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TAXONOMIC, ECOLOGIC, AND BIOGEOGRAPHIC STUDIES OF POLYMNIA (ASTERACEAE)

Courtney Ethel Gorman

TAXONOMIC, ECOLOGIC, AND BIOGEOGRAPHIC STUDIES OF *POLYMNIA* (ASTERACEAE)

A Thesis

Presented to

The College of Graduate Studies

Austin Peay State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science in Biology

Courtney Ethel Gorman

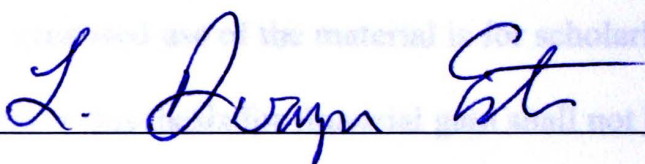
Masters candidate

August, 2012


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ABSTRACT

COURTNEY ETHEL GORMAN. Taxonomic, Ecological, and Biogeographic Studies in *Polymnia* (Asteraceae) (Under the direction of DR. DWAYNE ESTES).

The genus *Polymnia* L. (Asteraceae: Tribe Polymnieae) is endemic to eastern North American temperate, deciduous forests and currently includes four robust herbaceous species (*P. canadensis*, *P. cossatotensis*, *P. laevigata*, and *P. johnbeckii*) characterized by white ray corollas, glandular-aromatic foliage, and a chromosome number of $n=15$. This is a group that has remained unresolved due to confusion surrounding interspecific and infraspecific taxonomy, especially with regards to the widespread *P. canadensis*, a species that inhabits rocky, calcareous woods throughout most of temperate eastern North America. There has long been confusion regarding the infraspecific taxonomy of *P. canadensis* as varieties and forms within the species have been recognized based on distinctive morphological characteristics such as ligule length, achene morphology, and variations in pubescence.

This study seeks to determine whether there are morphologic entities within *P. canadensis* that are geographically defined or whether the variation within *P. canadensis* varies continuously without correlation to geography. Several hundred specimens of *P. canadensis* were borrowed from 12 institutions and critically examined for morphological variation. Principle Component Analysis (PCA) was used to identify distinct groups within *P. canadensis* and the morphological variables which best diagnose these groups. Using data obtained from herbarium specimens we plotted the distribution of distinct morphological groups on an outline map to examine geographic variation.

Results indicate the presence of at least three distinct morphological entities within *P. canadensis* that are geographically definable. One of the distinct morphological entities has a distribution centered in the Interior Highlands region of the United States. The second entity has a distribution centered on the Highland Rim of Tennessee. The third entity has a more widespread distribution throughout the northern United States. Based on these results, we have determined that *P. canadensis* is most likely a complex composed of several species or subspecies. Further research is necessary to determine the driving force behind this morphological divergence, and to describe and diagnose taxa. Future plans regarding this research involve analyzing populations across the entire geographic range using microsatellites and amplified fragment length polymorphism (AFLP) methods.

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CHAPTER I

Introduction

Overview of *Polymnia*—*Polymnia* is a North American genus of vascular plants endemic to eastern temperate, deciduous forests. This genus is included within Class Dicotyledonae, Subclass Asteridae, Order Asterales, and Family Asteraceae.

The Asteraceae (Compositae) is the largest family of flowering plants with upwards of 1,500 genera and 23,000 species worldwide (Stevens 2001, Panero and Crozier 2012). This family is composed of mostly herbaceous species, however, there are some tropical members that are trees and shrubs. Members of the Asteraceae comprise approximately 8% of all flowering plants and are distributed worldwide with the exception of Antarctica (Panero and Funk 2008). This family is characterized by having a specialized inflorescence called a capitulum, which is composed of one to several individual flowers (florets) that sit on a receptacle (Panero 2012). Florets exhibit six corolla types that are distinguished by differences in structure and sexuality (Panero 2012). There are five recognized capitulum types (radiate, discoid, liguliflorous, disciform, and radiant) that differ in the types of corollas exhibited by the florets that comprise the inflorescence (Panero and Crozier 2012).

There are multiple hypotheses regarding the origin of the Asteraceae. Muller (1981) suggested a late Oligocene origin based on fossil pollen records. Bremer and Gustafsson (1997) suggested that the Asterales originated in East Gondwana (Australasia) during the Late Cretaceous period and later expanded into West Gondwana prior to the final break-up of Pangaea. More recently, two pollen types exclusive to the Asteraceae were reported from the Upper Paleocene – Eocene of South Africa (Zavada

and de Villiers 2000). Based on this discovery they concluded that a West Gondwana origin was most likely (Zavada and de Villiers 2000). Kim et al. (2005) supported the hypothesis that the Asteraceae originated during the mid Eocene. Panero and Funk (2008) supported a South American origin based on their recent chloroplast phylogeny.

Recent phylogenetic research determined that Asteraceae contains 12 major lineages: Barnadesioideae Bremer & Jansen, Mutisioideae Lindley, Stiffioideae Panero, Wunderlichioideae Panero & Funk, Gochnatioideae Panero & Funk, Hecastocleidoideae Panero & Funk, Carduoideae Sweet, Pertyoideae Panero & Funk, Gymnarrhenioideae Panero & Funk, Cichorioideae Chevallier, Corymbioideae Panero & Funk, and Asteroideae Lindley (Panero and Funk 2008). These 12 lineages represent strictly monophyletic subfamilies that were determined using phylogenetic analyses of 10 chloroplast DNA loci (Panero and Funk 2008). These subfamilies are further classified into tribes based on morphological and molecular evidence (Panero and Funk 2008).

The taxa of focus in this study are included within the Asteroideae subfamily and represent a distinct lineage within the Heliantheae alliance; a clade composed of the following 13 tribes: Athroismeae J.L. Panero, Helenieae J.L. Panero, Coreopsidae J.L. Panero, Neurolaeneae J.L. Panero, Tageteae J. L. Panero, Chaenactideae J.L. Panero, Bahieae J.L. Panero, Polymnieae J.L. Panero, Heliantheae J.L. Panero, Millerieae J.L. Panero, Madieae B.G. Baldwin and J.L. Panero, Perityleae J.L. Panero, and Eupatorieae D.J.N. Hind and H. Robinson (Panero 2007, Panero and Funk 2008). The taxa of interest in the current study are included within the tribe Polymnieae, which is monotypic including only one genus, *Polymnia* L. Currently, there are four recognized species of

Polymnia (*P. canadensis* L., *P. cossatotensis* Pittman & V. M. Bates, *P. johnbeckii* D. Estes, and *P. laevigata* Beadle).

A taxonomic overview of *Polymnia* has not been attempted since the work of Wells (1965), which resulted in the recognition of 19 species of *Polymnia*. Conflicting opinions regarding *Polymnia* taxonomy prompted Robinson (1978) to re-evaluate the classification established by Wells (1965) and re-establish the genus *Smallanthus* Mackenzie ex Small. Robinson (1978) determined that all species recognized by Wells (1965) as *Polymnia*, with the exception of *P. canadensis* and *P. laevigata*, should be subsequently transferred to the genus *Smallanthus* based on distinctive achene characteristics, leaf venation, and chromosome number.

Polymnia is distinct in that the achene walls are smooth with three to five distinct ribs, versus striated achenes lacking distinct ribs in *Smallanthus* (Robinson 1978). Also, with the exception of *P. cossatotensis* which has simple, cordate leaves, *Polymnia* exhibits basic pinnate leaf lobing whereas *Smallanthus* exhibits primarily trinervate and palmately lobed leaves (Robinson 1978). Another important distinction between *Polymnia* and *Smallanthus* is chromosome number. Wells (1965) reviewed the cytological studies pertinent to his *Polymnia* research and compiled a table of gametic chromosome numbers for the 19 species he recognized. *Polymnia canadensis* and *P. laevigata* have $n=15$, while species of what is now recognized as *Smallanthus* have base numbers ranging from $n=16-17$ (Wells 1965, Robinson 1978). Also, the recently described *P. cossatotensis* and *P. johnbeckii* have a chromosome number of $n=15$ (Smith et al. 1992, Estes and Beck 2011).

A recent phylogenetic study sampled 65 taxa, including *P. canadensis*, *P. laevigata*, and *P. cossatotensis*, to assess the phylogenetic position of the subtribe Espeletiinae within the Heliantheae (Rauscher 2002). This study strongly supported the conclusion made by Robinson as it was found that the three sampled species of *Polymnia* form a distinct clade that is distant from *Smallanthus* (Rauscher 2002). *Polymnia johnbeckii* was not known at the time of the Rauscher (2002) study and was therefore not included.

The four currently recognized species of *Polymnia* are robust herbs native to eastern North American temperate, deciduous forests. They all possess radiate capitula where only the ray florets are fertile. These species are commonly referred to as Leaf-Cups and are characterized by white ray corollas, large glandular-aromatic foliage, and a chromosome number of $n=15$. All four species flower from summer to fall and can grow to over 5 feet tall. An overview of each species is provided here:

1. *Polymnia canadensis* L.

The most widespread species of *Polymnia* is *P. canadensis*, commonly referred to as the Canada or White-flower Leafcup. This species was first described by Linnaeus in *Species Plantarum* (1753) and serves as the type for the genus. It is characterized by a dense covering of stipitate-glandular to pilose trichomes and a 3-angled achene (Wells 1965). *Polymnia canadensis* occurs throughout deciduous forests in the eastern United States and extreme southern Ontario, Canada (Fig.1).

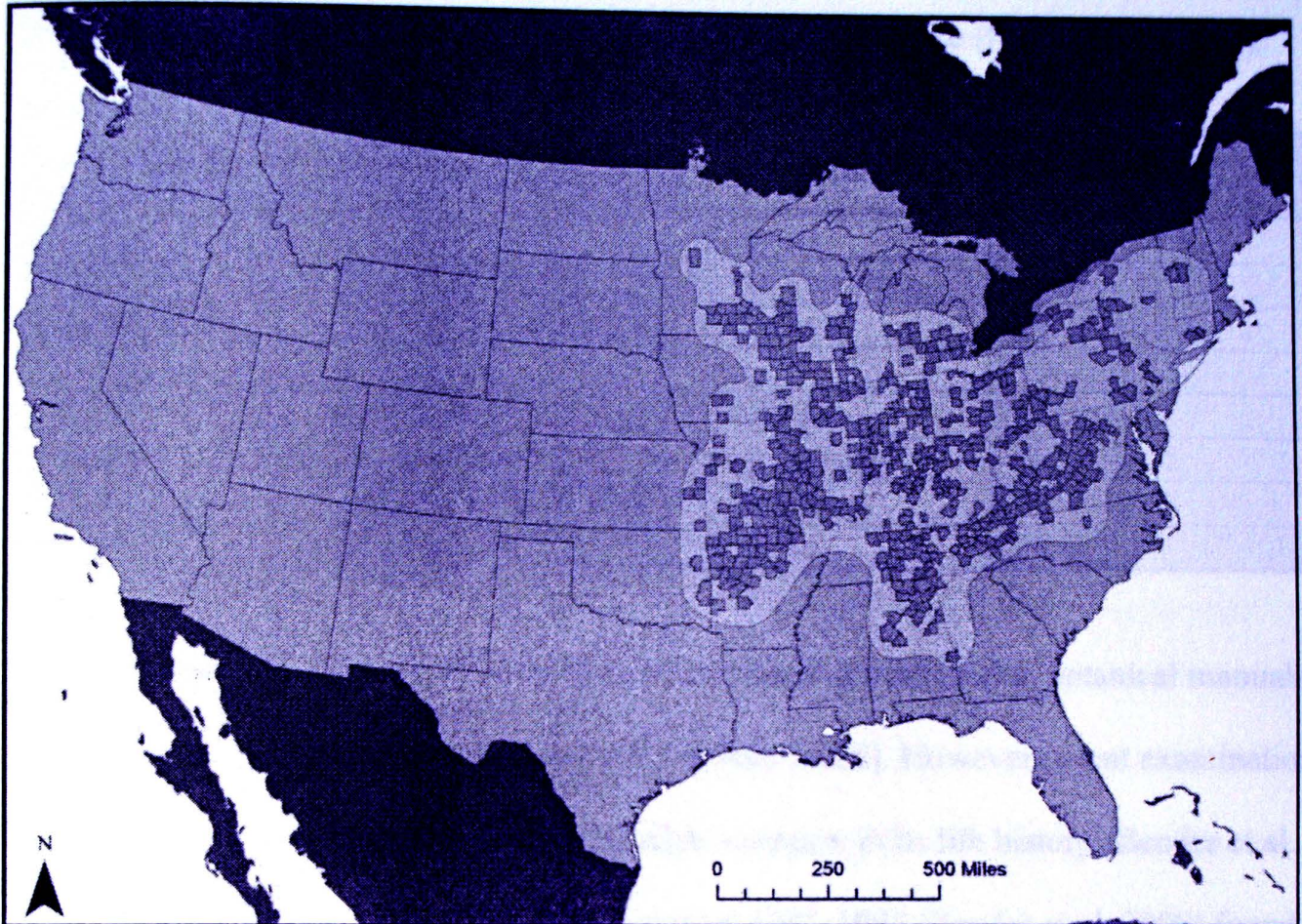


Figure 1. Distribution of *P. canadensis*.

This species is most often associated with dry calcareous woodlands, talus slopes, and bluff bases (Wells 1965), however it also is known from mesic habitats such as moist woods and stream banks (Wooden and Caplenor 1972, Bender 2002). *Polymnia canadensis* also has been reported from acidic soils underlain by sandstone and granitic bedrock in a quartzite glade in Sauk County, Wisconsin (Armstrong 1994). The global conservation rank of *P. canadensis* is listed by NatureServe as G5; however it is given the rank of S1 in the states of Connecticut, Kansas, and Vermont, and S3 in Iowa (NatureServe 2012). Table 1 illustrates the variation of rarity and conservation status throughout its range.

Table 1. Rarity and Conservation Status of *P. canadensis* by state (NatureServe 2012).

State	Conservation Rank	State	Conservation Rank
Alabama	Not Ranked	Missouri	Not Ranked
Arkansas	Not Ranked	North Carolina	Imperiled
Connecticut	Critically Imperiled	New York	Apparently Secure
Georgia	Secure	Ohio	Not Ranked
Iowa	Vulnerable	Oklahoma	Not Ranked
Indiana	Not Ranked	Pennsylvania	Not Ranked
Kansas	Critically Imperiled	Tennessee	Not Ranked
Kentucky	Secure	Virginia	Secure

Polymnia canadensis is most often referred to as a perennial in botanical manuals (Fernald 1950, Gleason and Cronquist 1991, Strother 2006). However recent examination of the demography of the species revealed wide variation in its life history (Bender et al. 2000). Based on demographic data collected from 1985-1994 (Bender et al. 2000) found that while the majority of plants exhibited a biennial life cycle, triennial, winter annual, monocarpic perennial, tricarpic perennial, and dicarpic perennial life cycles were also present (Bender et al. 2000). The life cycle of *P. canadensis* has been described as primarily monocarpic but with some members exhibiting winter annual to polycarpic perennial life cycles (Bender et al. 2000). More specifically, this species exhibits a primarily facultative biennial life cycle with some members taking longer than two years to reach sexual maturity (Bender et al. 2003). This type of life cycle is considered rare in deciduous forests (Bender et al. 2002).

In addition to the previously mentioned studies, much research has been done regarding the relationship between seed germination and the variable life history of *P. canadensis* (Bender et al. 2003). Seed dormancy and germination patterns characteristic of *P. canadensis* are common in deciduous woodland species (Bender et al. 2003). This

conclusion was based on the observation of three main properties common in herbaceous woodland species: 1) higher germination percentage after cold stratification, 2) greater germination success in light than in dark, and 3) the presence of a persistent soil seed bank (Bender et al. 2003). Based on these observations, seed dormancy and germination patterns do not contribute to the rarity of the facultative biennial life cycle (Bender et al. 2003). Another important observation reported in Bender et al. (2003) is that the germination phenology of *P. canadensis* differs from dry to mesic woodland sites, with germination occurring primarily in the fall at dry sites and in the spring at mesic sites.

2. *Polymnia laevigata* Beadle

Polymnia laevigata is commonly referred to as the Tennessee Leafcup. It is characterized by glabrous stems and glabrous-puberulent leaves, and a 5-ribbed achene (Beadle 1898, Wells 1965). This species is endemic to the southeastern United States, with the center of its range occurring in the southern Cumberland Plateau of Tennessee (Fig. 2; NatureServe 2012). The global conservation rank of *P. laevigata* is G3 as it is rare throughout its entire geographic range. It is presumed extirpated from Missouri and is considered critically imperiled in Florida, Georgia, and Kentucky (NatureServe 2012). Table 2 summarizes the conservation status of *P. laevigata* throughout its range.

Table 2. Rarity and Conservation Status of *P. laevigata* by state (NatureServe 2012).

State	Conservation Rank	State	Conservation Rank
Alabama	Imperiled	Kentucky	Critically Imperiled
Florida	Critically Imperiled	Missouri	Extirpated
Georgia	Critically Imperiled	Tennessee	Vulnerable

An unusual condition regarding pollen morphology has been documented from a population near Monteagle, Tennessee (Fisher and Wells 1962). *Polymnia laevigata* possesses a unique trait termed “heteromorphic pollen”, a condition in which the pollen occurs in multiple morphological forms and varies in the number of nuclei present (Fisher and Wells 1962). Five distinct morphological forms of pollen that could be differentiated into four distinct groups based on the number of nuclei in each pollen grain were noted in a single *P. laevigata* individual. The authors hypothesized that this condition resulted from either failure of wall formation at the completion of meiosis II or erratic divisions of the microspore nucleus (Fisher and Wells 1962). In either case, the resulting condition is uncommon in the plant kingdom.

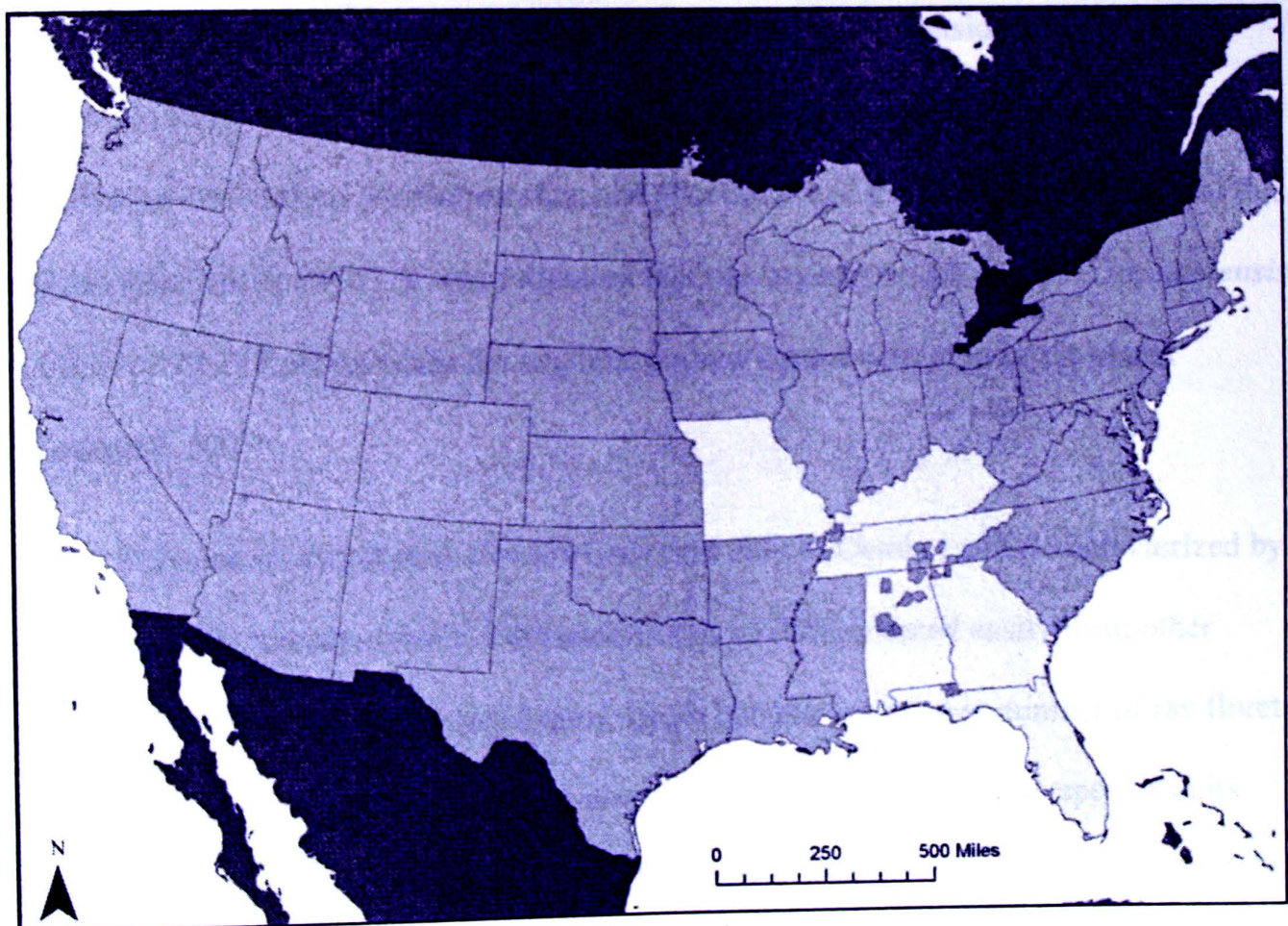


Figure 2. Distribution of *P. laevigata*.

3. *Polymnia cossatotensis* Pittman & V. M. Bates

Polymnia cossatotensis is a narrow endemic restricted to the Ouachita Mountains of the Interior Highlands region of Arkansas (Fig. 3; Pittman and Bates 1989). This species is known from only five populations, which all occur within 18 km of each other on the rugged mountain slopes within the Ouachita National Forest in Montgomery and Polk counties (Pittman and Bates 1989, Hardcastle 2007, NatureServe 2012, T. Witsell pers. comm. 2012). *Polymnia cossatotensis* is restricted to open talus slopes where it often grows epipetrically with its roots attached to bare rock (Hardcastle 2007, NatureServe 2012). *Polymnia cossatotensis* is given the global conservation rank of G1 along with a state rank of S1 and is considered critically imperiled (NatureServe 2012).

Because *P. cossatotensis* is so narrowly restricted it is considered to be highly vulnerable to extinction. To aid in conservation efforts, a recent study documented the distribution and current population size, and also described the habitat preferences of this species (Hardcastle 2007). It was estimated that the largest population of *P. cossatotensis* consists of 28,727 plants while the smallest population consists of only six plants (Hardcastle 2007).

Polymnia cossatotensis flowers from mid-July to October and is characterized by its large, simple, cordate leaves. This species can be differentiated easily from other species of *Polymnia* by its cordate leaves, larger achenes, and fewer number of ray florets (Pittman and Bates 1989). This species also differs from other *Polymnia* species in its annual life cycle (Pittman and Bates 1989, Hardcastle 2007).

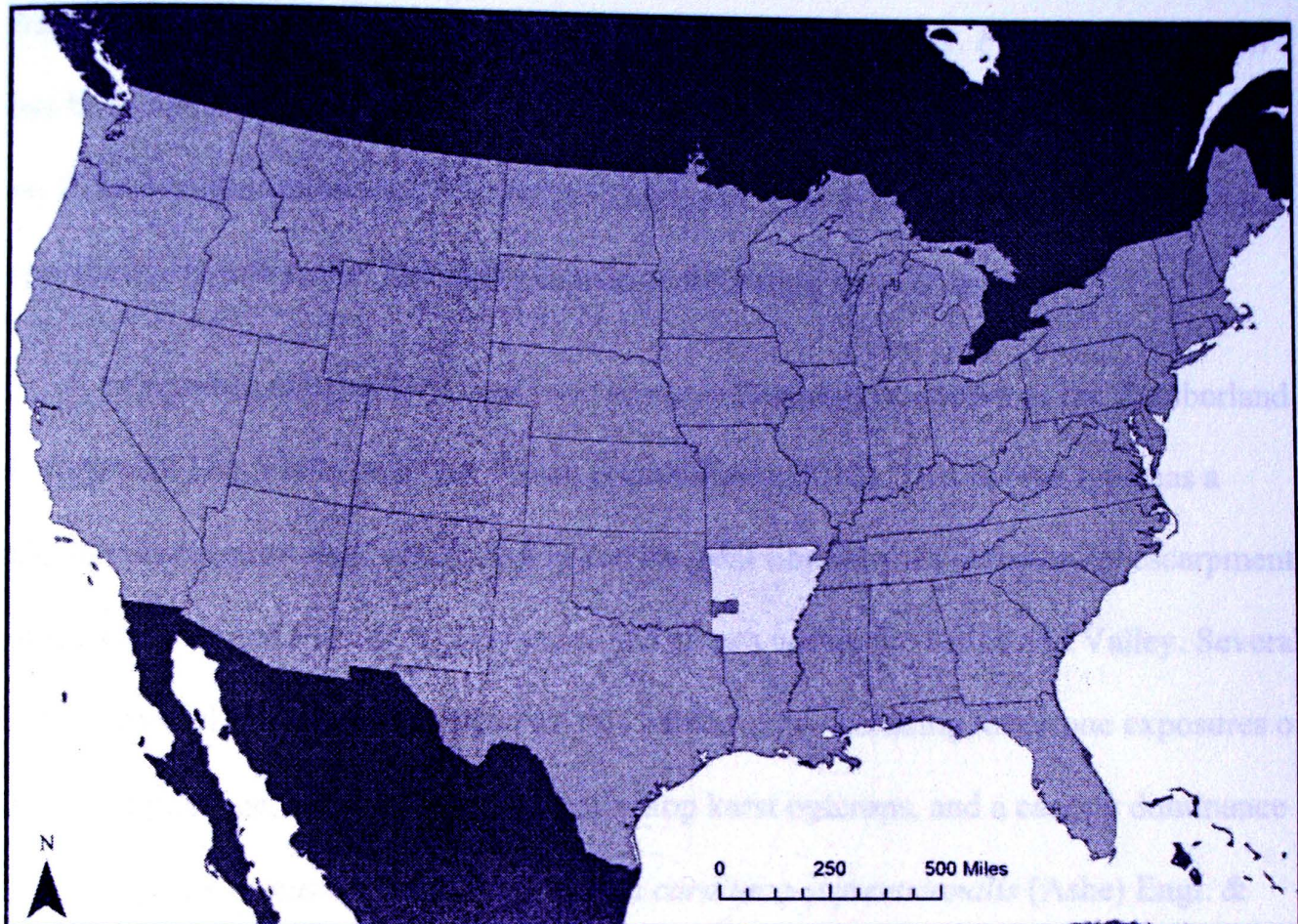


Figure 3. Distribution of *P. cossatotensis*.

4. *Polymnia johnbeckii* D. Estes

Polymnia johnbeckii is a newly described narrow endemic from the Sequatchie Valley of Tennessee (Fig 4; Estes and Beck 2011). This species was discovered in September 2008 and is currently known from only two populations that are located within 5 km of each other (Estes and Beck 2011). These populations occur on the slopes of two low mountains above Nickajack Lake, each named Little Cedar Mountain. To ease clarification of geographic location, these mountains have been referred to as “North” and “South” by Estes and Beck (2011) and therefore will be referred to as “North” and “South” Little Cedar Mountain throughout this study. The north and south mountains are separated by a larger mountain named Cedar Mountain that collectively form a

fragmented ridge along the eastern edge of the Sequatchie Valley. *Polymnia johnbeckii* has been assigned an S1 state conservation rank (D. Estes, pers. comm., 2012), however no federal conservation rank has yet been assigned. Due to its extreme rarity and habitat specificity, *P. johnbeckii* is a likely candidate for listing as a G1 species.

Polymnia johnbeckii is restricted to a rare habitat type known as the Cumberland Escarpment Dry Limestone Oak Forest (NatureServe 2012). This habitat type has a global conservation rank of G3, and is known from limited sites in the lower escarpment of the Cumberland Plateau in Tennessee and slopes within the Ridge and Valley. Several environmental conditions characterize this habitat type, including limestone exposures of Mississippian age, relatively shallow soils atop karst outcrops, and a canopy dominance consisting of *Cotinus obovatus* Raf., *Carya carolinae-septentrionalis* (Ashe) Engl. & Graebn., *Fraxinus americana* L., *Fraxinus quadrangulata* Michx., *Quercus stellata* Wangerh., and *Ulmus alata* Michx. (NatureServe 2012). The limestone outcrops within this habitat are covered with bryophyte mats and shallow soil deposits, allowing *P. johnbeckii* to grow in an epipetric habit (Estes and Beck 2011). This species exhibits a perennial life cycle.

Previous studies have determined *P. johnbeckii* to be diploid which indicates that it likely originated via one of two possible evolutionary pathways, cladogenesis or homoploid hybrid speciation (Estes and Beck 2011). This species could possibly be of hybrid origin from crosses between *P. canadensis* and *P. laevigata*. This possibility is supported in that both of these species occur within 23 kilometers of *P. johnbeckii*, and grow syntopically in at least two sites in adjacent Franklin County, Tennessee.

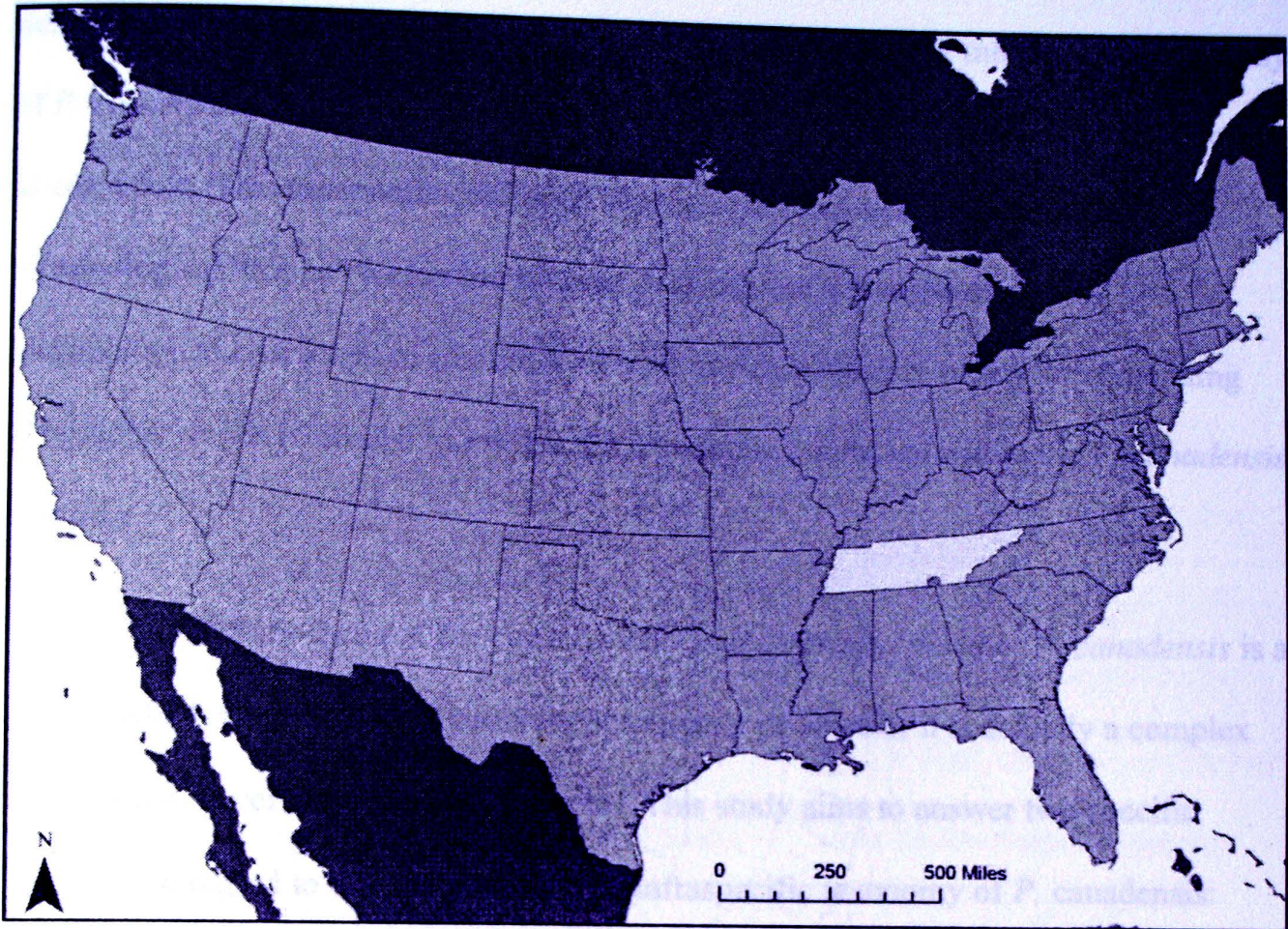


Figure 4. Distribution of *P. johnbeckii*.

Objectives— There has been long-standing confusion regarding the interspecific and infraspecific taxonomy of *P. canadensis*, as distinct morphological “types” have prompted recognition at the taxonomic levels of species, variety, and form. Gray (1881) first recognized a variety within this species, *P. canadensis* var. *discoidea*, that had ray florets “reduced to a mere ring around the base of the style”. Gray (1884) later recognized another variety within *P. canadensis* that was distinct in its long ray corollas, *P. canadensis* var. *radiata*. Small (1903) considered *P. radiata* a distinct species based on ligule length, pubescence, and achene characteristics. Fassett (1932) then recognized this taxon as *P. canadensis* f. *radiata*. Fassett described this form to be “strikingly different in appearance from the discoid form,” however these differences were never thoroughly

described. Wells (1965) noted that much confusion surrounds the infraspecific taxonomy of *P. canadensis* due to variation in length of the ray corollas; he chose to treat all forms as one taxon. This taxonomic confusion suggests the long-term awareness of morphological “forms” within the species. A critical examination of several morphological characters in combination with the identification of any corresponding geographic trends is needed to resolve the taxonomic confusion within the *P. canadensis* complex.

The primary focus of the current study is to determine whether *P. canadensis* is a species which exhibits highly variable morphology or whether it is actually a complex composed of several species or subspecies. This study aims to answer two specific questions in regard to the interspecific and infraspecific taxonomy of *P. canadensis*:

- 1) Are there diagnosable morphological entities within *Polymnia canadensis*?
- 2) If so, do they correspond with geographic patterns?

A multivariate analysis of the geographic variation in morphology will be employed to evaluate the morphologic diversity of *P. canadensis*. Based on past studies that have noted considerable variation and identified additional diversity in the species, I hypothesize that *P. canadensis* is likely a complex of several currently unrecognized species or sub-species.

CHAPTER II

Methods

Preliminary Observations—Populations of *P. canadensis* were observed at multiple locations throughout the eastern United States (Alabama, Arkansas, Georgia, Kentucky, Tennessee, Virginia, and West Virginia) from Fall 2010 to Summer 2012. Upon visiting 20+ populations across the eastern U.S., it became readily apparent that this species exhibits considerable morphological variation. Some of the most obvious variations were differences in pubescence types, density of pubescence, capitula size, number of rays, ray length, bract size, and number of lobes per leaf. Based on preliminary observations of populations that exhibited distinctly variable morphology, it appeared that these variations were possibly correlated to geography or specific sets of environmental factors.

The type specimen of *P. canadensis* was located and examined through digital images (LINN 1033.1 *Polymnia canadensis* (Herb Linn)) to investigate its morphology. Evaluation of the morphology of the type specimen allows for a direct comparison to the morphological variability observed within this species, therefore providing a baseline for what should be included within *P. canadensis sensu stricto*. Unfortunately the type specimen was not accompanied by a detailed physical description of the species. Without a detailed physical description it is difficult to determine the character states for characteristics that may be altered post preservation (i.e. pubescence characteristics such as types of hairs present and density).

Morphology—Morphological analysis is a popular method in taxonomy used to investigate observed differences among a group of visibly similar organisms (Naczi 1998, Naczi 2001, Cook 2002, Leonard et al. 2004, Cook et al. 2009). There are several advantages of these kinds of studies, and they can often be as revealing in regard to phylogenetic relationships as those that employ molecular methodology. This is illustrated in that many of the morphology-based classifications proposed by early botanists remain in use and are now supported by molecular evidence (Hillis 1987, Hillis and Wiens 2000, Scotland et al. 2003, Wiens 2004).

A major advantage of morphological analysis is the utility of herbarium specimens. The ability to access and utilize herbarium specimens from throughout the entire geographic range of the study species greatly reduces the time and cost of collecting the appropriate material. Another advantage is that morphological analyses are less destructive to herbarium specimens than some molecular techniques, as measurements can be taken with little to no specimen damage, while molecular techniques require the removal of tissue for DNA extraction. Morphological analyses are also less expensive than molecular analyses.

To investigate the geographical variation in morphology in *P. canadensis* several hundred herbarium specimens were examined. Specimens were borrowed from or examined at the following 12 herbaria: ASPC, BRIT, EKY, ISC, MICH, MO, MU, TENN, UARK, UNA, VPI, and WIS. Herbarium specimens were critically examined for morphological variation with the aid of a Meiji dissecting scope and sorted into *a priori* (preliminary) groups based on variations in pubescence, flower morphology, and cypselae characteristics. One specimen representative of each *a priori* group was selected

based on intactness to be photographed with a Canon Rebel digital camera mounted on a Meiji stereoscope to help visualize and compare the variation within *P. canadensis*. During the preliminary morphological evaluation, individual characters that exhibited variation were noted and used along with descriptions of *P. canadensis* in the taxonomic literature (Fernald 1950, Gleason and Cronquist 1991, Strother 2006) to aid in character selection for the quantitative analysis. Characters were chosen to represent the entire range of morphological variation of the plant. We chose 40 vegetative and floral characters to score for each specimen included in the data matrix. These characters along with a description of each are given in Table 3.

Polymnia is a challenging group with regards to morphometric study because of the way it is often preserved on herbarium sheets. Due to its large, robust habit, usually only the top half of the plant is collected. This makes it difficult to find complete specimens with adequate material for standardized sampling and measurements. The lack of overall specimen completeness is paired with the delicate nature of the foliage which is often highly wilted before it is preserved. This results in leaves that are crumpled and obscured, and further complicates the attempt to make accurate and standardized measurements. Given these challenges, specimens were considered complete if they had representative material of the lower, middle, and upper stem, leaves, and mature inflorescences.

Our preliminary groupings were used to aid in specimen selection to ensure that we chose specimens that exhibited the full range of morphological variation of the *P. canadensis* complex as well as specimens originating throughout the geographic range of this species. We then selected 55 complete specimens as a representative subset to

Table 3. Character abbreviations and descriptions for the morphological characters measured for the morphometric analysis of *P. canadensis*. Characters with values given in parentheses indicate a gradient.

Abbreviation	Character Description	Abbreviation	Character Description
LSPUB	Lower stem pubescence (0-6)	HEADH	Head height (mm)
MSPUB	Mid-stem pubescence (0-6)	HEADDIAM	Head diameter (mm)
USPUB	Upper stem pubescence (0-6)	SPHYLLPUB	Thin phyllary pubescence
MSLFLOB	# of lobes per leaf	SPHYLLL	Thin phyllary length (mm)
MSULFPUB	Pubescence of upper leaf surface	SPHYLLW	Thin phyllary width (mm)
MSLLPUB	Pubescence of lower leaf surface	FPHYLLPUB	Wide phyllary pubescence
MSLDENT	# serrations on the leaf margin per cm	FPHYLLL	Wide phyllary length (mm)
MSLL	Leaf length (mm)	FPHYLLW	Wide phyllary width (mm)
MSLW	Leaf width at widest point (mm)	NUMRAYS	# of rays per head
MSLTW	Leaf tip width (mm)	RAYL	Ray length (mm)
MSFLW	First lobe width (mm)	RAYW	Ray width (mm)
MSMSW	Distance between sinuses (mm)	RAYLOBL	Ray lobe length (mm)
MSSLW	Second lobe width at widest point (mm)	RAYLOBW	Ray lobe width (mm)
ULLS	Leaf shape	NUMDISKS	# of disks per head
ULL	Leaf length (mm)	DISKCORPUB	Disk corolla pubescence (0-5)
ULW	Leaf width (mm)	DISKCORTHRL	Disk corolla throat length (mm)
ULUSPUB	Pubescence of upper leaf surface (0-6)	DISKCORTUBL	Disk corolla tube length (mm)
ULLSPUB	Pubescence of lower leaf surface (0-6)	DISKCORLOBL	Disk corolla lobe length (mm)
NUMHEADS	# of heads on lateral branch	NUMCYPS	# of cypsela
PEDPUB	Peduncle pubescence (0-6)	CYPSPUB	Cypsela pubescence (0-5)

Geography—Using location data obtained from herbarium specimens, we plotted the distributions of the *a priori* group members on a county outline map (Fig. 5) to examine geographic trends. Each point on the map is representative of one herbarium specimen and each symbol represents a distinct morphological type. This allowed for preliminary observations and hypotheses regarding the correlation of geographic location to morphological variation in *P. canadensis* that served as the framework for additional data collection and analyses.

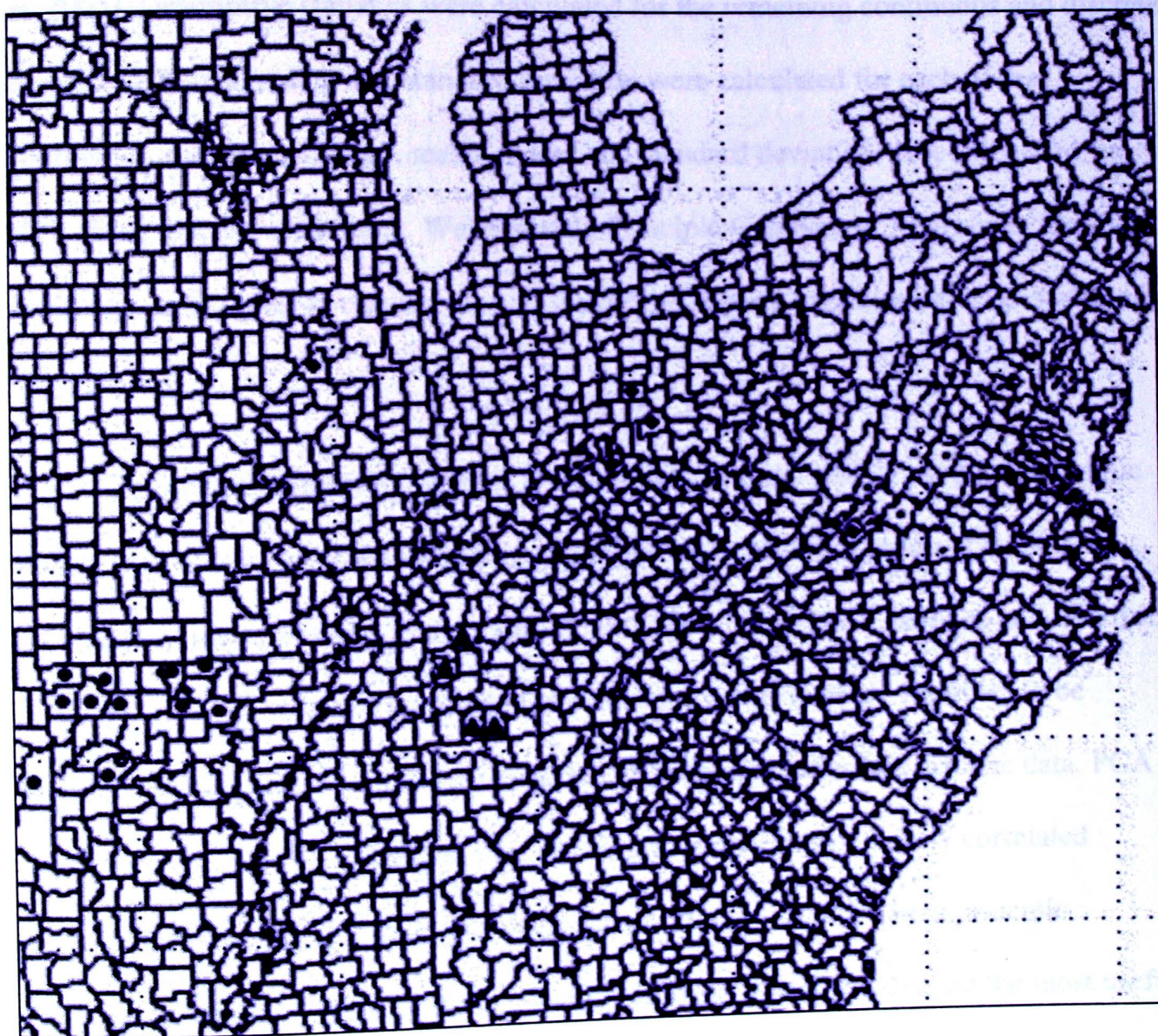


Fig 5. Preliminary distribution map of *a priori* group members where each symbol represents a distinct combination of morphological characters.

Statistical Analyses— A data matrix of the 55 specimens evaluated for the 40 discrete and continuous morphological characters given in Table 3 was constructed using Excel 2010. A Pearson's Correlation was performed to detect traits with a high degree of correlation ($|r| > 0.7$), i.e. genetically redundant traits (Cook et al. 2009). When a pair of characters was found to have a high degree of correlation, one character from the pair was then excluded from further statistical analyses as this condition could indicate that the phenotype of those two traits resulted from the regulation of a single gene (Leonard et al. 2005). Descriptive statistics were calculated for the remaining continuous and discrete characters. Range, mean, and standard deviation were calculated for each of the continuous characters. Range, mean, mode, and standard deviation were calculated for each of the discrete characters. We then used Principle Component Analysis to identify distinct groups within *P. canadensis* and the morphological variables which best diagnose these groups.

Principle Component Analysis is a mathematical tool widely used in taxonomic studies to quantify perceived morphological differences among a group of similar organisms (Aldasoro 1998, Naczi 1998, Levin 1999, Naczi 2001, Janovec and Harrison 2002). PCA is valuable as a taxonomic tool because it allows many variables to be analyzed at once, and allows for the analyses of both continuous and discrete data. PCA reduces a large number of variables by deriving combinations of closely correlated characters called principle components (Kucharczyk et al. 2002). These principle components are analyzed to identify the morphological characters that are the most useful for differentiating distinctive groups of organisms. We performed all statistical analyses with SYSTAT 8.0 and Excel 2010 (Wilkinson, 1996).

CHAPTER III

Results

Morphology—Results from the critical evaluation of morphology of *P. canadensis* indicate the presence of at least three distinct morphological entities within this species. It is important to note that the *a priori* groupings made in the preliminary morphological evaluation initially suggested four morphologically distinct groups as shown in Figure 5, however upon further examination it was determined that two of these groups represented a single entity so they were combined (Fig. 6).

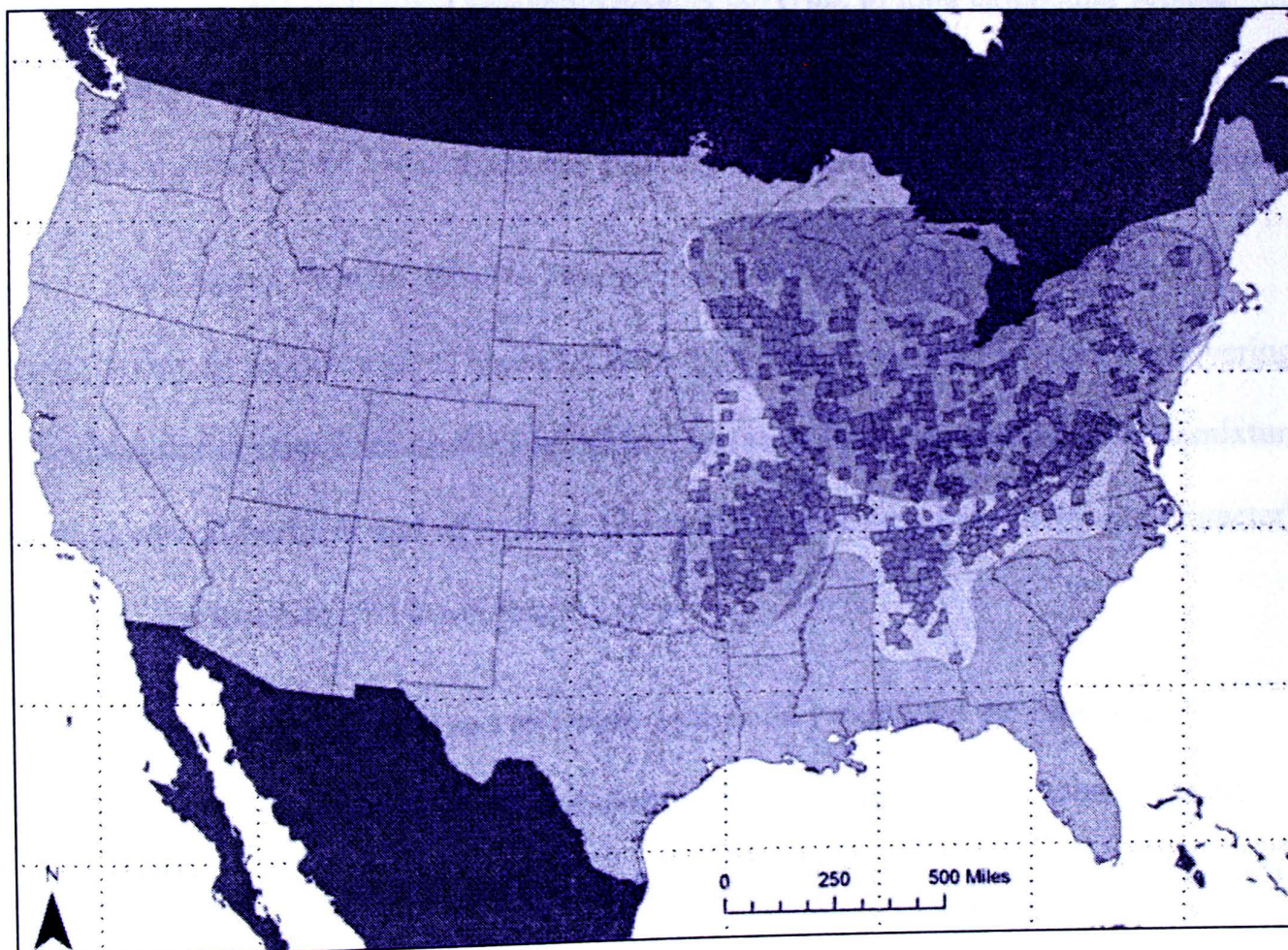


Fig. 6. Distribution of *a priori* group members where each shaded area represents a distinct combination of morphological characters. The blue shaded area represents the *a priori* groups that were combined.

It became readily apparent during critical examination of morphological characters that stem and leaf pubescence along with floral characters such as ray length were highly variable throughout the species (Fig. 7). It also became apparent that this variation was reliable in the sense that there were three distinct combinations of vegetative and floral characteristics that were commonly revealed through critical examination. These combinations of traits were used to identify three distinct groups (Group 1, Group 2, and Group 3) and identify specimens that belong with each grouping.

Specimens were assigned to Group 1 when they exhibited the following combination of morphological characteristics: A covering of long eglandular septate hairs; a dense glandular-puberulent underlayer on the stems and adaxial and abaxial leaf surfaces; medium (10-15 mm diameter) capitula; and medium (5-10 mm long) ray florets.

Specimens were assigned to Group 2 when they exhibited the following combination of morphological characteristics: glabrous lower and mid-stems; a covering of eglandular septate hairs on the adaxial leaf surface; abaxial leaf surface with a mixture of glandular-puberulent and eglandular septate hairs; small-medium (5-15 mm diameter) capitula; and medium (5-10 mm length) rays.

Specimens were assigned to Group 3 when they exhibited the following combination of morphological characteristics: long eglandular septate hairs covering stems and the adaxial and abaxial leaf surfaces; and small-medium (5-15 mm diameter) capitula and small (3-5 mm length or less) or absent ray florets. A summary of the observed morphological variation used to determine the distinct groups can be found in Table 4.

Table 4. Qualitative morphological differences that can be used to distinguish morphological groups.

Grouping	Stem Pubescence	Leaf Pubescence	Head Size	Ray Length
Group 1	Long eglandular septate hairs with a dense glandular-puberulent underlayer	Long eglandular septate hairs and short glandular-puberulent hairs on adaxial and abaxial surfaces.	Medium (10-15 mm)	Medium (5-10 mm)
Group 2	Glabrous	Adaxial surface with eglandular septate hairs and abaxial surface with a mix of glandular-puberulent and eglandular septate hairs	Small-Medium (5-15 mm)	Medium (5-10 mm)
Group 3	Long eglandular septate hairs	Eglandular septate hairs scattered on both adaxial and abaxial leaf surfaces	Small-Medium (5-15 mm)	Small (3-5 mm or less) or absent

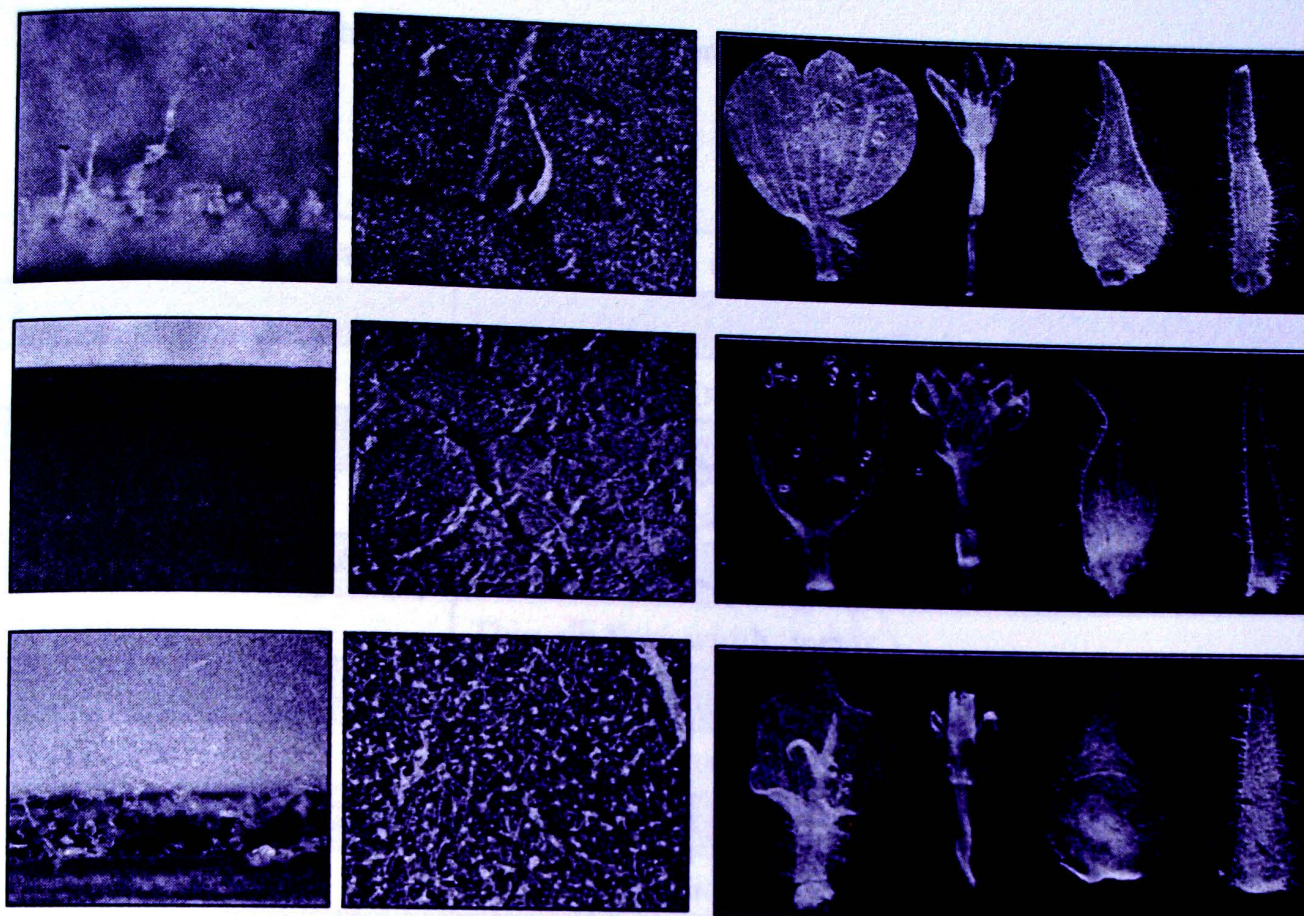


Figure 7. Photographs of specimens representing the range of morphological variation within the *P. canadensis* complex. The first row represents Group 1, the second row represents Group 2, and the third row represents Group 3. Characters photographed from left to right: mid-stem pubescence, lower surface of mid-stem leaf, ray floret, disc floret, inner phyllary, outer phyllary. Note: photographs not to scale. Specimens photographed from top to bottom: *Beer 93 UARK*, *Estes 03677 ASPC*, *Hartley 736 WIS*.

Statistical Analyses—Results of the Pearson's correlation indicated that 21 characters should be excluded from further statistical analyses due to a high degree of correlation between a pair of characters ($|r| > 0.7$). Table 5 summarizes the continuous and discrete characters that were included in further statistical analyses.

Table 5. Character abbreviations and definitions of the morphological characters included in the PCA.

Character Abbreviation	Character Description
MSLW	Leaf width at widest point (mm)
MSLTW	Leaf tip width (mm)
MSFLW	First lobe width (mm)
MSMSW	Mid-section width—distance between sinuses (mm)
HEADDIAM	Head diameter (mm)
SPHYLLW	Thin phyllary width (mm)
FPHYLLL	Wide phyllary length (mm)
FPHYLLW	Wide phyllary width (mm)
RAYL	Ray length (mm)
DISKCORTHRL	Disk corolla throat length (mm)
DISKCORTUBL	Disk corolla tube length (mm)
DISKCORLOBL	Disk corolla lobe length (mm)
MSPUB	Mid-stem pubescence (0-6)
MSULPUB	Pubescence of upper leaf surface
MSLLPUB	Pubescence of lower leaf surface
USLLPUB	Pubescence of lower leaf surface (0-6)
SPHYLLPUB	Thin phyllary pubescence
NUMRAYS	Number of rays per head
MSLDENT	Leaf dentation— # serrations on the leaf margin per cm

Results from the descriptive statistics of the continuous characters are summarized in Table 6. The results reveal particularly high standard deviations for measured leaf characteristics (MSLW, MSLTW, MSFLW, MSMSW), indicating that the data is widely variable. This is explained by the difference in size of the plants from which the leaves were measured, which is highly dependent on environmental conditions

such as light, water, and nutrient availability. The optimal way to standardize these data would be to analyze the ratio of the leaf characteristics to the height of the entire plant, however this was not possible in this case due to inadequate herbarium specimens. In this case, the first Principle Component was excluded to standardize the data. The means were similar for the majority of continuous characters, with the exception of RAYL, and there was significant overlap in the ranges of individual characters. This was expected as it was determined through critical analysis of morphology that ray length is an important characteristic for distinguishing the groups. When evaluating morphological variation it is often necessary to consider combinations of variables rather than relying on individual characters to illustrate differences.

Table 6. Descriptive statistics of continuous data.

Character Name	Group 1	Group 2	Group 3
MSLW	Range = 111.3-168.8 \bar{x} = 137.3 SD = 29.13	Range = 112.9-202.1 \bar{x} = 141.5 SD = 27.73	Range = 54.7-276.2 \bar{x} = 142.7 SD = 46.71
MSLTW	Range = 36.8-60.2 \bar{x} = 51.1 SD = 12.52	Range = 35.4-95.2 \bar{x} = 53.7 SD = 18.10	Range = 13.8-94.4 \bar{x} = 47.5 SD = 21.30
MSFLW	Range = 40.1-50.3 \bar{x} = 43.7 SD = 5.75	Range = 22.7-71.2 \bar{x} = 43.8 SD = 12.71	Range = 18.7-72.1 \bar{x} = 42.1 SD = 13.33
MSMSW	Range = 16.9-30.2 \bar{x} = 23.0 SD = 6.72	Range = 23.3-52.3 \bar{x} = 31.9 SD = 10.86	Range = 11.3-77.3 \bar{x} = 28.0 SD = 17.31
HEADDIAM	Range = 10.4-14.9 \bar{x} = 12.0 SD = 2.49	Range = 7.7-14.2 \bar{x} = 10.8 SD = 2.37	Range = 5.9-15.8 \bar{x} = 9.9 SD = 2.75
SPHYLLW	Range = 2.0-2.8 \bar{x} = 2.4 SD = 0.40	Range = 1.2-3.2 \bar{x} = 2.0 SD = 0.62	Range = 0.9-5.9 \bar{x} = 2.3 SD = 0.99
FPHYLLL	Range = 6.1-10.4 \bar{x} = 8.2 SD = 2.15	Range = 6.9-12.3 \bar{x} = 9.2 SD = 1.39	Range = 6.8-16.5 \bar{x} = 9.6 SD = 2.44
FPHYLLW	Range = 3.9-4.2 \bar{x} = 4.1 SD = 0.15	Range = 2.3-4.7 \bar{x} = 3.7 SD = 0.67	Range = 2.7-5.7 \bar{x} = 3.9 SD = 0.79
RAYL	Range = 3.3-10.3 \bar{x} = 6.0 SD = 3.74	Range = 4.7-10.5 \bar{x} = 7.6 SD = 2.06	Range = 0-7.1 \bar{x} = 3.6 SD = 1.86
DISKCORTHRL	Range = 2.1-3.4 \bar{x} = 2.9 SD = 0.72	Range = 2.2-3.2 \bar{x} = 2.7 SD = 0.36	Range = 1.8-3.9 \bar{x} = 2.7 SD = 0.55
DISKCORTUBL	Range = 3.3-4.0 \bar{x} = 3.6 SD = 0.36	Range = 3.3-5.6 \bar{x} = 4.4 SD = 0.78	Range = 3.1-7.4 \bar{x} = 4.6 SD = 0.92
DISKCORLOBL	Range = 0.5-1.1 \bar{x} = 0.7 SD = 0.32	Range = 0.7-1.1 \bar{x} = 0.9 SD = 0.16	Range = 0.4-1.4 \bar{x} = 0.8 SD = 0.24

Results from the descriptive statistics of the discrete characters are summarized in Table 7. The means were similar for the majority of discrete characters, with the exception of MSPUB, and there was significant overlap in the ranges of individual characters. This was expected as it was determined through critical analysis of morphology that mid-stem pubescence is an important qualitative character for distinguishing the groups. As was the case with the continuous data, it is necessary with discrete data to evaluate combinations of characters when assessing morphological variation.

Table 7. Descriptive statistics of discrete data.

Character Name	Group 1	Group 2	Group 3
MSPUB	Range = 5 $\bar{x} = 5$ (5-mode) $SD = 0$	Range = 0-5 $\bar{x} = 1.9$ (0-mode) $SD = 2.59$	Range = 3-6 $\bar{x} = 4.1$ (3-mode) $SD = 1.12$
MSULPUB	Range = 3-6 $\bar{x} = 3$ (3-mode) $SD = 0$	Range = 3-6 $\bar{x} = 3.9$ (3-mode) $SD = 1.25$	Range = 3-5 $\bar{x} = 3.4$ (3-mode) $SD = 0.84$
MSLLPUB	Range = 3-6 $\bar{x} = 3$ (3-mode) $SD = 0$	Range = 3-5 $\bar{x} = 4$ (3-mode) $SD = 1.07$	Range = 3-5 $\bar{x} = 3.5$ (3-mode) $SD = 0.88$
ULLSPUB	Range = 5 $\bar{x} = 5$ (5-mode) $SD = 0$	Range = 5 $\bar{x} = 5$ (5-mode) $SD = 0$	Range = 1-5 $\bar{x} = 3.9$ (5-mode) $SD = 1.15$
SPHYLLPUB	Range = 5 $\bar{x} = 5$ (5-mode) $SD = 0$	Range = 5-6 $\bar{x} = 5.4$ (5-mode) $SD = 0.52$	Range = 5-6 $\bar{x} = 5.1$ (5-mode) $SD = 0.36$
NUMRAYS	Range = 5-8 $\bar{x} = 6$ (5-mode) $SD = 1.73$	Range = 5-9 $\bar{x} = 6.4$ (6-mode) $SD = 1.41$	Range = 4-12 $\bar{x} = 6.2$ (5-mode) $SD = 2.01$
MSLDENT	Range = 2 $\bar{x} = 2$ (2-mode) $SD = 0$	Range = 2-3 $\bar{x} = 2.3$ (2-mode) $SD = 0.46$	Range = 1-3 $\bar{x} = 1.9$ (2-mode) $SD = 0.65$

Results of the Principle Component Analysis of continuous data show that a combination of characters drive separation between Group 1 and Group 2, however no combination of characters separate Groups 1 and 2 from Group 3. Table 8 summarizes the component loading scores from the second and third principle components from PCA of the continuous data. The first principle component was excluded as a function of size variables and to standardize the data. The characters with the highest loading scores on component 2 were MSLW, MSFLW, MSMSW, and DISKCORTUBL. The characters with the highest loadings on component 3 were SPHYLLW, and DISKCORLOBL. A component loading score > 0.5 was considered to be high. Variables with high loading scores contribute most to any separation observed on the plot of individuals in Fig. 8 and thus, represent the combination of characters that may be most useful when diagnosing the groups.

Character	Component 2	Component 3
DISKCORLOBL	0.262	0.776

The scatter plot (Fig. 8) of the factor scores of the second and third principle components of the continuous data illustrates nearly complete separation between Groups 1 and 2 (blue circles and red triangles) along PC2. This indicates that the variables with the highest loadings on PC2 (MSLW, MSFLW, MSMSW, and DISKCORTUBL) will be the most useful for distinguishing these groups. The scatter plot of the factor scores of Groups 1 and 2 from Group 3 is represented by the blue circles and red triangles. The separation between Groups 1 and 2 is nearly complete along PC2, indicating that the variables with the highest loadings on PC2 (MSLW, MSFLW, MSMSW, and DISKCORTUBL) will be the most useful for distinguishing these groups. The scatter plot of the factor scores of Groups 1 and 2 from Group 3 is represented by the blue circles and red triangles. The separation between Groups 1 and 2 is nearly complete along PC2, indicating that the variables with the highest loadings on PC2 (MSLW, MSFLW, MSMSW, and DISKCORTUBL) will be the most useful for distinguishing these groups.

Table 8. Component loadings for the second and third principle components from PCA of continuous data from herbarium specimens of *P. canadensis*.

Character	Component 2	Component 3
MSLW	-0.673	-0.158
MSLTW	-0.853	0.195
MSFLW	-0.836	-0.073
MSMSW	-0.686	0.276
HEADDIAM	0.094	-0.334
SPHYLLW	0.347	-0.525
FPHYLLL	0.091	-0.145
FPHYLLW	0.249	-0.251
RAYL	0.108	0.078
DISKCORTHRL	0.252	0.435
DISKCORTUBL	0.518	0.301
DISKCORLOBL	0.262	0.776

The scatter plot (Fig. 8) of the factor scores of the second and third principle components of continuous data illustrates nearly complete separation between Groups 1 and 2 (represented by the blue circles and red triangles) along PC3. This indicates that the variables that loaded most heavily on PC3 (SPHYLLW and DISKCORLOBL) will be the best combination of continuous characters for distinguishing those groups. The scatter plot also illustrates no separation of Groups 1 and 2 from Group 3 (represented by the black stars). This indicates that none of the continuous characters measured for the statistical analysis were useful for separating Groups 1 and 2 from Group 3. In this case, it is important to rely on a combination of quantitative data and qualitative data (i.e. morphological and geographical analyses) to evaluate separation.

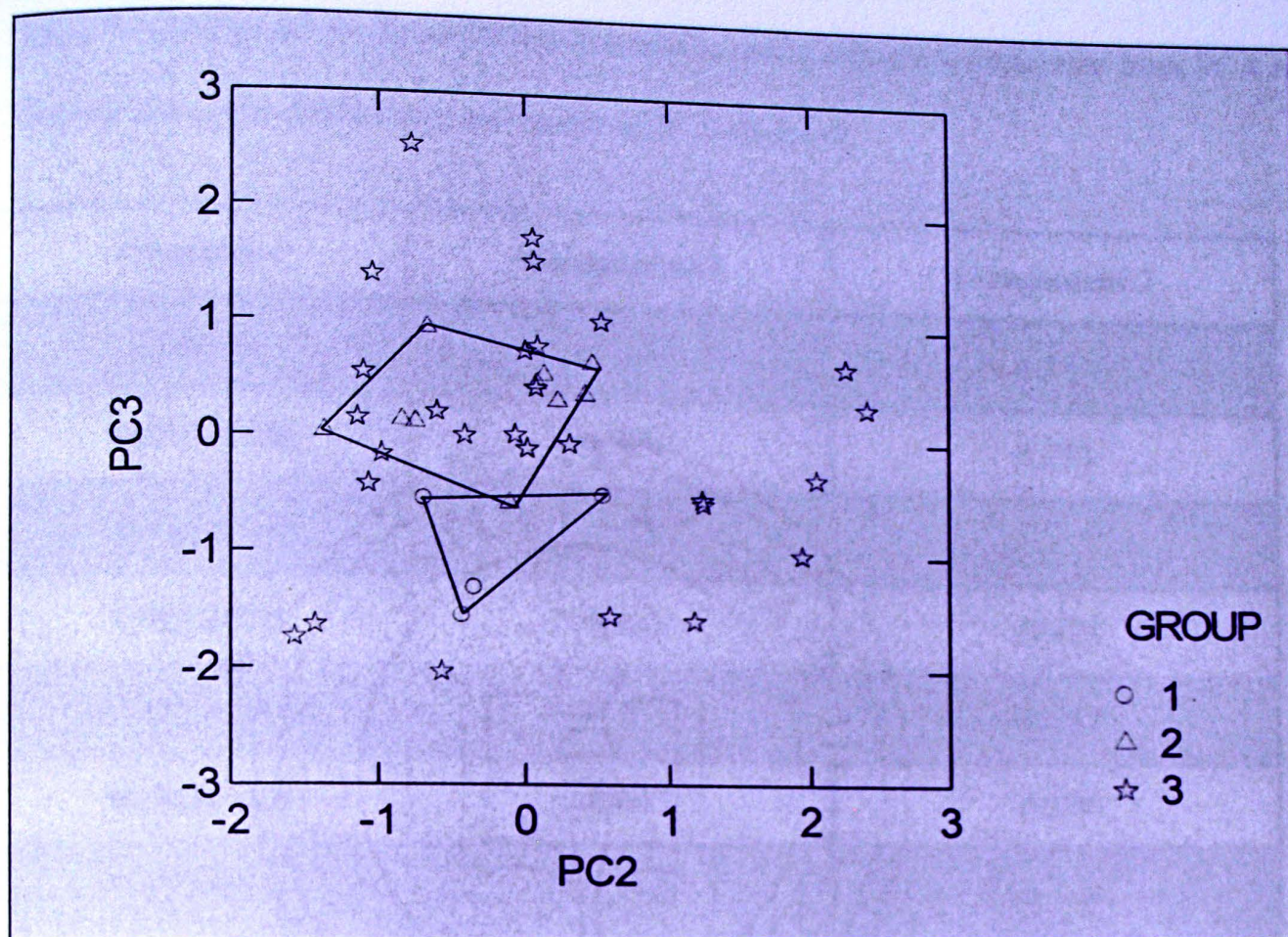


Figure 8. Principle Component Analysis of the continuous data from the morphological analysis of *P. canadensis*.

Results of the Principle Component Analysis of discrete data show that a combination of characters separate Group 1 from Group 2 and Group 2 from Group 3, however, no combination of characters separate Group 1 from Group 3. Table 9 summarizes the component loading scores for the first and second principle components from PCA of the discrete data. The characters with the highest component loading scores from component 1 are MSULPUB, MSLLPUB, USLLPUB, and MSLDENT. The characters with the highest component loading scores from component 2 are MSPUB, and SPHYLLPUB. These characters had high component loading scores (> 0.5), meaning that they are driving the separation seen on the scatter plot (Fig. 9).

Table 9. Component loadings for the first and second principle components from PCA of discrete data from herbarium specimens of *P. canadensis*.

Character	Component 1	Component 2
MSPUB	0.348	0.582
MSULPUB	0.799	0.253
MSLLPUB	0.816	0.159
USLLPUB	0.662	-0.423
SPHYLLPUB	-0.003	-0.757
NUMRAYS	-0.047	0.299
MSLDENT	0.594	-0.409

A scatter plot of the factor scores of the first and second principle components of the discrete data (Fig. 9) illustrates clear separation between Group 1 and Group 2 (represented by the blue circles and red triangles) along PC2. This indicates that the variables that loaded most heavily on PC2 (MSPUB and SPHYLLPUB) will be the most useful combination of discrete characters for distinguishing those groups. The scatter plot also illustrates near complete separation between Group 2 and Group 3 (represented by the black stars) along PC2, indicating that MSPUB and SPHYLLPUB will be the best combination of characters for distinguishing these groups as well. The scatter plot illustrates no separation between Groups 1 and 3, which makes it important to rely on a combination of quantitative and qualitative data to distinguish these two groups from each other.

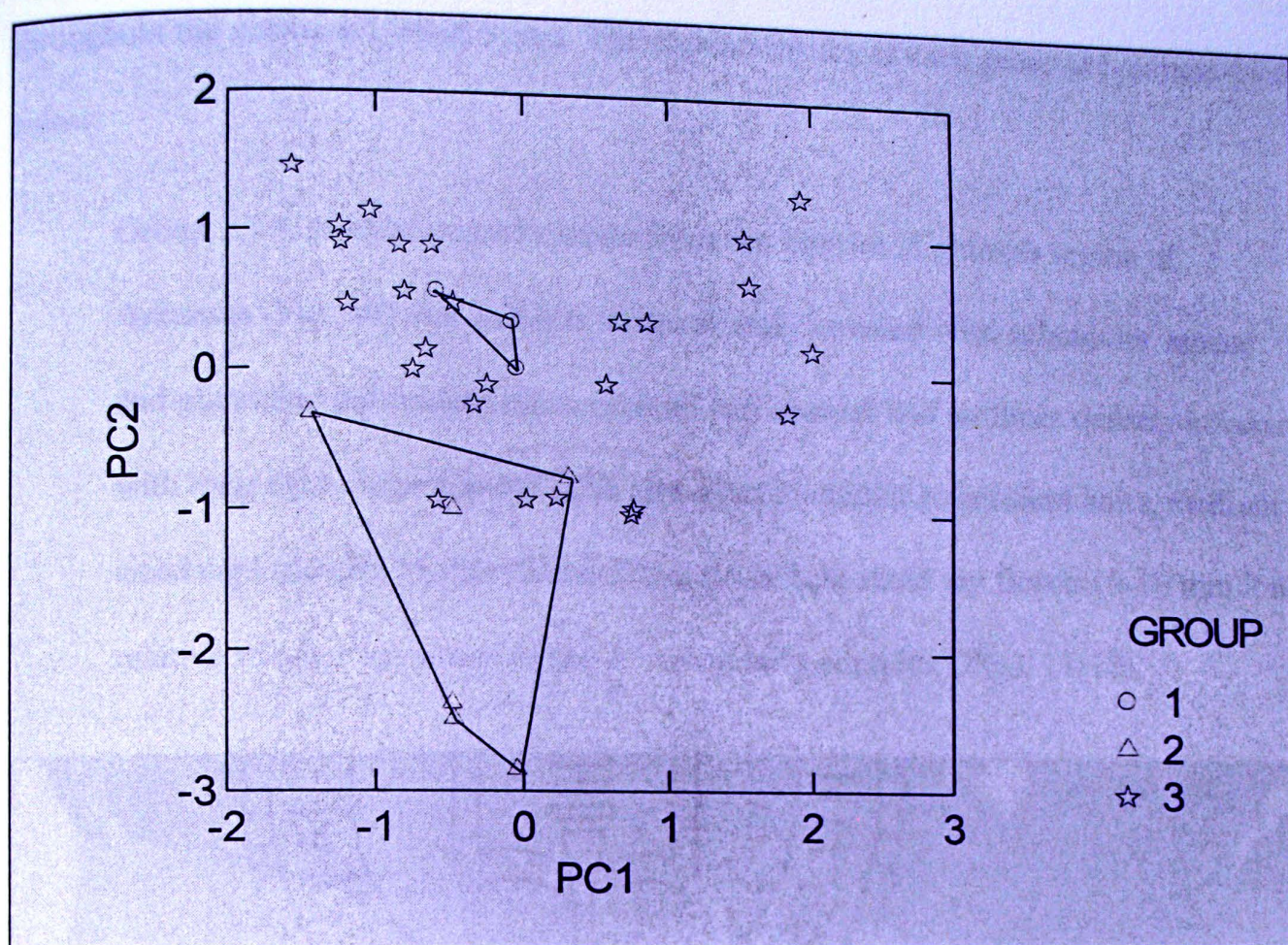


Figure 9. Principle Component Analysis of the discrete data from the morphological analysis of *P. canadensis*.

Geography—Results from the examination of the geography of *P. canadensis* support the hypothesis that there are at least three morphologically distinct entities within this species complex. Results also indicate that the three groups determined through morphological and statistical analyses are geographically separated and that this separation is reliable in that based on the combination of floral and vegetative characters present, the geographic origin of a given specimen can be reliably predicted.

The specimens from Group 1 originate from the Interior Highlands region of Arkansas. The specimens grouped within Group 2 originate from the Highland Rim of Tennessee. The specimens grouped within Group 3 originate from a broader range

throughout the northern United States. The characteristics of each group are summarized below:

Group 1.) A morphological variant from the Interior Highlands region of Arkansas (Fig. 10) that exhibits stems densely covered with eglandular septate and glandular-puberulent hairs, adaxial and abaxial leaf surfaces densely covered with long eglandular septate hairs and short glandular puberulent hairs, medium sized capitula (10-15 mm diameter), and medium sized ray florets (5-10 mm long) relative to other members of the *P. canadensis* complex (Figs. 11-12).

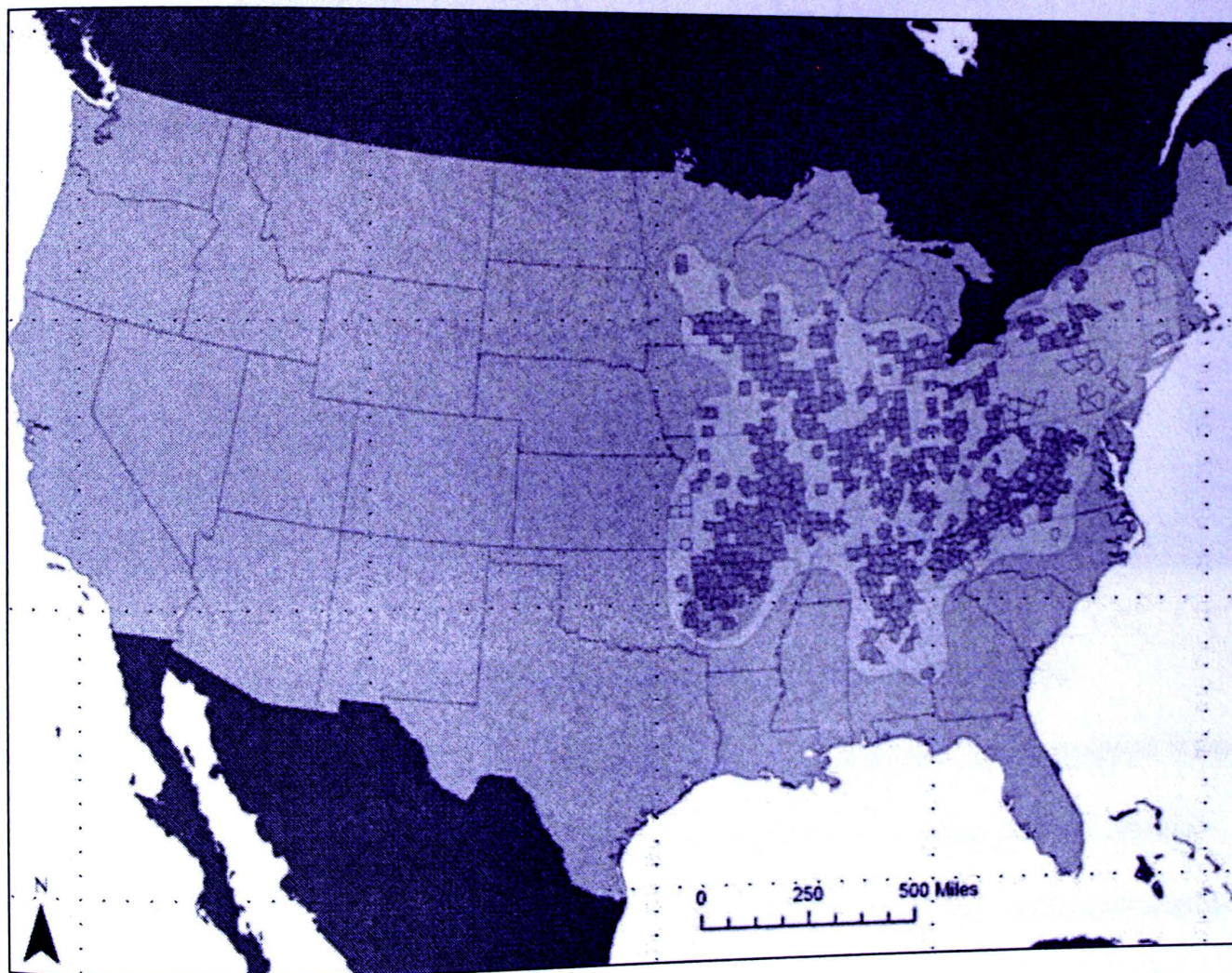


Fig. 10. Distribution map of the morphological entity from the Interior Highlands region (Group 1).

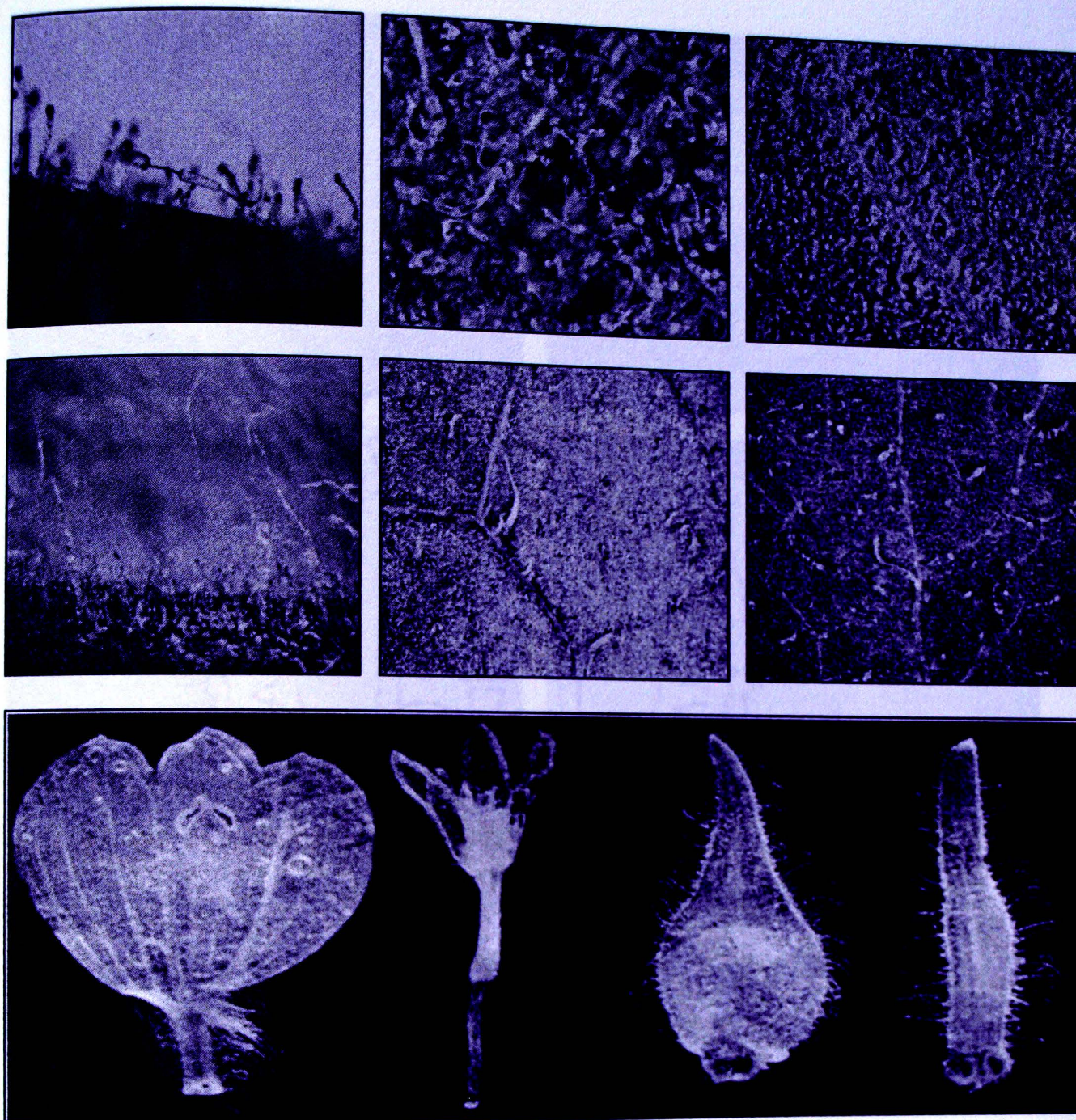


Fig. 11. Photographs of the morphological characters used to distinguish the morphological entity from the Interior Highlands region. Characters photographed from left to right: Row 1: Mid-stem pubescence, abaxial surface of a mid-stem leaf, abaxial surface of an upper stem leaf. Row 2: Upper stem pubescence, adaxial surface of a mid-stem leaf, adaxial surface of an upper stem leaf. Row 3: Ray floret, disc floret, wide phyllary, thin phyllary. Note: photographs not to scale. *Beer 93 UARK.*



Fig. 12. Photographs of live plants of the morphological entity that is distributed throughout the Interior Highlands region of the United States. Photographs were taken at Rich Mountain, AR by D. Estes.

Group 2.) A morphological variant from the Highland Rim of Tennessee (Fig. 13) with glabrous stems, small-medium sized capitula (5-15 mm), and medium sized ray florets (5-10 mm long) relative to other members of the *P. canadensis* complex (Figs. 14-15).

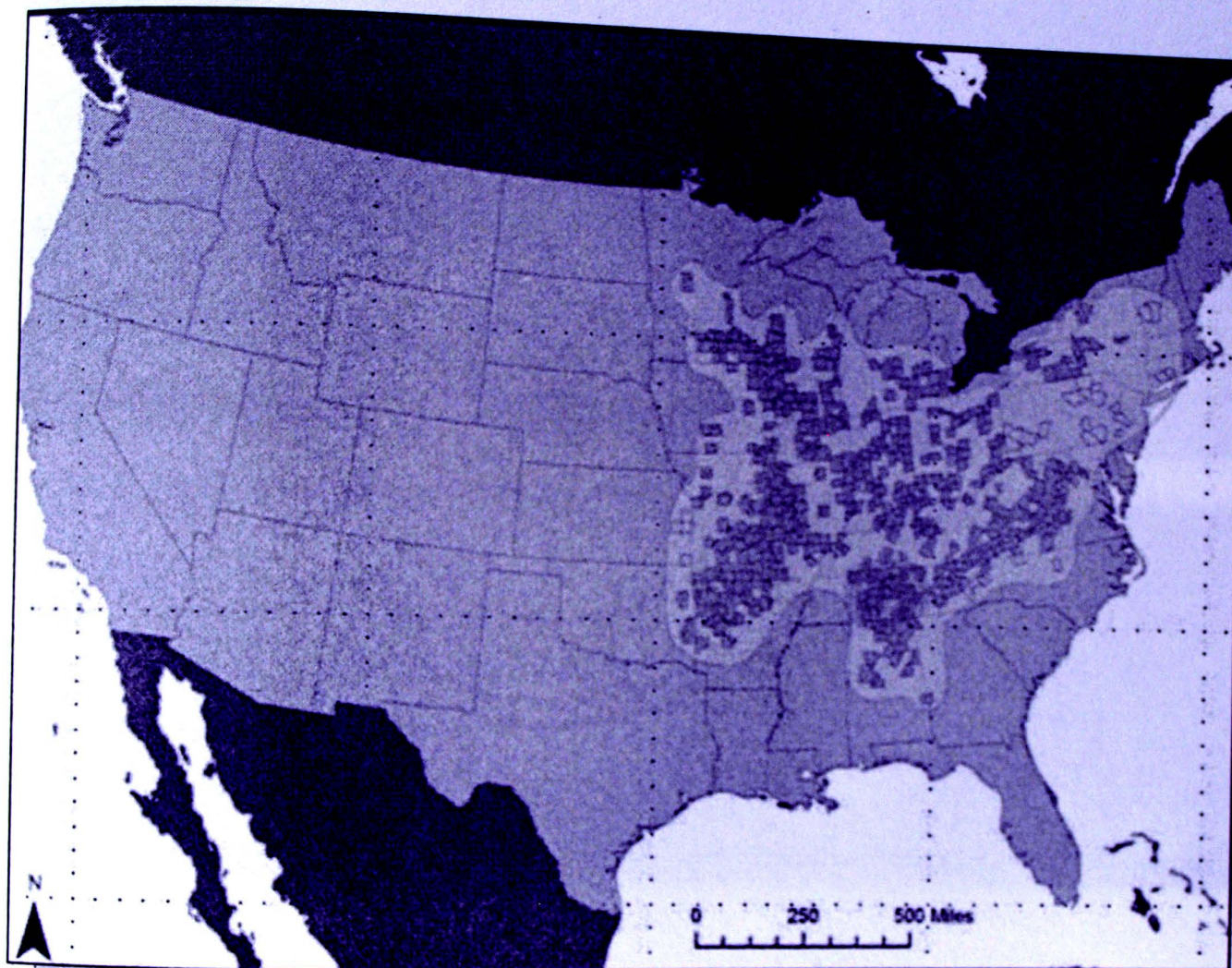


Fig. 13. Distribution of the morphological entity from the Highland Rim of Tennessee (Group 2).

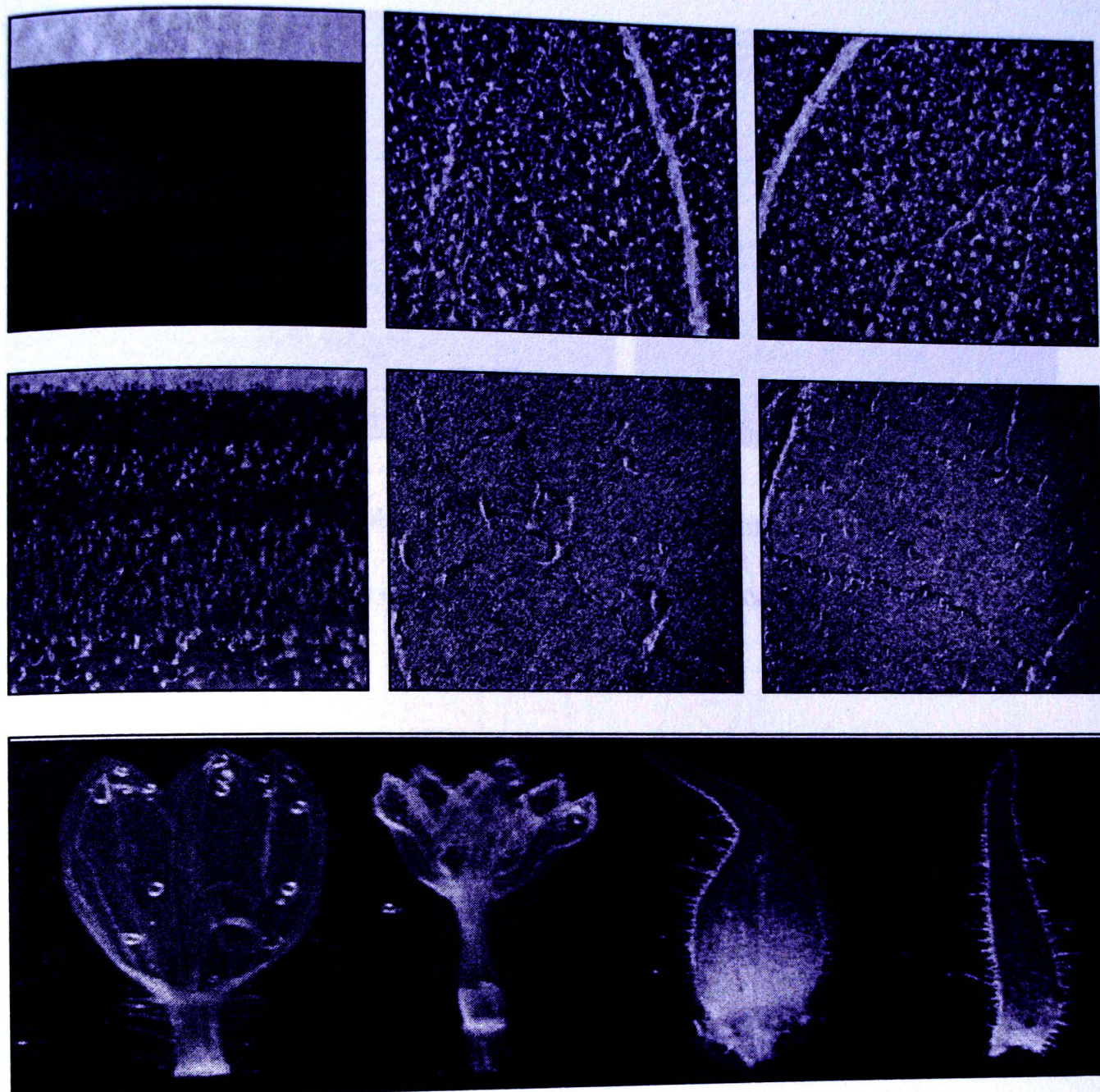


Fig. 14. Photographs of the morphological characters used to distinguish the morphological entity from the Highland Rim of Tennessee. Characters photographed from left to right: Row 1: Mid-stem pubescence, abaxial surface of a mid-stem leaf, abaxial surface of an upper stem leaf. Row 2: Upper stem pubescence, adaxial surface of a mid-stem leaf, adaxial surface of an upper stem leaf. Row 3: Ray floret, disc floret, wide phyllary, thin phyllary. Note: photographs not to scale. *Estes 03677* ASPC.

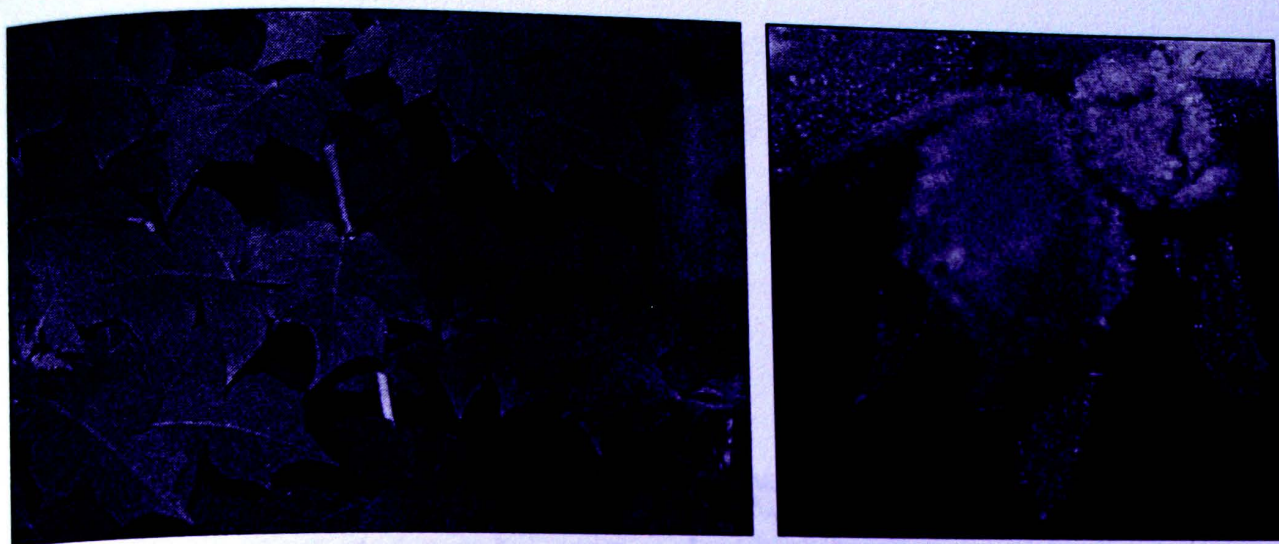


Fig. 15. Photographs of live plants of the morphological entity that is distributed throughout the Highland Rim of Tennessee. Photographs were taken in Cheatham County by D. Estes.

Group 3.) A morphological type with mostly eglandular septate hairs on the stems, eglandular septate hairs on the abaxial leaf surfaces as well as the adaxial leaf surfaces, small-medium heads (5-15 mm diameter), and small or absent ray florets (3-5mm or less in length) (Figs. 17-18) that is widespread in the northern United States and in southern Ontario, Canada (Fig. 16).

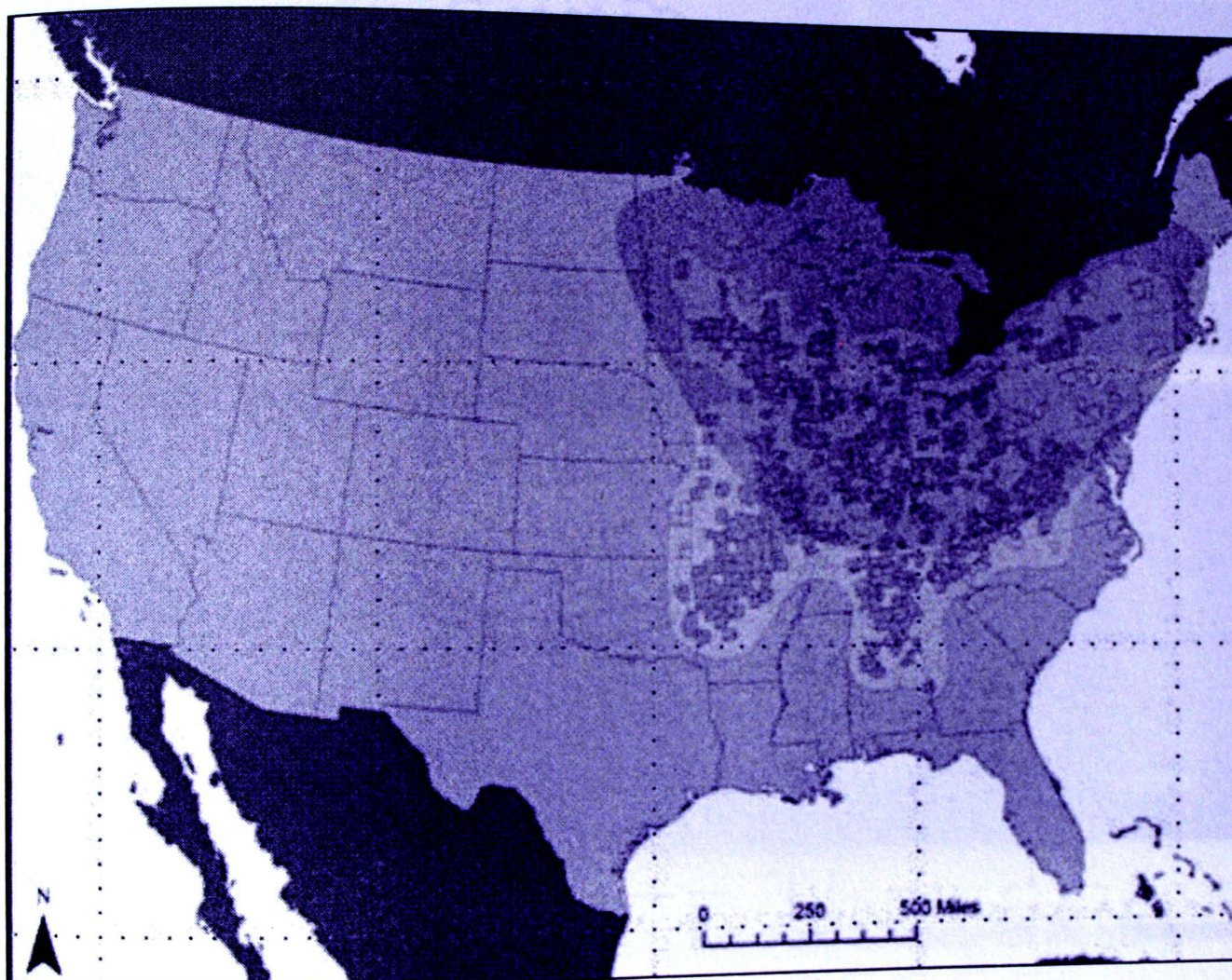


Fig. 16. Distribution map of the widespread morphological entity (Group 3).

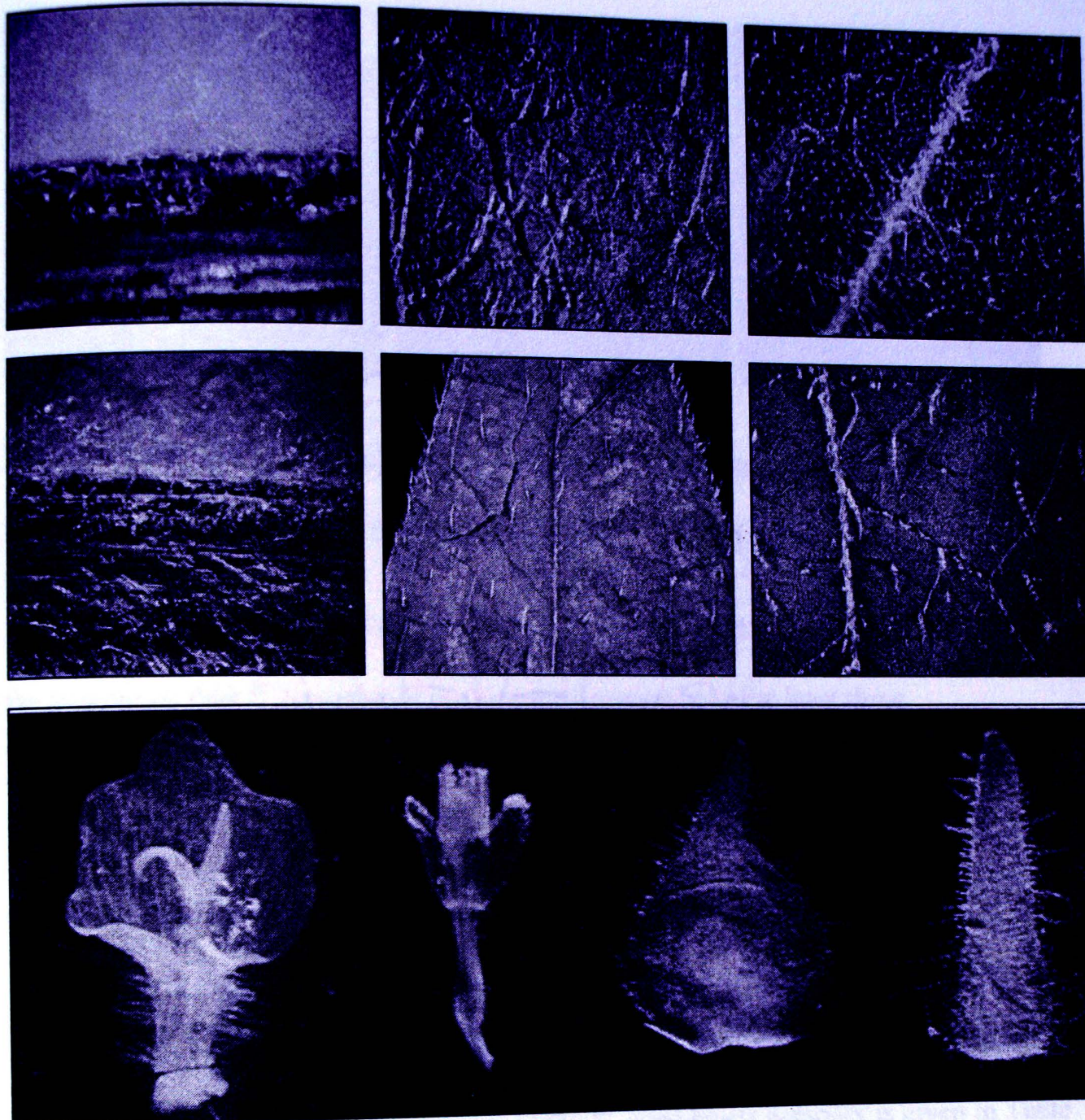


Fig. 17. Photographs of the morphological characters used to distinguish the widespread morphological entity. Characters photographed from left to right: Row 1: Mid-stem pubescence, abaxial surface of a mid-stem leaf, abaxial surface of an upper stem leaf. Row 2: Upper stem pubescence, adaxial surface of a mid-stem leaf, adaxial surface of an upper stem leaf. Row 3: Ray floret, disc floret, wide phyllary, thin phyllary. Note: photographs not to scale. *Hartley 736 WIS.*

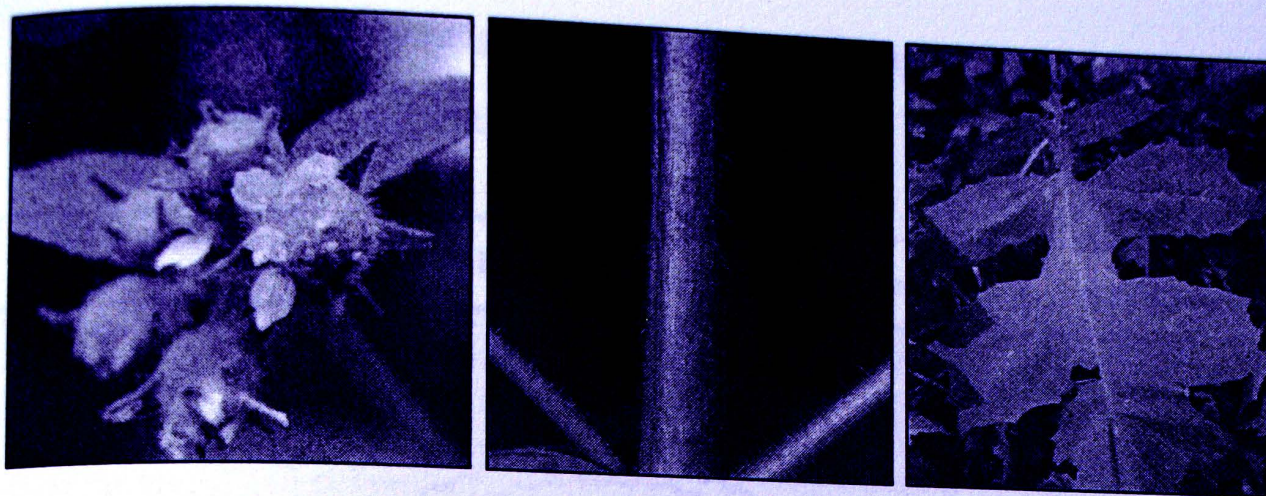


Fig. 18. Photographs of live plants of the widespread morphological entity. Photographs were taken at the Mississippi Palisades in Carroll County, IL by D. Estes.

Results of analyses of morphological, statistical, and geographical data support the hypothesis that *P. canadensis* is a complex comprised of at least three morphologically distinct entities. Two of the distinct forms are restricted to specific geographic regions, while the third form has a widespread distribution throughout the northern United States. Morphological and statistical data support the presence of these entities and illustrate distinction between each. The geographical data shows geographic separation between the distinct morphological groups. A summary of the morphology and geography used to distinguish members of the *P. canadensis* complex is given in Table 10.

Table 10. Summary of the geography and morphology of the distinct morphological groups determined through critical morphological analysis and PCA.

Geographic Distribution	Stem Pubescence	Leaf Pubescence	Ray Length
Interior Highlands (Group 1)	Long eglandular septate hairs with a dense glandular-puberulent underlayer.	Long eglandular septate hairs and short glandular-puberulent hairs on upper and lower surfaces.	Medium 3-10 mm $\bar{x} = 6.0$ mm
Highland Rim (Group 2)	Glabrous	Upper surface with eglandular septate hairs and lower surface with a mixture of glandular-puberulent and eglandular septate hairs.	Medium 4-11 mm $\bar{x} = 7.6$ mm
Widespread northern U.S. (Group 3)	Long eglandular septate hairs with glandular-puberulent hairs lacking to forming a dense underlayer.	Eglandular septate hairs scattered on both upper and lower leaf surfaces.	Small Absent-7 mm $\bar{x} = 3.6$ mm

CHAPTER IV

Discussion

Conclusions-- Data obtained from morphological, geographical, and statistical analyses supported the hypothesis that *P. canadensis* is a species complex composed of several species or subspecies. Three morphological forms have been identified within *P. canadensis*. Two of these forms are restricted to specific physiographic regions, the Interior Highlands (including Ozark and Ouachita Mountains) and Highland Rim of the Interior Low Plateau. The third form is more widespread, and occurs throughout a broad range in the northern United States. While further research is necessary to determine the driving force behind this morphological divergence and to describe and diagnose taxa, these findings provide valuable insight with regard to the observed taxonomic confusion and morphological variability within this species.

A distinct morphological entity distributed throughout the Interior Highlands of the United States (Fig. 10) is of interest because this region is proposed to have been an important refugium for eastern North American species during the Pleistocene glaciation (Redfearn 1986, Zamudio and Savage 2003). The Interior Highlands (Ozark-Ouachita Highlands) is a distinct physiographic region located mostly in Arkansas, but also extending to parts of Missouri and Oklahoma. This region is underlain by substrates of Paleozoic age, which are mostly composed of dolomite, limestone, sandstone, and chert (McFarland 1998) and is characterized by hilly to mountainous topography dominated by hardwood forests, however, it also includes some prairie habitat in southern Missouri (Foti and Bukenhofer 1998, Walker 1999).

This area is known to be inhabited by at least 36 endemic vascular plant taxa, as well as numerous endemic fauna (Mayden 1985, Redfearn 1986, Zollner et al. 2004). Of the 36 endemic vascular plant taxa identified from the Interior Highlands, 24 are endemic species while 12 are endemic subspecies or varieties of a more widely distributed species (Zollner et al. 2004). Given this information, it is suggested that the morphological entity identified in this study could possibly be an example of an Interior Highlands endemic taxon.

A distinct morphological form restricted to the Highland Rim (Fig. 13) is significant due to the relatively high levels of biological diversity observed in this area. The Highland Rim is a level IV ecoregion located within the Interior Low Plateau of Tennessee. This region is underlain by Mississippian-aged limestone, chert, and shale (Omernik 1987, DeSelm 1990) and is characterized by rolling hills, tablelands, and irregular plains (Omernik 1987). Although this area has been heavily impacted by past logging and mining operations (Omernik 1987, Hart et al. 2008), it continues to support a particularly diverse array of plant and animal species (Etnier and Starnes 1993, DeSelm 1990).

The Highland Rim is known for its high levels of biodiversity, and is inhabited by a significant number of endemic animal taxa (Etnier and Starnes 1993). This region is known to have one of the most diverse fish faunas in North America (Etnier and Starnes 1993), as well as high numbers of native amphibians, reptiles, crayfish, mussels, and snails (Isom et al. 1973, Miller, et al. 2005, Buhay et al. 2006). In addition to its highly diverse fauna, the Highland Rim is inhabited by at least 2,000 native vascular plant taxa (DeSelm 1990, Chester 1993, Estes and Walck 2005).

While the flora of this region is highly diverse, plant species strictly endemic to the Highland Rim are generally lacking. The distribution of this new entity is centered around the Central Basin, which was identified by Estill and Cruzan (2001) as one of the top centers of endemism in the Southeast. Many of the populations of this entity are located on the dissected escarpment of the Highland Rim in the transition zone to the Central Basin. This information suggests that the morphological form identified in this study is likely found in adjacent provinces such as the Central Basin or Cumberland Plateau.

The distinct morphological entity found to be widespread in the northern United States is distributed throughout a large area that was glaciated during the Pleistocene. During the Pleistocene, a large portion of North America experienced glaciation and therefore destruction of vegetation (Pielou 1991, Cox and Moore 2000). This pushed species into refugia south of the glacial boundary (i.e. Interior Highlands, Appalachian Mountains, etc). Post-glaciation there was a rapid recolonization of these areas by leading edge populations from the northern range of the refugia (Hewitt 2000). It is well documented that this rapid expansion over a long route lead to the loss of genetic diversity in these re-invading species (Lewis and Crawford 1995, Broyles 1998, Hewitt 2000, Griffin and Barrett 2004). This could explain the widespread distribution of the entity common in the northern United States in comparison to the more narrow distributions of the forms south of the last glacial maximum.

The variation observed in the widespread northern entity could also be significant in light of patterns of glaciation. Populations identified in southwest Wisconsin are located in the "Driftless Zone", a large region that was never glaciated and is very similar

to middle Tennessee and Kentucky geologically. This indicates that environmental conditions such as temperature, moisture, and sunlight availability could be driving the morphological divergence within this species.

The variants from the Interior Highlands and Highland Rim exhibited the tightest clustering in the PCA analyses and the most narrow ranges in the geographical analysis. This suggests that these entities have less morphological variation in comparison to the wide-ranging entity. It is possible that the tight clustering is an artifact of small sample size of the two southern groups, however specimens included in the analyses were from multiple localities. It is of interest that the two groups with the narrow geographic distributions are from the Interior Highlands and the Interior Low Plateau as these are areas known to have been important glacial refugia during the Pleistocene and harbor a relatively high concentration of endemic species (Estill and Cruzan 2001). Further phylogeographic analyses are needed to determine the complete distributional history of *P. canadensis*, however these results indicate that past climactic events play a large role in the current distribution of these distinct morphological entities.

The distribution patterns of the three morphological types identified in this study along with the wide morphological variability exhibited by the widespread entity suggest that *P. canadensis* re-colonized the glaciated region of North America from several southern refugia as the glaciers receded. There are many examples of this recolonization pattern in both plants and animals. Cywnar and MacDonald (1987) inferred from reduced allelic diversity and greater differentiation in northern populations than in southern populations of lodgepole pine (*Pinus contorta*) that this species underwent multiple long-distance founding events post-glaciation. In an examination of allozyme diversity in

Asclepias exaltata, Broyles (1998) proposed that a loss of rare alleles in northern regions of the United States may have resulted from several northward migrations from southern Appalachian refugia. A similar scenario is observed in the western North American spotted frog (*Rana pretiosa*) complex, which was determined to be comprised of two distinct species through analyses of allozyme variation and morphometrics (Green et al. 1996). In the case of the *Rana pretiosa* complex, one species had a distribution restricted to western Oregon and southwest Washington while the other species was more widespread throughout the western United States (Green et al. 1996). It was found that northern populations of the widespread species exhibited reduced allelic diversity and heterozygosity in comparison to southern populations (Green et al. 1996). While it is likely that the distribution patterns of the three morphological entities within *P. canadensis* identified in this study are a result of the recolonization of glaciated regions of North America from several southern refugia, further research including genetic analyses are needed to fully determine the evolutionary history of this species complex.

The results of this study provide valuable insight into the taxonomic confusion and morphological variability of *P. canadensis*. Early taxonomists recognized the presence of a long-rayed type (Gray 1884, Small 1903, Fassett 1932) as well as a type with absent-minute rays (Gray 1881) within this species. Since these early studies, *Polymnia* has largely been ignored by taxonomists, most likely due to the difficulties associated with its large, robust habit. While further research is needed to formally diagnose taxa, the current study provides a baseline data set for summarizing morphological variation in this complex and delineating the variation described by these

earlier works. It is expected that through further study of this complex, additional distinct morphological entities will be revealed.

Future Directions— Future directions of this research include expanding the sample size to 100-150 specimens, and collecting and examining specimens from areas where herbarium specimens are lacking. This includes sampling large rayed populations that fit the concept of *P. canadensis* var. *radiata*. After the sample size is increased, further statistical analyses could aid in delineating other distinct morphological entities within this species, and determining the level of taxonomic recognition of these groups. Future plans also involve analyzing populations across the entire geographic range using microsatellites and amplified fragment length polymorphism (AFLP) methods.

In addition to this research, there are several aspects of *Polymnia* systematics and ecology worth investigation. One aspect of *Polymnia* systematics that has important implications regarding the origin of one species and requires further investigation is the presence of naturally occurring hybrids between species, and the implications of any natural hybrids. The two species for which natural hybridization is most probable are *P. canadensis* and *P. laevigata*, as they have been found to occur syntopically in at least two sites (Gorman, pers. obs.). Naturally occurring hybrids between *P. canadensis* and *P. laevigata* could have broad implications regarding the understanding of the evolutionary origin of the newly described taxon, *P. johnbeckii*. There are two possible evolutionary pathways for the origin of *P. johnbeckii*: standard cladogenesis or homoploid hybrid speciation (Estes and Beck 2011). Natural hybrids found to possess morphology that is distinct from *P. johnbeckii* would support the null hypothesis that *P. johnbeckii* evolved as a result of standard cladogenesis, or phenotypic and genetic divergence due to physical

isolation or via adaptation to an alternate environment (Estes and Beck 2011). Naturally occurring hybrids found to have morphology that is similar to *P. johnbeckii* would imply homoploid hybrid speciation as the evolutionary pathway of origin of this species.

In addition to these systematic issues, the current status of the endemic *P. johnbeckii* should be investigated to help promote and assist conservation efforts for this species. Due to the recent description of *P. johnbeckii*, little is known of its ecological preferences and population size. To thoroughly document the ecological preferences of this species, a status survey including documentation of associated species, analysis of soil samples from the root zones of populations, and a census of the species are needed.

Future directions in *Polymnia* research include answering the following questions regarding taxonomic, ecologic, and biogeographic relationships within this genus:

- 1) What is the taxonomic status of *P. canadensis* populations that fit the concept of *P. canadensis* var. *radiata*?
- 2) Do *P. laevigata* and *P. canadensis* interbreed to produce naturally occurring hybrids?
- 3) If so, what do naturally occurring hybrids between *P. laevigata* and *P. canadensis* indicate about the origin of *P. johnbeckii*?
- 4) What is the current status of the newly described and endangered *P. johnbeckii*?

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APPENDICES



APPENDIX A

Explanation of State and Global Ranks

STATE RANK (TDEC 2008, p. iv)

The state rank is a numeric rating (S1 through S5) of relative rarity based primarily on the number of occurrences of the plant in the state. The state and global ranks are non-legal ranks and only indicate the rarity of a species. Other factors in addition to the number of occurrences are considered when assigning rank, so the number of occurrences suggested for each numeric rank below is not a hard and fast rule.

S1– Extremely rare and critically imperiled in the state with five or fewer occurrences, or very few remaining individuals, or

because of some special condition where the species is particularly vulnerable to extirpation from Tennessee.

S2– Very rare and imperiled within the state, six to twenty occurrences and less than 3000 individuals, or few remaining

individuals, or because of some factor(s) making it vulnerable to extirpation from Tennessee.

S3– Rare and uncommon in the state, from 21 to 100 occurrences.

S4– Widespread, abundant, and apparently secure within the state, though it may be quite rare in parts of its range, especially at

the periphery, and is of long-term concern.

SH– Of historical occurrence in Tennessee, i.e., known to occur in Tennessee in the past, with the expectation that it may be

rediscovered.

SU– Possibly in peril in Tennessee but status uncertain, need more information.

SX– Believed to be extirpated from Tennessee, with virtually no likelihood that it will be rediscovered.

S#S#– Denotes a range of ranks because the exact rarity of the element is uncertain (e.g., S1S2)

S?– Unranked within the state.

GLOBAL RANK (NatureServe 2012)

Global ranks are determined by the scientific staff of NatureServe, the non-governmental organization of national, state and provincial heritage programs. Global ranks provide the best available and objective assessment of a rare plant's rarity and the level of threat to its existence. The total number of individuals, the number of populations, and the threats to the populations are considered throughout the plant's range.

G1– Extremely rare and critically imperiled, generally with five or fewer occurrences in the world, or very few remaining individuals, or because of some special condition the species is particularly vulnerable to extinction.

G2– Very rare and imperiled, generally with six to twenty occurrences and less than 3000 individuals, or because of some factor(s), vulnerable to extinction.

G3– Very rare and local throughout its range or found locally in a restricted range, or, because of other factors, vulnerable to extinction throughout its range. Generally between 21 and 100 occurrences and fewer than 10,000 individuals.

G4– Apparently secure globally, though it may be quite rare in parts of its range, especially at the periphery. Thus, the plant is of long-term concern.

G5– Demonstrably secure globally, though it might be quite rare in parts of its range, especially at the periphery.

GH– Of historical occurrence throughout its range, i.e., formally part of the established biota, with the expectation that it may be rediscovered.

GU– Possibly in peril range-wide but status uncertain, need more information.

GX– Believed to be extinct throughout its former range, with virtually no likelihood that it will be rediscovered.

G#Q– Taxonomic status is questionable, numeric rank may change with taxonomy.

G#?– Inexact numeric rank.

Specimen	Physiographic Province	State	County
<i>Alcorn 662 TENN</i>	Interior Low Plateaus	TN	Sumner
<i>Bailey 8-20-1894 MICH</i>	Central Lowland	MI	Kent
<i>Beck 9273 TENN</i>	Interior Low Plateaus	TN	Wilson
<i>Browne 4617 BRIT</i>	Interior Low Plateaus	KY	Grant
<i>Chase 13485 BRIT</i>	Central Lowland	IL	Tazewell
<i>Cichan 77/195 TENN</i>	Ridge and Valley	WV	Hampshire
<i>Clebsch 881B ASPC</i>	Interior Low Plateaus	TN	Montgomery
<i>Cronquist 3997 BRIT</i>	Central Lowland	OH	Highland
<i>Deam 6-22-1905 MICH</i>	Central Lowland	IN	Wells
<i>Demaree 11524 BRIT</i>	Central Lowland	OH	Greene
<i>DeSelm 746 ASPC</i>	Interior Low Plateaus	TN	Rutherford
<i>Dreisbach 1087 MICH</i>	Central Lowland	OH	Ross
<i>Ellis 000675 ASPC</i>	Interior Low Plateaus	TN	Stewart
<i>Ellis 28931 TENN</i>	Appalachian Plateaus	TN	Anderson
<i>Estes 02718 TENN</i>	Interior Low Plateaus	TN	Maury
<i>Estes 03537 APSC</i>	Interior Low Plateaus	TN	Maury
<i>Estes 03677 ASPC</i>	Interior Low Plateaus	TN	Giles
<i>Estes 03723 ASPC</i>	Ridge and Valley	TN	Loudon
<i>Estes 03736 ASPC</i>	Interior Low Plateaus	TN	Lawrence
<i>Estes 09372 ASPC</i>	Appalachian Plateaus	TN	Cumberland
<i>Fassett 12641 WIS</i>	Central Lowland	WI	Grant
<i>Fassett 15219 WIS</i>	Central Lowland	WI	Dodge
<i>Fassett 7-16-34 WIS</i>	Central Lowland	WI	Brown
<i>Fritsch 1162 MICH</i>	Central Lowland	MI	Hillsdale
<i>Gunn SG786 ASPC</i>	Interior Low Plateaus	TN	Humphreys
<i>Hartley 736 WIS</i>	Central Lowland	WI	La Crosse
<i>Hermann 8436 MICH</i>	Central Lowland	IN	Vermillion
<i>Hinds 399 MU</i>	Central Lowland	OH	Warren
<i>Hyatt 1560.03 UARK</i>	Ozark Plateaus	AR	Baxter
<i>Johnson 7-12-1994 MICH</i>	Canadian Shield	ON	Grey
<i>Jones 1459 MU</i>	Central Lowland	OH	Huron
<i>Koelling 3370 TENN</i>	Central Lowland	IL	Monroe
<i>Kral 10966 VPI</i>	Appalachian Plateaus	VA	Smyth
<i>Kral 11484 VPI</i>	Appalachian Plateaus	VA	Montgomery
<i>Kral 47586 MICH</i>	Interior Low Plateaus	TN	Franklin

Kron 836 MICH	Central Lowland	MI	Berrien
Lisowski 161 MU	Central Lowland	IL	Vermillion
Lyon 7-4-1930 MICH	Central Lowland	IN	Kosciusko
McCormac 4854 MU	Appalachian Plateaus	OH	Lawrence
McFarland 3227 MICH	Interior Low Plateaus	KY	Jessamine
Mikula 7325 BRIT	Piedmont	VA	Madison
Minteer 226 UARK	Ozark Plateaus	AR	Washington
Nunn 6299 UARK	Ozark Plateaus	AR	Fulton
Pammel 8-18-1925 MICH	Central Lowland	IA	Winneshiek
Raven 26790 MICH	Ozark Plateaus	MO	Jefferson
Reznicek 6341 MICH	Canadian Shield	ON	Essex
Rill 3863 MU	Central Lowland	WI	Winnebago
Schmidt 1951 WIS	Central Lowland	WI	Clark
Stewart 89-174 UARK	Ozark Plateaus	AR	Madison
Steyermark 72325 BRIT	Central Lowland	MO	Lincoln
Sutton 824 MICH	Central Lowland	MI	Oakland
Tamulevich 30 WIS	Central Lowland	WI	Grant
Thomas 16458 BRIT	Ozark Plateaus	AR	Independence
Vincent 3130 MU	Central Lowland	OH	Clermont
Voss 7961 MICH	Central Lowland	MI	Washtenaw