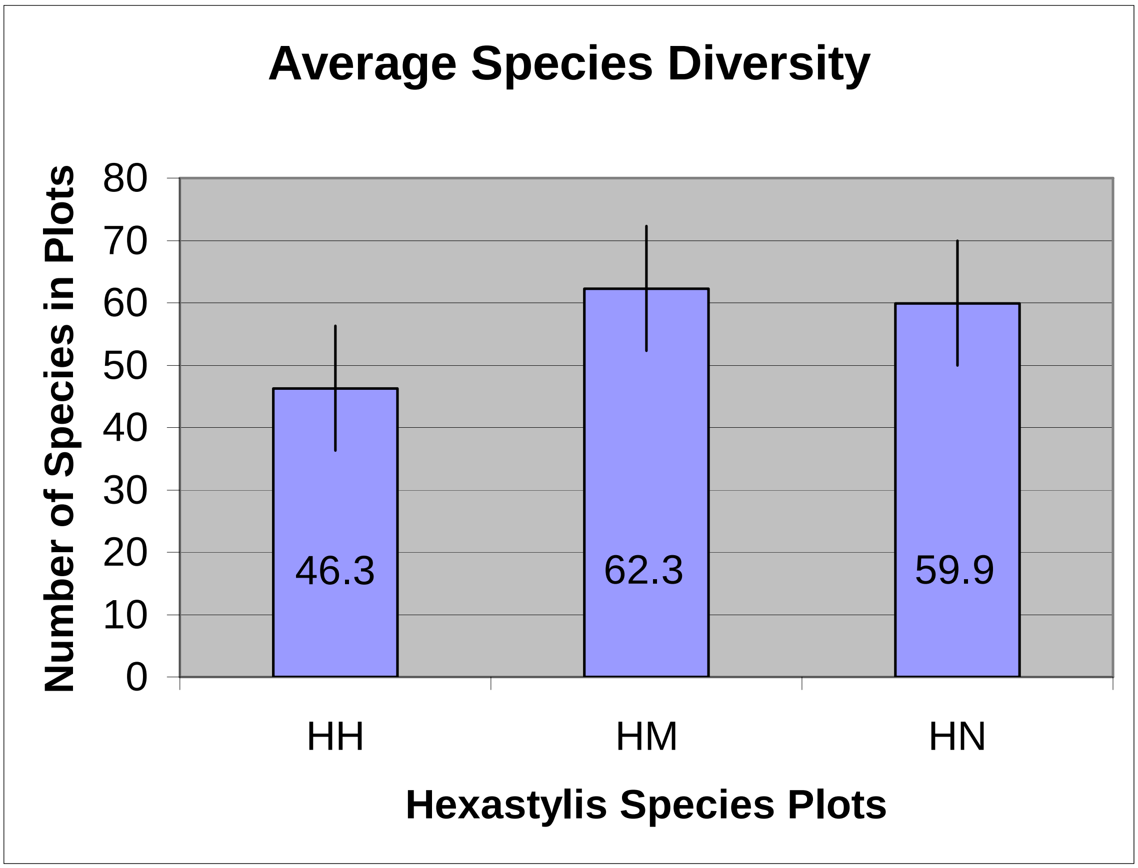

Average Species Diversity
Number of Species in Plots
80
70
60
50
40
30
20
10
0
46.3
62.3
59.9
HH
HM
HN
Hexastylis Species Plots

Figure 17. Sorenson's Index of Community Similarity to comparing the thirteen plots. A-C = *H. heterophylla*, D-F = *H. minor*, and G-M = *H. naniflora*.

	A	B	C	D	E	F	G	H	I	J	K	L	M
A	-												
B	0.5	-											
C	0.4	0.2	-										
D	0.3	0.3	0.5	-									
E	0.3	0.2	0.6	0.5	-								
F	0.3	0.3	0.5	0.5	0.6	-							
G	0.3	0.3	0.6	0.4	0.6	0.5	-						
H	0.3	0.3	0.4	0.4	0.4	0.4	0.6	-					
I	0.3	0.3	0.6	0.5	0.4	0.5	0.4	0.4	-				
J	0.2	0.3	0.5	0.5	0.6	0.5	0.6	0.6	0.5	-			
K	0.3	0.3	0.5	0.6	0.6	0.7	0.6	0.5	0.6	0.5	-		
L	0.3	0.3	0.5	0.5	0.5	0.5	0.5	0.6	0.6	0.7	0.6	-	
M	0.3	0.3	0.4	0.4	0.4	0.5	0.4	0.6	0.6	0.5	0.5	0.5	-

Figure 18. Dendrogram showing community relationships among the three species in the *H. heterophylla* complex for the thirteen plots. A-C = *H. heterophylla*, D-F = *H. minor*, and G-M = *H. naniflora*.

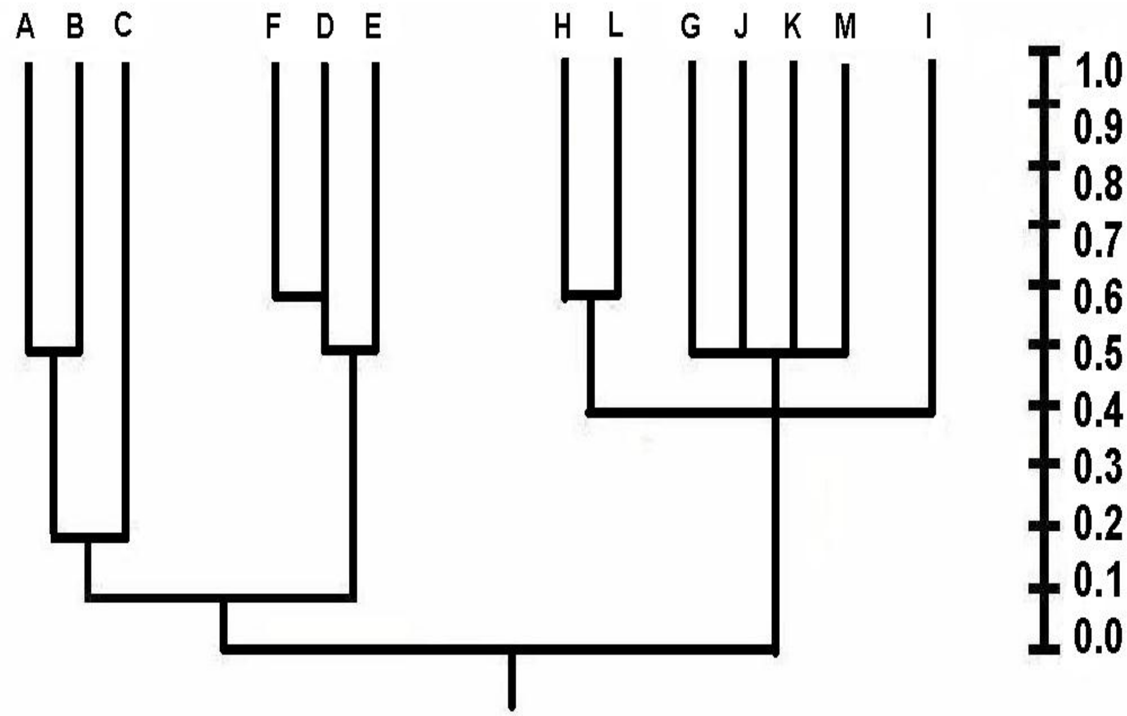

A
B
C
F
D
E
H
L
G
J
K
M
I
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0

Table 7. Graph showing species associates by *H. heterophylla* complex species for the thirteen plots. HH= *H. heterophylla*, HM= *H. minor*, and HN= *H. naniflora*.

	HH	HH	HH	HN	HN	HN	HN	HN	HN	HN	HM	HM	HM
	AT	HYFK	BHB	ALEX	LGPC	KFS	DAN	RHY	COW	PCR	BRG	CMSP	KMSP
ACERRUB	■	■			■	■	■	■		■	■	■	■
AMELARB						■		■	■				
BETUALE		■											
CALAFLO						■			■		■		
CAMPRAP			■										
CARPCAR			■							■	■		■
CARYGLA			■	■	■	■	■	■	■	■	■	■	■
CARYTOM			■		■	■			■			■	■
CORNFLO			■					■	■	■	■	■	■
CORYCOR						■	■	■					■
ILEXOPA					■					■	■	■	■
KALALAT	■	■			■			■				■	
LECUAXI		■									■		
LIQUSTY									■				
LIRETUL	■	■	■			■	■	■	■	■	■	■	■
METCREP		■			■	■	■	■	■	■		■	
OXYOARB	■				■	■	■	■			■	■	■
PINEECH										■	■		■
PINESTR					■								
QUERALB			■	■	■	■	■		■	■	■	■	■
QUERCOC						■		■		■			
QUERPRI								■					
QUERVEL				■		■			■				
RHODMAX		■											
SMIROT					■	■	■		■	■	■	■	
TSUGCAN		■											
VITOROT					■	■	■	■	■	■	■	■	
QUERNIG								■					
FAGUGRA		■				■				■			
VACCVAC					■	■					■		■
POLYACR	■	■	■			■		■	■		■		
HEXAHET	■	■	■										
HEXNAN				■	■	■	■	■	■	■			
HEXAMIN											■	■	■

Cornus florida. The colored bars in Table 7 indicate presence of an associate species occurring with the three species in the *H. heterophylla* complex.

Soils

The results of the soil samples analyzed at the Clemson Soil Lab suggest that major differences in soil chemistry exist between the species in the *H. heterophylla* complex. Statistical analysis of the soil samples showed that many of the basic elements were significantly different among the three species. Those significant differences occurred in Phosphorous (P), Potassium (K), Magnesium (Mg), Zinc (Zn), Manganese (Mn), (Na), Sodium, and Cation Exchange Capacity (CEC). Slightly significant differences were seen in Buffer pH (Bu pH), and Acidity (Table 8).

A Tukey's standardized range test demonstrated that several differences in soil chemistry were found between soils collected from populations of *H. minor* and *H. naniflora*, and fewer differences in soil chemistry were found between *H. heterophylla* and *H. naniflora* and between *H. heterophylla* and *H. minor* (Table 9).

Transplant analysis

Data collected from the transplant site located along Little Gunpowder Creek in Caldwell County, North Carolina revealed that in November 2000, 175 individuals, or 100% of the transplants were surviving. By April of 2002, 147 individuals, or 84% of the transplants were surviving. In April 2002, 119 individuals, or 68% of the transplants were surviving (Figure 19).

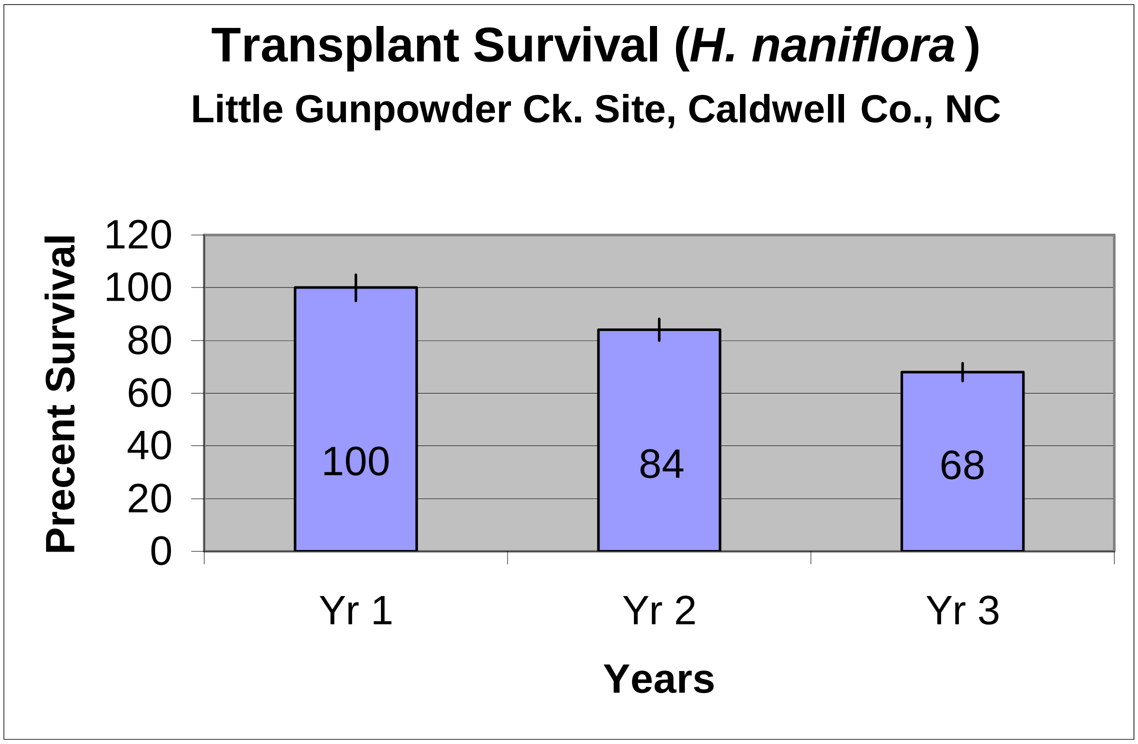
Table 8. Soil chemistry analysis for the three species in the *H. heterophylla* complex. P values are generated for proc GLM (SAS) where P < 0.05. Those values in red are significant with P < 0.05.

Test	**P > F**
Ph	0.850
Bu. pH	0.060
P	0.020
K	0.010
Ca	0.160
Mg	0.050
Zn	0.004
Mn	0.020
Cu	0.250
B	0.480
Na	0.004
CEC	0.007
Acidity	0.060
Base Sat	0.110

Table 9. Tukey's standard range test of soil chemistry in the species in the *H. heterophylla* complex. An X indicates significant differences within columns between the two species for the specific chemical measured. Those in Blue were slightly significant.

	HH/HM	HH/HN	HM/HN
Ph			
Bu. pH			
P			X
K			X
Ca			X
Mg			
Zn			X
Mn		X	X
Cu			X
B			
Na			
CEC	X		X
Acidity		X	X
Base Sat	X		

Figure 19. Graph showing percentage survival of *H. naniflora* over three years at Little Gunpowder Creek, Caldwell County, NC.



Transplant Survival (*H. naniflora*)
Little Gunpowder Ck. Site, Caldwell Co., NC
Precent Survival
120
100
80
60
40
20
0
100
84
68
Yr 1
Yr 2
Yr 3
Years

DISCUSSION

Biogeography

One of the major goals of this project was to explore species boundaries between *H. naniflora* and the most closely related species, *H. heterophylla* and *H. minor*. Since the first description of *H. naniflora* by Blomquist in 1957, field identification of this species has been difficult without fresh flowering material. Locality data has helped in recent years, as biologists gained a better idea of the range of the species, but misidentification of specimens has been a problem when trying to establish biogeographical maps from herbaria specimens and data obtained from the North Carolina Natural Heritage Program and the South Carolina Heritage Trust Program.

With examination of herbaria specimens and visits to field sites, I was able verify or eliminate previously documented *H. naniflora* sites. All the questionable sites were visited during flowering times so that flower materials could be collected and examined. Four sites in the North Carolina Heritage database were found not to be *H. naniflora*. Two sites were determined to be *H. heterophylla* (EO# NC-0041 and EO# NC-0042) and two sites were determined to be *H. minor* (EO# NC-0065 and EO# NC-0066). The locality map generated for this study provides an exact distribution map of all the known *H. naniflora* sites and this should be of benefit to regional biologists and land managers. Maps for the *H. heterophylla* complex were generated using presence or absence data at the county level. These maps show the overlap among the three species along contact zones and should generate future analyses of speciation and hybridization.

When dealing with biogeography, one must look at why a plant species is where it is and how it got there. The evidence suggests that species of *Hexastylis*, like many other plant species, migrated into river ravines in the south as the glacial ice moved southward and the climate cooled in 100,000 year cycles of the Pleistocene Epoch and then back northward during the interglacial periods (Davis 1976; Delcourt 1980; Pitelka 1997; Watts 1980; Webb 1981; Xiang and Soltis 1998). It would appear that this pattern of species distribution is due in part to past migrations, but this pattern is complicated by soil differences that separate many species in the genus. The only exception to this pattern of narrow species distributions in the group is *Hexastylis arifolia*, a species distributed throughout the entire southeastern United States. This exception suggests that *H. arifolia* is more tolerant of various soil types and moisture regimes than the *Hexastylis* species. The differences in soil type for the different species supports the hypothesis that speciation in the group has been driven by abiotic factors.

The mountains of the southeastern United States allow for migration of plant species southward due to their general north to south orientation. *Hexastylis* species, along with other plant species, would have had unimpeded migration routes to move south in colder climates and back north as the climate ameliorated. Some evidence of this migration northwards through mountain corridors can be seen in the morphological variation within *H. heterophylla*. It is the most variable of all the *Hexastylis* species and this variability is evident between mountain ranges from east to west along its distribution. The speed and direction of *Hexastylis* migration is unknown and uncertain, but it appears to be following river and mountain corridors from southwest to northeast in this complex.

Flower morphometrics

Flower data show that *H. naniflora* can be statistically differentiated from *H. heterophylla* or *H. minor*. The results from the PCA show clear separation between *H. naniflora* and the other two species in the complex. While *H. naniflora* can be separated from the other two species in a PCA analysis, no clear separation can be made between *H. heterophylla* and *H. minor*. This species pair is clearly in need of further study.

In obtaining flower measurements for use in analyses of this group, it is evident that fresh flower materials must be used. Data obtained from dried or preserved flower materials is unreliable due to flower distortion that occurs when the flower is pressed, dried, or preserved.

Flower size and shape have been at the crux of species identification in the genus (Blomquist 1957, Gaddy 1987). Flower size has been particularly important in distinguishing *H. naniflora* from *H. heterophylla* and *H minor*. The species of *Hexastylis* are very similar in leaf structure and overall morphology, so flowers are, in general, important in delineating species. Blomquist constructed a dichotomous identification key of *Hexastylis* species for using flower size and shape to identify species in the field. Gaddy (1987) also constructed a key for field identification *Hexastylis* species with flower measurements being important characters in his key.

Pollen

The need for some other means of species identification in the *H. heterophylla* complex was the reason we decided to look at pollen morphology. With fresh flowers as the only known means of species identification, we wanted to find a method to differentiate species using old flowers or herbarium specimens. Pollen work conducted by Traverse (1988) was examined for background information and general pollen structure. Kelly (2001) referenced a paper by Walker (1974), who examined a number of *Hexastylis* species pollen (one of which was *H. heterophylla*). I used a different SEM technique from Walker, a low vacuum capability, to examine the specimens. This method of SEM is relatively inexpensive when compared to older SEM methodology, which required critical point drying and sputter coating of specimens before examination.

The results from pollen analysis show that *H. naniflora* pollen surface is unlike that of *H. heterophylla* or *H. minor*. The lack of surface features in *H. naniflora* pollen appears unique to all the *Hexastylis* species. It appears that the *H. naniflora* pollen is more similar in micromorphology to *Aristolochia* species than other *Hexastylis* species. The size of the *H. naniflora* pollen is roughly the same as pollen from *H. heterophylla* and *H. minor*. Perhaps this pollen difference is a plesiomorphic character retained in *H. naniflora* or it might be a secondarily derived character state. Therefore, with SEM use, species differentiation can be made of questionable populations of *Hexastylis* in the *H. heterophylla* complex. With new digital capabilities and low vacuum SEM, the cost of examination of pollen, and in turn, unequivocal species identification, is now relatively inexpensive.

Vegetation Survey

It has been intuitively known for years that certain plant assemblages are found in association with various species of *Hexastylis*. Many associated plant species have been identified in various publications (Blomquist 1957; Gaddy 1981, Gaddy 1987; Henderson

2001). The NCVS analysis did not show statistically significant differences among the three species in the *H. heterophylla* complex. However, *H. naniflora* appears to have an association with three oak species that is lacking in the other two species in the complex. *Hexastylis heterophylla* was the only species in the *H. heterophylla* complex found to occur with Canadian Hemlock (*Tsuga canadensis*). *Hexastylis minor* is the only species in the complex that was found to grow in any aspect with respect to exposure to the sun, and was not restricted to a northern aspect, as are *H. naniflora* and *H. heterophylla.*

The examination of associate species occurring in the vicinity of species of the *H. heterophylla* complex, although not statistically significant, showed some interesting results in the case of *H. naniflora*. There are a number of oak species (*Q. coccinea*, *Q. prinus* (*Q. Montana*), and *Q. velutina*, [listed as QUERCOC, QUERPRI, and QUERVEL in Table 7]) that tend to co-occur with only *H. naniflora*, but are not present with the other two species in the complex. This may be the result of some microbial need or specific soil nutrient required for those species to occur in the same habitat.

Several studies looking at forest and plant community structure have utilized the NCVS method as a means of studying community structure. The reason for using such a vegetational survey comes from its flexibility in practically every level of ecological examination of forest structure. A few recent studies have utilized the NCVS in examining specific forest/plant perimeters. Like the research I conducted in the *H. heterophylla* complex, others have used the NCVS to look at particular plant species in a given environment. One such experiment examined the decline of oak and increase of maple in Piedmont forest communities (McDonald et al. 2002). Another study using the

NCVS examined the levels of invasive plants found in southern Appalachian plant communities (Brown and Peet, 2003).

Soils

Soil chemistry showed marked differences between the species in the complex. The results indicated that soil chemistry is very different between *H. naniflora* and *H. minor* localities. The results also show that *H. heterophylla* and *H. naniflora* are found in soils where the chemistry is more similar, but still showed significant differences. It would appear that differentiation in soil types could be used as a proxy for species delineation. The soil analysis also indicates that soils must be considered when trying to select sites for relocation of imperiled populations of *H. naniflora*.

Soil may have been the key component in speciation of *Hexastylis* species, especially those species in the *H. heterophylla* complex. Gaddy (1981) first made the suggestion that soils might be important in where one might find *Hexastylis* species in his report to the NCDA. He conducted a small number of soil tests on *H. naniflora* populations and on one *H. minor* site. He noted the differences between the soils of the two species, but stated that more work needed to be done to confirm soil importance. My study shows soil chemistry is important and the levels of certain elements found in soils associated with species of the *H. heterophylla* complex appear to play a key role in population location.

Transplant analysis

The NCDOT transplant site off Cedar Valley Road (SR1108) along Gunpowder Creek was very successful, with 68% survival over a three-year period. In those three years, there was an extensive period of drought in the region. This drought no doubt played a role in plant survivorship. It should be noted that this relocation was to an adjacent site. The similar soil type and slope aspect should be recognized as conditions conducive to successful transplants of *H. naniflora*.

Hexastylis naniflora appears to have a restricted range due to it habitat requirements. The habitat where *H. naniflora* exists is limited in size and scope due to a multitude of factors including soil type, moisture availability, and slope aspect. This unique combination of factors limits not only the range of *H. naniflora*, but also the size of a given population. With the limited range and size in populations, questions arise regarding gene flow among populations. How much is occurring and how often does it occur? It is due, in part, to narrow habitat requirements that conservation measures have been implemented for the protection of the species. Any efforts made to protect this species must consider giving protection to the available habitat.

According to U. S. Fish and Wildlife Service, the North Carolina Natural Heritage Program, and The North Carolina Department of Agriculture, a definable and discernable population of *Hexastylis* is one that is at least one-half mile from any existing population. If a new locality is found and it falls within the one half mile radius of another known population, that population then becomes a sub-population. A question that must be addressed in order to determine the value of this guideline is how readily these plants can transfer seeds or pollen one-half mile. *Hexastylis naniflora* populations are all generally small, with less than a few hundred individuals. Work has been done with seed dispersal in the genus. Wyatt (1955) suggested that the seeds are ant dispersed, which would indicate short dispersal distances. Gaddy (1986b) conducted a number of experiments with suspected ant dispersed species, with one of them being *H. naniflora*. His research supports the concept that ants play a critical role in dispersing *Hexastylis* species. Work on the pollination mechanisms of various species of *Hexastylis* suggests that a variety of insects and other invertebrates visit the flowers (Libby et al. 1996; Murrell and Carroll 1995; Wyatt 1955), but there is no information available on pollen movement between populations. The lack of information concerning pollen and seed dispersal in this species would suggest that the one-half mile distance for populations is arbitrary, at best, and it is likely that clusters of individuals that are no more than 100 m apart may be genetically isolated.

There are several sites where populations of different species of *Hexastylis* in the *H. heterophylla* complex are either in close proximity or growing together in the same habitat. Of all the known populations that overlap, none have been found to produce hybrids. To date, no known hybrids have been found in nature. Past attempts at hybridization have failed due the inability to remove the androecium without damaging the gynoecium (Gonzalez 1972; Otte, 1977).

A suggested species recovery plan for *H. naniflora* has been developed (Appendix D). The species recovery plan was modeled after a recovery plant for Heller's Blazing Star (*Liatris helleri*). The proposed recovery plan would put into motion a series of events and projects which would eventually lead to 1) better protection of known *H. naniflora* sites and 2) eventual delisting of *H. naniflora* from the Endangered Species List. Included are suggested recommendations for the number of populations that should be protected and measures that should occur before potential delisting of the species should proceed.

Recommendations for future of species

While the number of known populations of *H. naniflora* has been greatly increased in the past few years, conservation efforts should continue for the unforeseeable future. Because the plant's distribution overlaps one of the fastest growing areas in the southeastern U.S., it is imperative that enough populations are protected to maintain the genetic diversity of the species. The U. S. Fish and Wildlife Service suggested that delisting of the species is possible if enough populations are placed in protection.

In Appendix D, I have made recommendations in a species recovery plan for a set number of populations to be placed in protection and stabilized before moving towards a de-listing of the species. Evidence from my study, as well as studies conducted by Gaddy (1981, 1987), suggests that *H. naniflora* is not as rare as once suggested, and a large number of populations probably remain undiscovered. However, habitat requirements severely limit the range of this species. When specific soil and moisture needs are taken into consideration, the narrow habitat limitations indicate that the plant must have some type of protection in order to assure its survival and genetic diversity. Recent easements and land mitigations by the Broad River Greenway, NCDOT, the Natural Heritage programs of North Carolina and South Carolina, and the Spartanburg County Water Works have paved the way for greater protection of the habitat and plant that can eventually lead to removal of *H. naniflora* from the Endangered Species list as a Threatened species.

BIBLOGRAPHY

Amoroso, J. L. 2002. Natural Heritage Program List of Rare Plant Species of North Carolina. Raleigh, NC.

Araki, Y. 1937. The species of *Asarum* in the Santan District. Acta Phytotaxonomica et Geobotanica 6:122-135.

Araki, Y. 1953. Systema generis Asari. Acta Phytotaxonomica et Geobotanica 15: 33-36.

Ashe, W. W. 1897. The Glabrous-Leaved Species of *Asarum* in the Southeastern United States. Elisha Mitchell Society 14: 31-36.

Avise, J. C. and K. Wollenberg. 1997. Phylogenetic and the origin of species. Proc. Natl. Acad. Sci. 94: 7748-7755.

Barringer, K. 1993. New Combinations in North American *Asarum* (Aristolochiaceae). Novon 3: 225-227.

Blomquist, H. L. 1957. A Revision of *Hexastylis* in North America. Brittonia 8: 255-281.

Braun, A. 1861. *Index seminum Hotri Botanici Berolinensis*. Appendix. Plantarum novarum et minus cognitarum, quae in Horto Regio Botanico, Berolinensi coluntum. Berlin.

Bridle, J. R. and M. G. Richie. 2001. Assortative mating and the genetic view of speciation. Journal of Evolutionary Biology 14: 878-879.

Britton, N. L. and A. Brown. 1947. *Aristolochiaceae*. Pp. 641-645 in An Illustrated Flora of the Northern United States, Canada, and the British Possessions. New York Botanical Garden, New York, NY.

Britton-Davidian, J. 2001. How do chromosomal changes fit in? Journal of Evolutionary Biology 14: 872-873.

Brown, R. L and R. K. Peet. 2003. Diversity and Invasibility of Southern Appalachian Plant Communities. Ecology 84 (1): pp. 32-39.

Chester, E. W., B. E. Wofford, and R. Kral. 1997. *Hexastylis*. Pp. 19-20 in Atlas of Tennessee Vascular Plants. The Center For Field Biology. Austin Peay State University, Clarksville, TN.

Cronquist, A. 1981. *Magnolidae,* Order *Aristolochiales.* Chp. 4, Pp. 90-93 in An Intregrated System of Classification of Flowering Plants. New York Botanical Garden, New York, NY.

Davis, M. B. 1976. Pleistocene Biogeography of Temperate Deciduous Forest. Geoscience and Man. 13: 13-26.

De Jussieu, A. L. 1789. Genera piantarum secunduni ordines nahsrales disposita, juxta inethodum in horto regio Parisiensi exaratam. Paris: Apud vi-duam Hirissant.

Delcourt, P. A. 1980. Goshen Springs: Late Quaternary Vegetation Record for Southern Alabama. Ecology 61: pp. 371-386.

Delwiche, L D. and S. J. Slaughter. 1995. The Little SAS Book: A Primer, Cary, NC: SAS institute Inc. 228 pp.

Dodoens, R. 1574. Purgantium aliarumque eo facientum. Antverpiae: Offica C. Plantini.

Duchartre, P. 1864. *Aristolochiaceae*. Pp.421-498 in Prodromus systematis naturalis regnii vegtabilis, Vol. 15, ed. A. P. de Candolle. Paris: Truttel et Wurtz.

Fernald, M. L. 1943. Virginian botanizing under restrictions. Rhodora 45: 357-415.

Fernald, M. L. 1950. *Aristolochiaceae*. Pp. 562-566 in Gray's Manual of Botany. American Book Company, New York.

Gaddy, L. L. 1981. The Status of *Hexastylis naniflora* Blomquist in North Carolina. Unpublished Report. Plant Conservation Program, North Carolina Department of Agriculture, Raleigh, NC.

Gaddy, L. L. 1986a. A New *Hexastylis* (*Aristolochiaceae*) From Transylvania County, North Carolina. Brittonia 38: 82-85.

Gaddy, L. L. 1986b. Twelve new ant-dispersed species from the southern Appalachians. Bulletin of the Torrey Botanical Club 113: 247-251.

Gaddy, L. L. 1987. A Review of the Taxonomy and Biogeography of *Hexastylis*. Castanea 52: 186-196.

Gaddy, L. L. 2003. *Hexastylis heterophylla*. Electronic Mail.

Gonzalez, V. C. 1972. The Ecology of *Hexastylis arifolia*, An Evergreen Herb in the North Carolina Deciduous Forest. PhD Dissertation, Duke University, Durham, NC.

Gregory, M. P. 1956. A Phyletic Rearrangement in the Aristolochiaceae. American Journal of Botany 43: 110-122.

Harvill, A. M., T. R. Bradley, and C. E. Stevens. 1981. Atlas of the Virginia Flora. Virginia Botanical Associates, Farmville, VA.

Hayashi, N., K. Maeshima, and H. Komae. 1982. Phenol Ethers of Three North American *Hexastylis* Species. Phytochemistry 22: 299.

Henderson, A. 2001. Using ecological parameters to determine the location of *Hexastylis naniflora* (dwarf-flowered heartleaf) in the upper piedmont of North Carolina. Lenoir-Rhyne College, Hickory, NC.

Interior, Department of the. 1989. National Register Vol. 54: Pp.14964-14967 in the Federal Register, Washington, DC.

Judd, W. S., C. S. Campbell, E. A. Kellogg, P. F. Stevens, and M. J. Donoghue. 2002. Plant Systematics: A Phylogenetic approach. 2nd ed. Sinauer Associates, Inc.: Sunderland, MA.

Kelly, L. M. 1997. A Cladistic Analysis of *Asarum* (Aristolochiaceae) and Implications for the Evolution of Herkogamy. American Journal of Botany 84: 1752-1765.

Kelly, L. M. 1998a. Phylogenetic Relationships in *Asarum* (Aristolochiaceae) Based on Morphology and ITS Sequences. American Journal of Botany 85: 1454-1467.

Kelly, L. M. 1998b. Systematic in *Asarum* and Aristolochiaceae. PhD dissertation. Cornell University, Ithaca, NY.

Kelly, L. M. 2001. Taxonomy of *Asarum Section Asarum* (Aristolochiaceae). Systematic Botany 26: 17-53.

Kelly, L. M. 2002. *Asarum – Hexastylis* discussion. Electronic Mail.

Kral, R. 1983. Aristolochiaceae: *Hexastylis naniflora* Blomquist. Dwarf-flowered heartleaf. USFS, Region 8, Atlanta, GA. 318-321.

Libby, G. W., Z. E. Murrell, and P. E. Carroll. 1996. Inventory for *Hexastylis* *contracta* Blomquist (Southern Heartleaf) in Kentucky and Tennessee. Technical report to the U. S. Fish and Wildlife Department National Biological Survey.

Linnaeus, C. 1753. *Species Plantarum*. Stockholm: Impensis Laruentii Salvii.

Maekawa, F. 1936. *Asiasarum* Pp. 17-18 in *Flora Sylvatica Koreana*, vol. 21, ed. T. Nakai. Keijyo: Government General of Chosen.

Makwawa, F. and M. Ono. 1965. Karyotype analysis of the genus *Hexastylis* (Aristolochiaceae). Journal of the Faculty of Science. University of Toyko. Section III: Botany 9:151-159.

McDonald, R. L., R. K. Peet, and D. L. Urban. 2002. Environmental Correlates of Oak Decline and Red Maple Increase in the North Carolina Piedmont. Castanea 87: 84-95.

McMillian, P. D. 1995. The Ecological Phytogeography and Taxonomy of the *Asarum virginicum* Group. University of North Carolina at Chapel Hill. Senior Honor's Thesis.

Michaux, A. 1803. Flora Boreali-Americana. 2 vol. Paris.

Morren C. and J. Decaisne. 1834. Observations sur la flore du japon. Annales des Sciences Naturalles 2: 308-320.

Murrell, Z. E. and P. E. Carroll. 1995. Status survey of *Hexastylis contracta* in Tennessee. Technical report to the Kentucky Nature Preserves Commission.

Nakai, T. 1936. *Japonasarum*. Pp. 17-18 in *Flora Sylvatica Koreana*, vol. 21, ed. T. Nakai. Keijyo: Government General of Chosen.

Newberry, G. Unpublished. Notes on the Biology and Transplanting Techniques for the Federally Endangered Herb *Hexastylis naniflora* Blomquist (Aristolochiaceae).

Newberry, G. 1996. Technical and Agency Draft Recovery Plan for Dwarf-flowered Heartleaf (*Hexastylis naniflora*) Blomquist. U. S. Fish and Wildlife Service, Region 8, Atlanta, GA.

Noor, A. F. 2002. Is the biological species concept showing its age? Trends in Ecology and Evolution 17: 153-154.

Ormsby, T, Eileen Napoleon, Robert Burke, Carolyn Groessl, and Laura Feaster. 1998. Getting to Know ArcView GIS 3.X, ESRI, New York: 600pp.

Orr, H. A. 2001. Some doubts about (yes another) view of species. Journal of Evolutionary Biology 14: 870-871.

Otte, D. S. 1977. The Pollination Ecology of *Hexastylis Arifolia* (Mich.) Small Var. *arifolia* and *Hexastylis minor* (Ashe) Blomquist (Aristolochiaceae) in the Area of Chapel Hill, North Carolina. Master's Thesis, University of North Carolina, Chapel Hill, NC.

Parkinson, J. 1640. The Theatrum Botanicum. London.

Peattie, D. C. 1929. Flora of the Tryon Region. Elisha Mitchell Scientific Society 44: 141-229.

Peattie, D. C. 1940. How is Asarum Pollinated? Castanea 5: 24-29.

Peet, R. K, T. R. Wentworth, R. P Duncan, and P. S. White. 1998. A Flexible, Multipurpose Method for Recording Vegetation Composition and Structure. Castanea 63: 262-274.

Pitelka, L. F. 1997. Plant Migration and Climate Change. American Scientist 85: 464-473.

Radford, A. E., H. E. Ahles, and C. R. Richie. 1968. Manual of the Vascular Flora of the Carolinas. University of North Carolina Press, Chapel Hill, NC.

Rafinesque, C. S. 1825. *Neogenyton, or indication of sixty-six new genera of plants in North America.* 1-4.

Rayner, D. A. 1994. Inventory of the Natural Areas of the Pacolet Area. Heritage Program, N. C. Department of the Environment, Health and Natural Resources. Raleigh, NC.

Riesenberg, L. H. and J. M. Burke. 2001. A genetic view of species integration. Journal of Evolutionary Biology 14: 883-886.

Rundle, H. D. 2001. Hybridization without guilt: gene flow and the biological species concept. Journal of Evolutionary Biology 14: 868-869.

Schmidt, O. C. 1937. *Aristolochiaceae*. Pp 204-242 in Die Naturlichen Pflantzenfamilien ed. 2, Vol. 16b, Eds. Engler & Prantl, Liepzig: Verlag Wilhelm Engelmann.

Shaw, K. L. 2001. The genealogical view of speciation. Journal of Evolutionary Biology 14:880-882.

Small, J. K. 1933. Manual of Southeastern Flora. Published by Author, New York, NY.

Suiter, K. D., L. Mansberg, and J. Moore and . 1983. Endangered, threatened, and rare plant species of North Carolina: A revised list. Association of Southeastern Biologist. 30: 147-157.

Tournefort, J. P. 1694. Elmens de botanique; Inslitutiones Rei Herbariac. Paris.

Traverse, A. 1988. *Spore and Pollen Morphology*. Chp. 5 Pp 60-116 in Paleopalynology. Unwin Hyman, Boston, MA.

USDA. 1962. Soil Survey of Cherokee County, South Carolina, series 1958, No. 19. U.S. Government Printing Office, Washington, DC.

USDA. 1980. Soil Survey of Henderson County, North Carolina. U.S. Government Printing Office, Washington, DC.

USDA. 1995a. Soil Survey of Lincoln County, North Carolina. U.S. Government Printing Office, Washington, DC.

USDA. 1995b. Soil Survey of McDowell County, North Carolina. U.S. Government Printing Office, Washington, DC.

USDA. 2000a. Soil Survey of Rutherford County, North Carolina. U.S. Government Printing Office, Washington, DC.

USDA. 2000b. Soil Survey of Cleveland County, North Carolina. U.S. Government Printing Office, Washington, DC.

USDA. 2000c. Soil Survey of Spartanburg County, South Carolina. U.S. Government Printing Office, Washington, DC.

USFWS. 1990. Dwarf-Flowered Heartleaf. Department of the Interior, Washington, DC.

Vogler, A. P. 2001. The genic view: a useful model of the process of speciation? Journal of Evolutionary Biology 14:876-877.

Walker, J. W. 1974. Aperture Evolution in the Pollen of Primitive Angiosperms. American Journal of Botany 61:1112-1136.

Watts, W. A. 1980. The Late Quaternary Vegetation History of the Southeastern United States. Annual Reviews in Ecology 11: 387-409.

Webb, T. 1981. The Past 11,000 Years of Vegetational Change in Eastern North America. Bioscience 31(7) pp. 501-506.

Whittaker, R. H. 1975. Communities and Ecosystems. Collier-Macmillan; New York, NY.

Whittemore, A. T., and L. L. Gaddy. 1997. *Hexastylis*. Pp. 54-58 in *Flora of North America*, eds. Flora of North America Editorial Committee. New York: Oxford University Press.

Wofford, B. E. 1989. Guide to the Vascular Plants of the Blue Ridge. University of Georgia: Athens, GA.

Wu, C. 2001. The genetic view of the process of speciation. Journal of Evolutionary Biology 14: 851-865.

Wyatt, R. L. 1955. Floral morphology and phylogeny of the Aristolochiaceae. Master's Thesis, University of North Carolina, Chapel Hill, NC.

Xiang, Q. Y., and D. Soltis. 1998. The Eastern Asian and Eastern and western North American Floristic Disjunction: Congruent Phylogenetic Patterns in Seven Diverse Genera. Molecular Phylogenetics and Evolution 10: 178-190.

APPENDIX A

Herbarium Specimens Annotated

HERBARIA	SPECIES	STATE	COUNTY	COLLECTOR	DATE	ASC #
BOON	HAA	NC	BURKE	B CROUCH	4/18/1976	10307
BOON	HAA	SC	ABBEVILLE	ELLIS	6/11/1971	5626
BOON	HAA	NC	CABARRUS	L C BARRINGER	4/15/1979	12735
BOON	HAA	GA	LINCOLN	M GEIMAN	3/24/1977	11241
BOON	HAA	TN	ANDERSON	M L HICKS	4/24/1963	9075
BOON	HAA	NC	WATAGUA	MILLER & BAUCOM	5/4/1967	4639
BOON	HAA	NC	CHEROKEE	S MORROW	10/17/1979	13458
BOON	HAA	NC	CABARRUS	T DAGGY	5/29/1967	4164
BOON	HAC	LA	PERRY MILES	E LICKEY	4/4/2001	16047
BOON	HAR	NC	McDOWELL	D BUFF	5/4/1967	4638
BOON	HAR	TN	KNOX	M L HICKS	5/1/1968	8998
BOON	HAR	NC	CLAY	S W LEONARD & K MOORE	6/6/1968	3168
BOON	HH	NC	AVERY	GROUP V	6/20/1960	2202
BOON	HH	NC	CATAWBA	J PADGETT & E GILLESPIE	4/18/2002	16383
BOON	HH	NC	CALDWELL	K OAKLEY	4/7/1980	13547
BOON	HH	VA	VA	NICK DROZDA	6/19/1905	16598
BOON	HH	VA	FLUVANNA	NICK DROZDA	N/A	16591
BOON	HH	TN	UNICOI	NICK DROZDA	N/A	16593
BOON	HH	VA	FLUVANNA	NICK DROZDA	N/A	16594
BOON	HH	AL	WINSTON	ROBERT F C NACZI	5/21/1996	16596
BOON	HH	AL	CLEBURNE	ROBERT F C NACZI	5/23/1996	16595
BOON	HH	NC	BURKE	T D TAYLOR	5/22/1973	9905
BOON	HM	NC	GASTON	J PADGETT	3/24/2002	16378
BOON	HM	NC	GASTON	J PADGETT	3/24/2002	16381
BOON	HM	NC	ORANGE	RADFORD & STEWERT	4/4/1940	1279
BOON	HM	NC	RANDOLPH	S SMITH & ALLEN	3/14/1976	10308
BOON	HN	NC	RUTHERFORD	J PADGETT	4/2/2001	15993
BOON	HN	NC	RUTHERFORD	J PADGETT	4/4/2001	15992
BOON	HN	NC	RUTHERFORD	J PADGETT	4/4/2001	16001
BOON	HN	NC	RUTHERFORD	J PADGETT	4/4/2001	15994
BOON	HN	NC	RUTHERFORD	J PADGETT	4/6/2001	15995
BOON	HN	NC	LINCOLN	J PADGETT	4/6/2001	15991
BOON	HN	NC	RUTHERFORD	J PADGETT	4/6/2001	15990
BOON	HN	NC	CLEVELAND	J PADGETT	4/10/2001	15996
BOON	HN	NC	RUTHERFORD	J PADGETT	4/21/2001	16384
BOON	HN	NC	RUTHERFORD	J PADGETT	5/13/2001	15997
BOON	HN	NC	RUTHERFORD	J PADGETT	5/13/2001	15998
BOON	HN	NC	RUTHERFORD	J PADGETT	5/17/2001	15999
BOON	HN	NC	RUTHERFORD	J PADGETT	5/18/2001	16000
BOON	HN	NC	ALEXANDER	J PADGETT	4/3/2002	16376
BOON	HN	NC	RUTHERFORD	J PADGETT	4/20/2002	16385
BOON	HN	NC	RUTHERFORD	J PADGETT	4/19/2003	16599
BOON	HN	NC	RUTHERFORD	J PADGETT	4/19/2003	16600
BOON	HN	NC	RUTHERFORD	J PADGETT	4/19/2003	16601
BOON	HN	NC	RUTHERFORD	J PADGETT	4/20/2002	16388

BOON	HN	NC	BURKE	J PADGETT & E GILLESPIE	4/18/2002	16399
BOON	HN	NC	BURKE	J PADGETT & E GILLESPIE	4/18/2002	16377
BOON	HN	NC	CATAWBA	J PADGETT & E GILLESPIE	4/18/2002	16379
BOON	HN	NC	BURKE	J PADGETT & E GILLESPIE	4/18/2002	16380
BOON	HN	NC	CALDWELL	J PADGETT & E GILLESPIE	4/18/2002	16382
BOON	HN	NC	CATAWBA	J PADGETT & E GILLESPIE	4/18/2002	16387
BOON	HN	NC	LINCOLN	J ROBINSON	4/26/1970	5121
BOON	HN	NC	BURKE	JACKSON ET AL	5/5/1956	1522
BOON	HN	NC	CLEVELAND	NICK DROZDA	3/21/1996	16592
BOON	HN	NC	RUTHERFORD	NICK DROZDA	N/A	16597
BOON	HN	NC	BURKE	R D HARBISON	4/10/1971	7295
BOON	HN	NC	BURKE	SMITH ET AL	5/4/1956	1556
BOON	HR	NC	HENDERSON	K BERRY	4/8/1978	12513
BOON	HS	NC	AVERY	D BUFF	5/4/1967	4640
BOON	HS	NC	McDOWELLl	J PADGETT	4/18/2002	16386
BOON	HS	NC	McDOWELL	J PADGETT	4/18/2002	16391
BOON	HS	NC	McDOWELL	J PADGETT	4/18/2002	16390
BOON	HS	NC	McDOWELL	J PADGETT	4/18/2002	16400
BOON	HS	NC	MACON	M L HICKS	8/8/1978	12346
BOON	HS	NC	BURKE	R B HARBISON	5/8/1971	7294
BOON	HS	NC	BURKE	R B HARBISON	6/13/1971	5634
BOON	HS	NC	BURKE	R B HARBISON	6/13/1971	5639
BOON	HS	NC	BURKE	R B HARBISON	6/13/1971	6015
BOON	HS	NC	CHEROKEE	S R MORROW	10/21/1979	13063
BOON	HSp	AL	AUTAUGA	F C NACZI	5/28/1997	16491
BOON	HV	NC	WATAGUA	D BOONE	4/26/1977	11306
BOON	HV	NC	WILKES	D S GOFORTH	6/10/1971	5539
BOON	HV	NC	WATAGUA	GREER	6/22/1964	4302
BOON	HV	NC	STONE MT NC	HOLBROOK & GREER	5/2/1964	4407
BOON	HV	NC	CALDWELL	JACKSON ET AL	4/23/1956	1523
BOON	HV	NC	HENDERSON	P MORRISON	4/8/1978	13752
BOON	HV	NC	WATAGUA	W DOBY	3/29/1976	10393
BOON	HV	NC	WATAGUA	W HESTER	4/25/1977	11217
CONV	HAA	SC	SPARTANBURG	D L RICHARDSON	5/4/1978	5251
CONV	HAA	NC	DAVISON	LISA WILLIAMSON	3/30/1974	5020
CONV	HAA	SC	SPARTANBURG	T. ATWATER & G. WOOD	4/12/1988	5432
CONV	HAA	NC		TOM DASSY	5/29/1967	4419
CONV	HAA	SC	SPARTANBURG	W. MOORE & W. JOLLEY	4/12/1988	5516
CONV	HAR	TN	BLOUNT	H M JENNISON	5/1/1936	3060
CONV	HAR	NC	CLAY	S W LEONARD & K MOORE	6/6/1968	4310
CONV	HM	SC	SPARTANBURG	K MATZENGA & R VALDES	3/3/1976	5094
CONV	HM	VA	BOTETOURT	S S MOORE & K SIMMONS	5/6/1998	5094
CONV	HN	SC	SPARTANBURG	J BOWMAN & S HALEY	4/1/1968	3946
CONV	HN	SC	SPARTANBURG	M G McMILLANn	4/5/1967	3837
CONV	HN	SC	SPARTANBURG	P VOYLES	4/5/1967	2056
DUKE	HH	NC	ALEXANDER	CATHERINE KEEVER	5/10/1941	73375
DUKE	HH	SC	OCONEE	H L BLOMQUIST	6/16/1940	61099

DUKE	HH	SC	PICKENS	H L BLOMQUIST	5/3/1954	160588
DUKE	HH	NC	STOKES	H L BLOMQUIST	6/17/1945	160571
DUKE	HH	NC	STOKES	H L BLOMQUIST ET AL	4/24/1950	160605
DUKE	HH	NC	STOKES	H L BLOMQUIST ET AL	4/24/1950	160612
DUKE	HH	SC	PICKENS	LELAND RODGERS	5/4/1942	90679
DUKE	HH	SC	OCONEE	M R CROSBY & W R ANDERSON	4/25/1964	162907
DUKE	HH	VA	BUCHANAN	R KRAL	5/2/1965	178171
DUKE	HH	SC	OCONEE	R L WILBUR	6/2/1947	160604
DUKE	HH	NC	ALEXANDER	TOM DAGGY ET AL	5/16/1958	143017
DUKE	HH	NC	ALEXANDER	TOM DAGGY ET AL	5/16/1958	143018
DUKE	HH	NC	ALEXANDER	TOM DAGGY ET AL	5/16/1958	143001
DUKE	HH	NC	ALEXANDER	TUCKER CLINE & JAMES McNAIR	4/25/1972	362163
DUKE	HH	SC	OCONEE	W T BATSON	6/6/1950	138358
DUKE	HH	GA	UNION	WILBUR H DUNCAN	6/10/1942	N/A
DUKE	HM	NC	GRANVILLE	AHLES & RADFORD	4/26/1956	139119
DUKE	HM	NC	DURHAM	BETTY G BLACK	4/30/1954	160561
DUKE	HM	NC	WAKE	CARL MONK	5/16/1954	160537
DUKE	HM	NC	ORANGE	D S CORRELL	5/6/1935	134721
DUKE	HM	NC	RICHMOND	D S CORRELL	6/15/1935	136523
DUKE	HM	NC	WAKE	DEXTER HESS	1951-1952	151438
DUKE	HM	NC	DURHAM	H L BLOMQUIST	4/17/1932	19904
DUKE	HM	NC	DURHAM	H L BLOMQUIST	5/26/1945	160560
DUKE	HM	NC	NASH	H L BLOMQUIST	5/28/1945	160539
DUKE	HM	NC	PERSON	H L BLOMQUIST	4/9/1950	160538
DUKE	HM	NC	MOORE	H L BLOMQUIST	3/28/1952	160563
DUKE	HM	NC	GRANVILLE	H L BLOMQUIST	4/18/1953	160547
DUKE	HM	NC	PERSON	H L BLOMQUIST	4/10/1955	160711
DUKE	HM	NC	DURHAM	H L BLOMQUIST	N/A	489
DUKE	HM	NC	WAKE	H L BLOMQUIST	N/A	160562
DUKE	HM	NC	WAKE	M F BUELL	4/15/1956	37957
DUKE	HM	NC	ORANGE	MICHAEL W PALMER	4/28/1986	351682
DUKE	HM	NC	RANDOLPH	R K GODFREY ET AL	4/25/1948	126247
DUKE	HM	NC	STANLEY	R L WILBER	5/10/1963	154974
DUKE	HM	NC	WAKE	R L WILBUR	5/11/1965	169844
DUKE	HM	NC	WAKE	R L WILBUR	5/28/1974	237935
DUKE	HM	NC	CUMBERLAND	ROBERT A CLARK	5/1/1937	94629
DUKE	HM	NC	CHATHAM	TOM DAGGY ET AL	5/3/1958	143000
DUKE	HM	NC	DURHAM	W B DAVIS	3/13/1932	493
DUKE	HM	NC	MOORE	WILLIAM B FOX	5/13/1950	128501
DUKE	HM	NC	LEE	WILLIAM B FOX	5/13/1950	128503
DUKE	HM	NC	RICHMOND	WILLIAM B FOX	6/8/1950	128505
DUKE	HM	NC	FRANKLIN	Z E MURRELL	5/15/1991	339820
DUKE	HN	NC	CLEVELAND	AHLES & BELL	4/19/1956	166157
DUKE	HN	SC	CHEROKEE	AHLES & BELL	4/22/1956	166156
ETSU	HH	TN	CARTER	J C WARDEN	1977	12887
ETSU	HH	TN	WASHINGTON	W G PLESS	4/28/1959	11071
ETSU	HH	T	WASHINGTON	J PEARMAN	6/9/1956	11075

ETSU	HH	TN		J PAYNE	5/22/1966	154/488
ETSU	HH	TN		EE EASLY	4/30/1928	1543/11077
ETSU	HH	TN	UNICOI	F DAVISON	6/25/1961	1544/11094
ETSU	HH	TN	UNICOI	McGINLEIS	5/18/1956	1545/11098
ETSU	HH	TN	CARTER	A B BIGGERSTAFF	4/12/1956	1494/1081
ETSU	HH	TN	GREENE	R HOWE	5/15/1977	13085
ETSU	HH	TN	WASHINGTON	C Y LAFFITE	4/10/1957	1553/11068
ETSU	HH	TN	CARTER	C WILSON	5/18/1956	1547/11088
ETSU	HH	TN		J PAYNE	5/22/1966	486
ETSU	HH	TN	WASHINGTON	UNK	4/30/1952	1557/11078
ETSU	HH	TN	WASHINGTON	H SPARKS	4/24/1956	1556/11076
ETSU	HH	TN	WASHINGTON	G JOHNSON	4/24/1956	1558/11084
ETSU	HH	TN	WASHINGTON	GILBREATH	4/24/1956	1559/11086
ETSU	HH	TN	WASHINGTON	S CLINTON	4/24/1956	1560/11087
ETSU	HH	TN	WASHINGTON	TORRES	4/24/1956	1561/11089
ETSU	HH	TN	WASHINGTON	B J SAMS	4/15/1956	1562/11090
ETSU	HH	TN	SULLIVAN	L HOWARD	4/17/1972	1550/11081
ETSU	HH	TN	SULLIVAN	L HOWARD	5/5/1972	1549/11083
ETSU	HH	TN	UNICOI	T WILDS	6/30/1952	1570/11067
ETSU	HH	TN	UNICOI	E BAILCLIFF	5/21/1961	1569/11072
ETSU	HH	TN	UNICOI	HOUCHERS	4/2/1950	1568/11079
ETSU	HH	TN	WASHINGTON	J A WILLIAMS	4/24/1956	1567/11097
ETSU	HH	TN	WASHINGTON	P A WHITEHEAD	4/24/1956	1563/11091
ETSU	HH	TN	WASHINGTON	L LAWRENCE	4/24/1956	1564/11092
ETSU	HH	TN	WASHINGTON	PA PAYNE	4/24/1956	1565/11095
ETSU	HH	TN	WASHINGTON	M McCLELLAN	4/24/1956	1566/11096
ETSU	HH	TN	UNICOI	DELASHNIT	6/30/1952	1540/11065
ETSU	HH	TN		M MANNING	4/18/19??	1539/11066
ETSU	HH	TN		J SEAL	6/22/1958	1571/11085
ETSU	HH	TN	SULLIVAN	L KINKHEAD	3/7/1931	1530/11099
ETSU	HH	TN	GREENE	R HOWE	5/1/1977	16638
ETSU	HH	VA	SCOTT	R DAVIS	4/15/1978	N/A
GHH	HH	VA	BEDFORD	A H CURTIS	5/25/1877	N/A
GHH	HH	VA	ROANOKE	C E WOOD	6/14/1956	5996
GHH	HH	NC	MADISON	D E BOUFFORD ET AL	5/17/1974	N/A
GHH	HH	VA	HALIFAX	D E BOUFFORD ET AL	4/28/1982	22779
GHH	HH	SC	OCONEE	D E BOUFFORD ET AL	5/10/1982	22827
GHH	HH	VA	ROCKBRIDGE	E B BARTRAM	5/28/2009	N/A
GHH	HH	VA	ROANOKE	ET WHERRY & J W ADAMS	4/13/1936	N/A
GHH	HH	VA	RONOKE	ET WHERRY & J W ADAMS	4/13/1936	2651
GHH	HH	NC	JACKSON	F RUGEL	5/1844	24
GHH	HH	VA	ROCKBRIDGE	G G KENNEDY	4/28/1886	N/A
GHH	HH	NC	POLK	J R CHURCHILL	5/29/1899	N/A
GHH	HH	VA	BOTETOURT	R S FREER	3/31/1947	1194
GHH	HH	VA	ROCKBRIDGE	R S FREER	4/10/1947	1260
GHH	HH	SC	OCONEE	S R HILL & C N HORN	5/11/1989	20540
GHH	HH	TN	ROANE	V E McNEILUS	5/24/1987	N/A

GHH	HH	NC	ALEXANDER	W C GREGORY ET AL	6/15/1963	2639
GHH	HH?	TN	FENTRESS	H K SVENSON	6/17/1938	9017
GHH	HM	NC	ORANGE	A S PEASE	4/4/1939	27,014
GHH	HM	NC	N/A	D BOUFFORD & S SPONGBERG	4/26/1982	22773
GHH	HM	SC	CHEROKEE	J W HARDIN & R HARPER	4/16/1953	15491
GHH	HM	NC	WAKE	R K GODFREY	4/11/1938	3420
GHH	HM	NC	RANDOLPH	R K GODFREY ET AL	4/25/1948	48074
GHH	HN	SC	CHEROKEE	S R HILL	4/6/1989	20406
GHH	HN	SC	CHEROKEE	S W LEONARD & A E RADFORD	4/7/1969	2325
GHH	HV	VA	JAMES CITY	J T BALDWIN JR	2/17/1939	9
GHH	HV	NC	WILKES	S R HILL	4/12/1989	20426
GHH	HM	NC	DURHAM	R K GODFREY	5/4/1938	3800
GWU	HH	NC	RUTHERFORD	J COLE	4/20/1997	4741
GWU	HH	NC		M WRIGHT	3/8/1992	3252
GWU	HH	NC		P PRICE	3/21/1992	3268
GWU	HM	NC		B WILSON	4/12/1995	4225
GWU	HM	NC	CLEVELAND	C BAILEY	4/9/1997	4943
GWU	HM	NC	CLEVELAND	J SILVER	4/11/1997	4792
GWU	HM	NC		L LEE	3/7/9/2	3247
GWU	HM	NC		R L WRIGHT	4/12/1995	4357
GWU	HM	NC	CLEVELAND	S WARE	4/15/1997	5012
GWU	HN	NC	LINCOLN	B SAIN	4/14/2001	5917
GWU	HN	NC	RUTHERFORD	J BIGGERS	4/18/1992	3769
GWU	HN	NC		J PADGETT	3/15/1997	4515
GWU	HN	NC		J PADGETT	5/15/1998	6045
GWU	HN	NC		J PADGETT	4/2/2001	6039
GWU	HN	NC		J PADGETT	4/2/2001	6043
GWU	HN	NC		J PADGETT	4/4/2001	6044
GWU	HN	NC		J PADGETT	4/10/2001	6040
GWU	HN	NC		J PADGETT	5/10/2001	6042
GWU	HN	NC		J PADGETT	5/13/2001	6041
GWU	HN	NC		K McNEILY	5/3/1983	216
GWU	HN	NC		L SMITH	4/26/1975	219
GWU	HN	NC		M HOUSER	4/15/1995	3979
GWU	HN	NC		M LAIL	4/24/1983	217
GWU	HN	NC		T VINSETTE	1/3/1985	215
MOBOT	HH	NC	STOKES	S LEONARD & K MOORE	5/31/1968	2377113
MOBOT	HM	GA	STEPHENS	D E BOUFFORD ET AL	5/12/1976	2468716
MOBOT	HM	NC	UNK	W W ASHE	N/A	1985266
NYBG	HH	VA	BEDFORD	A BROWN ET AL	6/6/1890	N/A
NYBG	HH	VA	BEDFORD	A H CURTIS	5/15/1871	N/A
NYBG	HH	VA	BEDFORD	A H CURTIS	6/1868	N/A
NYBG	HH	VA	BEDFORD	A H CURTIS	6/1868	N/A
NYBG	HH	TN	GREENE	A J SHARP ET AL	5/17/1970	45209
NYBG	HH	TN	COCKE	B E WOFFORD	4/17/1979	79-44
NYBG	HH	VA	BEDFORD	CURTIS AND GARNER	5/1868	N/A
NYBG	HH	NC	MADISON	J S NEWBERRY	3/23/1891	N/A

NYBG	HH	NC	MADISON	J S NEWBERRY	5/1891	N/A
NYBG	HH	VA	SMYTH	N L & E BRITTON & M VAIL	6/22/1892	N/A
NYBG	HH	VA	N/A	N L BRITTON	9/2/1885	N/A
NYBG	HH	NC	STOKES	R KRAL	4/9/1960	9803
NYBG	HH	KY	HARLAN	T S PATRICK	4/5/1985	5556
NYBG	HH	TN	ROANE	V E McNEILUS	5/24/1987	N/A
NYBG	HM	VA	BEDFORD	A H CURTIS	5/15/1873	N/A
NYBG	HM	NC	MOORE	B FOX & S G BOYCE	5/13/1950	3609
NYBG	HM	NC	GASTON	C R BELL	4/19/1956	N/A
NYBG	HM	NC	ORANGE	LARRY A BURASKI ?	3/18/1976	553
NYBG	HM	NC	DURHAM	MARGARET P GREGORY	6/1/1944	N/A
NYBG	HM	NC	ORANGE	N/A	N/A	N/A
NYBG	HM	NC	WAKE	R K GODFREY	4/3/1937	N/A
NYBG	HM	NC	WAKE	STEVENS	3/16/1905	N/A
NYBG	HM	NC	ORANGE	W W ASHE	N/A	N/A
NYBG	HM	NC	ORANGE	W W ASHE	N/A	285575
NYBG	HN	SC	CHEROKEE	LENORD & RADFORD	4/7/1969	N/A
NYBG	HV	NC	WILKES	STEVEN R HILL	4/12/1989	N/A
UGH	HH	SC	PICKENS	D S CAMPBELL	4/24/1991	216331
UGH	HH	GA	HABERSHAM	G W McDOWELL & W DUNCAN	5/7/1950	50248
UGH	HH	NC	MADISON	H E AHLES & J A DUKE	4/26/1958	64092
UGH	HH	NC	STOKES	H L BLOMQUIST ET AL	4/24/1950	59580
UGH	HH	GA	STEPHENS	H M McKAY	4/15/1931	50247
UGH	HH	GA	UNION	L FOOTE	5/9/1964	102756
UGH	HH	GA	STEPHENS	M A GARLAND	5/28/1983	157397
UGH	HH	NC	BUNCOME	R WYATT	4/7/1991	200524
UGH	HH	TN	UNICOI	R WYATT	4/18/1993	202500
UGH	HH	NC	ALEXANDER	W C GREGORY ET AL	6/15/1963	92032
UGH	HM	SC	ANDERSON	H E AHLES & H E RADFORD	5/31/1956	64094
UGH	HM	SC	CHEROKEE	J HARDIN & R HARPER	4/16/1953	67685
UGH	HM	NC	GUILFORD	R KRAL	4/17/1966	107163
UGH	HM	NC	ORANGE	R WYATT	4/4/1970	158751
UGH	HM	NC	ORANGE	R WYATT	4/10/1970	158592
UGH	HN	NC	CLEVELAND	W C GREGORY ET AL	6/16/1963	92034
UGH	HV	VA	PULASKI	G P FRANK ET AL	5/1/1981	157247
UNCCH	HH	NC	MADISON	O M FREEMAN	6/5/1956	89893
UNCCH	HH	NC	STOKES	A E RADFORD	5/10/1953	56880
UNCCH	HH	NC	CALDWELL	A E RADFORD	5/12/1956	176089
UNCCH	HH	NC	ALEXANDER	A E RADFORD	5/13/1956	86899
UNCCH	HH	NC	STOKES	A E RADFORD	5/4/1968	176093
UNCCH	HH	TN	WASHINGTON	C E BEAUMONT & W W ASHE	5/22/1926	186371
UNCCH	HH	SC	SPARTANBURG	C R BELL	4/13/1957	176470
UNCCH	HH	NC	BURKE	C R BELL	4/27/1957	176087
UNCCH	HH	NC	CATAWBA	C R BELL	4/29/1957	176088
UNCCH	HH	NC	CATAWBA	C R BELL	6/12/1957	176123
UNCCH	HH	NC	POLK	D C PEATTIE	4/6/1937	14879
UNCCH	HH	NC	POLK	D C PEATTIE	4/13/1937	14888

UNCCH	HH	NC	POLK	D C PEATTIE	4/16/1937	14765
UNCCH	HH	NC	POLK	D C PEATTIE	4/16/1937	14917
UNCCH	HH	NC	MADISON	D E BOUFFORD ET AL	5/17/1974	307417
UNCCH	HH	NC	MADISON	D SATHER	5/28/1981	516491
UNCCH	HH	NC	HENDERSON	E R MEMMINGER	N/A	49426
UNCCH	HH	GA	STEPHENS	E W WOOD & D E BOUFFORD	6/19/1975	469940
UNCCH	HH	GA	STEPHENS	E W WOOD & D E BOUFFORD	6/30/1975	493054
UNCCH	HH	NC	GREENE	F BOWERS	5/17/1970	400860
UNCCH	HH	SC	ANDERSON	H E AHLES & A E RADFORD	3/31/1956	176121
UNCCH	HH	NC	MADISON	H E AHLES & J A DUKE	4/26/1958	176091
UNCCH	HH	NC	MICTHELL	H E AHLES & J A DUKE	6/16/1958	176090
UNCCH	HH	NC	IREDELL	H E AHLES & J McNEELY	4/19/1960	225123
UNCCH	HH	NC	IREDELL	H E AHLES & R BRITT	5/18/1958	184111
UNCCH	HH	SC	PICKENS	J E FAIREY III	5/22/1984	542063
UNCCH	HH	NC	TRANSYLVANIA	O M FREEMAN	4/24/1957	202355
UNCCH	HH	NC	STOKES	R KRAL	4/9/1960	217489
UNCCH	HH	NC	STOKES	R KRAL	4/9/1960	217785
UNCCH	HH	VA	PATRICK	R KRAL	4/20/1960	165406
UNCCH	HH	VA	CARROLL	R KRAL	4/22/1960	165937
UNCCH	HH	VA	MONTGOMERY	R KRAL	5/3/1960	161627
UNCCH	HH	VA	MONTGOMERY	R. KRAL	4/16/1960	217862
UNCCH	HH	VA	MONTGOMERY	R. KRAL	4/16/1960	127853
UNCCH	HH	NC	STOKES	RADFORD & STEWERT	6/1/1940	20728
UNCCH	HH	NC	STOKES	S LEONARD & A E RADFORD	4/23/1970	378299
UNCCH	HH	VA	APPOMATTOX	T F WIEBOLDT	5/9/1983	523692
UNCCH	HH	VA	MECKLENBURG	W D SEAMAN	4/10/1967	388850
UNCCH	HH	NC	HALIFAX	W E WES??? III	4/21/1967	296090
UNCCH	HH	TN	TOWN OF ERWIN	W W ASHE	5/1/1926	256037
UNCCH	HH	NC	POLK	W W ASHE	N/A	72633
UNCCH	HH	TN	WASHINGTON	W W ASHE	N/A	72629
UNCCH	HH	NC	STOKES	Y McCURDY	4/22/1974	473555
UNCCH	HM	NC	ORANGE	A E RADFORD	4/27/1946	31035
UNCCH	HM	NC	PERSON	A E RADFORD	4/4/1954	226411
UNCCH	HM	NC	RANDOLPH	A E RADFORD	4/13/1955	86921
UNCCH	HM	NC	WAKE	A E RADFORD	3/17/1956	86918
UNCCH	HM	NC	RANDOLPH	A E RADFORD	3/30/1956	86943
UNCCH	HM	NC	RANDOLPH	A E RADFORD	3/30/1956	86945
UNCCH	HM	NC	DAVIE	A E RADFORD	4/21/1956	173091
UNCCH	HM	NC	RANDOLPH	A E RADFORD	4/21/1956	173094
UNCCH	HM	NC	RICHMOND	A E RADFORD	5/19/1956	176469
UNCCH	HM	NC	DAVIDSON	A E RADFORD	6/16/1956	86944
UNCCH	HM	NC	MARTIAN	A E RADFORD	4/26/1958	176463
UNCCH	HM	NC	MONTGOMERY	A E RADFORD	5/24/1960	198160
UNCCH	HM	NC	MONTGOMERY	A E RADFORD	3/29/1961	249015
UNCCH	HM	NC	ANSON	A E RADFORD	5/20/1961	249025
UNCCH	HM	NC	GRANVILLE	A E RADFORD & A E AHLES	4/26/1956	86936
UNCCH	HM	NC	RICHMOND	A E RADFORD ET AL	4/3/1954	57377

UNCCH	HM	NC	N/A	B IVEY	3/31/1947	29582
UNCCH	HM	NC	GRANVILLE	B R DAYTON	4/23/1964	324377
UNCCH	HM	NC	ORANGE	B W WELLS	N/A	73902
UNCCH	HM	NC	GASTON	C R BELL	4/19/1956	176458
UNCCH	HM	SC	CHEROKEE	C R BELL	4/22/1956	174323
UNCCH	HM	SC	OCONEE	C R BELL	6/4/1956	176464
UNCCH	HM	NC	PERSON	C R BELL	4/22/1958	176365
UNCCH	HM	NC	RANDOLPH	C R BELL	5/27/1958	176468
UNCCH	HM	NC	POLK	D C PEATTIE	4/12/1937	14768
UNCCH	HM	NC	CUMBERLAND	D P JENSEN	3/30/1990	560748
UNCCH	HM	NC	ORANGE	E T BROWNE JR	4/10/1949	33776
UNCCH	HM	NC	ORANGE	G CHRISTENBERRY	3/22/1939	10651
UNCCH	HM	VA	FLUVANNA	G M DIGGS JR	4/20/1975	489867
UNCCH	HM	VA	APPOMATTOX	G W RAMSEY ET AL	6/20/1967	368214
UNCCH	HM	NC	CHATHAM	H E AHLES & M SEARS	3/19/1964	269593
UNCCH	HM	NC	VANCE	H E AHLES & C R BELL	4/15/1956	86920
UNCCH	HM	NC	NORTHAMPTON	H E AHLES & J A DUKE	3/31/1958	176169
UNCCH	HM	SC	CHEROKEE	H E AHLES & J G HEASLOOP	4/13/1957	174324
UNCCH	HM	NC	MONTGOMERY	H E AHLES & J G HEASLOOP	3/13/1965	271546
UNCCH	HM	NC	ORANGE	H HURLEY	4/15/XX	269563
UNCCH	HM	NC	HARNETT	H LAING	3/31/1957	176495
UNCCH	HM	NC	HARNETT	H LAING	3/31/1957	176103
UNCCH	HM	NC	HARNETT	H LAING	5/8/1957	176104
UNCCH	HM	NC	ORANGE	H SHERWIN	3/9/1943	73914
UNCCH	HM	NC	GUILFORD	J CAUSEY	N/A	12835
UNCCH	HM	NC	ORANGE	J G ULERIFRLY	4/9/1897	73907
UNCCH	HM	NC	ORANGE	J GLENN	4/11/1931	73913
UNCCH	HM	NC	MOORE	J H CARTER	4/8/1973	261808
UNCCH	HM	NC	ROWAN	J H HORTON	4/7/1957	198151
UNCCH	HM	NC	ORANGE	J LARKE	3/5/1990	558143
UNCCH	HM	NC	ORANGE	J R RAPER	4/15/1932	73903
UNCCH	HM	SC	LANCASTER	J W HARDIN & W H DUNCAN	4/21/1953	259271
UNCCH	HM	NC	GUILFORD	L MELVIN	3/24/1956	174800
UNCCH	HM	NC	GUILFORD	L MELVIN	3/29/1956	174799
UNCCH	HM	NC	MOORE	L MELVIN	5/13/1956	174802
UNCCH	HM	NC	ORANGE	L RUSH JR	5/6/1959	234625
UNCCH	HM	NC	LEE	L S BEARD	3/26/1955	176461
UNCCH	HM	NC	ORANGE	L W LYNCH	N/A	73911
UNCCH	HM	NC	ORANGE	L W OLSON	4/5/1964	255684
UNCCH	HM	NC	ORANGE	M MUNVH	3/13/1938	73912
UNCCH	HM	NC	ORANGE	N A BOATWRIGHT	4/11/1959	234617
UNCCH	HM	NC	ORANGE	N/A	4/3/1905	73905
UNCCH	HM	NC	ORANGE	N/A	N/A	73904
UNCCH	HM	NC	ORANGE	O WO HYMAN	4/5/1890	73910
UNCCH	HM	NC	ORANGE	P A KESSLER	2/21/1954	198120
UNCCH	HM	NC	CHATHAM	P A KESSLER	3/12/1956	86937
UNCCH	HM	NC	MOORE	P A KESSLER	5/16/1960	176462

UNCCH	HM	NC	ORANGE	P A WHITLOCK	2/27/1959	234630
UNCCH	HM	NC	CHATHAM	P J CRUTCHFIELD	5/30/1958	176122
UNCCH	HM	NC	MOORE	P KESSLER	4/2/1955	86932
UNCCH	HM	NC	MOORE	P KESSLER	4/17/1955	86938
UNCCH	HM	NC	ORANGE	R F BRITT	5/27/1957	178461
UNCCH	HM	NC	WAKE	R K GODFREY	4/4/1938	12036
UNCCH	HM	NC	ORANGE	RADFORD & STEWERT	4/4/1940	11467
UNCCH	HM	NC	STOKES	S W LEONARD & K MOORE	3/31/1968	323466
UNCCH	HM	NC	RICHMOND	T D NIFONG	4/17/1979	545244
UNCCH	HM	NC	N/A	TURRECTION ?	3/30/2001	73909
UNCCH	HM	NC	ORANGE	W C CONNER	4/11/1910	73908
UNCCH	HM	NC	ORANGE	W J KOCH	3/20/1943	32713
UNCCH	HM	NC	ROCKINGHAM	W MARTAIN	3/19/1966	275789
UNCCH	HM	NC	ORANGE	W W ASHE	4/1897	356927
UNCCH	HN	NC	LINCOLN	C R BELL	5/28/1957	176118
UNCCH	HN	NC	LINCOLN	C R BELL	9/9/1958	176119
UNCCH	HN	SC	GREENVILLE	O M FREEMAN	3/17/1956	86894
UNCCH	HR	NC	POLK	D C PEATTIE	N/A	73906
UNCCH	HR?	NC	POLK	D C PEATTIE	4/12/1937	14877
UNCCH	HV	NC	ROCKINGHAM	A E RADFORD	4/13/1956	86903
UNCCH	HV	NC	DAVIE	A E RADFORD	4/14/1956	86916
UNCCH	HV	NC	DAVIE	A E RADFORD	4/14/1956	86917
UNCCH	HV	NC	SURRY	A E RADFORD	4/16/1956	176092
UNCCH	HV	NC	SURRY	A E RADFORD	4/16/1956	87061
UNCCH	HV	NC	ALLEGHANY	A E RADFORD	5/2/1958	176094
UNCCH	HV	NC	STOKES	A E RADFORD	6/4/1958	176113
UNCCH	HV	NC	STOKES	A E RADFORD	6/4/1958	176112
UNCCH	HV	NC	ROCKINGHAM	A E RADFORD & H E AHLES	4/13/1956	176111
UNCCH	HV	NC	ROCKINGHAM	A E RADFORD & H E AHLES	4/13/1956	86907
UNCCH	HV	NC	FORSYTH	H E AHLES & R BRITT	5/17/1958	176097
UNCCH	HV	NC	WATAGUA	H E AHLES & R P ASHWORTH	5/4/1958	176114
UNCCH	HV	NC	WATAGUA	H E AHLES & R P ASHWORTH	5/4/1958	176504
UNCCH	HV	NC	PASQUOTANK	J W CHICKERING JR	4/1878	30108
UNCCH	HV	NC	GUILFORD	L MELVIN	4/12/1956	174804
UNCCH	HV	NC	GUILFORD	L MELVIN	4/25/1956	174801
UNCCH	HV	NC	ROBESON	R F BRITT	4/5/1958	184587
UNCCH	HV	VA	PATRICK	R KRAL	4/9/1960	217784
UNCCH	HV	VA	PATRICK	R KRAL	4/9/1960	217488
US	HH	VA	RONOKE	C E WOOD	6/14/1946	2051013
US	HH	VA	ALBEMARLE	E S RAWLINSON	5/10/1934	1622981
US	HH	NC	*ROAN MT*	J W CHICKERING JR	7/1/1880	796995
US	HH	TN	*RICH MT*	T H KEARNEY JR	4/25/1893	250060
US	HH	VA	AUGUSTA	W W EGGLESTON	N/A	1220664
USCH	HAA	SC	KERSHAW	A HOLLEY ET AL	4/10/1984	25742
USCH	HAA	SC	AIKEN	A M NIESEMANN	4/20/1969	7350
USCH	HAA	SC	KERSHAW	A T HOLLAND	7/16/1959	7362
USCH	HAA	SC	BAMBERG	B B BRANTLEY	3/31/1984	41691

USCH	HAA	SC	DALRINGTON	B E SMITH	5/28/1940	36349
USCH	HAA	SC	RICHLAND	BARTON & Kelley	3/8/1955	7386
USCH	HAA	SC	LEXINGTON	C A AULBACH-SMITH	3/31/1981	21698
USCH	HAA	SC	SALUDA	C A AULBACH-SMITH	4/17/1981	21687
USCH	HAA	SC	RICHLAND	C A AULBACH-SMITH	4/22/1982	25008
USCH	HAA	SC	JASPER	C A AULBACH-SMITH	3/16/1984	25451
USCH	HAA	SC	NEWBERRY	C H HORN	4/17/1987	40022
USCH	HAA	SC	BARNWELL	C L PORTER	8/17/1956	7357
USCH	HAA	SC	RICHLAND	C McCUTCHEN	4/23/1966	7388
USCH	HAA	SC	CALHOUN	C N HORN	4/18/1987	40021
USCH	HAA	AL	TUSCALOOSA	C N HORN	4/11/1992	64128
USCH	HAA	NC	COLUMBUS	C R BELL	4/25/1958	7393
USCH	HAA	SC	RICHLAND	COLUMBIA COLLEGE	2/12/1993	60966
USCH	HAA	SC	RICHLAND	COLUMBIA COLLEGE	4/16/1993	60665
USCH	HAA	SC	AIKEN	D A JOHNSON & J NELSON	4/6/1995	68678
USCH	HAA	NC	LEE	D CHEN	4/8/1965	36347
USCH	HAA	SC	LEXINGTON	D D DWEENEY	4/13/1992	59761
USCH	HAA	SC	RICHLAND	D H RENBERD?	4/26/1958	7371
USCH	HAA	NC	CLAY	D PITTILLO	6/12/1977	26220
USCH	HAA	SC	AIKEN	D SOBLO	3/14/1990	50506
USCH	HAA	SC	AIKEN	D SOBLO	3/14/1990	50507
USCH	HAA	SC	RICHLAND	ECOLOGY CLASS USCC	10/1/1927	7382
USCH	HAA	SC	KERSHAW	F McELVEEN	5/1/1964	7363
USCH	HAA	SC	PICKENS	G DOWNS	5/1/1975	2978
USCH	HAA	GA	DEKALB	G KEAFT	4/20/1965	7398
USCH	HAA	NC	ORANGE	G P SAWYER	4/3/1964	36348
USCH	HAA	SC	DARLINGTON	G P SAWYER	8/8/1975	2755
USCH	HAA	SC	RICHLAND	H HECHENBLEIKNER	3/7/1939	7375
USCH	HAA	SC	BERKELY	H TRAIT	4/30/1953	7358
USCH	HAA	SC	RICHLAND	J B NELSON	4/24/1984	32424
USCH	HAA	SC	LEE	J B NELSON	3/24/1989	49832
USCH	HAA	SC	GEORGETOWN	J B NELSON	3/28/1991	53913
USCH	HAA	SC	CLARENDON	J B NELSON ET AL	3/19/1986	33194
USCH	HAA	NC	ANSON	J B NELSON ET AL	3/28/1988	46722
USCH	HAA	SC	FAIRFIELD	J BASS	4/25/1965	7360
USCH	HAA	SC	SALUDA	J CROUCH	4/25/1965	7391
USCH	HAA	SC	CALHOUN	J E FAIREY ET AL	4/1/1961	7359
USCH	HAA	NC	GASTON	J E WARD & H J RICHARDS	4/13/1968	7392
USCH	HAA	SC	SUMTER	J F LUGUE	5/21/1982	40474
USCH	HAA	GA	PUTNAM	J H PYRON & R McVAUGH	4/2/1938	7394
USCH	HAA	SC	RICHLAND	J M BARRY	3/10/1967	7372
USCH	HAA	SC	LEXINGTON	J M BARRY	4/6/1967	7368
USCH	HAA	SC	RICHLAND	K R TREPANIER	4/21/1996	68851
USCH	HAA	SC	ALLENDALE	KELLEY & BATSON	3/30/1953	7352
USCH	HAA	SC	ALLENDALE	KELLEY & BATSON	4/6/1953	7351
USCH	HAA	SC	ALLENDALE	KELLEY & BATSON	4/6/1953	7356
USCH	HAA	SC	ALLENDALE	KELLEY & BATSON	5/11/1953	7353

USCH	HAA	SC	AIKEN	KELLEY & BATSON	4/1/1964	7349
USCH	HAA	SC	NEWBERRY	L H BUFF	8/12/1971	7370
USCH	HAA	SC	RICHLAND	L H ROBINSON	5/10/1966	7373
USCH	HAA	SC	LEXINGTON	L L SMITH ET AL	4/27/1966	7367
USCH	HAA	SC	SALUDA	L L SMITH ET AL	5/6/1966	7390
USCH	HAA	SC	LEXINGTON	L LOWENSTEIN	5/5/1960	7365
USCH	HAA	SC	RICHLAND	M J ROBINSON	4/23/1966	7383
USCH	HAA	SC	RICHLAND	M SAMPSON	4/15/1937	7377
USCH	HAA	SC	LEXINGTON	N/A	N/A	7387
USCH	HAA	SC	RICHLAND	N/A	N/A	7381
USCH	HAA	SC	RICHLAND	P J PHILSON	4/11/1936	7376
USCH	HAA	SC	RICHLAND	P J PHILSON	4/14/1936	7383
USCH	HAA	SC	RICHLAND	P J PHILSON	4/26/1940	7374
USCH	HAA	SC	RICHLAND	P J PHILSON	4/26/1940	7378
USCH	HAA	TN	COCKE	R D THOMAS	10/14/1989	50160
USCH	HAA	SC	DORCHESTER	R S HILL & D SOBLO	5/15/1988	50650
USCH	HAA	SC	AIKEN	R STICH	3/30/1992	57774
USCH	HAA	SC	LANCASTER	S CLYBURN	4/26/1958	7364
USCH	HAA	SC	FAIRFIELD	S GUERRY	4/19/1983	23366
USCH	HAA	SC	LEXINGTON	S L SMITH	5/8/1966	7366
USCH	HAA	SC	RICHLAND	T SMITH	N/A	7379
USCH	HAA	SC	RICHLAND	V UTSEY	N/A	7380
USCH	HAA	SC	AIKEN	W R KELLEY & W T BATSON	10/29/1951	7347
USCH	HAA	SC	AIKEN	W R KELLEY & W T BATSON	3/1/1952	7348
USCH	HAA	SC	McCORMICK	W T BATSON	4/16/1961	7369
USCH	HAA	SC	HAMPTON	W T BATSON	4/27/1988	45998
USCH	HAR	NC	CLAY	S W LEONARD & K MOORE	6/6/1968	7395
USCH	HH	SC	GREENVILLE	A E CRANDELL	9/18/1976	9119
USCH	HH	SC	GREENVILLE	A E CRANDELL	4/9/1977	9110
USCH	HH	SC	OCONEE	C H HORN	5/11/1989	52754
USCH	HH	SC	OCONEE	D MADSEN	3/3/1993	66017
USCH	HH	SC	OCONEE	D SOBLO	1/10/1991	52900
USCH	HH	SC	OCONEE	G P SAWYER ET AL	3/20/1965	36350
USCH	HH	SC	GREENVILLE	J B NELSON & S GREETER	3/30/1988	46718
USCH	HH	SC	OCONEE	J B NELSON & S MOFFAT	4/11/1989	48925
USCH	HH	SC	PICKENS	J E FAIREY III	5/22/1984	26472
USCH	HH	SC	PICKENS	J R CLONTS	8/21/1975	5643
USCH	HH	SC	PICKENS	W T BATSON	5/1/1954	7400
USCH	HM	SC	CHEROKEE	D A RAYNER	4/3/1986	48498
USCH	HM	NC	ORANGE	D CHEN	3/21/1965	36351
USCH	HM	SC	YORK	D E KENNEMORE & J B NELSON	5/18/1993	66287
USCH	HM	SC	YORK	D E KENNEMORE & K JSCKSON	3/28/1993	62648
USCH	HM	SC	YORK	D E KENNEMORE JR	4/19/1994	68475
USCH	HM	NC	RICHMOND	G P SAWYER & H AHLES	4/17/1964	36354
USCH	HM	SC	CHEROKEE	J B NELSON	3/17/1987	34754
USCH	HN	SC	GREENVILLE	D A RAYNER	4/21/1977	20729
USCH	HN	SC	GREENVILLE	D A RAYNER	5/19/1981	21514

USCH	HN	SC	GREENVILLE	D A RAYNER	5/21/1981	21511
USCH	HN	SC	GREENVILLE	D A RAYNER	6/1/1981	21516
USCH	HN	SC	SPARTANBURG	D A RAYNER	5/21/1985	47824
USCH	HN	SC	GREENVILLE	E A VERNON	5/10/1964	7361
USCH	HS	SC	PICKENS	C H HORN	5/9/1988	45627
USCH	HS	NC	BURKE	R HARBISON	6/13/1971	7397
USCH	HS	NC	TRANSYLVANIA	R JOHNSON	6/6/1993	62489
USCH	HS	SC	OCONEE	W T BATSON	5/29/1954	7401
UT	HH	TN	CARTER	A B GRINDSTAFF	4/12/1956	N/A
UT	HH	TN	COCKE	A J & EVELYN SHARP	10/12/1963	2170
UT	HH	TN	CARTER	A J & EVELYN SHARP	10/27/1963	2170
UT	HH	SC	PICKENS	A J SHARP	4/24/1955	2170
UT	HH	TN	CARTER	A J SHARP	5/5/1963	2170
UT	HH	TN	CARTER	A J SHARP	4/9/1967	2170A
UT	HH	TN	GREENE	A J SHARP	5/17/1970	2170A
UT	HH	TN	SULLIVAN	A J SHARP & C ELLIS	4/23/1979	2170A
UT	HH	TN	GREENE	A J SHARP & D K SMITH	9/23/1973	2170A
UT	HH	TN	CLAIBORNE	A J SHARP ET AL	6/10/1962	2170
UT	HH	TN	GREENE	A J SHARP ET AL	5/22/1986	2170A
UT	HH	TN	UNICOI	C LYLE	4/2/1955	2170
UT	HH	TN	UNICOI	E WOFFORD ET AL	7/13/1973	2170A
UT	HH	TN	COCKE	E WOFFORD ET AL	4/17/1979	2170A
UT	HH	TN	COCKE	E WOFFORD ET AL	4/17/1979	2170A
UT	HH	TN	COCKE	E WOFFORD ET AL	4/17/1979	2170A
UT	HH	NC	STOKES	H L BLOMQUIST ET AL	4/24/1950	2170A
UT	HH	TN	CARTER	J PEARMAN	6/9/1956	2170
UT	HH	NC	POLK	J R CHURCHILL	5/29/1899	2170A
UT	HH	TN	HAWKINS	J WOLFE	4/16/1955	N/A
UT	HH	TN	UNICOI	L L GADDY	10/7/1951	N/A
UT	HH	TN	CARTER	L L GADDY	6/12/1986	2170A
UT	HH	TN	UNICOI	L L GADDY	6/12/1986	2170A
UT	HH	TN	UNICOI	L L GADDY	6/12/1986	2170A
UT	HH	TN	UNICOI	L L GADDY	6/12/1986	2170A
UT	HH	TN	SULLIVAN	L L GADDY	6/12/1986	2170A
UT	HH	TN	SULLIVAN	L L GADDY	6/12/1986	2170A
UT	HH	TN	UNICOI	L L GADDY	6/13/1986	2170A
UT	HH	NC	MADISON	L L GADDY	6/30/1986	2170A
UT	HH	KY	BELL	L POUNDS	4/24/1985	2170A
UT	HH	TN	CARTER	R E SHANKS	9/11/1954	N/A
UT	HH	TN	CARTER	R E SHANKS & A J SHARP	7/24/1949	N/A
UT	HH	TN	UNICOI	R E SHANKS ET AL	9/7/1949	N/A
UT	HH	VA	LEE	R HINKLE	3/30/1974	2170A
UT	HH	KY	BELL	R HINKLE	4/12/1974	2170A
UT	HH	TN	UNICOI	R L JAMES	7/21/1952	2170
UT	HH	KY	BELL	T S PATRICK	5/4/1985	2170A
UT	HH	VA	SCOTT	T S PATRICK & B E PERKINS	4/18/1982	2170A
UT	HH	TN	ROANE	V E McNEILUS	4/7/1987	2170A

UT	HH	TN	ROANE	V E McNEILUS	4/9/1987	2170A
UT	HH	TN	ROANE	V E McNEILUS	5/24/1987	2170A
UT	HH	TN	ROANE	V E McNEILUS	4/8/1991	2170A
UT	HM	SC	LANCASTER	J HARDIN	4/21/1953	2170A
UT	HM	NC	ORANGE	W E KIRKLAND	3/2/1965	2170A
UT	HN	SC	CHEROKEE	S LEONARD & A E RADFORD	4/7/1969	2170A
UWI	HH	NC	MADISON	W W ASHE	3/11/1905	192
UWI	HH	NC	Boutes park CH	W W ASHE	4/5/1896	1372
UWI	HH	NC	POINT CREEK	W W ASHE	5/30/1898	192
UWI	HH?	TN	COCKE	A J & EVELYN SHARP	10/12/1963	32484
UWI	HM	NC	WINSTON-SALEM	W W ASHE	6/1897	192
UWI / US	HH	NC	CALDWELL	J K SMALL	6/20/1891	18262
VPI	HH	VA	RONOKE	L J UTTAL	4/13/1969	17715
VPI	HH	VA	BOTETOURT	A B MASSY	5/4/1940	36,873
VPI	HH	VA	MONTGOMERY	A B MASSY	5/16/1940	36876
VPI	HH	VA	MONTGOMERY	A B MASSY	4/22/1953	35,637
VPI	HH	VA	MONTGOMERY	A B MASSY	N/A	405
VPI	HH	VA	ROCKBRIDGE	B LONG	N/A	56722
VPI	HH	VA	MONTGOMERY	E A SMYTH	6/1893	35,635
VPI	HH	SC	ANDERSON	F EARLE	2/26/1905	35,640
VPI	HH	VA	ROCKBRIDGE	FREER	4/5/1966	44.637
VPI	HH	VA	BOTETOURT	FREER	4/21/1966	44,648
VPI	HH	VA	AMHERST	FREER ET AL	4/6/1966	44,532
VPI	HH	TN	UNICOI	G GONSOULIN	4/6/1974	N/A
VPI	HH	VA	PULASKI	G P FRANK ET AL	5/1/1981	68445
VPI	HH	VA	AMELIA	J B LEWIS	4/19/1905	3349
VPI	HH	VA	AMELIA	J B LEWIS	4/19/1905	36,875
VPI	HH	VA	AMELIA	J B LEWIS	4/12/1938	N/A
VPI	HH	VA	WASHINGTON	J C LUDWIG	5/7/1993	88425
VPI	HH	??	*BRUSH*MT	JSC	5-10-XX	8586
VPI	HH	VA	RONOKE	L J UTTAL	4/13/1969	39626
VPI	HH	VA	PULASKI	L J UTTAL	5/22/1969	27,048
VPI	HH	VA	RONOKE	L J UTTAL	5/5/1970	50158
VPI	HH	VA	PULASKI	L J UTTAL	5/7/1970	50127
VPI	HH	VA	PULASKI	L J UTTAL	5/7/1970	50145
VPI	HH	VA	LEE	L J UTTAL	6/5/1970	16,146
VPI	HH	VA	PATRICK	L J UTTAL	5/4/1971	51620
VPI	HH	VA	PATRICK	L J UTTAL	5/4/1971	25227
VPI	HH	VA	PULASKI	L J UTTAL	4/24/1975	60458
VPI	HH	TN	UNICOI	L J UTTAL	5/7/1985	77710
VPI	HH	NC	STOKES	R KRAL	4/9/1960	35,629
VPI	HH	VA	MONTGOMERY	R KRAL	4/16/1960	35,628
VPI	HH	VA	PATRICK	R KRAL	4/20/1960	35,636
VPI	HH	VA	CARROLL	R KRAL	4/22/1960	35,626
VPI	HH	VA	MONTGOMERY	R KRAL	5/1/1960	35,630
VPI	HH	VA	MONTGOMERY	R KRAL	5/3/1960	35,622
VPI	HH	VA	MONTGOMERY	R KRAL	5/23/1960	35,627

VPI	HH	VA	MONTGOMERY	R KRAL	4/26/1961	18,380
VPI	HH	VA	CAMPBELL	T F WIEBOLDT ET AL	5/13/1979	69977
VPI	HH	VA	SMYTH	T F WIEBOLDT ET AL	6/14/1980	79025
VPI	HH	VA	ALBEMARLE	T F WIEBOLDT ET AL	5/15/1982	72101
VPI	HH	VA	NELSON	T F WIEBOLDT ET AL	5/17/1982	72098
VPI	HH	VA	ROCKINGHAM	T F WIEBOLDT ET AL	5/17/1982	72099
VPI	HH	VA	PULASKI	T F WIEBOLDT ET AL	6/3/1982	72220
VPI	HH	VA	PULASKI	T F WIEBOLDT ET AL	6/3/1982	72219
VPI	HH	VA	FLOYD	T F WIEBOLDT ET AL	6/3/1982	72221
VPI	HH	VA	APPOMATTOX	T F WIEBOLDT ET AL	5/9/1983	73548
VPI	HH	VA	PRINCE EDWARD	T F WIEBOLDT ET AL	5/17/1984	76520
VPI	HH	VA	BUCKINGHAM	T F WIEBOLDT ET AL	5/17/1984	76519
VPI	HH	VA	MONTGOMERY	T F WIEBOLDT ET AL	5/20/1984	86380
VPI	HH	VA	PITTSYLVANIA	T F WIEBOLDT ET AL	4/23/1985	77929
VPI	HH	VA	ROCKBRIDGE	T F WIEBOLDT ET AL	6/9/1986	79273
VPI	HH	VA	CUMBERLAND	T F WIEBOLDT ET AL	5/11/1990	88026
VPI	HH	VA	WASHINGTON	W F RUSKA & S BENTLEY	5/10/1981	68512
VPI	HM	GA	RABUN	A E LANGLEY	4/27/1973	76099
VPI	HM	VA	APPOMATTOX	G W RAMSEY ET AL	6/20/1967	44,695
VPI	HM	VA	CHESTERFIELD	J C LUDWIG	4/9/1989	88397
VPI	HM	NC	MONTGOMERY	L J UTTAL	4/6/1976	62456
VPI	HM	NC	CHATHAM	P KESSLER	3/12/1956	17,323
VPI	HM	VA	MECKLENBURG	T F WIEBOLDT ET AL	5/12/1990	88071
VPI	HM	VA	MECKLENBURG	T F WIEBOLDT ET AL	5/12/1990	88072
VPI	HN	SC	CHEROKEE	S LEONARD & A E RADFORD	4/7/1969	60715
WOFF	HAA	SC	RICHLAND	DANNY HOLLIFIELD	4/1/1989	N/A
WOFF	HAA	SC	GREENVILLE	JAMAS GARDIN	4/16/1992	N/A
WOFF	HAA	SC	RICHLAND	SUZIE CHRISTOS	4/1/1989	N/A
WOFF	HH	SC	GREENVILLE	BRENDA WICHMANN	4/3/1998	N/A
WOFF	HH	SC	GREENVILLE	E E ELKINS	4/3/1992	N/A
WOFF	HH	SC	GREENVILLE	I B PARNELL	4/30/1998	N/A
WOFF	HH	SC	GREENVILLE	REGINA AYRES	5/14/1992	N/A
WOFF	HM	SC	YORK	SHAUNA D. CANNON	4/24/1990	N/A
WOFF	HM	SC	YORK	ZENOBIA L. COLLINS	5/10/1990	N/A
WOFF	HN	SC	SPARTANBURG	BRENDA WICHMANN	3/19/1998	N/A
WOFF	HN	SC	SPARTANBURG	D A RAYNER	4/15/1991	N/A
WOFF	HR	NC	POLK	GEORGE HUIZINGA	N/A	N/A
WOFF	HR	NC	POLK	HUGH BRADBURN	5/1/1993	N/A
WOFF	HR	NC	POLK	J E DOMBROSKI	4/4/1992	N/A
WOFF	HR	NC	POLK	MELISSA SHOULE	5/3/1990	N/A
WOFF	HS	NC	POLK	D A RAYNER	6/10/1991	N/A

APPENDIX B

Sites where flower and leaf materials were collected

***Hexastylis naniflora* sites visited 2001-2003**

County	State	EO #	Location
Alexander	NC	NA	US 64 and Hunter Bridge Road
Burke	NC	NC-005	Will Hudson Rd, SR 1910
Burke	NC	NC-011	Pleasant Grove Site, SR 1924
Burke	NC	NA	Corn Hill Rd off of Sugarloaf Rd
Caldwell	NC	NC-044	Little Gunpowder Creek, SR 1108
Catawba	NC	NC-021	Catawba River at US 321
Catawba	NC	NC-022	Murray's Mill at Balls Creek, SR 1003
Catawba	NC	NC-030	W of Tate Blvd., SR 1476
Catawba	NC	NC-031	Between I-40 and US 70 near Fairgrove
Catawba	NC	NC-039	Shiloh Church, Murray's Mill Lake, SR1824
Catawba	NC	NC-042	Bunker Hill Bridge
Catawba	NC	NA	Greedy Hwy and Hudson Road
Catawba	NC	NA	SR 1692 Fairgrove
Catawba	NC	NA	Conally Springs
Cleveland	NC	NC-001	Brushy Creek Bluff
Cleveland	NC	NC-008	Poundingmill Creek
Cleveland	NC	NC-014	Sandy Run Bluff Site, College Farm Road
Cleveland	NC	NC-017	Sandy Run Creek 1 miles west fo Boiling Springs
Cleveland	NC	NC-018	Sandy Run Creek, SR 1164
Cleveland	NC	NC-028	Cleveland County Landfill
Cleveland	NC	NC-046	Buffalo Creek, SR 1908
Cleveland	NC	NC-049	IP Tract (Now DOT-Greenway)
Cleveland	NC	NC-050	IP Tract (Now DOT-Greenway)
Cleveland	NC	NC-051	IP Tract (Now DOT-Greenway)
Cleveland	NC	NA	Along Leaman Gap Road just inside county
Cleveland	NC	NA	Dirty Ankle Road from Leaman Gap Road
Lincoln	NC	NC-002	Cat Square, Exerpated
Lincoln	NC	NC-015	Off US 274 3 miles N of Cherryville
Lincoln	NC	NA	SR 1104 Near new bridge
Polk	NC	NC-023	E of Kross Keys, N of NC 9 and E of SR1338
Rutherford	NC	NC-009	Henson's Creek Ravine
Rutherford	NC	NC-010	Kudzu Cow Farm Site
Rutherford	NC	NC-013	Sandy Mush Rock Outcrop
Rutherford	NC	NC-016	Off US 221 near Danieltown, Exerpated
Rutherford	NC	NC-037	Hunter Road, SR 1124, behind trailer
Rutherford	NC	NC-040	Jonas Rd. SR-1109
Rutherford	NC	NC-041	Pot Branch
Rutherford	NC	NC-052	Dills Creek Tributary
Rutherford	NC	NC-053	Broad River near SR 1111 from Bridge go North
Rutherford	NC	NC-054	Jebb Lamb Road, SR 1108 at McKinney Creek
Rutherford	NC	NC-055	Off SR 1111 below house on Dan River Prop.
Rutherford	NC	NC-056	Danieltown south to Henson Rd. to Floyd's Creek
Rutherford	NC	NC-057	Alexander Mills off 221A along RXR right of way
Rutherford	NC	NA	Dan River Property off SR 1111 at pond

Rutherford	NC	NA	Dan River Property across Richardson Creek
Rutherford	NC	NA	Duke Power-Crescent Industries along Broad River
Rutherford	NC	NA	Duke Power-Crescent Industries along Broad River
Rutherford	NC	NA	Broad River near Railroad Trestle
Rutherford	NC	NA	Harris NC off of Road along Floyds Creek
Rutherford	NC	NA	Harris NC off of Hogan's Road along Floyds Creek
Cherokee	SC	SC-016	Cowpens National Battlefield
Cherokee	SC	SC-017	Cowpens National Battlefield
Cherokee	SC	SC-018	Cowpens National Battlefield
Greenville	SC	SC-015	Bunched Arrowhead Preserve
Spartanburg	SC	SC-039	Landrum
Spartanburg	SC	SC-043	Landrum, back of 184 McKee Dr.
Spartanburg	SC	SC-027	Peters Creek Preserve
Spartanburg	SC	SC-028	Peters Creek Preserve
Spartanburg	SC	SC-032	Page Creek
Spartanburg	SC	SC-034	Arrowood Branch
Spartanburg	SC	SC-026	Peters Creek Preserve
Spartanburg	SC	SC-019	USCS Campus
Spartanburg	SC	SC-011	Peters Creek Preserve
Spartanburg	SC	SC-014	Peters Creek Preserve

***Hexastylis heterophylla* and *Hexastylis minor* sites visited 2001-2003**

Species	**County**	**State**	**Location**
HH	Caldwell	NC	HWY64/90
HH	Catawba	NC	Bunkerhill Bridge,
HH	Iredell	NC	Harris Bridge Rd.
HH	Madison	NC	Hickey's Fork
HH	Madison	NC	AT Trail near Hot Springs
HH	Polk	NC	Green River Cove
HH	Rutherford	NC	Luckadoo Mt 1
HH	Rutherford	NC	Camp McCall Road 2
HH	Rutherford	NC	Jonestown Road x Mt. Pleasant Church Rd.
HH	Wilkes	NC	Brocktown Rd 1
HH	Wilkes	NC	Brocktown Rd 2
HH	Wilkes	NC	Wilkes Community College
HH	Wilkes	NC	Brocktown Rd 3
HH	Buchanan	VA	Rd. 628
HM	Cleveland	NC	Broad River Greenway in plot
HM	Gaston	NC	Crowder’s Mt. St. Park.
HM	Moore	NC	HWY 22 on Deer River
HM	Randolph	NC	Randolph Co.
HM	Richmond	NC	Marshland off Hwy 1 near Masrton
HM	York	SC	Kings Mountain State Park.

APPENDIX C

Coordinates for *Hexastylis naniflora* populations

DD.DD N	DD.DD W
35.1914	81.9069
35.2028	81.9219
35.3067	81.9206
35.2081	81.8736
35.2089	81.6950
35.2103	81.8758
35.2108	81.9125
35.2114	81.8983
35.2119	81.8969
35.2125	81.8656
35.2136	81.8736
35.2150	81.6794
35.2153	81.6944
35.2161	81.6792
35.2167	81.8806
35.2192	81.8830
35.2217	81.6844
35.2222	81.9333
35.2247	81.6922
35.2253	81.0561
35.2267	81.6992
35.2289	81.8942
35.2292	81.6981
35.2292	81.9314
35.2317	81.0639
35.2317	81.9000
35.2319	81.9000
35.2333	81.9264
35.3333	81.9314
35.2620	81.9056
35.2667	81.8544
35.2686	81.8590
35.2800	81.6820
35.2825	81.5847
35.2847	81.5703
35.3075	81.8520
35.3086	81.9208
35.3189	81.6194
35.1264	81.3056
35.1816	81.9013
35.0503	82.0921

DD.DD N	DD.DD W
36.6125	81.4380
35.6347	81.4364
35.6403	81.5958
35.6447	81.3928
35.6686	81.3317
35.6697	81.5972
35.6700	81.0944
35.6728	81.5789
35.6742	81.1083
35.6786	81.0861
35.6836	81.3428
35.6972	81.1481
35.6975	81.4228
35.7022	81.2944
35.7047	81.3878
35.7175	81.2694
35.7194	81.1158
35.7408	81.8342
35.7597	81.5181
35.7611	81.3731
35.7711	81.6214
35.8189	81.4386
35.2043	81.9841
35.2124	81.9765
35.1571	82.2702
35.1443	82.1805
35.1818	82.0338
35.2243	82.0756
35.1269	81.8094
35.4353	81.2480
35.1230	81.7677
35.1075	82.2265
35.1766	82.1477
35.0195	82.4104
35.0227	82.3988
35.1063	81.9256
35.0221	82.3808
34.9882	81.8650
35.1572	82.1815
35.5120	82.1776
35.1809	82.1622

DD.DD N	DD.DD W
35.3306	81.4794
34.9709	81.9627
35.3514	81.4086
35.3792	81.6431
35.4203	81.4108
35.9952	81.8635
34.9718	81.9562
34.9952	82.4029
35.4203	81.2463
35.5375	81.4278
35.5375	81.4167
35.5406	81.4200
35.5464	81.1597
35.5519	81.7094
35.5594	81.5386
35.5764	81.5572
35.5819	81.5375
35.1267	81.8052
34.9991	81.9708
34.9002	81.9350
34.0406	82.2116
35.1004	82.0367
35.1017	82.0367
35.0907	81.8869
35.0681	81.0963
35.1067	81.9256
35.1742	81.1714
35.1264	81.3056
35.1816	81.9013
35.0503	82.0921
35.1572	82.1815
35.5120	82.1776
35.1809	82.1622
35.1909	82.1428
35.1279	81.4940
35.1139	81.7469
35.1328	81.4805
35.1279	81.4940
35.1139	81.7469
35.1328	81.4805
35.1909	82.1428

APPENDIX D

Recovery Plan for *Hexastylis naniflora*

PROPOSED RECOVERY PLAN FOR *Hexastylis naniflora*:

This recovery plan was written using the U. S. Fish and Wildlife Service (USFWS) recovery plan for *Liatris helleri* Porter as a template. In this recovery plan, taxonomy and ecology in *H. naniflora* are not addressed because they are addressed in other parts of the report.

It is worth mentioning here a few notable people who have contributed to the conservation efforts of *H. naniflora.* Blomquist (1957) described *H. naniflora* and stated that it was rare and restricted to a small area of North and South Carolina. It would be another twenty years before L. L. Gaddy (1980, 1981, and 1987) would address the conservation issues regarding *H. naniflora*

In April of 1989 the Department of the Interior formally listed *H. naniflora* as a federally Threatened species and afforded it some protection. In the late 1980s and early 1990s, Dr. Gillian Newberry (1995, 1996) made progress in developing techniques for moving and transplanting *H. naniflora* populations that were in danger of being destroyed. Her techniques have been used in recent moves of the plant from North Carolina Department of Transportation (NCDOT) construction sites. Dr. Newberry was instrumental in the location of a large number of new populations in South Carolina and a few new sites in North Carolina. With the number of known sites increasing over time and with a few sites already receiving some protection, conservation efforts have greatly improved the outlook for *H. naniflora* and afford the USFWS a rare opportunity to move towards delisting *H. naniflora*.

Current Status:

*Hexastylis naniflor*a is listed as a Federally Threatened plant species. It is currently known from approximately 150 populations and sub-populations in an eleven county area of North and South Carolina. Declines in known populations have occurred in Lincoln and Rutherford Counties in North Carolina as well as Spartanburg and Greenville Counties in South Carolina. The reasons for those declines range from highway construction and lake construction to urban sprawl and logging. Also, habitat destruction from pasture and small pond development has eradicated a number of populations.

Habitat Requirements and Limiting Factors:

Hexastylis naniflora is a very restricted species. Even with the seemingly high number of populations present, the actual numbers of individual plants vary greatly. Some populations have as few as twenty individuals while others may have upwards of 2000. The reason for this variance in population size is due mainly to the distribution of soil types on which *H. naniflora* is found to grow. *Hexastylis. naniflora* is restricted to acidic sandy-loam soils such as Pacolet, Madison, and Museulla soils. Recent soil analyses show that soil chemistry is very important to the location of *H. naniflora* (Padgett et al. 2003). Topography seems to play a part in *H. naniflora* location in any given habitat. It generally grows on the north facing side of slopes and ravines.

Recovery Objective:

Delisting of the species from the Endangered Species List.

Recovery criteria:

Hexastylis naniflora will be considered recovered when ten healthy populations are self-sustaining within its historical distribution and that the locality of those populations contains substantial genetic variability between them. If these conditions are met this species should no longer meet the criteria or definition of a Threatened species as outlined in the Endangered Species Act of 1973. A population that reproduces and is large enough to maintain genetic variability to survive and respond to natural changes in the habitat and environment will meet the criteria as a population healthy enough to receive protection. *Hexastylis naniflora* should be considered for delisting when the following criteria are met.

1. Of the 150 plus known populations and sub-populations of *H. naniflora* which are known to exist, at least twenty should be offered some sort of protection with ten populations receiving greater protection.

2. Management of those protected populations should be done in cooperation with the landowners and the necessary government agencies, and that any and all such actions should be well documented to ensure that future protection of those sites is not an issue.

3. With the location of new sites over time, at least one site per ten new sites found should be set aside and protected especially if they fall into locations where genetic variability might be of concern.

4. With the original ten sites placed under protection, ensure than any future human encroachments or natural threats are dealt with and that the survival of those sites is ensured.

Actions needed:

1. Survey of suitable habitats without *H. naniflora* present as possible transplant locations.

2. Monitor sites already under some protection.

3. Pollination studies.

4. Conduct research into threats on *H. naniflora* and its habitat, both biotic and abiotic.

5. Implement management practices at all key sites.

6. Involve the public though media and educational efforts.

7. Genetic analysis of intra-specific variation.

Date of Recovery:

The de-listing date is not known at this time.

Management and Recovery Plan for *Hexastylis naniflora*

With the status of *H. naniflora* being a Federally and State Threatened plant species in both North Carolina and South Carolina, efforts should be made to protect a set number of populations across the natural range to ensure its survival. The ultimate goal is to have *H. naniflora* de-listed, but to do that a substantial number of viable populations with intact plant communities must be set aside and given protection. Another consideration when setting aside protected sites should be the plant's ability to transfer genetic material in order to maintain a self-sustaining population. With the pollination mechanisms not well known, a study of pollination vectors might be required before any recovery plan can be successful. In order for the delisting and recovery of *H. naniflora* to be successful, the following criteria must be met.

1. Of the 150 plus known populations and sub-populations of *H. naniflora* which are known to exist, at least twenty should be offered some sort of protection with ten populations receiving greater protection.

2. Management of those protected populations should be done in cooperation with the landowners and the necessary government agencies, and that any and all such actions should be well documented to ensure that future protection of those sites is not an issue.

3. With the location of new sites over time, at least one site per ten new sites found should be set aside and protected especially if they fall into locations where genetic variability might be of concern.

4. With the original ten sites placed under protection, ensure than any future human encroachments or natural threats are dealt with and that the survival of those sites is ensured.

The timetable for a recovery and management plan of *H. naniflora* could proceed quickly if all the agencies and individuals involved can work towards getting critical habitat under protection either by outright purchase of property or by mitigation for sites. After ten good sites are protected, the USFWS could start the proceedings for a delisting of *Hexastylis naniflora* from the Endangered Species List.

Narrative Outline:

Hexastylis naniflora is an herbaceous evergreen perennial found in the western piedmont and foothills of North and South Carolina. It is limited in range due to its need for acidic sandy-loam soils and topographic locality. It is also generally restricted to stream heads and the moist ridges and hills adjacent to those streams, provided they are north facing and have suitable habitat. It's associated with a number of species that are found to be frequent in those same habitats, so when locating new populations, associate species information is very useful in locating favorable habitat. Of the 150 plus *H. naniflora* sites located in North Carolina and South Carolina, only a few are under any sort of protection. In the past, suitable habitat for *H. naniflora* was destroyed for use as pastureland, ponds, lakes, and peach orchards, which are all found frequently around the stream head habitats where *H. naniflora* is generally located. Only one site falls under Federal protection (Cowpens National Battlefield) and a few others fall under some sort of State or local protection. Spartanburg Waterworks currently has one of the largest populations of *H. naniflora* with some formal local protection. Other sites of interest with a large populations of *H. naniflora*, and has some protection with the Natural Heritage Program are Henson's Ravine in southern Rutherford County, NC and Peter's Creek Heritage Preserve in northern Spartanburg County, SC.

Management Issues:

1.1 The first step will be setting aside ten well-protected viable *Hexastylis naniflora* populations. There are currently four or five *H. naniflora* sites, which are receiving some sort of protection at the Federal, State, or Local level. With more sites protected across its historical range, a delisting of *H. naniflora* can proceed with little or no worry about long-term survival of the species.

1.2 Search for additional population should be encouraged and documented with the proper agencies. In recent years the number of known *H. naniflora* populations has increased dramatically, but those sites generally harbor small numbers of individuals due to the habitat restrictions of this plant. The historical range of *H. naniflora* has changed over time as well with several counties found to have small populations located in them. Additional populations might give rise in an increased number of protected sites over time, which further aids in the recovery of the species.

1.3 Habitat protection should be considered when setting aside *Hexastylis naniflora* populations for protection. Well-maintained habitats offer a higher species diversity and provide a more stable environment for *Hexastylis naniflora.*

1.4 North Carolina Department of Transportation (NCDOT) has to mitigate for *Hexastylis naniflora* when Highway right-of-way comes in contact with populations of *Hexastylis naniflora.* The process of mitigation cost the taxpayers millions of dollars each year when mitigation takes place. With mitigation dollars, NCDOT could help to place a number of *H. naniflora* sites into protection, which should allow the US Fish and Wildlife Service to consider the process of delisting.

1.5 The USFWS would benefit from a delisting by focusing their attentions on other more important issues at hand. The legal issues that USFWS faces from outside groups, which are in contest with them over their actions regarding the Endangered Species Act of 1973, would be reduced through delisting of *H. naniflora*.

1.6 Develop management plans and research programs at protected sites, which include, USFWS, NCDOT and the landowners. A working partnership between those agencies directly associated with *H. naniflora* and the landowners where protected sites may fall will be crucial for the future conservation of those sites. With this type of management practice now well developed, a close working relationship between those involved in management and protection should be maintained in order to promote the survival of *H. naniflora.*

1.7 Look at protection alternatives for *Hexastylis naniflora*. There are two areas of major interest here. The first would be to find suitable sites that are currently protected which might have no *H. naniflora* located on them, but might be used for re-location of populations in danger of being destroyed. Re-establishment or establishment of *H. naniflora* into an area must be looked at in further detail. Seed collection and propagation should be studied in order to have success in any such attempts. Another alternative to protecting sites is through transplanting. In the fall of 2000, 175 *H. naniflora* plants were transplanted onto an adjacent site along Little Gunpowder Creek in Caldwell County, North Carolina using a technique developed by Dr. Gill Newberry (1996) at the University of South Carolina. After three years and harsh drought conditions, 68% of the initial transplants were still alive. With this site, the conditions for *H. naniflora* were pre-existing because of plants growing adjacent to site which was to be destroyed. If a suitable site is not adjacent to a proposed site to be destroyed, then special attention must be paid to the soils and topography of any site thought to be favorable. The second initiative would entail cultivating a number of *Hexastylis naniflora* plants in greenhouse(s) for the purpose of providing a seed bank. This would ensure that genetic variability is maintained and if a protected site were destroyed by some natural occurrence, that replacement plants for that site would be available.

1.8 Populations that are protected or otherwise should be give a rating for size and habitat quality. Each existing known population of *H. naniflora* should be examined and a rate given for the number of individuals in that population and a score given for the quality of that habitat. Once each population has been scored, they can then be monitored for short and long term effects on them. The following are examples of scoring systems, which might be used.

Table 1. Class scoring that might be used for data collection regarding population size of *H. naniflora.*

Population Size	**Class for Population Size**
< 50	**1**
50-100	**2**
100-300	**3**
300- 500	**4**
500-1000	**5**
> 1000	**6**

Table 2. Habitat scoring that might be used in data collection regarding habitat quality of *H. naniflora* populations

Grades for Habitat
A - Excellent habitat. Mature forest with all the elements of the forest community present.
B - Very good habitat with maturing trees and all elements of the forest community present.
C - Above average habitat. Most of the elements of the forest community still in present.
D - Average habitat. Logging in the past 50 years evident by tree size and some elements of the forest community missing.
E - Below average habitat. Recent logging, erosion or urban sprawl apparent with a lot of the element of the forest community missing.
F – Poor habitat. Clear cut, or recent logging. Erosion massive and urban sprawl eminent. Most of the elements of the forest community are missing. Existing plants are imperiled.

Monitoring of *H. naniflora* populations can give information regarding abiotic and biotic factors within those sites. The effects of weather such as periods of drought and excess moisture can be examined. The effects of human impact on sites can be examined as well by looking at foot trample, the effects of logging, and burning (prescribed or natural) on existing sites. This information would be very valuable to individuals and agencies that are trying to develop and set up management plans on existing sites under protection or those proposed to be protected.

1.9 Designate and enforce laws to protect *Hexastylis naniflora* and its habitat. With protection comes enforcement of those protected site. North Carolina prohibits the taking of this species without a permit and the landowner's permission and regulates trade in the species. Signs should be placed in high-risk areas where collection might occur. Unwanted attention should not be given to the species in any location where it might be collected or removed. Law enforcement agents whose jurisdiction includes protected sites should be made aware of the status of *H. naniflora* and should be taught how to identify the species. Anyone caught digging; cutting, removing or destroying plants in knowing violation without a permit should be subject to any State law or regulation, including criminal trespass laws.

2.0 Information released through various media is important in the education of the public with regards to *Hexastylis naniflora*. In recent years, the public has become more aware of conservation issues, and many of them are willing to help, but they lack the knowledge or information to do so. Though news releases and informational brochures, the public can be made aware of the efforts being made to protect a species, which is federally and state endangered or threatened. Publications in popular magazines and science journals, regarding research being done with *H. naniflora*, the public as well as the scientific community can be made aware of conservation and protection efforts ongoing. A periodic review of recovery efforts should be given stating the current status of the managements implications and evaluations should be made regarding ongoing actions or ant re-directional changes which might be called for in assuring that the plans goals are being achieved is a quick and successful manner.

VITAE

James Edward Padgett was born in Rutherfordton, North Carolina on December 27, 1964. He attended elementary school in Harris, North Carolina and graduated from Chase High School in May 1983. In December 1984 he married the former Joyce Shelton of Golden Valley, North Carolina and have two children, Jessica and Brandon. In June of 1993 he entered Isothermal Community College in Spindale, North Carolina, and received an Associate of Arts degree in May 1995. In 1996 he entered Gardner-Webb University in Boiling Springs, North Carolina. In May 1999, he received a Bachelor of Science degree in Biology. In the fall of 2000 he accepted a teaching assistantship at Appalachian State University and began study towards a Masters' degree. This degree was awarded in May 2004.